Longitudinal Monitoring of Physiological, Psychological and Molecular Responses to Different Dietary Interventions: A Precision Health Approach

by

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Abstract

Diet and lifestyle choices play a crucial role in determining overall health and well-being. As chronic diseases become more prevalent and the increasing recognition of individual variability in response to dietary interventions, personalized approaches to healthcare are becoming increasingly important. Precision nutrition (PN) is a branch of nutrition science which aims to provide precise, customized dietary guidance based on an individual's needs, physiology, and molecular characteristics. However, PN has yet to deliver on its promise. By incorporating wearable devices (for physiological measurements), objective psychological testing and advanced, quantitative omics methods such as metabolomics, proteomics, genomics, and microbiomics (for molecular measurements), that it will be possible to deliver on the promise of PN.

The primary goal of this thesis is to investigate the feasibility of using wearable devices for physiological monitoring, objective psychological testing, and advanced, quantitative omics methods to enable precise characterization of the molecular, psychological, and physiological consequences of different diets. The secondary goals are to investigate the feasibility of N-of-1 self-monitoring and biosample self-collection for conducting PN studies, the costs associated with self-monitoring for PN purposes, the utility of daily mental and physical performance monitoring in providing useful physiological and psychological data, the relevance of quantitative omics data for guiding molecular-level analysis and interpretation, and the most effective data analysis techniques for interpreting dietary N-of-1 studies. To achieve these objectives, I utilized a comprehensive methodology involving multiple monitoring and testing systems along with well-defined, carefully controlled dietary interventions. The study included four different diets: a Fast Food Diet (FFD), a Mediterranean Diet (MD), a Ketogenic Diet (KD), and a Regular Diet (RD)

for two weeks. I also conducted detailed molecular-scale omics analyses, including metabolomics, proteomics, genomics, and gut microbiome profiling using blood, fecal and urine samples collected throughout the study period. Additionally, physiological data from various wearable devices measuring blood glucose (BG), body temperature (BT), heart rate (HR), and objective psychological test data were also collected.

The results of the study illustrate the tight relationship between diet, physiological health, psychological health, the gut microbiome, the proteome, and the metabolome. It was found that dietary changes led to differences in omic outcomes within a relatively brief timeframe. This rapid response highlights the value of quantitative omics methods in assessing the impact of diet and lifestyle choices on health. The integration of wearable device data (for quantitative physiological data), with objective psychological testing with omics measurements (for quantitative molecular-scale data) revealed several expected and unexpected connections. These results demonstrate that this combined approach could be helpful when implementing or designing a PN intervention.

The study also demonstrated that N-of-1 self-monitoring and biosample self-collection are feasible. Using the recent advancement in at-home sample collection kits and the possibility of sample shipment, sample collection and storage can be done with minimal training. Additionally, self-monitoring for PN purposes can be done inexpensively. While initial investments in wearable devices and biosample collection kits are required, the long-term benefits of personalized health insights outweigh the costs. The demonstrated cost-effectiveness makes PN accessible to a wider population, which may promote access to more equitable healthcare. Daily monitoring of mental and physical performance provided valuable data that complemented the physiological measurements. Metrics such as mood, cognitive function, and physical activity levels offered additional dimensions of health assessment, enriching the overall understanding of dietary impacts.

Quantitative omics data proved to be highly relevant and useful for guiding the analysis and interpretation of N-of-1 nutrition studies. In particular, metabolomics provided detailed insights into the metabolic shifts induced by different diets. This information was invaluable in identifying biomarkers of dietary response and can be used to facilitate personalized dietary recommendations. Various data analysis techniques were explored to interpret the complex datasets generated from N-of-1 studies. The large amount of data obtained from multiple wearable devices and omics measurements made the analysis quite challenging. The study highlighted the need for strong analytical methods to properly interpret and make use of the measured data.

Preface

This thesis is an original work by Dorsa Yahya Rayat. No part of this thesis has been previously published. The project was conducted in accordance with the ethical standards of, and approved by, the University of Alberta's Human Research Ethics Board (HREB) biomedical ethics committee (HREB #Pro00080942). I was responsible for data collection and analysis, as well as writing this thesis. Dr. David S. Wishart contributed philosophical, conceptual, and technical guidance and edited this thesis.

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As I conclude this chapter of my life, I am filled with gratitude for everyone who has supported me and look forward to the future with excitement and anticipation.

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2-HMBA	2-hydroxy-2-methylbutyric acid
3-NPH	3-Nitrophenylhydrazine
5-HT	5-Hydroxytryptamine
5-HTP	5-Hydroxytryptophan
AC	Acylcarnitine
ACE	Angiotensin-Converting Enzyme
ADG	Australian Dietary Guidelines
ADMA	Asymmetric Dimethyl Arginine
AGAT	L-Arginine: Glycine Amidinotransferase
AGEs	Advanced Glycation End Products
AHR	Aryl Hydrocarbon Receptor
AI	Adequate Intake
ALT	Alanine Aminotransferase
AMD	Age-Related Macular Degeneration
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
AP	High-Animal Protein
ASA24	The Automated Self-Administered 24-Hour Dietary Assessment Tool
BAIBA	3-Aminoisobutyric Acid
BCAA	Branched Chain Amino Acid
BFI	Biomarkers of Food Intake
BG	Blood Glucose
BHS	Biomarkers of Health Status
BHT	Butylated Hydroxytoluene
BMI	Body Mass Index
BMI	Body Mass Index

BP	Blood Pressure
BPM	Beats Per Minute
BT	Body Temperature
Cal	Calibration
CGM	Continuous Glucose Monitor
CMPF	3-Carboxy-4-Methyl-5-Propyl-2-Furanpropanoic Acid
CNV	Copy Number Variant
CoA	Acetyl Coenzyme A
CRP	C-Reactive Protein
CVD	Cardiovascular Diseases
ddH_2O	Double-Distilled Water
DFE	Dietary Folate Equivalent
DFI	Direct Flow Injection
DFI-MS	Direct Flow Injection Mass Spectrometry
DG	Diglyceride
DIA	Diastolic Blood Pressure
DKK1	Dickkopf WNT Signaling Pathway Inhibitor 1
DMG	Dimethylglycine
DOHaD	Developmental Origins of Health and Disease
DRI	Dietary Reference Intake
ECL	Electrochemiluminescence
EDTA	Ethylenediaminetetraacetic Acid
EGF	Epidermal Growth Factor
ELISA	Enzyme Linked Immunosorbent Assay
FAD	Flavin Adenine Dinucleotide
FC	Fold Change
FDR	False Discovery Rate
FFA	Free Fatty Acids

FFD	Fast-Food Diet
FFQ	Food Frequency Questionnaire
FMO3	Flavin Monooxygenase
FoodBAll	Food Biomarker Alliance
FOS	Fructooligosaccharide
FSS	Food Standards Scotland
GABA	Gamma Aminobutyric Acid
GC-MS	Gas Chromatography-Mass Spectrometry
GI	Glycemic Index
Glycosyl-Cer	Glycosylceramide
GO	Glucose Oxidase
GSA	Global Screening Array
GSH	Glutathione
HDAC	Histone Deacetylase Inhibitor
HDL	High Density Lipoprotein
HEFI	Healthy Eating Food Index
HEI	Healthy Eating Index
HES	Healthy Eating Score
hex 2 Cer	Dihexosylceramide
Hex Cer	Monohexosylated Ceramide
HLC	Healthy Low-Carbohydrate
HLF	Healthy Low-Fat
HMDB	The Human Metabolome Database
HMG CoA	3-Hydroxy-3-Methyl-Glutaryl-CoA
HP	High-Protein
HR	Heart Rate
HREB	Human Research Ethics Board
HRR	Heart Rate Recovery

HRV	Heart Rate Variability
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
ICAT	Isotope-Coded Affinity Tag
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IDO	Indoleamine 2,3-Dioxygenase
IDUA	Alpha-L-Iduronidase
IEM	Inborn Errors of Metabolism
IFN-γ	Interferon-γ
IGF	Insulin-Like Growth Factor
IL	Interleukin
IPA	Indole-3-Propionic Acid
iPOP	Integrative Personal Omics Profile
IRS	Insulin Receptor Substrate
ISS	The International Space Station
ISTD	Internal Standard
KD	Ketogenic Diet
LCHF	Low-Carbohydrate High-Fat
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LDL	Low-Density Lipoproteins
LDL-C	Low-Density Lipoprotein Cholesterol
LFHC	Low-Fat High-Carbohydrate diet
LPL	lipoprotein Lipase
LPL	Low-Protein
LSD	Least Significant Difference
LysoPC	Lysophosphatidylcholine
MCA	Mental Cognitive Assessment

MD	Mediterranean Diet
MedDiet	Mediterranean Diet Score
mGlyR	Metabotropic Glycine Receptor
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MSD	Meso Scale Discovery
mTOR	Mechanistic Target of Rapamycin
MUFA	Monounsaturated Fatty Acid
MyD88	Myeloid Differentiation Factor 88
NAFLD	Non-Alcoholic Fatty Liver Disease
NASA	National Aeronautics and Space Administration
NMR	Nuclear Magnetic Resonance Spectroscopy
NNMT	Nicotinamide-by-Nicotinamide N-Methyltransferase
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
OGCT	Oral Glucose Challenge Test
OTC	Ornithine Transcarbamylase
P100	Continuous Glucose Monitoring
PC	Phosphatidylcholine
PCA	Principal Component Analysis
PITC	Phenyl-Isothiocyanate
PLS-DA	Partial Least Square Discriminant Analysis
PN	Precision Nutrition
POMS	Profile of Mood State
PP	High-Plant Protein
PSH	Precision Space Health
PUFA	Polyunsaturated Fatty Acid
QC	Quality Control

RAE	Retinol Activity Equivalent
RCT	Randomized Controlled Trial
RD	Regular Diet
RDA	Recommended Dietary Allowance
REM	Rapid Eye Movement
ROS	Reactive Oxygen Species
SAM	S-Adenosylmethionine
SCFA	Short-Chain Fatty Acid
SCN	Suprachiasmatic Nucleus
SE	Sleep Efficiency
SFA	Saturated Fatty Acid
SGLT1	Sodium-Glucose Linked Transporter
SM	Sphingomyelin
SM(OH)	Hydroxy-Sphingomyelin
SNP	Single Nucleotide Polymorphisms
SRM	Single Reaction Monitoring
SYS BP	Systolic Blood Pressure
T2D	Type II Diabetes
TC	Total Cholesterol
TCA	Tricarboxylic Acid
TEG	Thermoelectric Generator
TG	Triglyceride
TGF	Transforming Growth Factor
TLR	Toll-Like Receptor
TMA	Trimethylamine
TMAO	Trimethylamine Oxide
TMIC	The Metabolomics Innovation Centre
TNF	Tumor Necrosis Factor

TNFRSF13b	TNF Receptor Superfamily Member 13B
TNF-α	Tumor Necrosis Factor-α
TST	Total Sleep Time
UFA	Unsaturated Fatty Acid
VLCFA-Cer	Very Long-Chain Fatty Acid Ceramides
VLDL	Very-Low-Density Lipoproteins

Chapter 1

Introduction

1.1. Importance of precision nutrition and advanced metabolomics technologies at informing nutritional intake, nutrition, and lifestyle in health:

The significance of lifestyle and nutritional habits cannot be overstated. This is especially true when it comes to maintaining health and preventing chronic diseases such as cardiovascular diseases (CVD), obesity, chronic kidney disease, type II diabetes (T2D), and diet-related cancers [1,2]. High-income countries, including Canada, the United States, and many European countries, have witnessed a substantial increase in the number of annual deaths attributed to these health conditions. For instance, non-communicable diseases, including diet and lifestyle-related diseases, are believed to account for the deaths of 41 million people each year, equivalent to 74% of all deaths globally [3]. Within Canada, these conditions together account for 65% of death [4,5] and more than two-thirds of all deaths in the United States [6].

A diet lacking in vegetables, fish, whole grains, nuts/seeds, and milk, coupled with excessive consumption of red meat is a major contributor to the Global Burden of Disease, accounting for 11.3 million deaths and 241.4 million disability-adjusted life-years worldwide [7]. It is estimated that as much as 80% of major cardiometabolic diseases and over one-third of cancer cases could be prevented by avoiding unhealthy dietary and lifestyle habits [8]. Healthy nutritional practices have been associated with a reduced risk of CVD by as much as 60% [9]. Likewise, it is estimated that improvements in diet, physical activity, and body composition could prevent 27-39% of the most prevalent cancers [10,11]. Smoking avoidance, regular physical activity, moderate or low alcohol consumption, and a diet rich in fruits and vegetables have been shown to lead to a fourfold reduction in mortality risk, which is equivalent to adding 14 years to one's lifespan [12,13].

As individuals age, their risk of acquiring chronic diseases increases significantly. So too does the burden of disability and frailty. Diet and lifestyle not only affect muscle, organ, and vascular function, but they also affect an individual's DNA (especially the epigenome). In a study conducted by Sailani and colleagues [14], a group of healthy men, with an average age of 62 years, was examined to explore the link between maintaining an active lifestyle and the overall DNA methylation patterns in skeletal muscle. Their findings indicated that a lifelong commitment to an active lifestyle is linked to specific, healthy DNA methylation patterns. These patterns have the potential to enhance insulin sensitivity and increase the expression of genes associated with energy metabolism, muscle development, contractile properties, and resistance to oxidative stress.

The molecular effects of diet and lifestyle are not limited to epigenetic changes in the DNA. They also affect the microbiome [15]. The human microbiome has been called the "forgotten organ" [16,17]. It is now realized that the microbiome, especially the gut microbiome, plays a critical role in the development of immune function (esp., with children), digestion, weight gain or weight loss, hormone function, inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), gastrointestinal cancers, gut health, and mental health [16,18–20]. Dietary and lifestyle habits can affect the type or abundance of "good" or "bad" microbes found in the gut. For instance, a diet that is high in fat and meat while low in fibre will encourage the growth of *Firmicutes* and *Proteobacteria* (i.e., "bad" bacteria), while a diet that is high in fruit, vegetables, and fibre is associated with higher amounts of *Bifidobacteria* and *Lactobacilli* (i.e., "good" bacteria) [21,22].

The bad bacteria typically produce a range of harmful food-derived metabolites such as cresol sulfate and indoxyl sulfate, while the good bacteria produce beneficial food-derived metabolites such as butyric acid and acetic acid [23,24]. Cresol sulfate and indoxyl sulfate, derived from dietary protein by gut bacteria, are uremic toxins associated with inflammation, cardiovascular conditions, and a variety of kidney problems [25–27]. Conversely, butyric acid and acetic acid, produced from dietary fibres by beneficial gut bacteria, are short-chain fatty acids crucial for gut health, providing energy to colon cells, exhibiting anti-inflammatory properties, and potentially regulating appetite and metabolism [24,27]. Similarly, as people age, their gut microbiome also ages. There is evidence that there is a gut microbiome that is characteristic of frail seniors and a gut microbiome that is characteristic of non-frail seniors [28].

While most of this discussion has focused on the effects of diet and lifestyle on the health of adults or the elderly, it is important to remember that young people can be affected too. Furthermore, these effects are not just limited to their short-term or immediate health, they appear to have long-term consequences. Inadequate nutrition for children and infants during crucial early developmental stages can elevate the risk of developing CVD, obesity, and other chronic metabolic health conditions that develop later in life [3]. A particularly compelling example of this is the Dutch Famine, also known as the Hunger Winter, which occurred in Holland during 1944-45 [29]. This event has been studied extensively to assess the effects of poor diet and inadequate nutrition on children, infants, and fetuses as they manifest later in life. Studies that focused on individuals exposed to malnutrition during this famine have revealed lifelong health implications, particularly for those whose mothers experienced malnutrition while carrying this offspring in utero or during early childhood [30,31]. These data linked famine exposure to increased risks of CVD, metabolic disorders, psychiatric conditions later in life, and even shorter adult height [29–31]. The famine's legacy extends beyond the immediate survivors to second and third generations post-famine, suggesting that the developmental impacts of poor nutrition during critical periods can persist across generations [32,33]. This highlights the importance of adequate nutrition during key developmental stages for long-term health outcomes [34,35].

Just as undernutrition during childhood, infancy, or *in utero* can have profound effects on long-term health, so too can overnutrition. The Developmental Origins of Health and Disease (DOHaD) hypothesis proposes that early-life environments, including nutrition, can influence health outcomes throughout a person's lifespan [36]. It has been proposed that when an individual experiences overnutrition, particularly during critical developmental windows, it can lead to programming alterations in the body's metabolism, immune system, and organ development [37]. These alterations can persist into adulthood, and may increase the susceptibility to conditions such as obesity, type 2 diabetes, CVDs, and metabolic disorders [37–39]. During fetal development, excessive nutrient exposure, especially high levels of sugars or fats, can disrupt the normal growth and development of organs and systems [40]. This disruption might not be immediately evident but could predispose individuals to metabolic dysregulation or metabolic reprogramming, making them more prone to obesity and related health problems in adulthood [40,41]. Promoting optimal nutrition during pregnancy and early childhood by reducing exposure to high-calorie, low-nutrient

foods may help mitigate the risks associated with overnutrition during crucial developmental periods, potentially reducing the burden of chronic diseases in later life [42,43].

Therefore, regardless of age, ethnicity, sex, gender or country of origin, diet, and lifestyle (i.e., the environment) play a critical role in determining not only lifespan but also healthspan. A good diet accompanied by a healthy lifestyle can add years to a person's life (lifespan) and years to a person's disease-free life (healthspan). A poor diet or poor lifestyle can reduce not only one's life expectancy but also bring on a wide array of debilitating chronic diseases at much younger ages. *Finding better ways to identify what constitutes a good diet and a healthy lifestyle, and to determine the best diet and lifestyle for an individual is the main aim of this thesis.*

However, prior to describing the exact aims, objectives, and experimental design of my thesis research, I will first provide a detailed literature review covering the main topics and technologies used in my thesis studies. These topics include diets and dietary guidelines, precision nutrition (PN), and the technologies associated with PN along with the merits of N-of-1 studies. Specifically, I will begin with a discussion of dietary guidelines and recommendations followed by a brief review of PN and how the field of PN has evolved. These introductory comments will segue into an overview of PN applications and various methods for diet monitoring. Subsequently, I will provide additional information on some of the key technologies underlying PN, including metabolomics, proteomics, microbiomics, and digital wearable technologies. Finally, I will describe how individual, or N-of-1 studies are evolving to facilitate PN/health research. This introductory chapter will conclude with a clear statement of my thesis aims and a brief outline of the content of my thesis chapters.

1.1.1. Healthy diet recommendations - general dietary guidelines:

Many government agencies in coordination with nutritionists and nutrition researchers from around the world have spent decades developing comprehensive, population-wide dietary guidelines. These efforts were aimed at providing key recommendations or guidance about the number of calories needed along with the recommended daily intake of vitamins, minerals, protein, fat, and carbohydrates to sustain life [44]. Initially, many national guidelines were developed specifically to prevent undernutrition and avoid nutrient deficiencies [45,46]. In many respects, these efforts have been remarkably successful. Nutrient deficiency diseases such as goiter (iodine

deficiency), pellagra (vitamin B deficiency), and rickets (vitamin D deficiency) have essentially vanished from the developed world [47]. However, now that most of the world can produce more food than it consumes and food manufacturing now includes vitamin or mineral supplementation, food guidelines have had to change.

Today many national dietary guidelines focus on suggesting foods or meals that reduce the intake of saturated fatty acids, sugars (and sugary drinks), and increase the consumption of vegetables, whole fruits, whole grains, and dietary fibres (Figure 1.1). As an example, Canada's Food Guide particularly emphasizes the importance of plant-based foods and recommends making water your drink of choice [48]. These recommendations are based on large-scale epidemiological studies that have shown strong correlations between sugar consumption and T2D risk [49], saturated fat consumption and CVD [50], and low fibre consumption with colon cancer [51]. Likewise, other large-scale dietary studies have shown that the consumption of high-fibre foods (fruits and vegetables) reduces not only T2D risk but also cancer and CVD risk [52] while the consumption of unsaturated fats and vegetable oils reduces the risk for many other chronic diseases, including cancer [53,54]. Unfortunately, despite concerted efforts to raise public awareness about healthy eating and fostering a health-conscious lifestyle, these endeavours have not yielded the desired results [55]. Indeed, over the past 20 years, there has been a steady increase in the prevalence of

Figure 1.1. The conventional dietary guidelines advocate for a balanced diet that prioritizes a diverse array of nutrient-rich foods across all food groups. This includes the consumption of fruits, vegetables, whole grains, lean proteins, and healthy fats.

chronic diet and lifestyle-related diseases, along with a corresponding rise in mortality rates associated with these conditions. This underscores the urgent need to reevaluate our approaches to addressing this global health challenge [45,56].

One of the problems with dietary guidelines revolves around "messaging." In particular, there is inconsistency between different countries about what constitutes healthy versus unhealthy eating patterns. A notable example is the classification of discretionary foods. These are foods that are characterized by their high levels of fat, added sugars, and salt. These foods are generally discouraged worldwide, yet there is a lack of uniformity in their international definition, leading to disparities in how healthy eating guidelines address their consumption [57]. Research on reducing the intake of discretionary foods and beverages is limited by the lack of consensus on defining and classifying these items, with divergent approaches based on energy density or snack-like characteristics complicating efforts to compare and reduce their consumption across populations [57].

While national guidelines, national education programs, widespread training, standardized labelling, and greater resource support are helpful in targeting a national audience, it is increasingly evident that there is substantial inter-individual variability in the body's response to diet or dietary changes. It is well known that some individuals maintain a fit, slim, and energetic physique despite following an unhealthy diet, and they appear to avoid common health issues. Conversely, others consume very little yet struggle with weight management and face chronic metabolic disorders [58,59]. It is clear that some population subgroups may benefit from certain diets more than others. These differences may be due to their genetic makeup, age, sex, epigenetics, habitual dietary patterns, gut microbiota, and environment. These individual factors may also influence the absorption, metabolism, and excretion of bioactive compounds. These inter-individual differences affect the bioavailability of these bioactives and their eventual biological impact [60]. Large randomized controlled trials have consistently demonstrated that not all participants respond in the same way to dietary interventions [61]. Therefore, while population-wide dietary measures or global dietary recommendations reduce disease risk at a population level, not all individuals will benefit to the same degree [62].

As noted earlier, genetics can play a role in interindividual variations in dietary response. Indeed, the response to beneficial plant bioactives such as carotenoids can vary due to specific

genetic variants in specific genes related to their absorption and metabolism. Two such examples are 1) variants in BCO1 (beta-carotene oxygenase 1) - a gene responsible for encoding the enzyme involved in the metabolism of carotenoids. This enzyme specifically converts beta-carotene into vitamin A. Variants in these gene can lead to alterations in the enzyme's activity or expression, potentially affecting the individual's ability to metabolize carotenoids; and 2) variants in SCARB1 (scavenger receptor class B member 1) - a gene that enzyme involved in the cellular uptake of fat-soluble vitamins and carotenoids. Variants in this gene can affect plasma concentrations of provitamin A carotenoids in different individuals [63,64].

Gut microbiota also plays a crucial role in the efficacy of certain bioactives, such as the conversion of soy isoflavone daidzein into a beneficial metabolite known as equol. The conversion efficiency varies significantly between Western populations and vegetarian/Asian populations and appears to be largely due to different gut bacteria in these two populations [65]. Moreover, sex-based differences in biological responses have also been observed for certain foods. One interesting example is a study involving the biological effects of flavan-3-ol-enriched dark chocolate, where it was found that dark chocolate reduced platelet aggregation in males but not in females [66]. This underscores the complex interplay of several factors in determining individual responses to specific dietary interventions and has led to the concept of precision nutrition or PN.

1.1.2. Precision nutrition:

Personalized nutrition or precision nutrition (PN), falls under the umbrella of precision medicine and precision health. Precision health, or precision medicine, takes an individualized approach to healthcare by considering an individual's unique background and characteristics. It employs omics technologies to gain a comprehensive understanding of an individual's molecular phenotype (via genomics, proteomics, transcriptomics, microbiomics) and complements this information with electronic wearables to track environmental impacts alongside regular clinical or omic measurements [67,68] (Figure 1.2). This comprehensive molecular and physiological phenotyping approach gives a much more detailed, personalized understanding of an individual's health status, aiding in the delivery of more personalized and effective treatments [69]. Moreover, precision health has the potential to detect deviations from a healthy state leading to the early identification of disease or the prediction of disease risks [68]. PN uses the same concepts as

precision medicine by exploiting the same kinds of multi-omics measurements to develop individualized treatments and tailored dietary recommendations [70]. PN is the opposite of relying on one-size-fits-all dietary recommendations.

PN can be applied to individuals or groups as distinct population groups, such as children, the elderly, pregnant women, athletes, and those with inborn errors of metabolism (IEMs) that have distinct nutritional needs [55]. PN, similar to precision health, is not solely focused on disease prevention or disease treatment. Rather both are intended to enable enhanced well-being, which encompasses enhancing mood, emotional health, attention, endurance, weight management, and the regulation of specific biochemical parameters. Many nutrition researchers point to the work of Zeevi et al. [71] as the first example of PN being used effectively. This study tracked the time-dependent changes of glycemic responses to the consumption of different foods, while at the same time measuring the microbiome of individuals. This work showcased the potential of combining omics (esp. microbiomics) data, with physiological data and machine-learning technologies to predict individualized post-prandial glycemic responses to specific foods or diets [71].

Since the Zeevi et al. study, several other PN studies have been published. For instance, Mendes-Soares et al. in 2019 [72] used a personalized approach to assess the variations in glycemic responses to a meal in a non-diabetic population. Their findings indicated significant differences in glycemic responses of participants to the same meal challenge, reflecting similar results to what was observed in previous studies [72]. Similar inter-individual variations have been observed in participant responses to weight loss treatments [73], highlighting the limitations of generalized dietary guidelines [74]. Other PN studies have shown that personalized dietary prescriptions, based on an individual's personal physiology or personal omics characteristics, can lead to more significant improvements in eating behaviours and weight loss compared to non-personalized recommendations [75–77]. Other studies have also explored the effectiveness of personalized nutrition compared to traditional randomized controlled trials (RCTs) in dietary outcomes. The Food4Me studies [77], for example, examined various levels of personalized or tailored dietary advice where participants were grouped based on either: 1) current diet, 2) current diet in combination with phenotypic data, and 3) current lifestyle combined with phenotype and genotype data. One Food4Me study showed promising results for the personalized diet groups compared to those receiving general dietary guidelines (control) in reducing consumption of discretionary foods

and promoting a healthier lifestyle [57]. This study, along with several other PN studies, is discussed in more detail later in this document.

PN studies are beginning to have an impact on the broader field of nutrition research. For instance, the "2020-2030 Strategic Plan for NIH Nutrition Research" [78] aims to modernize nutrition research and ensure consistency across studies that explore the role of diet in disease prevention and treatment. This plan proposes that nutrition research, particularly clinical trials, incorporate aspects of PN methodologies when possible. This could include gathering individual data encompassing genetic, phenotypic, medical, and nutritional aspects of participants, and considering how this information could inform nutritional recommendations at the individual and group level [55]. According to the NIH, PN holds potential for increasing the effectiveness in managing existing health conditions and possibly in helping to prevent the onset (or the risk of) some chronic diseases [79].

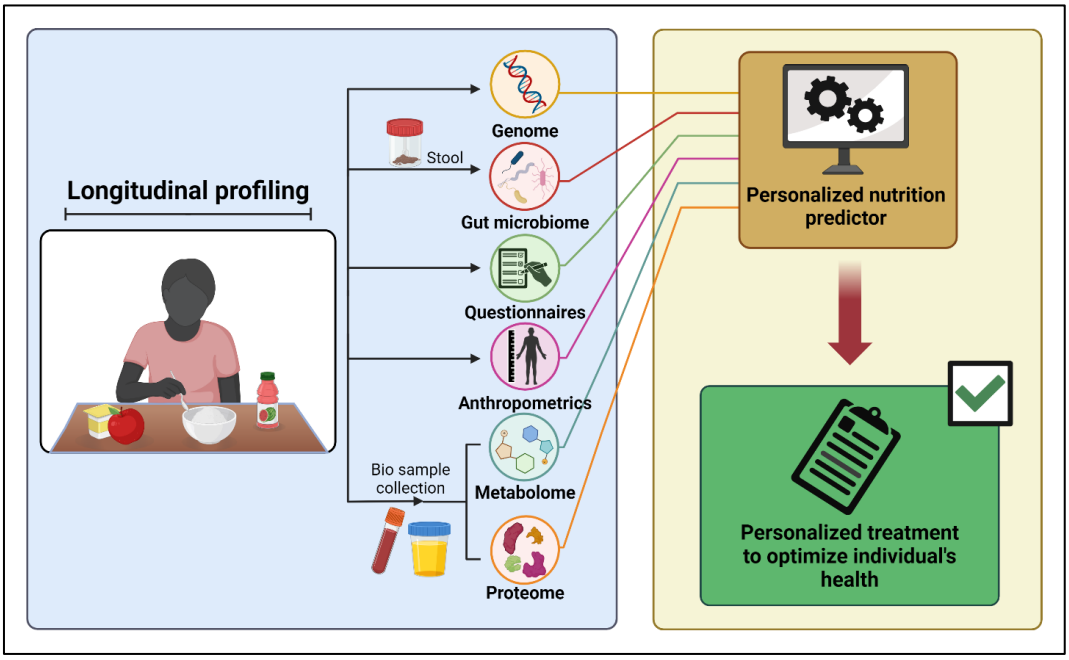

Figure 1.2. An overview of longitudinal monitoring for personal profiling. It includes multi-omics measurements such as genome analysis, gut microbiome assessment, proteomic analysis, and metabolomic profiling, in combination with general body measurements, questionnaires, and diaries. These collectively contribute to the characterization of an individual's profile. The collected data is then analyzed and considered collectively to try to describe a set of dietary intake recommendations with the objective of supporting an individual's health.

1.1.3. Specific applications of PN:

PN can be applied across numerous health domains. For instance, individuals with dietary restrictions or allergies may be able to leverage PN to gain a deeper understanding of what foods are suitable for their well-being or for avoiding poor health outcomes [80]. Until the last decade, individuals who were allergic to tree nuts received instructions to avoid all tree nuts to prevent allergic reactions and avoid the risk of cross-reactivity or cross-contamination during processing [80,81]. However, PN could allow for more nuanced guidance, enabling them to avoid only specific tree nuts rather than a blanket restriction [82]. PN also holds promise for helping to treat and manage specific health conditions, such as chronic inflammatory diseases (CVD, diabetes, obesity, asthma, arthritis) by targeting immunological pathways, microbiome dynamics, and human metabolism. PN has shown encouraging results in the treatment of patients with conditions such as IBD, metabolic syndrome, and certain autoimmune disorders [70,83]. Research in PN has also shown that tailored nutritional recommendations can significantly enhance glycemic control in individuals with diabetes [72,84–86].

To date, PN has been applied in two broad areas: 1) addressing the dietary needs of individuals with specific diseases or special nutritional requirements (e.g., T2D, inborn-errors of metabolism, during pregnancy or in old age) and 2) developing public health interventions [87]. While PN traditionally emphasizes maximizing individual benefits and minimizing adverse effects of dietary changes, there is hope that it could have a broader impact on a population [88]. Individuals may also seek PN to achieve personal goals such as attaining desired body sizes or shapes, excelling in competitive sports and being less prone to diet-induced metabolic diseases. For example, studies in the realm of athletic performance have highlighted substantial improvements when personalized dietary regimens compared to generic dietary recommendations were followed [89].

One particular PN study carried out by Livingstone et al. [77] offers valuable insights into the efficacy of PN methods. The study evaluated the effect of a personalized nutrition intervention on dietary changes associated with the Mediterranean diet (MD) by randomly assigning participants to receive either conventional dietary advice or personalized advice based on their current diet, phenotype, and genotype. Their research showed that personalized approaches yielded

superior rates of positive outcomes, contributing to the improvement of participants' or patients' health compared to traditional randomized control trials. Among those who received personalized dietary recommendations, there was a marked reduction in red meat and salt consumption, resulting in a general enhancement of the Healthy Eating Score (HES). This improvement stood in stark contrast to the control group which did not benefit from personalized dietary advice. Furthermore, the authors assessed the impact of varying levels of personalization within the study, namely, those rooted in diet (L1), phenotype (L2), and genotype (L3). Individuals randomly assigned to receive differing degrees of personalized guidance exhibited elevated Mediterranean Diet (MedDiet) scores. Although minor distinctions were discerned between these personalized groups, the overarching trend favored the personalized approach, demonstrating its beneficial influence on participants' dietary habits and overall health status [77].

In another PN example, Stanton et al. [90] monitored weight loss in adults with excess body weight and obesity over 12 months by randomly assigning different individuals to either a Healthy Low-Fat (HLF) or a Healthy Low-Carbohydrate (HLC) diet. The primary objective was to identify initial individual differences that could explain variations in weight loss outcomes among participants following the same weight loss plan. The study focused on potential genetic and insulin-glucose dynamic variations. However, it also collected extensive data on other relevant factors including physiology, psychology, diet, and behavior. It is important to note that the study's main goal was not to determine which diet was better for weight loss but to find out which diet worked best for each individual in achieving their weight loss goals. In both the HLF and HLC dietary interventions, short-term weight loss was primarily influenced by dietary adherence and diet quality rather than calorie restriction. Additionally, it was concluded that an individual's intrinsic fat oxidation rate plays a key role in determining the success of weight loss with different dietary strategies. These authors also suggested that inter-individual variations in the composition of the gut microbiome which may have contributed to inter-individual differences [90].

In yet another application of PN, Astrup and Hjorth [73] conducted a 10-week weight loss trial to compare the effects of a hypocaloric low-fat high-carbohydrate diet (LFHC) versus a low-carbohydrate high-fat (LCHF) diet in a cohort of 770 individuals with obesity. Among the insulin-sensitive participants with excess body weight in the study, those on the LFHC diet experienced a marginally greater weight loss of 0.4 kg compared to their counterparts on the LCHF diet.

Conversely, for insulin-resistant prediabetic individuals with obesity, both dietary approaches exhibited similar levels of effectiveness. However, the LCHF diet led to more weight loss (2.0 kg) when contrasted with the LFHC diet among those who had diabetes and obesity [73]. This highlights the applications and effectiveness of tailored dietary advice or group-based PN in weight loss.

A PN study conducted by Li et al. [91] explored the relationships between baseline molecular features of 609 individuals, who were randomized to either a healthy low-carbohydrate diet or a healthy low-fat diet, and the effectiveness of weight loss, particularly in a diet-specific context. Their results revealed that regardless of the diet type, caloric restrictions do not uniformly correlate with weight loss, and that adherence and the maintenance of a high-quality healthy diet are more impactful in short-term weight loss. However, they did find significant differences in the proteomic and microbiome characteristics of the individuals who successfully lost weight in a long-term regimen in comparison to those unsuccessful. The authors concluded that long-term weight loss is less dependent on dietary intake and more associated with individual molecular factors, including host-related protein levels of certain proteins such as Alpha-L-Iduronidase (IDUA), Tumor Necrosis Factor (TNF) receptor superfamily member 13B (TNFRSF13b), interleukin 16 (IL-16), Dickkopf WNT signalling pathway inhibitor 1 (DKK1), and lipoprotein lipase (LPL). Moreover, correlations between baseline respiratory quotient levels and regimen success highlighted the relevance of molecular characterization for effective weight loss treatments [91]. The work of Li et al. opens exciting possibilities for the development of tailored, individualized weight-loss strategies that can be finely tuned to suit each person's unique physiological makeup. However, these studies also highlight how it is imperative to embark on a mechanistic investigation of the underlying metabolic pathways that govern individual responses to diet-induced weight loss. This knowledge serves as the foundation upon which PN regimens can be built.

1.2. Food quality assessment tools and smart diet monitoring:

Understanding the chemical characteristics of foods, alongside their expected nutritional content, is crucial to nutritional studies, whether in personalized or non-personalized approaches. However, this food/nutrient content knowledge plays a particularly important role when

considering PN. It is widely acknowledged that PN requires robust tools for assessing dietary intake and nutritional status, not only before a PN intervention but also while on such an intervention [92,93]. Numerous dietary assessment tools are available for monitoring dietary intake, with Food Frequency Questionnaires (FFQs) and 24-hour recalls being commonly used methods [94]. Traditional 24-hour recalls involve capturing all food and beverage consumption within a 24-hour timeframe, including details such as portion size and preparation methods. The Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool [95], developed by NIH and NCI, streamlines this process. ASA24 is a web-based application allowing for multiple, automatically coded self-administered 24-hour recalls or multi-day food records, providing detailed data for assessing nutrient intake [56]. Furthermore, the nutritional values from these assessments can be used to calculate indices such as the Healthy Eating Index (HEI) which tries to facilitate the quantification of dietary intake [96,97].

However, traditional question-based or survey-based dietary assessments demand substantial time and effort from participants. As a result, they are prone to biases and often require adaptation for different populations [94]. The need for manual data input further compounds the participant burden, possibly affecting participants' honesty in their responses [98–101]. Additionally, self-reported dietary information is prone to significant random and systematic measurement errors. Both observational and interventional studies depend on self-reported data to determine primary exposures and evaluate adherence to interventions [102]. These challenges collectively hinder the practical implementation of dietary questionnaires for lifestyle monitoring and personalized nutrition.

To address these challenges, newer methods such as smartphone apps have emerged as practical tools for smart diet monitoring [100]. The convenience and accessibility of mobile phones and smartphone apps, in addition to their access to nutritional databases, enhance the accuracy and convenience of calculating and measuring nutritional values. Moreover, users worldwide can add and customize meals and foods to align with their local cuisines [100]. MyFitnessPal [103] is an example of a popular diet app and has over 14 million food items. Nutritionix [104] also has a database of ~900,000 grocery items, more than 190,000 restaurant foods, and more than 10,000 common food tags, all of which help streamline the tracking process [94]. These apps provide detailed breakdowns of dietary items, enhancing users' understanding of their choices. Barcode

scanners integrated into most apps facilitate the effortless addition of packaged items to food diaries. Some apps use machine learning algorithms to predict ingredients and portion sizes based on meal images [100,105].

Moreover, many of these apps connect to wearable health-monitoring devices, including fitness trackers, smart scales, heart rate (HR) monitors, and continuous glucose monitors (CGMs). This device integration provides users with a comprehensive overview of their nutritional status, intake, and energy expenditure. The combination of dietary intake tracking with other health monitoring methods can offer users a deeper understanding of their dietary intake and its possible effects on some health indicators. Heightened awareness of food intake and health indicators can be a motivator to change health habits. Indeed, consistency in recording food intake has been positively correlated with weight loss [106].

While food diaries, food apps and fitness monitoring apps provide macro-scale information about food and physiology, more detailed molecular information about diets and dietary consequences is also possible and potentially more revealing – especially as it relates to PN. In this regard, it is particularly important to discuss the impact that omics technologies, such as metabolomics, genomics and microbiomics are having on PN.

1.3. Metabolomics for monitoring diet and dietary effects:

Metabolomics is a discipline within analytical chemistry that is devoted to the characterization of small molecules (< 1500 Da) in biological samples such as tissues, cells, and biofluids [107]. These chemicals (i.e., metabolites) serve as substrates, intermediates, and end-products of metabolism, making them suitable targets for biomarker discovery [74,108]. Over the past decade, metabolomics has experienced significant growth, with applications extending to the exploration of physiological conditions, chronic diseases, and lifestyle biomarkers [109]. One of the most successful applications of metabolomics is newborn screening, which, as noted by LeVatte et al. [107], has improved the lives of up to 1 million children through early metabolic disease detection and intervention [108]. Nutritional studies have also benefited from metabolomics advances, resulting in the discovery of new food intake biomarkers [110,111]. These studies have

showcased the remarkable capabilities of metabolomics in detecting food intake biomarkers at the gram per day level [102,111,112]. The significance lies in the potential use of combinations of biomarkers to ascertain the consumption of specific foods in the diet. Furthermore, combining dietary biomarkers with conventional approaches has the potential to improve our ability to accurately assess dietary intake [99,102].

Metabolomics encompasses two primary approaches: untargeted metabolomics and targeted metabolomics. Untargeted metabolomics is aimed at detecting all measurable metabolites or metabolic features within a sample, while targeted metabolomics is intended for the quantitative measurement of pre-selected or high-priority metabolites in a sample [107,108,113]. Both approaches find applications across various scientific domains, but it's worth noting that untargeted metabolomics is generally biased toward detecting the most abundant molecules [114]. In contrast, there is growing interest in the application of targeted methods, given their ability to precisely determine metabolite concentrations and their utility in clinical applications [115]. However, targeted approaches require prior knowledge of the compounds of interest and their availability in purified form. This means that targeted metabolomics is not suitable for the discovery of new compounds [114].

Over the past two decades, four analytical technologies have emerged as the principal workhorses in metabolomics: nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) and inductively coupled plasma mass spectrometry (ICP-MS). Each technique offers extensive coverage of various classes of organic and inorganic compounds, encompassing lipids, amino acids, peptides, nucleotides, nucleosides, sugars, alcohols, biogenic amines, organic acids and in the case of ICP-MS, metals. NMR excels at identifying and quantifying high-abundance, highly water-soluble metabolites, while GC-MS and LC-MS are adept at detecting lower-abundance, more hydrophobic or lipophilic metabolites. ICP-MS is uniquely able to detect metals and certain anions, but not organic molecules. The combined use of multiple analytical technologies can significantly expand metabolite coverage and the range of samples that can be studied [107,114].

The limitations inherent in survey-based dietary measurement tools (discussed above) have led nutrition and nutrient researchers to seek alternative means of assessing dietary intake [112].

One of these alternative means includes the measurement of biomarkers of food intake (BFIs) via metabolomics. BFIs are chemicals or metabolites found in blood or urine that are characteristic of specific foods [102,111,116]. Certain well-established BFIs exist for various natural foods, such as urinary nitrogen for protein intake [117], daidzein for soy intake [118], proline betaine for citrus intake [119], lactose and trigonelline for milk consumption [118], and carotenoids for fruit and vegetable intake [120]. The detection of BFIs can offer valuable insights beyond self-reported food intake data, especially in scenarios where food composition data are limited or unavailable [121]. One of the most effective ways of identifying BFIs involves the use of metabolomics. Metabolomics is ideal for simultaneously identifying or measuring the abundance of hundreds of metabolites in biological samples. This broad chemical coverage allows the identification of specific metabolites or combinations of metabolites that rise or fall following the consumption of specific foods. The idea of using metabolomics to acquire a deeper understanding of BFIs led to the establishment of the Food Biomarker Alliance (FoodBAll) in 2015 [122]. FoodBAll was specifically tasked with developing novel metabolomics methods to aid in the identification and validation of BFIs commonly consumed in European foods [99]. FoodBAll identified more than 20 BFIs through various metabolomic methods and more than 50 BFIs through careful evaluation of the literature.

The identification and annotation of new BFIs via metabolomics requires a strong understanding of the chemical composition of human biofluids (urine or blood), the chemical composition of food, and the metabolic fate of food. To facilitate this, several online databases on human metabolites and food chemicals were developed or expanded by the FoodBAll consortium. The Human Metabolome Database (HMDB) is one such database [123]. It contains data on more than 250,000 chemicals or metabolites that can be found in human biofluids and tissues. The HMDB contains not only chemical structure information about metabolites but also detailed compound descriptions as well as known or predicted NMR, GC-MS, and LC-MS/MS spectra to facilitate compound identification via metabolomic methods. Other resources developed in coordination with FoodBAll, include FooDB, the world's largest repository of information on food constituents, chemistry, and biology [124], MarkerDB, an online database that consolidates information on clinical and selected pre-clinical biomarkers including some biomarkers of food consumption [125], Exposome-Explorer, a database dedicated to archiving data about biomarkers of exposure to environmental risk factors for diseases [126], and FoodComEx, a chemical resource

developed by the FoodBAll project to develop and improve the approach to developing standards for food-derived metabolites and promote the sharing of less accessible chemical standards [127].

In addition, given the vast range of individual responses to specific foods, which can encompass both inter-individual differences and changes within a single person over time, the need for longitudinal metabolomics monitoring of individuals is underscored. Metabolomics can also be used to evaluate how an individual reacts (physiologically or metabolically) to particular foods or dietary habits. In this regard, metabolomics assays can be used to measure endogenously produced (as opposed to exogenous or BFI-like) compounds. Indeed, a number of MS-based metabolomic assays have been developed or are offered by commercial testing labs that quantify some vitamins, minerals, and essential amino acids, present in the body. A list of the 40 most important essential nutrients is provided in Table 1.1.

Table 1.1. List of essential nutrients.

Phenylalanine	Choline	Thiamine (B1)	Potassium	Manganese
Tryptophan	Linoleic acid	Riboflavin (B2)	Chloride	Copper
Valine	a-Linolenic acid	Niacin (B3)	Sodium	Iodine
Threonine	Vitamin A	Pantothenate (B5)	Calcium	Selenium
Leucine	Vitamin C	Pyridoxine (B6)	Phosphorus	Cobalt
Isoleucine	Vitamin D	Biotin (B7)	Magnesium	Chromium
Lysine	Vitamin E	Folate (B9)	Iron	Molybdenum
Methionine	Vitamin K	Cobalamin (B12)	Zinc	Lithium

Simply measuring the concentrations of essential amino acid levels, essential minerals, or essential vitamin levels in blood via NMR, GC-MS, LC-MS or even ICP-MS-based metabolomic techniques and comparing those numbers to normal (healthy) values can help identify dietary deficiencies or excesses along with differences in metabolism among individuals. Those results might be used to guide diet modifications to prevent the development of vitamin-deficient conditions such as pellagra, scurvy, or rickets. Beyond the measurement of essential nutrients, metabolomics can also be used to measure health-related risk factors including blood glucose (BG), triglycerides (TGs), low-density lipoproteins (LDL), high-density lipoproteins (HDL), or cholesterol levels. Metabolomics may offer improved testing procedures, which could lead to earlier implementation of diet or medication interventions [107,116].

Likewise, metabolomics can also be used to detect the appearance of diet-derived metabolites that may have either beneficial or harmful physiological effects [107]. Metabolomics is particularly good at detecting small-molecule uremic toxins. Uremic toxins are diet-derived compounds found in the blood that cause damage (either directly or indirectly) to the kidneys, heart, liver, and brain [27]. Examples of uremic toxins include urea (which arises from protein metabolism) [128], uric acid (which comes from DNA/RNA metabolism) [129], hippuric acid (which comes from polyphenol metabolism) [130], indoxyl sulfate (which arises from tryptophan metabolism) [131] and cresol sulfate (which comes from aromatic amino acid metabolism) [24]. Some uremic toxins are generated from endogenous metabolism (urea, uric acid), while others come from gut microbial or host-microbial metabolism (hippuric acid, indoxyl sulfate, cresol sulfate).

Normally, uremic toxins are efficiently cleared by the kidneys (and appear in the urine) or are excreted in the feces. However, poor kidney function, a leaky gut, age >60 or other conditions are associated with an accumulation of uremic toxins in the bloodstream. This accumulation is associated with a host of chronic conditions including chronic kidney disease, liver disease, colitis, heart disease, as well as anxiety and depression [132,133].

Other diet-derived compounds can be quite beneficial. Specifically, short-chain fatty acids (SCFAs) such as acetic acid, propionic acid and especially butyric acid are known to exhibit immunomodulatory, anti-cancer and anti-inflammatory effects [133,134]. These SCFAs are generated by gut microflora that digest dietary fibre and other complex carbohydrates found in bran, fruits, and vegetables. The release of SCFAs within the large intestine ensures that these short-lived molecules can target specific cells within the intestine and strengthen the gut-blood barrier and enhance the maturation of various immune cells [135].

Metabolomics measurements also enable the classification of individuals into metabotypes. Metabotypes are metabolic phenotypes [136] that are determined by measuring the metabolic response of individuals to certain foods or diets or dietary challenges and grouping them into certain categories (Figure 1.3). An example of two metabotypes are equol producers and non-equol producers. Equol is a soy-derived metabolite that is generated by the presence of certain gut bacteria. People who are equol producers generally experience clear benefits from the consumption of soy products while non-equol producers generally experience no benefits [137]. The detection of the equol metabotype can only be done through the application of MS-based metabolomics

methods. Metabotypes can be assessed by specific food challenges, such as the oral glucose challenge test (OGCT), which measures how long it takes for a person's glucose levels to return to normal after a bolus consumption of a high glucose meal or drink. People who take a long time to return to normal are typically pre-diabetic or have poor oral glucose tolerance while those who return to normal quickly are generally healthy and have a good oral glucose tolerance. BG levels can be monitored through standard biochemical methods that generally measure a single metabolite. In contrast, a metabolomics approach can measure glucose and many other compounds (amino acids, lipids, etc.) in one assay. Indeed, the production of branched-chain amino acids or acylcarnitines during an OGCT test can be indicative of a number of metabolic disorders [138,139].

Other examples of metabotypes that have been detected by metabolomics methods are those who are fast metabolizers of caffeine and those who are slow metabolizers of caffeine [140]. Using MS-based metabolomics to longitudinally monitor the disappearance of caffeine or the appearance of 1-methylxanthine or trigonelline allows the determination of who is fast and who is a slow caffeine metabolizer [141]. Fast metabolizers experience little benefit from the consumption of caffeine-rich beverages while slow metabolizers can experience extended periods of stimulation or wakefulness [142].

As seen by these examples, metabolomics may offer accurate, often quantitative, approaches to measuring what foods individuals are consuming (via BFIs), how much (or how little) they are consuming (via quantitative BFI measurements, and only if the metabolites are proportional to intake), whether their diet is nutritionally adequate (via essential nutrient monitoring), how their diet is affecting their physiology and metabolism (measuring endogenous metabolites), and classifying the metabolic phenotype or metabotype to which a certain individual may belong. In other words, metabolomics can offer insights into an individual's health status, it can facilitate longitudinal monitoring during dietary interventions, can monitor metabolic reactions, and it can identify metabolic or physiological deviations from a healthy state. These insights are the reason why metabolomics is thought to be essential to the advancement of PN [60,108]. Ultimately, the incorporation of metabolomics into PN could significantly advance how dietary advice is personalized. [107].

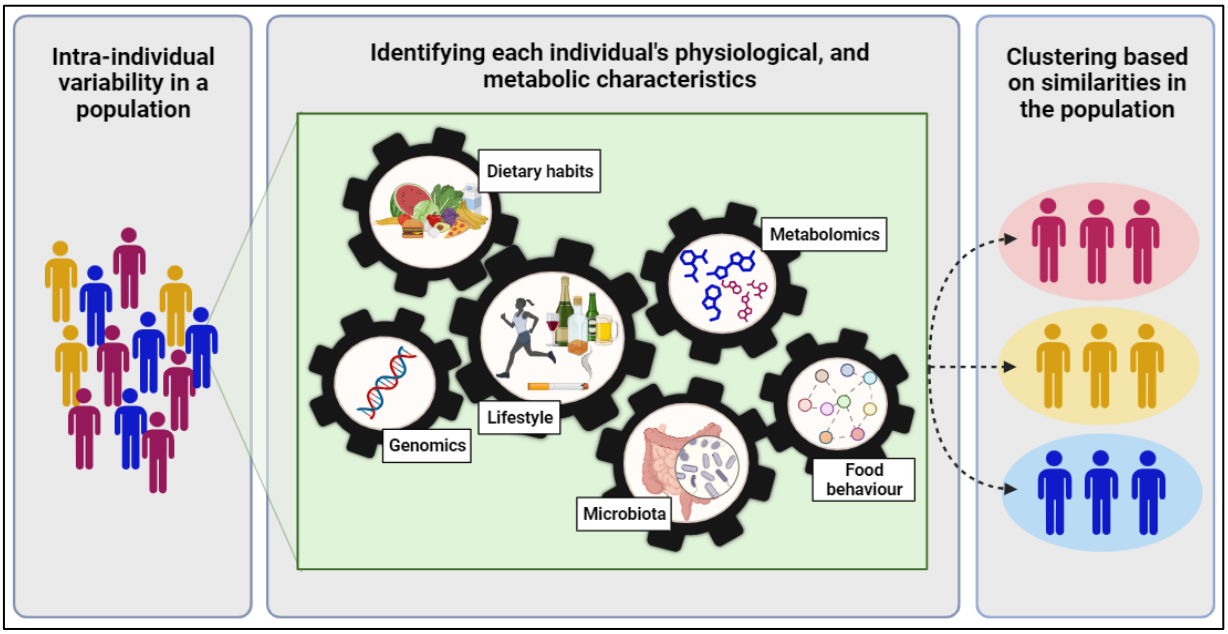

Figure 1.3. Metabotyping, clustering individuals with more similarities in their metabolic phenotypes, and physiological characteristics into subgroups (metabotypes) to provide optimum treatments. This figure outlines the process of metabotyping, where individuals are clustered into subgroups based on similarities in their metabolic phenotypes and physiological characteristics, to tailor optimal treatment strategies. The left panel illustrates the intra-individual variability within a population. The central panel identifies key factors such as dietary habits, genomics, lifestyle choices, microbiota composition, and metabolomics that are used to assess each individual's unique physiological and metabolic characteristics. The right panel shows the clustering of individuals into groups, or metabotypes, based on these similarities, highlighting the targeted approach to health management.

1.4. Other -omics methods in precision nutrition:

While metabolomics is particularly powerful in understanding the chemistry and biochemistry of foods and diets as well as their effects on human metabolism or physiology, other omics techniques such as proteomics, microbiomics and genomics can also offer important insights. This is because biological systems, especially at a molecular level, are highly interconnected. Indeed, a single base change at a genetic level can trigger cascading effects that are felt throughout the proteome, the transcriptome, the microbiome, and the metabolome.

Traditional single omics analyses tend to offer a rather narrow, single molecule-type perspective of biological systems. As a result, there is a growing preference across all domains in biology to include more than just one omics approach (i.e., multi-omics) to gain a broader, more holistic perspective of biological phenomena and their underlying mechanisms [83]. Multi-omics studies allow simultaneous examination and integration of various omics data, offering deeper insights into molecular scale events or measurements and their connections to health and disease. As a result, PN is beginning to expand its outlook from the almost singular use of metabolomics towards incorporating genomics (also called nutrigenomics), microbiomics and proteomics into the PN measurement paradigm.

1.1.4. Genomics:

The human genome consists of about 3 billion base pairs [143]. On average, each human genetically differs from any other human by about 3 million mutations or variants [143]. These variants include classic mutations, such as single base deletions, insertions or transversions (detectable in <1% of the population) as well as common single-base variants called single nucleotide polymorphisms (SNPs) that are detectable in >1% of the population [144]. Other (rarer) kinds of genetic changes can include copy number variants or CNVs [145]. CNVs occur when the number of copies of a particular gene varies from one individual to the next. CNVs represent a type of duplication or deletion event that affects a considerable number of base pairs. A representation of the difference between SNPs, mutations and CNVs is provided in Figure 1.4. The study of genomes as well as the study of how variations such as mutations, SNPs and CNVs affect physiology, biology or behavior is called genomics. Today, most genomics studies are based on a technology known as next-generation sequencing or NGS. NGS involves DNA fragmentation, library preparation, massive parallel sequencing (using specialized instrumentation that includes flow cells or sequencing chips), bioinformatics analysis, and variant/mutation annotation and interpretation. An outline of the NGS process is shown in Figure 1.5. Nutritional genomics is a branch of genomics that explores how genetic variation influences dietary response or preferences and how bioactive dietary components affect gene expression and function [146,147].

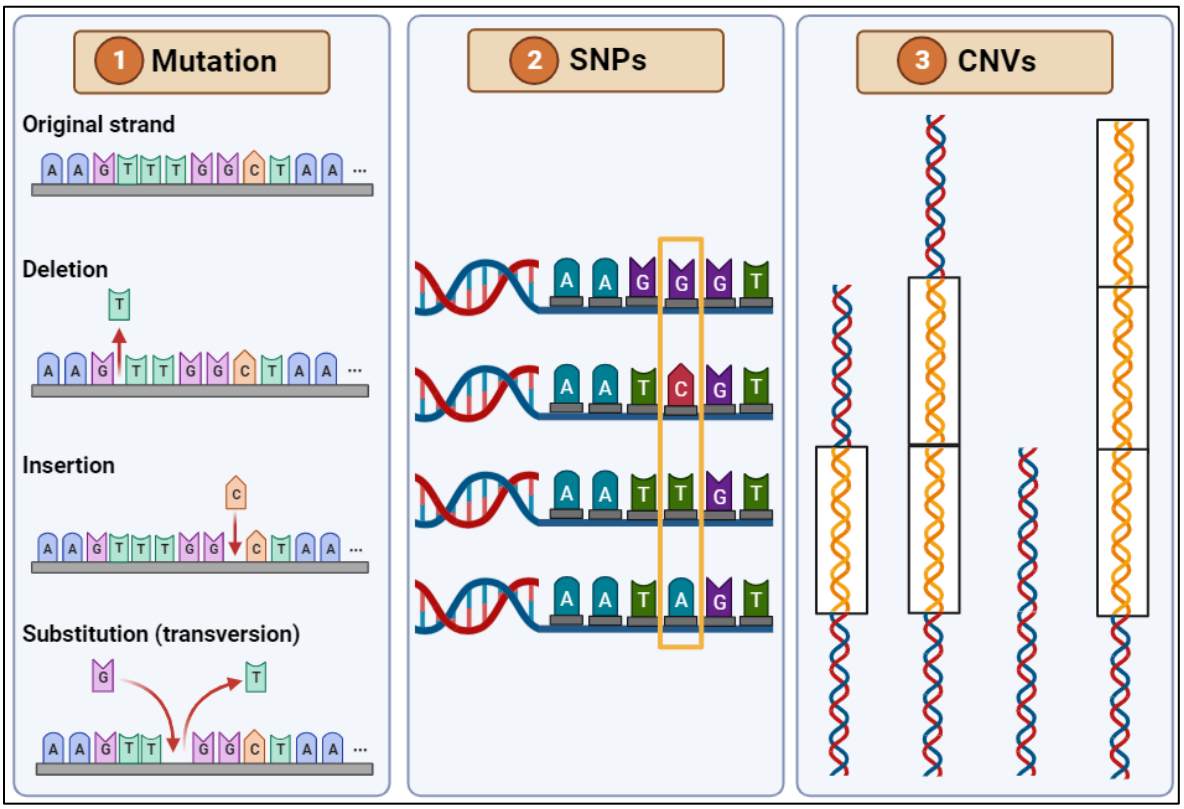

Figure 1.4. Comparative illustration representing 1) Mutations - Alterations in DNA sequence, 2) SNPs (Single Nucleotide Polymorphisms) - Variations at a single nucleotide position, and 3) CNVs (Copy Number Variations) - Changes in the number of copies of a particular gene.

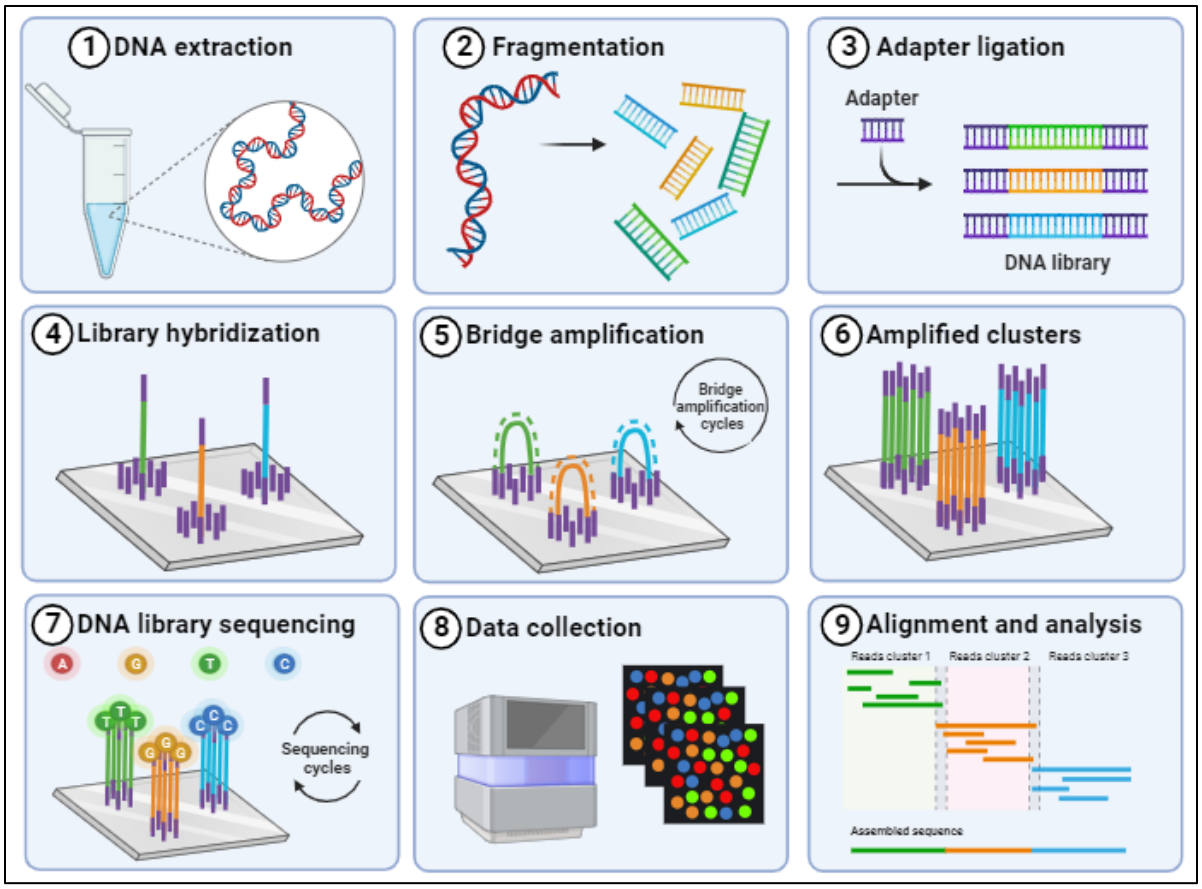

Figure 1.5. Stages of Next-Generation Sequencing: 1) DNA Extraction - Isolating DNA from a sample. 2) DNA Fragmentation - Breaking down DNA into smaller pieces. 3) Adapter Ligation - Attaching adapters to DNA fragments for sequencing. 4) Library Hybridization - Preparing the DNA library for sequencing. 5) Bridge Amplification - Creating multiple copies of each DNA fragment. 6) Amplified Clusters - Formation of clusters of identical DNA fragments. 7) DNA Library Sequencing - Determining the sequence of nucleotides in the DNA fragments. 8) Data Collection - Capturing the sequencing data. 9) Alignment and Analysis - Processing and interpreting the sequence data.

Nutrigenomics has most frequently been used to assess the impact of SNPs on dietary preferences [147]. It is well known that individuals who have adverse reactions to certain foods are unlikely to consume them and this will affect their dietary intake. Likewise, individuals who have cravings for certain foods will likely want to have their "designer" diets adjusted to include more frequent servings of these foods. For instance, those with lactose intolerance are often unable to consume milk products so milk or milk products should not be part of their designer diet. Lactose intolerance is linked to several SNPs in the introns of the *MCM6* (mini-chromosome maintenance) gene which appear to affect the expression of the lactase gene [148]. Lactase is an enzyme that breaks down lactose (a disaccharide) into glucose and galactose.

Another adverse food response is alcohol intolerance. Alcohol intolerance is associated with a SNP (rs671 [G>A]) in the mitochondrial *ADH2* gene (aldehyde dehydrogenase 2). This SNP makes this enzyme ineffective at metabolizing acetaldehyde, a toxic byproduct of alcohol metabolism [149]. Acetaldehyde can cause flushing, sweating, headaches, and nausea. Additionally, another SNP (rs1229984) in the alcohol dehydrogenase gene (*ADH1B*) has been found that leads to a similar aversion to alcohol for similar reasons [149]. Those with SNPs for alcohol intolerance experience flushing, sweating, headaches, and nausea after consuming even small amounts of alcohol so alcohol cannot or should not be part of any designed diet for these individuals (although it is worth noting that alcohol consumption is generally not recommended for anyone).

Other SNPs have been identified that are associated with individual intolerance or a strong distaste for certain foods. For instance, several SNPs on the *TAS2R38* (taste receptor) gene have been shown to affect the consumption of broccoli, cabbage, spinach, and related Brassica vegetables. Carriers of these *TAS2R38* SNPs detect a strong bitter aftertaste after consuming Brassica vegetables and are less likely to want these vegetables in their diet [150]. Likewise, a SNP on the *OR10A2* gene (olfactory receptor family 10 subfamily A member 2) (rs72921001 [A>C]) cause carriers to perceive cilantro (coriander) as tasting like soap. This taste perception makes carriers unable or unwilling to consume foods with cilantro in any designed diet [151].

In addition to SNPs that may lead to food avoidance, there are also SNPs that appear to increase an individual's preference or tolerance for certain foods. For instance, a SNP (rs11940694) in the *KLB* (β-Klotho) gene has been associated with increased alcohol consumption in both humans and mice [152]. Likewise, there are several SNPs located in or near genes likely

involved in caffeine metabolism that are associated with increased coffee consumption [153]. Furthermore, several polymorphisms in the *FGF21* gene (fibroblast growth factor 21) appear to be responsible for individuals with strong sugar cravings or a "sweet tooth" [154]. These data show that SNP characterization can and should be used to help design individualized diets.

In addition to these findings regarding SNPs and food preferences, nutrigenomics has also identified SNPs that determine how certain nutrients will affect an individual's health or physiology. For example, Wilson et al. [155] investigated the effect of riboflavin (also known as vitamin B2) on blood pressure (BP) in patients homozygous for the 677C→T polymorphism (TT genotype) in the gene encoding methylenetetrahydrofolate reductase (*MTHFR*) (rs1801133). They noted a significant reduction in systolic and diastolic BP levels compared to baseline measurements in patients with the TT genotype [155]. Riboflavin is a precursor to flavin adenine dinucleotide (FAD) which is a cofactor for MTHFR. Evidently, the TT polymorphism in *MTHFR* allows the FAD co-factor to function more effectively, thereby increasing the activity of MTHFR, which leads to reduced levels of homocysteine (a key contributor to hypertension).

In addition to this work on SNPs and vitamins B2/B9, another study, called the Vitamin D/Calcium Polyp Prevention trial, found a SNP (rs7968585) in the vitamin D receptor gene (*VDR*) could significantly increase the effectiveness of vitamin D in preventing colorectal cancer [156]. Among individuals with the AA genotype (26%) for rs7968585, vitamin D3 supplementation reduced the development of advanced colorectal adenoma risk by 64%. In 2016, the PREDIMED study demonstrated that a specific SNP on the *CLOCK* gene (rs4580704) (among G-allele carriers) was associated with a decreased incidence of Type 2 diabetes while CC homozygotes had an increased incidence of T2D. Furthermore, those with the G-allele were found to be more protected from T2D by adhering to the Mediterranean diet [157].

Taking a behavioural approach, Turnwald et al. [158] found that providing genetic risk information about certain SNPs and their impact on health lead to some individuals making positive lifestyle changes. This finding highlights the motivational potential of genetic information in driving positive results in personalized nutrition. Overall, it should be clear from these examples that individual genetic differences can significantly impact individual dietary responses or preferences, and that genetic testing will be important in crafting personalized nutrition strategies.

1.1.5. Microbiomics

The microbiome refers to the collection of all microbes, such as bacteria, fungi, viruses, and other microorganisms that naturally live on or within an organism. All animals have a microbiome, especially a gut microbiome, and it is considered essential for life, for digestion and proper immune development. The microbiome is sometimes called the "forgotten organ" [16]. The human gut contains the largest concentration of microbes in the body with up to 1 kg of gut bacteria housed (mostly) in the large intestine. Given the small size of bacterial cells and the large size of human cells, there are actually more microbial cells (38 trillion) than human cells (30 trillion) in the average human body [159]. In humans, more than 10,000 different microbial species are thought to exist on or within any given individual. Given this vast species diversity, it means there are 1000X more microbial genes than human genes in human beings. This microbial genetic diversity means that just as an individual's genome is unique, so too is their microbiome.

The study of the microbiome is called microbiomics. It is also called metagenomics [160] because the most common way of characterizing the microbiome is through high-throughput DNA sequencing of microbes isolated from a specific biosample (such as feces) or an environmental sample (wastewater, soil, etc.). The same NGS DNA sequencing methods used for humans (see Figure 1.5) are also used in most metagenomics studies. The gut microbiome, unlike the human genome, is profoundly influenced by one's dietary and lifestyle choices [161]. This is because the microbiome, especially the gut microbiome, plays a key role in digestion and metabolism including energy extraction, vitamin production, fermentation of dietary fiber, as well as the breakdown and conversion of non-nutrients/xenobiotics into nutrients or waste products [162]. How one lives or where one lives also affects the composition of the gut microflora and how the gut microbiome will ultimately "train" the immune system. The result can affect whether one develops asthma, dermatitis, IBD or a host of chronic inflammatory conditions [163–165].

The composition of the gut microbiome can also affect how certain foods are digested. For instance, the conversion of daidzein (a soy compound) to equol (a beneficial soy metabolite) is affected by the presence of *Coriobacteriaceae* in the gut [166]. Equol possesses antioxidative, anti-inflammatory, and vasodilatory properties and has been shown to reduce arterial stiffness and prevent atherosclerosis [167]. Similarly, the production of trimethylamine oxide (TMAO), which is

a known atherotoxin, has been shown to be dependent on the presence of certain gut microflora (*Anaerococcus hydrogenalis*, *Clostridium asparagiforme*, *Clostridium hathewayi, Clostridium sporogenes*, *Edwardsiella tarda*, *Escherichia fergusonii*, *Proteus penneri*, *and Providencia rettger*) [168]. TMAO arises from the bacterial conversion of dietary choline, betaine, and L-carnitine into trimethylamine (TMA) [168]. Choline and carnitine come primarily from eggs, other fatty foods, and meat. The production of butyrate, a highly beneficial metabolite, from the fermentation of non-digestible fibre (found in fruits and vegetables) is mediated by gut bacteria primarily from the *Firmicutes* phylum [169]. These include members of the *Ruminococcus, Clostridium, Eubacterium, and Coprococcus* genus. Butyrate is a histone deacetylase inhibitor (HDAC) and exhibits anti-inflammatory properties, as well as intestinal barrier function and mucosal immunity-enhancing functions [170]. In other words, these studies show that the foods we choose to consume have a profound impact on the composition and activity of our gut microbiota, which, in turn, can have a significant influence on our health outcomes. This emphasizes the importance of making informed dietary choices that align with the unique characteristics of our individual gut microbiomes.

Understanding more about the composition of an individual's microbiome and knowing how specific microbes respond to different foods might open the door to nutritional microbiomics or nutritional metagenomics [171]. Several groups have attempted to do this wherein an individual's specific gut microbiome characteristics, their dietary responses to different foods and their BG levels are analyzed using advanced machine-learning algorithms. This approach allows predictions to be made on how specific individuals will respond to specific dietary interventions or specific food types. Of particular interest are the studies wherein microbe measurements, daily BG readings and daily diet records could be used with machine learning to develop customized glucose-lowering diets for individuals [71,72,84]. Interestingly, the foods recommended in these personalized diets often differed substantially from those typically suggested in traditional glucose-lowering diets, like low-carbohydrate diets. This approach integrates various types of data, including microbiome, dietary, physiological, and others, along with artificial intelligence to craft diets that are highly specific to the individual's biological makeup. This integration signifies a significant advance in the field of nutrition, pointing toward a future where diets are fully personalized based on comprehensive biological data.

1.1.6. Proteomics:

Proteomics involves the high-throughput study or characterization of the proteome – the complete collection of proteins found in a cell, tissue, or organism [172]. The human proteome consists of nearly 20,000 genetically encoded proteins. However, because of the existence of splice variants and because of the post-translational modifications that can occur among proteins (such as phosphorylation, acetylation, glycosylation, etc.), it is thought that there are millions of different proteoforms in the human body [173]. The characterization of the proteome can be done using a variety of technologies, with mass spectrometry (MS)-based proteomics being the most widely used approach. In MS-based proteomics, protein mixtures are initially separated (by 2D gel or liquid chromatography), then individual peaks or gel spots are digested with trypsin to produce peptides. The peptides can then be characterized or sequenced by MS and the peptides identified by comparing their masses or sequences to protein sequence databases (Figure 1.6). The abundance of proteins can be measured by MS using isotope-coded affinity tags (ICAT) or isotopically labeled reference peptides measured with their single reaction monitoring (SRM) transitions [174]. MS-based proteomics methods allow the identification and quantification of 1000s of proteins in a sample in as little as one day [175]. Proteomics can also be done using DNA-tagged protein-specific antibodies, fluorescent-tagged antibodies or protein-specific RNA aptamers [175,176]. These reagents recognize and bind specific proteins. By isolating the target protein and reading off the RNA tags, and the DNA tags or measuring the fluorescence of the bound antibodies, it is possible to identify and quantify protein abundance. Platforms such as Luminex, SomaScan and O-Link are examples of these newer, non-MS-based proteomics methods or technologies (Figure 1.7).

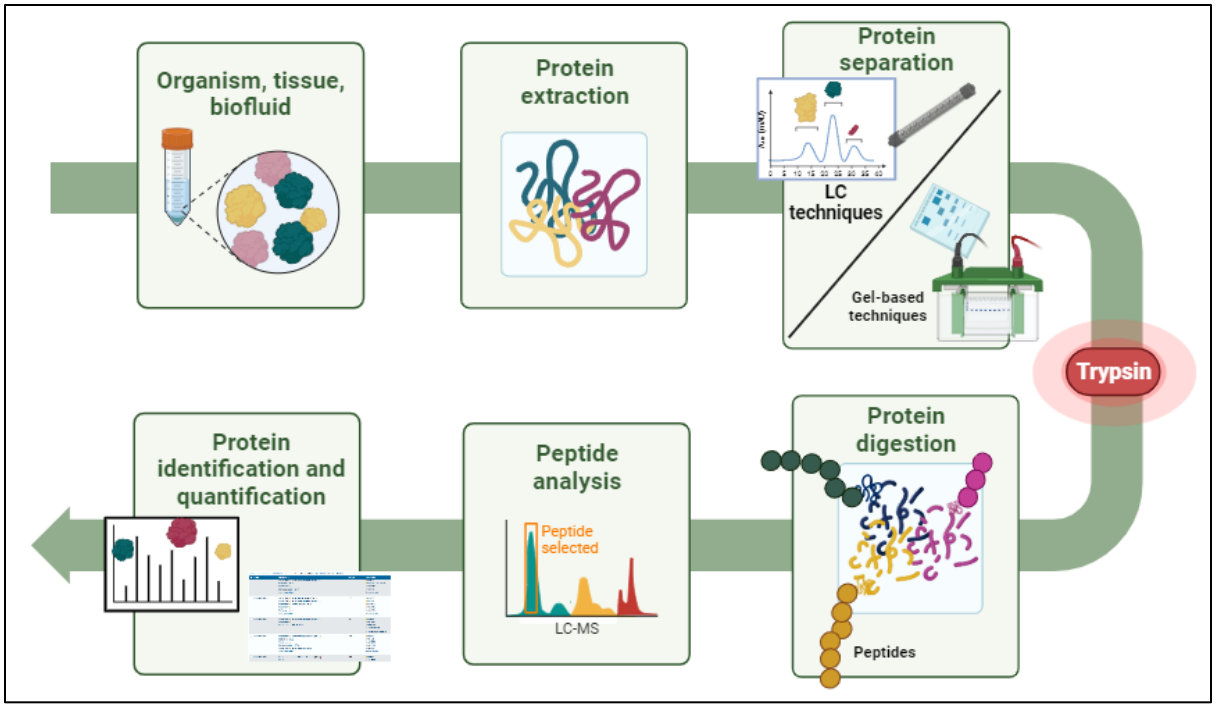

Figure 1.6. The figure illustrates key steps in MS-based proteomics, beginning with the separation of protein mixtures through techniques such as 2D gel electrophoresis or liquid chromatography after protein extraction from sample (organism, tissue or biofluid). Subsequently, individual peaks or gel spots are enzymatically digested with trypsin to generate peptides. Mass spectrometry is then employed to characterize and sequence the peptides, enabling identification by comparing their masses or sequences to protein sequence databases.

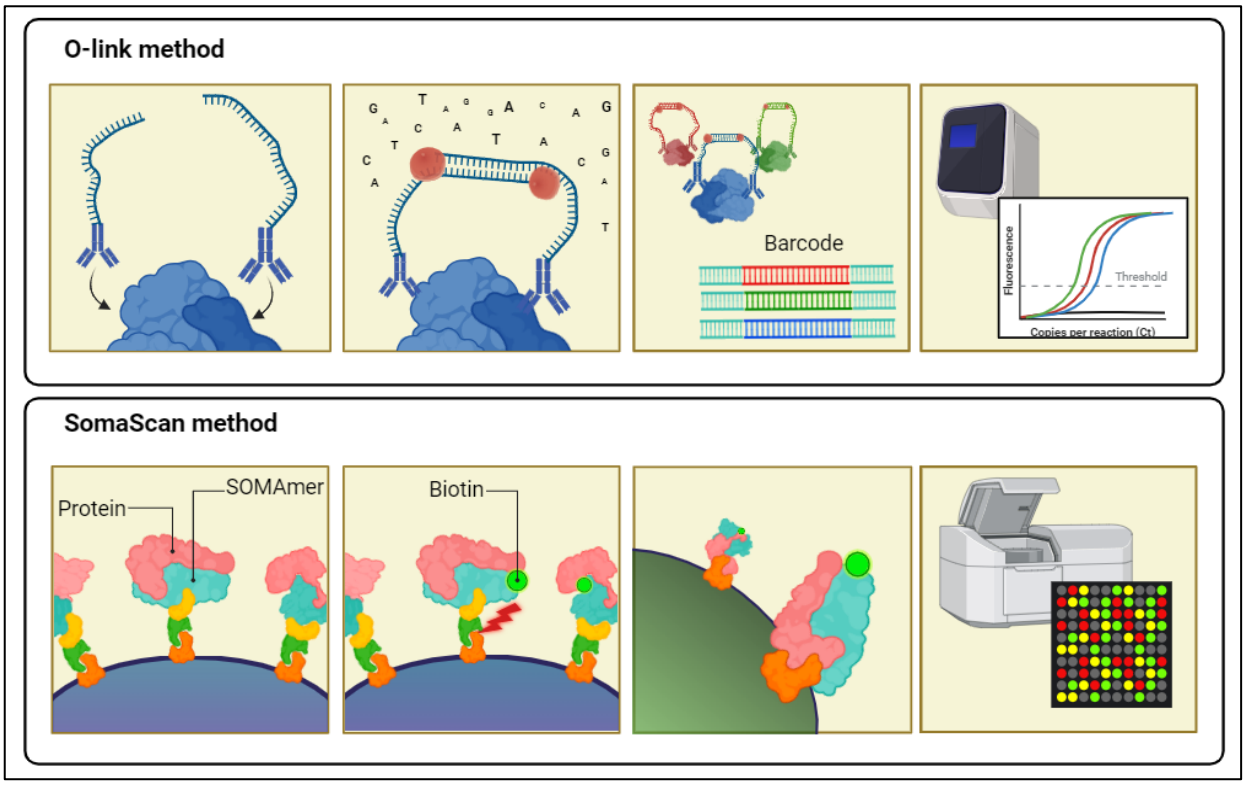

Figure 1.7. **The O-link method** utilizes DNA-linked antibodies to identify specific proteins in a given sample. The antibodies with complementary pairs bind to the target protein, enabling hybridization and extension with the help of DNA polymerase. Following PCR amplification, the DNA tags on properly matched antibodies are extended and amplified, with unique barcode regions distinguishing individual samples and proteins. Adaptors facilitate DNA amplification and readout. To represent different barcodes, distinct color codes are utilized. **The SomaScan assay** employs SOMAmers (light blue) that are synthesized with a photocleavable linker, a fluorophore, and biotin to immobilize the SOMAmers onto streptavidin beads. These SOMAmers can capture proteins (shown in light pink) from a solution and subsequently biotinylate the captured proteins. The photocleavable linker is disintegrated by external UV light, thereby releasing the SOMAmer/protein complex into the solution. Biotin-labeled proteins are then recaptured on secondary streptavidin beads. The denaturation of the captured protein leads to SOMAmer dissociation, allowing the SOMAmer reagents to hybridize with complementary sequences on a microarray chip. The amount of fluorescent intensity detected from each fluorophore is used to determine the abundance of each protein.

The study of how protein levels change in response to nutrients or nutrition is called nutriproteomics [177]. Most nutriproteomics studies are done longitudinally. This is because it is often of considerable interest to see how protein expression levels change over time (hours or days) as a consequence of food intake. Indeed, nearly everyone is familiar with the changes in insulin levels over time that arise from temporal changes in BG. Other key proteins involved in appetite control and satiety include leptin, ghrelin and insulin [178]. While proteins and peptide hormones involved in metabolism and appetite control are of interest in physiology, many nutriproteomic studies tend to focus on measuring a particular class of proteins – namely inflammatory proteins – to assess the impact of nutrients on the inflammatory response [179–181]. Some foods (esp. read meat, sugar-rich and fatty foods) are known to be pro-inflammatory while other foods (oily fish, fruits and vegetables) are known to be anti-inflammatory [182]. However, the level of inflammation can vary tremendously between individuals depending on their age, sex, genetics and underlying disease conditions [183]. The most commonly measured inflammatory or acute phase proteins are C-reactive protein (CRP), interferons, interleukins (IL), and various growth factors (such as transforming growth factor or TGF, epidermal growth factor or EGF and insulin-like growth factor or IGF). Using proteomics to measure these types of proteins has been valuable in assessing health by helping to unravel the relationship between infection, immunity, and nutritional well-being [182,183].

For example, in a study done by Koelman et al. [181], the effect of a high- and low-protein diet on inflammatory markers was tested on 18 individuals with obesity class III over 3 weeks. The levels of biomarkers were measured before and after the diet periods. Both high-protein (HP) and low-protein (LP) diets led to reduced levels of CRP and chemerin without significant differences between the two diets. However, the LP diet resulted in a more significant decrease in leptin and IL-6, along with an increase in total adiponectin. Additionally, the LP diet appeared to influence a broader spectrum of immune-inflammatory biomarkers compared to the HP diet in individuals with obesity, indicating its potential to modulate these markers. In another nutriproteomics study done by Markova et al. [184], 37 individuals with T2D were enrolled in a 2-week dietary trial. The participants were randomly assigned to either a high-animal protein (AP) or high-plant protein (PP) diet. The levels of the immune-inflammatory biomarkers such as IL-6 and cytokines, and proteins such as calprotectin and lactoferrin, were measured in their serum samples using enzyme-linked immunosorbent assays (ELISA). The findings indicate that both AP

and PP diets exhibit the potential to lower the concentrations of inflammatory adipokines, chemerin and progranulin. However, other immune-inflammatory markers did not show any statistically significant differences in either of the protein diet arms.

While nutriproteomics is a promising field, there are challenges with interpreting protein responses to nutrition or nutrient interventions. The "rapid response" acute phase inflammatory proteins are designed to combat pathogens, preserve safe levels of micronutrients, and initiate tissue repair. This acute response, however, can alter both nutrient concentrations and inflammatory protein levels, necessitating the differentiation between infection-induced changes and possible nutritional deficiencies [185]. For instance, in studies involving micronutrient interventions, the impact of inflammation can obscure supplement benefits or alter the utilization of absorbed nutrients. Inflammation may direct absorbed iron towards storage as ferritin rather than hemoglobin synthesis. Identifying and accounting for inflammation in nutrition research allows for deeper insights into nutritional requirements, aiding in designing effective intervention strategies against nutrient deficiencies or excesses and enhancing overall health outcomes.

The measurement of molecular changes at the metabolite, protein, or gene levels as well as measurements done at a physiological level (BG, electrocardiograms, BP, etc.) typically requires specialized biosample collection systems, very advanced and expensive instrumentation and specialized personnel or staff to run the instruments, analyze the data and interpret the measurement. This puts most molecular and physiological measurements out of reach of large numbers of people (including consumers) and has limited the application of omics or physiological testing techniques to low-cost, consumer-based PN applications. However, there is increasing movement towards making some of these high-cost measurements and devices more accessible and more affordable via wearable monitors and biosensors.

1.5. Wearable monitors and apps:

Wearable devices, often referred to simply as wearables, encompass a category of compact electronic gadgets equipped with sensors designed to be worn on the body, or seamlessly integrated into clothing and various other body-worn accessories. Wearable monitors, which take on various forms such as watches, rings, skin patches, headbands, or clothing, share a common core design principle: the ability to provide accurate and longitudinal measurements of physiological

characteristics [186]. These parameters include body temperature (BT), HR, BP, heart rhythm, and indicators of physical activity, such as steps, sleep cycles, BG, and many others. Wearables provide personalized, quantitative data for monitoring, and can substitute (or a complementary tool) clinical monitoring and diagnosis tools.

Beyond the standard physiological measures seen in today's wearables, some are being modified or having their data repurposed to detect signs of inflammation, forecast cardiometabolic health, and passively anticipate the onset of conditions like atrial fibrillation. Consumer-grade smartwatches have had their data collected and integrated to accurately generate clinical or pre-clinical indicators of inflammation, infection, and insulin sensitivity [187–190]. Studies, such as those performed by Li et al. [189] which investigated the use of portable devices for real-time physiological measurements, highlighted the effectiveness of wearables in understanding daily patterns, personalized baseline norms, and variations among individuals, contributing to early disease diagnosis. In response to the COVID-19 pandemic, wearable sensors have shown promise in the detection of respiratory infections. Duarte et al.'s study [191] revealed the potential of wearable sensors to detect SARS-CoV-2 infections before symptoms appear. In another application to COVID-19, Alavi et al. [192] engineered an algorithm for smartwatches to deliver notifications about potential presymptomatic and asymptomatic cases of SARS-CoV-2 infection through their application.

A recent study conducted to investigate the application and use of wearables for health monitoring and prediction of clinical laboratory test results revealed that wearable devices appeared to provide more reliable resting HR measurements than measurements made in clinic-based settings [193]. Furthermore, machine learning models have been used on data from wearable devices to predict cardiovascular conditions, diabetic states, and infection statuses [193]. These findings highlight the potential and reliability of modern wearables for continuous health monitoring. Not only can they offer a way to detect deviations from personal baseline measurements, but they also identify the need for further clinical laboratory testing [194]. Wearable digital sensors are not just limited to tracking physiological conditions. They also offer potential for tracking emotional stress and anxiety levels by measuring autonomic nervous system (ANS) metrics, such as electrodermal activity (galvanic skin response) and HR variability. These sensors aim to provide objective data on individual daily fluctuations in stress and anxiety levels.

Electrodermal activity, sleep cycles and sleep periods, along with HR variability, providing insights into individuals' emotional patterns and their impact on overall health and wellness [186,195].

Smartphone applications, developed alongside wearable monitors, are bringing a new dimension to what can be interpreted and learned via personal wearables. These integrated systems not only measure personal physiological or emotional parameters, but also collate and disseminate personalized guidance directly to individuals. In the field of nutrition, some applications provide personalized ratings for foods or recipes based on their potential impact on an individual's BG levels. Habit [196] takes this idea even further by offering comprehensive menu plans aligned with an individual's recommended nutrient intake, utilizing technology for personalized dietary advice on a large scale [2].

Wearables offer a less expensive (than clinical testing), continuous, longitudinal approach to measuring an individual's macroscale phenotype or physiological state. On the other hand, omics methods such as metabolomics, genomics, proteomics and microbiomics offer a somewhat more expensive, but more detailed approach to measure an individual's molecular phenotype. However, recent developments in metabolomics, metagenomics and genomics are driving the costs of sample analysis down, shortening analysis time and making sample collection so easy so that they are now affordable and accessible to most consumers [197–199]. Omics methods can be performed both longitudinally and cross-sectionally. Combining both approaches (wearables and omics measurements) offers an opportunity to relate personal molecular-scale changes to specific, customized health outcomes or action plans. Both wearables and omics methods allow a considerable degree of personalization, customization, and precision. Furthermore, both technologies are increasingly being used in N-of-1 studies to demonstrate their feasibility for PN/medicine.

1.6. N-of-1 studies, longitudinal personal monitoring:

As previously discussed, the traditional one-size-fits-all approach to healthcare and nutrition has shown limited efficacy, prompting a shift toward more personalized medicine and nutrition. Conventional methods, such as RCTs, often fail to consider individual diversity as most

people do not align with the "average" response. As a result, the single-patient trial, or "N-of-1 trial," has gained prominence, especially for monitoring, detecting, or treating chronic conditions such as T2D, CVD or a variety of metabolic disorders. The N-of-1 trial allows healthcare professionals to evaluate, monitor and tailor therapies to individual needs, with each person serving as their own control. This method offers a robust way to evaluate treatment effectiveness by rigorously looking at individual responses longitudinally rather than population or cross-sectional responses [200]. The success of N-of-1 trials in various healthcare settings has inspired a surge of interest in using N-of-1 trials for PN [87,201,202]. 'Omics' technologies, combined with comprehensive physiological data that can be obtained from wearables, enable large-scale individual-level "big data" collection. These big data sets, as shown by several authors and studies [68,203], can form the basis for personalized nutrition strategies.

Recent studies conducted by Zeevi et al. [71], and Mendes-Soares et al. [72] exemplify the application of N-of-1 studies using continuous glucose monitoring systems (CGMs) in single-subject nutritional interventions. N-of-1 studies can be scaled to larger groups. For instance, the Pioneer 100 Wellness Project (P100) [190] involved the individual tracking of 108 subjects over a period of nine months. During this time, whole genome sequencing, clinical tests, metabolomics, proteomics, microbiomics, and daily activity tracking, were used to identify potential disorders, develop personalized treatment plans, and monitor the results of these interventions. Other research, such as that conducted by Piening et al. [204] and Zhou et al. [205], have also shown how longitudinal multi-omics analysis of individuals via N-of-1 trials can be used to gain a personalized understanding of conditions such as obesity, T2D and daily physiological changes. Piening et al. used N-of-1 trials to gain insights into insulin resistance and sensitivity, while Zhou et al. used N-of-1 trials to identify early personalized molecular signatures preceding T2D onset.

Pioneering N-of-1 efforts by Michael Snyder have introduced the term "integrative personal omics profile" (iPOP), to the field of personalized health and PN. In Snyder's iPOP study, first described in 2012, extensive omics profiling of blood samples from a generally healthy individual (Dr. Snyder himself) was conducted over a period of 14 months. This comprehensive analysis encompassed whole-genome sequencing, transcriptomics, proteomics, metabolomics, and autoantibody profiles, resulting in the creation of an iPOP profile of Dr. Snyder [206]. The dataset obtained from various health or disease events and during viral infections underscored the potential

of estimating disease risk and detecting disease onset through continuous health monitoring via a multi-omics approach. In another study conducted in Dr. Snyder's lab, Shen et al. [92] collected micro-samples from a single participant over 24 hours for 7 days. This study led to the collection of enormous amounts of data from various monitoring devices like a smartwatch and CGM device, coupled with multi-omics data obtained from the blood samples. This work demonstrated the benefits of frequent and dense multi-omics microsampling in two applications. Firstly, it allowed detailed monitoring of individual responses to complex dietary interventions and led to the uncovering of unsuspected personalized inflammatory and metabolic reactions. Secondly, this approach was able to reveal extensive molecular fluctuations and numerous molecular connections linked to daily physiological changes (like HR), clinical biomarker levels (such as glucose and cortisol), and physical activity. This N-of-1 study showed that the combination of wearables and multi-omics microsampling offers a promising avenue for dynamic health profiling and biomarker discovery [92].

Another N-of-1 study conducted by Gao et al. [69] used longitudinal monitoring to obtain a comprehensive environmental health profile of a single individual. This study used longitudinal monitoring of both the personal exposome (external environmental exposures) and internal multi-omic profiles (genetic, metabolic, and proteomic information) in a single individual. This comprehensive approach was able to link thousands of external exposures with internal microbial, proteomic, and metabolic alterations, providing a more holistic view of the impact of the environment and environmental exposures to human health.

A precursor to the N-of-1 study by Gao et al [69] was a 15-person study published by Jiang et al. [207]. These authors conducted personalized exposome monitoring through a sensitive wearable device. The device was collected at different time points and the airborne chemicals adsorbed to the device were analyzed through MS-based exposome monitoring. Through this N-of-1 exposomics monitoring approach, these authors were able to track the personal exposomes of 15 individuals over nearly 890 days and across 66 diverse geographical locations. Their findings revealed that not only are humans exposed to a large array of airborne biological and chemical agents, including potentially harmful substances, but that it is possible to use wearable devices as portable monitoring stations for exposome research.

Another innovative application of N-of-1 studies led by the National Aeronautics and Space Administration (NASA) involved the personalized biomonitoring of astronauts during specific interventions and at baseline levels [208]. This approach has been implemented to ensure the health and well-being of astronauts during long, deep space missions. The Precision Space Health (PSH) System, as this is known, is an N-of-1 monitoring system that relies on longitudinal monitoring of individual astronauts. This system employs personalized biomonitoring and AI-driven data analysis to enhance astronauts' health and safety during space exploration [208].

A precursor to the PSH system was the NASA Twins Study [209]. This study investigated a pair of male monozygotic twins, one of whom spent 340 days aboard the International Space Station (ISS), while the other remained on Earth to function as a control. While technically an N-of-2 study, this study made use of the fact that monozygotic twins not only share identical genomes, but they also generally have near-identical physiology or physiological responses. So rather than conducting a study of one individual for 680 days, the study could be done over just 340 days. This study revealed a wide range of transient and enduring changes in various biological factors, including cell types, tissues, genetic profiles, and physical traits, in response to prolonged space travel. These insights are crucial for planning future deep-space missions and highlight the power of N-of-1 studies in understanding or monitoring individuals over extended periods.

A key limitation of many of these N-of-1 studies has been their substantial costs and the significant patient/subject burden required. Most of these studies required phlebotomists (to collect blood), doctors and nurses to monitor the subjects, as well as access to millions of dollars of expensive multi-omics platforms (DNA sequencers, mass spectrometers) and major investments of time and money by the sponsoring organization(s). Indeed, the P100 study was so expensive that it largely bankrupted the company that was conducting it. Furthermore, with the exception of those involving wearable devices, most of these studies did not or could not generate quantitative omics data. This lack of standardization in single-subject studies made omics comparisons between different N-of-1 studies essentially impossible. Likewise, as far as can be discerned, none of these N-of-1 studies looked at how these interventions affected individuals in terms of mental performance or mental health.

The success and limitations of these N-of-1 studies led me to consider conducting an N-of-1 dietary study that attempted to address the many limitations of other N-of-1 studies. In this study,

I would explore the effects of four different diets (a baseline or habitual diet, a fast-food, high-fat, high-sugar diet, a ketogenic diet, and a Mediterranean diet) on my microbiome, metabolome, and proteome. The diets would run for 2 weeks each (followed by 1 week wash-out periods) and I would employ wearables to measure a range of physiological parameters. Additionally, I would self-collect blood and urine samples on a daily basis and have these samples quantitatively analyzed using inexpensive, fully quantitative metabolomics and proteomics assays. Additionally, I would track and weigh all foods consumed and conduct daily mental and physical performance tests to assess changes in my mental/physical health and mental/physical capacity. Finally, I would integrate all the longitudinal multi-omic data and wearable data to assess how these dietary interventions affected my physical, mental, emotional, and molecular health indicators relative to my measured baseline health indicators.

1.7. Thesis objectives and thesis outline:

This thesis endeavors to delve into the relationships between dietary interventions, lifestyle changes, and their molecular and physiological impacts using an N-of-1 study design. Leveraging recent advances in self-administered biosample collection, omics technologies - including metabolomics, genomics, proteomics, and microbiomics – and high-performing, inexpensive wearable monitoring devices, this thesis seeks to explore the detailed physiological, physical, emotional, mental, and molecular effects of different dietary interventions on a single individual. The central objective of this thesis is to use an N-of-1 study design to assess the effects of four (2-week) dietary interventions using wearable and multi-omics monitoring methods. The working hypothesis for this thesis is to test whether the integration of wearables, quantitative omics techniques and careful mental/physical performance testing can provide quantitative and actionable information to identify the impact of dietary changes on individual health indicators.

This thesis also has five other specific objectives as it seeks to determine:

1) whether N-of-1 self-monitoring and biosample self-collection for PN studies is feasible

2) the costs of self-monitoring for PN purposes

3) whether daily mental/physical performance monitoring can provide useful data in an N-of-1 PN study

4) *whether quantitative omics data can provide information that is relevant and useful for guiding analysis and interpretation of N-of-1 nutrition studies*

5) *what kinds of data analysis techniques are most useful for interpreting dietary N-of-1 studies*

This thesis, its background, its rationale along with the results and implications of this N-of-1 study are presented over five separate chapters. The reasoning behind this study, combined with a literature review of the motivating concepts associated with PN, previous PN studies, the multi-omics technologies employed for this study and the achievements of other N-of-1 studies are described in chapter one (this chapter).

The second chapter focuses on the materials, methods, and study design, detailing the instruments, devices, and specific methodologies employed for data acquisition. The experimental design, including the rationale for the selection of diets, lifestyle modifications, and sample handling procedures, is further elaborated. In addition, the meal preparations, and physical and mental performance tests are also explained in this second chapter.

Chapter three, a pivotal section of this thesis, delves into the outcomes of the omics measurements. Using comprehensive bioinformatics and statistical analyses, this chapter interprets the results obtained from metabolomics, genomics, proteomics, and microbiomics assessments performed on the collected blood, fecal and urine samples. This chapter focuses on correlating or associating the measured molecular responses with my varying dietary patterns.

The synthesis and interpretations contained in chapter four, further amplify the dietary associative data described in chapter three. Chapter four examines and interprets the macro (physiological/mental), and micro-scale (molecular) correlations gleaned from the omics data, dissecting, and rationalizing the correlations and associations between physiological outcomes - as monitored by wearable technologies - and the detailed omics measurements. This chapter serves as a bridge between the physiological responses and the underlying molecular components, offering biological insights into personalized dietary implications and physiological/behavioral responses.

Concluding the thesis, the final chapter summarizes the findings and implications derived from this N-of-1 study. It highlights the effectiveness of this quantitative, integrated approach, combining wearable monitors and quantitative multi-omics methods. This section discusses the potential scalability of personalized nutrition strategies to a broader scope, suggesting future avenues for research and development in the field. Ultimately, this thesis aims to contribute to the advancement of PN methodologies by helping to elucidate the interplay between diet, molecular responses, and physiological outcomes at an individual level.

Chapter 2

Methods

2.1. Ethics approval:

The project was conducted in accordance with the ethical standards of, and approved by, the University of Alberta's Human Research Ethics Board (HREB) biomedical ethics committee (HREB #Pro00080942).

2.2. Study overview:

This is an N-of-1 study involving a healthy 25-year-old female subject (myself). I conducted a series of self-administered daily tests (physical, mental, physiological, and molecular) while adhering to four different isocaloric food patterns for 2 weeks each: a fast-food diet (FFD), a Mediterranean-type diet (MD), a Ketogenic diet (KD) and a "control" diet that was my habitual food pattern or Regular Diet (RD), and with washout periods interspersed amongst these patterns.

The FFD was chosen to represent a pattern that is high in processed or ultra-processed foods, with all foods purchased and consumed from a well-known fast food chain [210]. The foods consumed during the MD food pattern period were chosen to reflect the cuisines of Mediterranean countries, especially the regions of Crete, Greece and southern Italy [211]. The diet primarily consists of a variety of plant-based foods, including fruits, vegetables, grains, potatoes, beans, nuts, and seeds. Fresh fruit is commonly eaten as a daily dessert. Olive oil is the main fat source, with dairy products like cheese and yogurt also included. Fish and poultry are eaten occasionally in small to moderate quantities. Eggs are consumed infrequently, up to four times a week, while red meat is rarely consumed. Wine is typically enjoyed in moderate amounts during meals. However, none was consumed during this project [211,212]. The KD is a diet pattern that is low in carbohydrates and high in fats. Typical foods consumed on a KD include fatty cuts of meat, fish, eggs, avocados, nuts, seeds, and oils like coconut oil. Non-starchy vegetables like leafy greens, broccoli, and cauliflower are also common, while carbohydrate-rich foods like bread, pasta, and sugary foods

are avoided. The KD has been described in the literature as a diet pattern that may induce mild ketosis in people without diabetes and has been associated with improved metabolic outcomes such as glycemic control, lipid concentrations, and weight loss [213,214]. Finally, an analysis of my RD was conducted to have a better understanding of my regular dietary habits. I consumed food consistent with each of these patterns for two consecutive wewahout eks, followed by a one-week wash-out period. During the wash-out period, I returned to my RD (Figure 2.1). This wash-out period was used to minimize possible lingering effects from the previous diet pattern. Additionally, I followed the same data collection and self-monitoring protocols for a two-week period during my RD to establish a baseline for my typical physiological, mental, physical, and molecular responses.

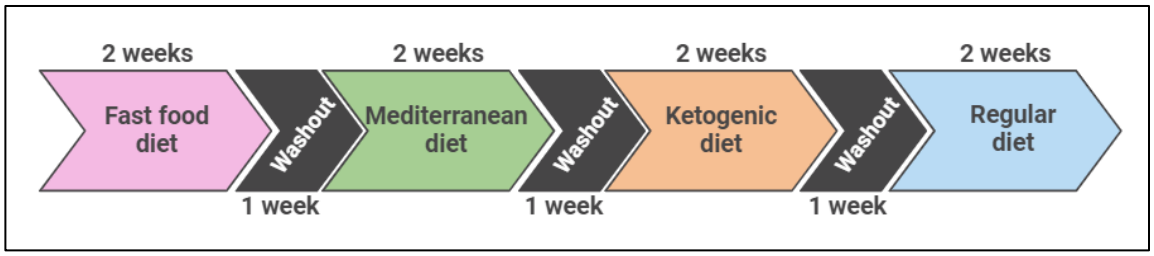

Figure 2.1. The timeline of dietary and washout periods. Each diet was for 2 weeks with a week of washout period (habitual diet) to ensure there are no lingering effects from the previous diet when initiating the next diet.

During the FFD phase, I consumed meals and snacks sourced exclusively from a local McDonald's restaurant. The sole criterion for food selection was adhering to the same type of meat, specifically red meat while avoiding fish or chicken. For the Mediterranean diet (MD), my diet featured an increased intake of seafood, poultry, fruits, and vegetables, and a low consumption of red meat [211]. Olive oil also played a prominent role as a dietary component. The ketogenic diet (KD), known for its high fat and high protein content and low carbohydrate intake, constituted my third dietary regimen. During this phase, I increased my consumption of meats, such as pork, and incorporated oils such as coconut oil. Carbohydrate intake was limited to less than 20 grams per day. My regular diet (RD) consisted of mainly whole wheat bread, peanut butter, pasta, basmati rice, chicken, red meat, or fish, vegetables (e.g. beans, carrots, and corn), fruits (e.g. apples and bananas) and coffee with 2% fat milk. All diets were carefully tracked in terms of their food or component composition, the weight of individual ingredients (except for the FFD, for which

weighed ingredient data was published) and calorie count (using the built-in Fitbit food log) [215] (Figure 2.2).

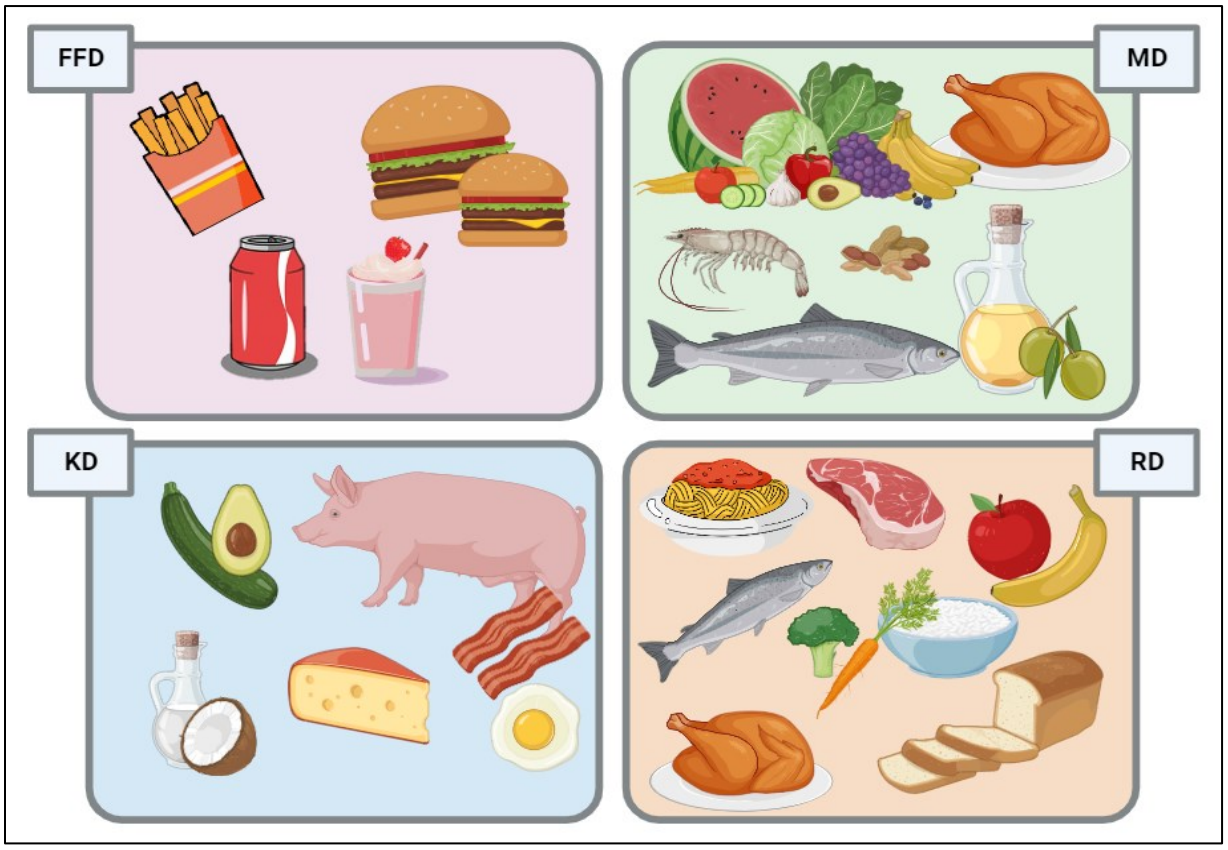

Figure 2.2. A summary of the foods and ingredients consumed during each diet. The FFD consisted of beef burgers, fries, sugary drinks, and milkshakes. The main ingredients for the MD were seafood, poultry, olives and olive oil, and nuts and legumes. The KD consisted of a higher consumption of pork (bacon and pork chops), eggs, cheese, fatty vegetables such as avocadoes and low-carbohydrate vegetables such as zucchini. To increase the fat intake, coconut oil was also added to my diet. The RD consisted of a moderate consumption of fruits and vegetables, whole wheat bread, pasta, and rice in addition to red meat, poultry and fish.

2.3. Food and constituents:

For all the diets, except for the FFD, I weighed each ingredient and food prior to consuming it, using an Etekcity Digital Kitchen Scale, model EK6015. For the foods sourced from McDonald's, it was not possible to weigh each ingredient. However, because detailed information about the ingredients, nutrient composition, cooked weight, and calories was available from McDonald's (via online data), ingredient weighing was not necessary. Each meal was weighed before consumption. All meals were consumed fully, and in a few incidences where I was not able to finish the meal, the leftover portions were weighed and consumed later in the day. For the MD and KD, all meal ingredients were delivered weekly through a meal kit delivery company [216]. All food components or ingredients compatible with the dietary recipes provided by the meal kit company were measured and used in preparing the meals. All foods were weighed and logged into the Fitbit application food log (Fitbit Inc., version 3.42) [215]. Daily food consumption was isocaloric and between 1800-2000/day kcals based on the Fitbit database. Fitbit device estimates energy expenditure using Basal Metabolic Rate (BMR), activity data, and HR measurements. Fitbit uses a proprietary formula to calculate total daily calorie burn, though its foundation is based on well-established scientific principles. BMR represents the number of calories expended by the body at rest to maintain essential physiological functions such as breathing, circulation, and cellular processes. Fitbit estimates BMR using personal data including gender, age, weight, and height. For this estimation, Fitbit applies the Mifflin-St Jeor equation [1], which is considered a reliable method for calculating resting energy expenditure (REE):

$$BMR\ for\ males = 10 \times weight\ (kg) + 6.25 \times height\ (cm) - 5 \times age\ (years) + 5$$

$$BMR\ for\ females = 10 \times weight\ (kg) + 6.25 \times height\ (cm) - 5 \times age\ (years) - 161$$

This equation accounts for individual differences in body size and composition, providing an estimate of the daily calories burned at rest, assuming no physical activity. Additionally, Fitbit tracks physical movement throughout the day using accelerometers and other sensors to monitor step count, distance traveled, and minutes spent being physically active. For each activity, Fitbit assigns a Metabolic Equivalent of Task (MET) value. MET values represent the energy cost of physical activities as a multiple of BMR. For example, sitting quietly has a MET of 1.0, indicating

energy expenditure equivalent to the BMR. More intense activities such as brisk walking or running have higher MET values, indicating greater energy expenditure. Fitbit multiplies the individual's BMR by the appropriate MET value for the activity being performed, adjusting for the duration and intensity of the activity. The general formula for caloric burn from an activity is:

$$Calories\ burned = MET\ value \times BMR \times Duration\ (hours)$$

For Fitbit devices with heart rate monitors, caloric burn estimates during physical activity are refined using heart rate data. Fitbit uses the heart rate reserve (HRR) method, which incorporates both the maximum heart rate and resting heart rate of the user. The formula for estimating heart rate reserve is as follows (and the maximum HR is typically calculated as 220 – age (years)):

$$Heart\ Rate\ Reserve\ (HRR) = Maximum\ Heart\ Rate - Resting\ Heart\ Rate$$

Fitbit uses this reserve to assess the intensity of physical activity and calculates calories burned more accurately based on heart rate during exercise. Higher heart rates during physical activity correspond to greater energy expenditure. The formula for caloric burn based on heart rate data is as follows:

$$Calories\ burned\ per\ minute = HRR\ \% \times BMR \times Duration\ (minutes)$$

Fitbit calculates total daily caloric expenditure by combining the BMR and the calories burned from physical activities throughout the day. This includes BMR (calories burned at rest over 24 hours), Activity Calories (calories burned through day-to-day movements such as walking, climbing stairs, or standing), and Exercise Calories (additional calories burned during intentional physical activities such as running, cycling, or gym workouts). By summing these components, Fitbit provides an estimate of the total calories burned each day.

For tracking calorie intake, Fitbit relies on the user manually entering or logging the food they consume into the Fitbit app. The process involves drawing from a large food database, which contains nutritional information, including calorie counts for thousands of food items. This database includes commonly consumed foods, restaurant meals, and packaged items. When logging food, the user enters the food type and portion size, and Fitbit calculates the corresponding

calorie intake based on the stored nutritional data. Additionally, the app allows users to scan barcodes of packaged foods, automatically pulling the relevant nutritional information from Fitbit's database. In cases where foods are not listed, users can manually input the nutritional values, allowing for flexibility with custom recipes or unique meals. Once the food data is logged, Fitbit compares the calorie intake (from logged foods) against the calories burned (estimated by the device) to provide insights into energy balance, which can help users monitor weight management goals.

Each meal and snack food item, timing of food consumption, and food weight were recorded using the Fitbit diary (Fitbit Inc., version 3.42) [215]. A record of all the foods and beverages I consumed each day was also entered into the Automated Self-Administered 24-Hour (ASA24®) Dietary Assessment Tool [95] (ASA24-Canada-2018), developed by the National Cancer Institute, Bethesda, MD. This is a web-based platform used to capture food and beverage intake, as well as some eating characteristics, and is widely considered to be the gold standard for collecting dietary intake data. The comparison results between ASA24 and Fitbit showed a moderate alignment in calorie intake estimates, as indicated by the correlation coefficient of 0.53. This suggests that, while the two methods generally follow the same trend, there are noticeable differences in their daily calorie estimates. The average daily calorie intake recorded by ASA24 was 1959.8 ± 132.0 kcal/day, while Fitbit estimated a slightly higher average of 1966.6 ± 241.2 kcal/day. The Bland-Altman plot further illustrates this by showing the differences between the two methods (Figure 2.3). The plot demonstrates that, although the majority of the points fall within the limits of agreement, there are some fluctuations, particularly at higher intake levels. The mean difference between the methods is small, indicating no strong systematic bias, but the variation at individual data points suggests that the methods may not always align perfectly for certain days or calorie ranges (Figure 2.3).

For this study, I used the Canadian 2018 version of ASA24, which links the foods entered to the Canadian Nutrient File [217]. I also calculated the Healthy Eating Index (HEI) score (Healthy Eating Food Index (HEFI)-2019, Canada) [218,219]. This score is used to quantify how closely my daily dietary intake aligned with recommendations from Health Canada. As mentioned earlier for the FFD, all the meals were consumed fully during the other three dietary interventions, and in a few incidences where I was not able to finish the meal, the leftover portions were weighed and

consumed later in the day. An example of a typical day during the interventions, daily calorie intakes across all diets and a sample menu is presented in Appendix 1 Tables 1 - 3.

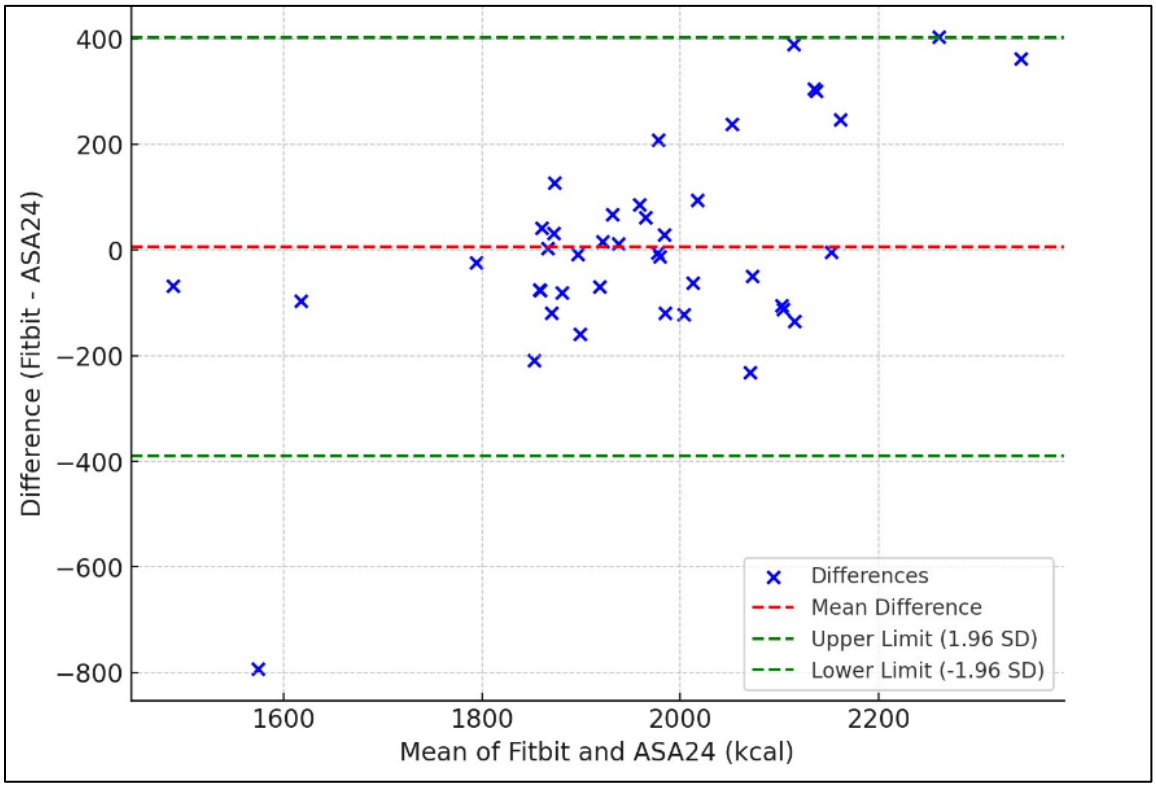

Figure 2.3 - Bland-Altman plot comparing calorie estimates from ASA24 and Fitbit. The plot displays the mean of the two methods on the x-axis and the difference between Fitbit and ASA24 calorie estimates on the y-axis. The red dashed line represents the mean difference between the two methods, while the green dashed lines indicate the limits of agreement (±1.96 standard deviations). The majority of points fall within the limits, indicating general agreement between the two monitoring methods.

2.4. Anthropometric Assessments:

I conducted daily measurements of general anthropometric information, including my weight (in kilograms, Etekcity Digital Body Weight Bathroom Scale, model EB4887), my body mass index (BMI), and percentage of body fat. BMI was calculated by dividing my measured weight in kilograms by the square of my height in meters (using Body Measuring Tape, White yaochi-200). Body fat percentage was determined using the US Navy method [220], which considers gender, age, height, and weight (in kilograms), as well as daily measurements of neck, waist, and hip circumferences (in centimeters). Measurements were made using a white Yaochi-200 Body Measuring Tape. To ensure accuracy while self-measuring, a flexible measuring tape was used, avoiding elastic or cloth tapes that might stretch. The tape was correctly positioned and consistently placed at each measurement site, with the aid of a mirror to maintain level and straight placement around the body. Additionally, measurements were taken at the same time each day and under similar conditions, to minimize variations caused by factors such as food intake and hydration levels.

2.5. Biological sample collection:

I collected blood and urine samples each day during the entire study. Fasting blood samples were obtained each morning using a Tasso+ device [221]. The Tasso+ is a single-use blood-lancing device that extracts small whole blood samples using a micro-capillary device. The device consists of a button for releasing the microblade and a detachable microtube (Figure 2.4A). For blood collection, the device was placed on the upper arm following the application of a heat pack (provided in the package) to enhance blood circulation, and then sterilization of the area. Upon pressing the device's lancing button, the microblade is released, creating a small incision on the arm. Within 5 minutes, 500-600 μL of blood can be collected with little or no pain or follow-on soreness. After collection, the microtube (containing EDTA (Ethylenediaminetetraacetic acid)) is detached from the device and closed using the tube cap and inverted several times. After incubating for 30 minutes at room temperature under quiescent conditions, these sample tubes were centrifuged at 810 g for 10 minutes at room temperature to separate the plasma. The separated

plasma was then carefully transferred into Eppendorf tubes and stored at -20°C until they could be transported to a long-term storage facility at -80°C.

Urine samples were collected twice daily into sterile urine collection container (Corning 50 mL centrifuge tubes), once in the morning upon waking and once at night (Figure 2.4B). These urine samples were aliquoted using disposable plastic pipettes into 1.5 mL tubes and promptly frozen at -20°C until they could be transported for final storage in the -80°C freezer.

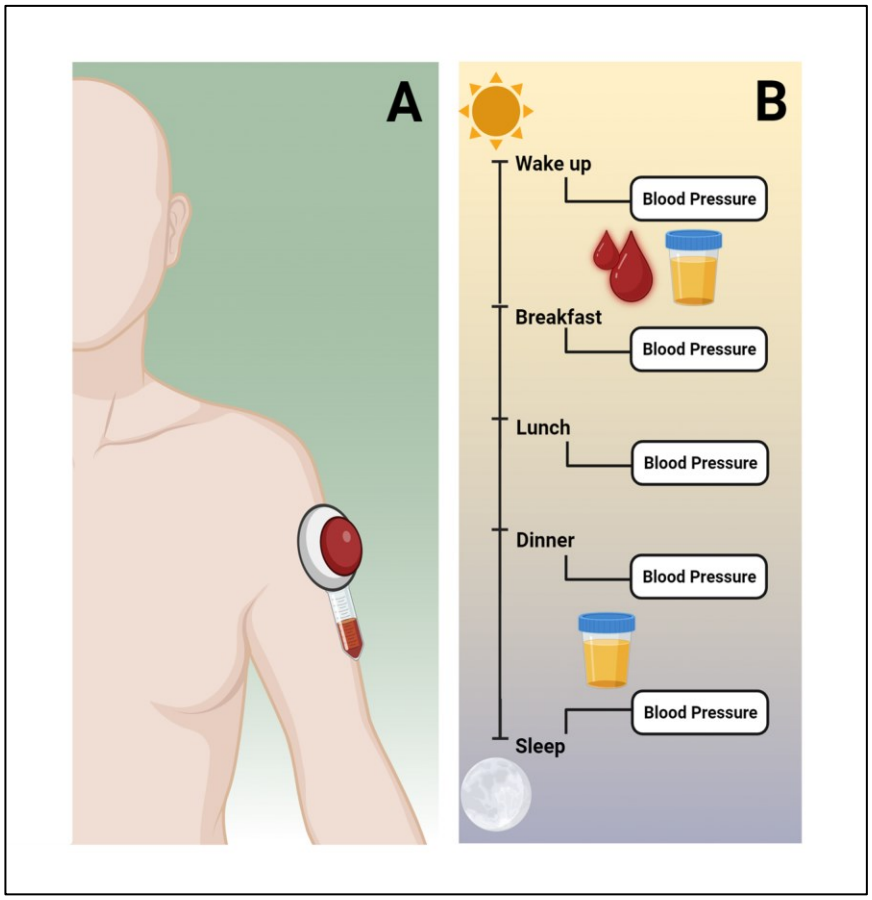

Figure 2.4. A: Tasso+ device attached to the upper arm. Upon pressing the red button, the microblade is released which creates an incision on the arm and is expected to collect 500-600 µL of blood within 5 minutes. **B:** The daily timeline of sample collection (blood and urine), and blood pressure monitoring.

2.6. Blood pressure (BP) monitoring:

BP readings were obtained using a smart wearable BP monitor (YHE BP Doctor Pro BP Smartwatch (Yanhe Intelligent Technology Ltd, Hangzhou, China)). BP Doctor Smartwatch employs a downsized version of the elements used in standard oscillometric BP measurement. It integrates an inflatable cuff into the watch band similar to the process of a typical upper arm oscillometric device. I measured my BP five times daily: upon waking in the morning, following each main meal (breakfast, lunch, and dinner), and just before bedtime (Figure 2.4B). All data acquired by the BP Doctor Smartwatch was exported to a computer using the BP Doctor App and saved as a *.csv file. A picture of the BP Doctor Smartwatch is shown in Figure 2.5.

Figure 2.5. The smart wearable BP monitor (YHE BP Doctor Pro Blood Pressure Smartwatch). The inner layer of the watch strap (air cuff) slowly inflates, increasing the tightness around the wrist with the SYS (systolic) number going up. Once the measurement is complete, the SYS/DIA (diastolic) measurements in mmHg as well as the HR (bpm) are presented on the screen.

2.7. Physiological monitoring via a smartwatch:

To track a number of physiological measures continuously and quantitatively, I wore a Fitbit Sense Smartwatch (Fitbit Inc., San Francisco, USA) on my non-dominant (left) wrist. This model continuously records HR (Heart Rate, in beats per minute, bpm), breathing rate (expressed as breaths per minute), HR variability (measured in milliseconds), and sleep-related metrics such as sleep quality, sleep cycles, sleep stages, and sleep duration. Periods of restlessness during sleep and instances of snoring were also noted. Variations in BT (body temperature) from the baseline were also recorded during each sleep cycle using the smartwatch, along with the percentage of

time spent with a HR above the resting HR during sleep (examples of the Fitbit dashboard and Fitbit app information are provided in Figure 2.6). All data acquired by the smartwatch was exported via the Fitbit API using the Python programming language or through the Fitbit dashboard associated with my Fitbit account and saved as a *.csv file.

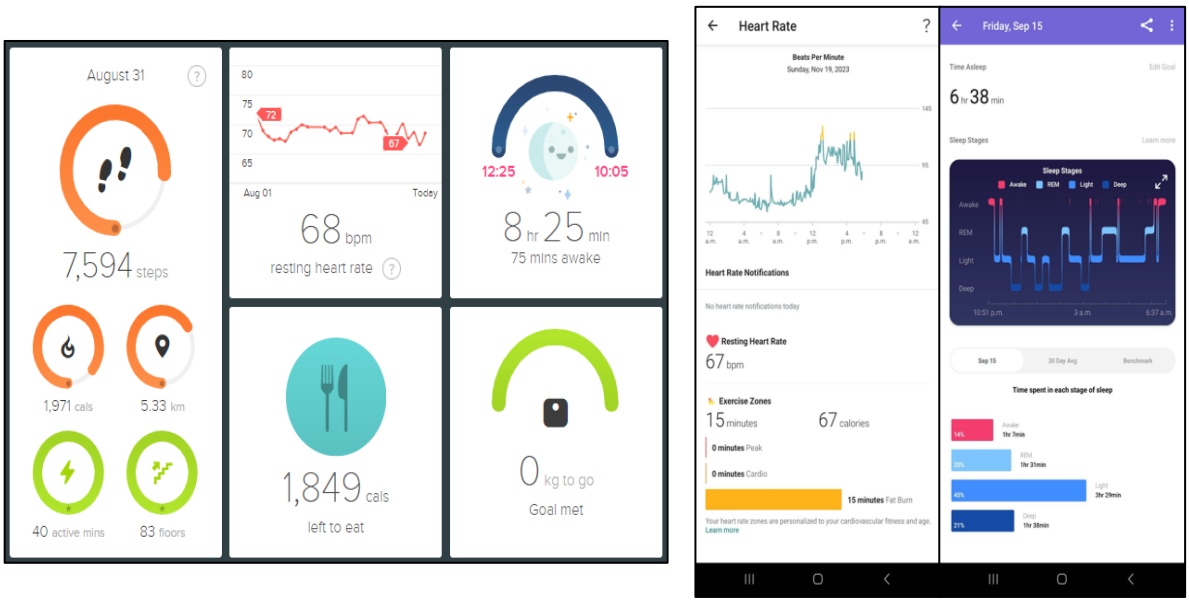

Figure 2.6. A: An example of the Fitbit data obtained from my personal Fitbit dashboard. A summary of physical activity, sleep, HR (Heart Rate) and the calorie intake are shown in this view. B: A screenshot of the Fitbit app showing HR variations of a day and a screenshot of sleep data, including the sleep stages and the time spent in each stage.

2.8. Blood glucose (BG) monitoring:

I continuously monitored my BG levels using a Dexcom G6 (DexCom Inc., California, USA) continuous glucose monitor (CGM), which provided BG values in mmol/L at five-minute intervals. The Dexcom system is a battery-powered CGM that uses a tiny sensor wire inserted just underneath a person's skin using an automatic applicator. This sensor wire uses a glucose oxidase (GO) chemical reaction to generate an electrical signal that is converted into an estimated glucose value [222]. An adhesive patch holds the CGM sensor housing in place, so the sensor can measure glucose readings in interstitial fluid throughout the day and night. A small, reusable transmitter connects to the sensor wire and sends real-time readings wirelessly to a receiver so that the user can view glucose information. To gain insights into my glucose response to specific foods, I conducted glucose challenge tests. I noted the time of meal consumption and avoided eating for the following two hours to monitor fluctuations in my glucose levels. The Dexcom G6 monitor recorded BG values and transmitted this data to my smartphone. The collected data was accessible through the Dexcom G6 mobile application, including cloud-based storage, and provided alerts for low and high glucose levels, sent directly to my smartphone. All data acquired by the Dexcom G6 monitor was exported via the Dexcom App and the cloud-based storage associated with my Dexcom account and saved to my computer as a *.csv file.

2.9. Body temperature (BT) monitoring:

BT was tracked using a wearable and non-invasive system designed for continuous core BT monitoring (CORE, greenTEG, Rümlang, Switzerland) (Figure 2.7). The sensor was affixed to my non-dominant (left) arm using an arm strap and remained in place throughout each of the diet periods, with brief periods where it was removed for recharging. It recorded skin temperature values at five-minute intervals, with the collected data accessible through a dedicated mobile application and cloud-based storage. CORE's sensor detects heat transfer within the body, delivering real-time core BT. Specifically, the CORE sensor uses a thermoelectric generator (TEG) that takes advantage of the Seebeck effect to measure temperature. The Seebeck effect is a phenomenon in which a temperature difference between two dissimilar electrical conductors or semiconductors produces a voltage difference between the two substances [223]. When (body) heat

is applied to one of the two conductors or semiconductors, heated electrons flow toward the cooler one. In this way, the TEG sensor generates a proportional voltage signal based on heat flow. CORE uses miniaturized TEGs, such as green TEG's, which were designed specifically for compact wearables. All data acquired by the CORE monitor was exported and saved as a *.csv file.

Figure 2.7. CORE sensor wearable body thermometer, worn on the upper arm using the arm strap.

2.10. Emotional and mood monitoring:

Food and diets are known to affect an individual's mood and emotions [224]. As few other PN studies had ever studied mood or emotions and the effects that foods or diet had on mood or emotions, I decided to track this for my N-of-1 study. This emotional/mood surveillance was done at three-day intervals by administering a comprehensive questionnaire known as the Profile of Mood State (POMS) [225]. This questionnaire provided a structured framework to evaluate my emotional state and to gain insights into the dynamic nature of my mood changes. I recorded introspective accounts of my emotional experiences on specific days, days 1, 4, 7, 10 and 14 during each diet trial. This self-administered survey was intended to capture both immediate and evolving emotional responses to dietary changes. In addition to these three-day evaluations, I also implemented a daily emotional/mood tracking system. This entailed the maintenance of a detailed written diary, which allowed me to record my emotions and feelings daily throughout the entire duration of each diet trial. This daily diary approach ensured that I did not miss nuances or fluctuations in emotional states that were missed by the three-day POMS survey. The combination of periodic questionnaires and daily diaries provided a rich collection of mood and emotional data, which was intended to explore the connections between diet, mood, and emotions with mental, molecular, or physiological data.

2.11. Physical performance monitoring:

Food and diets are known to affect an individual's physical performance and physical well-being [226]. As few other PN studies had ever studied physical performance and the effects that foods or diets had on physical performance, I decided to track this for my N-of-1 study. Physical performance monitoring was achieved through the implementation of a consistent exercise regimen spanning five days per week during the diet trials. The core of this exercise routine involved treadmill walking sessions lasting between 40 to 45 minutes. These treadmill sessions involved alternating between walking at 5 km/h and 7 km/h with three-minute periods at the slower pace followed by two minutes at the higher pace, continuing in cycles until the designated exercise duration was met. Throughout the exercise period, BT was monitored, as was HR. Additionally, the time required for my HR to return to the resting rate after exercise (commonly referred to as

the recovery time) was also tracked. This physiological evaluation provided valuable insights into my cardiovascular fitness, endurance, and the impact of different diets on my physical performance.

2.12. Mental performance monitoring:

Food and diets are known to affect an individual's mental performance and cognitive function [227,228]. As few other PN studies had ever studied mental performance and the effects that foods or diet had on mental performance, I decided to track this for my N-of-1 study. Five different mental tests were developed, and each was delivered and taken electronically via simple computer programs that I wrote in Python (the code for these programs is provided in Appendix 1A - 1E). The tests were: 1) a Serial Subtraction Test; 2) a Reaction Time Test; 3) a Trail-Making Test; 4) a Stroop Effect test and 5) a Digit Span test. More details about each test and what it entailed are given below. It is important to note that these mental tests were specifically chosen and specifically designed so that they would assess different aspects of my mental performance and cognition. The tests were also selected because they were the type of test that could not easily be mastered through practice or repetition. Although it is possible to improve on these tests with repeated administration, the rate of learning generally diminishes over time, thanks to the plateauing of learning curves associated with such tests [229]. To minimize the influence of these learning effects on the study results, all assessments were evaluated before the start of the dietary interventions. Alternate forms of the tests were used, longer intervals between test sessions were enforced, and repeated measures were employed in the data analysis to adjust for potential learning effects. This ensured that the observed changes in cognitive performance were not merely due to repeated exposure to the tests and would better reflect or assess mental performance effects as a consequence of each diet regimen. The fact that the tests were conducted through a computer and were fully quantitative ensured high precision and consistency over the assessment process. These tests, which were conducted daily, provided information about my cognitive function and mental performance throughout the study.

The Serial Subtraction Test [230] is a mental performance test wherein I was presented with a random starting number between 100 and 999. A second random number between 3, 4, 6, 7, or 8 was then provided, and generated through a random number generator. My task was to perform

these subtractions within a 60-second time frame. Upon completion, the program assessed both the number of subtraction calculations completed and the accuracy of those calculations. This test was conducted three times, and the average results provided a comprehensive snapshot of my mental agility.

The Reaction Time Test [231] is a mental performance test designed to evaluate a participant's reflexes and response time. During this test, the screen displayed a prompt after a random number of seconds, and upon seeing the prompt I had to press the spacebar as quickly as possible. The program records the time it takes to react to the prompt in seconds (with 0.1-second accuracy). This test was performed five times, with the average reaction time from these five trials serving as the metric of response time.

The Trail-Making Test [232] is a mental performance test designed to assess cognitive processing speed, attention, and executive function. In this test, a random combination of letters and numbers was generated using the computer and a Python script. The final random combination was transformed to *.pdf format and then printed. My challenge was to connect the numbers and letters sequentially in the correct order. The time taken to complete the task as well as the number of errors made during the process were recorded. The trail-making test was only conducted once. This test provided useful insights into my cognitive flexibility and attention span.

The Stroop Effect Test [233] is a mental performance test designed to evaluate cognitive processing speed, selective attention, and cognitive flexibility. In this test, the computer generated a list of 100 color names, with the words written in various colors. My task was to read aloud the color in which each word is written (ink color), not the actual color names. The test recorded the time taken to complete the task (in seconds) and the percentage of correct answers as the final result. The Stroop effect test was only conducted once. This test assessed my ability to manage cognitive conflicts and attentional control.

The Digit Span Test [234] is a mental performance test designed to assess working memory and attention span. In this test, 10 random numbers between 0 and 9 were generated using a random number generator and displayed on the computer. The numbers were displayed one after the other. After the sequence was presented, it was cleared from the screen, and I had to recall and type the

numbers in the exact order they appeared. This test measured my ability to retain and manipulate information in working memory.

The typical time to complete all five tests was approximately 30 minutes. All data for all mental performance tests throughout all diet periods was recorded and kept as a *.csv file for subsequent data analysis.

2.13. Metabolomics analysis:

2.13.1. Plasma analysis

Metabolomic analyses were conducted on the collected plasma and urine samples using the cutting-edge assay developed by The Metabolomics Innovation Centre (TMIC) known as the TMIC MEGA assay. This assay uses a combination of direct flow injection mass spectrometry (DFI-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) to separate and identify metabolites. This LC/DFI-MS/MS assay can perform targeted, absolutely quantitative metabolic profiling of up to 726 endogenous metabolites. An additional ~200 metabolite ratios and sums can be calculated based on those metabolite concentrations. Within the context of this study, the MEGA assay yielded quantitative results for an average of 636 metabolites in my plasma samples. The MEGA assay covers a wide range of metabolite categories, spanning biogenic amines, amino acids, amino acid-related metabolites, phosphatidylcholines (PCs), lysophosphatidylcholines (LysoPCs), sphingomyelins (SMs), hydroxy-sphingomyelins (SM(OH)s), acylcarnitines (ACs), triglycerides (TGs), diglycerides (DGs), organic acids, and more. Combined with an average of 188 combinations of metabolite sums and ratios, this assay could yield a total of 824 absolutely quantitative metabolite values.

The TMIC MEGA assay uses a combination of chemical derivatization, analyte extraction and separation, and selective mass-spectrometric detection using multiple reaction monitoring (MRM). This allows the assay to not only identify but also accurately quantify metabolites. The assay utilizes chemical derivatization methods reverse-phase high-performance liquid chromatography tandem mass spectrometry (RP-HPLC-MS/MS) for organic acids, amino acids, amino acid derivatives, and biogenic amines, as well as direct flow injection (DFI) analysis for the

quantification of lipids and ACs. To ensure precise metabolite quantification, the assay uses isotope-labeled internal standards (ISTDs) in conjunction with other ISTDs. For optimal high throughput analysis, the assay was performed in 96-well plate format. It is also worth noting that this assay requires specific standard reagents and solvents, with stock solutions of each standard prepared by dissolving accurately weighed solids in double-distilled water (ddH_2O). Seven different calibration curve standards (Cal 1 - Cal 7) were obtained by mixing and diluting these stock solutions with appropriate solvents, covering different concentration ranges for different analytes according to their known or expected normal/pathological concentrations in human biospecimens. For analysis of amino acids, amino acid derivatives, biogenic amines, nucleotide/nucleosides, and organic acids, 3 quality control (QC) standards with different concentrations were prepared by diluting the Cal 7 standard solution with the same solvents as the calibration standards. This comprehensive analytical framework was extended to all metabolite categories, with stock solutions of isotope-labeled compounds prepared in a similar fashion for carbohydrates, ACs and their derivatives, and lipids and their derivatives. A working internal standard (ISTD) solution mixture in ddH_2O was also created by mixing all the prepared isotope-labeled stock solutions. For organic acids, stock solutions of isotope-labeled compounds were prepared using 75% aqueous methanol. A working ISTD solution mixture in 75% aqueous methanol was then created by mixing and diluting all the isotope-labeled stock solutions.

The TMIC MEGA assay uses a 96 deep-well plate configuration, with a separate filter upper plate securely attached on top of a lower plate via sealing tape. A total of 14 wells of the 96 wells are dedicated to performing various calibration, QC, and reference checks, including wells for a blank sample, three zero-point samples, seven standard-containing or calibration samples, and three QC samples. To prepare the plasma samples for analysis, they were thawed on ice after removal from the freezer, followed by thorough vortexing and centrifugation at 18,000 x g for 5 min at 4ºC temperature. Subsequently, 10 µL of each plasma sample was loaded onto the center of the filter paper (placed in each well) on the upper 96-well plate and dried under a stream of nitrogen. To derivatize amine-containing metabolites, phenyl-isothiocyanate (PITC) was added to the dried plasma, and the filter spots were dried once more using an evaporator. The metabolites were then extracted by introducing an ammonium acetate/methanol mixture (5 mM ammonium acetate dissolved in 300 µL methanol), and the extracts were subsequently centrifuged into the lower 96-deep well plate before being diluted with the MS running solvent.

For organic acid analysis, 150 µL of ice-cold methanol and 10 µL of an isotopically-labeled ISTD mixture were added to 50 µL of the plasma sample and incubated at -20 °C overnight to precipitate protein. Then the sample was centrifuged at 13,000 x g for 20 minutes at 4 °C. Following centrifugation, 50 µL of the supernatant was loaded onto the center of a selected well of the 96-deep well plate. To derivatize organic acid-containing metabolites, 3-nitrophenylhydrazine (3-NPH) was added as well as butylated hydroxytoluene (BHT), used as a stabilizer, along with water, before LC-MS injection. All chemically-derivatized samples were subsequently delivered to a Sciex Qtrap® 5500 tandem MS (Agilent Technologies, Palo Alto, CA), equipped with an Agilent 1290 series UHPLC system and an Agilent Zorbax C18 column (Agilent Technologies, Palo Alto, CA). Furthermore, to detect and quantify lipids and ACs, samples were delivered directly to the MS via DFI. Data analysis was performed using Analyst 1.7.2 software (Applied Biosystems/MDS Analytical Technologies, Foster City, CA), ensuring the accuracy and precision of our metabolomic assessments.

2.13.2. Urine analysis

Nearly the same metabolomics methodology described above was applied to the analysis of urine samples. The one major difference is that the protein precipitation step was not necessary for the urine samples. The urine assay also differs from the plasma assay in terms of the final dilution factor and the volume of sample (20 µL) analyzed. The urine analysis produced quantifiable results for 262 metabolites and more than 150 sums and ratios were calculated using the measured metabolites.

2.14. Microbiome analysis:

To assess my microbiome, I employed a specialized gut microbiome test provided by a commercial supplier known as EasyDNA (EasyDNA Canada, Ontario, Canada). The gut microbiome test kit includes all the materials needed to collect and ship the fecal sample, including rubber gloves, a test tube, and a shipping envelope. The microbiome tests were conducted at the end of each 14-day diet trial, with samples being collected and sent to EasyDNA on the 14th day and data returned within 2-3 weeks. The EasyDNA test uses 16s rRNA sequencing to identify the type and abundance of specific bacteria in a fecal sample. An example of an EasyDNA microbiome

test report is provided in Figure 2.8. The gut microbiome test allowed me to conduct a systematic evaluation of how each diet trial influenced the composition and richness of my gut microbiome. The assessment of microbial diversity provided a way to connect dietary changes with changes in my gut microbiota. It also helped to pinpoint specific shifts, adaptations, or alterations within my gut microbial communities that were induced by dietary changes.

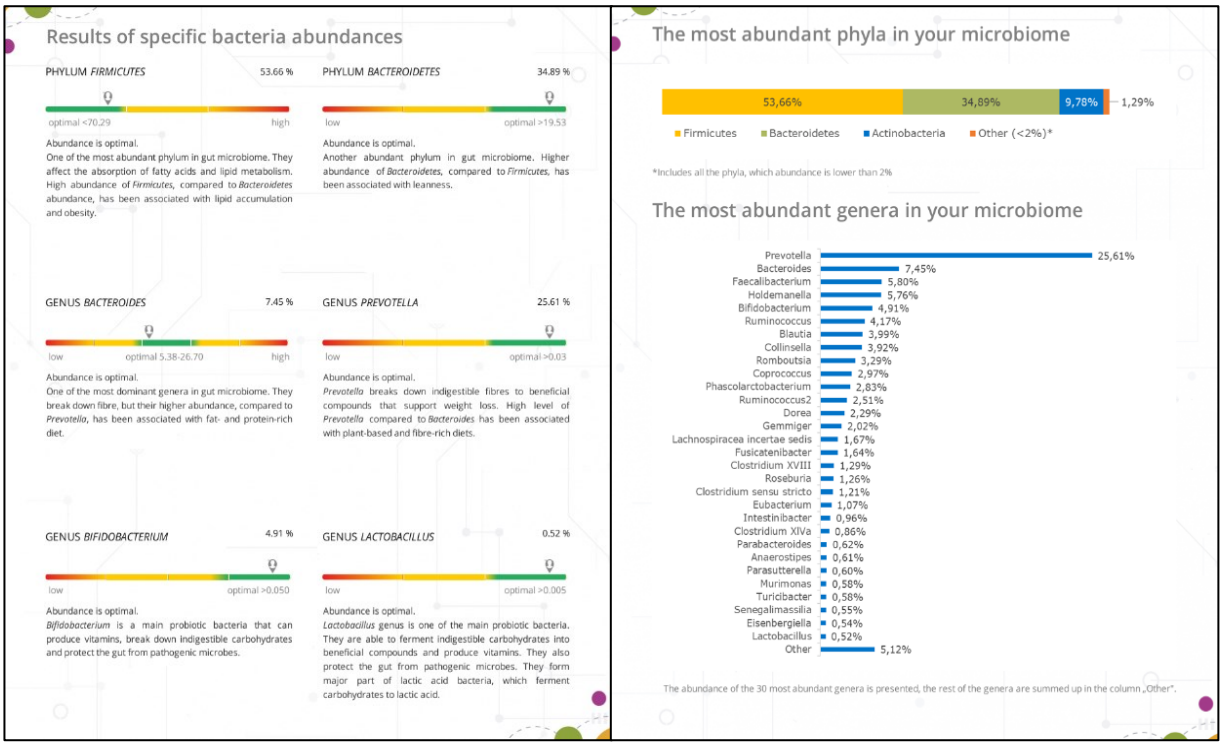

Figure 2.8. An example of the Easy DNA gut microbiome report which indicates the detected and quantified abundances of various bacterial phyla and genera.

2.15. Proteome analysis:

Proteomic analysis was conducted on collected plasma samples (day 1, 3, 5, 7, 11, 14), employing the advanced Meso Scale Discovery (MSD, Rockville, Maryland, USA) V-PLEX Proinflammatory Panel 1 (human) Kit. This kit quantifies ten specific immune proteins including IFN-γ (interferon-γ), IL-1β (interleukin-1β), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF-α (Tumor Necrosis factor-α). The V-PLEX method is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) that uses electrochemiluminescence (ECL) as a detection technique as opposed to a colorimetric reaction employed by more conventional ELISAs. The V-PLEX Proinflammatory Panel 1 (human) Kit contains MULTI-SPOT plates pre-coated with capture antibodies, calibrators, individual labeled detection antibodies and optimized diluents. All ELISAs were performed following manufacturer instructions which involved these general steps. First, the plate was washed 3 times with PBS followed by an addition of 50 µL the calibrator dilutions into the wells. Then 25 µL of each plasma sample was added. The plate was sealed and incubated for 2 hours while shaking at room temperature. The plate was washed with PBS and 25 µL of the Sulfo-Tag labelled Detection Antibody Blend Solution was dispensed to the plate, followed by another 2-hour incubation, and shaking at room temperature. After the incubation period, the plate was washed and 150 µL of the GOLD Read Buffer B was added. After completing the ELISA steps, the plate was read using an MSD plate reader (QuickPlex SQ 120MM Imager) located on the 6th floor of the Li ka Shing building - the University of Alberta. The longitudinal measurement of this subset of pro-inflammatory proteins over each dietary period was intended to complement the metabolomic information and provide additional details about more subtle diet-induced inflammatory changes that may not have been detectable via metabolomics or physiological monitoring.

2.16. Genome analysis:

To gain a comprehensive understanding of my genetic makeup, I performed a genetic test using the 23andMe (23andMe, California, USA) genetics profiling kit. The kit comes with instructions, a specially designed tube for capturing and preserving saliva and a shipping envelope. 23andMe performs genetic testing using the saliva sample, employing the Illumina Global

Screening Array (GSA), version 5.0 SNP chip to measure genetic variations. Version 5.0 of the GSA is a next-generation genotyping array for population-scale genetics, variant screening, pharmacogenomics studies, and precision medicine assessment. This version of the chip has 650,000 SNPs suitable for both ancestry and health testing. This genetic test was performed only once, at the beginning of the study, and was used primarily to identify any potential genetic propensities, food preferences, food aversions or other risks or potential responses that might affect the planned dietary interventions. This genetic data was intended to help complement the physiological, dietary, and biochemical information collected throughout the study.

A visual representation of the methods and monitors used in this study is presented in Figure 2.9.

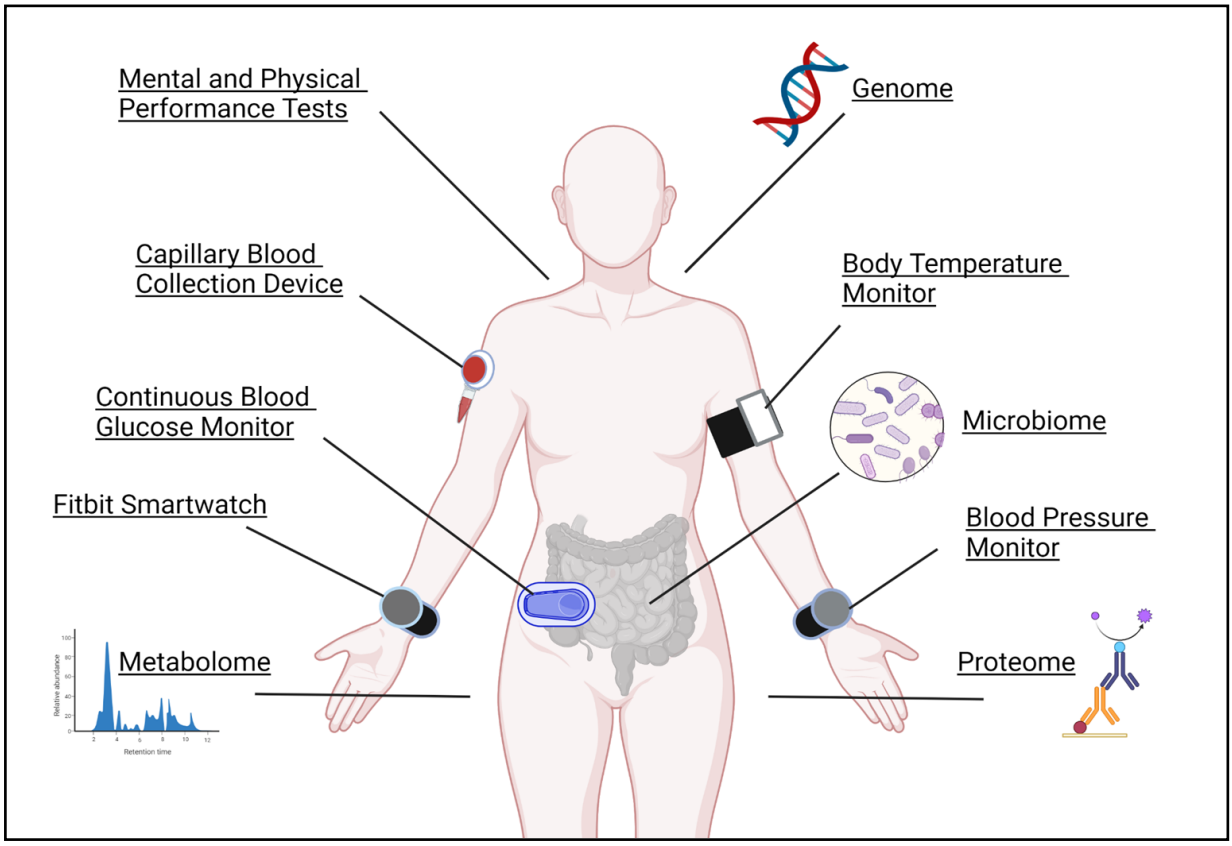

Figure 2.9. A general overview of the monitors, tests and omics measurements used in this study.

2.17. Statistical Analysis:

Throughout this project, I utilized a range of statistical methods to ensure comprehensive data analysis. To compare means between different groups, I employed t-tests for pairwise comparisons and Analysis of Variance (ANOVA) for multiple group comparisons. To check for equal variances, I applied Brown-Forsythe and Bartlett's tests. Univariate analysis was conducted to explore individual variables, while multivariate analysis examined the relationships between multiple variables simultaneously. Chemometrics analysis was used to process and interpret complex chemical data, aiding in the identification of significant patterns. Cluster analysis helped to group similar data points, revealing natural clusters within the dataset. Across all these analyses, a p-value of 0.05 was used as the threshold for statistical significance, ensuring that the results were reliable and robust. It is worth noting that all urinary metabolomics data were normalized to creatinine levels (μmol/mmol creatinine).

Chapter 3

Results

3.1. Diet Analysis: Food Categories

To calculate the intake for each food or food group, menu items were converted into the number of standard servings per day using the ASA24 - 2018, Canada serving size guidelines. The food intake variables were then estimated by averaging the intake over 14 days to derive mean servings per day. A summary of the food groups, food items, and the average amount consumed during each dietary intervention is presented in Tables 3.1 and Figures 3.1 – 3.3.

3.1.1. Fruits and vegetables:

Fruits or fruit juices were consumed during the MD and RD, while none were consumed while on the FFD and KD (Table 3.1). The highest level of dark green vegetables consumption was while on the KD, while dark green vegetables were consumed at a lower rate while on the RD and MD. No dark green vegetables were consumed while on the FFD (Table 3.1). Red and orange vegetables, such as tomatoes and tomato products, were consumed at an average rate of 346.9 ± 288.3 g/day during the MD. In contrast, the RD, KD, and FFD averaged 196.5 ± 151.5 g/day, 117.6 ± 102.2 g/day, and 29.9 ± 32.2 g/day of red/orange vegetable consumption, respectively. The consumption of starchy vegetables was the highest while on the FFD (113.0 ± 83.3 g/day) which was fully sourced from potatoes. In contrast, just 29.6 ± 41.7 g of potatoes and 24.7 ± 51.2 g of other starchy vegetables were consumed during the MD. No potatoes were consumed during the RD. However, 45.7 ± 34.5 g of other starchy vegetables such as carrots, corn, and green peas were consumed during the RD. No starchy vegetables were consumed during the KD. On average, 33.6 ± 70.4 g/day of legumes were consumed during the MD, while none were consumed while on the FFD, KD, and RD. (Table 3.1, Figure 3.1).

3.1.2. Grains, nuts, and seeds:

On average, 199.0 ± 35.4 g/day of refined grains were consumed while on the FFD, while no whole grains were consumed. An average of 25.4 ± 19.7 g/day of whole grains and 87.9 ± 31.1 g/day of refined grains were consumed while on the RD. The MD had the highest consumption of grains with an average of 45.2 ± 56.7 g/day of whole grains and 92.7 ± 48.6 g/day of refined grains. On the other hand, no grains were consumed while on the KD. 28.3 ± 28.3 g/day of nuts and seeds were consumed while on the RD, 5.9 ± 12.1g/day were consumed while on the KD, and 2.1 ± 2.4 g/day were consumed while on the MD, while no nuts or seeds were consumed while on the FFD (Table 3.1, Figure 3.1).

3.1.3. Meat and eggs:

The KD had the highest levels of meat consumption, while the lowest amount of meat consumption occurred while I was on the RD (Table 3.1, Figure 3.2). The KD and FFD had the highest level of red meat (including beef, veal, pork, lamb, and game meat) consumption with an overall average of 141.2 ± 82.3 g/day, and 116.9 ± 38.4 g/day, respectively, while the RD and MD had the lowest levels of red meat consumption (5.5 ± 12.8 g/day, and 2.5 ± 6.8 g/day, respectively). An average of 5.4 ± 16.3 g/day and 22.1 ± 10.8 g/day of cured meat were consumed while on the RD and KD, respectively. The FFD had the highest levels of cured meat and sausage consumption (31.1 ± 23.5 g/day). No cured meat (including sausages, corned beef, and luncheon meat) was consumed while on the MD. On average, 57.3 ± 63.8 g/day of poultry was consumed while on the MD, 55.4 ± 83.1 g/day on the KD, and 19.8 ± 34.1 g/day while on the RD. No poultry or seafood were consumed while on the FFD. No consumption of organ meat from beef, veal, pork, lamb, game, and poultry was recorded for any of the diets. Eggs were consumed the most while on the KD (55.7 ± 14.2 g/day), while eggs were consumed much less frequently while on the other diets (4.4 ± 11.3 g/day, 11.5 ± 26.3 g/day, 0.6 ± 2.3 g/day while on the FFD, RD, and MD, respectively). No food containing soy was consumed across any of the diets.

3.1.4. Dairy:

The highest consumption of dairy was while on the FFD which was mainly sourced from cheese (410.0 ± 150.1 g/day), and milk (63.7 ± 80.8 g/day). The MD had the second-highest

consumption of dairy with an average of 358.7 ± 186.3 g/day of cheese, 95.2 ± 73.4 g/day of milk, and 16.4 ± 28.2 g/day of yogurt. In contrast, just 208.3 ± 208.3 g/day of cheese and 17.4 ± 6.6 g/day of milk were consumed while on the KD, while no yogurt was consumed. An average of 129.8 ± 133.5 g/day of cheese, 49.52± 83.8 g/day of milk and no consumption of yogurt was recorded while on the RD (Table 3.1, Figure 3.2).

3.1.5. Oils and solid fats:

Fats that are naturally present in nuts, seeds, and seafood. Unhydrogenated vegetable oils, coconut oils, and the fat in avocado and olives (above the allowable amount) were recorded as "oils" which were highest while on the KD (52.1 ± 28.5 g), and MD (45.3 ± 21.5 g). The lowest level of oils was consumed with the FFD (17.9 ± 5.6 g). In contrast, solid fats (fats naturally present in meat, poultry, eggs, and dairy; hydrogenated/partially hydrogenated oils; shortening, palm, palm kernel, coconut oils; coconut meat, and cocoa butter), were highest with the FFD (58.3 ± 13.3 g/day), and lowest with the RD (9.6 ± 5.7 g/day) (Figure 3.3).

3.1.6. Beverages:

The consumption of unsweetened drinks such as coffees were 441.3 ± 146.0 g/day while on the KD, 391 ± 96.5 g/day while on the MD, 393 ± 76.2 g/day while on the FFD and 264.9 ± 73.7 g/day while on the RD. No other kinds of drinks such as teas or alcoholic beverages were consumed while on any of the four diets.

3.1.7. Added sugars:

The highest sugar consumption with an average of 55.0 ± 14.1 g/day of sugar was recorded while on the FFD. Expectedly, the lowest sugar consumption was with the KD (2.8 ± 7. 8 g/day) (Figure 3.3). An average of 297.5 ± 123.1 g/day of sugar in the form of sugary drinks were consumed while on the FFD which consisted of Coke and other sodas, milkshakes, and sweetened iced coffees and coffee creamers. The quantity of sugary drinks consumed for all other diets was zero. However, an average of 24.4 ± 6.6 g/day of sugar in the form of unsweetened fruit juice was consumed while on the MD.

Table 3.1 - The mean 14-d intake of consumed foods, beverages, and dietary supplements measured by weighed food across all dietary interventions

Food Group	Diet exposure	FFD	MD	KD	RD
Fruits	Intact fruits of citrus, melons, and berries (grapefruit, blueberry) (g/day)	0.0 ± 0.0	88.3 ± 153.7	0.0 ± 0.0	35.6 ± 97.6
	Intact fruits; excluding citrus, melons, and berries (apple and banana) (g/day)	0.0 ±0.0	404.4 ± 354.7	0.0 ± 0.0	235.9 ± 234.7
	Fruit juices (lemon and orange juice) (g/day)	0.0 ± 0.0	152.6 ± 119.5	0.0 ± 0.0	3.8 ± 9.8
Vegetables	Dark green vegetables (broccoli, romaine, spinach, kale, parsley, other dark green leafy vegetables) (g/day)	0.0 ± 0.0	19.8 ± 52.3	340.3 ± 478.2	184.4 ± 191.3
	Tomatoes and tomato products (g/day)	29.9 ± 32.2	196.6 ± 156.4	108.3 ± 107.6	94.1 ± 144.3
	Other red and orange vegetables, excluding tomatoes and tomato products (sweet potato, red pepper,	0.0 ± 0.0	150.2 ± 159.3	9.3 ± 31.9	102.5 ± 85.1

	cabbage, carrots) (g/day)				
	White potatoes (g/day)	113.0 ± 83.3	29.6 ± 41.7	0.0 ± 0.0	0.0 ± 0.0
	Other starchy vegetables, excluding white potatoes (corn) (g/day)	0.0 ± 0.0	24.7 ± 51.2	0.0 ± 0.0	45.7 ± 34.5
	Other vegetables not in the vegetable components listed above (onion, bell pepper, avocado, squash, garlic, green beans, cucumber and pickles, olives, brussels sprouts, sweet peas) (g/day)	89.3 ± 25.5	363.7 ± 157.7	361.7 ± 301.0	201.2 ± 158.8
	Legumes (g/day)	0.0 ± 0.0	33.6 ± 70.4	0.0 ± 0.0	0.0 ± 0.0
Grains	Grains defined as whole grains and contained the entire grain kernel, the bran, germ, and endosperm (whole wheat bread) (g/day)	0.0 ± 0.0	45.2 ± 56.2	0.0 ± 0.0	25.4 ± 19.7
	Refined grains that do not contain all of the components of the entire grain	199.0 ± 35.4	92.7 ± 48.6	0.0 ± 0.0	87.9 ± 31.1

	kernel (bun, spaghetti, couscous, white rice) (g/day)				
Protein	Beef, pork, bison; excludes organ meat and cured meat (g/day)	116.9 ± 38.4	2.5 ± 6.8	141.2 ± 82.3	5.5 ± 12.8
	Cured meat (bacon, bologna) (g/day)	31.1 ± 23.5	0.0 ± 0.0	22.1 ± 10.8	5.4 ± 16.3
	Poultry (chicken, turkey) (g/day)	0.0 ± 0.0	57.3 ± 63.8	55.4 ± 83.1	19.8 ± 34.1
	Seafood (fish and shrimp) (g/day)	0.0 ± 0.0	150.6 ± 126.2	17.1 ± 45.2	69.5 ± 94.7
	Eggs (g/day)	4.4 ± 11.3	0.6 ± 2.3	55.7 ± 26.5	11.5 ± 26.3
	Peanuts, tree nuts, and seeds; excludes coconut (peanuts, sesame seeds and walnuts) (g/day)	0.0 ± 0.0	2.1 ± 2.4	5.9 ± 12.1	28.4 ± 28.4
	Legumes computed as protein foods (beans, peas, and lentils) (g/day)	0.0 ± 0.0	15.9 ± 33.4	0.0 ± 0.0	0.0 ± 0.0
Dairy	Fluid milk and cream (g/day)	63.7 ± 80.8	95.2 ± 73.4	17.4 ± 6.6	49.5 ± 83.8
	Yogurt (g/day)	0.0 ± 0.0	16.4 ± 28.2	0.0 ± 0.0	0.0 ± 0.0
	Cheese (g/day)	410.0 ± 150.1	358.7 ± 186.3	208.3 ± 208.3	129.8 ± 133.5

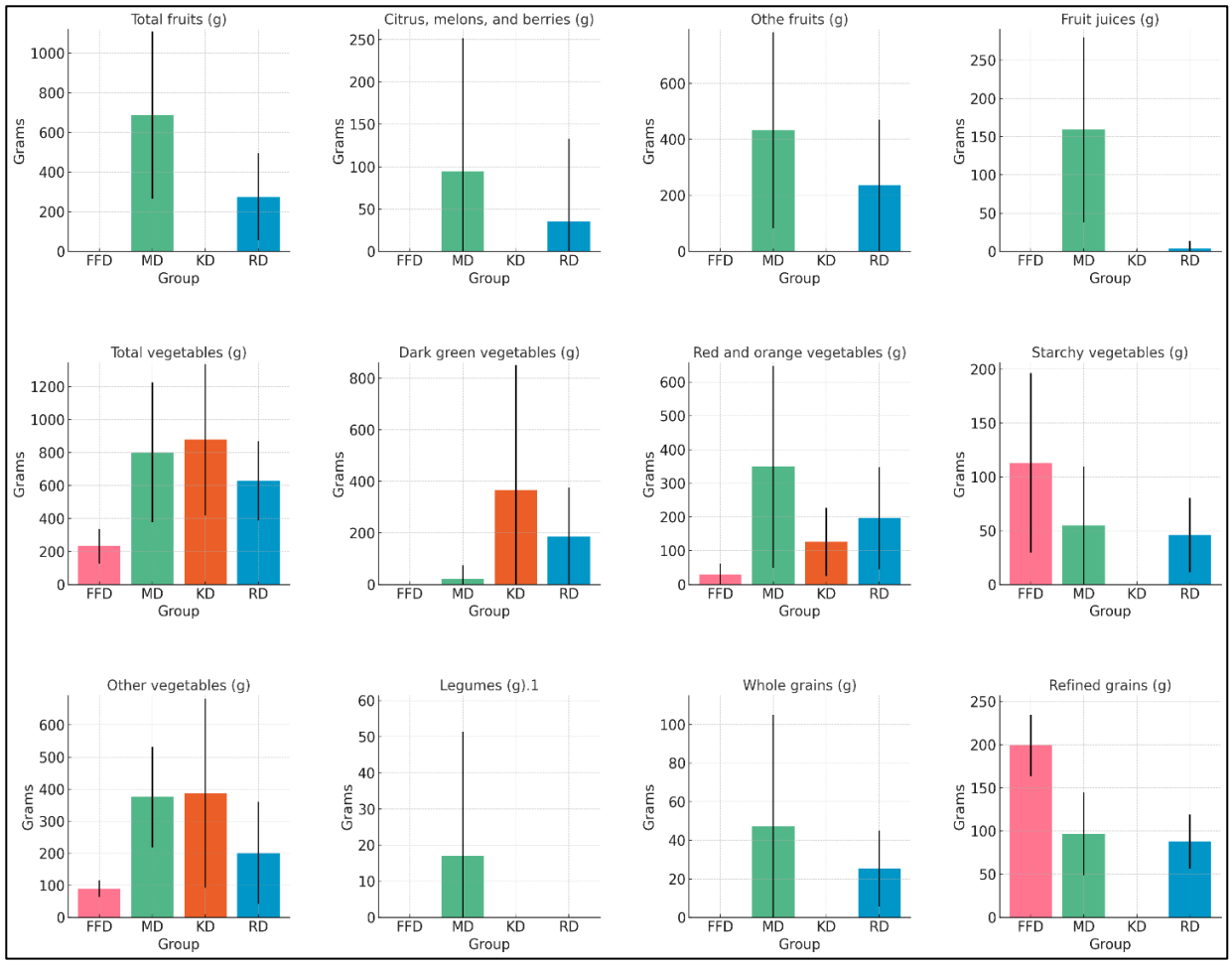

Figure 3.1 - Bar charts of the averaged amount (g)/day of fruits, vegetables and grains consumed during each dietary intervention.

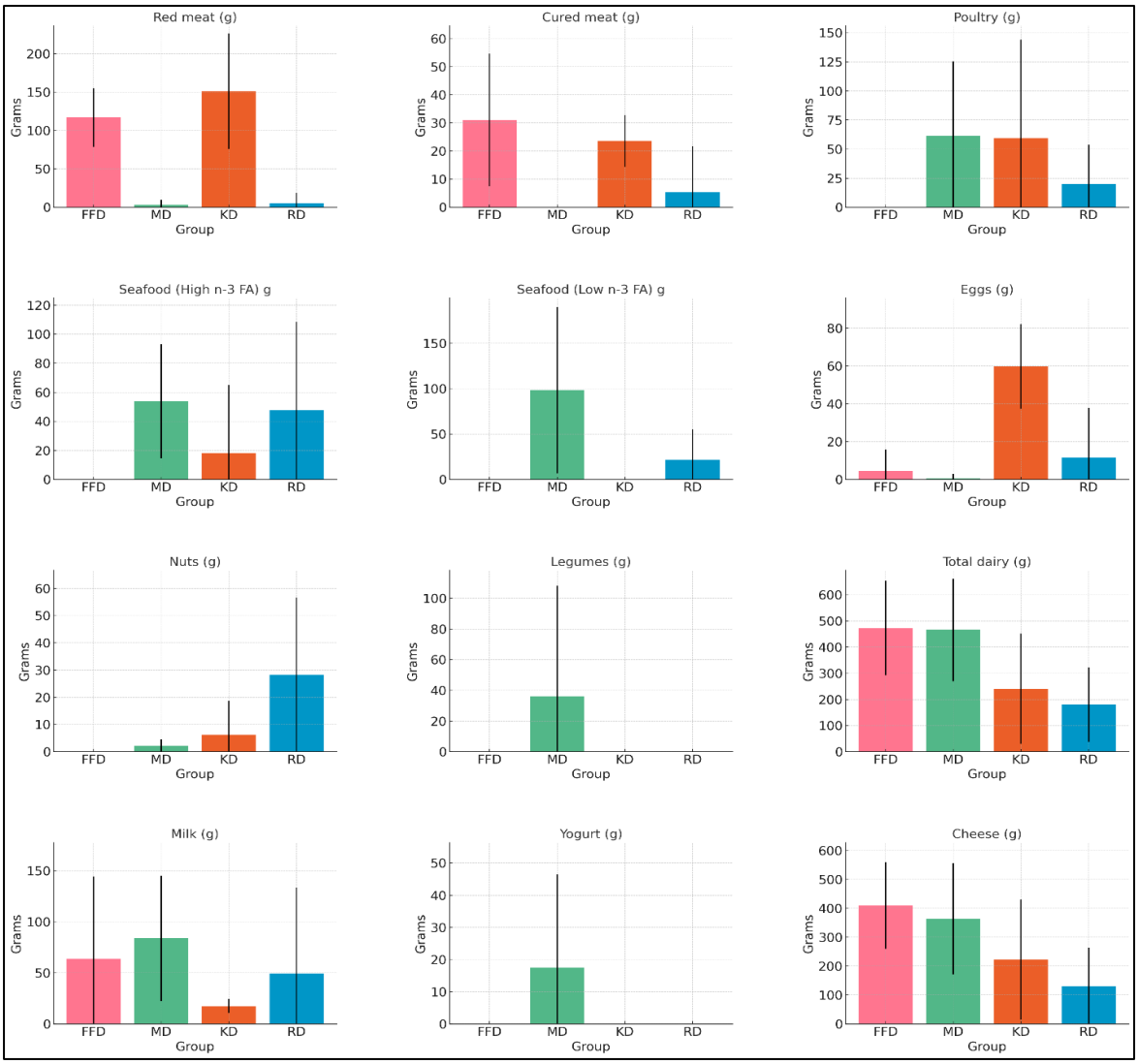

Figure 3.2 - Bar charts of the averaged amount (g)/day of protein and dairy consumed during each dietary intervention.

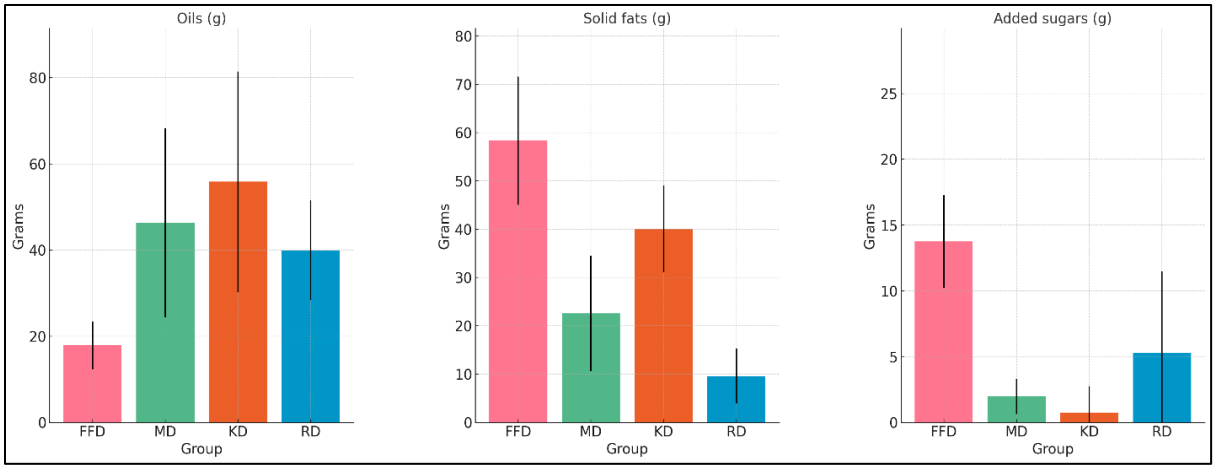

Figure 3.3 - Bar charts of the average amount (g/day) of oils, fats and added sugars consumed during each dietary intervention.

3.1.8. Meals

An analysis of the ingredients for each meal consumed for each dietary intervention was also conducted. The food intake variables were then estimated by averaging the intake over 14 days to derive the mean servings per day of each meal. A summary of the food groups, food items, and the average amount consumed with breakfasts, lunches, dinners, and snacks during each dietary intervention is presented in Appendix Tables 3A – 3D.

Breakfasts

During the dietary interventions, no fruits were consumed with breakfasts while on the FFD, KD, and RD. In contrast, the MD included fruit juices with breakfasts. No dark green vegetables were eaten with any breakfast across all diet groups. Red and orange vegetables were included with breakfasts while on the MD and FFD diets, but not on the KD or RD diets. Starchy vegetables and legumes were absent from breakfasts in all dietary interventions. Other types of vegetables such as avocados were consumed with breakfasts while on the RD and MD. Whole grains and refined grains were not part of breakfasts while on the KD. However, the FFD included refined grains, and the MD and RD included both whole and refined grains. Protein-rich foods were mostly consumed with breakfasts on the KD, primarily from cured meats (bacon) and eggs,

with no nuts included. The FFD had the second highest protein intake with breakfasts, from meat, cured meat (sausages), and eggs, but no nuts. The breakfast protein during the RD was sourced exclusively from nuts (peanut butter). No protein foods like eggs, nuts, or meats were included in breakfasts on the MD diet. No milk or yogurt was consumed with breakfasts with any of the dietary interventions. However, cheese was a part of breakfasts on the MD, FFD, and RD, with no cheese consumed while on the KD diet. Additionally, added sugars were included in breakfasts while on the FFD, MD, and RD, but not on the KD diet. Table 3.2 represents a summary of the food groups consumed with breakfasts across all dietary interventions, and Figure 3.4 shows a PCA plot of breakfasts and their averaged constituents (micro, macro, and non-nutrients) showing the differences in breakfasts' composition across all diet groups.

Table 3.2 – The mean 14-d intake of consumed foods with breakfasts across all dietary interventions

Food groups	**FFD**	**MD**	**KD**	**RD**
Fruits (g/day)	0.0 ± 0.0	123.7 ± 133.5	0.0 ± 0.0	0.0 ± 0.0
Vegetables (g/day)	8.4 ± 26.9	67.3 ± 100.8	0.0 ± 0.0	74.0 ± 121.0
Grains (g/day)	62.4 ± 16.0	87.5 ± 47.4	0.0 ± 0.0	51.3 ± 16.7
Proteins (g/day)	39.7 ± 16.3	0.0 ± 0.0	68.3 ± 10.4	28.4 ± 28.4
Oils and fats (g/day)	18.9 ± 7.8	14.0 ± 10.0	17.6 ± 2.3	14.6 ± 9.1
Dairy (g/day)	109.9 ± 67.4	339.5 ± 170.9	0.0 ± 0.0	77.7 ± 123.3
Foods with added sugars (g/day)	3.0 ± 2.4	4.0 ± 1.9	0.0 ± 0.0	11.5 ± 12.2

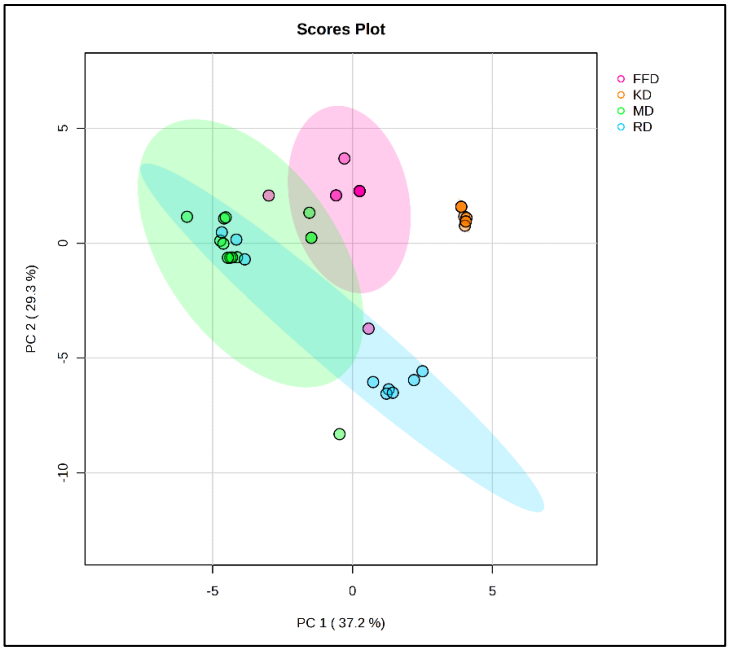

Figure 3.4 - PCA plot of breakfasts' averaged micro, macro, and non-nutrients showing the differences in breakfasts' composition across all diet groups.

Lunches:

Fruit intake with lunch was the highest while on the MD and RD diets in the form of fruit juices, while no fruits or fruit juices were consumed with lunches while on the FFD and KD. Dark green vegetables were not consumed with lunches while on the FFD but were included in varying forms such as spinach and bell pepper for the RD, KD, and MD. Red and orange vegetables such as tomato and tomato products were part of lunches on all diets, with the highest intake while on the MD and the lowest while on the FFD. Starchy vegetables, primarily in the form of potatoes, were consumed with lunches on the FFD and MD, and other starchy vegetables such as corn were included while on the RD, but none were consumed on the KD. No legumes were included in lunches across all four diet interventions. Refined grains were consumed with lunches on the FFD and MD, and in smaller amounts on the RD, with no consumption during the KD. Protein consumption with lunches was highest during the KD, including beef and pork, poultry, seafood, and eggs, whereas the RD diet had the lowest meat consumption. The MD diet included protein-rich foods such as meats, poultry, seafood, and nuts, while the RD featured a similar variety with smaller amounts. Dairy consumption during lunches was primarily in the form of cheese across all diets, with additional yogurt intake while on the MD. Added sugars were highest with lunches on the FFD, and minimal on the KD, RD, and MD. Table 3.3 represents a summary of the food groups

consumed with lunches across all dietary interventions, and Figure 3.5 shows a PCA plot of lunches and its averaged constituents (micro, macro, and non-nutrients) showing the differences in lunches' composition across all diet groups.

Table 3.3 - The mean 14-d intake of consumed foods with lunches across all dietary interventions

Food groups	FFD	MD	KD	RD
Fruits (g/day)	0.0 ± 0.0	12.7 ± 17.0	0.0 ± 0.0	3.8 ± 9.8
Vegetables (g/day)	113.9 ± 64.0	190.0 ± 124.4	220.9 ± 142.7	212.6 ± 124.5
Grains (g/day)	70.5 ± 5.0	24.4 ± 14.8	0.0 ± 0.0	33.4 ± 8.2
Proteins (g/day)	64.9 ± 18.2	1.7 ± 2.8	134.4 ± 128.7	1.8 ± 5.5
Oils and fats (g/day)	25.2 ±7.2	28.4 ±15.7	35.5 ±10.7	20.3 ±10.8
Dairy (g/day)	207.0 ± 87.7	68.9 ± 67.0	160.6 ± 173.7	51.5 ± 108.1
Foods with added sugars (g/day)	15.7 ± 12.8	0.05 ± 0.1	1.0 ± 2.1	0.4 ± 0.8

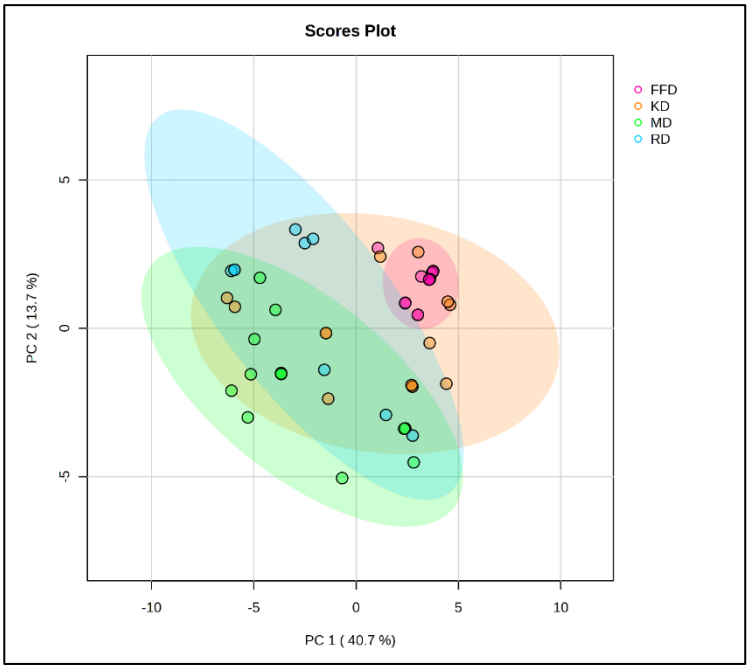

Figure 3.5 - PCA plot of lunches averaged micro, macro, and non-nutrients showing the differences in lunches' composition across all diet groups.

Dinners:

No fruits or fruit juices were consumed with dinners while on the FFD or RD. However, some fruit juice was consumed with dinners while on the MD. The highest vegetable consumption with dinners occurred while on the MD, which included a variety of red and orange vegetables such as sweet potato and tomato, starchy vegetables such as corn, legumes, and other vegetables. Dinners with the KD and RD featured dark green vegetables such as spinach and zucchini, red and orange vegetables, and other vegetables such as avocado. The FFD had a significantly lower vegetable intake with only small amounts of red and orange vegetables sourced from tomato and tomato products, starchy vegetables sourced from potatoes, and other vegetables including onions and pickles. No grains were consumed with dinners on the KD. Refined grains were included in dinners while on the FFD and MD, and both refined and whole grains were consumed with dinners during the RD. Dinners with the KD had the highest protein intake with a variety of meat, cured meat, poultry, eggs, and nuts. Dinners with the RD had the second highest protein intake, sourced from poultry, seafood, and cured meat. The MD featured poultry, seafood, nuts, and legumes, while the FFD diet had the lowest overall protein intake, primarily from meat and cured meat. No yogurt was consumed for dinner on any of the diets. Dairy intake, in the form of cheese, was highest on the FFD, followed by the KD, with minimal consumption on the RD diet. The MD diet included both cheese and milk. Added sugars were most prevalent with dinners while on the FFD, with significantly lower intake during the other three diets. Figure 3.6 and Table 3.4 provide a detailed breakdown of the nutrient compositions of dinners across all diets. Table 3.4 represents a summary of the food groups consumed with dinners across all dietary interventions, and Figure 3.6 shows a PCA plot of the different dietary group dinners and their averaged constituents (micro, macro, and non-nutrients) showing the differences in dinner composition across all diet groups.

Table 3.4 - The mean 14-d intake of consumed foods with dinners across all dietary interventions

Food groups	FFD	MD	KD	RD
Fruits (g/day)	0.0 ± 0.0	8.5 ± 18.2	0.5 ± 1.6	0.0 ± 0.0
Vegetables (g/day)	75.4 ± 73.5	471.9 ± 254.9	361.5 ± 210.4	332.1 ± 215.6
Grains (g/day)	77.4 ± 42.4	24.5 ± 39.0	0.0 ± 0.0	23.8 ± 26.2
Proteins (g/day)	55.4 ± 25.1	95.2 ± 62.8	149.5 ± 74.8	116.6 ± 45.4
Oils and fats (g/day)	21.8 ±12.7	28.5 ±12.4	38.5 ±16.5	20.8 ±10.4
Dairy (g/day)	122.3 ± 113.7	39.1 ± 125.6	67.4 ± 116.6	1.0 ± 1.9
Foods with added sugars (g/day)	22.4 ± 19.9	3.3 ± 4.4	0.9 ± 2.0	1.7 ± 1.4

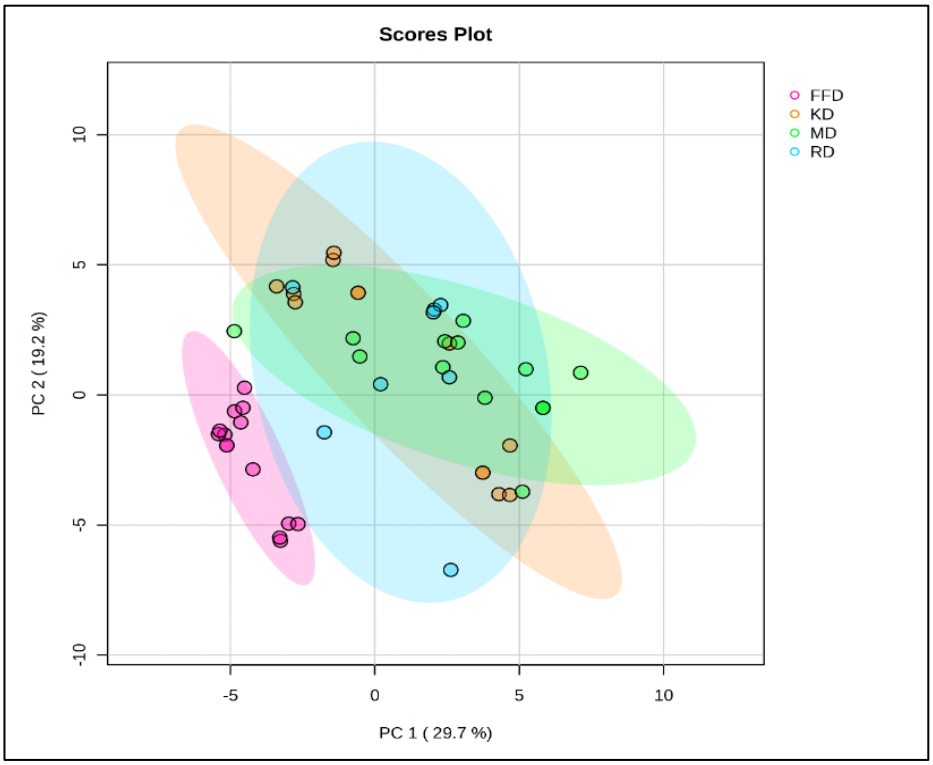

Figure 3.6 - PCA plot of dinners averaged micro, macro, and non-nutrients showing the differences in dinners' composition across all diet groups.

Snacks:

No fruits were consumed with snacks while on the FFD and KD interventions. However, while on the MD, various fruits, including grapefruit, apples, and bananas as well as fruit juices, were consumed as snacks. The RD intervention also included grapefruit, apples, and bananas with snacks but in different quantities. The RD snacks had the highest vegetable intake, including red or orange vegetables such as carrots and other vegetables such as sweet peas. In contrast, the FFD and KD snacks had small amounts of vegetables. Snacks with the MD had the lowest vegetable intake, with only a small amount of avocado consumed. No grains were consumed with snacks while on the KD. However, refined grains were consumed with snacks while on the FFD and RD, and both refined and whole grains were consumed with snacks while on the MD. No proteins, such as meat, eggs, poultry, or seafood, were consumed with snacks while on the MD. Snacks while on the FFD included red meat, while the RD included eggs, and the KD included small amounts of cured meat (bacon) and eggs. The highest dairy intake with snacks occurred during the MD, with milk and cheese being consumed. Dairy intake with snacks during the FFD and RD included milk, while the KD had the lowest dairy intake, consisting of milk and cheese. No added sugars were consumed with snacks during the KD intervention, while added sugars were consumed with snacks during the FFD, RD, and MD interventions. Table 3.5 represents a summary of the food groups consumed with snacks across all dietary interventions, and Figure 3.7 shows a PCA plot of snacks and their averaged constituents (micro, macro, and non-nutrients) showing the differences in snacks' composition across all diet groups.

Table 3.5 - Mean 14-d intake of consumed foods with snacks across all dietary interventions

Food groups	FFD	MD	KD	RD
Fruits (g/day)	0.0 ± 0.0	698.1 ± 428.2	0.0 ± 0.0	371.6 ± 216.7
Vegetables (g/day)	59.4 ± 98.8	15.1 ± 52.4	26.4 ± 53.3	91.0 ± 110.1
Grains (g/day)	10.8 ± 21.8	21.1 ± 33.7	0.0 ± 0.0	7.4 ± 22.3
Proteins (g/day)	10.2 ± 21.9	0.0 ± 0.0	12.3 ± 8.8	10.0 ± 24.8
Oils and fats (g/day)	21.1 ± 11.8	4.4 ± 8.5	27.8 ± 8.7	5.3 ± 7.4

Dairy (g/day)	141.1 ± 165.5	169.9 ± 210.8	23.3 ± 21.4	49.5 ± 83.8
Foods with added sugars (g/day)	36.0 ± 17.2	0.9 ± 1.5	0.0 ± 0.0	8.0 ± 13.8

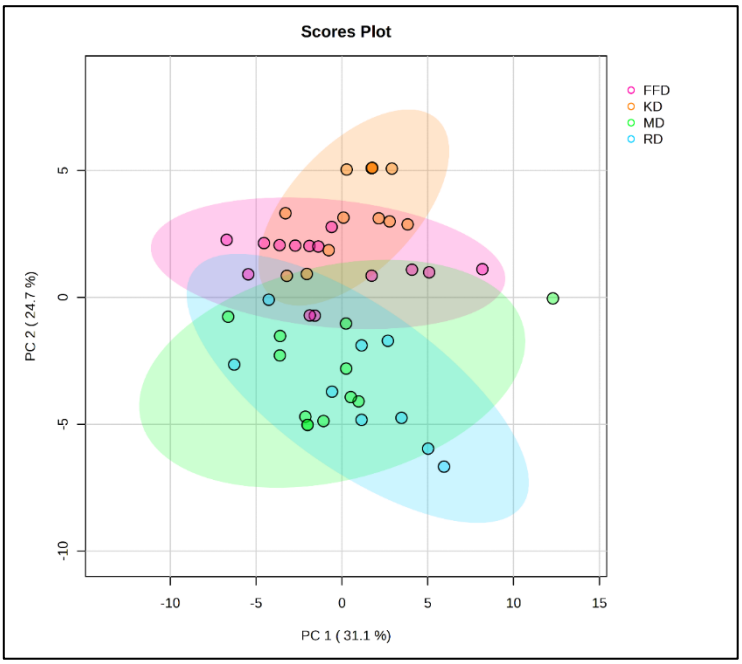

Figure 3.7 - PCA plot of snacks averaged micro, macro, and non-nutrients showing the differences in snacks' composition across all diet groups.

3.2. Diet analysis: calories, macro- and micro-nutrients:

In this section, a detailed summary of the caloric intake as well as the macro and micro-nutrients consumed for each of the four diets is provided. This is broken down according to meal types (breakfast, lunch, dinner, snacks) and the daily average. The intent is to provide a measure of the composition of the foods consumed for these diets and to allow better chemical comparisons to be made with the metabolomic data and wearables data (described later). The caloric content, macro and micro-nutrient analysis was determined by comparing the weighed food consumption records with the ASA24 tables and calculating the nutrient profiles. Overall, the results obtained from this analysis showed that the daily energy received from the four diets was, on average,

isocaloric (Table 3.6). An ANOVA analysis indicated that no statistically detectable difference was evident in terms of caloric consumption between the diets. Also shown in Table 3.7 is the average caloric content for each of the three meals (plus snacks) for each of the four diets. From this table, it is evident that the lowest calorie meal for the MD, KD and RD was snacks and breakfast for the FFD, and the highest calorie meal was lunch for the FFD, KD and RD and dinner for the MD.

In addition to analyzing the total energy content, four other macronutrient features were analyzed, both on a daily basis and on an average meal basis (Tables 3.6, and 3.7). These macronutrients included protein, fat, carbohydrates, water, and sugar. The average amount of protein consumed per day was highest with the KD (94.7 ± 34.1 g/day), and lowest with the FFD (81.0 ± 17.7 g/day). The MD and RD had an average protein intake of 92.2 ± 13.3 g/day and 91.9 ± 20.8 g/day, respectively. Each of the four diets exceeded the minimum Recommended Dietary Allowance (RDA) for protein intake, which is 46 grams per day [235]. In terms of meals, breakfasts with the KD had the highest protein intake (20.4 ± 2.6 g/day) while the lowest intake was with the RD (12.1 ± 3.6 g/day). Lunches with the highest protein intake were with the KD (55.9 ± 25.3 g/day) while the lowest was with the RD (33.2 ± 10.0 g/day). Dinners with the KD had the highest protein intake (46.0 ± 19.8 g/day), and the lowest intake was with the FFD (26.6 ± 12.9 g/day). The average amount of fat consumed per day was highest with the KD (118.0 ± 43.6 g/day). The FFD had the second-highest average amount of fat intake at 97.5 ± 18.8 g/day. The MD and RD had lower levels of fat content (Table 3.6). In terms of meals, breakfasts with the FFD had the highest fat intake (25.2 ± 8.9 g/day) while the lowest intake was with the MD (13.4 ± 10.9 g/day). Lunches with the highest fat intake were with the KD (53.3 ± 21.8 g/day) while the lowest was with the RD (23.6 ± 14.5 g/day). Dinners with the KD had the highest fat intake (45.5 ± 19.0 g/day), and the lowest intake was with the FFD (24.6 ± 15.9 g/day). (Note: the RDA of fats is marked as not determinable in the reference values provided by the government of Canada [235]). The average carbohydrate intake was at its lowest with KD (21.1 ± 11.2 g/day), and highest during MD (208.8 ± 54.9 g/day). The FFD and RD had middling levels of average carbohydrate intake at 180.5 ± 28.1 g/day and 180.5 ± 28.1 g/day, respectively. All the diets except for the KD exceeded the RDA of 130 g/day for carbohydrates [235]. In terms of meals, breakfasts with the MD had the highest carbohydrate intake (61.8 ± 32.7 g/day) while the lowest intake was with the KD (1.4 ± 0.2 g/day). Lunches with the highest carbohydrate intake were with the FFD (51.4 ± 21.6 g/day)

while the lowest was with the KD (10.9 ± 5.4 g/day). Dinners with the FFD had the highest carbohydrate intake (72.2 ± 37.9 g/day) and the lowest intake was with the KD (11.9 ± 6.0 g/day).

Table 3.6 - A summary of the macronutrients across all diet groups

Food groups	**FFD**	**MD**	**KD**	**RD**
Energy (kcal/day)	1985.5 ± 106.3	1940.4 ± 104.3	1967.9 ± 136.5	1942.0 ± 66.6
Protein (g/day)	81.0 ± 17.7	94.6 ± 13.3	94.7 ± 34.1	91.9 ± 20.8
Total Fat (g/day)	97.5 ± 18.8	75.6 ± 24.0	126.2 ± 43.6	63.9 ± 11.9
Carbohydrate (g/day)	180.5 ± 28.1	208.8 ± 54.9	21.1 ± 11.2	159.6 ± 38.9

Table 3.7 - A summary of the average calorie intake (kcal/day) as well as average daily protein, fat, carbohydrate and added sugars intake across each diet type for different meals (breakfast, lunch, dinner, and snacks)

	Meal	**FFD**	**MD**	**KD**	**RD**
Energy (kcal/day)	Breakfast	431.0 ±140.7	439.2 ± 241.0	304.4 ± 40.6	349.2 ± 129.9
	Lunch	688.4 ± 194.5	525.3 ± 159.2	747.4 ± 296.6	591.5 ± 176.7
	Dinner	534.0 ± 329.8	619.0 ± 159.2	641.9 ± 172.9	609.4 ± 113.8
	Snack	454.1 ± 313.1	354.9 ± 223.7	277.9 ± 95.5	384.7 ± 183.5
Protein (g/day)	Breakfast	19.1 ± 9.0	18.7 ± 8.5	20.4 ± 2.6	12.1 ± 3.6
	Lunch	35.0 ± 10.3	39.4 ± 8.3	56.0 ± 25.3	33.2 ± 10.0
	Dinner	26.6 ± 12.9	34.1 ± 12.4	46.0 ± 19.8	33.4 ± 6.5
	Snack	12.1 ± 9.6	9.8 ± 8.2	2.8 ± 3.2	6.9 ± 9.0
T o t	Breakfast	25.2 ± 8.9	13.4 ± 10.9	23.5 ± 3.2	17.2 ± 9.4

	Lunch	37.6 ± 10.0	32.2 ± 14.6	53.3 ± 21.8	23.6 ± 14.5
	Dinner	24.6 ± 15.9	30.6 ± 8.7	45.5 ± 19.0	26.3 ± 12.0
	Snack	22.5 ± 13.0	5.7 ± 8.9	18.0 ± 9.3	6.4 ± 7.4
Carbohydrate (g/day)	Breakfast	32.4 ± 8.2	61.8 ± 32.7	1.4 ± 0.2	40.2 ± 16.7
	Lunch	51.4 ± 21.6	34.2 ± 13.0	10.9 ± 5.4	49.2 ± 19.6
	Dinner	72.2 ± 37.9	60.2 ± 20.3	11.9 ± 6.0	27.8 ± 23.3
	Snack	67.5 ± 44.8	72.8 ± 39.4	2.9 ± 2.6	46.7 ± 27.6

The average daily consumption for 56 micronutrients was determined from the weighed food records for each of the four diets. Nutrients were assessed in relation to the DRI's [235] to determine whether they exceeded the recommended or required levels throughout each diet. These are presented in Tables 3.8, 3.9 and 3.10, and Figures 3.8 and 3.9.

The FFD exhibited several significant deviations from the Dietary Reference Intakes (DRIs). Fiber intake was notably low at 7.3 ± 1.8 g, which is significantly below the recommended 25-38 g per day. Calcium intake, at 893.7 ± 226.9 mg, fell close to the DRI range of 1000-1200 mg for adults. Iron intake at 13.5 ± 2.3 mg was slightly below the DRI for women, which is 18 mg. Magnesium intake of 160.9 ± 33.6 mg was considerably lower than the DRI of 310-420 mg. Potassium intake was also low at 1887.8 ± 515.6 mg, far below the recommended 2600-3400 mg. Conversely, sodium intake significantly exceeded the recommended limit, with an intake of 3541.4 ± 625.1 mg compared to the advised maximum of 2300 mg. Zinc and selenium intakes exceeded their respective DRIs, with values of 13.5 ± 3.2 mg and 104.6 ± 20.6 µg. Vitamin C intake was significantly low at 19.8 ± 11.5 mg, far below the recommended 75-90 mg. Intakes of thiamine, riboflavin, niacin, and vitamin B-6 were within or above the DRI ranges, indicating a sufficient intake of these B vitamins (Table 3.8).

The MD presented a contrasting nutrient profile. Fiber intake was at the lower end of the DRI (25-38 g/day), with an intake of 24.9 ± 12.6 g. Calcium intake was below the DRI (1000-1200 mg/day) at 804.1 ± 215.2 mg. Iron intake of 10.9 ± 3.9 mg was lower than recommended for

women. Magnesium intake met the DRI at 362.1 ± 106.2 mg, and potassium intake significantly exceeded the DRI at 3704.9 ± 1241.9 mg. Sodium intake was exceedingly high, at 5259.8 ± 2489.1 mg, well above the recommended limit. Zinc intake met the DRI at 8.6 ± 1.7 mg, and selenium intake exceeded the DRI at 142.2 ± 33.9 µg. Vitamin C intake was significantly high at 285.1 ± 176.9 mg, surpassing the DRI. Thiamine, riboflavin, niacin, and vitamin B-6 intakes were all above their respective DRI ranges, indicating robust intake levels of these essential vitamins (Table 3.8).

The KD showed a different distribution of nutrients. Fiber intake was significantly low at 8.6 ± 6.4 g, well below the DRI of 28-35 g/day. Calcium intake was notably insufficient at 558 ± 258 mg. Iron intake was insufficient for women at 9.4 ± 5.9 mg. Magnesium intake was low at 225.1 ± 163 mg, and potassium intake was near the lower end of the DRI at 2495.4 ± 1167.2 mg. Sodium intake was high at 3259.9 ± 1442 mg, exceeding the recommended limit. Zinc intake met the DRI at 11.2 ± 3.8 mg, and selenium intake exceeded it at 139.8 ± 54.5 µg. Vitamin C intake met the DRI at 79.1 ± 73.8 mg. Thiamine, riboflavin, niacin, and vitamin B-6 intakes were all above their respective DRI ranges, indicating sufficient intake levels of these vitamins (Table 3.8).

The RD also had notable deficiencies and excesses. Fiber intake was below the DRI at 19.7 ± 2.8 g. Calcium intake was significantly low at 432.4 ± 219.3 mg. Iron intake was significantly below the requirement for women at 8.4 ± 2.3 mg. Magnesium intake was low at 253.9 ± 36.7 mg, and potassium intake was below the DRI at 2402.3 ± 357.3 mg. Sodium intake was moderately high at 2523.8 ± 787.4 mg, exceeding the recommended limit. Zinc intake was below the DRI at 6.3 ± 1.7 mg. Selenium intake exceeded the DRI at 104.6 ± 42.5 µg. Vitamin C intake met the DRI at 84.5 ± 47.7 mg. Thiamine intake was slightly below the DRI at 1.0 ± 0.3 mg, while riboflavin, niacin, and vitamin B-6 intakes met or exceeded the DRI ranges (Table 3.8).

Caffeine intake was highest in the KD (159.8 ± 50.5 mg), followed by the FFD (122.4 ± 57.5 mg) and the MD (122.2 ± 52.9 mg), with the RD having the lowest intake (115.6 ± 66.1 mg). Theobromine intake was present in the FFD (56.1 ± 36.5 mg) and the RD (16.4 ± 33.6 mg) but was absent in the MD and KD. The intake of caffeine across all diets was within the general safety guidelines which suggest that up to 400 mg per day is considered safe for most adults (Table 3.8).

Table 3.8 - Average daily intake of other components of food for each of the dietary interventions

Nutrients (per day)	FFD	MD	KD	RD
Caffeine (mg/day)	122.4 ± 57.5	122.2 ± 52.9	159.8 ± 50.5	115.6 ± 66.1
Theobromine (mg/day)	56.1 ± 36.5	0.0 ± 0.0	0.0 ± 0.0	16.4 ± 33.6
Fibre, total dietary (g/day) [#1]	7.3 ± 1.8	24.9 ± 12.6	8.6 ± 6.4	19.7 ± 2.8
Calcium (mg/day) [#2]	893.7 ± 226.9	804.1 ± 215.2	558 ± 258	432.4 ± 219.3
Iron (mg/day) [#3]	13.5 ± 2.3	10.9 ± 3.9	9.4 ± 5.9	8.4 ± 2.3
Magnesium (mg/day) [#4]	160.9 ± 33.6	362.1 ± 106.2	225.1 ± 163	253.9 ± 36.7
Phosphorus (mg/day) [#5]	1104.9 ± 265.8	1696.1 ± 368.9	1211.4 ± 481.9	1190.5 ± 519.8
Potassium (mg/day) [#6]	1887.8 ± 515.6	3704.9 ± 1241.9	2495.4 ± 1167.2	2402.3 ± 357.3
Sodium (mg/day) [#7]	3541.4 ± 625.1	5259.8 ± 2489.1	3259.9 ± 1442	2523.8 ± 787.4
Zinc (mg/day) [#8]	13.5 ± 3.2	8.6 ± 1.7	11.2 ± 3.8	6.3 ± 1.7
Copper (mg/day) [#9]	0.8 ± 0.2	1.4 ± 0.4	0.8 ± 0.6	0.9 ± 0.1
Selenium (µg/day) [#10]	104.6 ± 20.6	142.2 ± 33.9	139.8 ± 54.5	104.6 ± 42.5
Vitamin C (mg/day) [#11]	19.8 ± 11.5	285.1 ± 176.9	79.1 ± 73.8	84.5 ± 47.7
Thiamine (mg/day) [#12]	1.6 ± 0.3	1.6 ± 0.6	1.2 ± 0.7	1.0 ± 0.3
Riboflavin (mg/day) [#13]	1.4 ± 0.3	1.7 ± 0.3	1.9 ± 0.6	1.3 ± 0.5
Niacin (mg/day) [#14]	21.7 ± 4.3	26.4 ± 7.9	21.8 ± 10	26 ± 10.6
Vitamin B-6 (mg/day) [#15]	1.1 ± 0.4	2.5 ± 0.7	1.9 ± 1.1	1.6 ± 0.4
Folate, total (µg/day)	280.0 ± 45	377.8 ± 106.3	287.5 ± 188.7	357.9 ± 126.5
Folic acid (µg/day)	145.9 ± 24.2	102.1 ± 61.8	0.0 ± 0.0	92.3 ± 49.4
Folate, food (µg/day)	134.1 ± 35.3	275.8 ± 128.1	287.5 ± 188.7	265.7 ± 100.7
Folate, DFE (µg DFE/day) [#16]	382.1 ± 57.2	449.3 ± 110.8	287.5 ± 188.7	422.6 ± 151.6

Choline, total (mg/day) [#17]	240.3 ± 77.4	355 ± 57.1	561.3 ± 187.9	259.4 ± 124.3

#1 - All four diets were below the AI levels for fibre (25 g/day). However, the MD was significantly higher and closer to this level.

#2 - None of the diets passed the RDA levels of calcium (1000 mg/day)

#3 - None of the diets passed the RDA levels of iron (18 mg/day)

#4 - All the diets except for the MD were below the RDA for magnesium (310 mg/day)

#5 - None of the diets passed the RDA levels of phosphorus (700 mg/day)

#6 - All the diets except for the MD were below the AI of potassium (2600 mg/day)

#7 - All the diets were above the AI of sodium (1500 mg/day)

#8 - All the diets except for the RD were above the AI for zinc (8 mg/day)

#9 - The FD and the KD were below the RDA for copper (0.9 mg/day)

#10 - All the diets were above the RDA for selenium (55 µg/day)

#11 - The vitamin C levels were lower than the RDA levels (75 mg/day) during the FFD and significantly higher during the MD

#12 - All the diets except for the RD were above the RDA levels for thiamin (1.1 mg/day)

#13 - All the diets were higher than the RDA for riboflavin (1.1 mg/day)

#14 - All the diets were higher than the RDA for niacin (14 mg/day)

#15 - All the diets except for the FFD were higher than the RDA for vitamin B-6 (1.3 mg/day)

#16 - The FFD and the KD were lower than the RDA of folate (400 µg/day) – **DFE**: Dietary Folate Equivalents

#17 - All the diets except for the KD were below the AI levels of choline (425 mg/day)

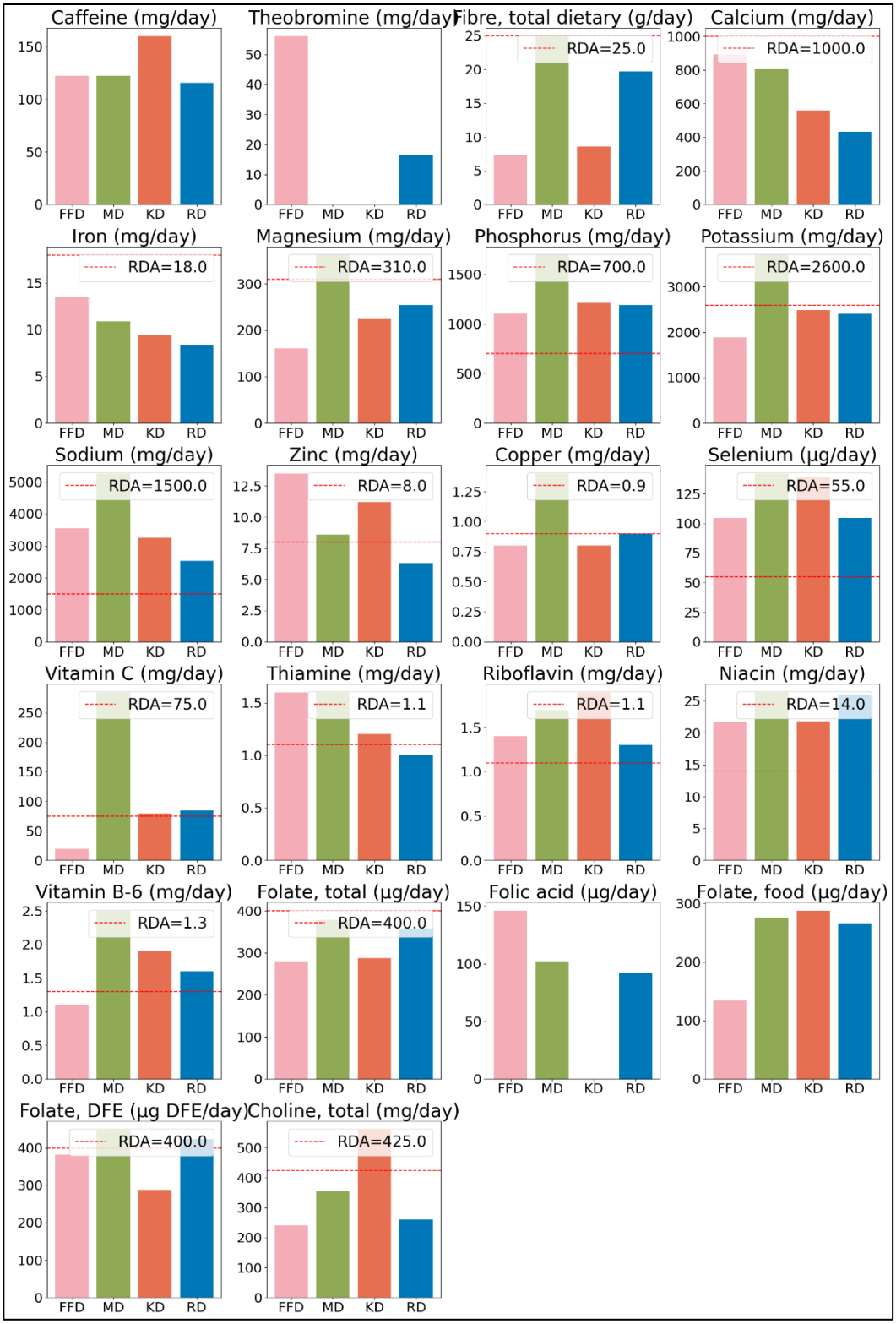

Figure 3.8 - This figure presents the daily intake of various nutrients across all diets: Fast Food Diet (FFD), Mediterranean Diet (MD), Ketogenic Diet (KD), and Regular Diet (RD). Each subplot represents a specific nutrient with bars showing the intake values for each diet. The red dotted line indicates the Recommended Dietary Allowance (RDA) where applicable.

The FFD showed significant variations in daily vitamin and carotenoid intake. Vitamin B-12 intake was high at 6.3 ± 1.8 µg, significantly exceeding the DRI of 2.4 µg. In contrast, daily vitamin A intake was low at 274.1 ± 108.9 µg RAE (Retinol Activity Equivalents), below the recommended 700-900 µg. Daily retinol intake was similarly low at 252.4 ± 107.6 µg. Daily carotenoid intakes, such as beta-carotene (267.5 ± 89.5 µg) and alpha-carotene (3.4 ± 1.2 µg), were minimal. Daily lycopene intake was relatively higher at 3752.2 ± 3832.6 µg.

The MD showed significantly higher daily intakes of carotenoids and vitamins (Table 3.9). Daily beta-carotene intake was high at 7632.8 ± 7185.9 µg, and alpha-carotene intake was 88.4 ± 60.4 µg. Daily vitamin A intake met the DRI at 966 ± 595.6 µg RAE. Daily vitamin E intake was close to the DRI at 13.8 ± 6.1 mg, and daily vitamin K intake significantly exceeded the DRI at 144.5 ± 154.6 µg. However, daily vitamin D intake was below the DRI at 10 ± 5.6 µg.

For the KD, daily vitamin A intake was significantly high at 1449.3 ± 1675.5 µg RAE, exceeding the DRI. Daily beta-carotene intake was also notably high at 12504.2 ± 20113.2 µg. Daily vitamin E intake was close to the DRI at 13.6 ± 6.9 mg, and daily vitamin K intake significantly exceeded the DRI at 1143 ± 1897.2 µg. Daily vitamin D intake was below the DRI at 6.9 ± 7.2 µg.

In the RD, daily vitamin A intake met the DRI at 831.3 ± 481.5 µg RAE. Daily beta-carotene (7137.3 ± 4188.1 µg) and alpha-carotene (1992.4 ± 1535.3 µg) intakes were higher than the FFD but lower than the MD and KD. Daily vitamin E intake was below the DRI at 10.4 ± 2.8 mg, while daily vitamin K intake exceeded the DRI at 248.5 ± 136.4 µg, and daily vitamin D intake was below the DRI at 7.3 ± 8 µg (Table 3.9).

Table 3.9 - A summary of the average daily vitamin intake from foods for each of the four dietary interventions

Nutrients (per day)	FFD	MD	KD	RD
Vitamin B-12 (µg) [#1]	6.3 ± 1.8	5.8 ± 2.2	5.3 ± 2.1	3.3 ± 2.2
Vitamin A, RAE (µg _RAE) [#2]	274.1 ± 108.9	966 ± 595.6	1449.3 ± 1675.5	831.3 ± 481.5

Retinol (μg)	252.4 ± 107.6	313.2 ± 50.7	395.2 ± 130.6	152.2 ± 132.2
Carotene, beta (μg) [#3]	267.5 ± 89.5	7632.8 ± 7185.9	12504.2 ± 20113.2	7137.3 ± 4188.1
Carotene, alpha (μg) [#3]	3.4 ± 1.2	88.4 ± 60.4	264.5 ± 591.7	1992.4 ± 1535.3
Cryptoxanthin, beta (μg)	10.4 ± 7.2	344.8 ± 343.6	27.7 ± 19.6	35 ± 36.7
Lycopene (μg)	3752.2 ± 3832.6	8558.2 ± 7269.2	1968.4 ± 1954.8	9238.4 ± 19412.9
Lutein + zeaxanthin (μg)	242.8 ± 149.1	3457 ± 3632.8	24419.2 ± 44186.3	3161.8 ± 2234.4
Vitamin E, alpha-tocopherol (mg) [#4]	4 ± 1.2	13.8 ± 6.1	13.6 ± 6.9	10.4 ± 2.8
Vitamin K, phylloquinone (μg) [#5]	48 ± 15.1	144.5 ± 154.6	1143 ± 1897.2	248.5 ± 136.4
Vitamin D (D2 + D3) (μg) [#6]	1.5 ± 1.4	10 ± 5.6	6.9 ± 7.2	7.3 ± 8

#1 - All the diets were above the RDA levels of vitamin B-12 (2.4 μg/day)

#2 - The FFD was lower than the RDA levels of vitamin A (700 μg/day)

#3 - No dietary recommended intakes are established for beta-carotene or other carotenoids.

#4 - All the diets (especially the FFD) were below the RDA levels of vitamin E (15 mg/day)

#5 – the FFD was lower than the AI levels for vitamin K (90 μg/day)

#6 – All four diets were below the RDA levels for vitamin D (15 μg/day)

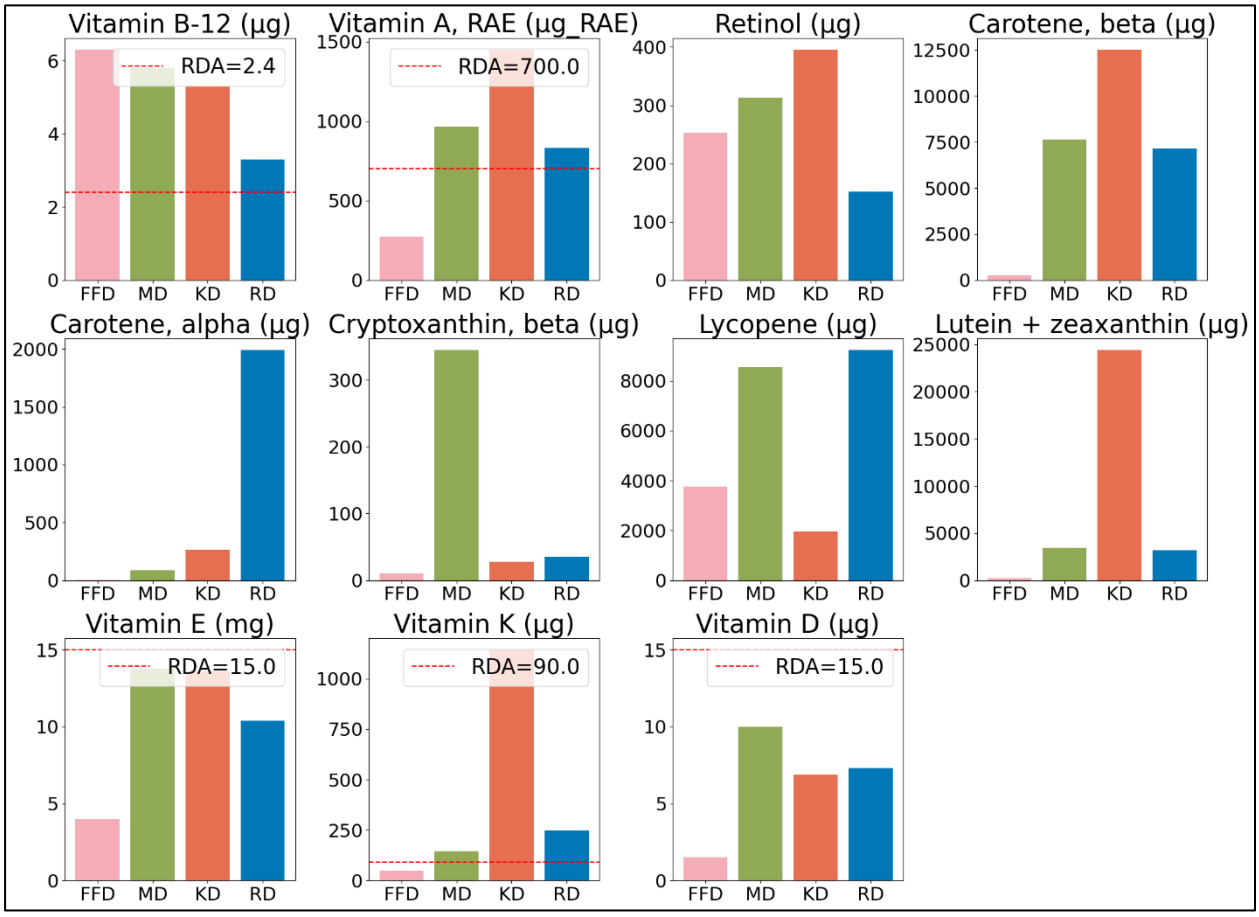

Figure 3.9 - This figure displays the daily intake of several micronutrients across four dietary patterns: Fast Food Diet (FFD), Mediterranean Diet (MD), Ketogenic Diet (KD), and Regular Diet (RD). Each subplot represents a specific micronutrient, with bars indicating the intake values for each diet. The red dotted line signifies the Recommended Dietary Allowance (RDA) where applicable.

The FFD was characterized by a high daily intake of cholesterol (253.9 ± 83.4 mg), approaching the upper limit of the recommended 300 mg/day. Daily saturated fatty acid intake was also high at 33.7 ± 8.3 g, whereas daily intakes of monounsaturated (38 ± 8.5 g) and polyunsaturated fatty acids (18 ± 3.6 g) were relatively balanced but did not align with the recommendation to keep these as low as possible.

The MD showed a moderate daily cholesterol intake of 262 ± 94.1 mg. Daily saturated fatty acid intake was lower than in the FFD and KD at 18.3 ± 4.7 g (Table 3.10). Daily monounsaturated fatty acid intake was high at 34.8 ± 16 g, consistent with the characteristics of the MD. Daily polyunsaturated fatty acid intake was moderate at 15.2 ± 3.9 g.

For the KD, daily cholesterol intake was significantly high at 664.1 ± 226.2 mg, far exceeding the recommended limit. Daily saturated fatty acid intake was also high at 30.6 ± 9.6 g. Daily monounsaturated fatty acid intake was the highest among the diets at 46.9 ± 16.6 g, and daily polyunsaturated fatty acid intake was also relatively high at 30.5 ± 17.8 g (Table 3.10).

The RD had a daily cholesterol intake of 157.8 ± 164.1 mg, which was within the recommended limit. Daily saturated fatty acid intake was lower than the FFD and KD at 14.6 ± 3.4 g. Daily monounsaturated fatty acid intake was 26.7 ± 5.2 g, and daily polyunsaturated fatty acid intake was 17.9 ± 6.9 g, indicating a more balanced lipid profile compared to the FFD and KD. Overall, the MD and RD showed a more balanced intake of lipids compared to the FFD and KD, with lower levels of saturated fats and moderate levels of monounsaturated and polyunsaturated fats (Table 3.10).

Table 3.10 - A summary of the average daily fatty acid intake from foods across each of the four diet types

Nutrients (per day)	FFD	MD	KD	RD
Cholesterol (mg/day)	253.9 ± 83.4	262 ± 94.1	664.1 ± 226.2	157.8 ± 164.1
Fatty acids, total SFA (g/day) [#2]	33.7 ± 8.3	18.3 ± 4.7	30.6 ± 9.6	14.6 ± 3.4
4:0, Butanoic acid (g/day) [#2]	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.2
6:0, Hexanoic acid (g/day) [#2]	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
8:0, Octanoic acid (g/day) [#2]	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
10:0, Decanoic acid (g/day) [#2]	0.4 ± 0.2	0.5 ± 0.3	0.3 ± 0.2	0.2 ± 0.1
12:0, Dodecanoic acid (g/day) [#2]	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	0.4 ± 0.4
14:0, Tetradecanoic acid (g/day) [#2]	2.8 ± 0.9	1.6 ± 0.5	1.9 ± 0.8	0.9 ± 0.6
16:0, Hexadecanoic acid (g/day) [#2]	19 ± 4.5	11.1 ± 3.1	19.3 ± 6.2	9.3 ± 1.9

18:0, Octadecanoic acid (g/day) [#2]	9.3 ± 2.3	3.4 ± 0.9	7.8 ± 2.7	2.8 ± 1
Fatty acids, total MUFA (g/day) [#3]	38 ± 8.5	34.8 ± 16	46.9 ± 16.6	26.7 ± 5.2
16:1, Hexadecenoic acid, undifferentiated (g/day) [#3]	1.8 ± 0.5	1.1 ± 0.5	2 ± 0.6	1.1 ± 0.6
18:1, Octadecenoic acid, undifferentiated (g/day) [#3]	28.9 ± 9.8	32.7 ± 15.4	44.6 ± 16.7	24.6 ± 5.2
20:1, Eicosenoic acid, undifferentiated (g/day) [#3]	0.3 ± 0.1	0.6 ± 0.2	0.6 ± 0.4	0.5 ± 0.3
22:1, Docosenoic acid, undifferentiated (g/day) [#3]	0.1 ± 0	0.2 ± 0.3	0.2 ± 0.4	0.3 ± 0.3
Fatty acids, total PUFA (g/day) [#4]	18 ± 3.6	15.2 ± 3.9	30.5 ± 17.8	17.9 ± 6.9
18:2, Octadecadienoic acid (g/day) [#4]	15.4 ± 3.2	12.1 ± 3.4	25.8 ± 15.4	15.5 ± 7.1
18:3, Octadecatrienoic acid (g/day) [#4]	1.9 ± 0.4	1.7 ± 0.6	3.7 ± 2.5	1.2 ± 0.4
18:4, Octadecatetraenoic acid (g/day) [#4]	0.1 ± 0	0.1 ± 0.1	0.1	0.1 ± 0.1
20:4, Eicosatetraenoic acid (g/day) [#4]	0.1 ± 0	0.1 ± 0.1	0.4 ± 0.2	0.1 ± 0.1
20:5 n-3, Eicosapentaenoic acid [EPA] (g/day) [#4]	0	0.3 ± 0.3	0.1 ± 0.3	0.3 ± 0.4
22:5 n-3, Docosapentaenoic acid [DPA] (g/day) [#4]	0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1

22:6 n-3, Docosahexaenoic acid [DHA] (g/day) [#4]	0	0.6 ± 0.4	0.3 ± 0.5	0.5 ± 0.6

#1 - The RDA for saturated fatty acids, trans fatty acids and dietary cholesterol is as low as possible while consuming a nutritionally adequate diet.

#2 – Saturated Fatty Acids (SFA)

#3 – Monounsaturated Fatty Acids (MUFA)

#4 – Polyunsaturated Fatty Acids (PUFA)

3.2.1. Healthy Eating Index (HEI)

The ASA24 results were further analyzed by calculating the Healthy Eating Index (HEI) for each diet based on the micro and macronutrients consumed during the dietary periods using the Canadian 2019 Healthy Eating Guidelines [97]. The code for this calculation is provided in Appendix 3E. Interestingly, the FFD received no points for any of the components defined in the HEI guideline. The HEI has 10 components: 1) fruits and vegetables, 2) whole grain foods, 3) grain foods ratio, 4) protein foods, 5) plant-based protein foods, 6) sugary foods and beverages, 7) the ratio of unsaturated fat to saturated fats, 8) the percentage of energy received from saturated fats, 9) the percentage of energy intake from free sugars and 10) sodium consumption. The other three diets received full points for consumption of fruits and vegetables (component 1). None of the diets received any points for the consumption of whole grain foods (component 2). However, the MD received the maximum number of points for the consumption of grain foods ratio. On the other hand, the KD did not receive any points for the consumption of grain foods (component 3).

The MD, KD and RD received full points for overall consumption of protein foods (component 4). However, none of the diets met the criteria for receiving points for plant-protein consumption (component 5). Full points for component 6 were given to the MD, KD and RD diets that had very little-to-no consumption of sugary drinks and the main beverages consumed were unsweetened low-fat milk, and unsweetened tea or coffee. Components 7 and 8 are related to fat consumption. The ratio of unsaturated fat to saturated fats, and the percentage of energy received from saturated fats defined whether the diets received full marks for these components or not. The MD and RD received full marks for these components, while KD did not pass any of the HEI criteria for fat consumption.

The percentage of energy intake from free sugars determined whether a given diet was qualified to receive full points for component 9, and three diets (MD, KD, and RD), received the maximum points for this component. Finally, for component 10, points were given to the diets that had adequate consumption of sodium based on the daily calorie intake. Only the MD received the marks for this section. In the end, all the components were summed to calculate a HEI score for each of the diets. The FFD had an HEI of zero as it did not receive any points for any of the 10 components. The KD had an HEI of 45, while the RD and MD had an overall score of 60 and 70, respectively. The maximum HEI score achievable is 100. In conclusion, the MD scored the highest for HEI among all four diet groups while the FFD scored the lowest. Table 3.11 shows the distribution of scores for each component across all interventions.

Table 3.11 – The Healthy Eating Index components across all interventions

Components	**FFD**	**MD**	**KD**	**RD**
1. Fruits and vegetables	0	20	20	20
2. Whole grain foods	0	0	0	0
3. Grain foods ratio	0	5	0	0
4. Protein foods	0	5	5	5
5. Plant-based protein foods	0	0	0	0
6. Sugary foods and beverages	0	10	10	10
7. The ratio of unsaturated fats to saturated fats	0	5	0	5
8. E% from saturated fats	0	5	0	5
9. E% from free sugars	0	10	10	10
10. Sodium consumption	0	10	0	0
Healthy Eating Index	0	70	45	55

3.3. Physiological measurements: weight, BMI, and percent body fat estimation:

General anthropometric information, including weight (in kilograms or kg), was measured daily to track any weight loss or gain during the dietary interventions. Body weight (kg) was measured daily at the beginning of the day, upon waking up and before having breakfast. In addition, body mass index (BMI), and percentage of body fat were calculated on a daily basis to have a better understanding of gross physiological effect of diets on my body. A general summary of the mean and variations of my diet-induced body weight, BMI and percent body fat is presented in Figure 3.10. Based on one-way ANOVA, there was at least one pair of diet-induced average body weight changes that were significantly different (p = 1.42 E-18) (Figure 3.10). In particular, the most significant difference between diet-induced body weight changes was between the FFD and the KD with a difference of 2.3 kg (p =2.2156E-14) between the end of the FFD and the end

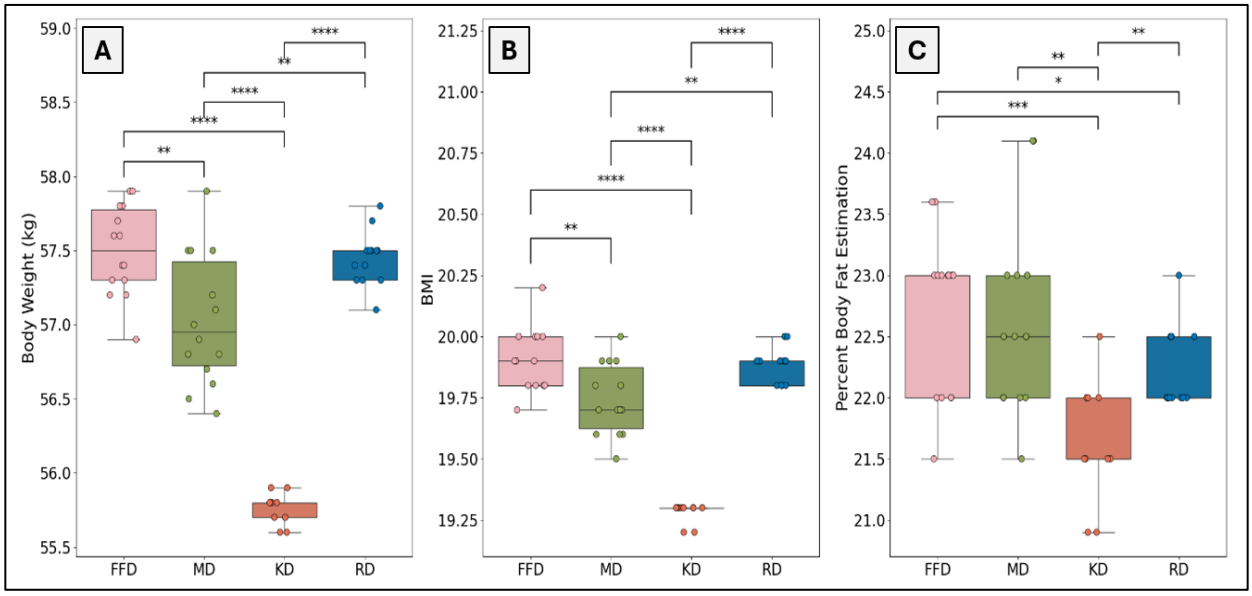

Figure 3.10 - Summary and comparison of the body weight (kg, A), BMI (B) and percent body fat estimation (C) measured during each diet period. Lowest weight and the highest variation in weight throughout the diet was observed during the KD (over the last 10 days), with the highest weight in the FFD. Similar pattern was observed for the BMI compared between the groups. Highest variations in percent body fat estimation were observed during the MD and KD, and the least variation was observed during RD (p-value ≤ 0.05 → *, p-value ≤ 0.01 → **, p-value ≤ 0.001 → ***, p-value ≤ 0.0001 → ****)

of the KD. In general, my body weight exhibited very minimal fluctuations while on the FFD (mean ± SD = 57.50 ± 0.30), the MD (mean ± SD = 57.03 ± 0.45), and the RD (mean ± SD = 57.40 ± 0.20) diets. However, there was a significant drop in body weight (~2 kg) observed within the first few days of the KD (mean ± SD = 56.10 ± 0.75) (Figure 3.11). Note that the weight loss was maintained until the last day of the KD (Figures 3.10 and 3.11). The lost weight was regained within 2-3 weeks after completing the KD intervention.

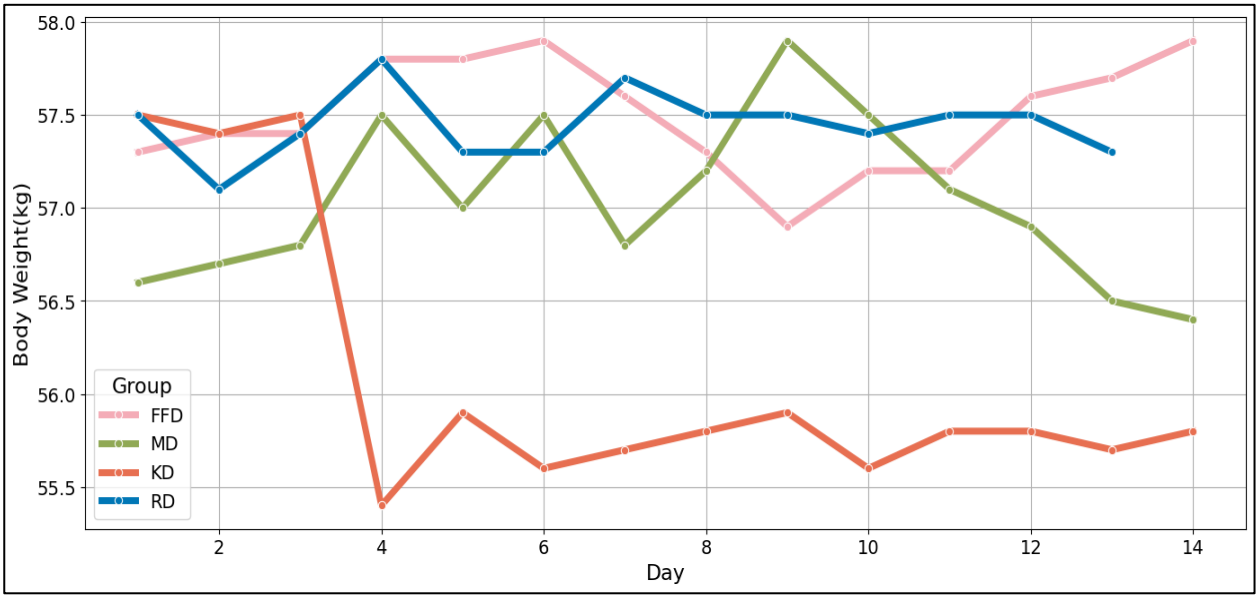

Figure 3.11 - Body weight (kg) variations during each diet. Note: the FFD (fast food diet) was the first diet followed by the MD (Mediterranean diet), then the KD (ketogenic diet) and finally my RD (regular diet). Please refer to Figure 2.1.

Since my body weight varied between the different diet groups, so too did my BMI. Using one-way ANOVA, at least one pair of average BMIs was deemed to be significantly different (p =1.7381E-18) (Figure 3.10). In particular, there was a statistically significant difference between the BMI of the FFD and the KD with a difference of 0.3 BMI units (kg/m2) (p =3.8247E-13). The highest BMI was observed while on the FFD, and essentially the same BMI was maintained while on the MD and RD. However, my BMI levels were lower while on the KD and by the end of the KD (19.41 ± 0.27). A similar pattern in percent body fat estimation was seen, and a statistically significant difference was detected between the diet groups (p = 2.9632E-4). As before, the largest difference in body fat was between the FFD and the KD with a difference of 1% at the end of both

diets (p =3.0419E-4). The highest body fat percentage was with the FFD (mean ± SD = 22.69% ± 0.66), and the lowest was with the KD (22.11% ± 0.95).

3.4. Blood pressure (BP):

BP remained in the normal range throughout all four diet treatments. Based on one-way ANOVA, there was at least one pair of diet-induced average systolic (SYS) BP that was statistically different (p = 5.8705E-4). In terms of the BP change, the FFD intervention induced an average SYS BP value that was 4.2 points higher than the RD-induced value (p = 0.0024). No significant differences were found between the average diastolic (DIA) BP among the four diet groups. A summary of the BP measurements across all diets is provided in Table 3.11. The FFD had the highest SYS BP value upon waking up in the morning, and the RD showed the lowest SYS BP value in the morning (difference of 9.6 points with a p = 8.2174E-5). Apart from the FFD, a general increase in the BP was observed after eating breakfast (Table 3.11). Furthermore, an ANOVA test revealed that there was a significant difference between the SYS BP values among the diet groups after breakfast (p = 0.0014), and no significant differences were observed between the diet groups in terms of the DIA BP after breakfast consumption. In addition, no significance was detected in the measured SYS and DIA BP after lunch or after dinner. The measured BP value before going to bed and at the end of the day of each diet revealed a significant average SYS BP difference between at least one pair of average SYS BP measurements in the diet groups (p = 0.0117). The FFD had the highest SYS BP values (111.0 ± 6.10), while the RD led to the lowest SYS BP values (104.3 ± 4.29) at the end of the day. No significant differences were observed between the DIA BP values measured before going to bed. A general summary of the mean and variations of my diet-induced daily average BP, and the BP values measured in the morning, after breakfast, and at the end of the day is presented in Figure 3.12. It is worth noting that the BP measurements did not have an exact time during the days as wake up time and the times of meal consumption varied from one day to another.

Table 3.12 - A summary of the measured BP values for the four different diets at different times of the day highlighting the significant difference seen using one-way ANOVA

BP measurement time	FFD	MD	KD	RD
SYS (daily average) ***	108.5 ± 3.12	106.49 ± 2.40	104.83 ± 2.36	104.17 ± 3.22
DIA (daily average) ns	73.80 ± 1.06	74.00 ± 2.46	73.79 ± 2.34	72.97 ± 2.04
Morning SYS ****	110.21 ± 6.28	107.64 ± 4.70	104.5 ± 5.45	100.62 ± 3.78
Morning DIA ns	75.96 ± 4.31	74.86 ± 5.78	75.86 ± 3.23	72.92 ± 3.09
After breakfast SYS ***	107.79 ± 4.28	108 ± 3.46	103.14 ± 3.72	103.31 ± 4.92
After breakfast DIA ns	72.57 ± 5.85	73.79 ± 3.24	72.43 ± 6.07	72.23 ± 3.83
After lunch SYS ns	104.79 ± 4.28	107.5 ± 6.39	104.5 ± 5.03	106.50 ± 7.55
After lunch DIA ns	74.21 ± 3.14	74.86 ± 4.20	73.36 ± 2.84	75.23 ± 5.20
After dinner SYS ns	108.71 ± 5.38	103.79 ± 4.59	105.5 ± 5.65	105.08 ± 4.21
After dinner DIA ns	74.57 ± 5.49	73.93 ± 5.18	73.93 ± 5.64	72.15 ± 6.09
Night SYS **	111.00 ± 6.10	105.5 ± 4.26	106.5 ± 5.59	104.31 ± 4.29
Night DIA ns	72.29 ± 4.23	72.57 ± 4.65	73.36 ± 3.20	72.31 ± 3.71
p-value > 0.05 → ns, p-value ≤ 0.05 → *, p-value ≤ 0.01 → **, p-value ≤ 0.001 → ***, p-value ≤ 0.0001 → ****				

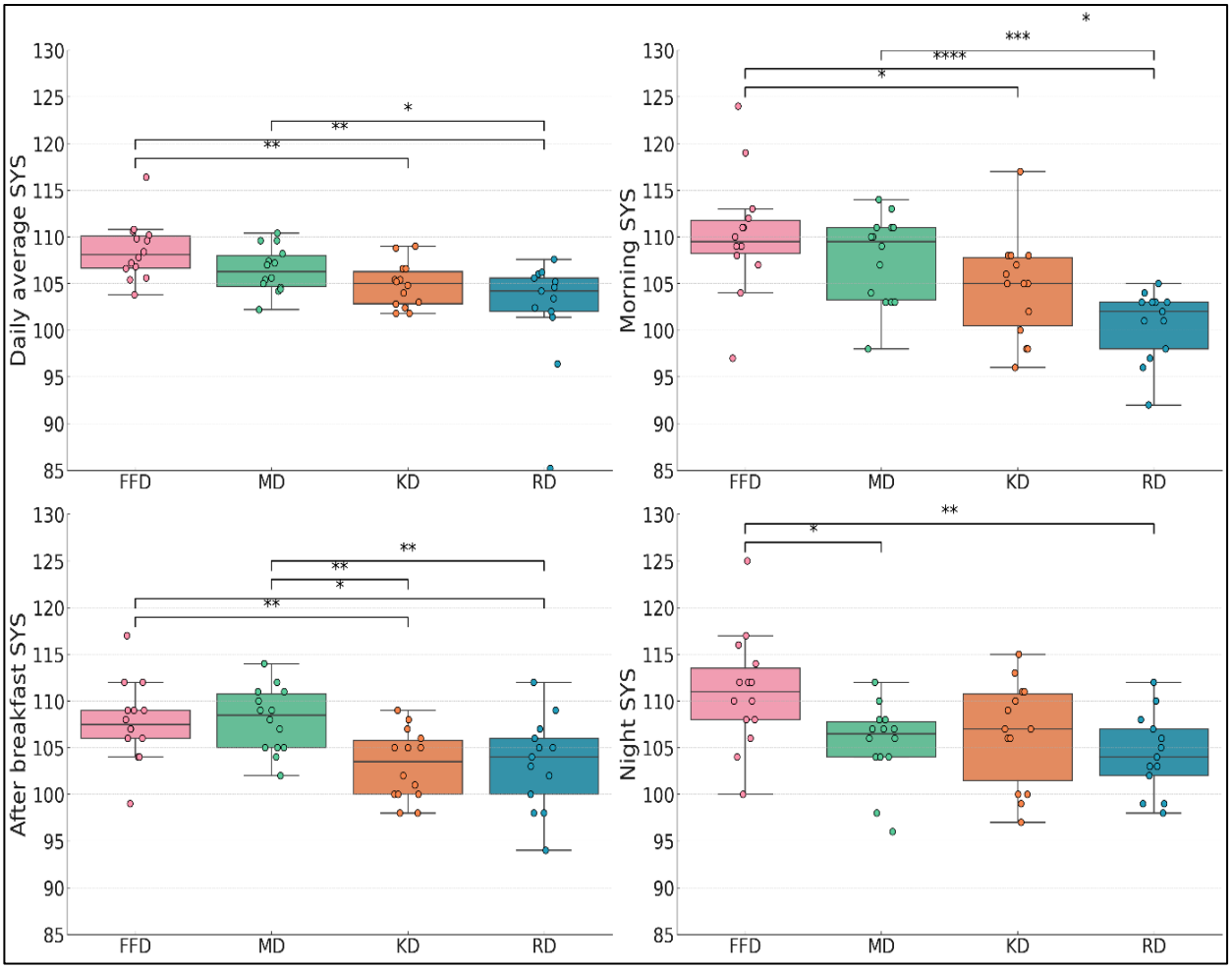

Figure 3.12 - Summary and comparison of the significantly different blood pressure (BP) values (SYS or systolic) measured during each diet period. As there were no significant differences in the diastolic (DIA) BP for any of these times, these DIA differences were excluded from this figure. p-value ≤ 0.05 → *, p-value ≤ 0.01 → **, p-value ≤ 0.001 → ***, p-value ≤ 0.0001 → ****

3.5. Body temperature (BT) (°C):

My BT varied slightly across the dietary interventions. It ranged from 36.30 °C to 37.81 °C while on the FFD, 36.20 °C to 37.80 °C while on the MD, 36.44 °C to 37.87 °C while on the KD, and 36.20 °C to 37.84 °C while on the RD, excluding the temperatures recorded during exercise. The one-way ANOVA test performed on the average daily BT values (°C) did not reveal any statistically significant differences in BT between different diet interventions. In terms of the

most evident diet-induced BT change, the KD intervention had an average BT that was 0.12 °C higher than the RD-induced values. More specifically, the highest average BT was observed while on the KD at 37.04 °C ± 0.15 °C. The second highest BT was observed while on the FFD (36.99 °C ± 0.10), and then MD (36.96 °C ± 0.12). The lowest average BT was observed while on the RD (36.91 °C ± 0.16). An overview of the BT changes measured throughout the day and for each diet is provided in Figure 3.13. While averages and variations in BT are modestly revealing, the longitudinal profile of BT changes is somewhat more interesting. Among all four diets, there was an expected drop in temperature while sleeping (between 0:00 to 8:00 hrs) and a corresponding increase in BT while awake. However, between the diets, there were differences over the extent and duration of the temperature drop. The KD had the highest average sleep temperature (36.81°C ± 0.18) while the FFD had the shortest low-temperature duration (~3 hrs), while the MD had both the lowest average sleep temperature and the longest low-temperature duration (36.76°C ± 0.20, ~5 hrs) (p = 0.183). During the waking period, the RD had the lowest average temperature (37.05°C ± 0.07) while the KD had the highest average temperature (37.18°C ± 0.10) (p = 0.03). These BT differences (during waking and sleep), and temperature durations appear to correlate well with BG, sleep duration, mood, and mental performance, as will be discussed later.

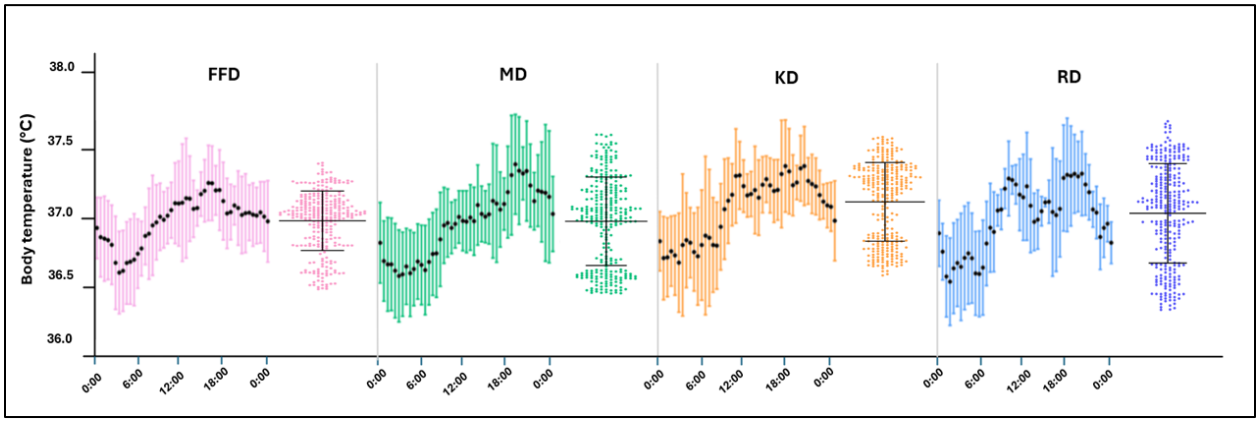

Figure 3.13 - A comparison of the body temperature (BT) values (°C) measured during the diets. The overall variations throughout a day, in addition to the mean, upper and lower interquartile are marked for each data group. On average, the lowest BT (°C) was observed during the MD and the highest was observed during the KD. The highest variation from the mean BT (°C) was marked during the RD and the lowest variation was observed during the FFD.

Because my BT was being continuously monitored, it was possible to look at how other factors such as meal consumption, exercise, menstrual cycle, or mental testing affected it. Overall, it was found that BT changes did not provide a clear indication of when a meal or snack was consumed, as other factors such as room temperature and the level of physical activity at that moment would more significantly affect BT. On the other hand, it was possible to track the occurrence and duration of any exercise with BT. Over the course of each diet (~10 exercise or walking periods) BT changes had a sensitivity of 0.9 and specificity of 0.9 for detecting physical activity for the diets (using a threshold of 37.3 °C) (Figure 3.14). However, over the course of each diet (15-25 mental test periods), BT changes did not show a significant change across any diet groups with regard to intense mental testing.

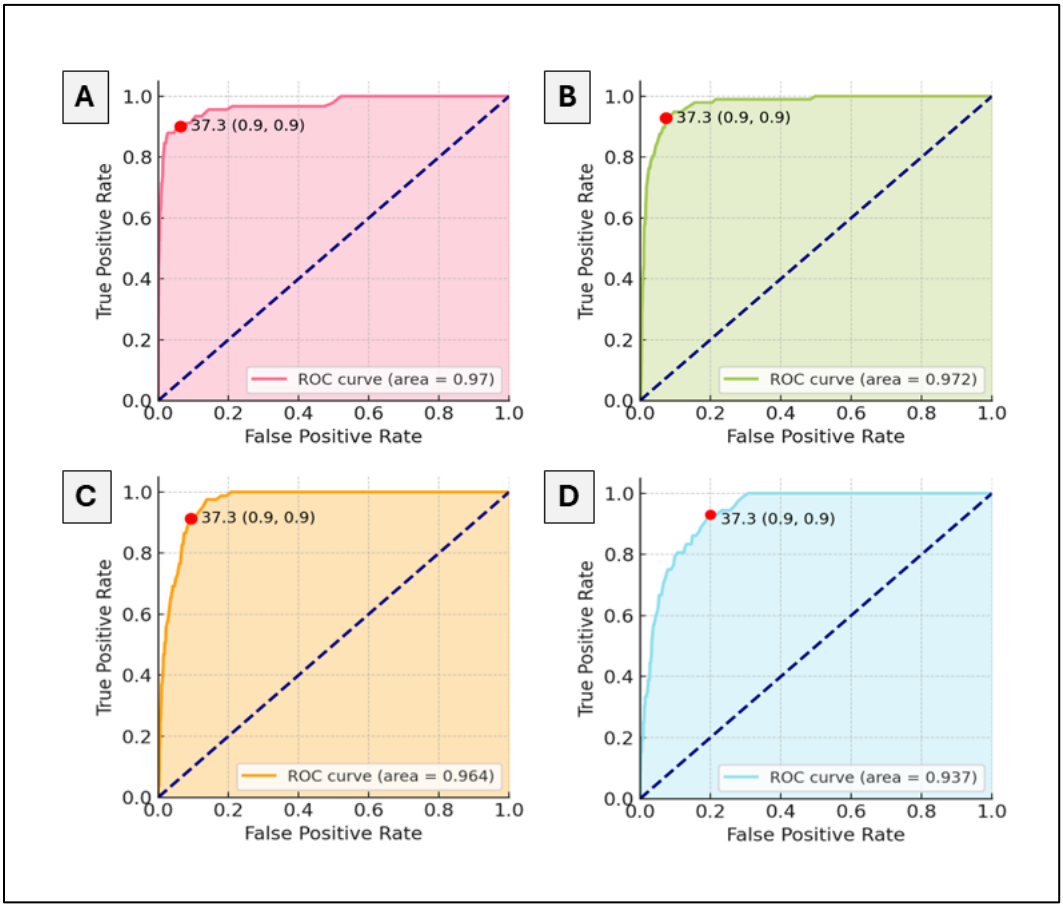

Figure 3.14 - Sensitivity and specificity of the body temperature (BT) monitor during exercises. A: ROC curves and area under the curve for the FFD, B: ROC curves and area under the curve for the MD, C: ROC curves and area under the curve for the KD, and D: ROC curves and area under the curve for the RD

3.6. Blood glucose (BG) (mmol/L):

The one-way ANOVA analysis on the BG datasets revealed a significant difference between mean glucose values for at least two of the diet-induced measurements ($p<0.05$). The FFD intervention led to an average BG level that was 1.27 mmol/L higher than the KD-induced values (p =3.4568E-8). In addition, the Brown-Forsythe test and Bartlett's test showed significant differences between the standard deviations. Overall, the highest average BG levels were observed while on the FFD (6.42 ± 0.49 mmol/L). The MD and RD with average BG levels of 6.11 ± 0.36 mmol/L, and 5.97 ± 0.32 mmol/L, respectively, were slightly lower. On the other hand, the KD had the lowest average BG levels (5.15 ± 0.17 mmol/L). An overall depiction of the BG data as measured throughout the day and across diets is presented in Figure 3.15.

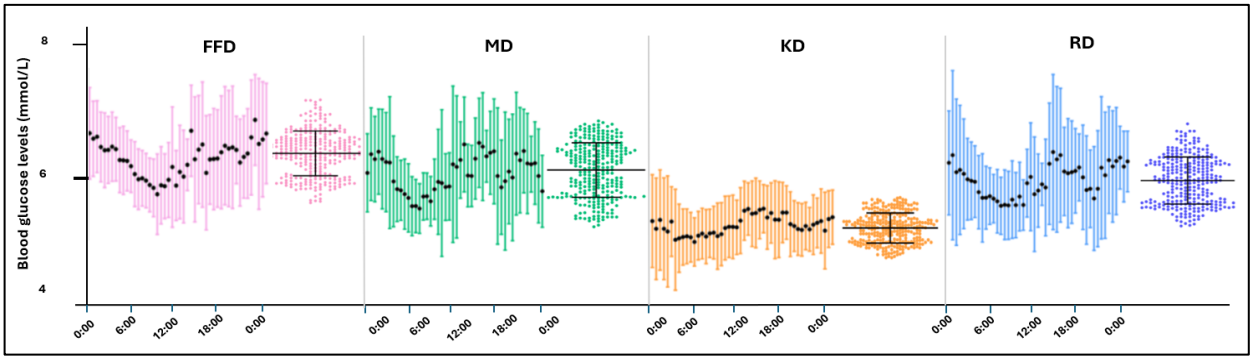

Figure 3.15 - A visual summary of the variations in BG levels (mmol/L) across all diet groups. The overall variations throughout a day, in addition to the mean, upper and lower interquartile, are marked for each data group. On average, the lowest BG levels were measured during the KD, while the FFD caused the highest BG levels. The MD and RD had more similar variations and average in BG.

While averages and variations in BG are quite revealing, the longitudinal profile of BG changes is also quite interesting. Among all four diets, there is a general drop in BG levels while sleeping (between 0:00 to 8:00 hrs) and a corresponding increase in BG while awake and eating. However, between the diets, there are significant differences over the extent and duration of the glucose drop. The KD had the most modest drop in BG while the MD had the shortest low-glucose duration (while sleeping). While sleeping, the FFD had the most significant drop in BG while the RD had the longest low-BG duration (~ 0.9 mmol/L, ~4.5 hrs). During the waking period, the KD had the lowest BG while the FFD had the highest average BG (6.35 mmol/L ± 0.47 while on FFD,

and 5.24 mmol/L ± 0.37 while on the KD, p = 2.3032E-7). Figures 3.16 and 3.17 illustrate the BG variations during sleep across all dietary interventions. These BG differences (during waking and sleep), and low/high BG durations appear to correlate well with BT, sleep duration, mood, and mental performance, as will be discussed later.

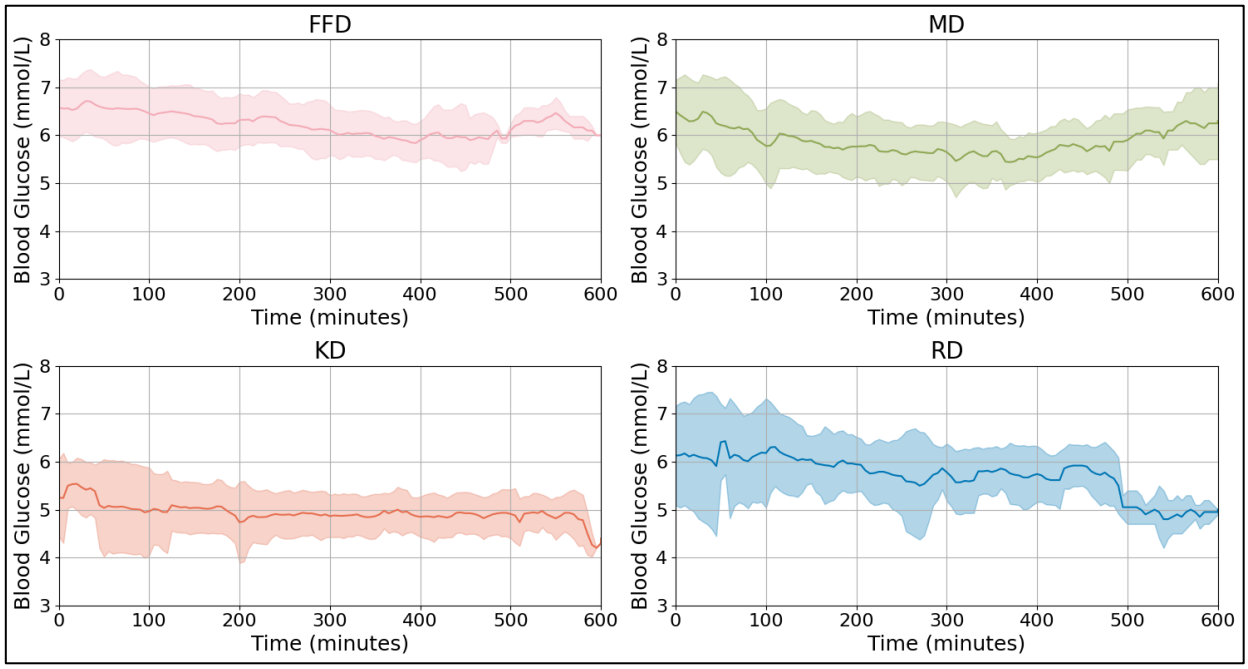

Figure 3.16 - Nighttime BG variations across all four dietary interventions

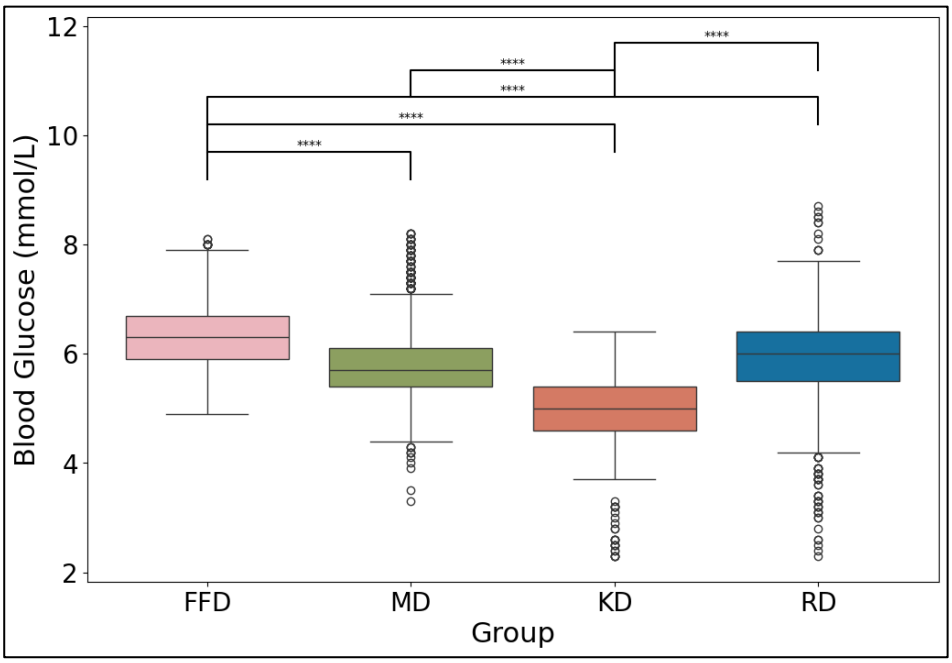

Figure 3.17 - Boxplots illustrating the mean, upper, and lower interquartile of nighttime BG changes during sleep. p-value ≤ 0.05 → *, p-value ≤ 0.01 → **, p-value ≤ 0.001 → ***, p-value ≤ 0.0001 → ****

Because my BG was being continuously monitored, it was possible to look at how other factors such as meal consumption, exercise, menstrual cycle, or mental testing affected it. In particular, it was found that my BG would consistently spike within 20 minutes after eating a meal or snack and reach the maximum postprandial BG within an hour after the meal (Figure 3.18). The rise over baseline post-prandially was greatest with the FFD (38.85%) and lowest with the KD (21.11%). While on the MD and KD, it was highest after breakfasts (51.94%, and 22.35%, respectively) and lowest after snacks (27.90%, and 20.37% respectively). While on the FFD, the rise over baseline post-prandially was highest after dinners (49.52%) and lowest after breakfasts (26.99%), while with the RD, it was highest after lunch (36.77%) and lowest after dinner (29.80 %) (Figure 3.18). In terms of using BG to predict meal or snack consumption over the course of each diet (~40 meals, ~25 snacks), BG changes had a sensitivity of 0.9 and specificity of 0.9 for FFD (using an elevation over baseline of 20%) but only a sensitivity of 0.5 and specificity of 0.8 for KD (using an elevation over baseline of 20%).

It was also possible to track the occurrence and duration of any exercise event via BG. Over the course of each diet (~10 exercise or walking periods), BG changes had a sensitivity of 0.8 and specificity of 0.8 for the MD (using an optimal cutoff of 5.35), but only a sensitivity of 0.6 and specificity of 0.6 for the KD (using an optimal cutoff of 4.95) (Figure 3.19). Additionally, over the course of each diet (14 mental test periods), BG changes did not show significant variations between the testing and non-testing periods.

Through BG monitoring, it was also possible to identify which foods led to the largest changes in BG. I found that high-sugar foods/beverages, such as soft drinks (Coke) and milkshakes consumed while on the FFD, led to a rise over baseline (BG level before meal consumption) of 3.7 mmol/L and 4.1 mmol/L respectively, and orange juice consumed during the MD led to a rise over baseline of 4.7 mmol/L (Figure 3.20). These beverages consistently caused the largest increases in BG levels (my normal range of BG level was between 3.9 and 10 mmol/L). Foods such as bananas and mixed vegetables (carrots and sweet peas) resulted in an increase in the BG (an average rise over baseline of 2.13 mmol/L and 1.6 mmol/L respectively) (Figure 3.20). Interestingly, the consumption of high glycemic-index foods such as rice, bread, pasta, potatoes, and other carbohydrate-rich foods did not lead to a detectable elevation in BG.

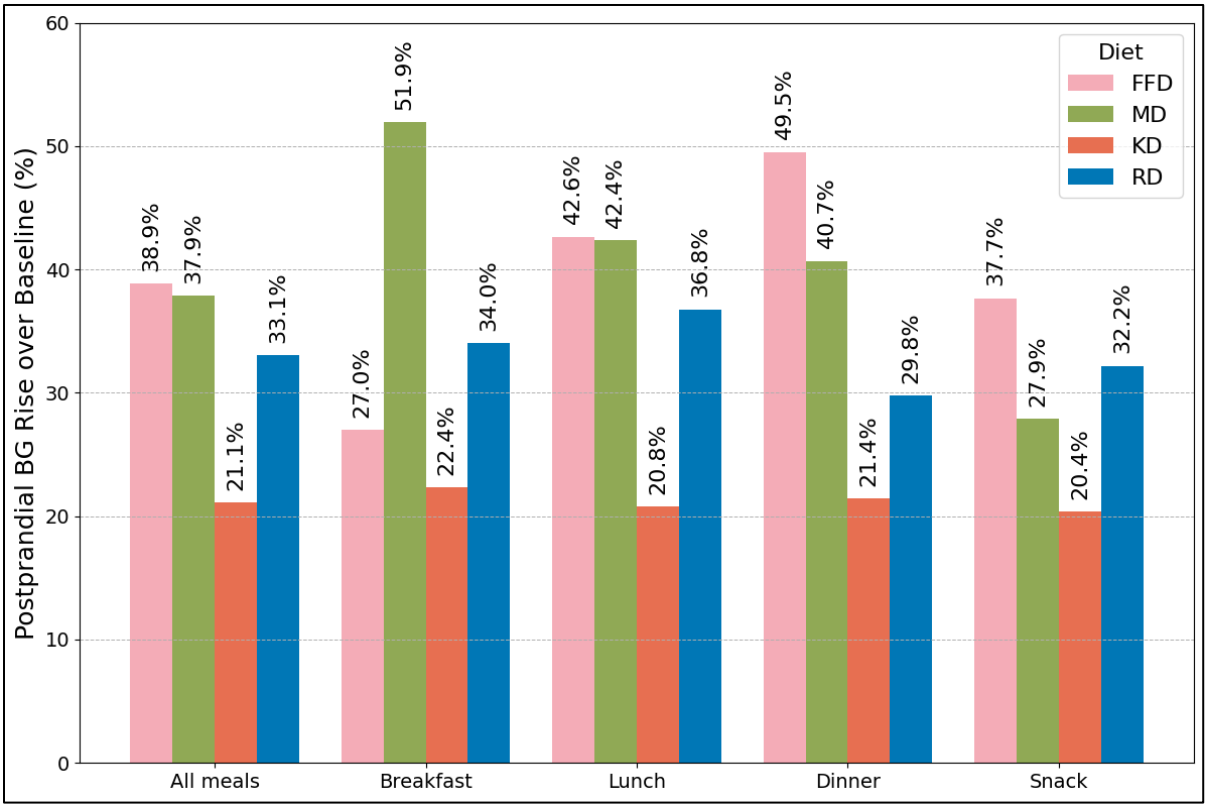

Figure 3.18 - The average percentage of rise of blood glucose (BG) over baseline after each meal consumption during the dietary interventions.

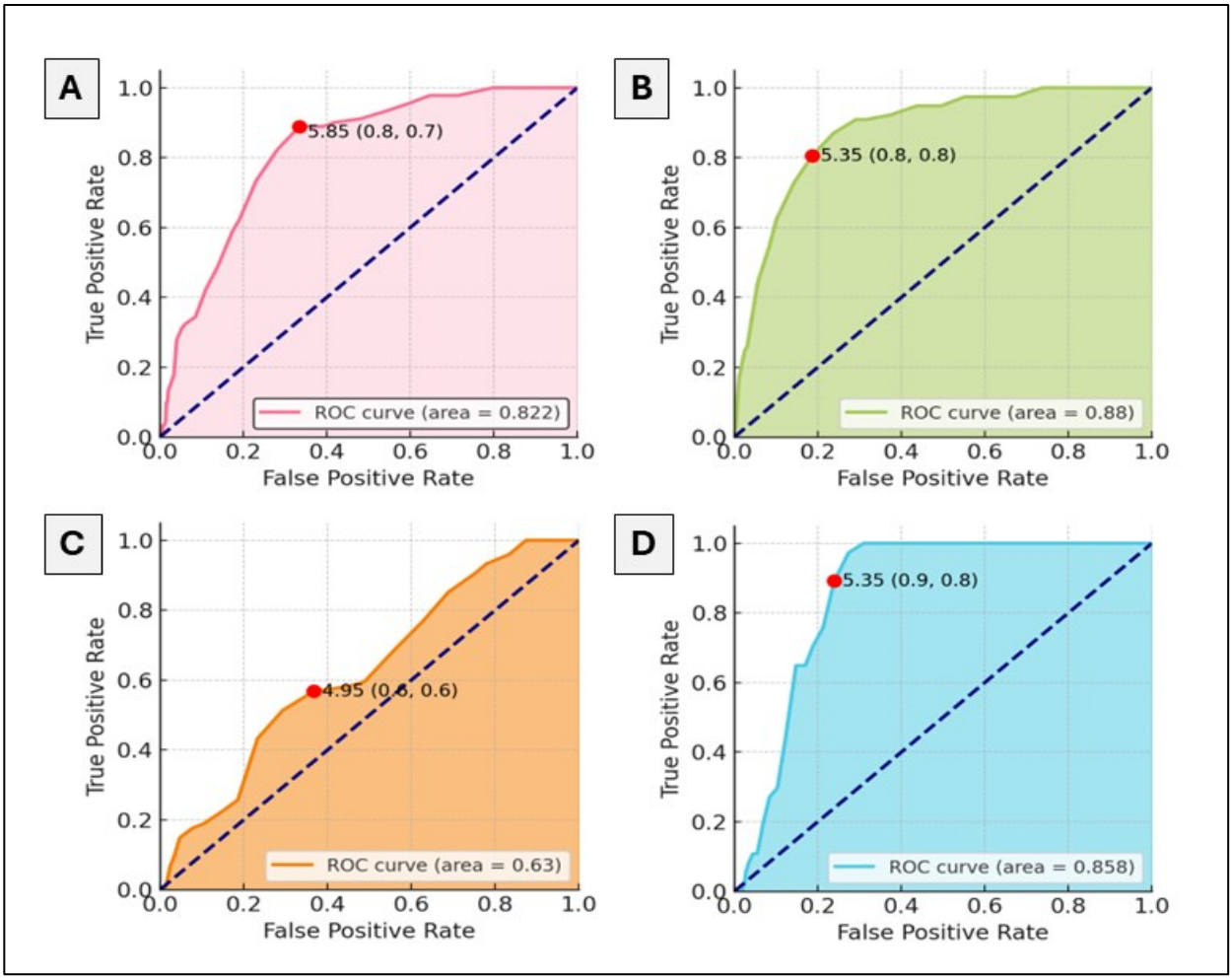

Figure 3.19 - Sensitivity and specificity of the blood glucose (BG) monitor during exercises. A: ROC curves and area under the curve for the FFD, B: ROC curves and area under the curve for the MD, C: ROC curves and area under the curve for the KD, and D: ROC curves and area under the curve for the RD.

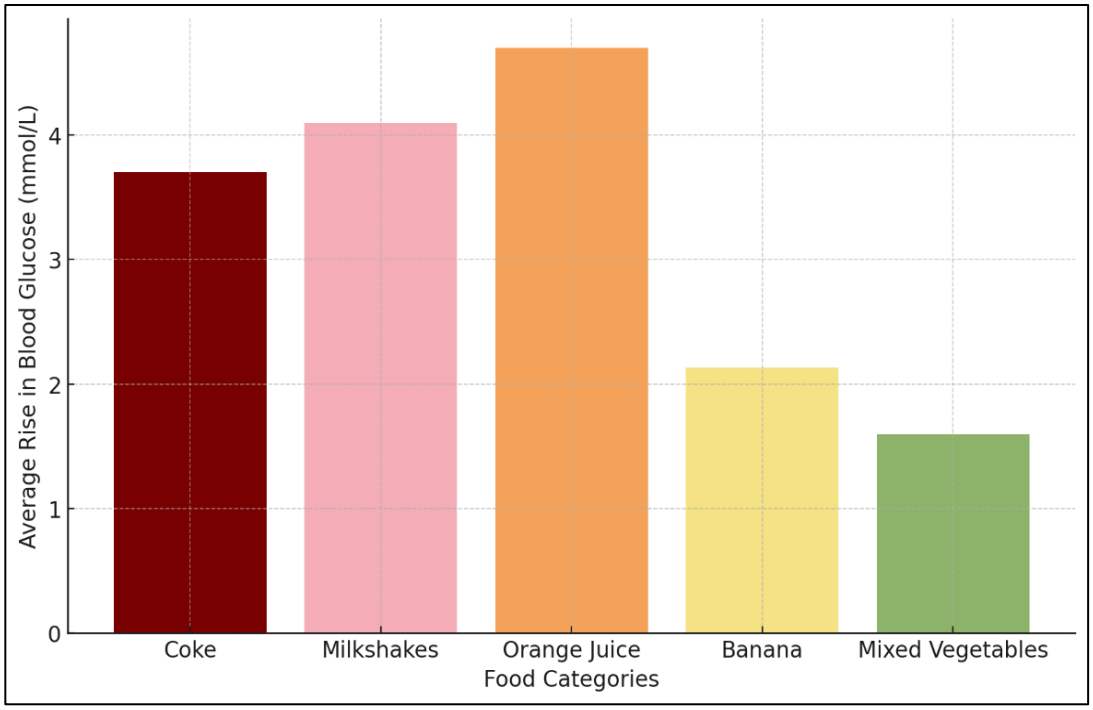

Figure 3.20 – Average rise in blood glucose (BG) levels (mmol/L) above baseline after consumption of specific foods.

3.7. Heart rate (HR) (bpm):

HR in beats per minute (bpm) was monitored every minute of each day using a Fitbit smartwatch. The average daily HR (with both active and resting) dropped over the length of the MD intervention (from 82.08 ±14.42 bpm to 64.41 ± 8.57 bpm). This trend is highly significant compared to the other diet groups (p = 0.0012). On the other hand, the KD had an average daily HR that was 6.97 bpm higher than the MD-associated values (p = 0.0017). The KD intervention led to the highest average daily HR (79.41 ± 5.61 bpm). A slight increase in the average daily HR was observed during the first few days of the KD diet (74.32 ± 17.94 bpm on day one and 92.53 ± 12.17 bpm on day 5). However, a modest downward trend was evident throughout the rest of the KD intervention (79.62 ± 15.51 bpm on day 14). The FFD registered the second-highest daily HR at 77.03 ± 3.39 bpm. The RD and MD had the lowest daily HR values among all diet groups (74.11 ± 4.43 bpm, and 72.44 ± 4.92 bpm, respectively). A visual representation of the HR variations throughout the day and across the diets is presented in Figure 3.21.

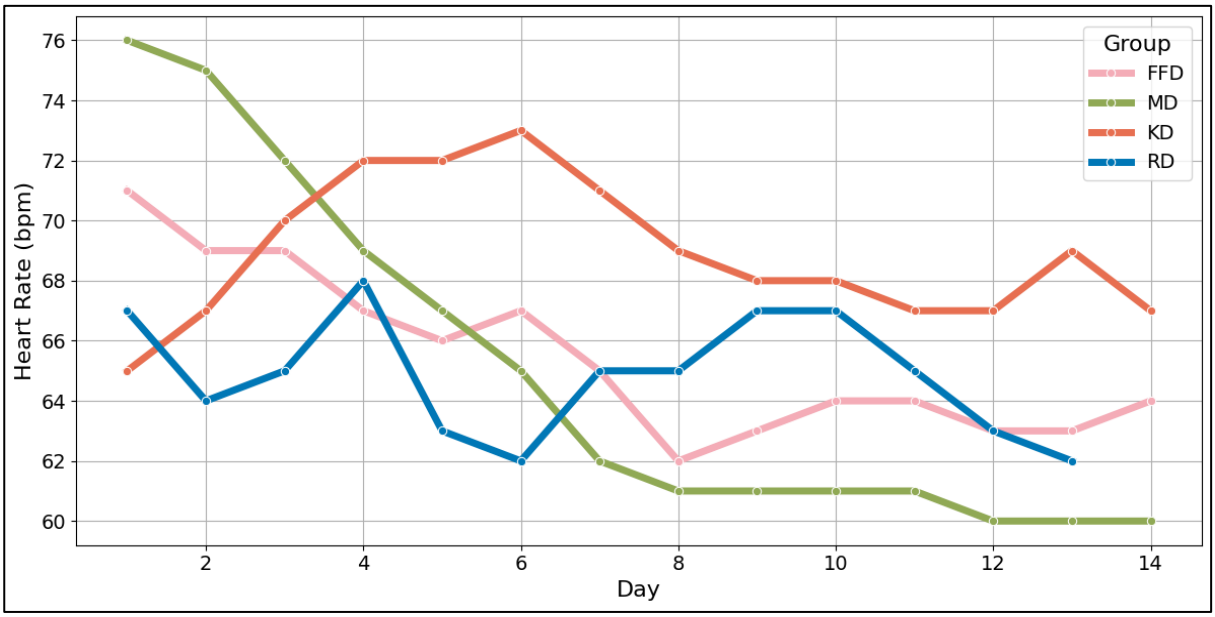

Figure 3.21 - The average daily heart rate (HR, in beats per minute, bpm) variations across all diets from the beginning of the diet period until the final day. A significant decreasing trend was observed for the HR values measured during the MD, and overall, MD had the lowest HR levels in comparison to the other diets. The KD increased the HR within the first few days and this increased level was maintained until the end of the diet period. The FFD and RD had fluctuations during the diet period which was within the normal range of my HR variations.

In addition to the comparison of the average daily HR changes throughout the dietary interventions, it is also possible to compare the average resting HR across diets. This comparison showed a significant difference between at least one pair of diet-induced HR across the four diets (p = 0.012) (Figure 3.22). The decreasing trend during the MD was also observed with the average daily resting HR (Figure 3.22). In terms of the most evident difference in diet-induced resting HR, the KD had an average resting HR that was 4 bpm higher than the RD-associated values (p = 0.012). Overall, the KD had the highest average resting HR throughout the diet period at 68.93 ± 2.37 bpm. On the other hand, the RD had the lowest average resting HR (mean ± SD = 64.85 ± 1.99 bpm). The FFD and MD had resting HR between those values of 65.50 ± 2.74 bpm, and 65.00 ± 5.80 bpm, respectively.

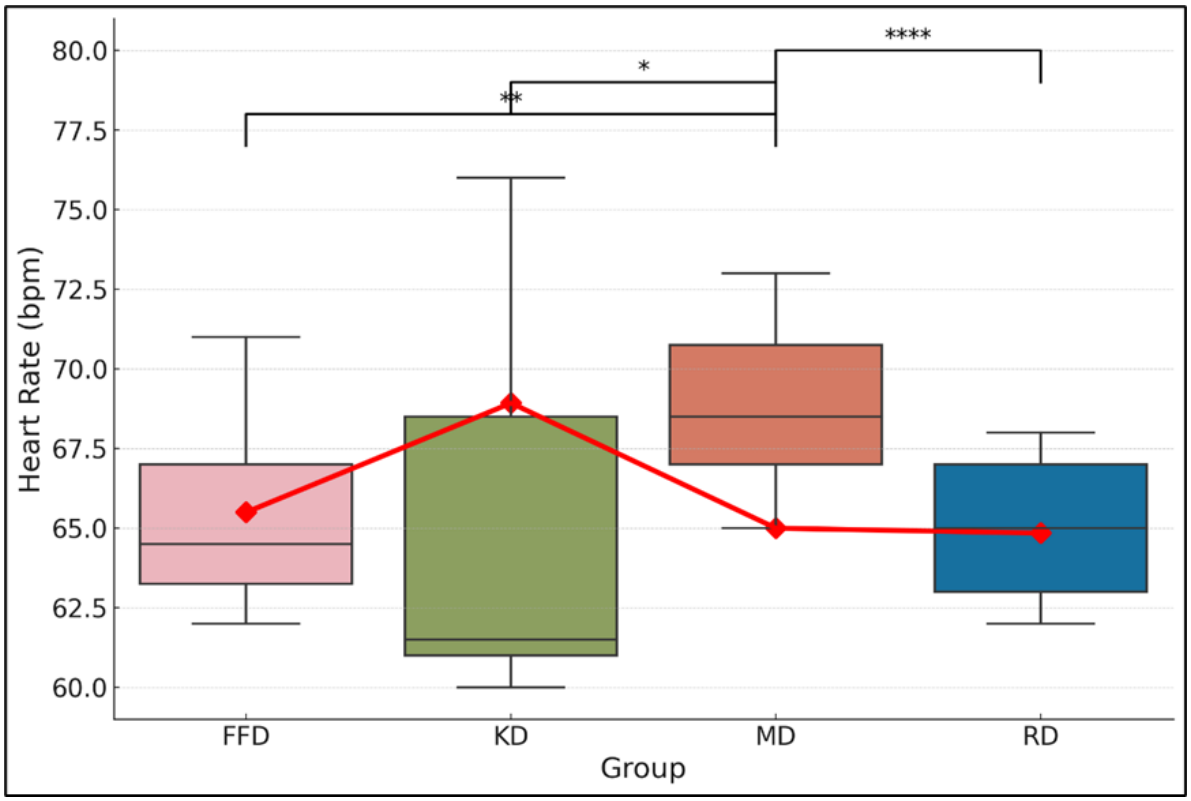

Figure 3.22 - Boxplot displaying the distribution of the resting heart rate (HR) measurements (in beats per minute, bpm) for different groups. The median HR for each group is indicated by a black line, and the means are highlighted by the red line. p-value ≤ 0.05 → *, p-value ≤ 0.01 → **, p-value ≤ 0.001 → ***, p-value ≤ 0.0001 → ****

While averages and variations in HR are modestly revealing, the longitudinal profile of HR changes over the course of the day was potentially more revealing. As might be expected, among all four diets, there was a consistent drop in the HR while sleeping and a corresponding increase in the HR while awake and eating. However, between the diets, there were significant differences over the extent and duration of the HR depression. I had the most modest drop-in HR during sleep while on the RD, while the MD had the shortest low-HR duration (Figure 3.23). The FFD had the most significant drop in HR while the RD had the longest low-HR duration (~12 bpm, ~5 hrs). During the waking period, the MD had the lowest HR while the KD had the highest average HR (78.67 bpm ± 6.10 versus 86.92 bpm ± 5.24, p = 9.3795E-4) (Figures 3.23 and 3.24).

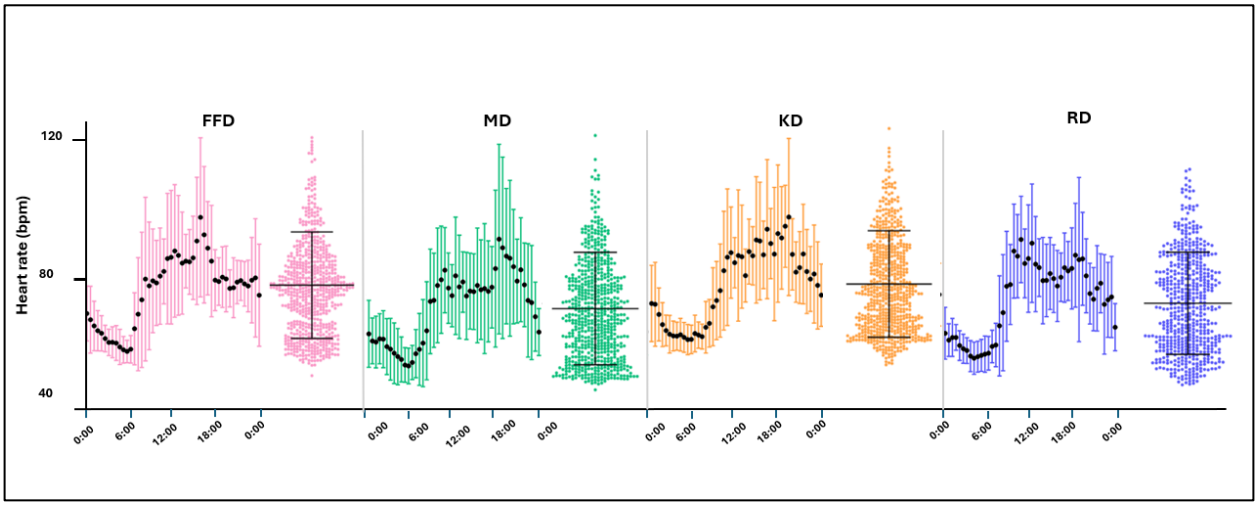

Figure 3.23 - A visual summary of the variations in heart rate (HR) levels (bpm) across all diet groups. The overall variations throughout a day, in addition to the mean, upper and lower interquartile, are marked for each data group. On average, the lowest HR levels were measured during the MD, while the KD caused the highest HR levels. HR levels during the RD was close to the MD, while FFD showed higher HR levels.

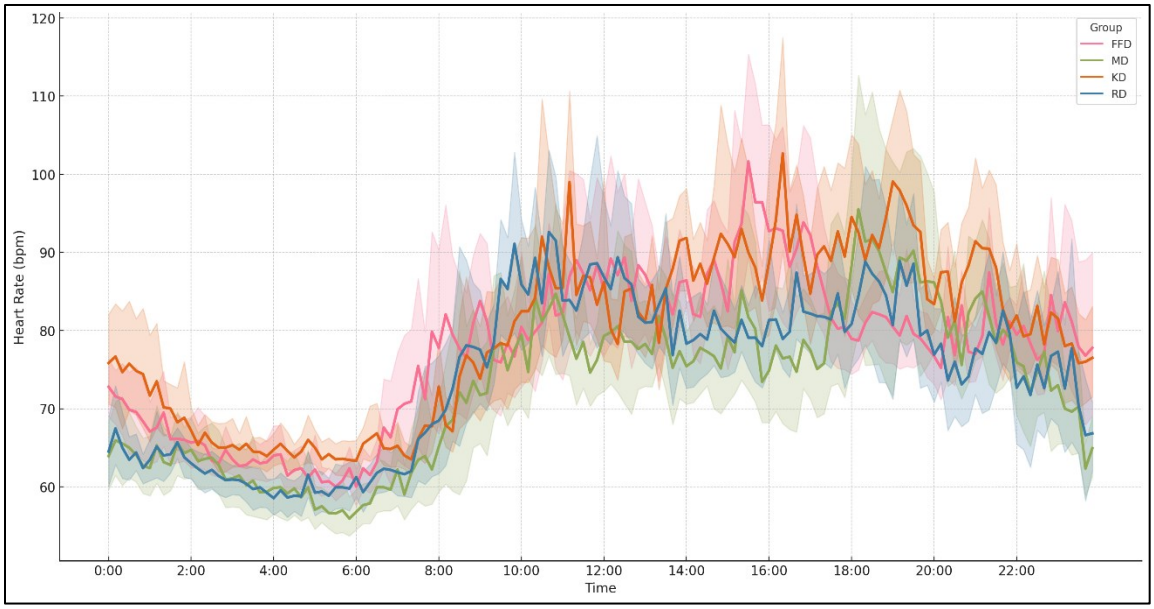

Figure 3.24 – Heart rate (HR) trends over the entire day for each group. Each line shows the average HR trend over the course of the day for each group, highlighting the variations and patterns in HR throughout the day.

The longitudinal analysis of HR during sleep across different diet groups revealed several interesting findings. The FFD intervention had an initial average HR of approximately 63.07 ± 6.23 bpm, the MD intervention had an average HR of 65.18 ± 7.17 bpm, the KD intervention averaged 68.24 ± 6.41 bpm, and the RD intervention had an average HR of 62.31 ± 6.32 bpm. It is worth noting that the results presented here were obtained by averaging and comparing the total HR levels that were recorded during sleep times. The exact average sleep HR levels reported by Fitbit are discussed later in the Sleep section (see Section 3.10). Trend analysis using linear regression models further elucidated these patterns. For the FFD intervention, the HR decreased significantly over time with a slope of -0.0053 bpm per minute ($p < 0.001$). The MD and RD interventions also showed negative trends with more modest negative slopes of -0.0011 bpm ($p = 0.0007$) and -0.0019 bpm per minute ($p = 0.0029$), respectively. The KD intervention exhibited a stronger negative trend with a slope of -0.0034 bpm per minute ($p < 0.001$). These trends suggested a general decline in HR over the sleep period across all groups, with the most significant decrease observed in the FFD and KD interventions. Figure 3.25 shows the changes in HR during sleep over time for each dietary intervention.

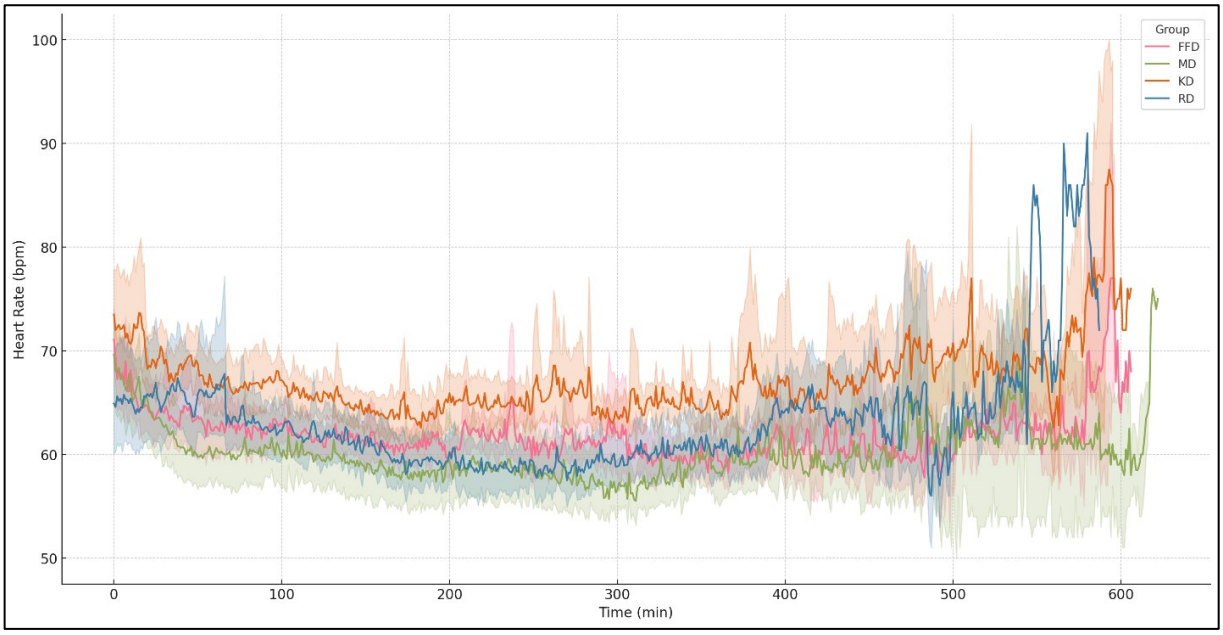

Figure 3.25 – Heart rate (HR) during sleep over time for each group. The x-axis represents sleep time in minutes, while the y-axis represents the HR in beats per minute (bpm). The darker colored lines show the average HR trend over time for each group, indicating how HR changes throughout the sleep periods

Since my HR was being continuously monitored, it was possible to look at how other factors such as meal consumption, mental testing or exercise affected it. By looking at the longitudinal changes after each meal consumption across all diets, my HR did not show a consistent spike after eating a meal or snack. Additionally, over the course of each diet (15-25 mental test periods), HR changes did not show a correlation to the mental testing times. However, HR elevations and duration during any exercise changed consistently compared to daily HR levels (which excluded the exercise periods). Over the course of each diet (~10 exercise or walking periods), HR changes had a sensitivity of 1.0 and specificity of 1.0 for the MD and RD (using an optimal cutoff of 104 and 106 bpm, respectively) but only a sensitivity of 0.9 and specificity of 0.9 for the KD (using an optimal cutoff of 104 bpm) (Figure 3.26).

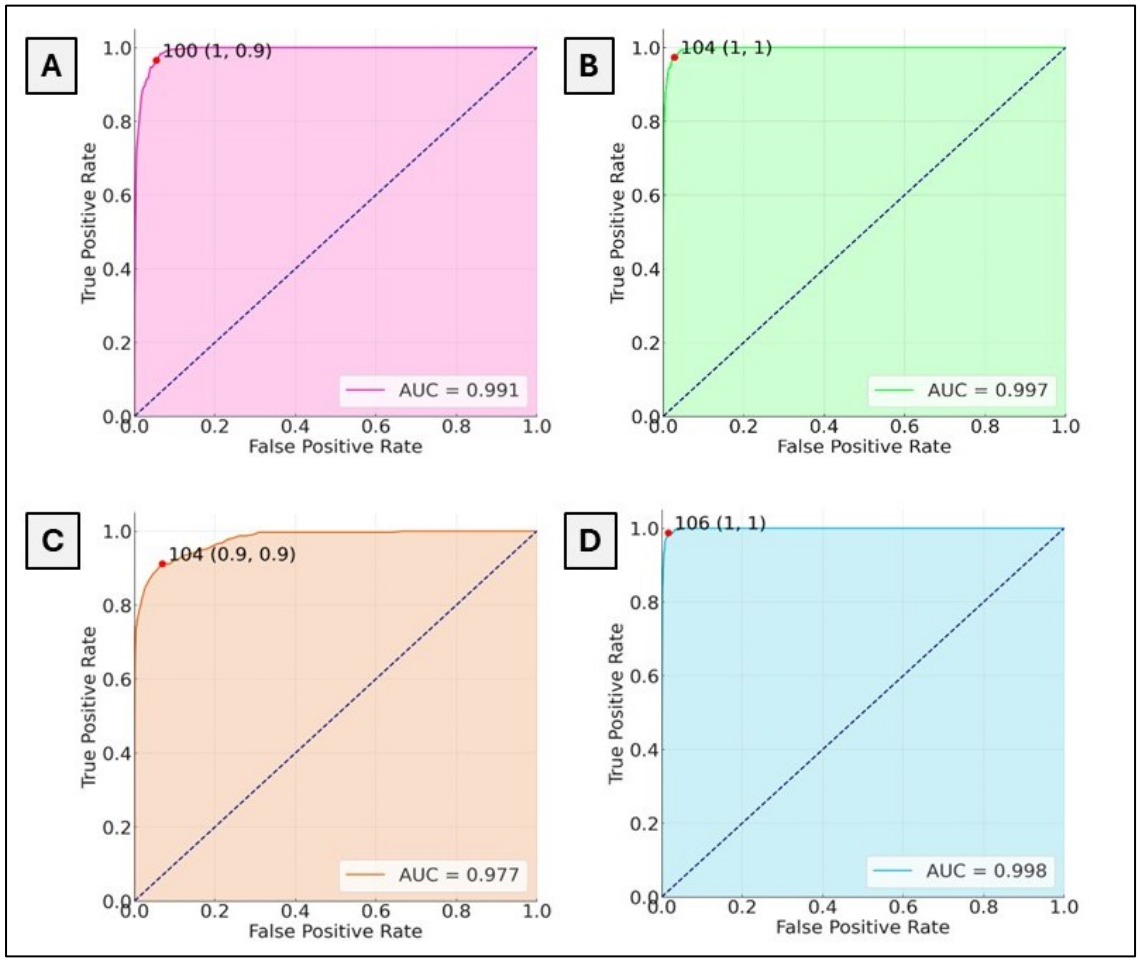

Figure 3.26 - Sensitivity and specificity of heart rate (HR) changes in prediction of exercise versus non-exercise HR levels. A: ROC curves and area under the curve for the FFD, B: ROC curves and area under the curve for the MD, C: ROC curves and area under the curve for the KD, and D: ROC curves and area under the curve for the RD.

3.8. Mental performance:

Serial Subtraction Test: There was a statistically significant difference between the means of the percent of correct serial subtractions among diet groups (p =0.027). The best scores were obtained with the MD (98.62 ± 1.43 % correct subtractions) and the RD (97.81 ± 3.67 % correct subtractions). The KD and FFD showed the worst results in terms of serial subtraction accuracies (94.99 ± 5.47, and 93.51 ± 7.0 % accuracy, respectively) (Figure 3.27).

Reaction Time Test: The best mean reaction time scores were obtained while on the RD (0.55 ± 0.08 s), and the slowest reactions were observed while on the FFD (0.69 ± 0.11 s). The MD had similar results to the RD (0.57 ± 0.10 s), and the KD had slightly slower reactions (0.62 ± 0.07 s) (Figure 3.27). A statistically significant difference was observed between the reaction times across the diet groups (p =0.0008).

Trail-making Test: No significant differences were observed among the diet groups in terms of making correct connections between letters and numbers (trail-making test). However, the average time to finish the test varied among the diet groups (p = 8.27 e-07). The biggest difference was seen between the time to complete the test while on the RD versus the FFD (11.4 sec, p = 5.78E-6). On average, the time to finish the test was 24.7 ± 1.9 seconds while on the RD, and 28.28 ± 4.1 seconds while on the MD. The average time to finish the test was highest while on the FFD and KD at 36.1 ± 6.9 seconds and 33.1 ± 5.3 seconds, respectively (Figure 3.27).

Stroop Effect and Digit Span Test: No significant differences were observed between the diet groups in terms of accuracy in finishing the Stroop Effect test or the time to finish the test. On the other hand, the digit span test revealed significant variations in mental performance, among the diet groups (p = 1.96 E-10). On average, the highest digit span accuracy was obtained while on the RD (91.0 ± 7.2). The KD and MD revealed similar averages (83.1 ± 11.9, and 87.1 ± 7.2, respectively). The FFD had the lowest scores at 63.9 ± 7.6 (Figure 3.27). The difference between the RD and FFD performance was 27.1 (p = 1.02E-8).

Overall, the results of the mental capability assessment tests revealed that the performance was relatively better while on the RD (higher accuracy, shorter time), and second best while on the MD. The FFD produced significantly worse results in every mental test.

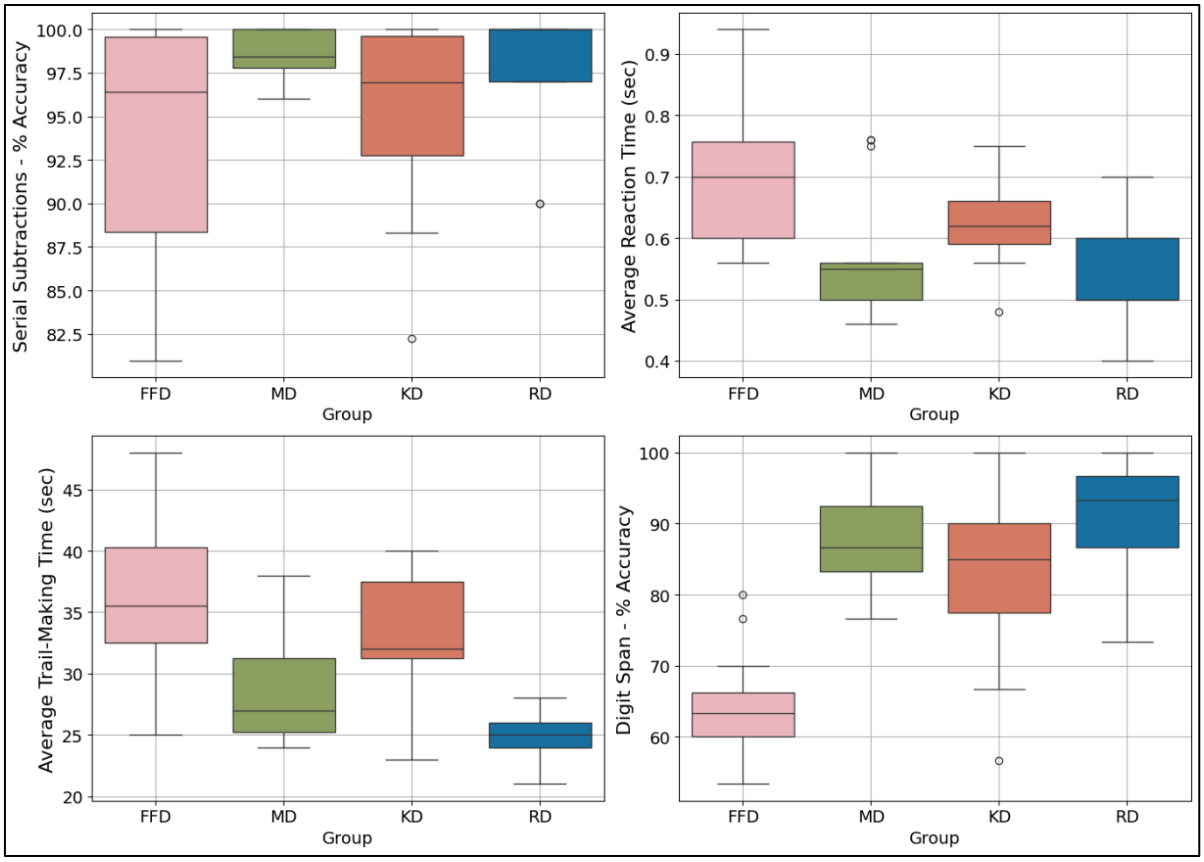

Figure 3.27 - Boxplots of mental capability assessment test results. Overall, the FFD marked the lowest mental capability level across all diets, as the highest variations and lowest accuracy of answers in the serial subtraction test, slowest reaction, slowest trail making, and lowest accuracy in the digit span test was observed. On the other hand, better accuracy, faster reactions, and faster problem solving were marked during the MD and RD.

3.9. Mood changes:

Using the Profile of Mood State (POMS) standard form, moods and feelings were converted into quantitative values or mood scores to facilitate the process of data analysis and comparison. POMS is composed of 65 adjectives that can be rated by the user on a 5-point scale. The final results can be summarized in 6 factors: Tension (scored 0-36), Depression (scored 0-60), Anger (scored 0-48), Fatigue (scored 0-28), Confusion (scored 0-28), and Vigor (scored 0-32). A general total mood disturbance score (scored -32 to 200) was calculated by adding the Tension, Depression, Anger, Fatigue, and Confusion scores, subtracted by the Vigor score.

Overall, significant differences were observed between the mood factors among the four diet groups. A detailed summary of POMS results is provided in Table 3.12. A higher score of Tension was observed while on the FFD, and the KD, while the lowest level of tension was detected while on the MD. The KD resulted in the highest scores of depression (6 ± 1.4) while the lowest depression scores were observed while on the MD (1 ± 0). The feeling of anger peaked while on the FFD and was at its lowest while on the MD and RD. Fatigue was most evident while on the FFD and KD, while I felt more energized while on the RD and MD. Confusion was also very high while on the FFD, while the second highest confusion score was obtained while on the MD. Meanwhile, the KD and RD interventions showed lower scores of confusion. Vigor was optimal while on the MD and RD interventions, and the lowest Vigor levels were observed while on the KD. Overall, the total mood disturbance score was the highest (worst) while on the FFD (26.2 ± 6.4) while the second highest (worst) score was while on the KD (4.8 ± 16.1). The lowest (best) scores were measured while on the MD and RD (-19.0 ± 2.2, and -17.2 ± 3.0, respectively). These differences were highly significant between the diet groups (p = 3.64 e-06), with the most significant difference being between the MD and FFD scores (total difference = 45.2, p = 5.44E-7).

Table 3.13 - Summary of Profile of Mood States (POMS) test results

Mood factors	FFD	MD	KD	RD
Tension ****	7.2 ± 2.5	1 ± 0	3 ± 1.2	1.8 ± 0.8
Depression ***	3.2 ± 2.5	1 ± 0	6 ± 1.4	1 ± 0.7
Anger ****	4 ± 1.2	0 ± 0	1.4 ± 1.7	0 ± 0
Fatigue ****	13 ± 3.2	0.5 ± 0.6	7.2 ± 5.3	0.4 ± 0.5
Confusion ****	7.6 ± 1.1	2.5 ± 1	1.2 ± 0.4	0.6 ± 0.9
Vigor ****	11.6 ± 2.2	24 ± 1.4	11.2 ± 6.1	20.8 ± 1.3
p-value > 0.05 → ns, p-value ≤ 0.05 → *, p-value ≤ 0.01 → **, p-value ≤ 0.001 → ***, p-value ≤ 0.0001 → ****				

3.10. Sleep:

Sleep was monitored using a Fitbit smartwatch. The number of occurrences of each sleep stage, the number of minutes spent in each stage, the sleeping HR (bpm), tossing and turning (restlessness), the percentage of the sleeping HR measurements that was above the resting HR, and the temperature variance from baseline was measured using the Fitbit smartwatch and collected through the Fitbit API or the mobile phone application. A general summary of the sleep data and the comparison between the diets is presented in Figure 3.28 and Tables 3.13 and 3.14.

Qualitatively, I experienced a number of persistent sleep problems and had difficulty falling asleep while on the FFD. The least amount of sleep occurred while on the FFD with 342.8 ± 108.5 minutes/night (Table 3.13). On the other hand, the longest average sleep time was obtained while on the KD (435.4 ± 65.9 minutes/night). I felt very tired (along with headaches and significant mood changes) while on the KD and generally, I slept more. The second longest sleep time was observed while on the RD, and the overall time spent sleeping was similar to my usual sleep patterns. On the other hand, the longest awake times during my sleep periods were observed with the KD (78.0 ± 28.7 minutes/night), followed by the RD (64.8 ± 17.7 minutes/night), followed by the MD (56.6 ± 15.7 minutes/night), and finally, the FFD (50.7 ± 22.2 minutes/night) which was significantly different between the diet groups (p = 0.01). Interestingly, the overall awake time positively correlated with the overall sleep time (more sleep, more waking) (Figure 3.28, Table 3.13).

Deep sleep was on average the longest while on the RD, and the second longest was observed while on the KD. The shortest amount of deep sleep occurred when on the MD, and FFD, respectively. Another factor that was monitored by the Fitbit smartwatch was the number of times each sleep stage occurred during sleep, which here is referred to as the "stage (deep/light/REM/wake) count." The average deep count was highest while on the RD and MD. The frequency of the deep sleep stage when on the KD was lower which showed a negative correlation with the amount of sleep. This is because my longest sleep times occurred while on the KD. As indicated in Table 3.13, the least amount of deep sleep occurred while on the FFD.

The frequency of the number of light sleep stages and the average time spent in this stage were significantly different between the diets (Table 3.13). The light sleep stage (light count) was the most frequent during the RD at 23.5 ± 6.0 times, with an average of 211.6 ± 50.6 minutes/night of light sleep. The KD intervention was characterized by the longest light sleep stage. The MD had a mean of 215.9 ± 32.9 minutes/night of light sleep which happened 18.9 ± 5.03 times on average (Table 3.13). The least amount of light sleep and lowest frequency of the light sleep stage occurred while on the FFD (168.7 ± 53.4 minutes/night, 14.4 ± 4.01 times) (Figure 3.28, Table 3.13). The most amount of REM sleep occurred while on the KD (104.4 ± 19.14 minutes/night), and RD (101.1 ± 36.1 minutes/night). However, the MD and FFD showed lower levels of average REM sleep with 96.3 ± 23.8 minutes/night at 9.3 ± 3.6 times for the MD, and 95.7 ± 37.9 minutes/night at 11.1 ± 4.4 times for the FFD. The FFD was associated with the lowest amount of REM sleep (Figure 3.28, Table 3.13).

The sleeping HR (reported by Fitbit) was significantly different between the diet groups (p = 0.002), where a similar pattern to the daily resting HR was observed across all diets. The MD had the lowest HR during sleep (58.6 ± 4.7 bpm), and the KD had the highest HR during sleep at 64.5 ± 2.8 bpm. The RD and FFD had moderate sleeping HR of 60 ± 4.9 bpm, and 61.3 ± 2.8 bpm, respectively. The same pattern was also observed for the percentage of HR during sleep that was above the resting HR (Table 3.14). It is worth noting that the sleeping average HR reported by Fitbit was different from what was calculated from the raw data from Fitbit. This might be due to the processing algorithms Fitbit uses in the reported data. Additionally, Stucky et al. [236] have reported a general underestimation of 0.9 bpm for sleeping HR levels by Fitbit compared to sleep polysomnography. However, their overall results revealed that Fitbit device reports reasonably accurate sleep time and HR estimates.

The Fitbit company does not disclose its precise formula for measuring restlessness during sleep, but the algorithm incorporates sleeping HR, tossing, and turning, and snoring to calculate a quantitative value for restless sleep. A number is assigned to each sleep night with a higher number indicating more restlessness. My overall level of sleep restlessness while on the RD was the lowest, as minimal tossing and turning or snoring was recorded during the RD sleep periods (5.5 ± 2.4%). The sleep restlessness score peaked while on the KD where 7.0 ± 2.3% of the sleep was recorded as being restless (Table 3.14). In addition, my highest BT at night was observed while on the RD

(0.05 ± 0.3 °C above the baseline). However, the largest deviation from my baseline BT was observed while on the FFD (-0.2 ± 0.4 °C), and KD (-0.1 ± 0.5 °C) (Table 3.14).

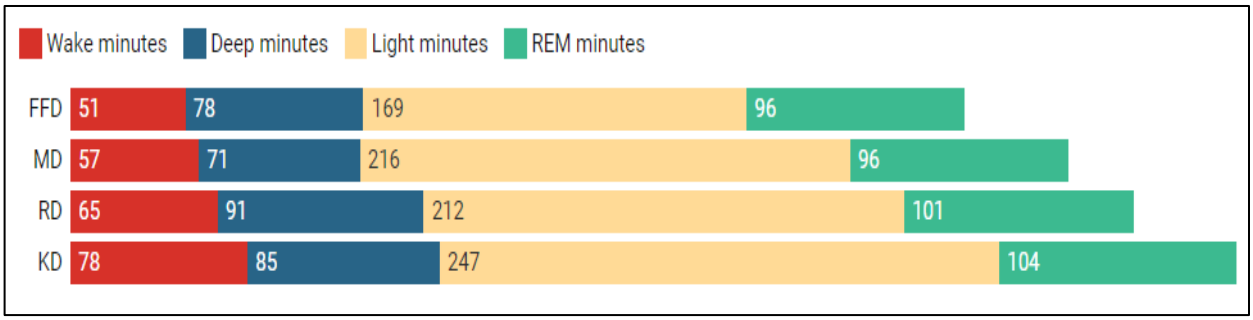

Figure 3.28 - A general overview of the average time spent in each sleep stage (minutes) across all diets.

Table 3.14 - A summary of the sleep stage variations (number of times occurred and the time spent in each stage) between each of the four diets.

	FFD	MD	KD	RD
Asleep minutes ^{ns}	342.8 ± 108.5	383.0 ± 72.7	435.38 ± 65.9	403.5 ± 102.8
Wake minutes *	50.7 ± 22.2	56.6 ± 15.7	78.0 ± 28.7	64.8 ± 17.7
Wake count *	19.8 ± 6.55	20.8 ± 5.8	26.1 ± 8.8	25.7 ± 6.4
Deep count ^{ns}	2.6 ± 1.0	3.2 ± 1.2	2.8 ± 1.3	3.8 ± 1.6
Deep minutes ^{ns}	78.36 ± 25.6	70.78 ± 30.2	84.5 ± 20.6	90.77 ± 32.0
Light count ****	14.4 ± 4.01	18.9 ± 5.03	23.2 ± 6.7	23.54 ± 6.0
Light minutes ***	168.7 ± 53.4	215.9 ± 32.95	246.5 ± 52.3	211.6 ± 50.6
REM count ^{ns}	11.1 ± 4.4	9.28 ± 3.6	10.4 ± 3.6	9.08 ± 4.4
REM minutes ^{ns}	95.75 ± 37.9	96.3 ± 23.8	104.38 ± 19.14	101.1 ± 36.1
p-value > 0.05 → ns, p-value ≤ 0.05 → *, p-value ≤ 0.01 → **, p-value ≤ 0.001 → ***, p-value ≤ 0.0001 → ****				

Table 3.15 - A summary of other sleep quality parameters measured using the Fitbit smartwatch compared across all four diets.

	FFD	MD	KD	RD
Sleeping HR (bpm) **	61.3 ± 2.8	58.6 ± 4.7	64.5 ± 2.8	60 ± 4.9
Percentage of HR above resting HR [ns]	11.3 ± 9.4	7.1 ± 7.3	17.8 ± 14.96	9.4 ± 8.1
Restlessness [ns]	6.8 ± 2.8	6.1 ± 1.4	7 ± 2.3	5.5 ± 2.4
Temperature at night (deviations from the baseline) [ns]	-0.2 ± 0.4	-0.05 ± 0.5	-0.1 ± 0.5	0.05 ± 0.3
p-value > 0.05 → ns, p-value ≤ 0.05 → *, p-value ≤ 0.01 → **, p-value ≤ 0.001 → ***, p-value ≤ 0.0001 → ****				

3.11. Physical activity:

Physical activity was monitored daily to determine any possible effect of each diet on my overall physical performance. During each of the diet periods, I performed the same physical activity for five days per week which consisted of jogging on a treadmill for 40-45 minutes at 5-7 km/h. My performance criteria were monitored using body temperature (BT) (°C) via the CORE body temperature monitor (worn on the upper left arm), blood glucose (BG) (mmol/L) using the Dexcom G6 blood glucose monitor, and HR (bpm) which was done using the Fitbit smartwatch (worn on my non-dominant hand). The differences between the diet groups in terms of BT, BG and HR during my scheduled physical activities matched well with the overall changes observed in daily BT, BG, and HR levels across the diets. My BT rose to its highest during exercise while I was on the MD with a temperature of 37.67 ± 0.35 °C. Exercise-induced temperature elevations were more modest with the FFD and RD (37.65 ± 0.29 °C, and 37.65 ± 0.32 °C respectively), and the lowest body temperature occurred while on the KD (37.63 ± 0.27 °C). However, no statistical significance was detected in the exercise temperature across all four dietary interventions. The highest average BG levels during physical exercise were observed while on the FFD (mean ± SD = 5.39 ± 0.62 mmol/L), and the lowest levels were observed with the KD (mean ± SD = 4.88 ± 0.54 mmol/L). The MD and RD showed similar BG levels of 4.95 ± 0.61 mmol/L and 4.96 ± 0.41

mmol/L, respectively. Overall, as expected, a drop in BG levels was observed during exercises for all diet interventions. However, the level of this drop varied between the diet groups. The MD and RD had the largest drop in BG levels during exercise with -0.97 mmol/L ± 1.50, and -0.85 mmol/L ± 0.35, respectively. On the other hand, a moderate drop in BG levels was observed while on the KD (-0.67 mmol/L ± 0.80). Most notably, the FFD had the lowest BG drop during exercise (-0.41 mmol/L ± 1.30). However, because the BG variations were so large, no statistically significant differences were detected between the four dietary interventions.

I had the highest HR (bpm) during my exercise routines while on the MD (137.41 ± 16.02 bpm – resting HR = 65.00 ± 5.80 bpm), and the lowest exercise HR was on the FFD (134.28 ± 16.94 bpm – resting HR = 65.50 ± 2.74 bpm). I had an average exercise HR of 136.16 ± 13.84 bpm while on the RD (resting HR = 64.85 ± 1.99 bpm) which was more similar to the MD, and the KD had an average exercise HR of 135.48 ± 21.11 bpm (resting HR = 68.93 ± 2.37 bpm). The HR returned to normal within 10-60 minutes after finishing the exercise (which will be discussed further in the next chapter). However, because the HR variations were so large, no statistically significant differences were observed between the diets in terms of the average time spent in cardio or fat burn (minutes) or the average calories burned during the exercises (Table 3.15).

Table 3.16 - A summary of the average blood glucose (mmol/L), body temperature (°C), and heart rate (bpm), measured during physical activity.

	FFD	MD	KD	RD
Blood glucose (mmol/L)	5.39 ± 0.62	4.95 ± 0.61	4.88 ± 0.54	4.96 ± 0.41
Body temperature (°C)	37.65 ± 0.29	37.67 ± 0.35	37.63 ± 0.27	37.65 ± 0.32
Heart rate (bpm)	134.3 ± 16.9	137.4 ± 16.0	135.5 ± 21.1	136.2 ± 13.8
Minutes in cardio HR	16.1 ± 4.5	15.3 ± 2.8	15.9 ± 3.2	15.2 ± 3.1
Minutes in fat burn HR	22.1 ± 5.9	20.1 ± 3.3	19.8 ± 3.8	21.4 ± 2.5
Average calories burnt (kcals)	307.5 ± 35.6	304.9 ± 33.6	293.9 ± 36.7	301.8 ± 35.9

3.12. Plasma metabolites:

After collecting plasma samples while on each of the diet interventions, the TMIC MEGA assay was run to detect and quantify the plasma metabolites in all 46 collected plasma samples. An average of 544 metabolites (and 228 sums and ratios) were measured in each sample. The results were analyzed using MetaboAnalyst 5.0, with log transformation and mean centering applied for data scaling and normalization. Overall, the plasma metabolome analysis revealed several noteworthy changes in my metabolite levels across all diets and over the time course of the dietary interventions.

The one-way ANOVA and Fisher's least significant difference (LSD) *post-hoc* analysis using a p-value (false discovery rate or FDR) cutoff of 0.05, identified 210 plasma metabolites that were significantly different between at least one pair of the diet groups. Figure 3.29 shows the differences in levels of the top 20 most significantly different metabolites or calculated sums and ratios between the dietary interventions. The full ANOVA table of the most significantly different plasma metabolites or sums/ratios is presented in Appendix Table 3-F. The most significantly different metabolites (or the sum/ratio calculated using the metabolite concentrations) between the diet groups were 2-hydroxyisovaleric acid, acetoacetic acid, the ratio of carnitine to total acylcarnitines, the ratio (also known as the Fischer ratio) of isoleucine, leucine and valine (BCAAs or branched chain amino acids) to phenylalanine, tryptophan, and tyrosine (AAAs or aromatic amino acids), and the concentration of 3-hydroxyisobutyric acid. Principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) analyses were performed on the plasma samples, which revealed a clear separation between the diet groups in terms of their plasma metabolomic composition (Figure 3.30).

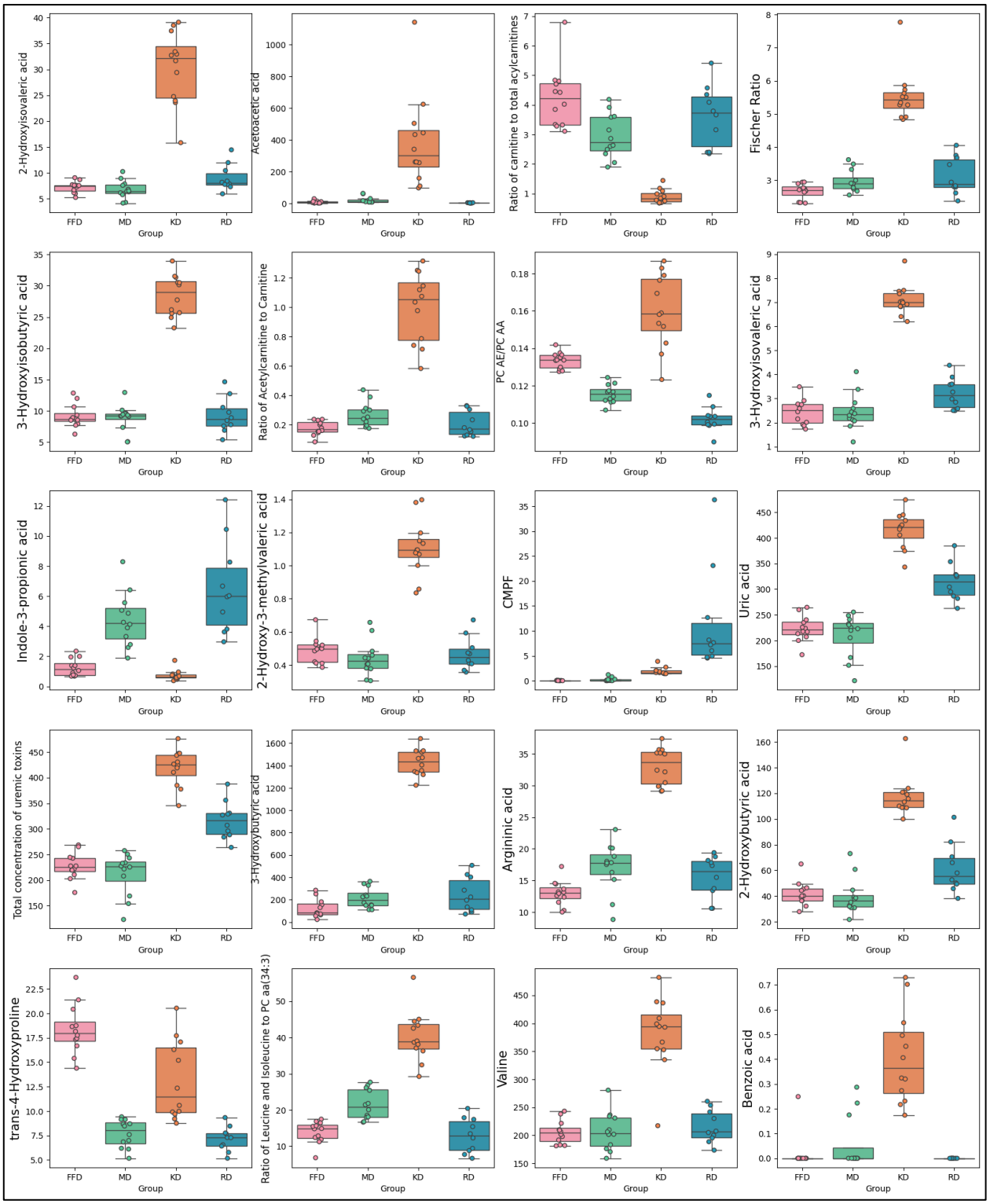

Figure 3.29 - General variations in mean and SD (in µmol/L) of the top 20 significantly different plasma metabolites across all four diet groups.

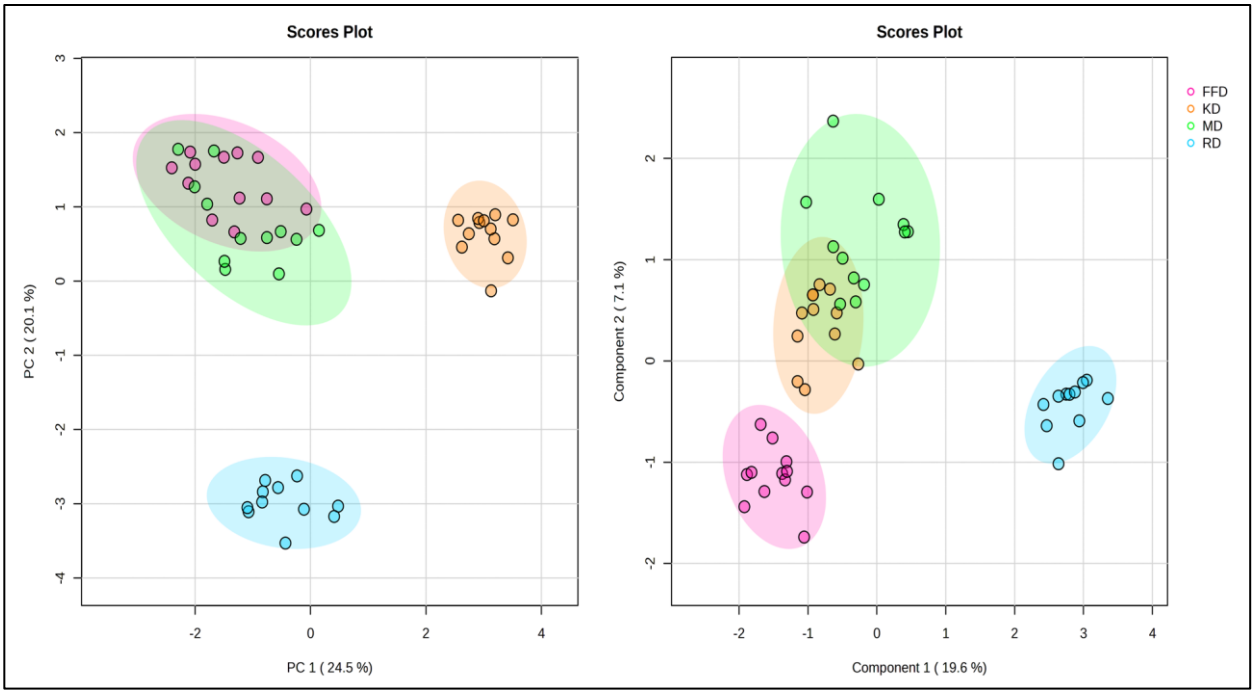

Figure 3.30 - The PCA (left) and PLS-DA (right) plots of the plasma samples from all four diets.

The plasma metabolomics data were further analyzed by comparing each of the diets in pairs using the same statistical method on MetaboAnalyst 5.0. PCA score plots of the paired comparisons are provided in Figure 3.31. In addition, volcano plots were used to display the distribution of metabolite changes between the FFD, MD, KD, and RD (Figures 3.32 and 3.33). Metabolites located on the far right and left of the x-axis indicated significant upregulation or downregulation, respectively, based on a Fold Change (FC) threshold of 2 and a *p*-value threshold of 0.05. Several significant metabolites had a log-fold change that exceeded the threshold.

The FFD had a higher abundance of trans-4-hydroxyproline, and trimethylamine N-oxide compared to the MD, and a higher ratio of carnitine to total ACs compared to the KD. The MD had an increased abundance of N2-acetyl-ornithine, indole-3-propionic, and hippuric acid in comparison to the FFD, a higher ratio of carnitine to total ACs compared to the KD, and a lower ratio of betaine to choline compared to the RD.

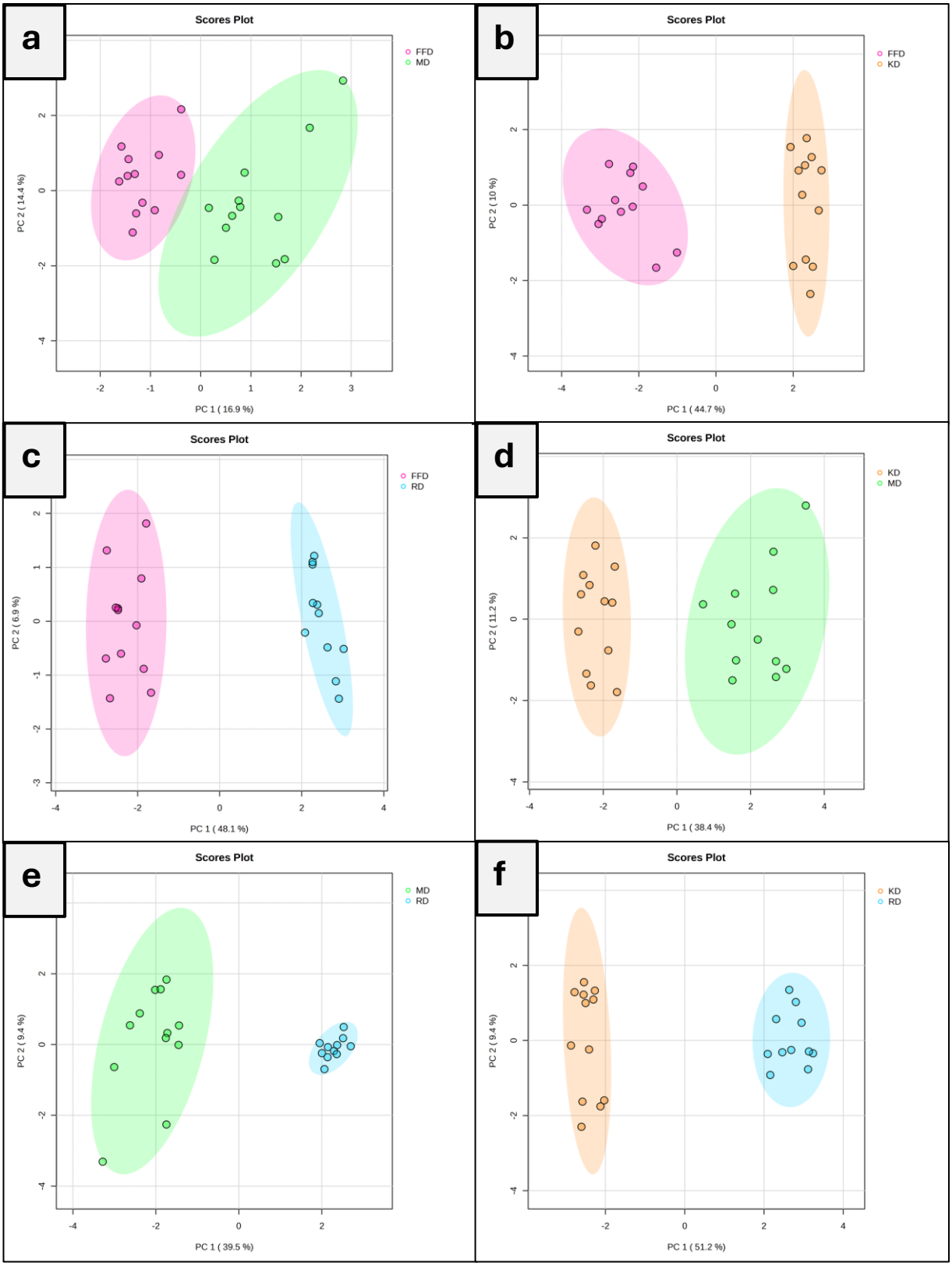

Figure 3.31 - PCA score plots of FFD and MD (a), FFD and KD (b), FFD and RD (c), KD and MD (d), MD and RD (e), and KD and RD (f)

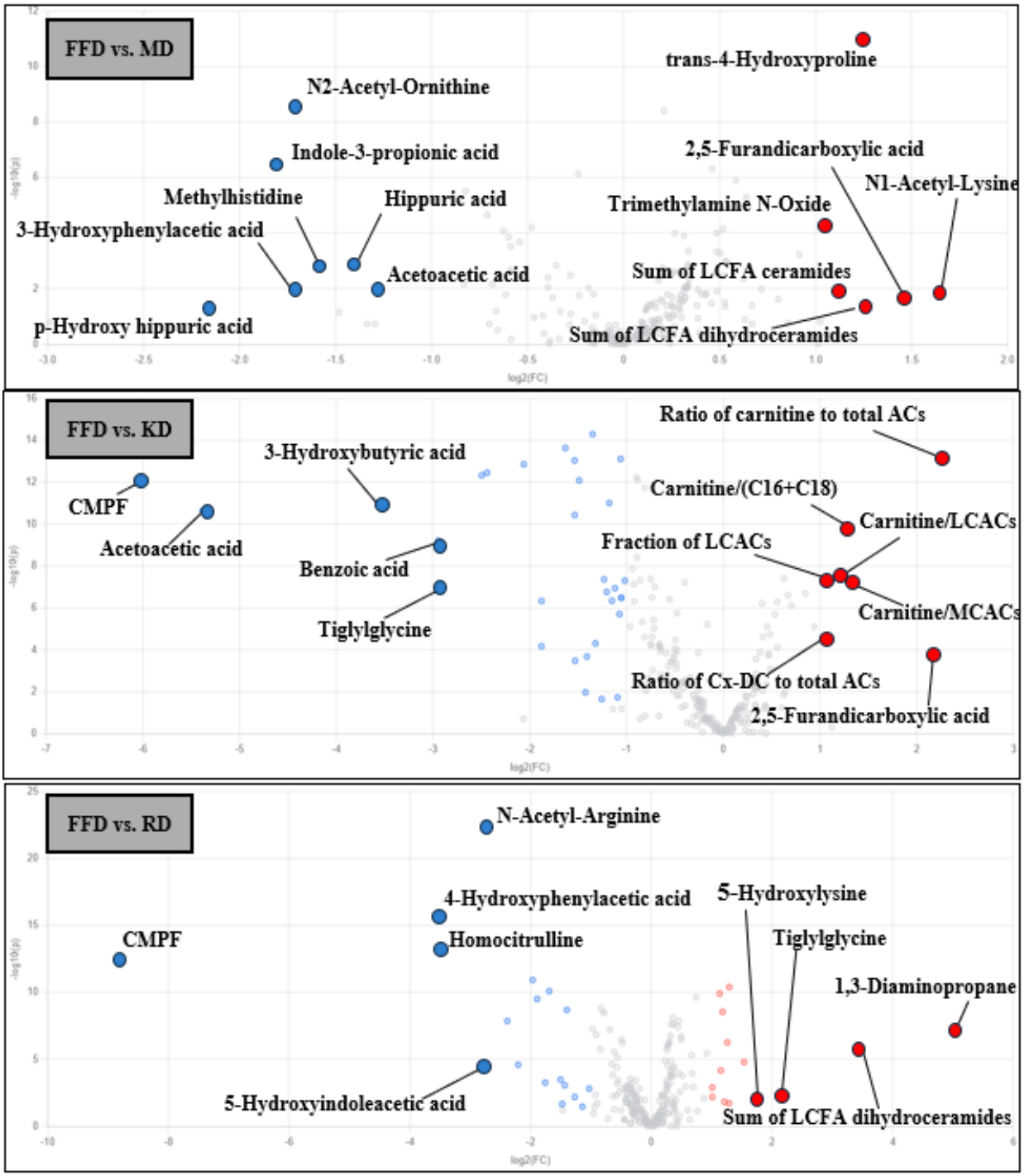

Figure 3.32 - Volcano plot analysis performed on the FFD and MD, FFD and KD, and FFD and RD plasma samples with a *p*-value threshold of 0.05, and a FC threshold of 2. Upregulated (red) or downregulated (blue) significantly different metabolites are marked in the figure. Abbreviations: ACs - Acylcarnitines; CMPF - 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; Cx-DC - dicarboxylic acylcarnitines; LCAC - long-chain acylcarnitines; LCFA – long-chain fatty acids; MCFA – medium-chain fatty acids

A significant increase in the levels of 3-hydroxyisovaleric acid and an elevated Fischer Ratio (ratio of isoleucine, leucine and valine to phenylalanine, tryptophan, and tyrosine) were observed with the KD in comparison to the FFD. The Fischer Ratio is important for assessing liver metabolism, hepatic functional reserve, and the severity of liver dysfunction, with high values indicating liver dysfunction. Moreover, the KD exhibited higher levels of 3-hydroxybutyric acid, 2-hydroxyisovaleric acid, 3-hydroxyisobutyric acid, and 3-hydroxyisovaleric acid (ketone bodies) in comparison to the MD. Additionally, tiglylglycine, acetoacetic acid, and the total concentration of the long-chain fatty acid dihydroceramides were significantly higher on the KD in contrast to the RD.

Similar to the other diets, the RD showed significant differences when compared to the other three diets via pairwise comparisons. While on the RD intervention, N-acetyl-arginine, 4-hydroxyphenylacetic acid, homocitrulline, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF), and the ratio of homoarginine to arginine and lysine were found to be significantly higher than the FFD. Similarly, N-acetyl-arginine and 4-hydroxyphenylacetic acid were elevated while on the RD in comparison to the KD. Additionally, when compared to the MD, N-acetyl-arginine, 4-hydroxyphenylacetic acid, homocitrulline, and N1-acetyl-lysine were significantly higher while on the RD.

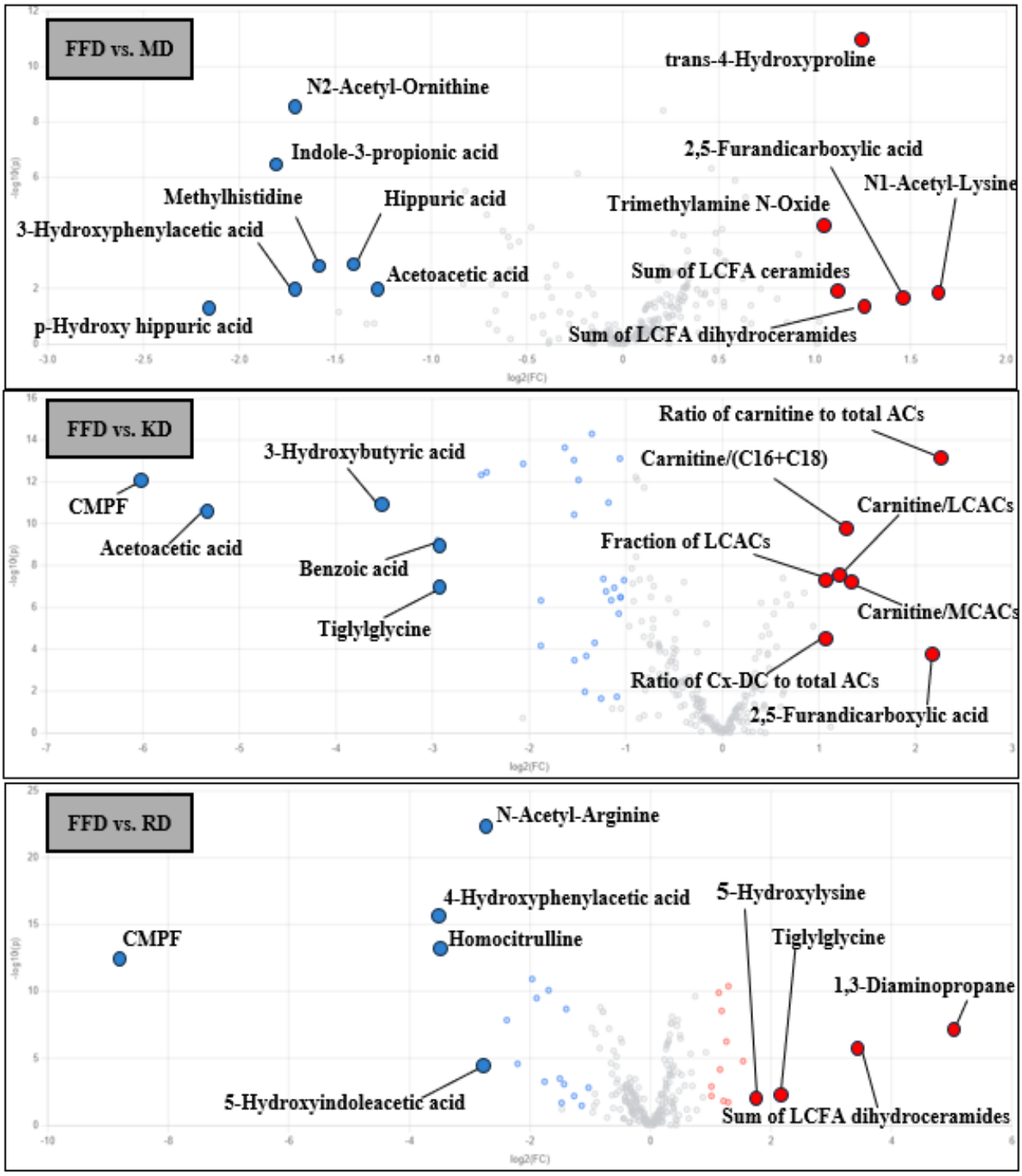

Figure 3.33 - Volcano plot analysis performed on the KD and MD, MD and RD, and KD and RD plasma samples with a p-value threshold of 0.05, and a fold change (FC) threshold of 2. Upregulated (red) or downregulated (blue) significantly different metabolites are marked on the figure. Abbreviations: ACs - Acylcarnitines; CMPF - 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; LCAC - long-chain acylcarnitines; LCFA – long-chain fatty acids; MCFA – medium-chain fatty acids

3.13. Urine metabolites:

A total of 107 urine samples, which were collected twice daily during each of the dietary interventions, were analyzed using the same TMIC MEGA assay methodology and the same web tool, MetaboAnalyst 5.0, as was done for the plasma samples. On average,192 metabolites (and 176 sums and ratios) were detected for each urine sample. The dataset was normalized by creatinine, and log transformation and auto-scaling were used as data transformation and data scaling methods, respectively. Utilizing a one-way ANOVA with a *p*-value cutoff of 0.05, I identified 161 urinary metabolites or calculated sums/ratios displaying statistically significant differences among at least one pair of the four diet groups. The full ANOVA table of the significantly different urinary metabolites or sums/ratios is presented in Appendix Table 3-G. Notably, acetoacetic, guanidinopropionic acid, 3-hydroxybutyric acid, 3-hydroxyisobutyric acid, and 3-methoxytyramine were highlighted as the most significant metabolites between the four diet groups (Figure 3.34). PCA and PLS-DA analysis were performed on the urine metabolomics datasets to have a better understanding of the similarities or dissimilarities between the diets (Figure 3.35). It is worth noting that all urinary metabolites were normalized to creatinine before further analysis.

The metabolomics data obtained from the urine samples were also analyzed by comparing the diet groups in pairs. T-test, volcano plot and PCA analyses were performed on each paired diet group to better indicate the similarities or dissimilarities between the urinary metabolites. The dataset was normalized by creatinine, and log transformation and auto-scaling were used as data transformation and data scaling methods respectively using MetaboAnalyst 5.0. The PCA and volcano plots analyses are provided in Figures 3.36 - 3.38, and Figure 3.39 represents a hierarchical clustering heatmap of the urine metabolites. As seen with these tables and figures, the FFD had a higher abundance of trans-4-hydroxyproline, the total concentration of hydroxyproline, N-acetyl-histidine, and ethanolamine compared to the MD. The FFD also had a higher abundance of phenylalanine, and compared to the KD, the FFD had a higher abundance of trans-4-hydroxyproline, the total concentration of hydroxyproline, N-acetyl-histidine, and the concentration of hydroxylated carnitines compared to the RD.

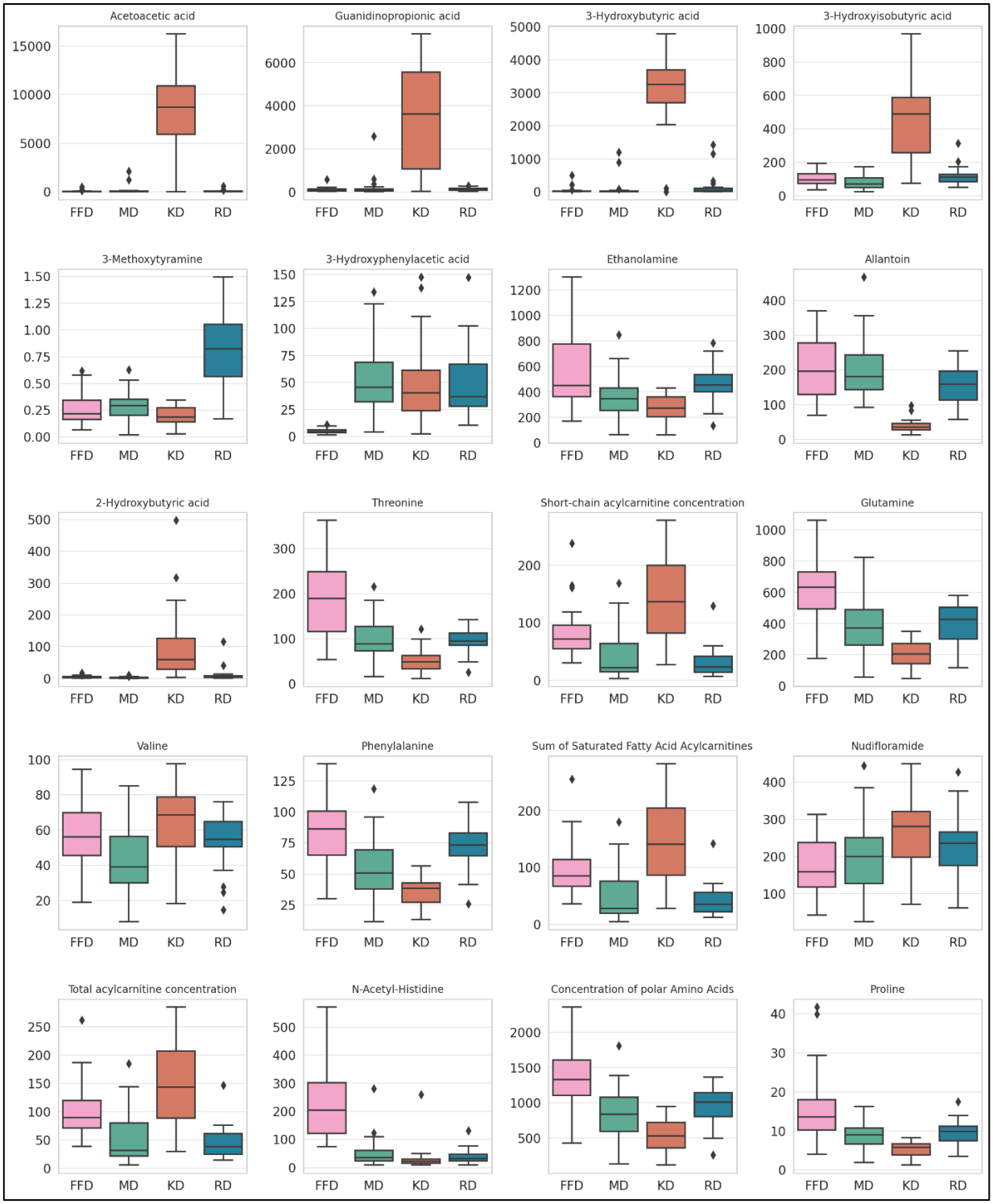

Figure 3.34 - General variations in mean and SD (in µmol/mmol creatinine) of the top 20 significantly different urinary metabolites across all four diet groups.

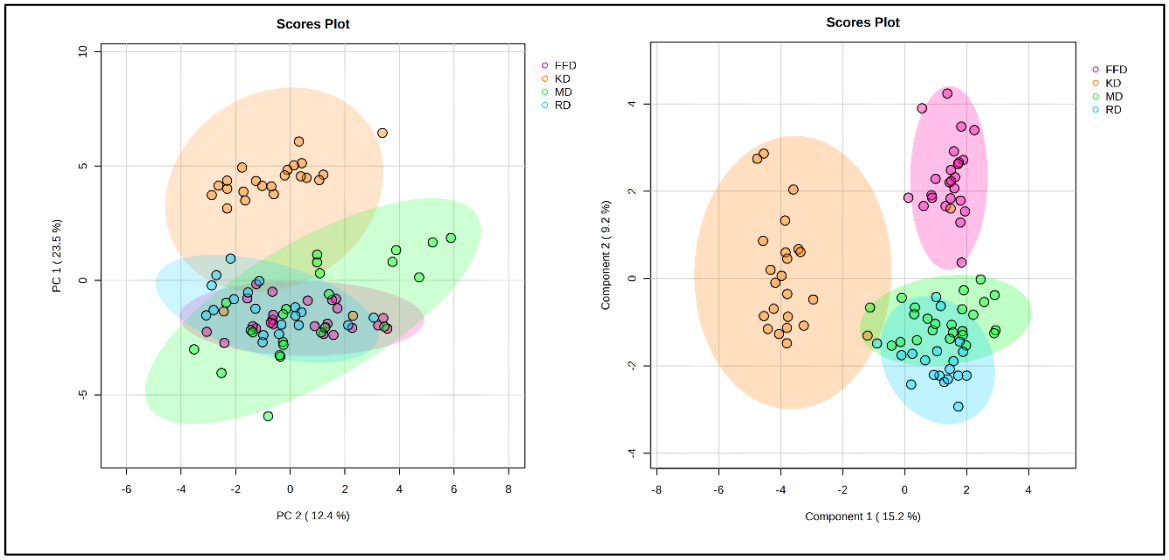

Figure 3.35 - PCA (left) and PLS-DA (right) scores plot of all the diets - urine samples.

Significant differences were observed between the metabolomic composition of my urine samples concerning each dietary intervention. For instance, 3-hydroxyphenylacetic acid, and nudifloramide were significantly higher with the MD in comparison to the FFD. In addition, the MD had significantly higher levels of threonine, citric acid, allantoin, and asparagine compared to the KD, and the MD had a higher abundance of ornithine, and cytidine, and a higher level of the ratio of kynurenine to tryptophan compared to the RD. Compared to the FFD, the KD had a higher abundance of acetoacetic acid, guanidinopropionic acid, and 3-hydroxybutyric acid. The concentrations of acetoacetic acid, creatine, and guanidinopropionic acid were also significantly higher with the KD compared to the RD. The abundance of 3-methoxytyramine was significantly higher with the RD compared to the other diets. Additionally, the RD had higher levels of 3-hydroxyphenylacetic acid when compared to the FFD.

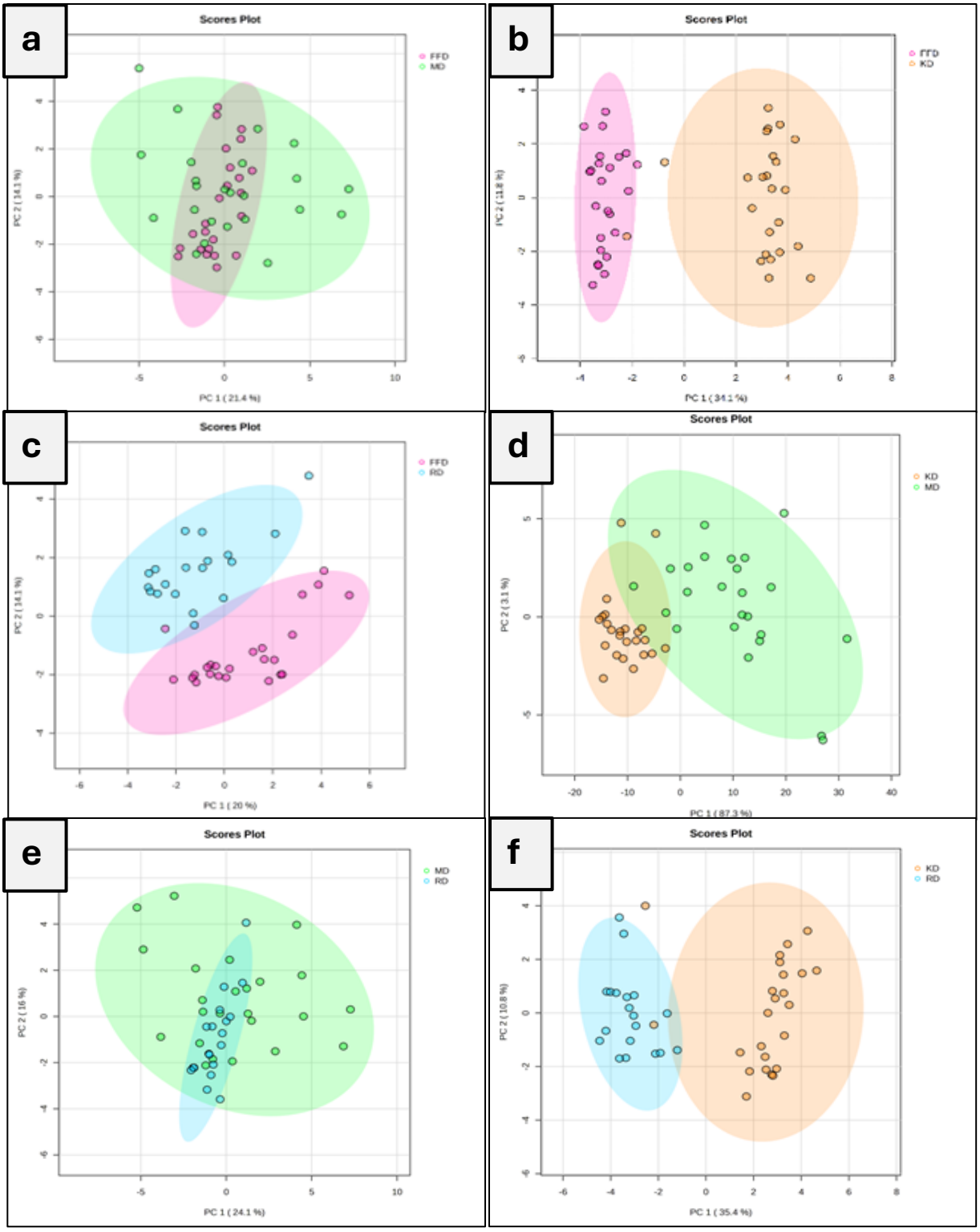

Figure 3.36 - PCA plots of the urine metabolomics datasets. a) FFD and MD, b) FFD and KD, c) FFD and RD, d) KD and MD, e) MD and RD, and f) KD and RD

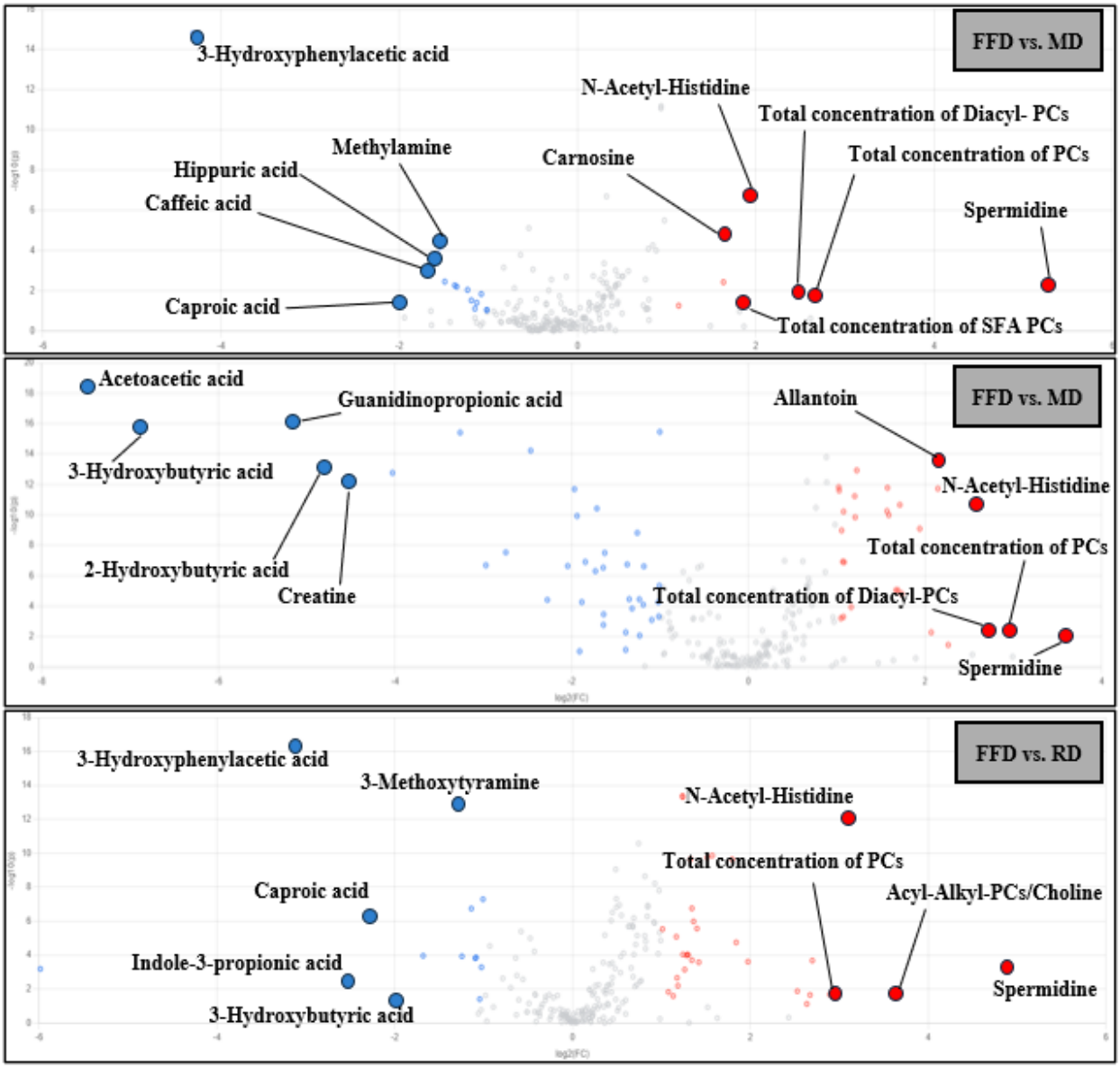

Figure 3.37 - Volcano plots of the urinary metabolites comparing the FFD and the other three diets. Significantly different upregulated (red) or downregulated (blue) metabolites are marked on the figure. Abbreviations: PCs – phosphatidylcholines; SFA – saturated fatty acids

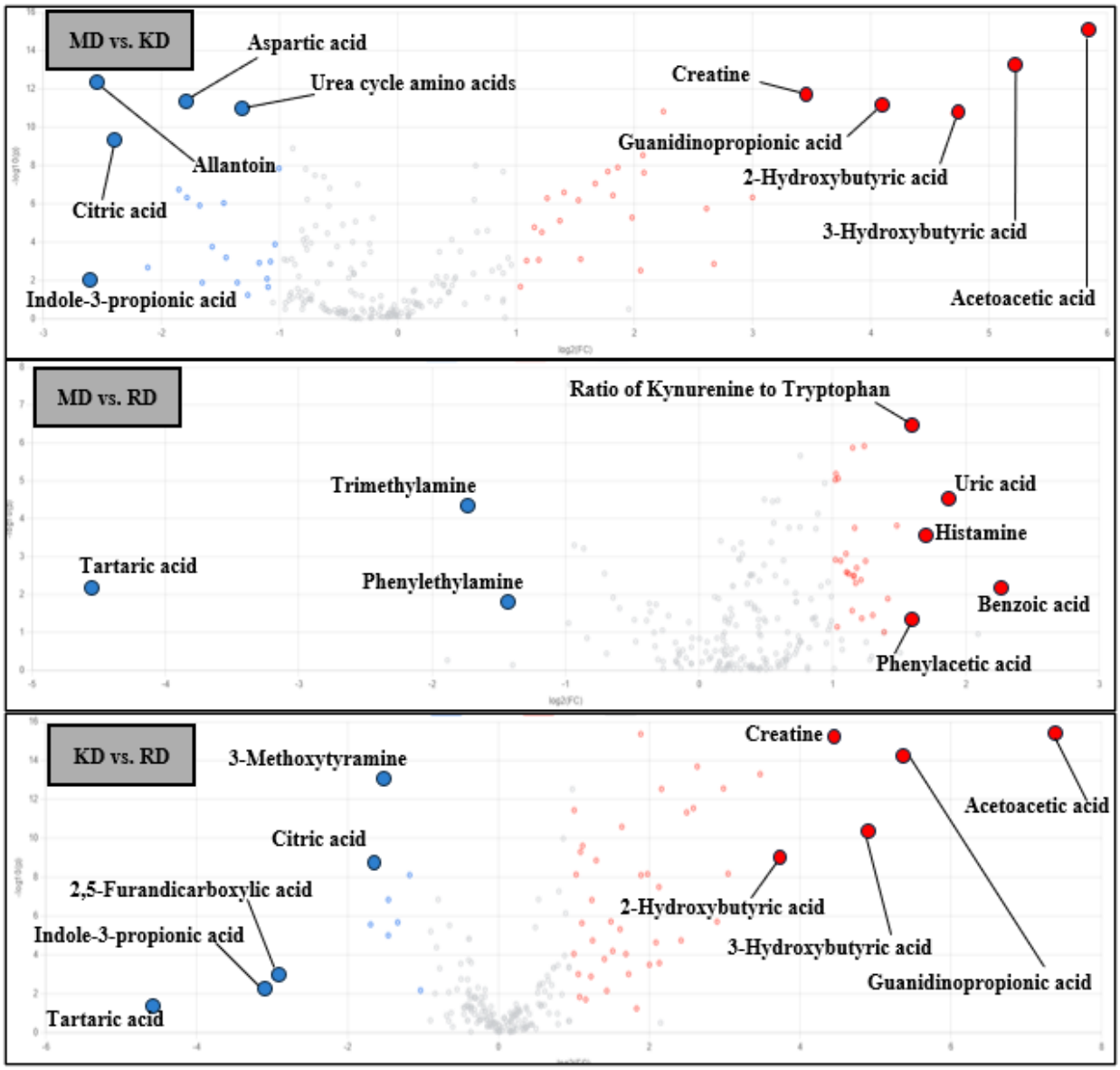

Figure 3.38 - Volcano plots of the urinary metabolites of MD, KD, and RD. Significantly different upregulated (red) or downregulated (blue) metabolites are marked on the figure.

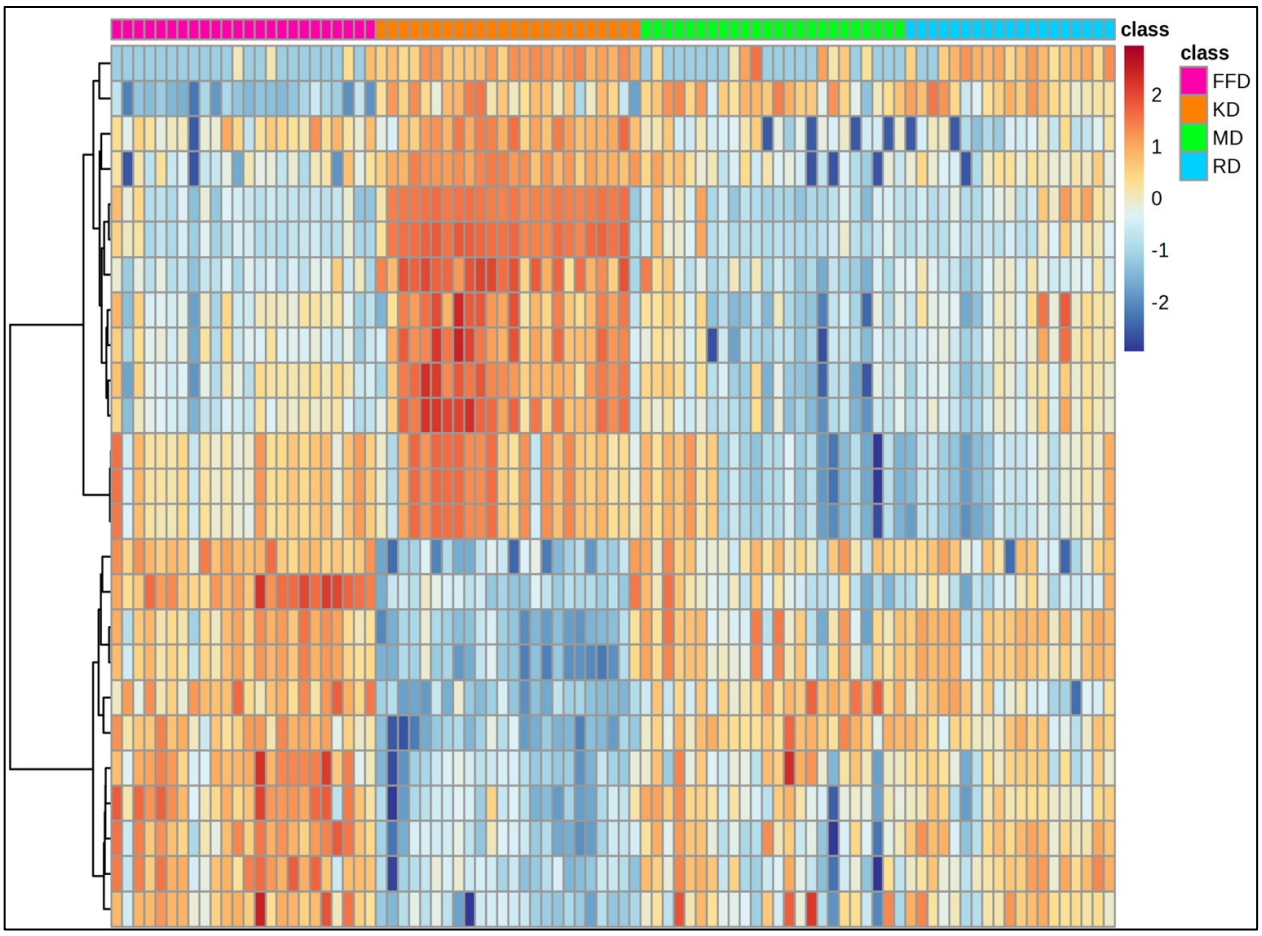

Figure 3.39 - The hierarchical clustering heatmap of the creatinine-normalized concentrations of urine metabolites. The figure indicates that although dissimilarities are observed across all diet groups, the KD is the most significantly different compared to the other diets. FFD, MD and RD show more similarities in terms of their urinary metabolite compositions.

3.14. Plasma immunoassay-based protein quantification:

The plasma samples were analyzed for quantification of cytokines using the MSD V-PLEX Proinflammatory Panel 1 Human Kit. The data was further analyzed using programs written in the Python programming language, and MetaboAnalyst 5.0. After normalization, IL-10, IL-12p70, and IL-2 were identified as the most significantly differentially expressed inflammatory markers in at least one pair of the diet groups (Figure 3.40). Of all groups, the KD had the highest concentration of IL-10 (0.48 ± 0.2 pg/mL), while the RD, MD, and FFD had lower levels of IL-10 (0.35 ± 0.03 pg/mL, 0.31 ± 0.04 pg/mL, and 0.25 ± 0.03 pg/mL, respectively; p = 0.011208 comparing the FFD and KD). The longitudinal change in IL-10 levels while on the KD intervention was striking and this was the only protein that was seen to increase over the course of any diet intervention (Figure 3.41).

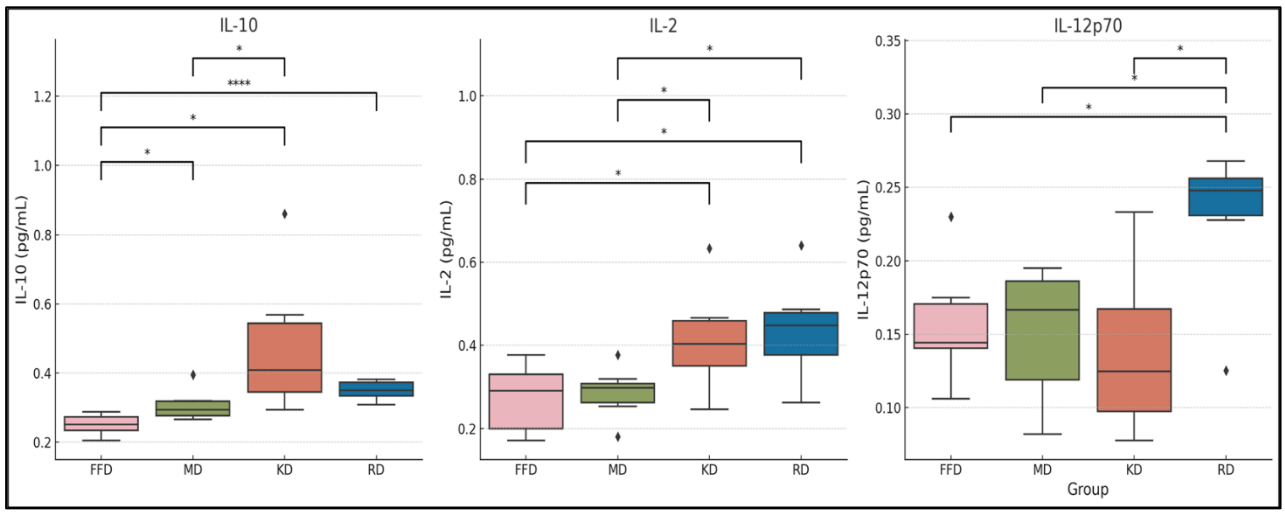

Figure 3.40 - A general summary of the variations in IL-10, IL-2, and IL-12p70 concentrations of all the diet groups. The plot is changed (p values added)

The results show that the RD had the highest average levels of IL-12p70 (0.23 ± 0.05 pg/mL), while the KD exhibited the lowest levels (0.14 ± 0.07 pg/mL). IL-12p70 remained consistently low and stable throughout the FFD and MD interventions, with average levels of 0.16 ± 0.04 pg/mL and 0.15 ± 0.04 pg/mL, respectively (p = 0.0039149 comparing the KD and RD). The highest levels of IL-2 were observed while on the RD and KD, with average values of 0.44 ±

0.13 pg/mL and 0.41 ± 0.12 pg/mL, respectively. In contrast, the FFD and MD had significantly lower levels of plasma IL-2 (0.21 ± 0.08 pg/mL and 0.28 ± 0.06 pg/mL, respectively; $p = 0.014526$ comparing the FFD and RD). No significant differences were found in the abundance of the other inflammatory cytokines detected in the plasma samples in any other diets. Table 3.16 provides a summary of the mean abundances of the 10 cytokines during different diets.

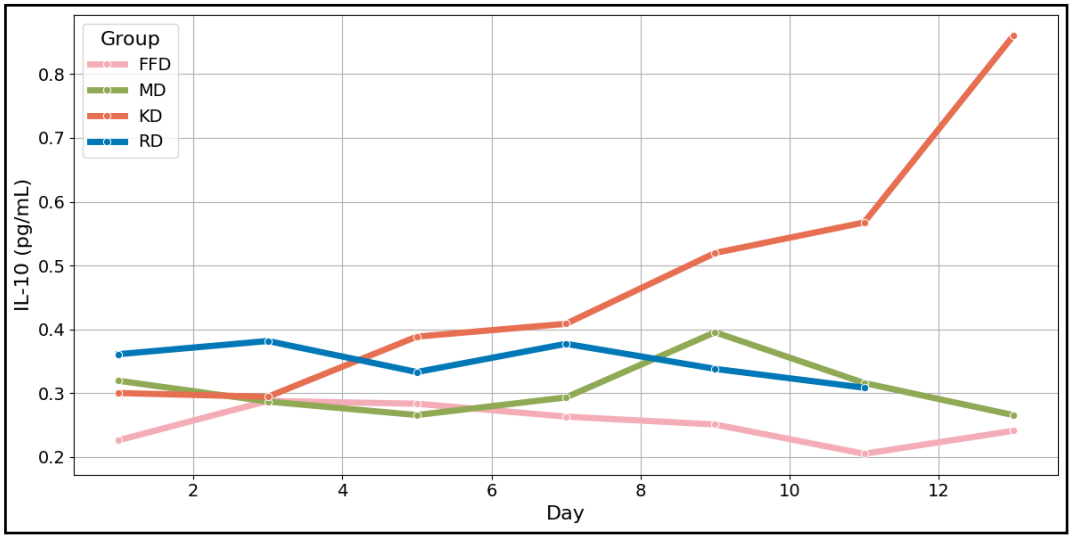

Figure 3.41 - An increase in the IL-10 levels in plasma samples was observed from the beginning to the end of the KD period. The other three diets maintained a relatively steady and lower level of IL-10.

Table 3.17 - A summary of the cytokines detected and quantified in plasma samples in pg/mL.

	FFD	MD	KD	RD
IFN-γ ^{ns}	4.25 ± 0.82	5.41 ± 1.72	7.85 ± 1.64	5.83 ± 0.84
IL-10 ***	0.25 ± 0.03	0.31 ± 0.04	0.48 ± 0.2	0.35 ± 0.03
IL-12p70 **	0.16 ± 0.04	0.15 ± 0.04	0.14 ± 0.07	0.23 ± 0.05
IL-13 ^{ns}	2.22 ± 0.57	1.83 ± 0.41	1.37 ± 0.68	2.24 ± 0.7
IL-1β ^{ns}	0.18 ± 0.16	0.2 ± 0.22	0.05 ± 0.03	0.59 ± 0.66
IL-2 **	0.27 ± 0.08	0.29 ± 0.06	0.41 ± 0.12	0.44 ± 0.13
IL-4 ^{ns}	0.07 ± 0.03	0.05 ± 0.02	0.04 ± 0.04	0.06 ± 0.03
IL-6 ^{ns}	1.25 ± 0.5	1.81 ± 1.01	1.22 ± 0.14	1.1 ± 0.25
IL-8 ^{ns}	17.58 ± 14.46	12.28 ± 2.47	10.53 ± 2.17	12.22 ± 2.01
TNF-α ^{ns}	1.78 ± 0.11	2.02 ± 0.27	2.05 ± 0.21	2.23 ± 0.38
p-value > 0.05 → ns, p-value ≤ 0.05 → *, p-value ≤ 0.01 → **, p-value ≤ 0.001 → ***, p-value ≤ 0.0001 → ****				

3.15. Gut microbiome:

A fecal sample was collected after completing each 14-day dietary intervention and the sample was sent to EASY DNA Laboratory Inc. as per their instructions. Within 2-3 weeks of shipment, the microbiome results were received. The results included data regarding the overall bacterial species population, with information on the most abundant phyla and genera present in my microbiome following the conclusion of each diet intervention. Overall, the diversity of my gut microbiome, and the abundance of fibre-degrading and vitamin-producing bacteria remained within the optimal range following all four diets. The overall condition of my gut microbiome was similar after the RD, FFD, and MD interventions, while the KD yielded somewhat different results. According to EASY DNA's microbiome assessment, my microbiome demonstrated a slight preference for obesity and inflammatory conditions after the FFD, MD, and RD, whereas no such preference was observed following the KD. Conversely, the abundance of probiotic bacteria was slightly lower following the KD, but within the optimal range after the FFD, MD, and RD. Significantly, an increased abundance of pathogenic bacteria was observed after the KD. However, this was not observed following the other three diets. A summary of specific bacterial abundances can be seen in Table 3.17. Figure 3.42 represents the most abundant phyla and top 10 most abundant genera of the gut microbiome after each dietary intervention.

Table 3.18 - A summary of the percentages of specific bacteria abundances detected in the gut microbiome after each diet.

	FFD	MD	KD	RD
Akkermansia muciniphila (%)	0	0.004	0.02	0.01
Oxalobacter formigenes (%)	0	0	0	0
Butyric acid-producing bacteria (%)	14.48	18.26	17.90	14.20
Fiber-degrading bacteria (%)	52.83	66.6	52.86	57.26

To highlight some of the key differences in gut microbiome between the dietary interventions, the most abundant genera in my gut microbiome were *Prevotella,* except after the KD where it was the second most abundant and *Bacteroides* replaced it as the most abundant genera detected (Figure 3.42B, Table 3.18). *Faecalibacterium* was detected in all diets. However, it was increased in abundance after the KD. *Holdemanella,* abundant in the gut microbiome after the FFD and KD, were higher compared to the gut microbiome after the MD and RD (Figure 3.42B, Table 3.18). The same decreasing pattern with *Prevotella* was observed with the genus *Bifidobacterium*, which was lower after the RD, the FFD, and the MD, but drastically dropped after the KD (Figure 3.42B, Table 3.18).

Ruminococcus was at its highest levels after the KD and the RD interventions yet formed just 1-2% of the microbiome after the FFD and MD (Table 3.18). *Blautia* was most abundant after the MD, in comparison to the other diets. The abundance of the genus *Lactobacillus* after the RD was significantly lowered after the FFD. *Lactobacillus* was also quite low after the MD and constituted only 0.05% of the microbiome after the KD (Table 3.18). Low levels of *Enterobacter*, *Haemophilus*, *Clostridium IV* and *Odoribacter* were detected in my microbiome after the KD, while these genera were always absent in the microbiome after the other three diets (Table 3.18). The FFD caused an increase in the abundance of *Insolitispirillum,* and *Barnesiella* while these genera were not detected after the other diet interventions. The microbiome after the MD intervention consisted of 0.31% *Oscillibacter* while it was not detected after the other dietary periods. A full overview of the species or genera abundances in the gut microbiome is provided in Table 3.18.

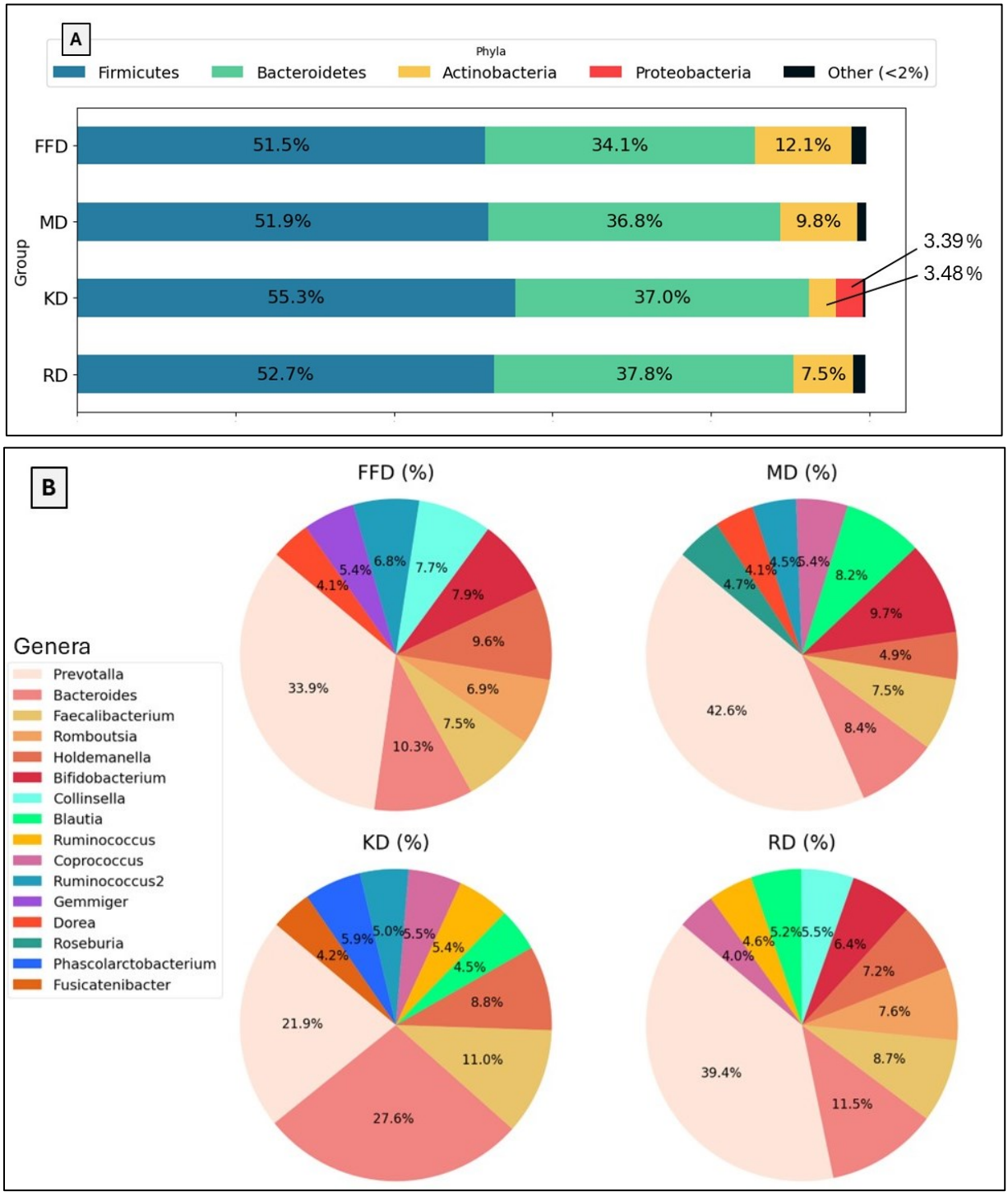

Figure 3.42 - Gut microbiome test results - **A**: The most abundant phyla in the microbiome after finishing each diet. **B**: Top 10 most abundant genera of the gut microbiome after finishing each dietary intervention.

Table 3.19 - A summary of the abundance (%) of the top 20 different microbiome genera after each diet

Phylum	Genera	FFD (%)	MD (%)	KD (%)	RD (%)
Bacteroidetes	*Prevotella*	24.5	29.77	15.28	25.88
	Bacteroides	7.42	5.87	19.26	7.58
	Parabacteroides	0.4	-	0.53	0.62
	Alistipes	0.62	0.27	0.84	0.90
	Barnesiella	0.43	-	-	-
	Odoribacter	-	-	0.66	-
Firmicutes	*Faecalibacterium*	5.42	5.27	7.7	5.69
	Holdemanella	6.92	3.42	6.12	4.74
	Ruminococcus	1.08	1.81	3.79	3.05
	Blautia	2.63	5.73	3.12	3.43
	Romboutsia	4.97	2.14	1.05	5.00
	Coprococcus	1.33	3.75	3.85	2.61
	Phascolarctobacterium	1.7	2.12	4.13	2.56
	Ruminococcus2	4.93	3.16	3.51	2.56
	Dorea	2.96	2.85	1.94	2.00
	Gemmiger	3.89	2.35	1.77	2.35
	Lachnospiracea incertae sedis	1.17	1.2	1.42	1.84
	Fusicatenibacter	0.88	1.79	2.96	1.90
	Clostridium XVIII	1.75	1.63	1.81	1.20
	Roseburia	1.62	3.27	0.91	1.24
	Clostridium sensu stricto	2.75	0.45	-	2.02
	Eubacterium	0.81	1.54	1.11	0.89
	Intestinibacter	1.33	0.84	0.53	0.77
	Clostridium XIVa	0.57	0.86	1.21	0.74
	Anaerostipes	-	0.6	-	0.72
	Murimonas	-	0.37	0.88	0.59
	Turicibacter	-	1.24	-	0.58
	Senegalimassilia	0.53	0.43	0.68	0.49
	Eisenbergiella	0.55	0.76	0.97	0.59

	Lactobacillus	-	-	-	0.95
	Streptococcus	0.43	-	-	0.74
	Intestinimonas	-	-	-	0.54
	Insolitispirillum	0.51	-	-	-
	clostridium IV	-	-	0.71	-
	Oscillibacter	-	0.31	-	-
Proteobacteria	*Enterobacter*	-	-	1.03	-
	Haemophilus	-	-	0.93	-
	Parasutterella	0.72	0.45	1.04	0.71
Actinobacteria	*Bifidobacterium*	5.74	6.75	-	4.19
	Collinsella	5.54	2.25	2.28	3.59
Other	*Other*	3.96	3.91	5.5	4.57

Cells left empty (-), indicate that the bacterium did not rank among the top 30 in abundance for that group.

3.16. Genomics:

After shipping off a saliva sample to be analyzed by 23andMe, I received the results in just a few weeks. The health predisposition report showed that I had one variant detected in the ARMS2 gene for Age-Related Macular Degeneration (AMD), but I am not likely at increased risk of developing AMD based on my genetic results. It was also highlighted that I have an average likelihood of developing T2D. No other disease-associated variations were identified for genetic conditions such as Alpha-1 Antitrypsin Deficiency, Late-Onset Alzheimer's Disease, Parkinson's Disease, and others. Additionally, no variants were detected concerning carrier-tested diseases, including Beta Thalassemia and Related Hemoglobinopathies, Bloom Syndrome, Cystic Fibrosis, Hereditary Fructose Intolerance, Maple Syrup Urine Disease Type 1B, Phenylketonuria and other related genetic disorders.

The “wellness and traits” report from 23andMe provided additional information about possible associations between my genome and dietary preferences, my tolerance of some food, and my sleep habits. For instance, it highlighted a low probability of experiencing facial flushing with alcohol consumption (G/G genotype on ALDH2 gene - rs671), a likelihood of lactose intolerance (G/G genotype - rs4988235, MCM6 gene), and a lower likelihood of being a deep sleeper (C/C genotype - rs73598374, ADA gene). The predicted lactose intolerance was confirmed by my own experience with consuming dairy products. It is also likely for me to have a similar weight on diets high or low in saturated fat as long as they are isocaloric (A/A genotype - rs5082, APOA2 gene). Moreover, it indicated I have a predisposition to other traits such as being able to detect asparagus odor in urine and perceiving cilantro as having a soapy taste. Both traits are ones that I can confirm. I am also predicted to be less likely than average to be afraid of heights, and more likely to experience motion sickness, which I can also confirm.

3.17. Menstrual cycle:

Due to my health complications, my menstrual cycle could not be monitored in a standardized way. However, I conducted a comparison of my luteal and follicular phases based on physiological measurements. Over the course of each diet (2 menstrual periods) changes in BG, BT, HR, BP, weight, and BMI were detectable during the luteal phase and premenstrual phases of my cycle (Figure 3.43), however, no statistically significant differences were detected between the two phases. On average, the BG levels were slightly higher during the luteal phase (6.05 mmol/L ± 0.68) compared to the follicular phase (5.77 mmol/L ± 0.60). Body weight, BMI, and percent body fat estimation were also slightly higher during the luteal phase compared to the follicular phase (Figure 3.43). On the other hand, the average resting HR was higher during the follicular phase compared to the luteal phase (66.88 bpm ± 4.13, and 65.14 bpm ± 3.28, respectively). Similarly, the daily average HR (including resting and non-resting HR) was higher during the follicular phase compared to the luteal phase (76.68 bpm ± 5.29, and 74.66 bpm ± 5.18, respectively). Both systolic and diastolic BP was higher during the follicular phase (106.79 ± 3.07, and 74.07 ± 1.96, respectively) compared to the luteal phase (103.83 ± 5.16, and 73.03 ± 2.05). BT levels were very similar between the two phases with 36.97 ºC ± 0.14 during the luteal phase and 36.98 ºC ± 0.13 during the follicular phase (Figure 3.43).

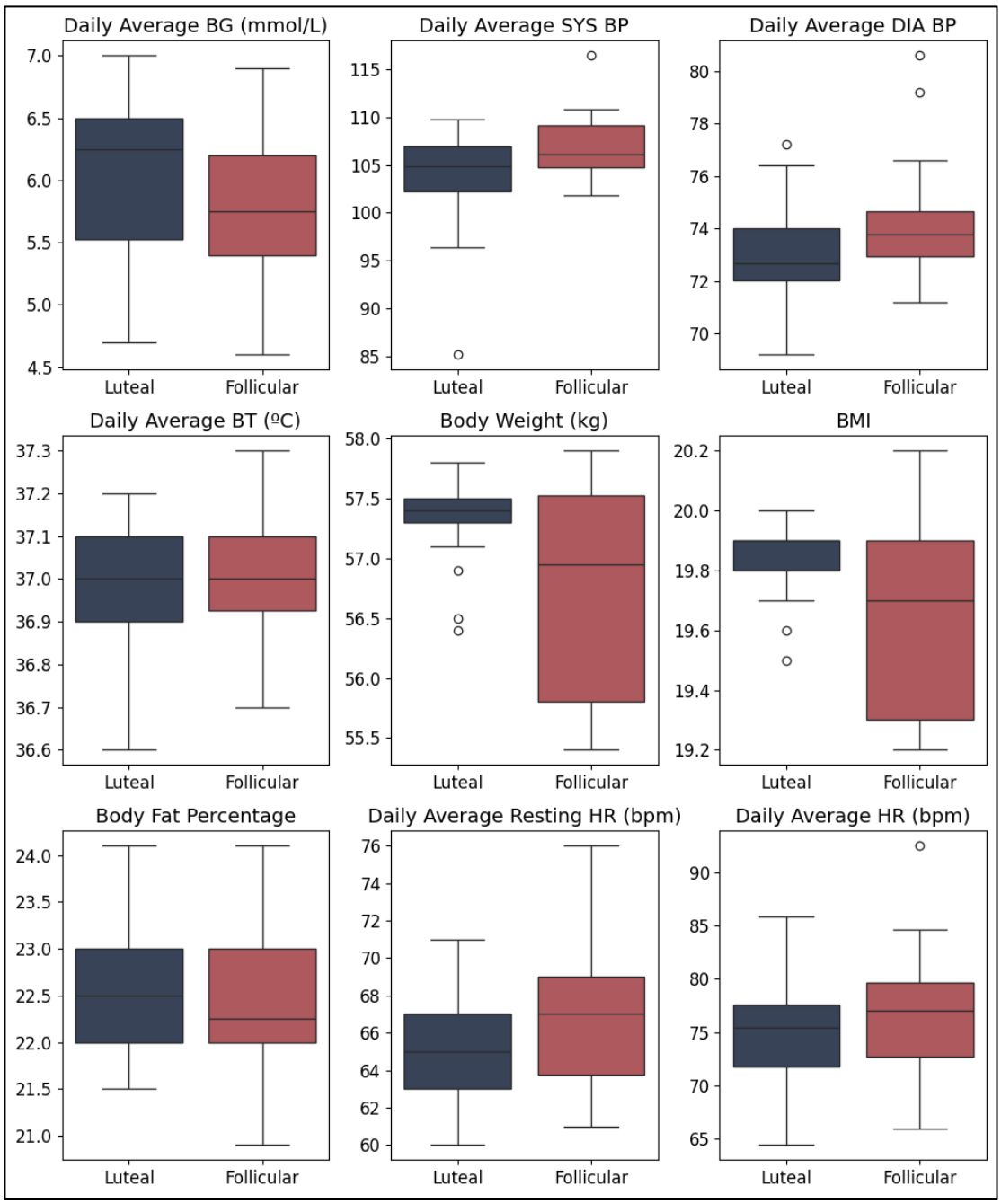

Figure 3.43 - Box and whisker plot illustrating the distribution of different physiological measures during the Follicular phase (marked in red) and the Luteal phase (marked in blue) of the menstrual cycle. Abbreviations – BG: blood glucose (mmol/L); SYS BP: systolic blood pressure; DIA BPS: diastolic blood pressure; BT: body temperature (ºC); BMI: Body Mass Index; HR: heart rate (bpm)

No significant differences were observed between the menstrual cycle phases in terms of physical performance. The BG, BT and HR levels during the exercises showed a similar pattern to daily averages where BG was slightly higher during the luteal phase, while HR was slightly higher during the follicular phase, and BT was similar during the exercises in both phases. Interestingly, the average calories burnt during exercises were higher during the follicular (307.00 kcal ± 43.82), compared to the luteal phase (282.52 kcal ± 47.25) (Figure 3.44). This is in parallel with the higher daily average HR, BT and BP levels, and lower body weight and BMI levels observed during the follicular phase. Studies have shown that during the (late) follicular phase, higher estrogen levels enhance exercise performance by improving muscle function, increasing energy expenditure, and influencing substrate metabolism [237]. Additionally, lower progesterone levels during this phase

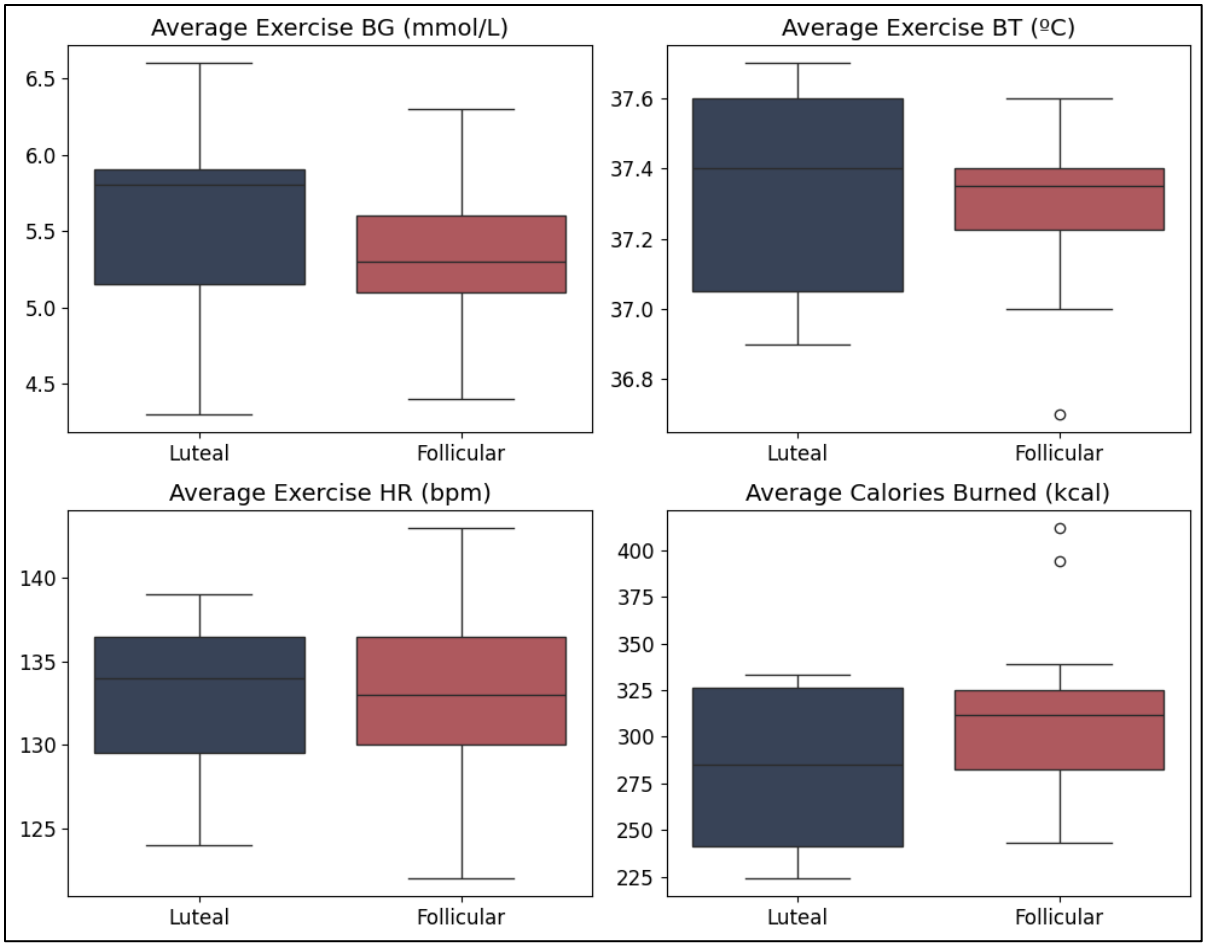

Figure 3.44 - Box and whisker plot illustrating the distribution of the average blood glucose (BG), body temperature (BT), heart rate (HR) and burned calories during physical exercises on the Follicular phase (marked in red) and the Luteal phase (marked in blue) of the menstrual cycle.

contribute to a lower BT and reduced thermogenic effects [237]. Higher HR, BT, and BP levels indicate a heightened cardiovascular response, improving oxygen delivery and nutrient transport to muscles, which can increase calorie burn. Additionally, lower body weight and BMI during the follicular phase, likely due to reduced water retention and bloating which are common in the luteal phase, can enhance exercise efficiency. Estrogen also promotes fat utilization as a fuel source, potentially prolonging exercise duration or intensity. Despite a slightly lower basal metabolic rate, the overall metabolic response to exercise is more pronounced, resulting in higher calorie expenditure during physical activity.

No significant differences were detected between the two phases in terms of sleep metrics. Overall, more time asleep and less time awake was observed during the luteal phase. However, the frequency of the wake stage was higher during the luteal phase. On the other hand, REM stage and light stage sleep were slightly longer during the follicular phase. Additionally, higher sleeping HR levels and restlessness during sleep were observed during the follicular phase (Figure 3.45). The differences in sleep patterns and quality between the follicular and luteal phases of the menstrual cycle are influenced by hormonal fluctuations. During the luteal phase, higher levels of progesterone, which has sedative properties, contribute to more time asleep and less time awake overall [238]. However, this phase also sees an increase in the frequency of wake stages due to progesterone's effects on the central nervous system, leading to more fragmented sleep [238]. In contrast, during the follicular phase, higher estrogen levels and lower progesterone levels enhance REM and light sleep stages, resulting in slightly longer durations of these sleep stages. Estrogen also increases sympathetic nervous system activity, which can lead to higher sleeping HR and increased restlessness during sleep [238]. These hormonal changes throughout the menstrual cycle impact various aspects of sleep, leading to the observed differences in sleep patterns and quality between the follicular and luteal phases.

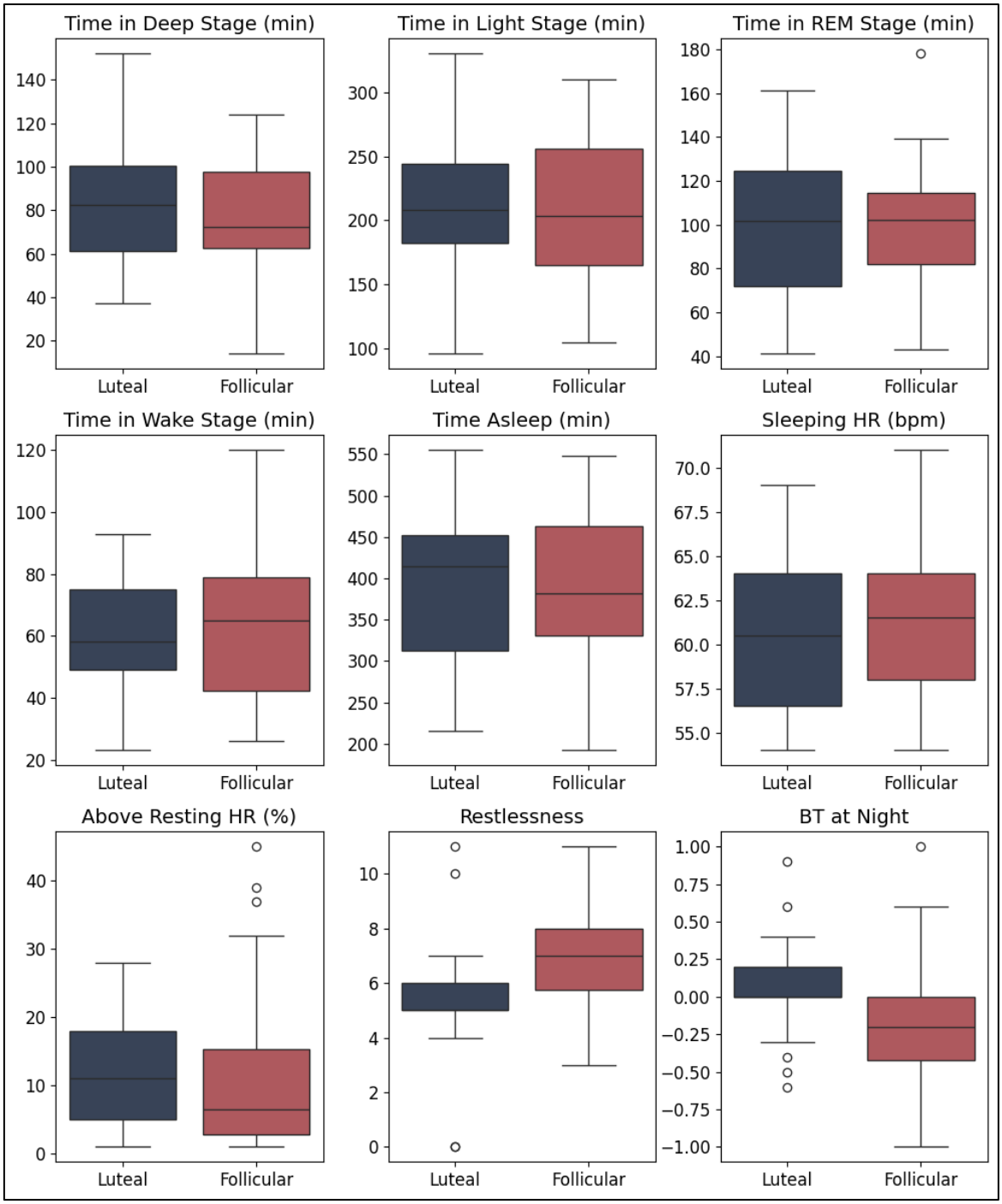

Figure 3.45 - Box and whisker plot illustrating the distribution of different sleep metrics during the Follicular phase (marked in red) and the Luteal phase (marked in blue) of the menstrual cycle.

Chapter 4

Data Analysis and Discussion

Introduction

The impact of diet and lifestyle on an individual's overall health is very clear, as research has consistently shown their profound influence on physiological and metabolic processes [162]. It has also been emphasized both in Chapter 1 and in many other published studies [65,67,68,70–72,239] that in order to advance healthcare, a shift to individualized or personalized treatments is essential. The traditional one-size-fits-all approach to healthcare and nutrition fails to account for individual genetic predispositions, physiological propensities, individual metabolic profiles, and individual lifestyle choices that are unique to each person [68,107]. Lifestyle factors such as physical activity, stress management, and sleep hygiene further underscore the interplay between environmental factors and health outcomes. PN when combined with omics technologies such as genomics, proteomics, and metabolomics along with the use of wearable monitors, allows for an improved understanding of an individual's health status. By embracing this combined, molecular + clinical approach, it has been shown that it is possible to both optimize and enhance the overall health status of individuals [69].

This chapter focuses on data analysis, with formatting designed to ensure the results remain closely connected to their interpretations. It discusses the interpretation of the multi-omics, physiological and mental/mood testing results of the four diet (FFD, MD, KD, and RD) intervention study that I conducted on myself. The diets and measurement methods were described in detail in Chapter 2 while the results were discussed in detail in Chapter 3. This chapter aims to highlight, correlate and rationalize the effects of different diet interventions and their molecular consequences (such as metabolome, proteome, and microbiome changes) as well as physiological/psychological impacts (such as HR, BT, BG, mood, cognition, and sleep quality) on my body. Ultimately, the main goal in this chapter is to assess and identify the most effective data analysis techniques for interpreting quantitative, multi-omic dietary N-of-1 studies.

4.1. Physiological Responses:

It has been shown that diet can have an association with common physiological measures such as blood glucose (BG), heart rate (HR) and sleep quality. As detailed in Chapter 2, in order to monitor the physiological effect of each of the dietary interventions, multiple monitoring devices were used. These tracked HR, BG, BT, and BP across the different dietary phases. In addition, routine anthropometric measurements were made throughout the study. Moreover, a series of physical and mental cognitive tests were conducted to assess the broader "performance" impacts of these diets. The data collected from these devices and tests were analyzed to identify correlations between different physiological responses. The objective was to highlight the specific effects of each dietary intervention on my body's physiological parameters and performance, thereby contributing to the understanding of how dietary choices can influence overall health.

Diet Effects and Body Weight

As each of the diets I consumed was isocaloric, no weight gain or weight loss was expected. For the most part, this is exactly what was seen. No statistically significant change to body weight occurred while on the FFD, the RD or the MD. However, this was not the case for the KD. My body weight was strongly changed while on the KD. More specifically, a significant decrease in body weight was observed while on the KD within a few days of the start of the diet. Overall, the KD led to a significant decrease in body weight (r = -0.66 and p = 0.011), body mass index (BMI) (r = -0.66 and p = 0.011), and percent body fat estimation (r = -0.77, p = 0.0012) within the 2 weeks of the diet intervention. Additionally, higher average levels of HR (r = 3.57, p = 7.84E-4) and BT (although not statistically significant) were observed while on the KD compared to the other three dietary interventions (Figure 4.1).

When an individual is on a KD, the reduction in carbohydrate consumption leads to decreased insulin levels, initiating several metabolic shifts such as enhanced glycogen breakdown, glucose production through gluconeogenesis, and fat breakdown. As glucose reserves are exhausted, the body shifts to using fats as the main energy source [240]. This process involves the breakdown of free fatty acids into acetyl coenzyme A (CoA) via beta-oxidation in the liver's mitochondria. Acetyl CoA is then transformed into 3-hydroxy-3-methyl-glutaryl-CoA (HMG

CoA), which subsequently produces the ketone body acetoacetate, marking the beginning of ketogenesis. Acetoacetate may further transform into either acetone or 3-β-hydroxybutyric acid, providing an alternative energy source from stored fats for vital organs such as the heart, brain, and muscles [241,242]. Previous studies have shown that the KD may initially cause an increase in energy expenditure (and subsequently in HR and BT) due to increased hepatic oxygen consumption for gluconeogenesis and triglyceride-fatty acid recycling [241]. However, a subsequent decrease in 24-hour energy expenditure could occur due to slowed gluconeogenesis and increased fatty acid oxidation [241] resulting in a more stable weight.

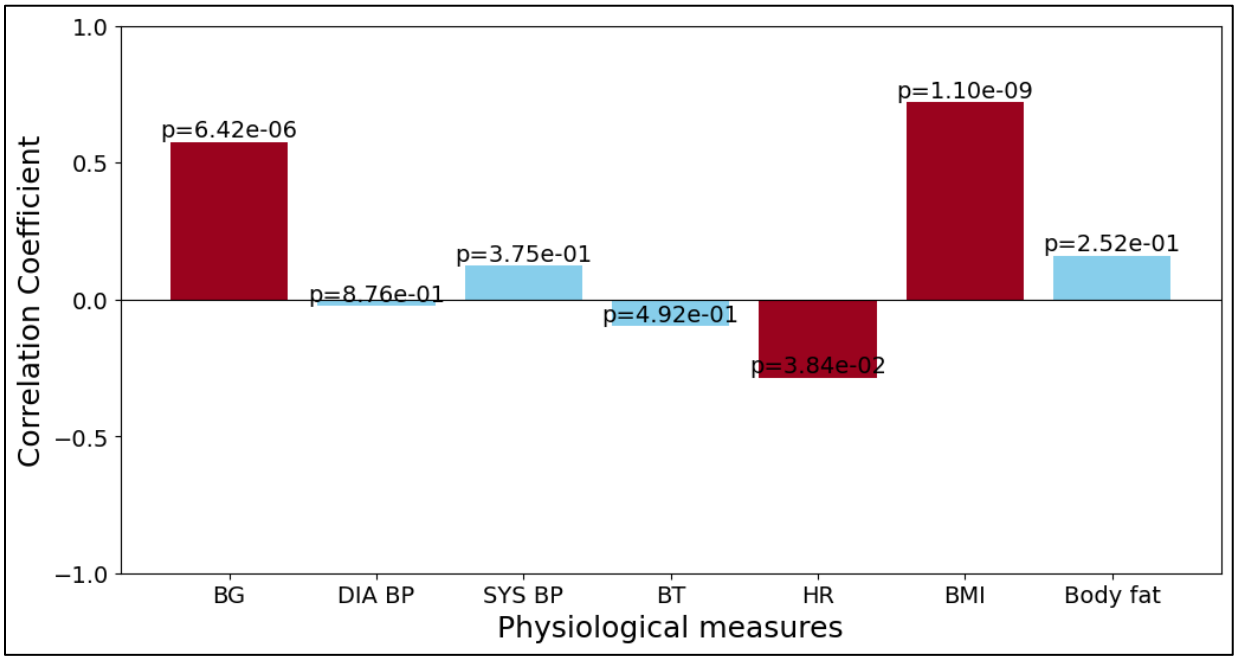

Figure 4.1 – The correlation coefficient and p-values of weight (kg) versus other physiological measures (BG, DIA and SYS BP, BT, HR, BMI and body fat estimation). P-values are represented on each bar. The significant ($p<0.05$) comparisons are colored in dark red.

In a study by Kennedy et al. [243] the effects of different diets on the physiology and weight of mice were examined. The diets included a KD, a high-fat and carbohydrate diet (similar to a FFD), and unrestricted eating. The mice on the KD consumed as much or more calories than those on the high-fat diet yet did not gain weight despite the high-calorie count. Furthermore, compared to mice on a regular diet, those on the KD initially lost weight and then maintained a lower weight similar to those on a calorie-restricted diet. The mice on the KD also exhibited unique bodily responses such as increased energy expenditure and a notably low respiratory quotient. These

results largely mirrored what I saw and measured while I was on the KD. In another study conducted by Zajac et al. [244] to assess the effects of a KD on the physical performance of off-road cyclists, it was concluded that the KD may influence HR and oxygen uptake, as these parameters were found to be significantly higher in the athletes at rest and during the initial stages of exercise after adopting a KD. This could indicate a change in how the body responds to physical activity under ketosis. While the association between KD on BT is less directly studied, the modulation of energy expenditure and the metabolic shift to utilizing fat as a primary energy source could theoretically influence BT regulation. Both the mouse study and the off-road cyclist studies appear to replicate the physiological findings for my N-of-1 study on the KD.

Postprandial Changes in Blood Glucose (BG), Body Temperature (BT), and Heart Rate (HR) – Temporal and Diet Effects

The comparison of postprandial BG changes within 2 hours of meal consumption, demonstrated highly significant differences between the four dietary groups (p = 1.97e-287). The FFD had a higher average BG level postprandially compared to the other dietary interventions, while the KD had the lowest average BG levels (Figure 4.2). Moreover, the RD had higher average BG levels after meal consumption compared to the MD (Figure 4.2). Overall, for most meals a spike in BG levels was observed within 30 to 40 minutes after meal consumption which typically returned to baseline within 1 to 2 hours of eating. This postprandial BG pattern was absent while on the KD.

The analysis of postprandial BT revealed distinct trends for each dietary intervention. The FFD exhibited a relatively stable BT with minor fluctuations, maintaining an average of around 37.1 °C across the observed time points, with a smaller SD of 0.16 °C. The MD showed a slight, consistent decline in BT after meal consumption from approximately 37.2 °C to 37.1 °C over the 60-minute post-meal period, with a larger SD of 0.23 °C. The KD began with a higher initial BT of 37.2 °C, and essentially maintained it over the 60-minute post-meal period, with a smaller SD of 0.17 °C. The RD maintained a fairly stable temperature, with slight variations but generally hovering around 37.1 °C, and a larger SD of 0.2 °C (Figure 4.3). Overall, while all diet interventions except the KD led to some degree of temperature change post-meal, the MD and

FFD interventions showed the most significant decline, However, these differences were not statistically significant between the dietary interventions.

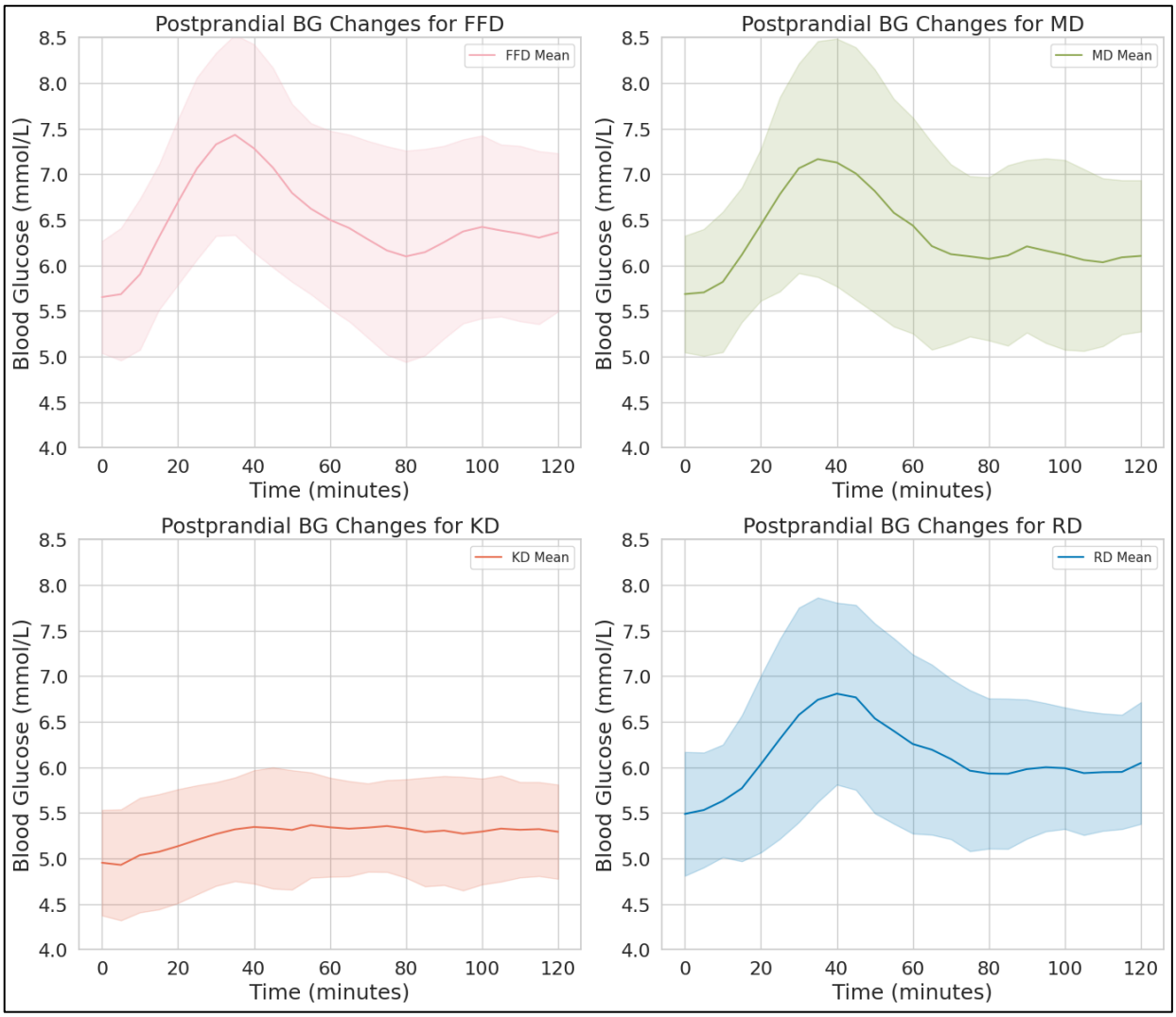

Figure 4.2 - Postprandial BG changes within 2 hours of meal consumption (including breakfasts, lunches, dinners and snacks).

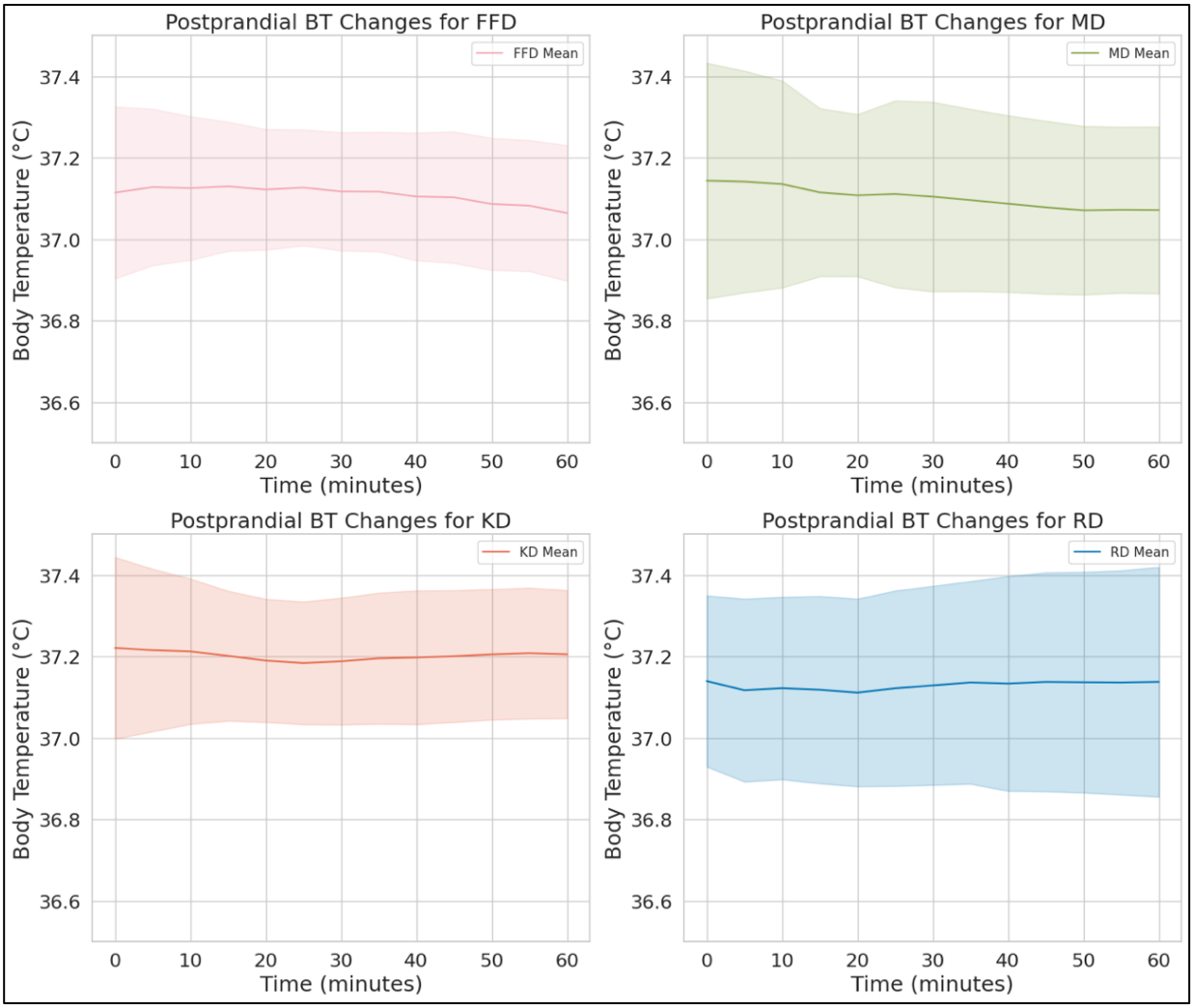

Figure 4.3 – Postprandial BT changes within an hour after meal consumption (including breakfasts, lunches, dinners and snacks)

A comparison of HR changes after meal consumption showed significant differences between the dietary interventions (p = 0.0003). The FFD showed higher average HR levels postprandially compared to the MD (p = 0.00097) and the RD (p = 0.016), while there was no significant difference in HR changes between the FFD and the KD. Similarly, the KD had higher average HR levels after meal consumption compared to the MD (p = 0.0003), and the RD (p = 0.006). On the other hand, there was no significant difference in HR changes between the MD and RD after meal consumption (Figure 4.4). The higher average HR values for the KD reflected the higher overall energy expenditure or metabolic rate that characterized the KD. Additionally, the

KD had a higher fat content than the other diets (detailed in Chapter 3) which likely led to a higher HR. This is likely due to increased digestive effort, activation of the sympathetic nervous system, and the release of hormones like adrenaline. These responses can cause the heart to work harder temporarily, raising the HR and blood pressure. Previous studies have shown that high-fat meals lead to increased BP and increased cardiovascular reactivity [245].

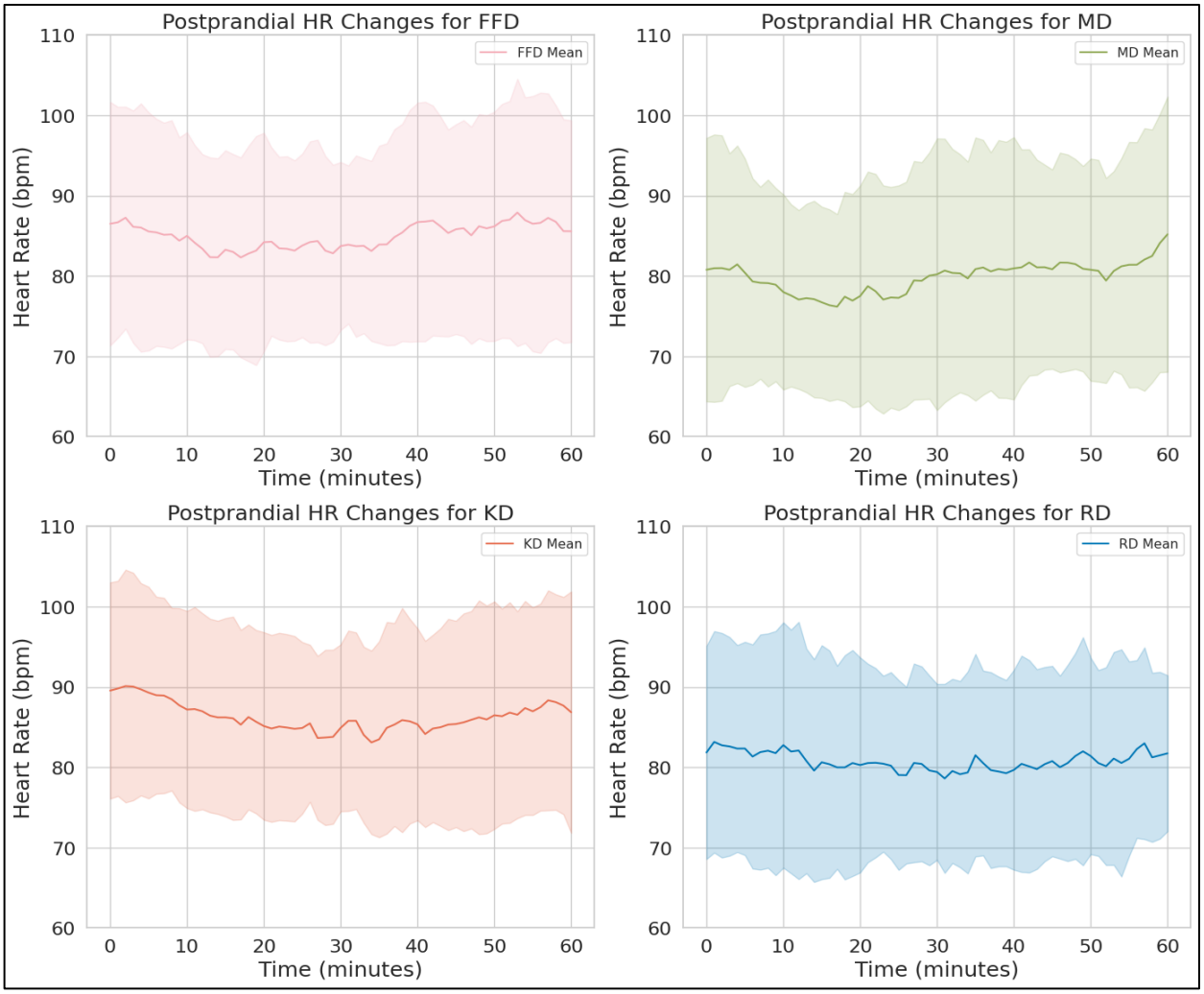

Figure 4.4 - Postprandial HR changes within an hour of meal consumption (including breakfasts, lunches, dinners and snacks).

Changes in Blood Glucose (BG), Body Temperature (BT), and Heart Rate (HR) After Exercise – Temporal and Diet Effects

Comparison of BG changes within an hour post-exercise, demonstrated a significant difference in BG level across all dietary interventions (p =0.037). BG levels spiked after finishing aerobic exercise within ~30 minutes while on the FFD, and MD, ~40 minutes while on the RD, and less than ~30 minutes while on the KD. BG levels mostly returned to normal within an hour after finishing the exercise period (Figure 4.5). A rise in BG is expected during and after moderate exercise as the stress hormones cortisol and adrenaline, which are released during exercise, cause BG to increase to better supply the muscle tissue with glucose to support the extra activity in

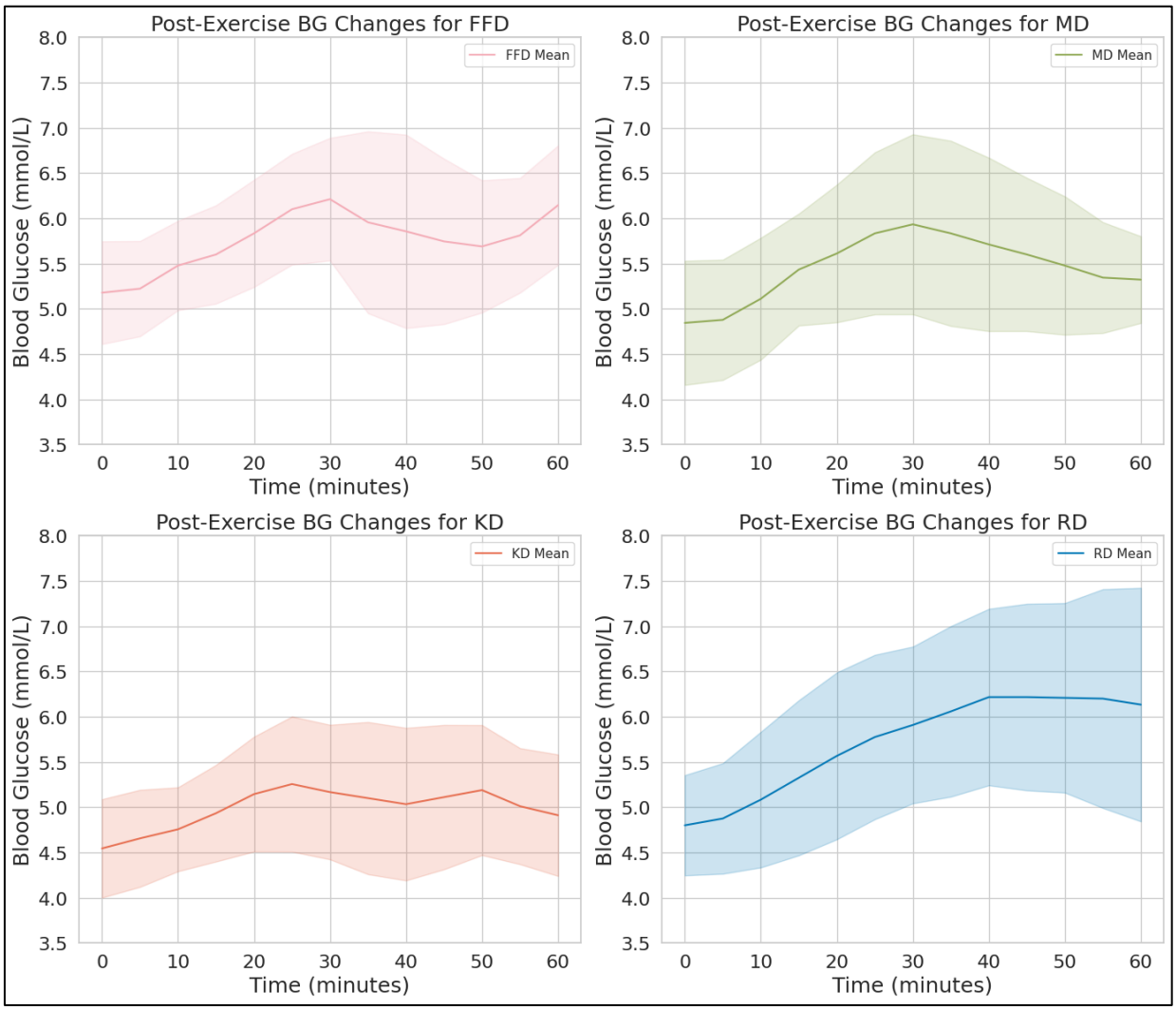

Figure 4.5 - BG changes within an hour of physical activity

muscle tissue during exercise. The time it takes for BG to spike, and later fall is a function of how quickly the muscle cells can restore themselves to normal, resting aerobic metabolism (fitness) or how quickly the liver can start converting the glucose to glycogen (liver function). The long delay for BG to peak and to fall to normal levels while on the RD may reflect lower fitness levels or poorer liver function – or both. However, given that I attempted to control for fitness levels on all four diet interventions by exercising daily about the same amount (intensity and time), it would suggest that the RD was impairing my liver function, especially its ability to convert glucose to glycogen.

A similar comparison was conducted for post-exercise BT levels. Although not statistically significant, interesting differences were observed between the dietary interventions. The starting BT after finishing the exercise period was consistently ~0.3 ºC lower while on the FFD compared to the other three diet interventions ($p = 0.17$). However, the decreasing pattern in BT levels after finishing the exercise was relatively similar across all interventions. In particular, the BT typically decreased to ~37.2 ºC within one hour of finishing the exercise period (Figure 4.6). The reason for the lower BT post-exercise while on the FFD may be due to the higher BG levels that were seen throughout the FFD (Figure 4.5). The greater availability of BG while on the FFD may have allowed the muscles to access energy sources more easily thereby requiring less work by the liver (to convert glycogen to glucose) or other tissues, which would therefore require less ATP to be used, and less thermal heat to be generated. This would also argue that exercise while on the FFD was less effective at "burning calories" or reducing weight as the low BT post-exercise indicated that fewer calories were being burned and less thermal activity was occurring while on the FFD. An elevated BT is an indication of greater thermal activity and more calories (and ATP) being consumed. Higher post-exercise BTs were observed in all other diets (KD, RD, and MD).

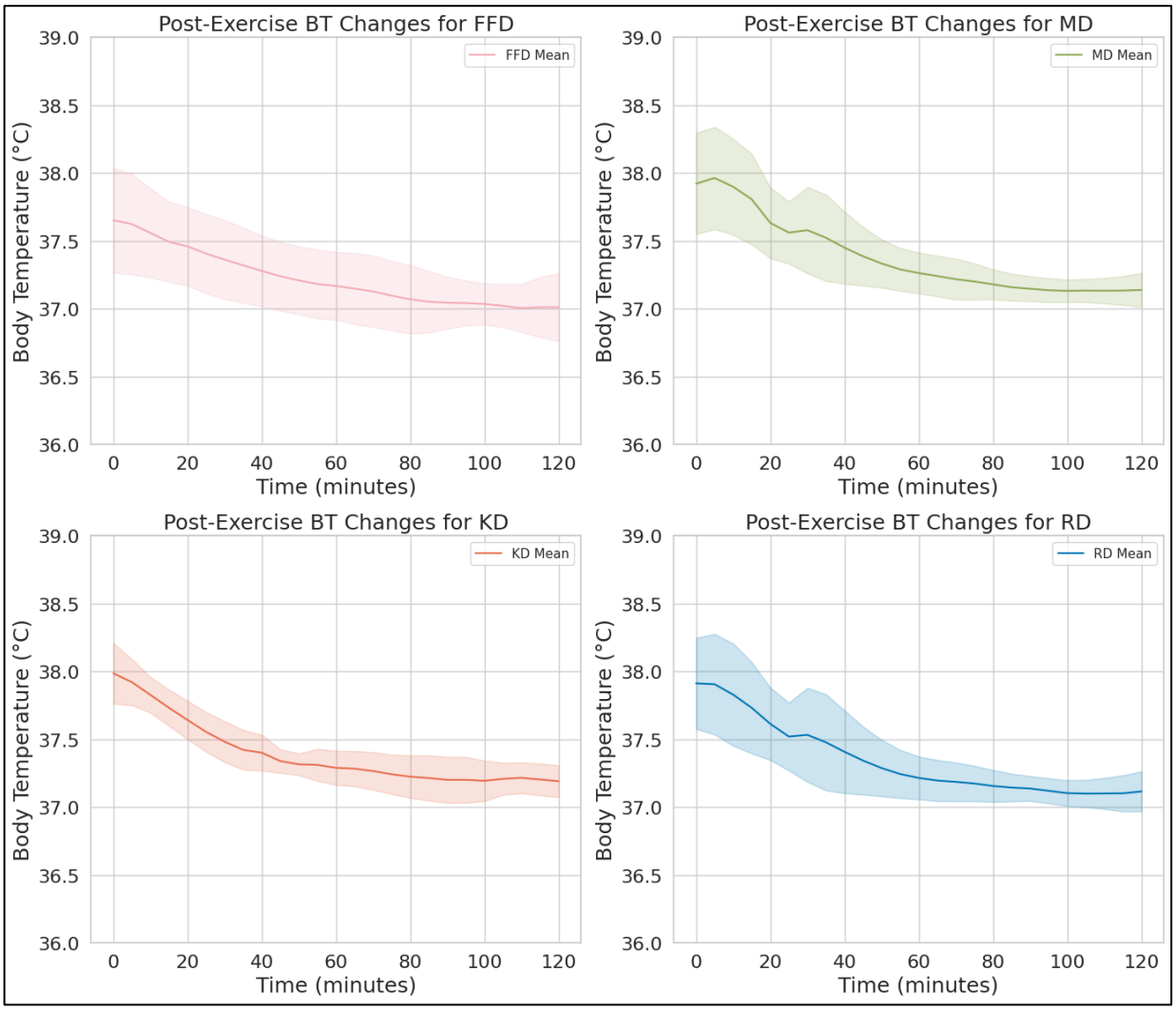

Figure 4.6 - BT changes within an hour of physical activity

A comparison of the post-exercise HR levels between the dietary interventions also revealed significant differences (p = 4.532e-07). The HR levels after finishing the exercise was significantly lower while on the FFD compared to the other diet interventions (p = 0.018). The decline in HR to baseline was fastest while on the MD (~10 minutes), while it was the slowest while on the FFD (~60 minutes). The time to restore to baseline HR while on the KD and RD was ~ 20 minutes (Figure 4.7). The time it takes for the HR to drop resting or near-resting levels is called heart rate recovery (HRR) [246]. The longer it takes for the HR to recover, the lower one's level of fitness or the poorer one's cardiovascular health may be. Because I was exercising regularly with approximately the same intensity and length of time each day, it could be argued that the level of fitness was being controlled. Therefore, the HRR is a better measure of

cardiovascular health. The rapid decline in post-exercise HR (i.e., the fast HRR) while on the MD appears to be indicative of the improved cardiovascular health effects that the MD is known to impart [247]. On the other hand, the slow HRR or the slow decline in post-exercise HR while on the FFD appears to be indicative of reduced cardiovascular health. This is consistent with studies showing that FFD reduces cardiovascular health [248].

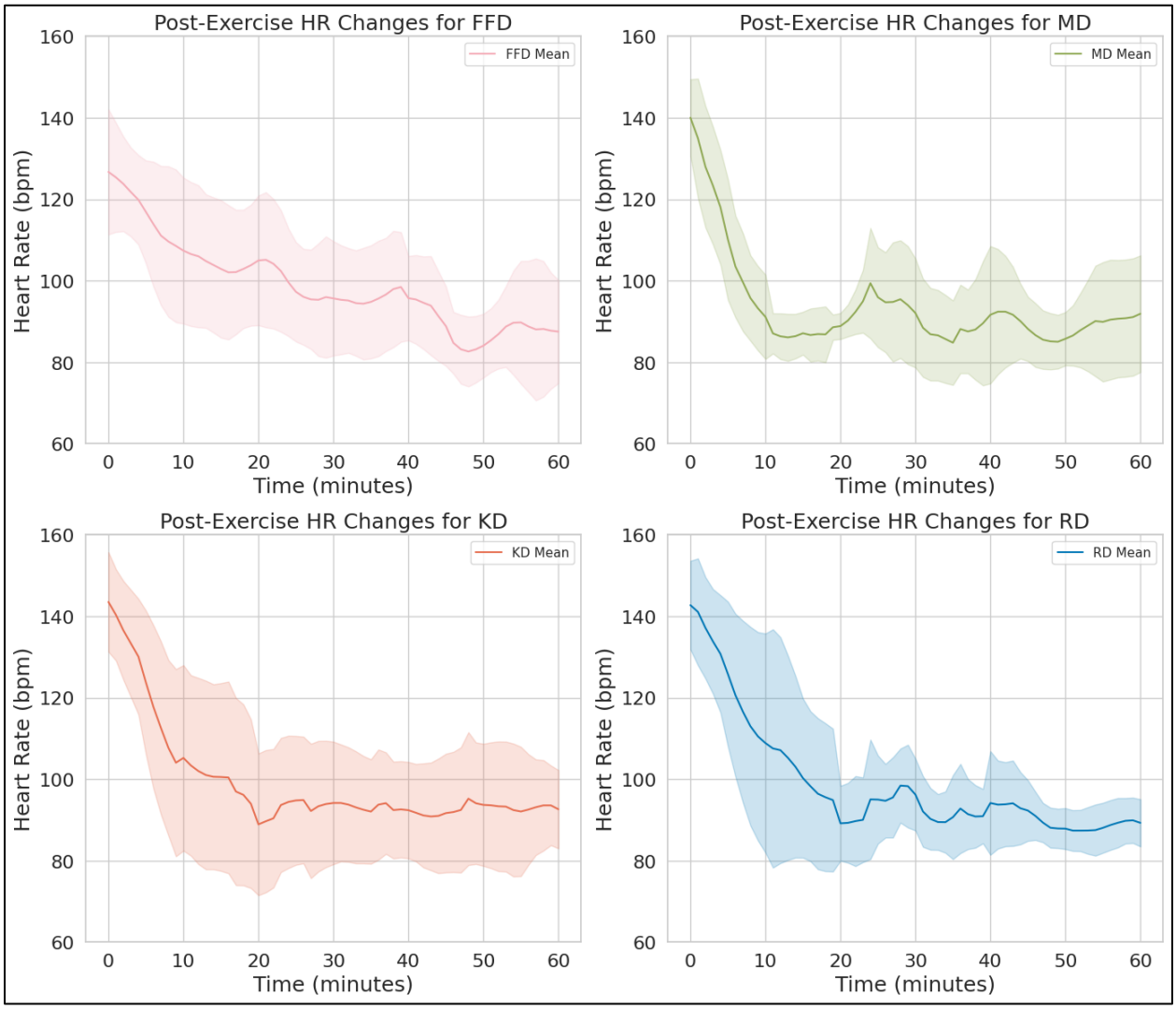

Figure 4.7 - HR changes within an hour of physical activity

Blood Glucose (BG) versus Heart Rate (HR) – Temporal and Diet Effects

Blood glucose (BG) levels are known to affect heart activity [249]. Therefore, longitudinal hourly HR and BG levels were compared to observe any correlations between them. Specifically, I looked at the relationship between BG and HR for different diets and over different times. The correlation analysis (Figure 4.8) between hourly HR levels and BG revealed varying relationships throughout the day. The 24-hour histogram illustrated in Figure 4.8 shows the correlation coefficient between HR and BG, with the P-value listed above each histogram bar. Significant ($p<0.05$) positive correlations were observed in the early morning hours (2 to 3 am), and significant ($p<0.05$) negative correlations were observed in the late afternoon to early evening hours (5 to 6 pm). Figure 4.9 displays the average HR and BG levels at different times of the day, along with the polynomial trendline to illustrate the overall pattern for each dietary intervention. Broadly speaking, these variations indicate that during sleep when HR levels are lower and no food is consumed, BG levels are also lower. On the other hand, during the day, when HR levels are higher and meals are consumed, BG levels tend to be higher. On a more detailed level it is evident that the MD and KD exhibit strong sinusoidal characteristics with a short periodicity (a clear maximum and a clear minimum within each day) with BG and HR being nicely "in phase." On the other hand, the FFD has only a single maximum or minimum with a longer periodicity than the MD or KD. Likewise, with the FFD the HR and BG are clearly out of phase. The RD only shows a weak periodicity with a much lower amplitude than any of the other diets. These differences suggest that the RD was putting less stress on the heart and pancreas (and likely other organs), while the other diets were more physiologically stressful, especially the KD, which had the highest amplitude in HR changes.

The out-of-phase character between the BG and HR for the FFD suggests that the heart was having to work more with less available energy (glucose) or that some internal metabolic signaling was potentially out of synch. The low BG levels during mid-day with the FFD likely contributed to the general malaise and poor physical/mental performance while I was on this diet. At an even finer level of detail, it is clear that there is shorter periodicity seen during the waking period for the RD, KD, and MD with regard to HR. In particular, two strong HR maxima lasting

~2 hours are seen at 10:00 hrs and at 18:00 hrs for all the diets. These are not correlated with any spikes in BG. By cross-checking the daily diaries and physical activity records, I determined that these times corresponded to the regular exercise periods for the study or regular walking periods.

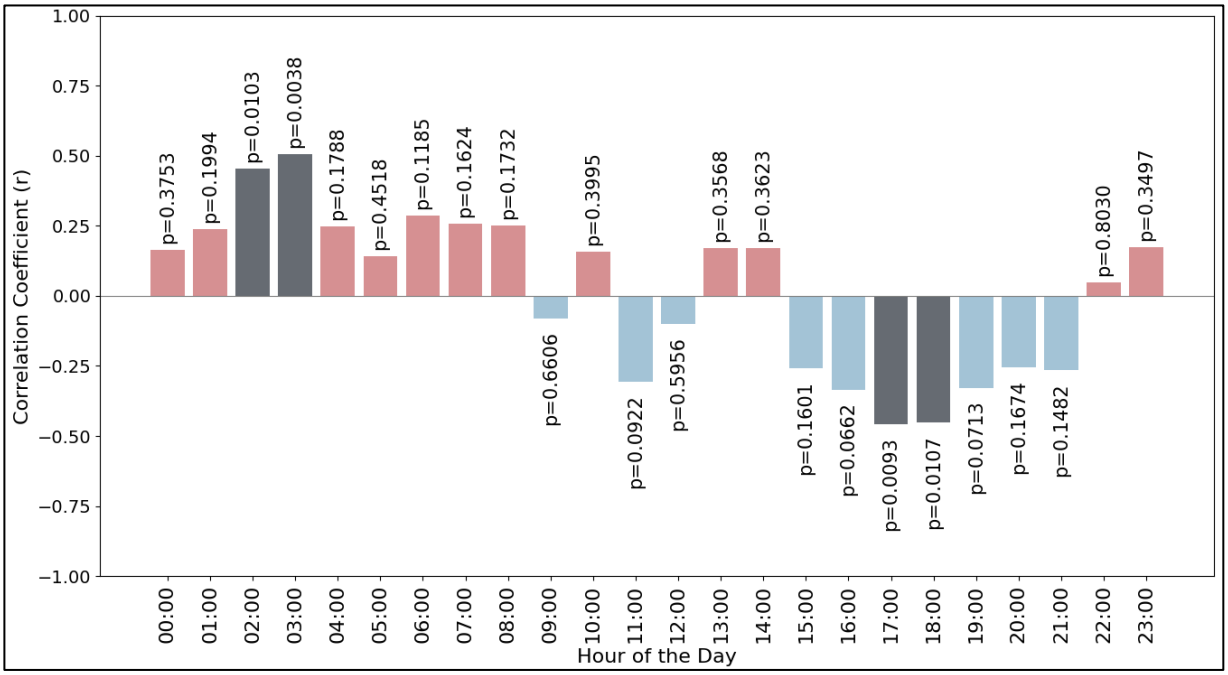

Figure 4.8 - The correlation coefficients (y axis) between hourly BG and HR changes across all diets. P- values are presented on each histogram bar and the significant correlations ($p<0.05$) are colored dark grey.

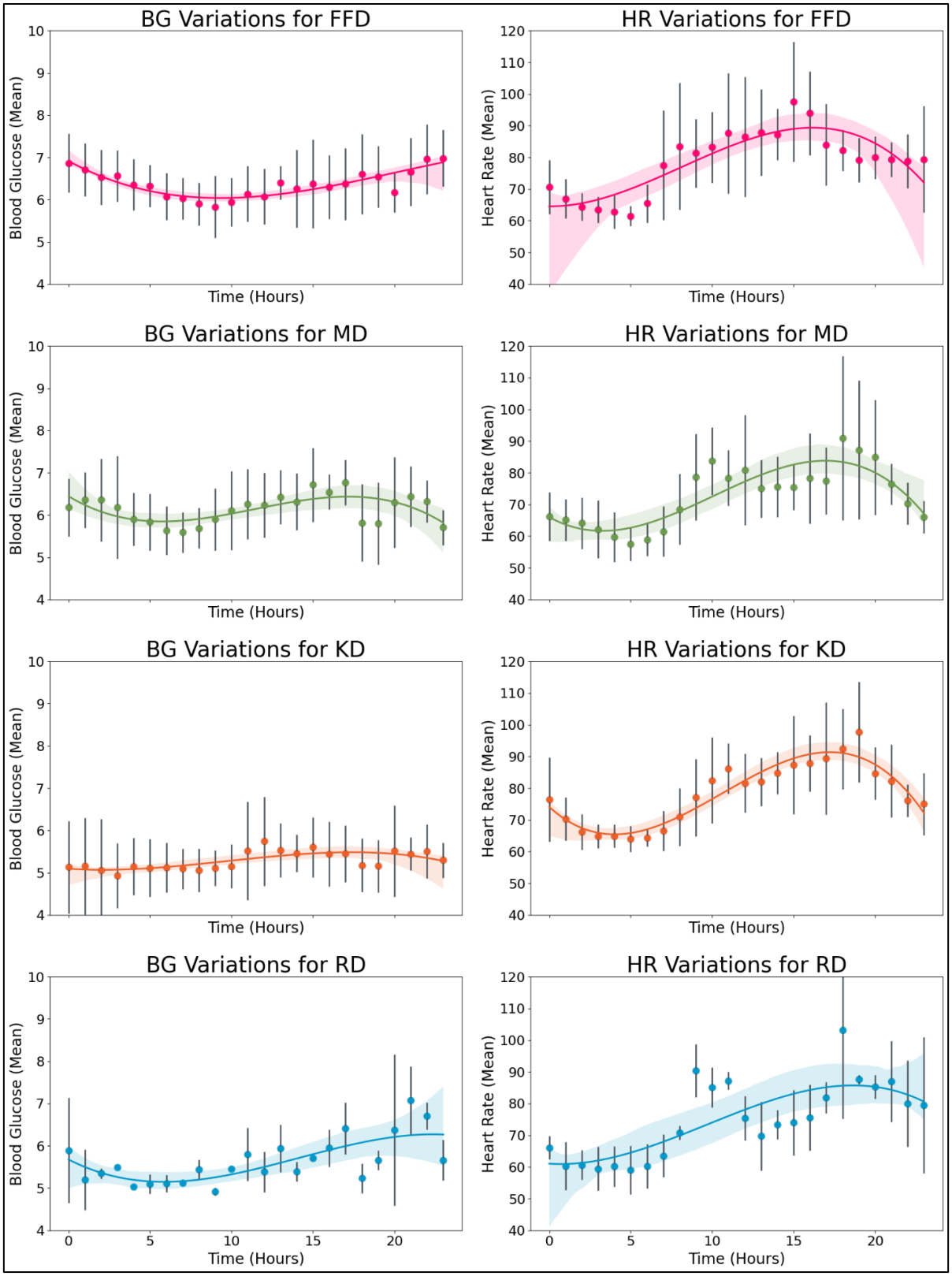

Figure 4.9 - The average HR and BG changes, along with the polynomial trendline for each dietary intervention. The x axis is time in hours and the error bars are highlighted for each time point.

Blood Glucose (BG) versus Blood Pressure (BP) – Temporal and Diet Effects

It has been observed that high BG increases blood pressure (BP) and that consumption of high glycemic index foods also increases BP [250]. To explore this effect further, I looked at the relationship between BG and BP for different diets and over different times. Different diets led to different week-to-week variations in BG. For instance, the average daily BG levels were higher during the first week on the FFD (6.7 ± 0.8 mmol/L), and lower during the second week on the FFD (5.9 ± 0.8 mmol/L). On the other hand, BG increased during the second week on the KD compared to the first week on the KD (5.0 ± 0.8 mmol/L, and 5.3 ± 0.6 respectively). Similarly, different diets led to different within-day variations in BP. For instance, BP levels were higher in the morning and at night while on the FFD. In contrast, BP was higher in the morning, after breakfast and lunch, and lower at night during the MD. The KD also led to higher levels of BP after dinner and at night while it was lower in the morning and after breakfast. During the RD, BP was lower in the morning and higher after lunch, dinner and at night.

As a general rule, SYS BP was found to be more sensitive to BG than DIA BP (Figure 4.10). Specifically, average daily SYS BP and average daily BG levels showed a significant weak positive correlation ($r = 0.269$, $p = 0.049$). SYS BP and DIA BP levels were compared to the BG levels at the time of BP measurement. SYS BP levels in the morning showed a positive correlation to BG levels after lunch ($r = 0.33$, $p = 0.02$). Likewise, after breakfast SYS BPs were positively correlated to the after-breakfast BG levels ($r = 0.28$, $p = 0.049$), BG levels after lunch ($r = 0.28$, $p = 0.05$), and BG levels after dinner ($r = 0.30$, $p = 0.03$). SYS BP levels after dinner showed a moderate correlation to BG levels in the morning ($r = 0.35$, $p = 0.01$), and BG levels after lunch ($r = 0.32$, $p = 0.02$). Additionally, DIA BP levels at night were positively correlated to BG levels at night ($r = 0.34$, $p = 0.02$) (Figure 4.10). Comparing the longitudinal BG measurements to BP changes suggests that the increased BG levels at night during the FFD led to higher BP levels at night and the following morning.

A similar analysis was performed on BP and BG levels at the time of BP measurement for each diet separately. Interestingly, during the FFD, the BG levels at night were negatively correlated to DIA BP levels after dinner ($r = -0.57$, $p = 0.03$). Although not quite statistically

significant, the BG levels after lunch during the FFD showed a moderate positive correlation with SYS BP levels after dinner (r = 0.50, p = 0.068).

Much stronger correlations between BG and BP were seen with the MD and RD. For instance, while on the MD, nighttime BG levels were strongly correlated to DIA BP levels after dinner (r = 0.64, p = 0.019), and at night (r = 0.68, p = 0.011). While on the KD, nighttime BG levels were significantly correlated to SYS BP values after dinner (r = 0.61, p – 0.020). While on the RD, after breakfast BG levels showed a positive correlation to SYS BP levels measured after dinner (r = 0.70, p =0.037). Additionally, after lunch BG levels were positively correlated to DIA BP levels after dinner (r = 0.68, p = 0.046), and nighttime BG levels were negatively correlated to after dinner DIA BP (r = -0.77, p =0.015). Figure 4.11 shows the changes in SYS BP, DIA BP, and BG levels at the time of BP measurements for all the dietary interventions.

The connection between BG and BP has been studied extensively by many researchers [250–253], revealing that high BG can indeed lead to elevated BP through multiple mechanisms. Elevated BG levels, particularly in diabetes, can induce insulin resistance, causing the body to retain sodium and water, which increases blood volume and BP. Insulin resistance also affects blood vessel function, reducing their elasticity and promoting the accumulation of fatty deposits, which further raises BP [254]. Chronic hyperglycemia stimulates the sympathetic nervous system, leading to vasoconstriction and higher BP [254]. Additionally, high BG levels can damage the endothelium, impairing its ability to produce NO, a crucial molecule for blood vessel relaxation, thereby increasing vascular resistance and BP [255]. Advanced glycation end products (AGEs) formed due to prolonged high BG stiffen blood vessels, exacerbating hypertension. In a longitudinal study of 3985 Chinese women, for a duration of 15 years, it was also concluded that higher fasting BG levels were significantly correlated with the risk of new-onset hypertension [256].

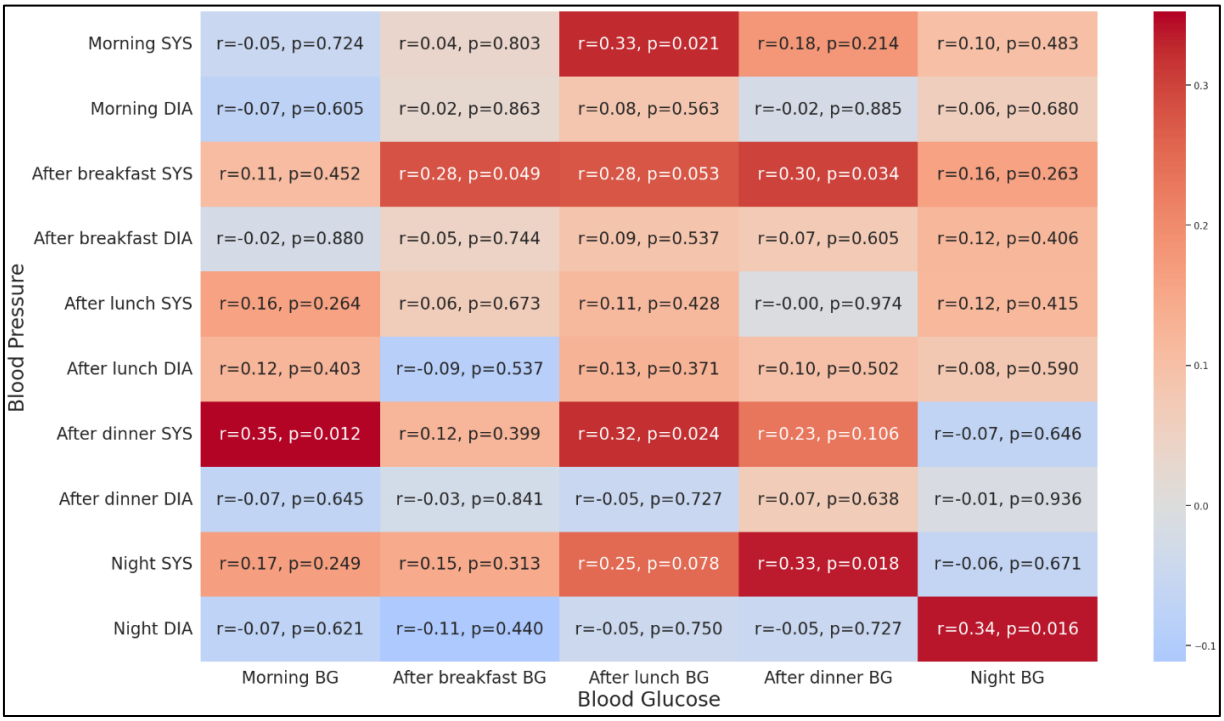

Figure 4.10 – Correlation heatmap of overall BP and BG levels at the time of BP measurement.

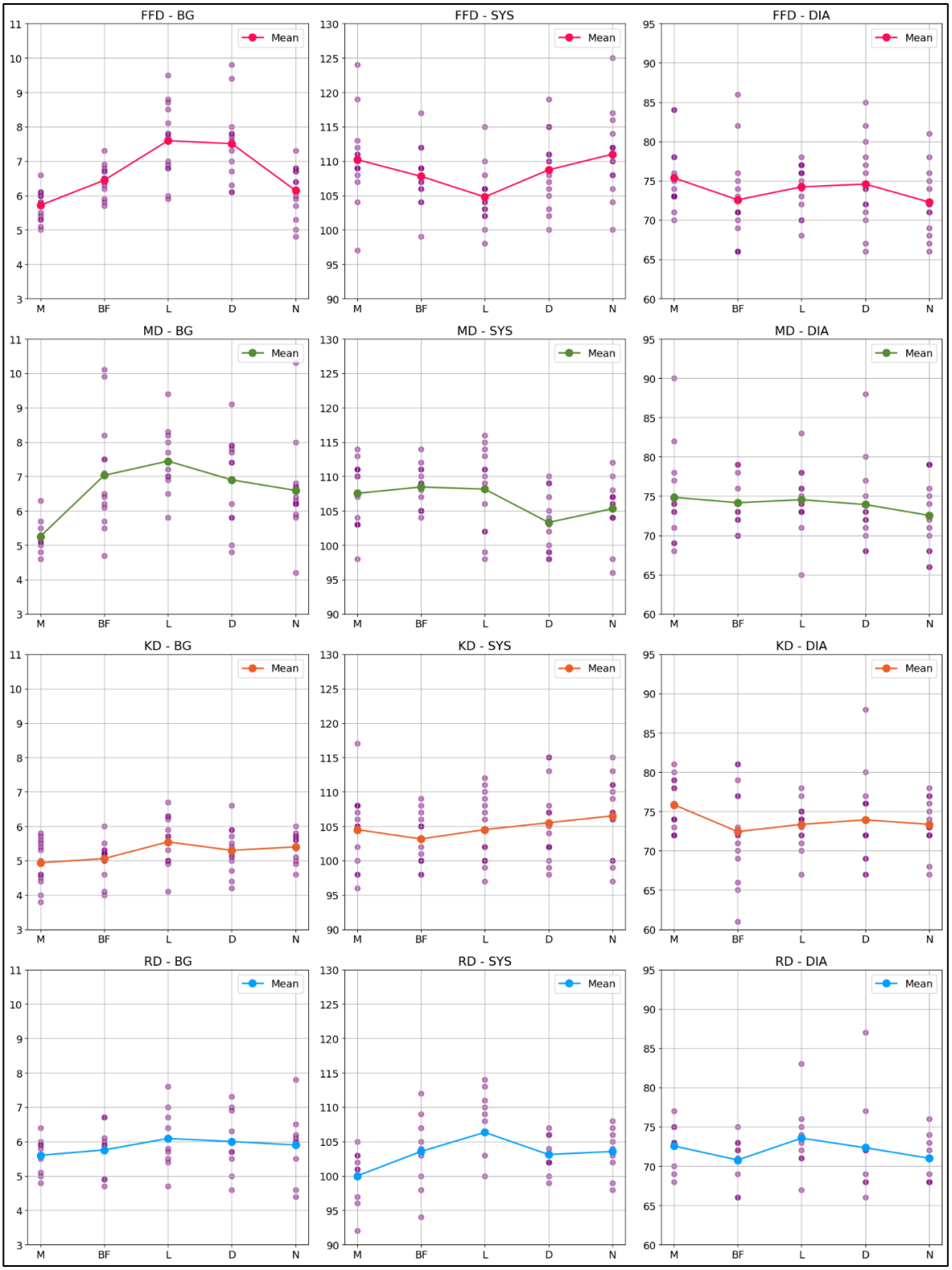

Figure 4.11 - SYS and DIA BP and BG changes at the time of BP measurements. M: morning, BF: after breakfast consumption, L: after lunch consumption, D: after dinner consumption, and N: nighttime

Blood Glucose (BG) versus Body Temperature (BT) – Temporal and Diet Effects

Body temperature (BT) is known to be affected by food intake via the so-called thermic effect of food [257,258]. To explore this further, I compared BG to BT and analyzed this relationship for different diets and over different times. The correlation analysis between hourly BG levels and hourly BT readings revealed varying relationships throughout the day. Some time periods show moderate negative correlations, notably at 11:00 (r = -0.416, p = 0.022), 14:00 (r = -0.474, p =0.008), 17:00 (r = -0.513, p = 0.004), and 19:00 (r = -0.446, p = 0.014), indicating statistically significant inverse relationships at these times (Figure 4.12). 14:00 corresponded to about 1 to 2 hours after lunch (lunch was typically consumed at 12:00 -13:00), while 17:00-19:00 corresponded to the dinner and/or post-snack period (dinner was typically consumed at 19:00, while an afternoon snack was consumed at 18:00). A similar negative (but not statistically significant) correlation was detected one hour after breakfast consumption (at 10:00).

Other hours exhibit weak or insignificant correlations, both positive and negative, demonstrating that the relationship between BG and BT is not consistent throughout the day. Typically, after a larger meal is consumed, BG levels increase while at the same time, surface BT is decreased as blood is directed away from the skin or body surface towards the stomach and other digestive organs to support their digestive or peristaltic activity. Since my BT sensor was a surface sensor, it detected the expected post-meal drop in skin temperature. Outside of these expected findings, the lack of correlation between BG and BT at other times (night or day) highlights the complexity of the interaction between BG and BT, which are modulated by circadian rhythms, metabolic processes, and daily activities [259,260]. Figure 4.12 displays the average BG and BT levels at different times of the day, along with Figure 4.13 which represents the polynomial trendline to illustrate the overall pattern for each dietary intervention. Figure 4.13 shows that for all diets, BT steadily and significantly increases by about 0.5 ºC from 0:00 to 23:00, while BG shows only a very modest increase from 0:00 to 23:00 for three of the four diets. The FFD exhibits a parabolic trend in BG with a minimum at 10:00.

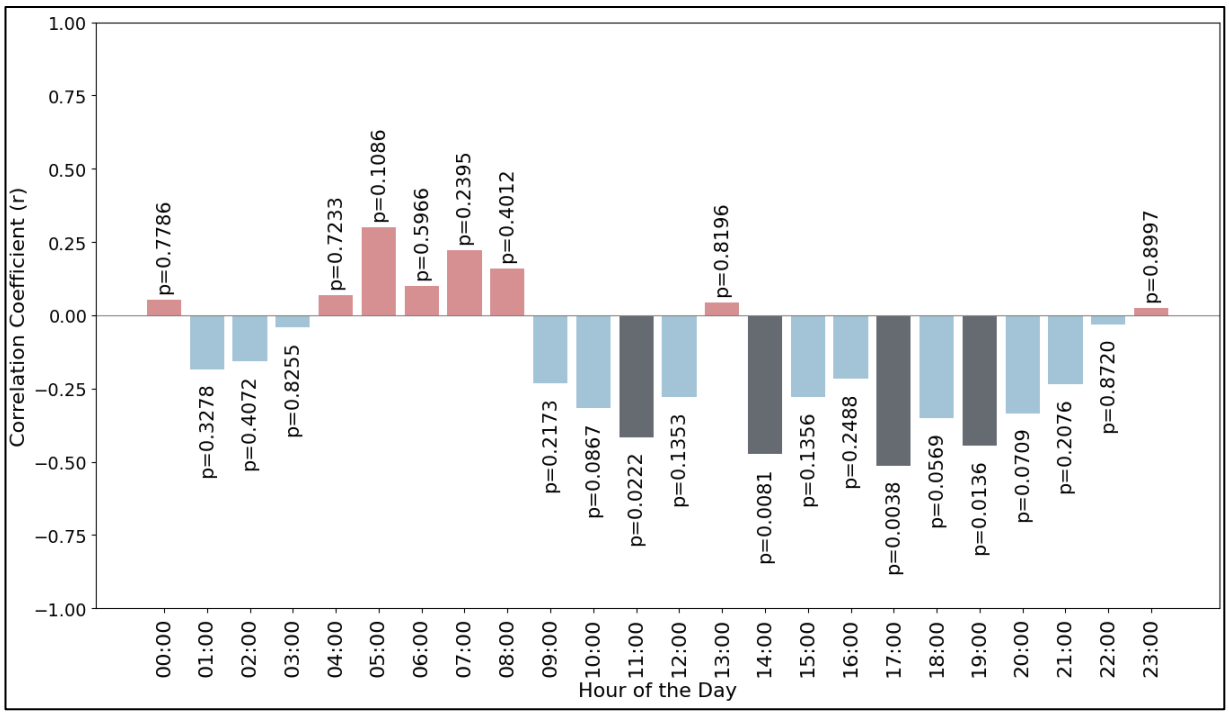

Figure 4.12 - The correlation coefficients (y axis) between hourly BG and BT changes across all diets. P-values are presented on each histogram bar and the significant correlations ($p<0.05$) are colored dark grey.

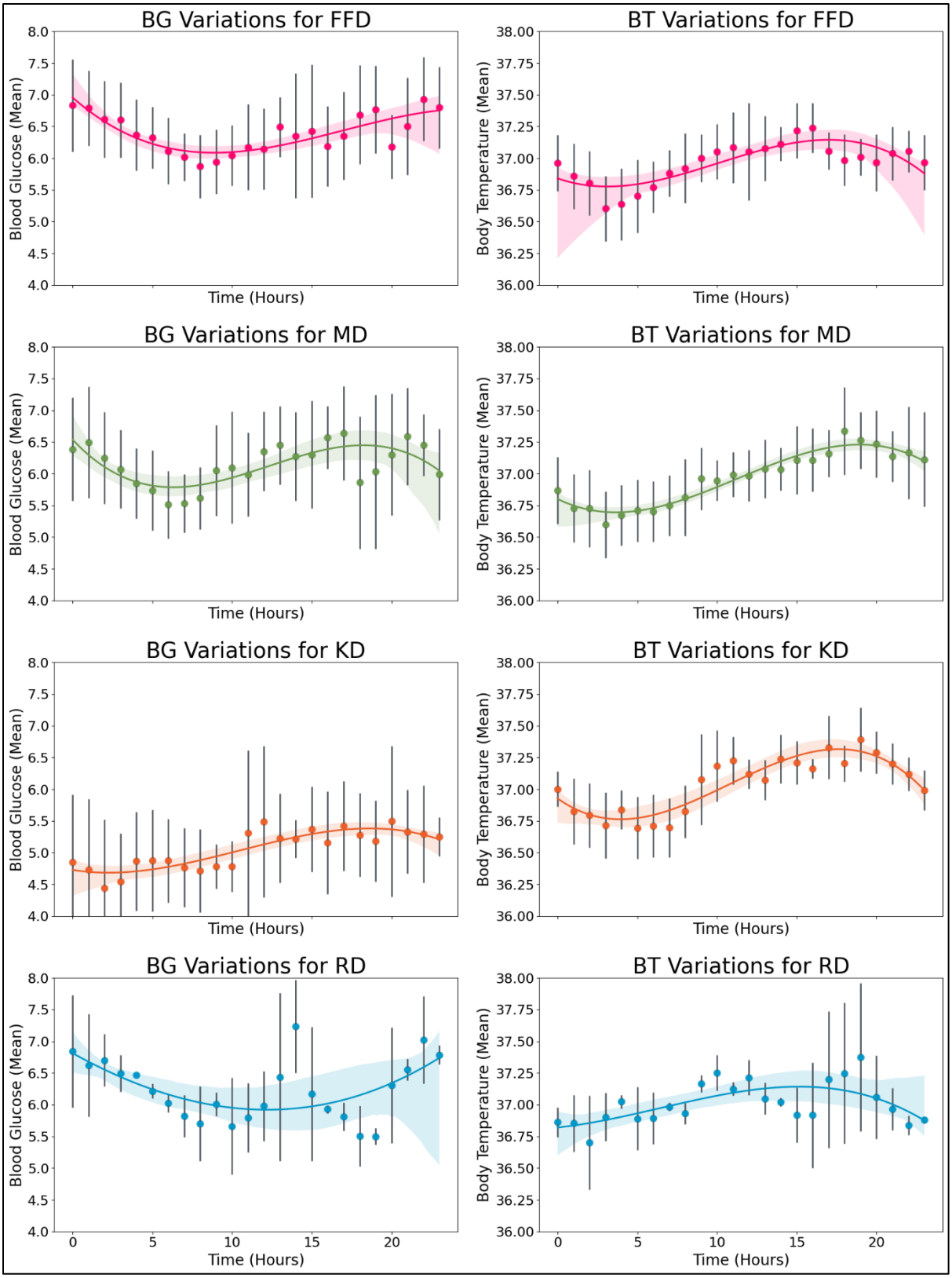

Figure 4.13 - The average BG and BT levels at different times of the day, along with a polynomial trendline to illustrate the overall pattern

Blood Glucose (BG) versus Profile of Mood State (POMS) – Temporal and Diet Effects

Hyperglycemia and hypoglycemia are known to affect mood and mental states [261,262]. To explore these effects further, I looked at the relationship between BG and POMS for different diets and over different times. The mood questionnaires were completed on days 1, 4, 7, 10, and 14 of each two-week diet intervention and the final scores in different categories (anger, confusion, depression, fatigue, tension, vigor, and the total mood disturbance scores) were compared to the daily average BG levels to uncover possible connections. Interestingly, confusion had a moderate positive correlation (r = 0.467, p = 0.05) with average BG levels, indicating that higher BG levels might be associated with increased confusion. Additionally, depression showed a moderate negative correlation (r = −0.552, p =0.018) with average BG levels, suggesting that lower BG levels might be associated with higher depression scores. Other mood metrics showed weaker correlations with BG levels (Figure 4.14). Additionally, a correlation analysis was performed on average BG levels and mood metric scores for each dietary intervention. None of the mood metrics showed a significant correlation to the average BG levels, except for depression, which showed a near-perfect negative correlation with average BG levels while on the FFD (r = -0.881, p =0.049).

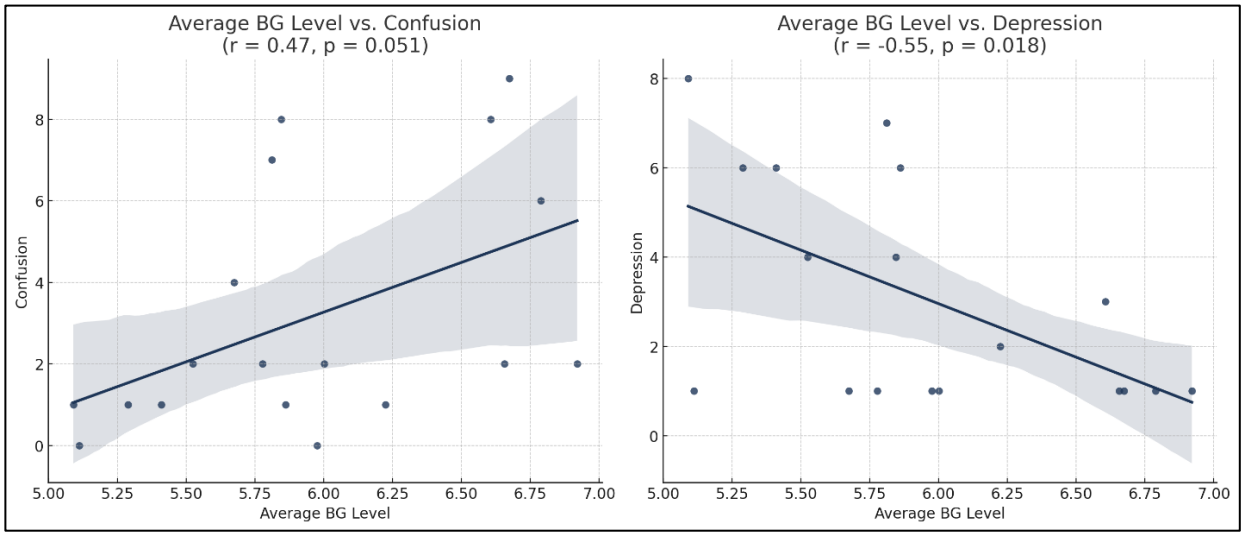

Figure 4.14 - The relationships between average BG levels and mood metrics confusion and depression across all dietary interventions.

A number of studies have demonstrated that glycemic variability significantly impacts mood and quality of life, highlighting the psychological impact of BG fluctuations [261]. These studies emphasize that while effective glycemic control can mitigate depressive symptoms, poor glycemic management can worsen them [262,263].

Hypoglycemia, or low BG levels, can lead to symptoms such as irritability, anxiety, and confusion because glucose is a primary energy source for the brain, and its deficiency impairs cognitive function and emotional regulation. On the other hand, hyperglycemia, or high BG levels, is often associated with fatigue, irritability, psychotic and depressive symptoms [264]. One interesting case report identified a woman with type I diabetes who suffered severe psychotic episodes and periods of confusion when experiencing acute hyperglycemia [265]. Chronic hyperglycemia also induces stress and inflammation, which negatively affect mood. Insulin resistance, commonly found in individuals with T2D and obesity, further complicates this relationship by impairing the brain's ability to utilize glucose efficiently, contributing to mood disturbances. Insulin itself plays a role in brain function and mood regulation, linking metabolic health directly with emotional well-being [264]. Additionally, chronically high BG levels can trigger systemic inflammation, which affects neurotransmitter regulation in the brain, particularly serotonin and dopamine, which are key players in mood regulation [266]. Inflammation has been implicated in the development of depression and other mood disorders [266]. Elevated BG also stimulates the release of stress hormones such as cortisol, leading to increased anxiety and depressive symptoms. Clinical evidence supports the assertion that individuals with better BG control often report improved mood and quality of life [261]. Specifically, it has been shown that individuals with more stable BG levels, achieved through continuous glucose monitoring (CGM) or other management strategies, often report feeling more cheerful, energetic, and relaxed [267]. These improvements in mood can be linked to the physiological benefits of maintaining BG within a healthy, normal range (4.0 – 6.0 mM), which reduces the frequency of both hyperglycemia and hypoglycemia, conditions known to negatively affect mental well-being [261,268]. While most studies on hypo- and hyperglycemia have focused on diabetic patients (because their BG levels are more frequently abnormal or extreme) it is not unreasonable to assume that abnormal BG levels can also affect healthy, normal individuals as well, since brain chemistry is thought to be identical between diabetics and non-diabetics.

Blood Glucose (BG) versus Mental Cognitive Assessment (MCA) – Temporal and Diet Effects

Hyperglycemia and hypoglycemia are also known to affect mental cognition and mental test scores [268]. To explore this effect further, I looked at the relationship between BG and MCA for different diets and over different times. To further investigate the effect of BG changes on mental health and cognitive performance, the daily average BG levels were compared to the MCA test results as well as the overall mental performance score to check for possible connections. Two statistically significant and interesting patterns emerged. Specifically, the number of times serial subtraction was performed, was negatively correlated to average BG levels (r = -0.44, p =0.0008), and the overall mental capability score was negatively correlated with BG levels (r = -0.36, p =0.007). Other MCA comparisons did not show any strong or significant correlations. Likewise, the weekly averages of BG did not show strong or significant correlations. However, the weekly average of the total mental capability score showed a trend for a negative correlation with BG levels (r = -0.66, p =0.077). The same analysis was performed for each diet group separately for each of the mental performance test scores and BG levels. While on the FFD, only the number of times serial subtractions were performed showed a statistically significant negative correlation (r = -0.643, p = 0.013). Additionally, the time to finish the trail-making test showed a positive correlation with BG levels during the RD (r = 0.631, p =0.027). The positive correlation between high BG levels and poor mental performance would be expected as BG levels are usually elevated when exposed to adrenalin or cortisol, leading to the "flight or fight" response, which is not characterized by careful, planned, or rational thinking [ref]. No other significant correlations were observed across dietary interventions. Overall, these correlations between BG and MCA highlight the connections between BG and mental performance.

These results are also aligned with what is known about BG and brain function because glucose is the primary energy source for the brain. The brain, although it comprises only about 2% of the body's weight, consumes around 20% of the body's energy [269]. Glucose is essential for maintaining neuronal function, neurotransmitter synthesis, and overall brain activity. Low BG levels can impair cognitive functions such as attention, memory, and executive functioning. When BG levels drop, the brain's energy supply diminishes, leading to symptoms like confusion and

difficulty concentrating, which reduce overall cognitive performance. Additionally, periods of acute hyperglycemia have shown impacts on mood and cognitive function in T2D patients [268].

Blood Glucose (BG) versus Sleep Quality – Temporal and Diet Effects

The brain is the largest consumer of glucose in the body and it is well-known that BG levels can affect an individual's state of consciousness [270,271]. To study this effect further, I looked at the relationship between BG and sleep quality for different diets and over different times. The average nighttime BG levels were compared to different sleep metrics. Notably, there were statistically significant negative correlations between BG at night and the frequency of light stage sleep (r = -0.410, p = 0.0026), time spent in light stage sleep (r = -0.464, p = 0.0005), frequency of wake stages (r = -0.366, p = 0.0076), time spent in wake stages (r = -0.437, p = 0.0012), and the total time asleep (r = -0.414, p = 0.0023). These results suggest that higher BG levels at night relate to less time spent in light sleep stages, less awake time during the night, and overall reduced sleep duration. Several studies have indicated a significant relationship between nighttime BG levels and various aspects of sleep [272,273]. For instance, elevated BG levels have shown associations with non-REM sleep, whereas REM sleep was associated with more stable BG levels [274].

A comparison was performed between sleep metrics and the next day (wake-time) BG average – across all diets. There were significant negative correlations between next-day BG averages and sleep metrics from the night before (frequency of light stage (r = -0.340, p = 0.0156), time in light stage (r = -0.388, p = 0.0054), time in wake stage (r = -0.482, p = 0.0004), total time asleep (r = -0.328, p = 0.020), and the percentage of HR above resting HR (%) (r = -0.305, p = 0.031). These results suggest that higher BG levels measured on the next day are associated with less time spent in light sleep stages, less time awake during the night, overall reduced sleep duration, and lower levels of HR above resting levels during sleep of the night before (Figure 4.15). Figure 4.16 shows a heatmap of the correlation analysis performed between various sleep metrics. These findings were observed across all diets. Therefore, these data suggest a possible association between poor sleep quality and elevated BG, regardless of diet.

Studies have shown that individuals who do shift work tend, which have disrupted circadian rhythms and relatively poor quality sleep, to have a higher risk for diabetes and higher overall levels of BG [275]. Studies have also shown that disrupted sleep leads to higher levels of stress, higher levels of stress hormones (cortisol) and a corresponding increase in BG [276]. Disrupted circadian rhythms from sleep deprivation or flying across multiple time zones also increase wake-time BG levels, reduce glucose tolerance and cuts-down insulin sensitivity during the day [277]. These findings simply underline the fact that poor sleep hygiene leads to increased BG and that chronically poor sleep hygiene can lead to chronic conditions such as heart disease and diabetes.

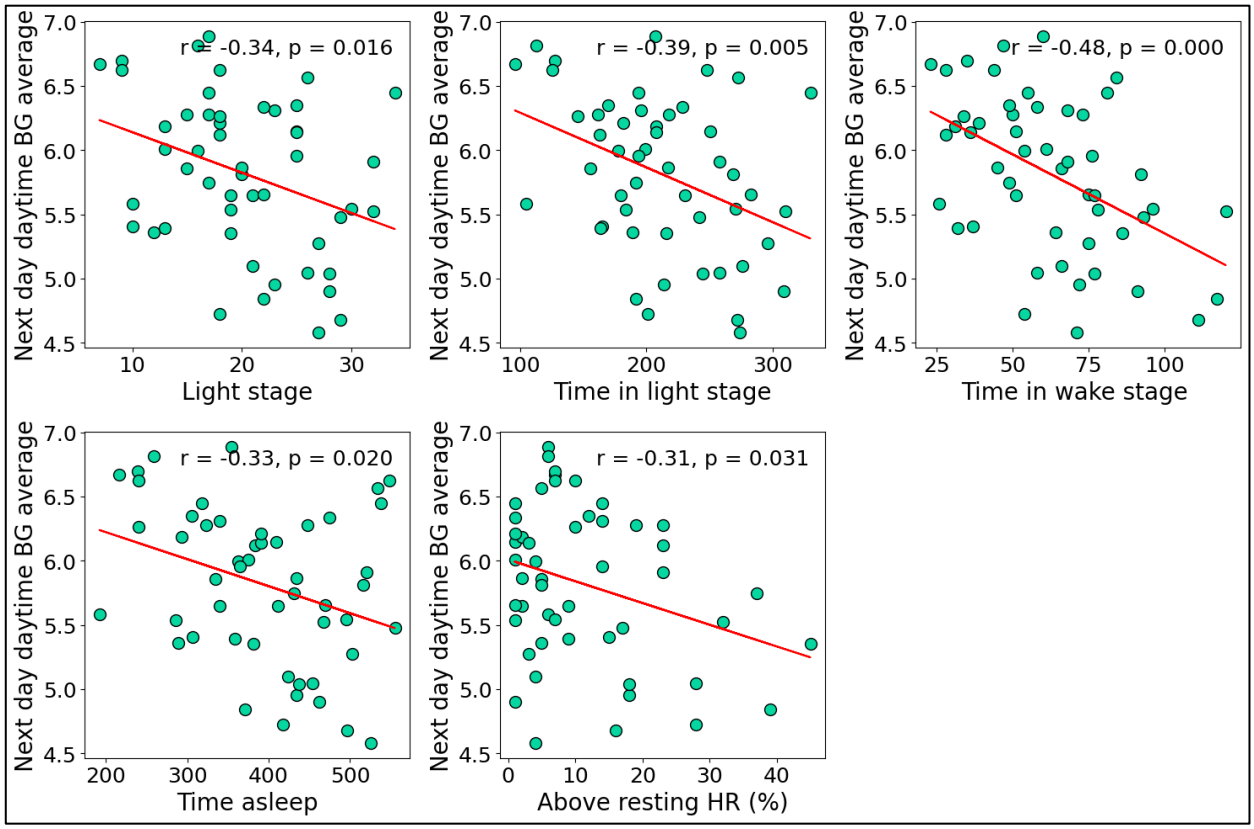

Figure 4.15 - The correlation analysis between daytime BG levels and sleep metrics from the night before. The average BG levels returned significant correlations with the time spent in the light stage, wake stage, the overall time asleep, the frequency of light stage and the sleep HR levels above resting HR.

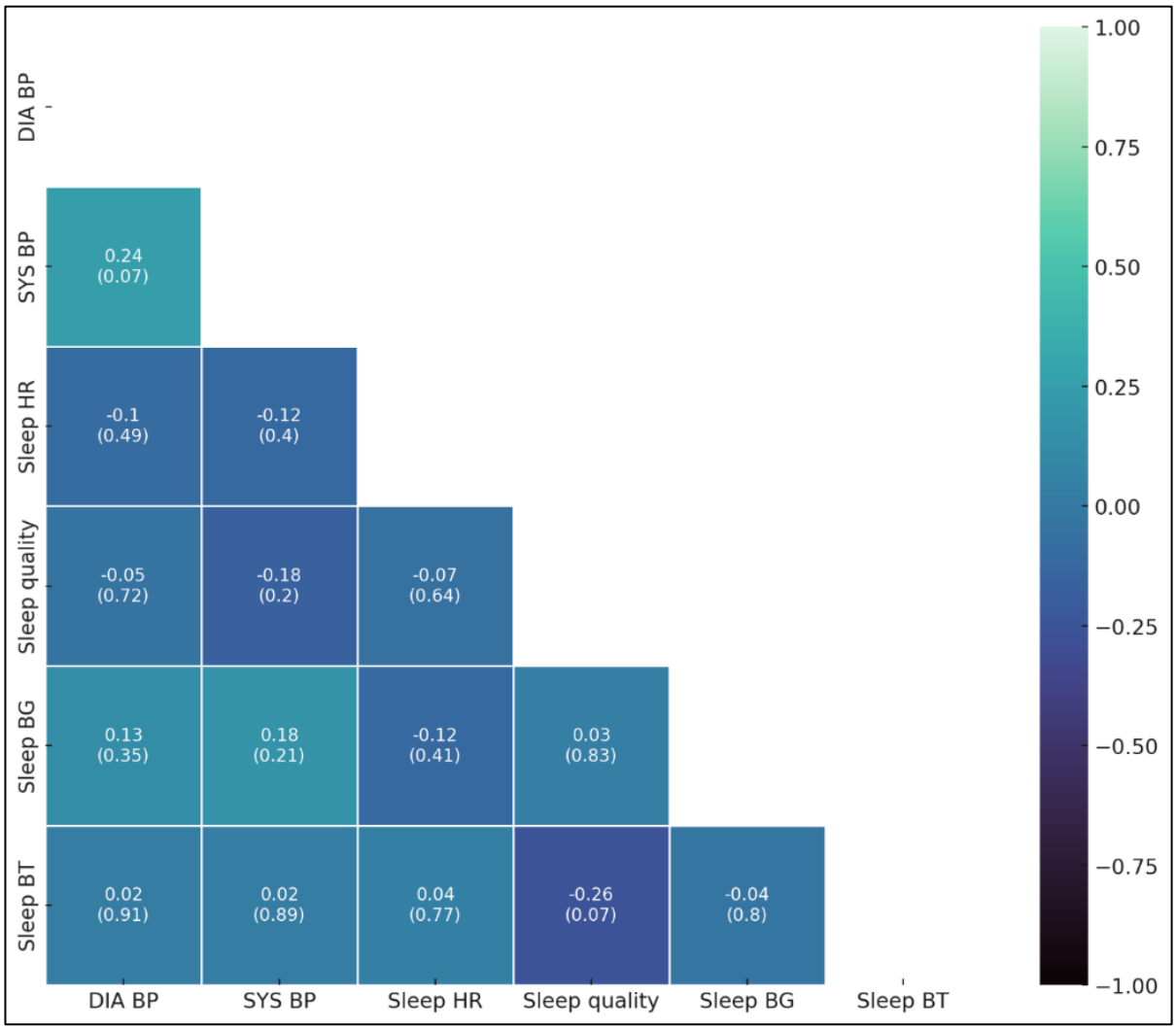

Figure 4.16 - The correlation heatmap of sleep metrics. The correlation coefficient and (p values) of each comparison is presented in each cell.

Heart Rate (HR) versus Blood Pressure (BP) – Temporal and Diet Effects

It is well known that an increased HR elevates BP while a reduced HR decreases BP [278]. Likewise, it is known that certain diets can change BP and HR [279,280]. To explore this effect further, I looked at the relationship between HR and BP for different diets and over different mealtimes. Overall, the relationship between HR and BP as mediated by diet and meals was relatively small.

The comparison between the daily average HR levels and BP levels measured at different times only showed a statistically significant correlation between the daily HR average and SYS BP measured after lunch (r = -0.378, p =0.007), and SYS BP measured at night (r = 0.280, p =0.049). Other meals, other diets, and other measures of BP (namely DIA BP) did not show any statistically significant correlations. The lack of a strong "signal" between BP and HR from dietary or mealtime effects was not entirely unexpected. This is because shorter-term diets (1-2 weeks) are not known to affect BP in very substantive ways [281], while shorter term diets, and even individual meals or foods, are known to affect HR [282]. Figure 4.17 shows SYS and DIA BP and HR changes at the time of BP measurements along with the polynomial trendline to illustrate the overall pattern for each dietary intervention. Inspection of this Figure shows that there is a general decreasing trend over the day for DIA BP for all diets. On the other hand, for SYS BP different minimums occur at different times for different diets. For the FFD the minimum SYS BP occurs after lunch, for the MD, the minimum SYS BP occurs after dinner, for the KD, the minimum SYS BP occurs after breakfast while for the RD, the minimum SYS BP occurs early in the morning. Diurnal blood pressure (BP) trends normally exhibit a 24-hour fluctuation with BP rising in the early morning, peaking mid-morning (8-10 a.m.) then stabilizing at a high level during the day, potentially declining slightly in the afternoon, then rises again in the early evening before bedtime. During sleep, BP drops, known as the "nocturnal dip," reflecting reduced sympathetic activity. Only the MD and the RD led to SYS BP trends that followed the "typical" diurnal cycle. The altered SYS BP trends with the KD and FFD suggest that a fundamental change in cardiovascular function had occurred. In particular, for the FFD, the SYS BP temporal trend exhibited a form of "reverse dipping," where the BP seemed to rise through the afternoon and evening and stay high at night, where it normally should have fallen. Reverse dipping is indicative of cardiovascular problems [283]. On the other hand, the KD diet led to a very blunted daytime (and nighttime) variation in SYS BP. Blunted diurnal BP variation is also a risk indicator of cardiovascular problems [284].

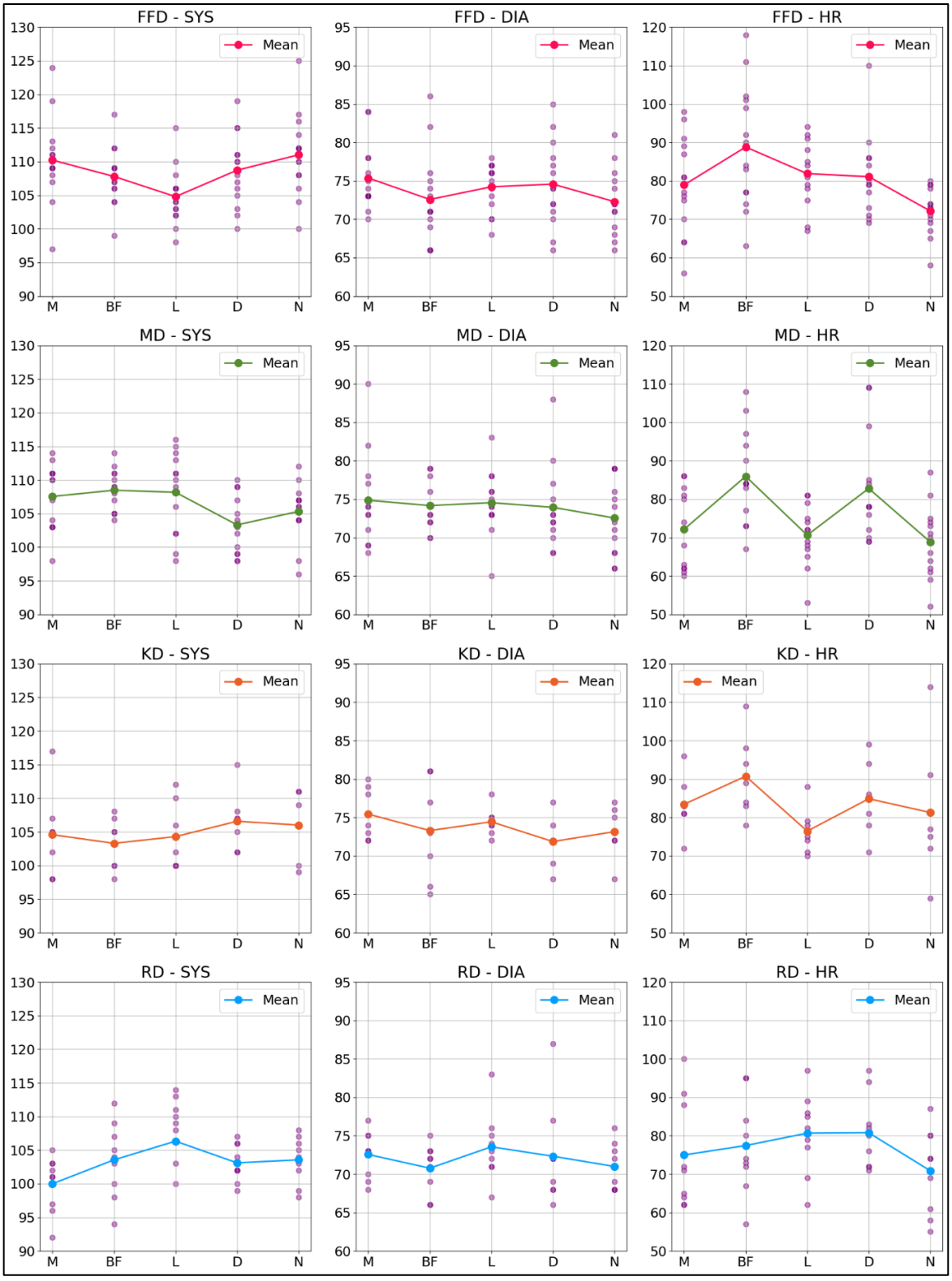

Figure 4.17 - SYS and DIA BP and HR changes at the time of BP measurements. M: morning, BF: after breakfast consumption, L: after lunch consumption, D: after dinner consumption, and N: nighttime

Heart Rate (HR) versus Body Temperature (BT) – Temporal and Diet Effects

Increased HR which usually arise from increased metabolic or physiological activity will lead to increased BT [285]. To explore this effect further, I looked at the relationship between HR and BT for different diets and over different times. Specifically, longitudinal hourly HR and BT levels were compared across diets to observe any correlations between them. The cross-diet correlation analysis between hourly HR levels and BT revealed varying relationships throughout the day. For example, strong positive correlations were found at 00:00 hr (r = 0.606, p = 0.0028), 08:00 (r = 0.626, p = 0.0018), 09:00 hr (r = 0.551, p = 0.0079), 10:00 hr (r = 0.560, p = 0.0067), 11:00 hr (r = 0.615, p = 0.0023), and 12:00 hr (r = 0.679, p = 0.0005) (Figure 4.18). Additionally, a correlation coefficient of 0.62 was calculated between HR and BT (measured hourly, across all diets) which indicates a strong positive relationship, confirming the well-known fact that as BT increases, HR tends to increase as well. The extremely low p-value (p =1.76×10 −57) signifies that this correlation is highly statistically significant (Figure 4.19). These findings confirm the expected relationship between BT and HR, particularly around midday. As BT increases or decreases, HR tends to follow a similar pattern during these times. As BT increases, the metabolic rate rises, requiring more oxygen and nutrients, which forces the heart to pump more blood more quickly leading to an elevated HR. Thermoregulation processes also contribute, as the body increases HR to dissipate heat through the skin. Physical activity, which generates heat in muscles and raises BT, also increases HR to supply oxygen-rich blood to the muscles. Additionally, the autonomic nervous system regulates both HR and BT, with sympathetic activation during stress or exercise increasing both parameters [286,287].

Figure 4.20 displays the average HR and BT levels at different times of the day, along with the polynomial trendline to illustrate the overall pattern for each dietary intervention. As seen previously, there is a clear sinusoidal dependence with HR and BT over the course of the day for both the MD and the KD, with a minimum near 05:00 hr and a maximum near 18:00 hr. On the other hand, for the FFD and RD, the period is longer, with just a single maximum at 18:00 hr. The larger amplitude changes for KD, FFD and MD suggest greater metabolic variation or metabolic

stress over the course of the day, while the smaller amplitude changes with the RD suggest that the body is more adapted to this diet and experiences less metabolic stress.

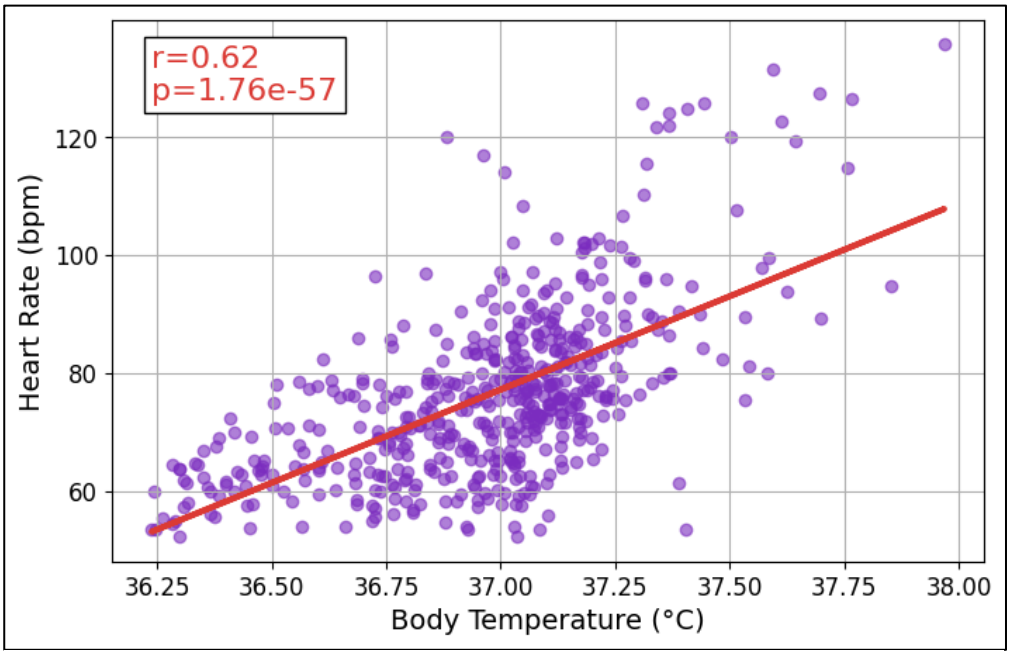

Figure 4.19 - Correlation between HR and BT levels across all dietary interventions.

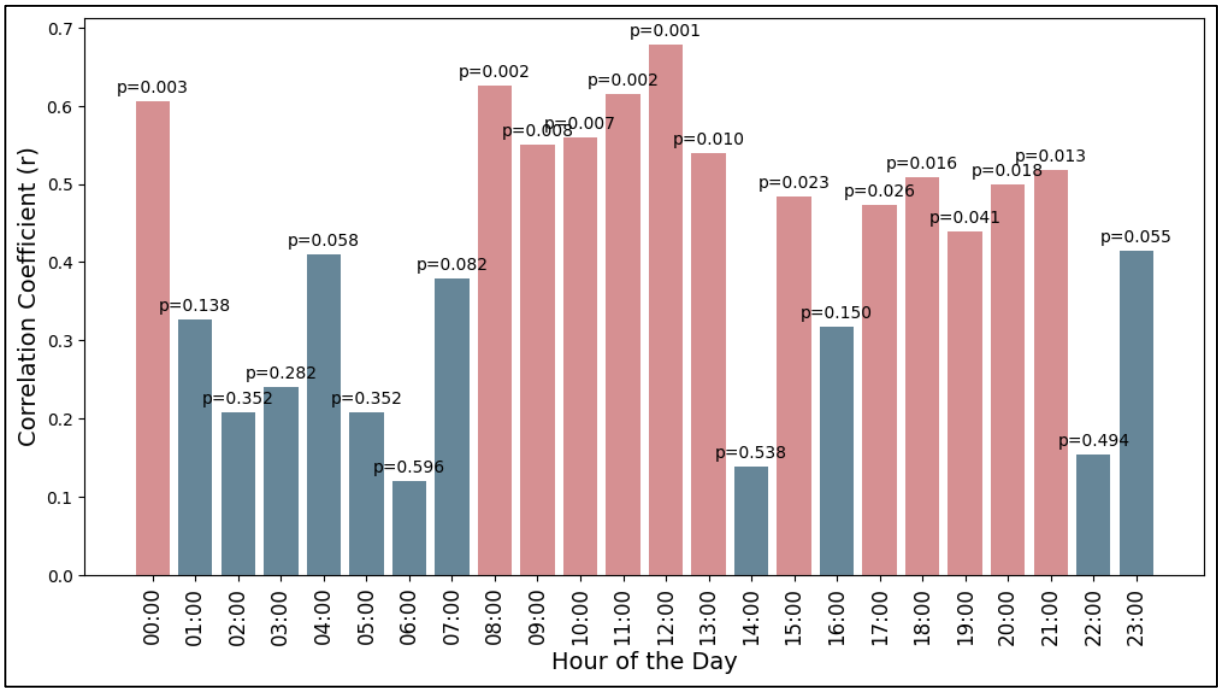

Figure 4.18 - Correlation coefficients and p values of BT vs HR at different times of day. P-values are presented on each histogram bar and the significant correlations ($p<0.05$) are colored red.

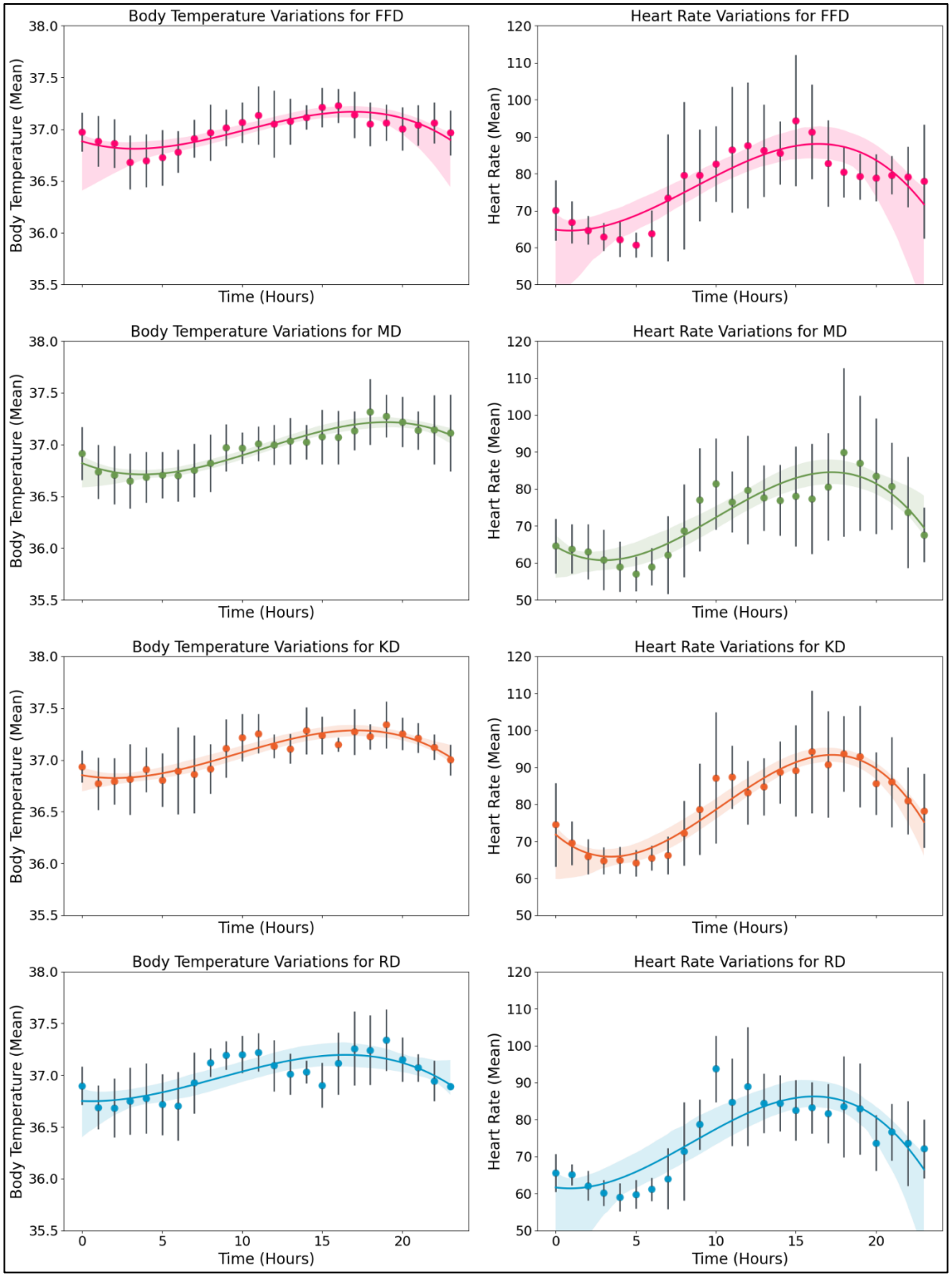

Figure 4.20 - The average HR and BT levels at different times of the day, along with the polynomial trendline to illustrate the overall pattern for each dietary intervention

Heart Rate (HR) versus Profile of Mood State (POMS)

Studies have shown that when individuals are exercising (therefore having significantly increased HRs) their moods change to be less tolerant, more reactive and less thoughtful [288]. However, there is relatively little data on how modest HR changes affect mood. To explore this effect further, I looked at the relationship between HR and POMS for different diets and over different times. Specifically, HR levels were compared to mood scores obtained from POMS, where a higher score in each mood category indicates a greater presence of that mood. Although not significant, vigor had a moderate negative correlation (r = -0.408, p =0.074) with average HR levels, indicating that higher HR levels might be associated with decreased vigor levels. The other mood metrics showed weaker and less significant correlations with HR levels. Studies on how emotions like anger, fear, and amusement affect HR and heart rate variability (HRV) have shown that a higher HR is often associated with lower feelings of positive energy and vigor. For instance, during emotional states that increase heart rate, such as anger or fear, people often report lower vigor compared to more neutral or positive states [289]. In a study by Duncan et al. [290], it was found that moderate-intensity cycling whilst viewing a green environment had significant effects on mood states. Specifically, after the exercise, the mood scores for fatigue were higher, and scores for vigor were lower, indicating that increased heart rate post-exercise correlated with decreased feelings of vigor. In addition, in a study conducted by Panchal et al. [291] that utilized the POMS to assess changes in specific mood factors, including vigor, before and after a meditation session, it was concluded that reduced HRV, often associated with a higher HR, was correlated with lower vigor scores, illustrating a negative correlation between HR and vigor.

Heart Rate (HR) versus Mental Cognitive Assessment (MCA)

Studies have shown that when individuals are exercising (therefore having significantly increased HRs) their ability to solve complex problems is reduced [292]. However, there is relatively little data on how modest HR changes affect MCA. Daily average HR levels were compared to MCA test results as well as the overall mental performance score to check for possible connections. As expected, the results from the serial subtraction test (r = -0.336, p =0.013) and digit span test (r = -0.354, p =0.009) had significant negative correlations with HR levels. Additionally, the time to finish the trail-making test had a positive correlation with HR levels (r = 0.508, p =0.00009)

which is also indicative of poor mental performance. These tests are commonly used to assess working memory and attention, which can be influenced by physiological states such as HR. Studies indicate that increased HR, often a marker of stress, can impair performance on cognitive tasks such as the serial subtraction test. Higher stress levels, reflected by increased HR, can reduce the capacity of working memory and processing speed required for tasks like serial subtraction [289,293]. It has been shown that increased sympathetic activity and decreased parasympathetic activity are associated with worse performance in cognitive tasks. This includes tasks like the digit span test, where a higher HR correlates with lower performance due to stress or autonomic imbalance [293].

Heart Rate (HR) vs Sleep Quality

Individuals with infections or fevers (characterized by very high HR) or who are highly stressed (also higher HR) tend to have poor sleep quality [294]. However, there is relatively little data on how modest HR changes affect MCA. Night-time sleeping HR levels were compared to sleep metrics. Night-time HR levels showed a positive correlation with the time spent in the deep sleep stage (r = 0.292, p =0.036), and the frequency of light sleep stage episodes (r = 0.367, p =0.007). Sleeping HR levels and other sleep metrics did not reveal any other significant correlations. These data confirm the general observation that sleep quality is affected by modest changes in HR with an elevated HR leading to increased periodicity between deep sleep and light sleep.

Body Temperature (BT) vs Blood Pressure (BP) – Temporal and Diet Effects

It has been suggested that lower BT increases blood viscosity, leading to an increase in BP, while higher BT decreases blood viscosity, leading to a decrease in BP [295]. However, there is relatively little data on how modest BP changes are correlated with BT. To explore this issue further, I looked at the relationship between BT and BP for different diets and over different times. Comparing the daily average BT values to SYS and DIA BP levels did not reveal any significant correlations. However, when analyzed over more extended periods of time, a trend did emerge. For instance, the average over two days of BT and SYS BP showed a weak negative correlation (r

= -0.31, p =0.027), and the weekly DIA BP average showed a strong positive correlation to the weekly BT average (r = 0.782, p = 0.022). By comparing the BP levels with BT at the time of BP measurement, it was observed that BT in the morning was negatively correlated with SYS BP levels after breakfast consumption (r = -0.35, p = 0.020), while the BT after breakfast was positively correlated with DIA BP levels after breakfast consumption (r = 0.32, p =0.034). BT and SYS BP levels after lunch showed a significant negative correlation (r = -0.41, p = 0.006) (Figure 4.21).

Comparing the BP levels and the BT at the time of BP measurement for each dietary intervention, revealed that while on the KD, SYS BP levels after dinner were strongly and negatively correlated to nighttime BT (r = -0.68, p =0.015). While on the RD, morning DIA BP levels were almost perfectly correlated to BT in the morning (r = 0.98, p =0.004), and BT after breakfast consumption (r = 0.93, p =0.023). In addition, while on the RD, SYS BP, and BT levels after lunch consumption, were strongly and negatively correlated (r = -0.90, p =0.040) (Figure 4.22). Figure 4.23 shows the changes in SYS BP, DIA BP, and BT levels at the time of BP measurements for all the dietary interventions.

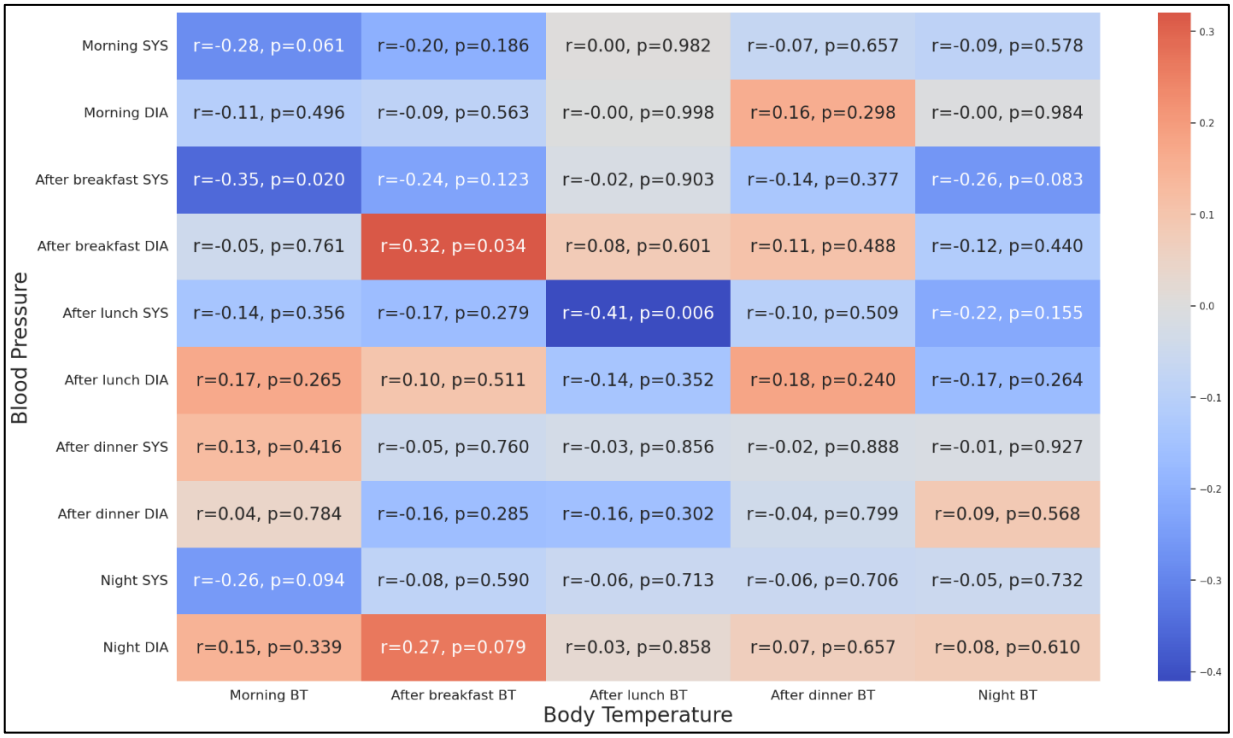

Figure 4.21 - The correlation between SYS and DIA BP and BT at the time of BP measurement

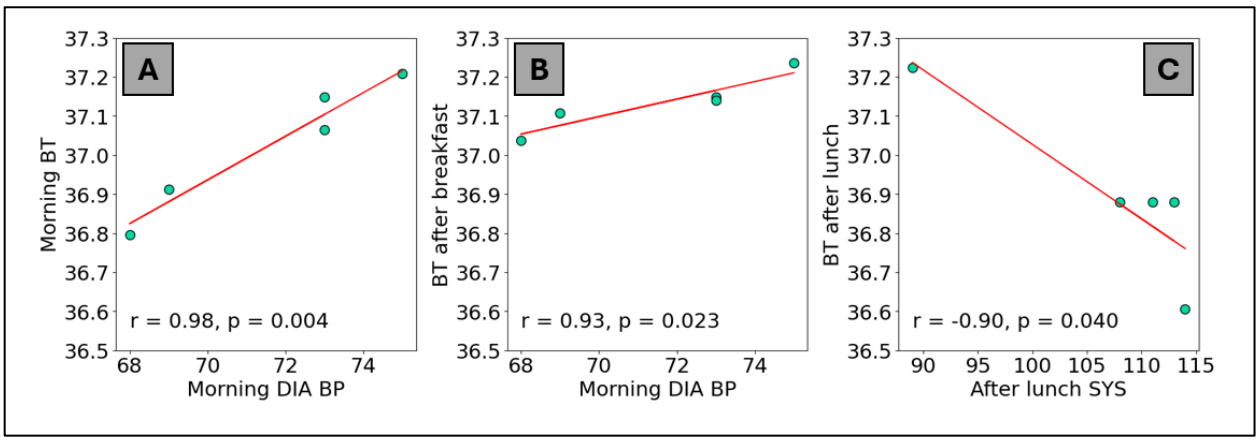

Figure 4.22 – Scatter plots illustrating the correlations between various blood pressure (BP) measurements and body temperature (BT) levels while on the RD. (A) The correlation between Morning DIA BP levels and Morning BT (r = 0.69, p = 0.039). (B) The correlation between Morning DIA BP levels and BT after breakfast (r = 0.57, p = 0.112). (C) The correlation between After lunch BP levels and BT after lunch (r = 0.16, p = 0.673). Each plot includes a trendline and annotation showing the correlation coefficient and p-value.

A number of studies have investigated the connection between BP and BT to help better understand how changes in body and ambient temperatures can affect BP levels. A study by Shinji [296] found that a significant SYS BP difference was associated with higher HR, moderate fever (≥37.5°C), and hypertension. These connections indicate a physiologic as well as pathologic association between BT and BP. In another study, it was demonstrated that lower BT increases blood viscosity, leading to a decrease in blood flow rate and a compensatory increase in BP. Conversely, higher BT decreases blood viscosity and lowers BP [295]. Studies have also shown that changes in home temperature, as well as seasonal and daily variations in temperature, are connected to changes in BP levels [297,298]. This suggests that external or environmental factors may also be involved in the correlation between BP and BT. However, further investigation on the effect of modest BT changes and BP variations is certainly needed.

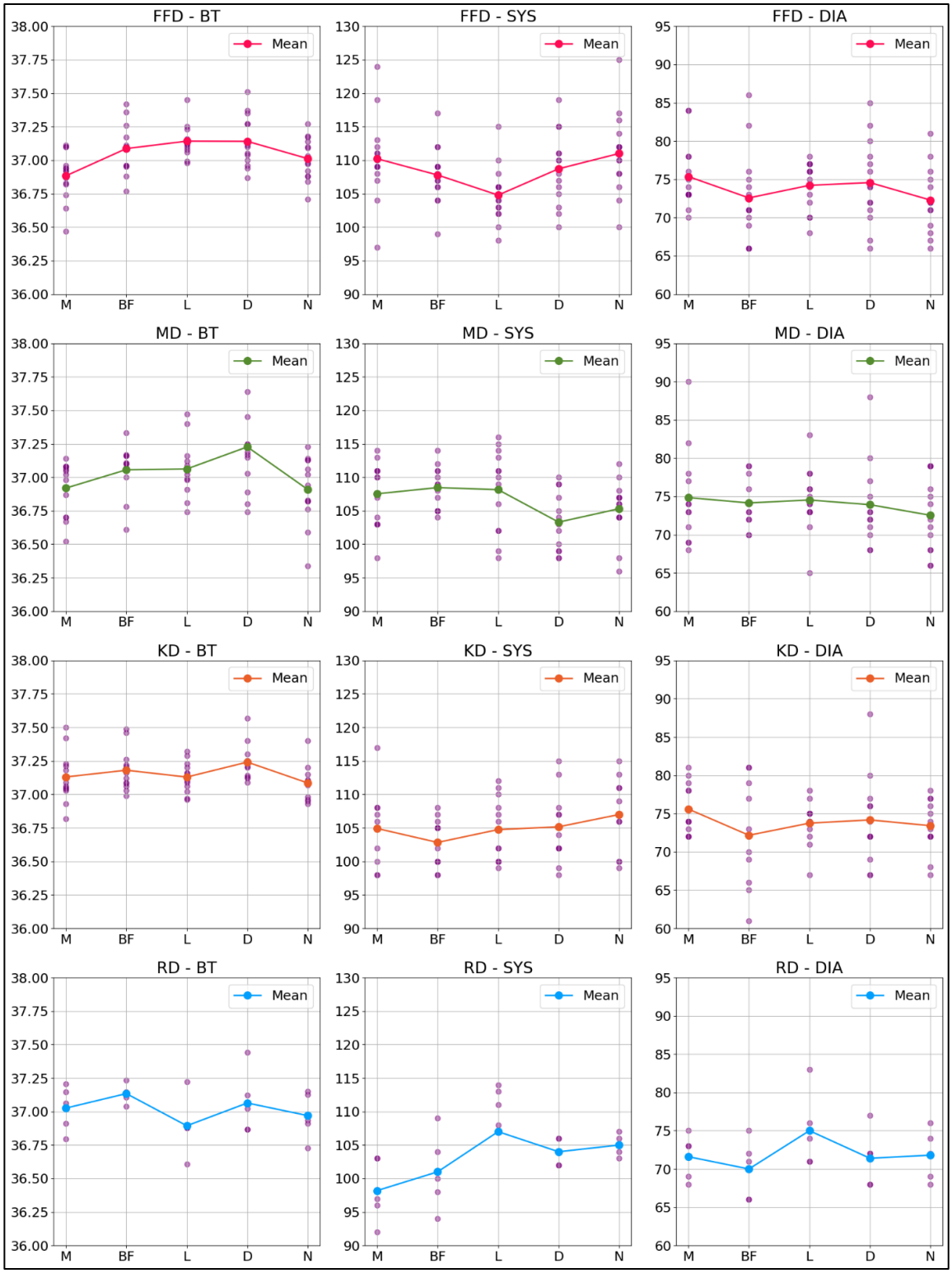

Figure 4.23 - Changes in SYS and DIA BP and BT at the time of BP measurement across all dietary interventions. M: morning, BF: after breakfast consumption, L: after lunch consumption, D: after dinner consumption, and N: nighttime

Body Temperature (BT) vs Profile of Mood States (POMS) – Temporal and Diet Effects

Given that fevers (characterized by high BT) and exercise/exertion (also characterized by high BT) can lead to feelings of exhaustion, malaise, and lethargy, I decided to explore whether modest changes in BT would affect mood. To explore this issue further, I looked at the relationship between BT and POMS for different diets and over different days. Specifically, the results from the daily mood questionnaires were compared to the daily average BT levels to uncover possible connections. Overall, the correlations between POMS and average BT were found to be weak and not statistically significant across all diet groups. The analysis was repeated for each dietary intervention separately. The correlations remained weak or insignificant across all dietary interventions and mood categories.

Body Temperature (BT) vs Mental Cognitive Assessment (MCA) – Temporal and Diet Effects

Body temperature can significantly impact cognitive function. Both hyperthermia (elevated BT) and hypothermia (reduced BT) are known to affect cognitive processes such as memory, attention, and executive function [299]. To explore this effect further, I looked at the relationship between BT and MCA test results. The comparison between daily average BT levels and various MCA scores showed weak and statistically insignificant correlations with BT suggesting that there may not be a strong direct relationship between these variables. A correlation analysis was also performed between the two variables for each dietary intervention separately. While on the MD the average BT levels showed a significant negative correlation with the time (in seconds) to finish the trail-making test ($r = -0.564$, $p = 0.036$), and the total mental assessment score ($r = -0.667$, $p = 0.009$). Likewise, while on the RD, the average BT was negatively correlated to the number of times to do subtractions in the serial subtraction test ($r = -0.608$, $p = 0.027$). None of the other test scores showed a significant correlation to BT levels during different dietary interventions. There is relatively little data on how increased BT is correlated with decreased mental performance, however, several studies have investigated the effect of environmental temperature changes on mental cognitive function, and it has been shown that different environmental conditions (such as

unusually cold, or hot environments) can negatively impact cognitive function [299–301]. In addition, elevations in core body temperature, as seen when one has a fever, can also impact cognitive function [302]. The release of inflammatory cytokines during a fever can cross the blood-brain barrier, leading to disruptions in normal brain processes. This neuroinflammation can result in cognitive impairments such as confusion, memory problems, and attention deficits [302]. Fever also increases the body's metabolic rate, including in the brain. This heightened metabolic activity can lead to the production of modestly toxic metabolic byproducts and oxidative stress markers, which may impair cognitive functions. Additionally, fever-induced dehydration and electrolyte imbalances can further exacerbate these cognitive impairments, impacting overall brain function [303], suggesting that extreme temperature changes whether in the environment or the body temperature changes, can affect mental performance. However, further investigation on the effect of modest BT changes and mental performance is certainly needed.

Body Temperature (BT) vs Sleep Quality – Temporal Effects

Fever can lead to fragmented and less restful sleep. The body's elevated temperature can cause discomfort, making it difficult to fall asleep and stay asleep throughout the night. This disruption can lead to multiple awakenings and reduced overall sleep duration [302]. Likewise, elevated room temperatures that are uncomfortably above room temperature (20-25 ºC) can also lead to disrupted sleep. For this reason, I decided to investigate the effect of modest BT changes on sleep. Sleep time BT was compared with different stages of sleep to monitor for possible associations. No significant correlations were observed between the nighttime BT and the time spent in deep, REM, light, or wake stages. Additionally, no significant correlations were observed between BT and the sleeping HR, or restlessness levels. These results suggest that modest changes in BT at night do not have strong correlations with most sleep stages or metrics, indicating that nighttime BT might not be a significant predictor of the structure or quality of sleep based on the current dataset.

However, there was a modest negative correlation between BT at night and sleep quality score with a p-value nearing significance (r = -0.247, p = 0.072), suggesting a potential trend where an elevated BT at night could be associated with lower sleep quality. Additionally, a comparison was performed between sleep metrics and average daily BT levels of the next day.

Unfortunately, this analysis revealed no statistically significant correlations between the next day daytime BT average and any of the sleep stages or metrics.

Fever, characterized by an elevated BT, significantly affects sleep quality through several interconnected mechanisms involving the body's immune and metabolic responses. The body's immune response to fever triggers the release of cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α). These cytokines play a dual role in sleep regulation: while they promote sleepiness, they also contribute to sleep fragmentation and disturbances in the sleep cycle [304,305]. This can result in more frequent awakenings and less restorative sleep. In addition to the inflammatory response, fever increases the metabolic rate, leading to higher production of metabolic byproducts and oxidative stress. These changes can impair sleep by disrupting normal metabolic processes essential for maintaining sleep integrity [306]. Furthermore, the physical symptoms associated with fever, such as sweating, chills, and body aches, add to the discomfort, making it even harder to achieve and maintain quality sleep. Studies have shown that fever may decrease the amount of slow-wave sleep (deep sleep) and REM sleep, both of which are crucial for physical and cognitive restoration. This alteration in sleep architecture can result in less restorative sleep, contributing to feelings of fatigue and cognitive impairment during waking hours [302]. Additionally, while fever disrupts sleep, it can also increase overall sleepiness due to the body's need to conserve energy and fight infection. However, this increased sleepiness often results in more time spent in lighter stages of sleep, which are less restorative than deeper sleep stages [change the ref]. All of this information highlights the physiological connection between BT and sleep quality. However, further investigation is needed to understand the association between subtle BT changes on sleep quality.

Blood Pressure (BP) vs Profile of Mood States (POMS) – Temporal and Diet Effects

Hypertension and BP variability, which refers to fluctuations in BP over time, have been linked to mental health [307]. To explore this effect further, and to investigate the possible effects of modest BP changes on mood, I looked at the relationship between BP and the results from the POMS for different diets and over different times. When I compared the daily average SYS BP and DIA BP levels to POMS mood metric scores, no significant correlations were detected.

However, when the correlation analysis was performed on average BP levels and mood metrics for each dietary intervention, a near-perfect positive correlation between the average SYS BP and confusion (r = 0.998, p =0.002) was observed while on the MD, and a significant, near-perfect negative correlation between the average DIA BP and tension (r = −0.980, p =0.034) while on the KD (Figure 4.24). While the observed correlations are significant, it is important to note that the small number of data points (n = 5/intervention) may limit the robustness of these findings. The mood questionnaires were completed on days 1, 4, 7, 10 and 14 of each intervention, limiting the number of data points for each intervention. These results should be interpreted with caution, and further investigation with larger datasets would be needed to confirm these trends.

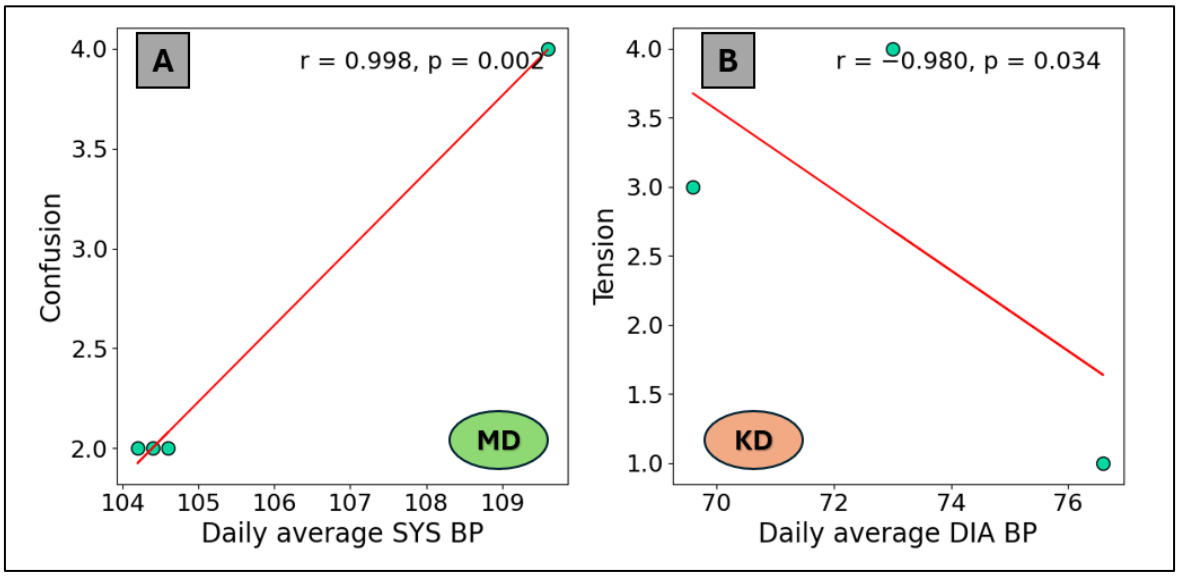

Figure 4.24 - Scatter plots illustrating the significant relationships between blood pressure and mood metrics. A: The relationship between Daily Average Systolic Blood Pressure (SYS BP) and Confusion during the **MD** diet group. B: The relationship between Daily Average Diastolic Blood Pressure (DIA BP) and Tension during the **KD** diet group. The scatter plots show individual data points, with a trend line (in red) indicating a strong positive correlation

It has been shown that high BP (hypertension) is associated with an increased risk of anxiety and depression [308]. The positive correlation observed between SYS BP and confusion can be due to its impact on cerebral autoregulation and ANS balance [287]. The ANS regulates bodily functions, including BP and stress responses. Dysregulation in the ANS can lead to both hypertension and increased anxiety or depression. For instance, high BP can result from chronic

activation of the stress response, which also predisposes individuals to anxiety and depression [307]. Additionally, hypertension is associated with chronic inflammation and alterations in hormonal levels, such as cortisol (the stress hormone). Elevated cortisol levels can lead to mood disorders, including depression and anxiety [307]. These data confirm the general observation that mood and mental health are affected by changes in BP. However, the modest changes observed in this dataset might not be the best predictors of these effects on mood.

Blood Pressure (BP) vs Mental Cognitive Assessment (MCA) – Temporal Effects

Research has shown that blood pressure (BP) significantly impacts cognitive function [309,310]. For instance, high BP (hypertension) can negatively impact thinking by damaging blood vessels and causing arterial stiffness, leading to reduced blood flow to the brain [311]. Conversely, low BP (hypotension) can cause inadequate blood flow to the brain, leading to symptoms like dizziness, confusion, and fainting. Chronic low blood pressure can result in poor oxygen delivery to brain tissues, affecting cognitive performance and causing issues with focus and mental clarity [312]. To investigate the association between modest BP changes on mental cognitive function, the average SYS BP and DIA BP values were compared to daily mental cognitive assessment scores. Only one notable finding was made. In particular, the reaction time (measured in seconds) showed a positive correlation to average daily DIA BP values (r = 0.614, p =0.020). No other correlation was observed between the mental assessment scores and average SYS BP or DIA BP values.

However, by comparing the test results to the BP measurements at different times of each day, it was revealed that the trail-making test results showed a strong negative correlation to after-breakfast DIA BP (r = -0.827, p = 0.00026) and a good positive correlation to the DIA BP after dinner (r = 0.623, p = 0.017). Additionally, the time to finish the trail-making test was negatively correlated to after-dinner SYS BP levels (r = -0.534, p = 0.049), and the overall mental assessment score was positively correlated to after-dinner DIA BP (r = 0.547, p =0.043). The comparison between the daily mental assessment test results and daily average SYS BP levels showed a significant negative correlation (r = -0.42, p = 0.0031), while no correlation was observed with daily average DIA BP levels. A near-perfect negative correlation was also observed over the weekly averages of mental capability assessment results and SYS BP (correlation coefficient = -

0.89, p = 0.0027). A comparison of SYS BP and DIA BP levels and the mental performance test scores for each group separately did not return any significant results.

Past research has indicated that high SYS BP can lead to reduced cerebral blood flow, which impairs cognitive functions such as attention and memory, contributing to confusion. SYS BP variability has also been associated with cognitive impairments due to the stress it places on cardiovascular and nervous systems [310,313,314]. Moreover, the increased cognitive load and confusion associated with high SYS BP can be attributed to the elevated workload on the brain and the body's attempt to regulate these fluctuations [313,314]. The positive correlation between higher nighttime HR levels and sleep stages further supports the idea that autonomic nervous system imbalances during sleep can influence cognitive states.

Blood Pressure vs Sleep Quality – Temporal Effects

Blood pressure changes, especially hypertension, have been associated with decreased quality of sleep [315]. To investigate the effect of modest BP changes on sleep quality, the average SYS BP and DIA BP values were compared to daily sleep quality scores and other sleep metrics. The daily average DIA BP levels showed no significant correlation to sleep quality, or the time spent in deep sleep, in REM stages, light stages, wake stages, sleeping HR, or restlessness levels. However, average daily SYS BP values were weakly and negatively correlated with the time spent in light stage sleep (r = -0.287, p = 0.035), and the frequency of light stage sleep (r = -0.311, p = 0.022). Additionally, a comparison was performed between various sleep metrics and nighttime BP levels. Nighttime DIA BP levels did not show any significant correlations; however, nighttime SYS BP levels were modestly and negatively correlated to time spent in light stage sleep (r = -0.403, p =0.0037), time spent in REM stage sleep (r = -0.292, p =0.0396), and the total time asleep (r = -0.341, p = 0.015).

A similar correlation analysis was performed to check for possible connections between different sleep metrics and BP levels on the following day. Daily average BP SYS and DIA levels were compared to various sleep metrics from the night before. This analysis revealed good negative correlations between the next day's SYS and several sleep metrics. Specifically, the frequency of light stage sleep (r = -0.455, p = 0.0013), time in light stage sleep (r = -0.403, p =

0.0050), frequency of wakeful stages (r = -0.417, p = 0.0035), and total time asleep (r = -0.381, p = 0.0083) all showed statistically significant negative correlations with next day SYS BP levels. These results suggest that higher SYS BP occurs after a restless night characterized by shorter durations in light sleep stages, less wake time during the night, and overall reduced sleep duration. However, this analysis did not find any significant correlations between the next day's DIA BP and other sleep metrics.

BP has been shown to affect sleep through various mechanisms. Autonomic nervous system (ANS) dysfunction, characterized by imbalances between sympathetic and parasympathetic activity, disrupts BP regulation and sleep patterns. This leads to poor sleep quality and increased BP [315,316]. Hormonal regulation also plays a crucial role. Elevated levels of stress hormones such as cortisol, often resulting from poor sleep, can increase BP and further disrupt sleep architecture [315,317]. Additionally, insufficient sleep duration and poor sleep quality are linked to heightened sympathetic nervous system activity and reduced parasympathetic activity, contributing to elevated BP and cardiovascular risk [316,317]. These interconnected mechanisms create a feedback loop where disturbed sleep worsens hypertension, which in turn impairs sleep further, highlighting the complex interplay between sleep and cardiovascular health.

Sleep Stages versus Physiological Parameters

Research has shown that there is a connection between sleep and various physiological measures such as blood pressure (BP), body temperature (BT), heart rate (HR), and blood glucose (BG) levels [318–320]. As explained earlier, connections were found between various physiological measures and sleep. A correlation analysis was performed on different sleep metrics to further explore relationships among stages and physiological indicators during sleep. Figure 4.25 is the heatmap providing a visual representation of the correlation coefficients (r values) and their corresponding p-values between various sleep-related variables. Notably, variables such as the time spent in light stage sleep and the total time asleep show a strong positive correlation with each other (r = 0.88, p = 3e-18), suggesting that as the total sleep time increases, the time spent in light sleep tends to increase. Interestingly, variables related to HR during sleep showed weaker correlations with other sleep stages, suggesting that HR variability might be influenced by additional factors not captured solely through sleep stages. In addition, a positive correlation (r =

0.43, p = 0.0012) was observed between sleep quality and the time in deep-stage sleep, suggesting that longer durations spent in deep sleep are associated with better perceived sleep quality. On the other hand, a weak negative correlation (r = -0.26, p = 0.058) was observed between sleep quality and restlessness score. This indicates that higher levels of restlessness during sleep, which could involve frequent awakenings, movements, or snoring, corresponded to poorer sleep quality ratings. Correlations with other stages such as REM and light sleep were less pronounced.

The association between sleep and various physiological measures likely happens through several mechanisms. As mentioned earlier, during deep sleep, BP typically drops, a phenomenon known as "nocturnal dipping," which is crucial for cardiovascular health [321]. Disrupted sleep patterns, such as those seen in sleep apnea, can prevent this dipping, leading to hypertension [321]. BT also follows a circadian rhythm, decreasing during sleep and reaching its lowest point in the early morning. Poor sleep can disrupt this pattern, causing elevated nighttime temperatures [319]. Additionally, HR decreases during deep sleep, allowing the heart to rest and recover, but sleep deprivation can result in a consistently higher HR, adding stress to the cardiovascular system [313]. Furthermore, sleep plays a critical role in glucose metabolism and insulin sensitivity; insufficient sleep can impair insulin response, elevating BG levels and increasing the risk of T2D. These physiological disruptions highlight the essential role of sleep in maintaining overall health and preventing chronic conditions [318,320].

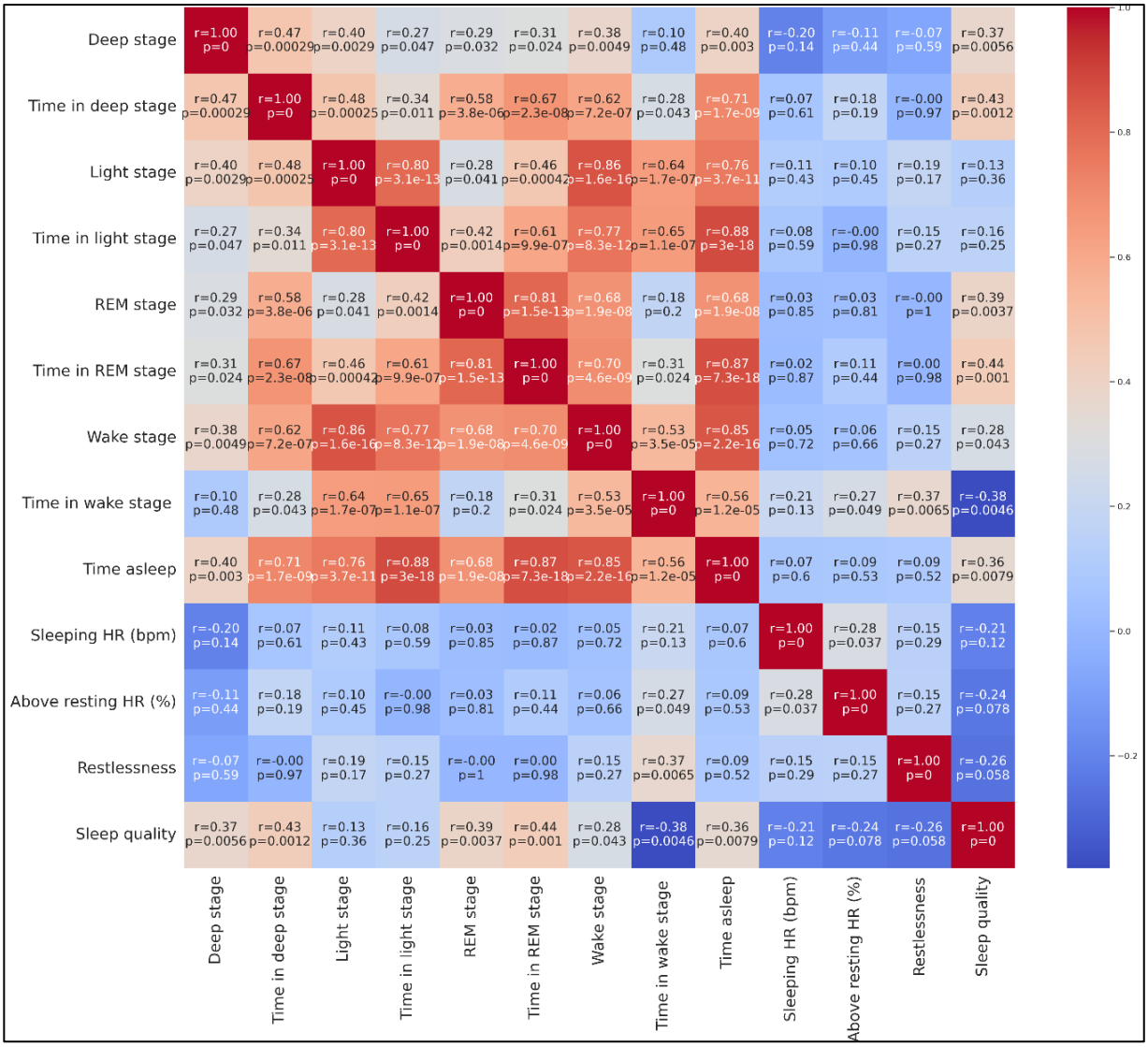

Figure 4.25 - The correlation heatmap between different sleep stages and metrics, showing several significant correlations. Each cell represents correlation coefficient (p value).

4.2. Cytokine Responses to Physiological Variables:

Diet and lifestyle choices significantly influence the body's inflammatory responses, a crucial factor in overall health and disease prevention. To explore this relationship, I analyzed cytokine levels in plasma samples using a Proinflammatory Proteomics Kit, as detailed in Chapter 2. The proinflammatory proteome offers a comprehensive snapshot of the proinflammatory proteins (largely cytokines) expressed in the body at a given time, reflecting dynamic physiological changes and responses to external factors like diet. By examining the proinflammatory proteome, we can identify specific cytokines and cytokine pathways associated with different dietary patterns. This approach provides a deeper understanding of the relationships between the dietary period and cytokine concentrations in various biological fluids. I compared the proinflammatory (i.e., cytokine) proteome with physiological data to correlate specific protein changes with observable health outcomes. This dual analysis was expected to help uncover more of the molecular mechanisms behind dietary effects on inflammation and can guide personalized nutrition strategies to optimize health and manage inflammation-related conditions.

Cytokine Proteome versus BG, BT, BP, and HR

It has been suggested that inflammatory protein biomarkers are correlated with physiological responses such as BG, BT, BP, and HR. To investigate the effects of short-term dietary interventions on cytokines and the correlation between inflammation biomarkers and physiological responses, the concentrations of IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, IL-8, and TNF-α cytokines were compared against the daily average BG, BT, BP and HR levels. Among the more interesting findings were that BG levels showed a significant positive correlation with IL-13 levels (r = 0.510, p = 0.030), and a significant negative correlation with TNF-α (r = -0.495, p = 0.037). Additionally, the average SYS BP was negatively correlated with plasma concentration of IFN-γ (r = -0.507, p = 0.032), IL-10 (r = -0.695, p = 0.0014), and IL-2 (r = -0.648, p = 0.0036), while DIA BP levels did not show any significant correlations with any cytokines. Likewise, HR and BT levels did not reveal any strong or significant correlations with any of the cytokine levels. Figure 4.26 shows a heatmap of the correlations between the cytokine data and average BG, BT, BP, and HR levels.

The results concerning BG levels and cytokine changes are partly explained by an earlier study conducted by Eik et al. [322]. These authors evaluated the effect of the acute increase and decrease of glycemia on circulating proinflammatory and anti-inflammatory cytokines in non-diabetic individuals using an oral glucose tolerance test. Plasma levels of BG, pro-inflammatory (e.g., IL-1β, IL-6, TNF-α, IFN- γ) and anti-inflammatory cytokines (e.g., IL-10, IL-4, IL-13) were measured at different time points following ingestion of the glucose solution. BG levels peaked within 30 minutes and returned to normal levels within 120 minutes. IL-13 (anti-inflammatory) levels showed a similar pattern to BG levels in increasing and decreasing within 180 minutes of receiving the glucose solution, while TNF-α levels (pro-inflammatory) peaked at 120 minutes. These authors noted that the observed decrease in blood levels of pro-inflammatory cytokines in their study could be partially attributed to the anti-inflammatory effects of insulin, which showed increased blood levels for 120 minutes following glucose ingestion. Conversely, the increase in pro-inflammatory cytokine levels might be partly due to the progressive decline in cortisol levels during the oral glucose tolerance test [322].

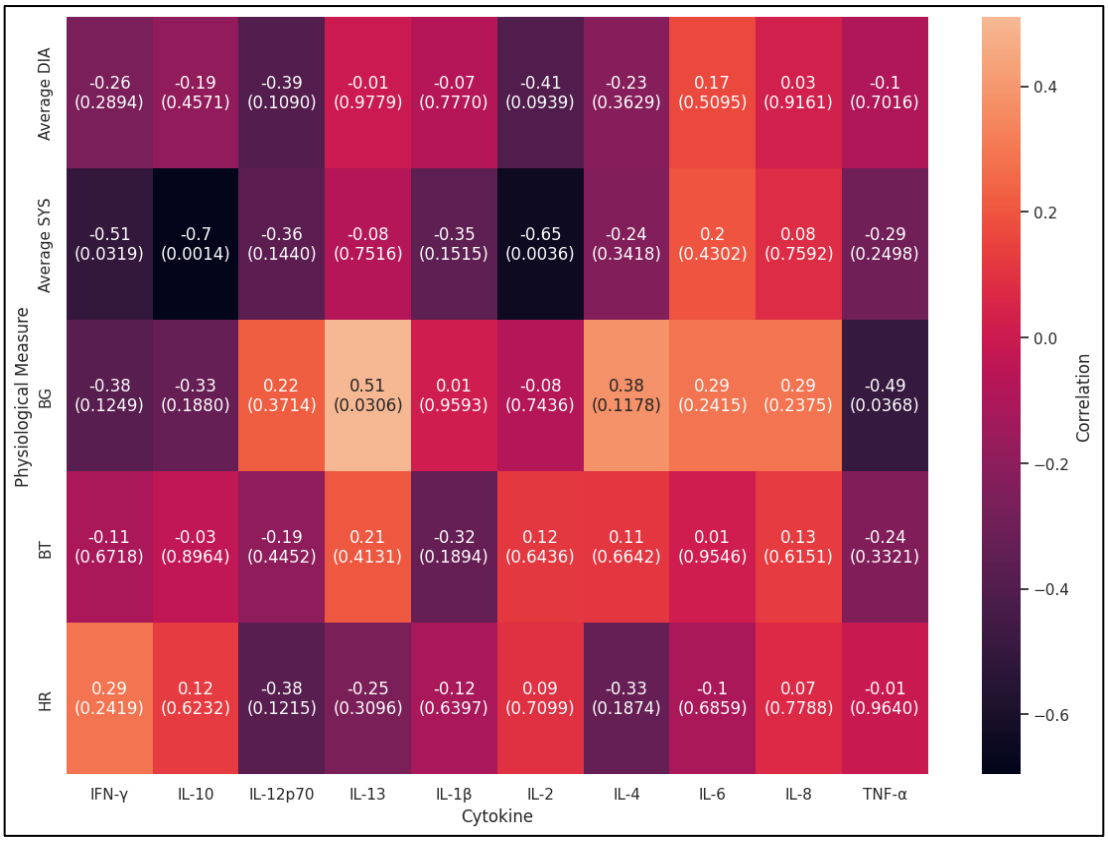

Figure 4.26 - The correlation heatmap of various cytokines and physiological measures. Each cell represents correlation coefficients (p values).

Cytokine Proteome versus Profile of Mood States (POMS) and Mental Cognitive Assessment (MCA)

Research has shown a bidirectional relationship between immune activity and inflammation and mood disorders, mental health and cognitive performance, suggesting that an activated inflammatory response is linked to the onset and persistence of mood symptoms and mental performance [323–325]. For this reason, in addition to the physiological responses, I investigated the association between inflammation protein biomarkers on mood and mental health changes. When I compared the plasma cytokines levels with different mood categories, I found that anger showed a negative correlation with IL-10 (r = -0.59, p = 0.03), while confusion was significantly correlated with IFN-γ, IL-10, and TNF-α (r = -0.69, p = 0.01; r = -0.68, p = 0.01; and r = -0.58, p = 0.03, respectively). Fatigue and tension were negatively correlated with IFN-γ (r = -0.55, p = 0.04; and r = -0.54, p = 0.05, respectively). The total mood disturbance score showed a nearly significant correlation with IFN-γ (r = -0.53, p = 0.05), and TNF-α r = -0.50, p = 0.07). However, vigor and depression did not show any significant correlation with plasma cytokines. Figure 4.27 shows a heatmap of these correlations with the corresponding r and p values. Additionally, a correlation analysis was performed on the plasma cytokines levels and MCA results. The Stroop effect test and the serial subtractions test results did not show any significant correlations with cytokine levels, while the number of times serial subtractions were performed was positively correlated with Il-10, IL-12p70, IL-2, and TNF-α (r = 0.55, p = 0.01; r = 0.46, p = 0.05; r = 0.49, p = 0.04; and r = 0.74, p = 0. 0.0003, respectively). Likewise, the reaction time was negatively correlated with TNF-α (r = 0.50, p = 0.03). The trail-making test was not significantly correlated with any of the cytokines while the time it took to finish the trail-making test was negatively correlated with IL-12p70 levels (r = -0.51, p = 0.03), and positively correlated to IL-8 levels (r = 0.50, p = 0.03). The overall mental cognitive function test results were strongly correlated to IFN-γ, Il-10, IL-13, and TNF-α (r = 0.78, p = 0.000092; r = 0.67, p = 0.0018; r = 0.50, p = 0.03; r = 0.51, p = 0.03, respectively). Figure 4.28 shows a heatmap of these correlations with the corresponding r and p values.

These POMS results and cytokine measurements are somewhat aligned with results from earlier studies. In a study conducted by Reichenberg et al. [323] to investigate the association

between cytokine levels and emotional and cognitive disturbance, 20 male healthy individuals with no history of depressive disorders were endotoxin-infused which triggered the release of cytokines, leading to the onset of classic depressive symptoms. The research found a direct correlation between elevated levels of IL-6 and TNF-alpha and symptoms of depression and anxiety. This indicates that pro-inflammatory cytokines contribute to the development of anxiety and depression [326]. Additionally, cytokines, including those produced by macrophages, can impact mental cognition through neuroinflammation [327]. This process involves the activation of immune cells within the central nervous system, which releases pro-inflammatory cytokines. These cytokines can affect neuronal function by disrupting synaptic signaling and altering neurotransmitter systems [328]. The overall impact can lead to changes in behavior and cognitive functions. Additionally, prolonged exposure to high levels of inflammatory cytokines can contribute to neurodegenerative diseases by promoting neuronal damage and inhibiting repair mechanisms [327]. This evidence suggests a link between pro-inflammatory cytokines and neurocognitive impairments, supporting the hypothesis that chronic inflammation might be a significant contributing factor to both psychological disorders and neurodegenerative diseases.

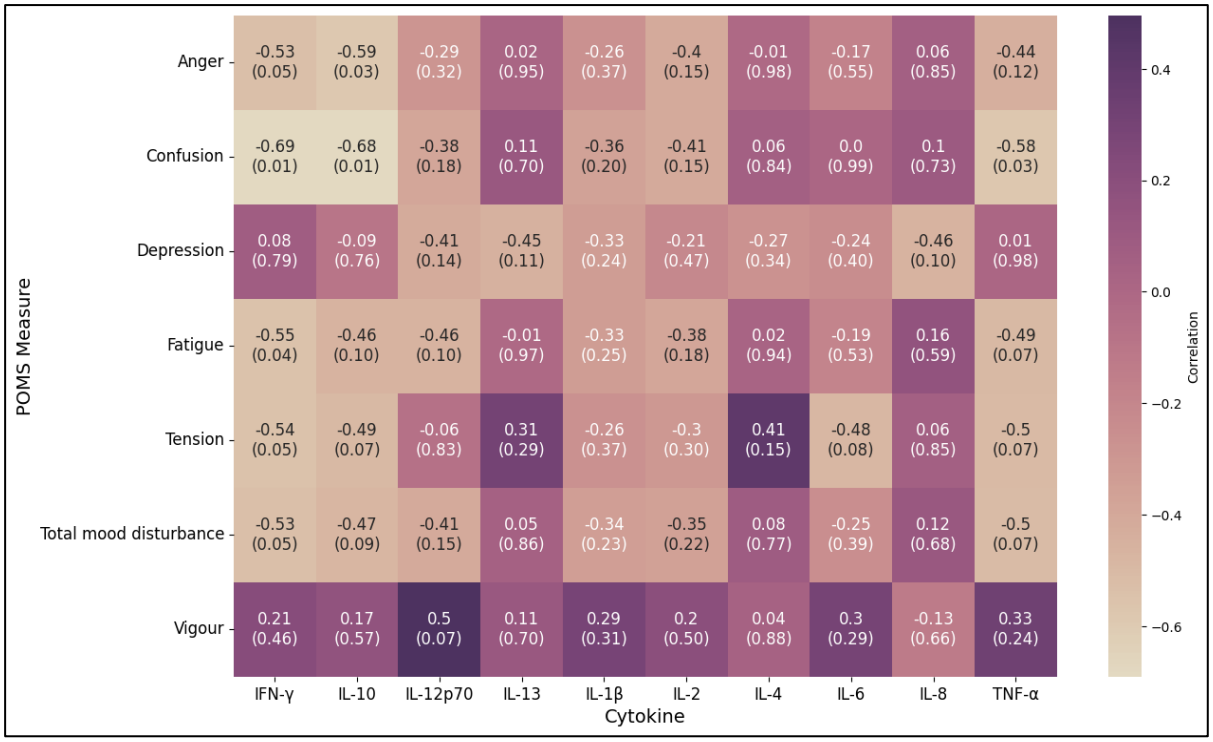

Figure 4.27 - The correlation analysis between cytokines and POMS measures. Each cell represents correlation coefficient (p value).

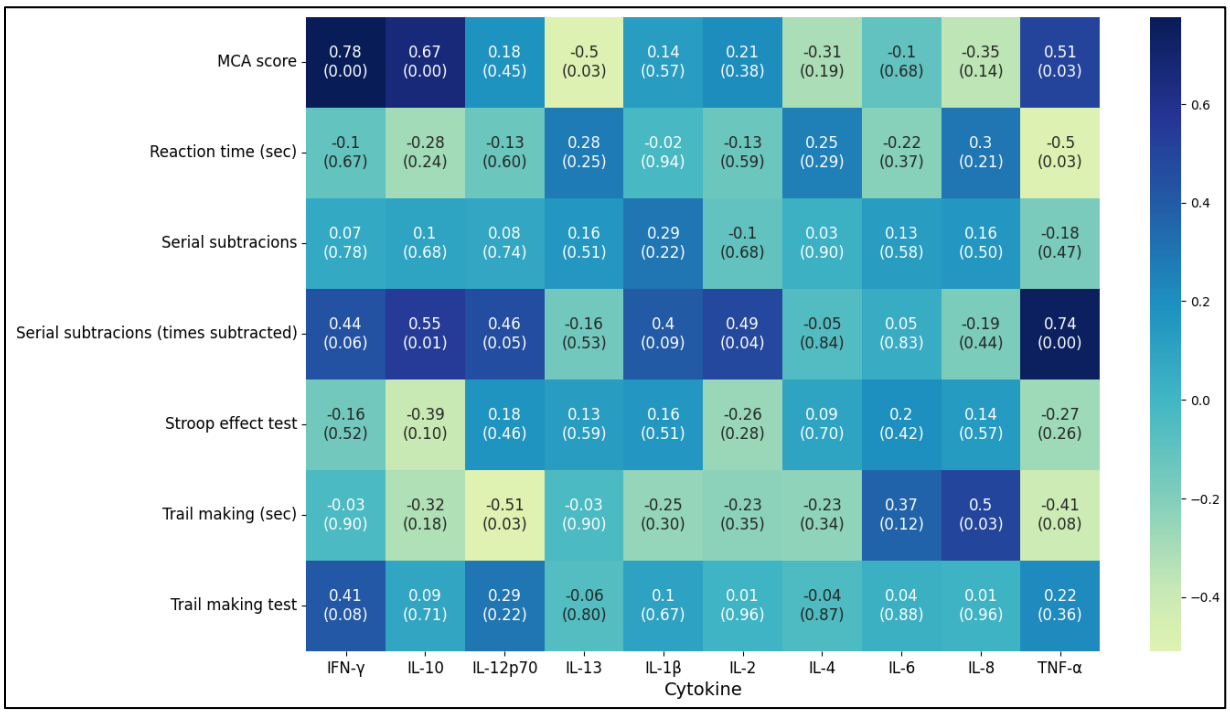

Figure 4.28 - The correlation heatmap of MCA test results and various cytokines. Each cell represents correlation coefficient (p value).

Cytokine Proteome versus Sleep Quality

It has been shown that there is a bidirectional relationship between cytokines and sleep, where changes in inflammatory biomarkers can affect sleep quality, and changes in sleep hygiene, such as sleep deprivation, can impact the concentration of inflammatory biomarkers [327,329,330]. To investigate this connection further, plasma cytokines levels were compared to sleep metrics and BG, BT, and HR levels during sleep. I found that IFN-γ was positively correlated with the frequency of the wake stage during sleep (r = 0.64, p = 0.01), and negatively correlated with BG levels during sleep (r = -0.65, p = 0.01). IL-13 was positively correlated with BG and BT levels during sleep (r = -0.59, p = 0.01, and r = -0.50, p = 0.04, respectively). Il-10, IL-12p70, IL-1β, Il-2, and IL-4 did not return any significant correlations with any of the sleep metrics. Figure 4.29 shows a heatmap of these correlations with the corresponding r and p values.

These results appear to align with previous studies on cytokines and sleep. Kwak et al. [331] used rat models to investigate the long-term effects of IFN-γ on the neurons of the suprachiasmatic nucleus (SCN), the brain's central clock regulating circadian rhythms. They concluded that IFN-γ can interfere with the normal electrical activity and function of clock genes in the neurons of the suprachiasmatic nucleus. In particular, they observed that prolonged exposure to IFN-γ reduced the average firing rate of these neurons and made their activity patterns more erratic. Additionally, IFN-γ weakened the rhythmic expression of the *Period 1 (Per1)* gene, which is crucial for maintaining circadian rhythms, and may be linked to sleep and rhythm problems seen in some infections and in older individuals [331].

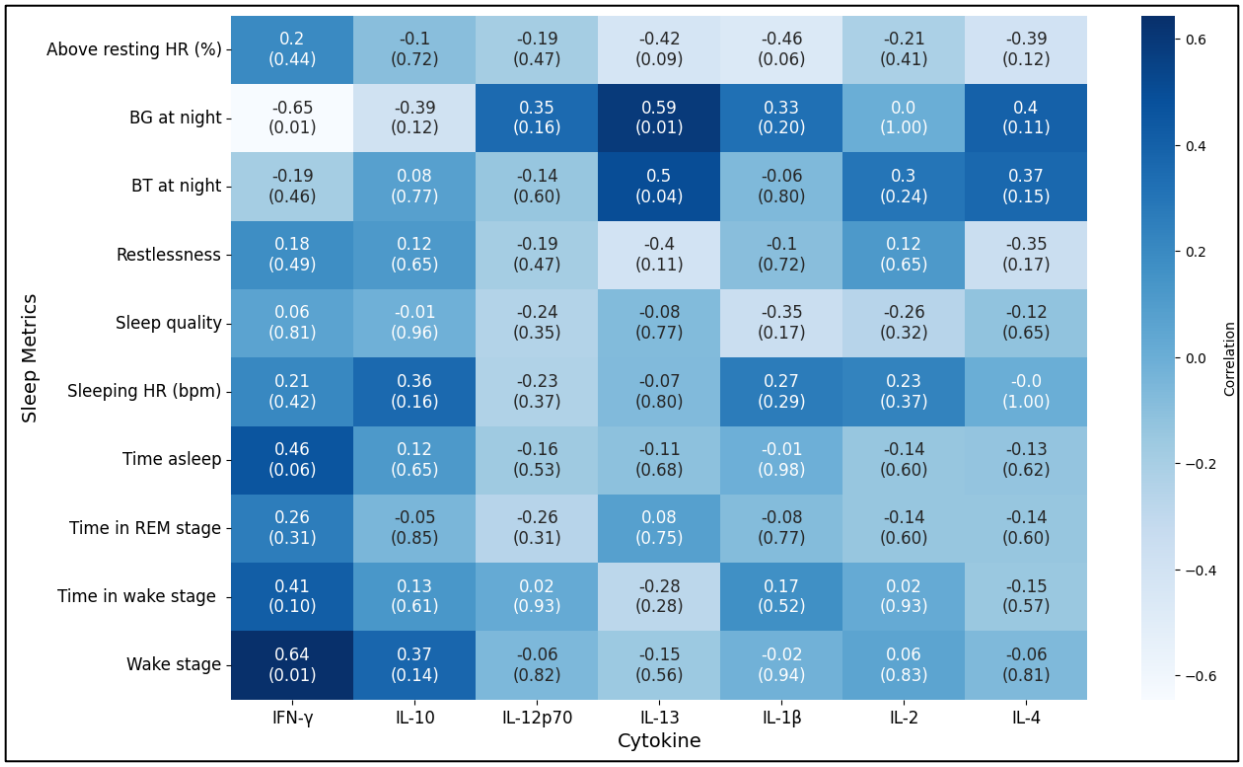

Figure 4.29 - Correlation coefficients and p values of cytokines vs sleep metrics. Each cell represents correlation coefficient (p value).

4.3. The Gut Microbiome Responses to Physiological Variables:

The gut microbiome, which is composed of trillions of bacteria belonging to >2000 different species, plays a significant role in modulating various physiological processes, including metabolism, immune responses, and even mood regulation. For instance, certain bacterial taxa have been shown to produce short-chain fatty acids (SCFAs), which play a critical role in modulating inflammation, glucose metabolism, and gut barrier integrity [332,333]. Likewise, other bacteria such as *Lactobacillus sp*. and *Bifidobacteria* produce gamma aminobutyric acid (GABA), a neurotransmitter that helps reduce anxiety and promotes relaxation [334], while others such as *Clostridia* and *Bacteroides* species produce indole metabolites such as indoxyl sulfate, which causes depression and anxiety [335]. In this section, I explain the correlation analysis performed to explore the significant relationships between specific bacterial counts and physiological measurements, indicating potential interactions between gut microbiota and bodily functions. The analysis table is available in Appendix Table 4-O.

Gut Microbiome versus BG, BT, BP, and Sleep Quality

My BG levels showed a strong negative correlation with several bacteria found in my gut, including *Faecalibacterium*, *Phascolarctobacterium*, *Fusicatenibacter*, *Clostridium IV*, *Enterobacter*, *Haemophilus*, and *Odoribacter*. *Faecalibacterium prausnitzii* is known for its anti-inflammatory properties, producing SCFAs like butyrate that maintain gut barrier integrity and regulate systemic inflammation [336,337]. Butyrate enhances insulin sensitivity by modulating gut hormone release and reducing inflammation, which can lower BG levels [336]. The negative correlation with BG suggests that higher levels of these bacteria were possibly helping to lower my BG levels by enhancing insulin sensitivity and reducing inflammatory cytokines. *Enterobacter* and *Haemophilus* are often associated with dysbiosis and metabolic disorders [338]. Their negative correlation with my BG levels indicates that reducing the abundance of potentially pathogenic bacteria may be beneficial for glucose homeostasis. Dysbiosis involving these bacteria can lead to increased intestinal permeability ("leaky gut"), systemic inflammation, and insulin resistance, all of which can raise BG levels [338].

Prevotella showed a strong negative correlation with my BT, while *Bacteroides*, *Faecalibacterium*, and *Senegalimassilia* were positively correlated with BT. *Prevotella* species are known to be involved in carbohydrate metabolism. Their negative correlation with BT might be linked to their role in enhancing metabolic efficiency and energy expenditure [339]. *Prevotella* can ferment carbohydrates to produce SCFAs, which can influence systemic metabolism and energy balance, thereby potentially lowering BT [339]. *Bacteroides* species contribute to polysaccharide breakdown and SCFA production. SCFAs like acetate and propionate can be absorbed and utilized in the liver and other tissues to generate heat (thermogenesis), thereby influencing systemic metabolism and increasing BT [340]. A significant negative correlation was observed between the abundance of *Lactobacillus* and *Intestinimonas* with DIA BP. *Lactobacillus* species are known to produce lactic acid and SCFAs, which can modulate vascular tone and reduce BP through various mechanisms, including the enhancement of nitric oxide (NO) production [341]. NO is a vasodilator that relaxes blood vessels, improving blood flow and reducing diastolic DIA BP [341].

My sleep quality scores showed a significant negative correlation with levels of *Clostridium XVIII*. The negative correlation with sleep quality suggests that this bacterial group might produce metabolites that disrupt sleep or that its presence is indicative of a dysbiotic state that affects sleep [342]. *Clostridium* species can produce various metabolites, including uremic toxins such as indoxyl sulfate, p-cresol sulfate and phenylacetylglutamine, which may affect central nervous system function and sleep regulation. Dysbiosis involving *Clostridium* may also lead to increased production of pro-inflammatory cytokines and metabolites that disrupt normal sleep patterns [342]. Overall, the correlation analysis highlights the significant interactions between gut microbiota and physiological measurements, emphasizing the complex biochemical pathways involved. These findings also underscore the exciting potential of using gut microbiome profiling to identify biomarkers for metabolic and physiological health, offering insights into individual health status and guiding personalized interventions.

4.4. The Metabolome, Diet and Physiological Responses:

As highlighted in my previous chapters, the relationship between diet, metabolomics, and physiological responses is fundamental to understanding the biochemical association between nutritional intake and health. Metabolomics, which involves the comprehensive analysis of metabolites in biological samples, enables the investigation of metabolic changes influenced by dietary habits. Characterization of the plasma and urine metabolomes provides valuable data reflecting one's metabolic state and how it is modulated by dietary inputs and existing or emerging physiological conditions. In this section, I will compare metabolomic data from my daily plasma and urine samples with various physiological responses. The quantity of data analyzed was enormous and the results are quite expansive, but to keep the discussion more focused only a few of the more important findings are discussed. This is intended to help highlight the more interesting or significant correlations between specific metabolites and various physiological parameters such as glucose and heart rate levels, the consumption of various food components as well as various inflammatory markers. Integrating metabolomic data with physiological measures allows for a more detailed understanding of how dietary components influence metabolic pathways and physiological functions. It is worth noting that as this study was an N-of-1 with a single case study, it is not possible to draw firm conclusions about how these factors are inter-related.

4.4.1. The Metabolome versus Blood Glucose (BG) – Dietary Effects

Several significant diet-induced metabolite differences were detected between the four dietary interventions. As expected, the reduced carbohydrate consumption during the KD led to lower BG levels, while the elevated intake of carbohydrates and added sugars in the FFD caused increased BG levels. Using a different (categorical) analytical approach, the physiological information gathered through wearable tracking devices was categorized into two classes such as "good" versus "bad" physiological responses or "low" versus "high" physiological responses, without considering the type of diet. The metabolomic data from plasma and urine samples on

those days were compared to each other (and to "normal" values) to uncover any potential links between the metabolomic profiles and the physiological results.

For the analysis of BG, plasma metabolomics data were grouped into two groups of high (average BG >6.5 mM) and low BG (average BG <5.5 mM), and 84 metabolites were detected as significantly different between the two groups. 3-hydroxyisovaleric acid (p = 4.0086E-8), 3-hydroxybutyric acid (p = 1.2487E-6), the Fischer ratio (ratio of isoleucine, leucine and valine to phenylalanine, tryptophan, and tyrosine which serves as an indicator of liver damage, chronic

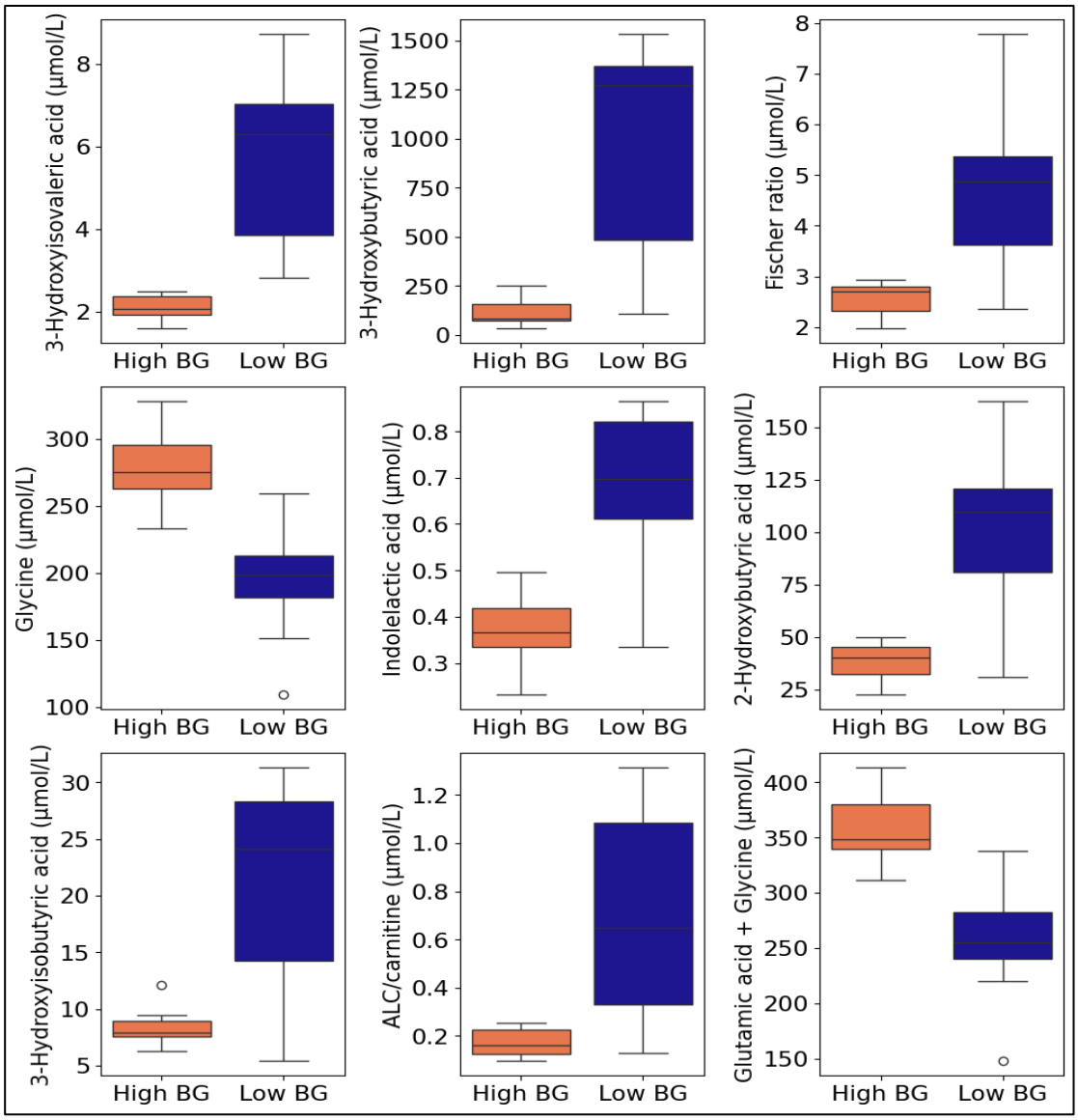

Figure 4.30 - Top 9 significantly different blood metabolites for low BG vs high BG.
ALC (Acetylcarnitine)

hepatitis, cardiac events, and hepatocellular carcinoma [343–345]) (p = 7.7246E-6), and indolelactic acid (p = 8.3746E-6) were higher in the plasma of my low BG days. On the other hand, glycine (p = 7.8174E-6), and the total concentration of glutamic acid and glycine were higher in the plasma of my high BG days (Figure 4.30). Figure 4.31 shows a PCA and PLS-DA analysis performed on the plasma samples and Figure 4.32 displays a heatmap of plasma metabolome of high and low BG days.

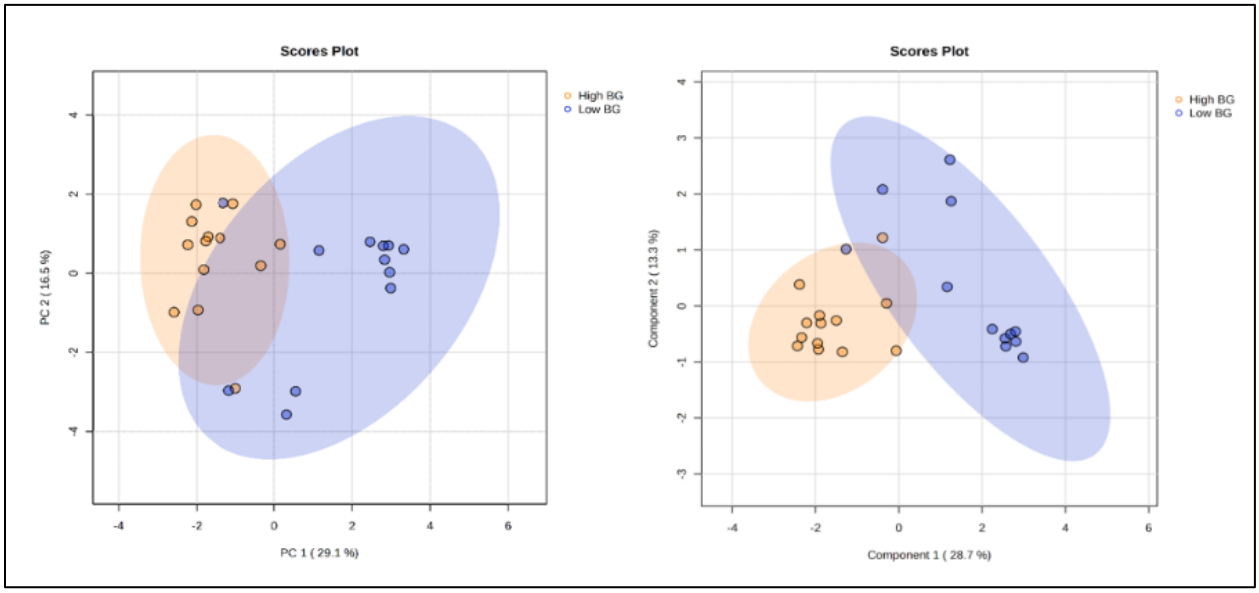

Figure 4.31 - PCA (left) and PLS-DA (right) of the plasma samples showing a clear separation between the high BG and low BG

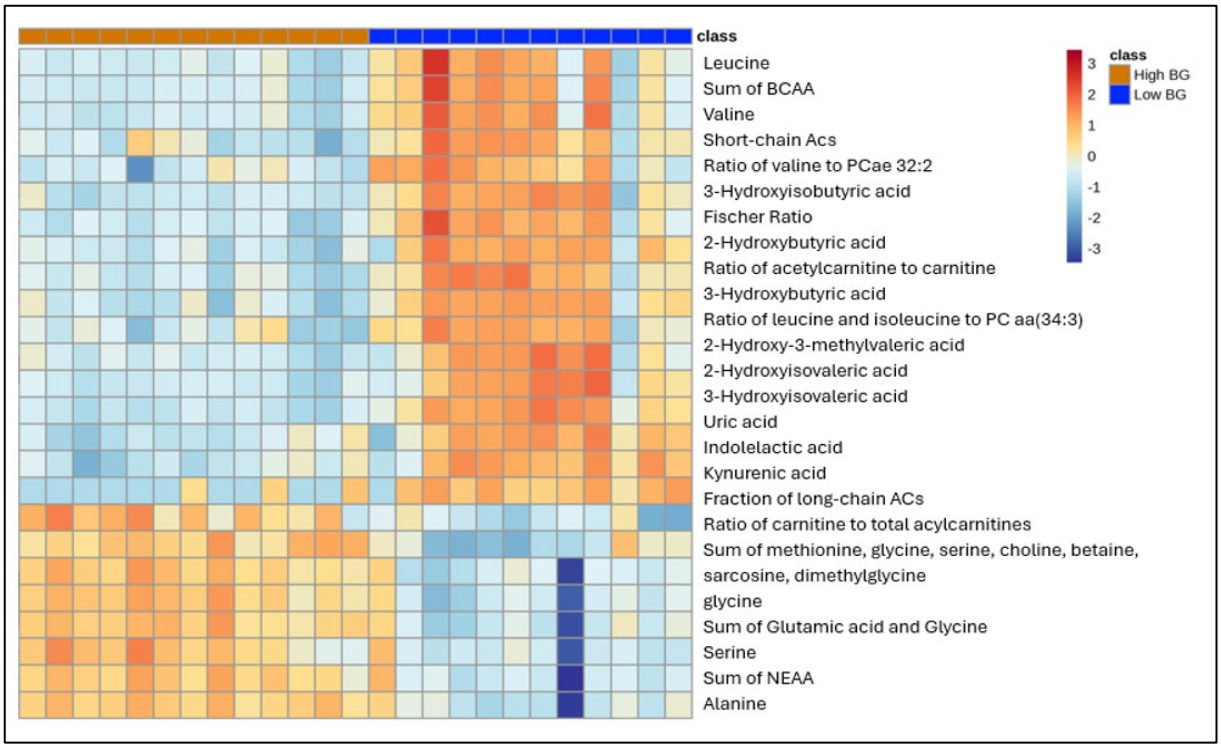

Figure 4.32 - Hierarchical Clustering Heatmap of plasma samples of low and high BG days

A similar analysis was performed with the urine samples from the days with high and low BG levels. Figure 4.33 presents PCA and PLS-DA analysis of urinary metabolites of both high and low BG days. Following an ANOVA analysis, 82 urinary metabolites were detected as significantly different between the two groups (Figure 4.34). Metabolites such as guanidinopropionic acid (p = 5.4534E-12), creatine (p = 2.7012E-10), nudifloramide (p = 8.0206E-10), valine (p = 4.0211E-9), and acetoacetic acid (p = 6.5075E-9) were significantly higher in the urine samples of my low BG days, while the concentration of allantoin (p = 5.7839E-5), and the ratio of carnitine to short-chain acylcarnitines (p = 4.2712E-6), were higher in the urine samples of the days with high BG levels. The carnitine to short-chain acylcarnitines ratio has been used as an indicator of various disorders of organic acid metabolism associated with an intramitochondrial accumulation of acyl-CoA intermediates. In particular, an increase has been observed in the urine abundance of short-chain acylcarnitines, and free carnitine in these individuals [346].

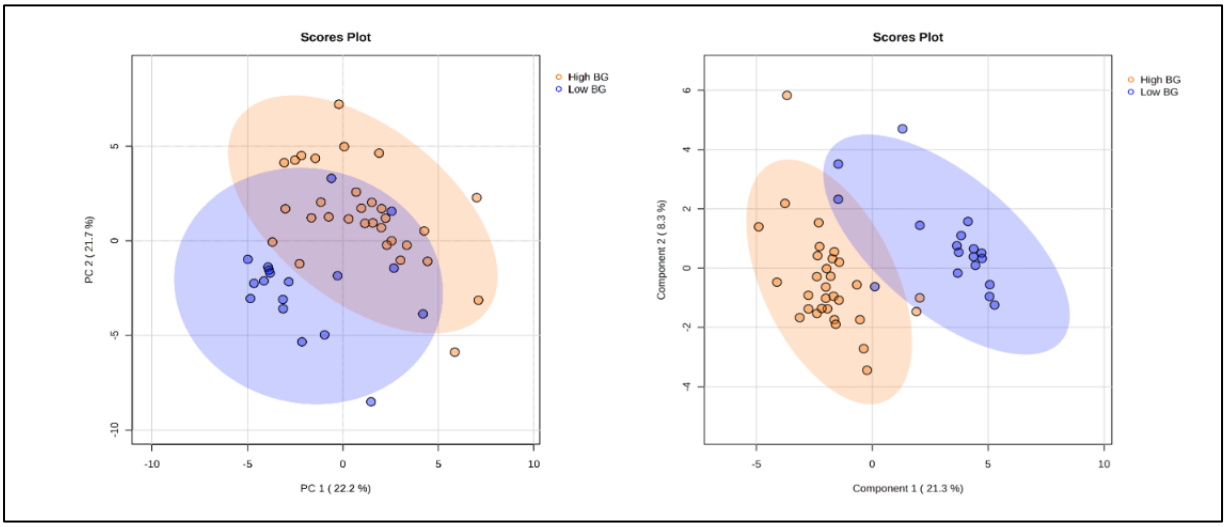

Figure 4.33 - PCA (left and PLS-DA (right) of urine metabolite of low and high BG

Higher levels of 3-hydroxyisovaleric acid were also observed in both plasma (p = 4.0086E-8) and urine (p = 1.8187E-6) of my low BG days. Elevated levels of this metabolite can be associated with high protein diets, amino acid supplementation, biotin deficiency, or ketoacidosis which are factors that can influence blood sugar levels and overall metabolism [347–349]. Additionally, a positive correlation between the levels of 3-hydroxyisovaleric acid and blood ketones such as 3-hydroxybutyric acid has been previously reported [347]. Both of these organic acids are involved in the metabolic breakdown of branched-chain amino acids (BCAA) [350], which is crucial for energy production in cells. Serum concentrations of 3-hydroxyisobutyric acid, which is structurally related to 3-hydroxyisovaleric acid, have been found to be significantly higher in ketogenic conditions, as it can be formed from the metabolism of ketone bodies [349]. Additionally, levels of 3-hydroxyisobutyrate have been observed to rise significantly and progressively in the blood of individuals with prediabetes and T2D, indicating an advancement in systemic insulin resistance [351]. Notably, urinary and plasma levels of BCAA were higher during my low BG days. Elevated levels of certain amino acids, specifically BCAAs such as isoleucine, leucine, valine, and aromatic amino acids including tyrosine and phenylalanine, have been associated with an increased risk of developing obesity and T2D. It has been suggested that these amino acids may impair beta-cell function or insulin signaling, leading to an increased risk of insulin resistance and diabetes . In a study conducted by Wang et al. [353], a significant association between five specific amino acids and the future development of diabetes among normoglycemic individuals was established. Their findings suggested that individuals with elevated levels of these amino acids had a more than

fivefold increased risk of developing diabetes, underscoring the predictive potential of amino acid profiles in diabetes risk assessment [353]. Further research by Wang et al. [356] expanded on these findings by examining the association between plasma levels of 20 amino acids and the risk of diabetes in a middle-aged and elderly populations. It was found that individuals with the highest tertile levels of plasma BCAAs and cysteine were at a significantly increased risk of diabetes and elevated BG levels. Moreover, baseline levels of BCAAs were strong predictors of developing new-onset diabetes, reinforcing the link between amino acid profiles and diabetes risk. Interestingly, animal studies have shown that meat-rich diets, such as KD, that are high in proteins and fats and low in carbohydrates increase the concentration of BCAA and the risk of insulin resistance as well as leading to increased glucose output [357,358]. All of these findings underline the connection observed between elevated 3-hydroxyisovaleric acid, 3-hydroxybutyric acid and the overall BCAA levels and the drop in BG levels.

Significantly higher levels of carnitine were detected in the samples from higher BG days (p = 0.001). Carnitine, an essential metabolite for the metabolism of fatty acids and lipids. Lipids serve as a crucial energy source for the body's metabolic processes. These lipids, including fatty acid derivatives and cholesterol, come from food or are synthesized by the body in the liver and adipose (fat) tissue. In conditions such as obesity, where there is an accumulation of fat in the body, there is often an increase in the levels of plasma free fatty acids (FFAs) [359]. Carnitine plays a significant role in obesity due to its involvement in breaking down fatty acids for energy. Providing FFAs can enhance the oxidation of fatty acids, a process essential for converting fats into energy [360,361]. However, fatty acids can only be converted into energy through a process called beta-oxidation, which occurs in the mitochondria and requires carnitine. This process involves the esterification of fatty acids and their transport into the mitochondrion, necessitating carnitine [362]. Therefore, with obesity leading to higher levels of plasma FFAs, more carnitine is needed to facilitate efficient beta-oxidation. The availability of carnitine within cells is thus a key factor in regulating this energy-converting process.

A correlation analysis was performed to understand the possible connections between the blood and urine metabolites and my high/low BG levels. The Pearson correlation coefficients and p-values are presented in Appendix Table 4-A (for plasma and BG) and Appendix Table 4-B (for urine and BG). The most significant positive correlation was observed between the BG levels and

the plasma levels of the ratio of carnitine to the total concentration of acylcarnitines (r = 0.684, p = 8.00E-05), which highlights the association between these two factors. Lower levels of free carnitine have been linked to metabolic disorders and abnormal lipid profiles in the plasma [363]. Moreover, when the ratio of acylcarnitine to free carnitine exceeds 0.4, it is deemed atypical,

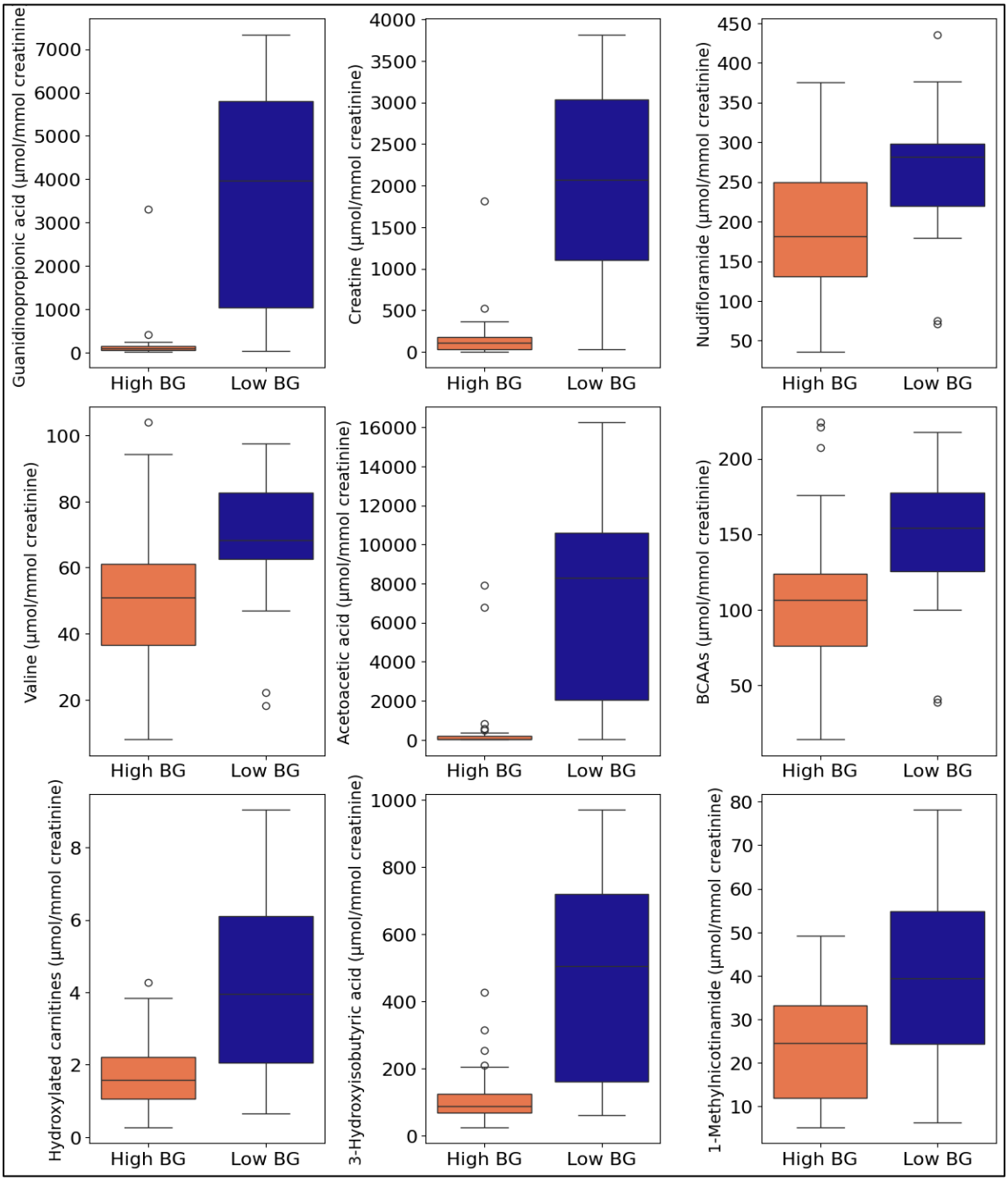

Figure 4.34 - Top 9 significant of urine metabolites from high and low BG days; BCAAs (Branched-chain amino acids)

indicating a lack of carnitine. Interestingly, elevated concentrations of free carnitines have been detected in the fasting plasma of individuals with obesity and T2D [364–366].

A positive correlation was observed between my BG levels and the total plasma concentration of the tripeptide glutathione (GSH) constituents, glutamic acid, and glycine (r = 0.665, p = 3.09E-08). The urine levels of these metabolites were also positively correlated to the BG levels, but the association was quite weak (r = 0.237, p = 0.024). GSH is an antioxidant capable of preventing damage to cellular components caused by reactive oxygen species [367]. The biosynthesis of glutathione involves two stages: first, the rate-limiting enzyme glutamate-cysteine ligase combines glutamate with cysteine; then, glutathione synthetase adds glycine to the compound [367]. Elevated glucose levels, as seen in diabetes, can lead to increased oxidative stress, potentially depleting GSH levels. As GSH is a key antioxidant, its deficiency can exacerbate oxidative damage and contribute to diabetes-related complications. The relationship between glutathione and BG levels indicates that low glutathione is associated with poor glycemic control, suggesting a link between glutathione synthesis and BG regulation [368]. Maintaining proper GSH levels could support cellular protection against oxidative stress, which is relevant for those with high BG levels. Moreover, insulin resistance and diabetes can affect GSH metabolism, indicating a complex interplay between glucose regulation and antioxidant capacity [369]. This connection was further demonstrated in a study conducted by Zenil-Vega et al. [370], who concluded that a significant correlation existed between glycine and insulin plasma levels in patients with pulmonary tuberculosis and T2D mellitus, suggesting a potential reference test for evaluating both conditions.

As seen in Appendix Table 4-A the plasma ratio between long-chain acylcarnitines and total acylcarnitines was significantly and positively correlated with BG levels (r = 0.632, p = 2.30E-07). This ratio reflects the relative abundance and utilization of long-chain fatty acids as an energy source. Alterations in this fraction may indicate shifts in fatty acid metabolism, mitochondrial function, or specific metabolic disorders [371]. Studies have shown associations between acylcarnitine profiles and the risk of developing T2D [372,373]. Elevated concentrations of short- and long-chain acylcarnitines, in particular, have been linked to an increased risk of insulin resistance, obesity, and T2D, especially in individuals already at high cardiovascular risk [373]. Long-chain acylcarnitines are being investigated as potential biomarkers for insulin resistance and

T2D, as they may reflect disruptions in lipid metabolism, which are tied to impaired glucose homeostasis [372]. Given insulin's critical role in suppressing lipolysis and promoting glucose uptake, an accumulation of these fatty acid metabolism intermediates, such as long-chain acylcarnitines, could indicate reduced insulin sensitivity [372]. Studies have shown that acute and chronic administration of palmitoylcarnitine (a long-chain acylcarnitine) can inhibit insulin signaling and decrease insulin-dependent glucose uptake in murine muscle, further highlighting the mechanistic link between long-chain acylcarnitines and glucose metabolism [372]. Additionally, during pregnancy, certain acylcarnitines have been associated with an increased risk of gestational diabetes. For example, higher levels of C14:1-OH at 10-14 weeks gestation were linked to a 55% increased risk of gestational diabetes, and at 15-26 weeks, elevated levels of several acylcarnitines (C4, C8:1, C16:1-OH) were associated with increased gestational diabetes risk [374]. Therefore, the observed positive correlation between LCACs and total acylcarnitines with BG levels is reasonable, as these compounds indicate changes in FA metabolism and insulin sensitivity.

As seen in Appendix Table 4-A, the total plasma concentration of non-essential amino acids was positively correlated with BG levels (r = 0.628, p = 2.89E-07). Previous studies have shown a significant positive correlation between plasma levels of amino acids and BG levels, suggesting that the dysregulation of these amino acids could contribute to the development of acute diabetic syndrome [375]. For instance, in a study by Nakamura et al. [376], specific non-essential amino acids, including glutamate, were correlated with markers of insulin resistance and glycemic control in patients with T2D, highlighting how alterations in amino acid levels can reflect changes in BG levels. The concentration of glucogenic amino acids was positively correlated with BG levels (r = 0.441, p = 0.0007). Glucogenic amino acids, including alanine, serine, glycine, glutamine, and others, are a subset of amino acids that play a crucial role in maintaining BG levels through the process of gluconeogenesis. This metabolic pathway occurs primarily in the liver and involves the conversion of non-carbohydrate substrates into glucose. When dietary glucose is scarce, such as during fasting, prolonged exercise, or low carbohydrate intake (such as a KD), these amino acids are deaminated [377]. Their carbon skeletons are then utilized in the gluconeogenesis pathway to produce glucose. This endogenous glucose production is essential for maintaining BG levels, ensuring a continuous supply of this critical energy source for glucose-dependent tissues, especially the brain and red blood cells, which rely heavily on glucose for energy [377,378]. The good correlation seen between glucogenic amino acids and BG levels, therefore, is perfectly reasonable

as glucogenic amino acids do not need to be converted to glucose when BG levels are high. Plasma concentrations of ketogenic amino acids were negatively correlated with BG levels (r = -0.416, p = 0.0016). Ketogenic amino acids are a unique class of amino acids that can be converted into ketone bodies rather than glucose. These amino acids include leucine and lysine, which are exclusively ketogenic, as well as isoleucine, phenylalanine, tryptophan, and tyrosine, which are both ketogenic and glucogenic. The primary metabolic pathway for ketogenic amino acids involves their conversion into acetoacetate or beta-hydroxybutyrate, which are key ketone bodies produced during periods of low carbohydrate intake, such as fasting or adherence to a KD [379]. The conversion of ketogenic amino acids to ketone bodies instead of glucose has significant implications for BG levels. Unlike glucogenic amino acids, which contribute to gluconeogenesis and can increase BG levels, ketogenic amino acids provide an alternative energy source that does not directly impact BG levels. During states of carbohydrate restriction or prolonged fasting, the body shifts its primary energy source from glucose to ketone bodies, sparing glucose for tissues that are entirely dependent on it, such as the brain and red blood cells [379]. This metabolic shift can help maintain stable BG levels and prevent hypoglycemia. The negative correlation seen between ketogenic amino acids and BG levels, therefore, is perfectly reasonable as ketogenic amino acids need to be converted to ketone bodies when BG levels are low.

As seen in Appendix Table 4-A, plasma levels of 2-hydroxybutyric acid were significantly negatively correlated with BG levels (r = -0.66, p =3.16E-08). 2-hydroxybutyric acid is a metabolite that increases in response to oxidative stress and is considered an early marker for insulin resistance and impaired glucose regulation [380,381]. Given that the FFD had the most elevated levels of BG, an elevated abundance of this “early warning” metabolite would not be unexpected. Elevated levels of 2-hydroxybutyric acid appear to signal early onset of metabolic disease and have been linked with a positive causal effect on glycemic traits, making it a useful biomarker for identifying individuals at risk of developing diabetes and insulin resistance [381].

The integration of BG monitoring with metabolomic profiling helps provide a more comprehensive understanding of the biochemical pathways influenced by dietary intake and metabolic health. The significant correlations observed between BG levels and various metabolites, such as acylcarnitines, glutathione constituents, certain amino acids, and neurotransmitter metabolites, underscore the connections between glucose metabolism and

broader metabolic processes. These findings highlight the potential of using metabolomics to identify biomarkers for glucose regulation and metabolic health, offering insights into individual responses to dietary interventions. While I have only explored some of the connections found between BG and metabolites in this study, the results demonstrate the value of combining wearable BG monitoring with metabolomics.

4.4.2. The Metabolome versus Heart Rate (HR) – Dietary Effects

As mentioned earlier, the average resting HR and the daily average HR were significantly higher during the KD while the MD caused a striking decrease in both resting and daily average HR within the 2 weeks of the diet intervention. The same analysis was performed for the plasma samples being grouped based on the HR levels (low HR – corresponding to an average daily HR of <70 bpm and high HR – corresponding to an average daily HR of >80 bpm). The PCA and PLS-DA analysis is shown in Figure 4.35, and the heatmap analysis is provided in Figure 4.36. 12 metabolites were found to be significantly different between the two HR groupings. Caprylic acid (p = 6.2449E-5), N-acetyl-aspartic acid (p = 1.46E-4), trans-4-hydroxyproline (p = 3.3673E-4), total concentration of long-chain fatty acid glycosyl-ceramides (p = 8.6701E-4), and creatine (p = 0.0011249) were significantly higher in the high HR days, while the concentration of indole-3-propionic acid (IPA) (p = 0.0018354), and methylhistidine (p = 0.0023076) was higher in the low HR days (Figure 4.37).

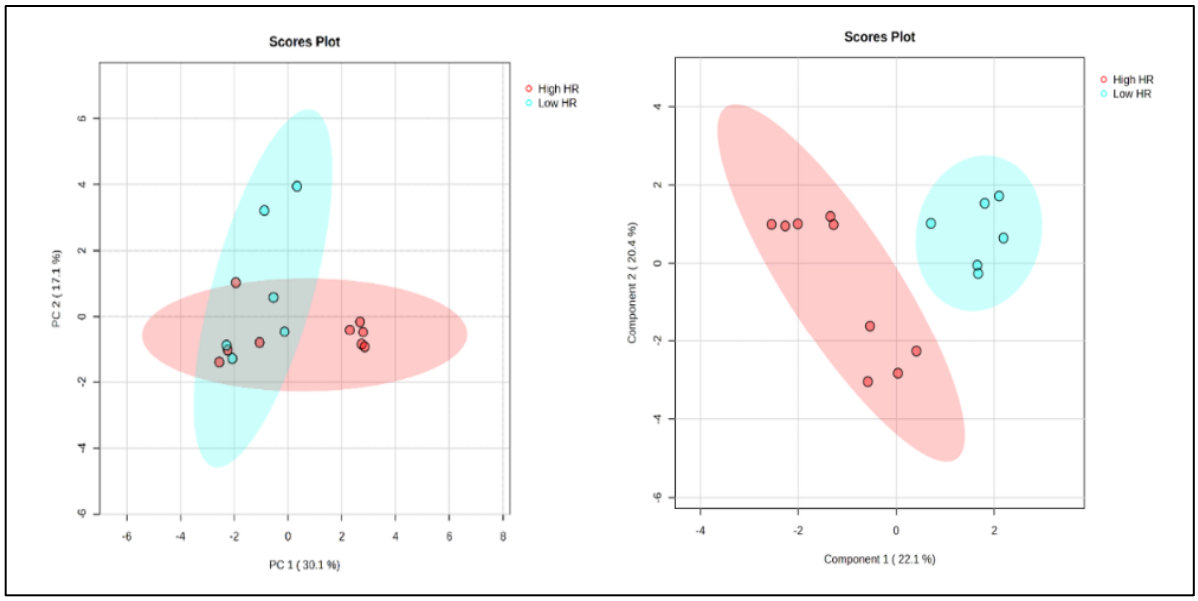

Figure 4.35 - PCA (left) and PLS-DA (right) of the plasma samples showing a clear separation between the high HR and low HR days

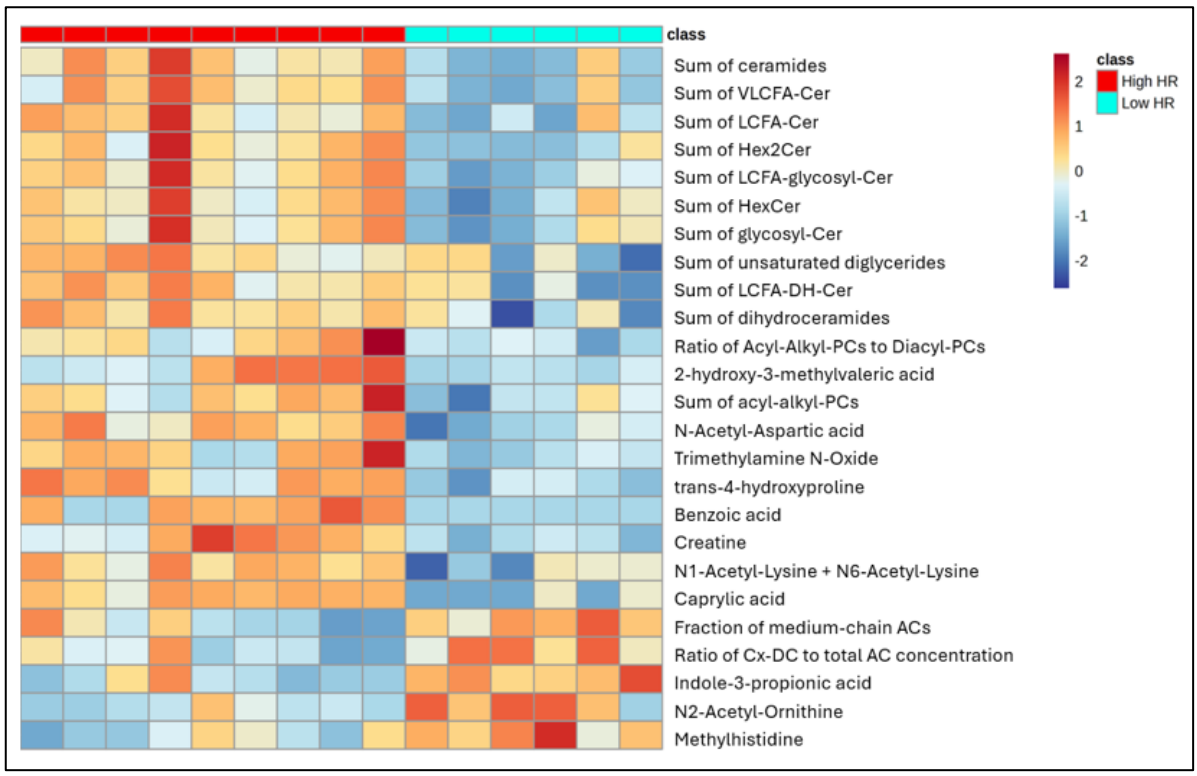

Figure 4.36 - Hierarchical Clustering Heatmap of plasma samples of high and low HR days

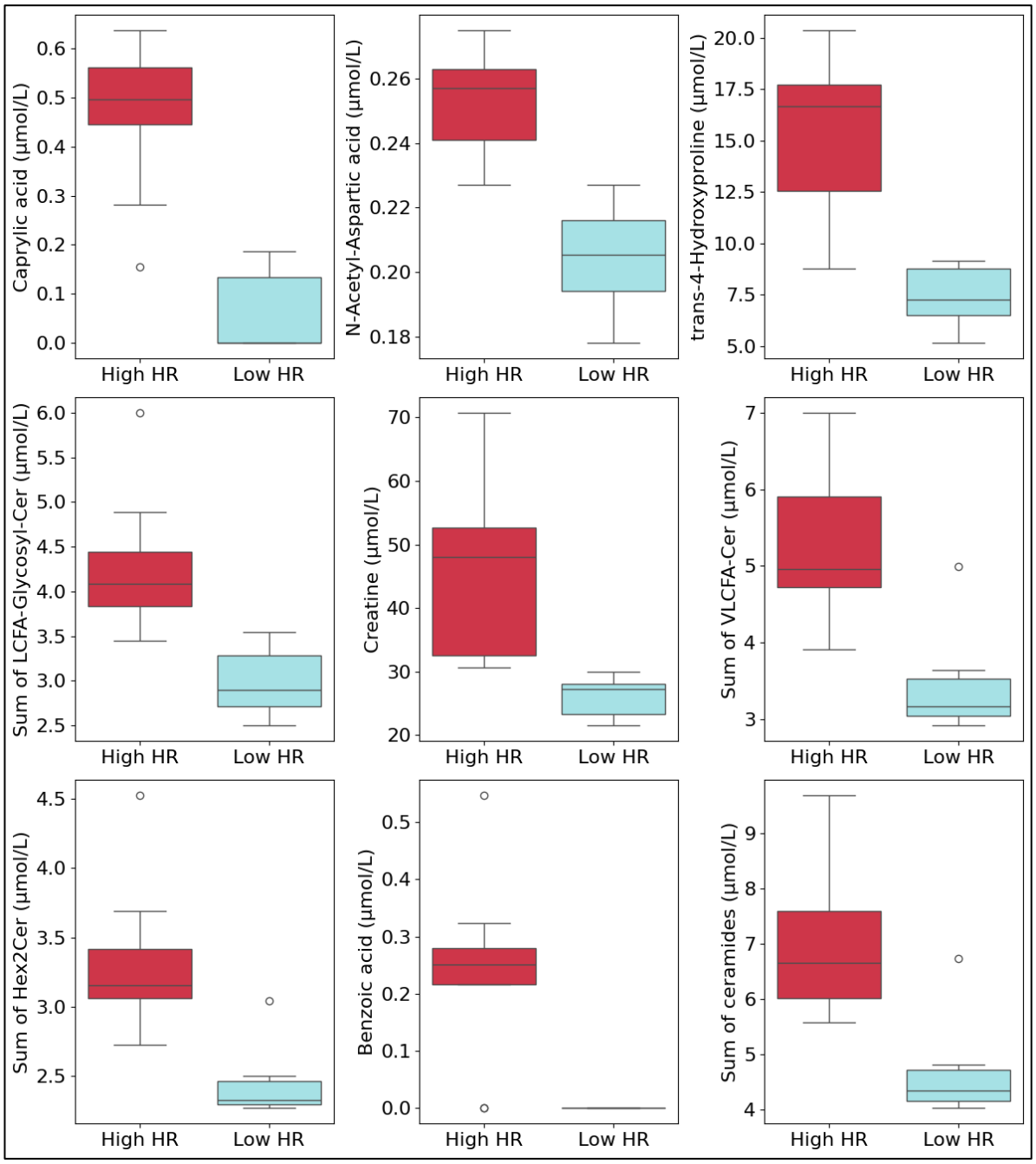

Figure 4.37 - Boxplots of the top 9 plasma metabolites between the High HR and low HR groups; LCFA-Glycosyl-Cer (long-chain fatty acid glycosyl ceramides); VLCFA-Cer (very-long chain fatty acid ceramides); Hex2Cer (dihexosylceramides)

A similar analysis conducted on the urine samples of the days with low/high HR levels revealed significant differences between the two HR groups. Figure 4.38 shows PCA and PLS-DA analyses performed on the urine samples for the two groups. Short-chain acylcarnitine concentration (p = 1.0468E-5), the total concentration of saturated fatty acid acylcarnitines (p =1.1428E-5), the total acylcarnitine concentration (p = 1.3153E-5), the hydroxylated carnitines (p = 3.9369E-4), trans-4-hydroxyproline (p = 5.5034E-4) and the concentration of hydroxyproline (p = 5.6793E-4) were significantly higher in the urine samples of the days with higher HR levels. On the other hand, methylamine (p = 5.7186E-4), arginine (p = 0.0012), and the ratio of phosphatidylcholines to lyso-phosphatidylcholines (p = 0.0012) were higher in the urine samples of the days with lower HR levels (Figure 4.39).

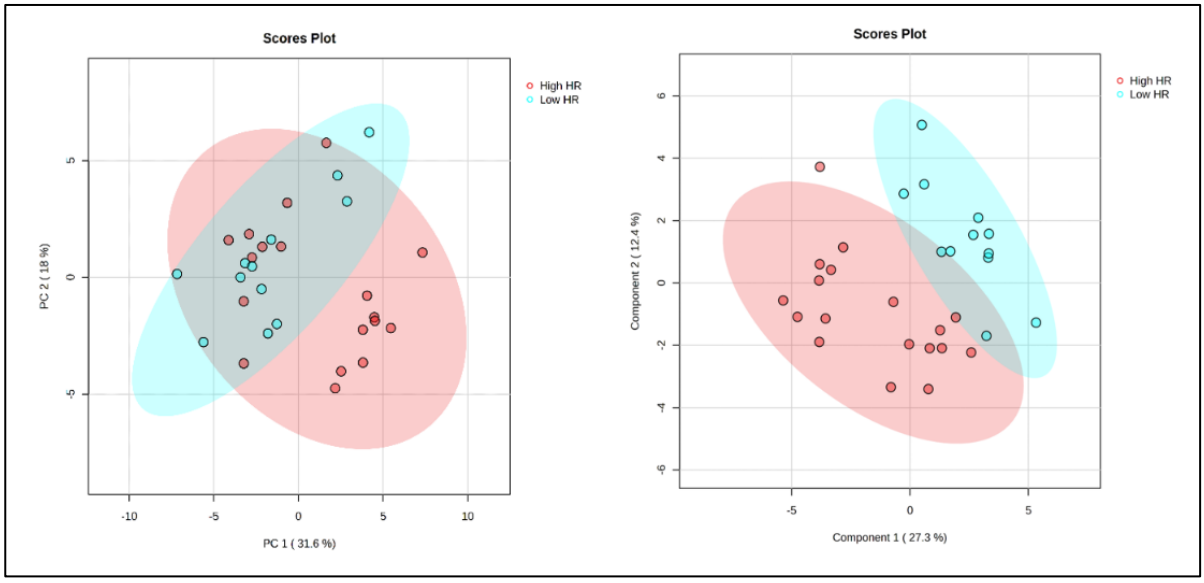

Figure 4.38 - PCA and PLSDA (right) analysis on the urine samples of high and low HR

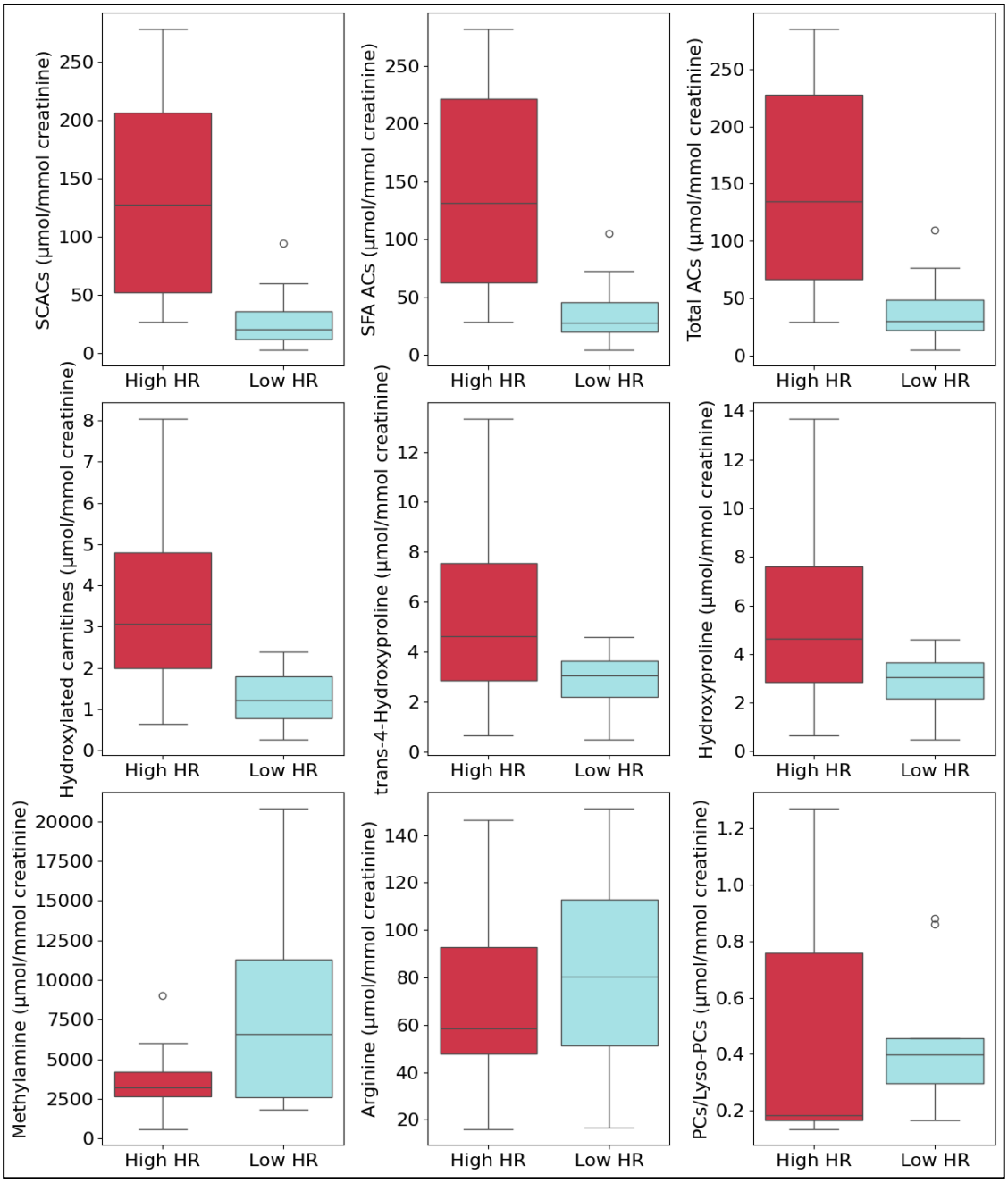

Figure 4.39 - Boxplots of the top 9 significantly different urinary metabolites between the High HR and low HR groups - SCACs (short-chain acylcarnitine); SFA ACs (saturated fatty acid acylcarnitines); ACs (acylcarnitine); PCs/Lyso-PCs (ratio of phosphatidylcholines to Lyso-phosphatidylcholines)

A correlation analysis was performed to understand the possible connections and associations between the blood and urine metabolites and the monitored HR levels. The Pearson correlation coefficients and p-values are presented in Appendix Table 4-C (for plasma and HR)

and Appendix Table 4-D (for urine and HR). The most significant and strongest correlations between plasma metabolites and HR levels was the positive correlations observed with the total concentration of ceramides (r = 0.503, p = 9.17E-05), very long-chain fatty acids ceramides (r = 0.501, p = 9.86E-05), dihexosylceramides (r = 0.481, p = 0.0002), and the total concentration of long-chain fatty acid glycosyl-ceramides (r = 0.473, p = 0.0003). Ceramides are sphingolipids involved in critical cellular processes, including apoptosis and inflammation. Elevated plasma ceramide levels are associated with adverse cardiovascular outcomes and may influence heart function, impacting HR and other cardiac-related conditions [382]. High plasma ceramide concentrations have been predictive of coronary artery disease and heart failure, conditions that can affect HR and overall heart function. [382]. Furthermore, ceramides have been associated with insulin resistance, T2D, and other cardiometabolic diseases, which also affect HR by disrupting normal cellular functions [383].

As seen in Appendix Table 4-C, plasma acylcarnitines and hydroxylated carnitine concentrations were also positively correlated with BG levels (r = 0.447, p = 0.00063; and r = 0.446, p = 0.00064, respectively). More specifically, short-chain acylcarnitine concentrations were also positively correlated with HR levels (r = 0.457, p = 0.0004). Studies have shown that the elevation of blood concentrations of short and medium-chain acylcarnitines is related to the risk of cardiovascular disease, particularly in T2D patients [384,385]. Specifically, elevated levels of C2 and C4 acylcarnitines have shown associations with an increased risk of CVD. These metabolites were elevated during the KD and might be associated with the elevated HR levels observed during the KD.

As seen in Appendix Table 4-C, plasma levels of IPA were negatively correlated with HR levels (r = -0.487, p =0.00016). This compound has gained attention due to its potent antioxidant properties, neuroprotective effects, and potential anti-inflammatory benefits. Studies have shown that IPA can significantly reduce oxidative stress, which is a key factor in cardiovascular health [386]. Oxidative stress impairs heart function and contributes to higher HR. By mitigating oxidative stress, IPA helps improve overall heart function, which can lead to a lower HR. Additionally, its anti-inflammatory effects might reduce systemic inflammation, often associated with cardiovascular diseases and elevated HR. Furthermore, studies have highlighted IPA's involvement

in the gut-brain axis, particularly in modulating vagal tone, which influences HR regulation [386–388].

As shown in Appendix Table 4-D, higher urine arginine levels were observed in the lower HR days. This can be indicative of increased plasma arginine availability, which is linked to lower HR through enhanced NO synthesis. NO promotes vasodilation, reduces vascular resistance, and improves endothelial function, easing the heart's workload and slowing HR. Improved vascular health and reduced sympathetic nervous system activity also contribute to this effect. A study conducted by Lucotti et al. [389] showed that arginine supplementation improves endothelial function and reduces HR in patients with metabolic syndrome.

The integration of HR monitoring with metabolomic profiling provides a much more detailed understanding of the biochemical pathways affecting cardiovascular health. Significant correlations were observed between HR levels and various metabolites, such as ceramides, carnitines, IPA, and arginine, highlighting the links between lipid metabolism, oxidative stress, inflammation, and heart function. These findings highlight the value of using metabolomics to identify biomarkers that can predict cardiovascular outcomes and provide insights into individual responses to dietary interventions. This study demonstrated that elevated levels of ceramides and acylcarnitines are associated with adverse cardiovascular effects, while IPA and arginine are linked to beneficial impacts on HR and overall heart health. Overall, although this section has investigated only a few of the links between HR and metabolites, the findings underscore the significance of integrating wearable HR monitoring with metabolomic studies.

4.4.3. The Metabolome versus Systolic Blood Pressure (SYS BP) – Dietary Effects

A correlation analysis was performed to understand the possible connections and correlations between the blood and urine metabolites and the monitored SYS BP levels. The Pearson correlation coefficients and p-values are presented in Appendix Table 4-E (for plasma and SYS BP) and Appendix Table 4-F (for urine and SYS BP). While most plasma metabolites showed weak and relatively statistically insignificant correlations ($r < 0.4$, $p > 0.05$) with BP, several urinary metabolites exhibited strong correlations with SYS BP levels. The ratio of ornithine to

citrulline was significantly correlated to SYS BP levels (r = 0.386, p =0.004). This ratio is a crucial indicator of the balance impacting NO synthesis and, consequently, BP regulation [390]. In the urea cycle, ornithine combines with carbamoyl phosphate to form citrulline, which is then converted to arginine [391,392]. Arginine serves as a precursor for NO production, a molecule essential for vasodilation and vascular health. A high ornithine/citrulline ratio suggests an abundance of ornithine relative to citrulline, potentially indicating a bottleneck in arginine production [391,392]. This imbalance can lead to reduced NO synthesis, resulting in decreased vasodilation and increased vascular resistance, which elevates SYS BP. Studies such as those by Schwedhelm et al. [391] and Rashid et al. [393] have shown that enhancing citrulline levels can boost arginine production and improve NO-mediated endothelial function, subsequently lowering BP. Conversely, a disrupted ornithine-to-citrulline ratio, reflecting inefficient urea cycle function, can lead to insufficient NO production. This insufficiency can impair endothelial function, a critical factor in maintaining vascular tone and preventing hypertension.

As shown in Appendix Table 4-E, plasma levels of essential amino acids, such as lysine (r = 0.372, p =0.0052), and threonine (r = 0.367, p =0.0058), also showed positive correlations with SYS BP. Lysine plays a critical role in protein synthesis and various metabolic processes [394]. The impact of lysine on BP can be understood through several mechanisms. Lysine is known to influence the cardiovascular system and has been studied for its effects on hypertension. One key mechanism involves the balance between lysine and arginine, another essential amino acid. As mentioned earlier, arginine is a precursor for NO, a potent vasodilator that helps regulate BP [395]. Studies have shown that a high lysine-to-arginine ratio can affect NO production and subsequently BP [394–396]. For instance, a study on Wistar rats indicated that adjusting the arginine-to-lysine ratio affected hypertension by modulating NO levels and angiotensin-converting enzyme (ACE) activity, which plays a role in blood vessel constriction and BP regulation [395]. Additionally, lysine supplementation has been shown to reduce stress-induced BP spikes. A study on hypertensive individuals in Ghana demonstrated significant reductions in SYS BP with lysine supplementation, highlighting its potential in modulating cardiovascular responses to stress [394]. Another study by Vuvor et al. [396] suggested that lysine's role in stress and anxiety reduction might contribute to its BP-lowering effects.

As seen in Appendix Table 4-F, several metabolites in urine were also found to be correlated with SYS BP changes observed over the course of the study. For instance, urinary levels of 2-hydroxyisobutyric acid were positively correlated with SYS BP levels (r = 0.728, p =4.44E-16). 2-hydroxyisobutyric acid has been associated with hypertension and other cardiovascular conditions, but the evidence is limited. Elevated levels of 2-hydroxyisobutyric acid have shown association with an increased risk of developing hypertension [381]. Additionally, urinary 2-hydroxyisobutyric acid levels were significantly higher in patients who had suffered a myocardial infarction (heart attack) compared to healthy controls [397], suggesting elevated 2-hydroxyisobutyric acid may be a biomarker for cardiovascular disease, although a direct link to BP was not established.

Also seen in Appendix Table 4-F is the fact that urinary levels of orotic acid showed a significant and strong correlation with SYS BP (r = 0.723, p = 4.44E-16). In a study conducted by Choi et al. [398], it was observed that rats fed a diet containing 1% orotic acid developed systemic insulin resistance and hypertension, with a 25% increase in SYS BP compared to controls. Orotic acid affects insulin signaling pathways such as PI3K-Akt and AMPK, leading to reduced NO production and endothelial dysfunction, which subsequently affects BP.

Additionally, urinary adenosine levels showed a negative correlation to SYS BP levels (r = -0.710, p =3.11E-15). Adenosine concentrations in urine have a complex relationship with BP regulation, with evidence suggesting both hypertensive and hypotensive effects depending on the context. Systemic or intrarenal adenosine elevations could raise BP via renal nerve stimulation [399], while adenosine receptor activation may lower BP by reducing renin release [400], and oxidative stress [401]. Urinary adenosine levels reflect its production and metabolism, but the direct effects on BP likely depend on local tissue concentrations and receptor activation in specific organs like the kidneys.

While this section has focused only on a selection of relationships between BP and metabolites, the findings highlight the benefits of integrating BP monitoring with metabolomic analysis. The significant correlations observed between SYS BP levels and a number of metabolites, such as the ornithine to citrulline ratio, lysine, threonine, 2-hydroxyisobutyric acid, orotic acid, and adenosine, underscore the connections between amino acid metabolism, nitric

oxide synthesis, and cardiovascular health. These findings emphasize the value of using metabolomics to identify biomarkers that can predict BP regulation and provide insights into individual responses to dietary interventions.

4.4.4. The Metabolome versus Body Temperature (BT) – Dietary Effects

Grouped analysis of high (daily average BT above 37.1 ºC) and low BT (daily average BT below 36.8 ºC) against blood and urine metabolites did not reveal any statistically significant metabolites between the two groups. However, a correlation analysis was performed to understand the possible connections and associations between the blood and urine metabolites and the monitored BT levels. The Pearson correlation coefficients and p-values are presented in Appendix Table 4-G (for plasma and BT) and Appendix Table 4-H (for urine and BT). While plasma concentrations of several metabolites showed moderate to weak correlations with BT levels, urinary metabolites showed more significant and strong correlations with BT. For example, urinary 2-hydroxyglutaric acid levels showed a significant positive correlation with BT (r = 0.7319, p = 2.22E-16). Small amounts of 2-hydroxyglutaric acid may be produced under regular metabolism but its accumulation to higher levels tends to indicate that the mitochondrial enzyme responsible for converting 2-hydroxyglutarate back to α-ketoglutarate (2-hydroxyglutarate dehydrogenase) is deficient or impaired. This suggests that high BT levels may impair mitochondrial function and that 2-hydroxyglutarate dehydrogenase is a particularly sensitive body temperature sensor. For instance, in conditions where metabolic rates are disrupted, as with metabolic disorders such as L-2-hydroxyglutaric aciduria, BT regulation can be impaired [402]. Additionally, analysis of urinary excretions in metabolic conditions often reveals significant changes in metabolites like 2-hydroxyglutaric acid, which could be linked to altered thermogenesis and heat production [402].

The correlation observed between BT levels and urinary metabolites such as 2-hydroxyglutaric acid, highlights the interesting connections between metabolic processes, mitochondrial function, oxidative stress, and thermoregulation. These findings emphasize the value of using metabolomics to identify biomarkers that can predict thermoregulatory responses and provide insights into individual metabolic health. Overall, while this section only explored a

very small number of relationships between BT and metabolite levels, the results highlight the potential benefits of combining wearable BT monitoring with metabolomic research.

4.4.5. The Metabolome versus Body Weight – Dietary Effects

A correlation analysis was performed to understand the possible connections and correlations between the blood and urine metabolites and the monitored body weights. The Pearson correlation coefficients and p-values are presented in Appendix Table 4-I (for plasma and weight) and Appendix Table 4-J (for urine and weight). The most significant correlation between my body weight and plasma metabolites was a negative correlation observed with salicylic acid concentrations (r = -0.57173, p = 5.13E-06). In other words, higher salicylic acid levels were associated with weight loss and lower salicylic acid levels were associated with weight gain. Salicylic acid is a plant-derived phenolic compound known for its anti-inflammatory properties and is used in various medications, including aspirin. Salicylic acid is naturally found in fruits and vegetables and is involved in plant defense mechanisms [403,404]. In humans, it has been linked to reduced inflammation and may play a role in metabolic health. Lower serum salicylic acid levels have been observed in individuals with obesity, and interventions that increase salicylic acid levels can improve metabolic profiles, suggesting its potential role in managing metabolic disorders and managing weight conditions [405].

As seen in Appendix Table 4-I, the total plasma concentration of unsaturated diglycerides (r = 0.52277, p =4.23E-05) and total diglycerides (r = 0.48483, p =0.000176) showed a significant positive correlation with my body weight. In other words, high levels of unsaturated diglycerides and total diglycerides were associated with increased weight while low levels were associated with decreased weight. This correlation is likely due to the role of diglycerides in lipid metabolism and energy storage. Diglycerides, particularly those with unsaturated fatty acids, can influence the metabolic pathways that regulate fat accumulation and energy expenditure [406]. Elevated levels of these metabolites can enhance the incorporation of fatty acids into triglycerides, leading to increased fat accumulation when dietary intake exceeds energy expenditure [406]. These findings are in alignments with research involving both animal models and human subjects that has shown that higher concentrations of unsaturated diglycerides are associated with greater average daily weight gain and changes in lipid profiles that favor fat storage [406,407].

Also seen in Appendix Table 4-I is the fact that plasma levels of long-chain acylcarnitines showed a significant positive correlation to weight changes (r = 0.54849, p =1.46E-05). In other words, high levels of long-chain acylcarnitines were associated with increased weight while low levels were associated with decreased weight. Long-chain acylcarnitines are metabolites involved in fatty acid oxidation, which can accumulate in plasma due to metabolic inefficiencies or imbalances. Elevated levels of long chain acylcarnitines in plasma have shown an association with insulin resistance and obesity [408]. This accumulation can lead to impaired fatty acid oxidation, contributing to weight gain and metabolic disorders [408].

As seen in Appendix Table 4-J, urinary levels of 2-hydroxy-2-methylbutyric acid (2-HMBA) were positively correlated with weight changes (r = 0.73203, p =2.22E-16), indicating that higher levels of this metabolite were associated with increased body weight. 2-hydroxy-2-methylbutyric acid is a product of BCAA degradation, particularly valine, leucine, and isoleucine, and is associated with ketogenesis [409]. This correlation suggests that increased dietary intake of BCAAs or heightened BCAA catabolism may be contributing to the observed weight changes. High protein intake, especially from sources rich in BCAAs, stimulates metabolic pathways involved in amino acid oxidation, potentially reflecting a state of excess caloric intake. The metabolite 2-HMBA has also been linked to insulin resistance and alterations in lipid metabolism, which are both factors that can contribute to weight gain and metabolic dysfunction [410]. Similarly, 2-hydroxyisobutyric acid levels in urine showed a positive correlation to weight changes (r = 0.72782, p =4.44E-16), indicating that higher levels of this metabolite are associated with increased body weight. Elevated levels of urinary 2-HIBA have been associated with increased body weight and adiposity [411]. This compound may be involved in metabolic pathways linked to lipid metabolism and energy homeostasis. Additionally, bariatric surgery-induced weight loss has been shown to significantly alter urinary levels of 2-hydroxyisobutyric acid, further establishing its link to weight changes [412].

Also seen in Appendix Table 4-J is the fact that urinary concentrations of cis-aconitic acid, an intermediate in the citric acid cycle, showed a significant positive correlation to body weight (r = 0.71427, p =1.78E-15). This metabolite is intricately linked to several metabolic processes that impact body weight. As part of the tricarboxylic acid (TCA) cycle, cis-aconitic acid is formed from citrate and converted to isocitrate via aconitase, playing an important role in cellular energy

production and metabolic regulation [413]. Interestingly, the total concentration of TCA acids involved in the TCA cycle (citrate, cis-aconitic acid, alpha-ketoglutarate, succinate, fumarate, and malate) was positively correlated to body weight changes (r = 0.56857, p =4.12E-09). The positive correlation between plasma levels of TCA cycle intermediates and body weight may indicate early metabolic changes that increase the risk of developing obesity. Elevated levels of TCA cycle organic acids, especially in the urine, may indicate metabolic inefficiencies, such as impaired energy metabolism and mitochondrial dysfunction. This can lead to inefficient calorie burning, increased fat storage, and insulin resistance, all contributing to weight gain. Additionally, these disruptions may exacerbate oxidative stress and inflammation, further promoting weight gain and metabolic disorders. Indeed, slight weight gain can initiate mitochondrial stress and mild insulin resistance, which disrupt normal glucose metabolism [414]. This disruption can cause higher circulating levels of glucose and other substrates that feed into the TCA cycle, thereby increasing the plasma concentrations of its intermediates. These early metabolic alterations can set the stage for more significant metabolic dysfunction if weight gain continues, potentially leading to obesity. Furthermore, slight weight gain can trigger low-grade inflammation and oxidative stress, which are precursors to more severe metabolic disturbances associated with obesity [415,416].

The significant correlations observed between body weight and various metabolites, such as salicylic acid, diglycerides, long-chain acylcarnitines, 2-hydroxy-2-methylbutyric acid, and cis-aconitic acid, underscore the many connections between body weight and many metabolic processes, lipid metabolism, oxidative stress, and energy regulation. These findings emphasize the value of using metabolomics to identify biomarkers that can either predict weight changes or drive weight change and provide insights into individual metabolic health. While this section has limited its discussion to only a small selection of the relationships between body weight and metabolites, the findings highlight the benefits of integrating body weight monitoring with metabolomic analysis.

4.4.6. The Metabolome versus Sleep Quality – Dietary Effects

A low quality of sleep, more specifically insufficient sleep, significantly impacts metabolic health, particularly affecting lipid metabolism, as revealed in several studies. Weljie et al. [417]

found that restricting sleep to four hours for five days in a row in healthy adults led to significant alterations in blood levels of fats and molecules such as lysophosphatidylcholines, triglycerides, phosphatidylcholines, and acylcarnitines. Importantly, these alterations persisted even after a night of recovery sleep, with a notable decrease in long-chain acylcarnitines and an increase in amino acids such as tryptophan and phenylalanine not returning to baseline levels [417]. Similarly, a study involving 16 normal-weight individuals demonstrated that periods of insufficient sleep (5 hours) compared to adequate sleep (9 hours) resulted in pronounced changes in the lipid metabolome [418]. These changes were associated with processes crucial for fat metabolism and the functioning of cell membranes, including shifts in levels of phospholipids and sphingolipids [418]. Further research demonstrated that even minor sleep reductions could significantly affect individuals with a familial history of diabetes, showing an increase in various blood lipids and changes in carbohydrate and amino acid metabolism. These studies highlight that insufficient sleep adversely affects metabolic health, including reducing beneficial high-density lipoproteins, underscoring the importance of adequate sleep in maintaining metabolic health and preventing disorders [419].

To investigate the possible connection between metabolomics measurements and sleep patterns, the sleep data obtained from my Fitbit smartwatch was analyzed to determine the quality of the sleep on each night. Several factors were considered to assess the quality of sleep. Short total sleep time (under 6 hours), low sleep efficiency (indicating a significant portion of time in bed is spent awake), minimal deep or REM sleep (suggesting insufficient restorative sleep), high wakefulness after sleep onset, significant time spent awake after initially falling asleep, high levels of restlessness or movements during sleep, and a higher percentage of time with the HR above the resting HR during sleep (indicating possible stress or less restful sleep) were classified as poor sleep quality. As most of the nights resembled a moderate sleep quality and overall similar sleep patterns, the nights with the most significant differences were chosen as either “good” or “poor”-quality sleep. The sleep data were grouped into good and poor-quality sleep based on a scoring system that evaluated key sleep metrics [420]. Objective measures included Total Sleep Time (TST), Sleep Efficiency (SE), and time spent in various sleep stages (light, deep, and REM sleep). TST was measured to ensure it aligned with recommended durations, generally 7-9 hours for adults. SE was calculated as the percentage of time spent asleep while in bed, with 85% or higher was considered good quality sleep. The time spent in deep and REM sleep stages is crucial, with deep sleep ideally comprising 13-23% and REM sleep 20-25% of TST. Wakefulness After Sleep Onset

(WASO) was another key metric, indicating sleep fragmentation, lower WASO values signify better sleep continuity [420]. The plasma metabolomes of those days were compared with each other. Figure 4.40 indicates a PCA and PLS-DA analysis performed on these plasma samples revealed the metabolomic separation between the two groups of high and low-quality sleep. Additionally, heatmap analysis confirmed differences in the plasma metabolomic composition of the good sleep and poor sleep quality groups (Figure 4.41).

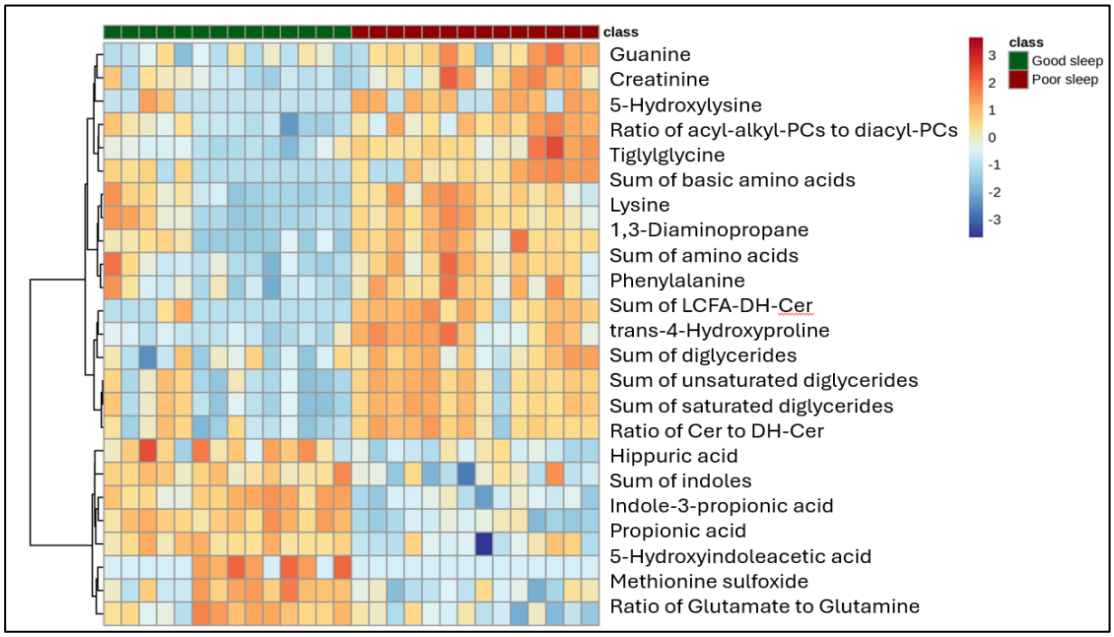

Figure 4.40 - Hierarchical Clustering Heatmap of the plasma samples of good and poor sleep quality

Similar to the plasma samples, urine samples were also grouped according to good and poor sleep quality. After normalizing for creatinine, 29 metabolites (or calculated sums and ratios) were detected as significantly different between the two groups. PCA and PLS-DA analysis performed on these urine samples reveal the metabolomic separation between the two groups of high and low-quality sleep (Figure 4.43). Metabolites such as 3-methoxytyramine (p = 2.3759E-7), cystathionine (p = 1.4677E-4), and 3-hydroxyphenylacetic acid (p = 1.7234E-4) were higher in the urine samples of the good sleep quality group. Conversely, trans-4-hydroxyproline (p = 1.4385E-5), the total concentration of hydroxyproline (p = 1.543E-5), betaine (p = 7.8551E-5), and valine (p = 1.4411E-4) were higher in the poor sleep quality group. Figure 4.44 represents the top 9 significantly different metabolites between the urine samples of both groups.

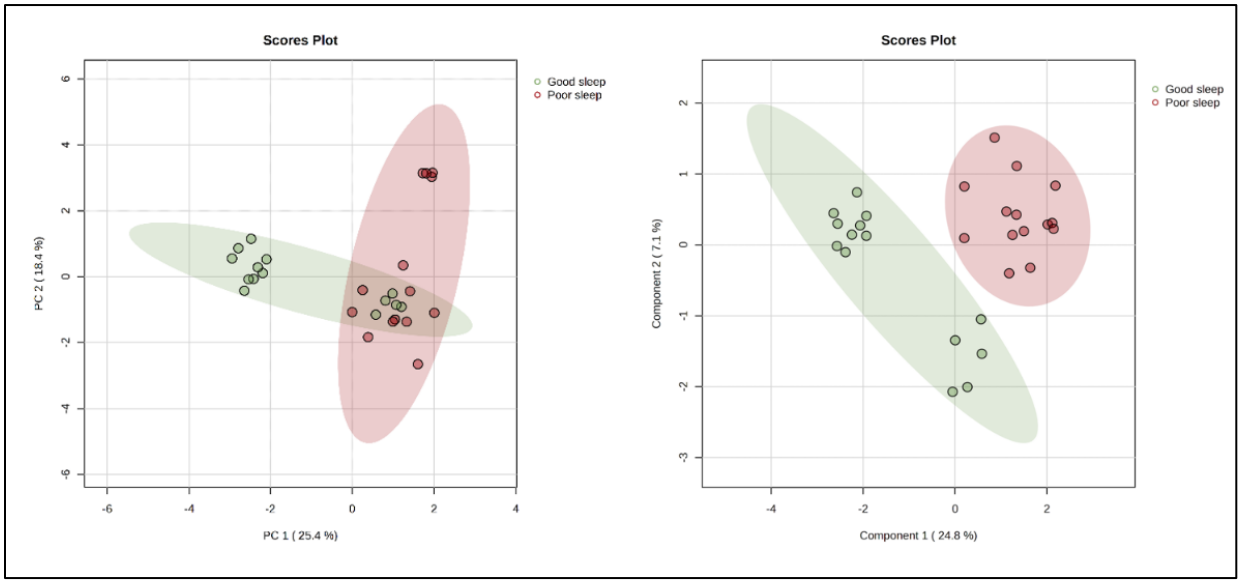

Figure 4.41 - The PCA (left) and PLS-DA (right) of the plasma samples, showing a clear separation between the metabolomic composition of different sleep qualities.

Overall, a notable shift in the composition of amino acids, lipids, and various organic acids was noted between the two groups. Research using metabolic profiling under conditions of sleep and sleep deprivation in humans has revealed differences in plasma metabolites linked to amino acid, lipid, and neurotransmitter metabolism between the two groups [422]. In particular, increased plasma levels of lipids and acylcarnitines were noted during periods of sleep deprivation [423]. Higher plasma levels of IPA were identified in samples collected on days/nights with a better sleep quality. IPA is a potent antioxidant that exceeds melatonin in its ability to neutralize harmful hydroxyl radicals, which are crucial for preventing oxidative stress [424]. IPA promotes sleep primarily through its interactions within the gut-brain axis and its influence on neurotransmitter systems, rather than its antioxidant properties [425]. IPA is both a plant hormone (found in high abundance in fruits such as bananas) and a naturally produced metabolite synthesized by certain gut bacteria from the amino acid tryptophan. The presence of IPA reflects a healthy and active gut microbiota, which is crucial for optimal brain function [425,426]. The gut-brain axis is a bidirectional communication system where metabolites such as IPA can influence brain activity and mood. One of the key pathways involved is the serotonin pathway. Tryptophan, the precursor of serotonin, is

metabolized in the gut, and the presence of IPA indicates active tryptophan metabolism. Increased serotonin levels in the brain promote relaxation and well-being, which is then converted into melatonin, the hormone responsible for regulating the sleep-wake cycle [426]. Additionally, IPA may modulate the levels of other neurotransmitters such as GABA (gamma-aminobutyric acid), which has calming effects and promotes sleep. By influencing neurotransmitter systems, IPA helps create a balanced neurochemical environment conducive to restful sleep. Furthermore, IPA's ability to regulate circadian rhythms, the body's internal clock, plays a crucial role in maintaining a stable sleep pattern [425].

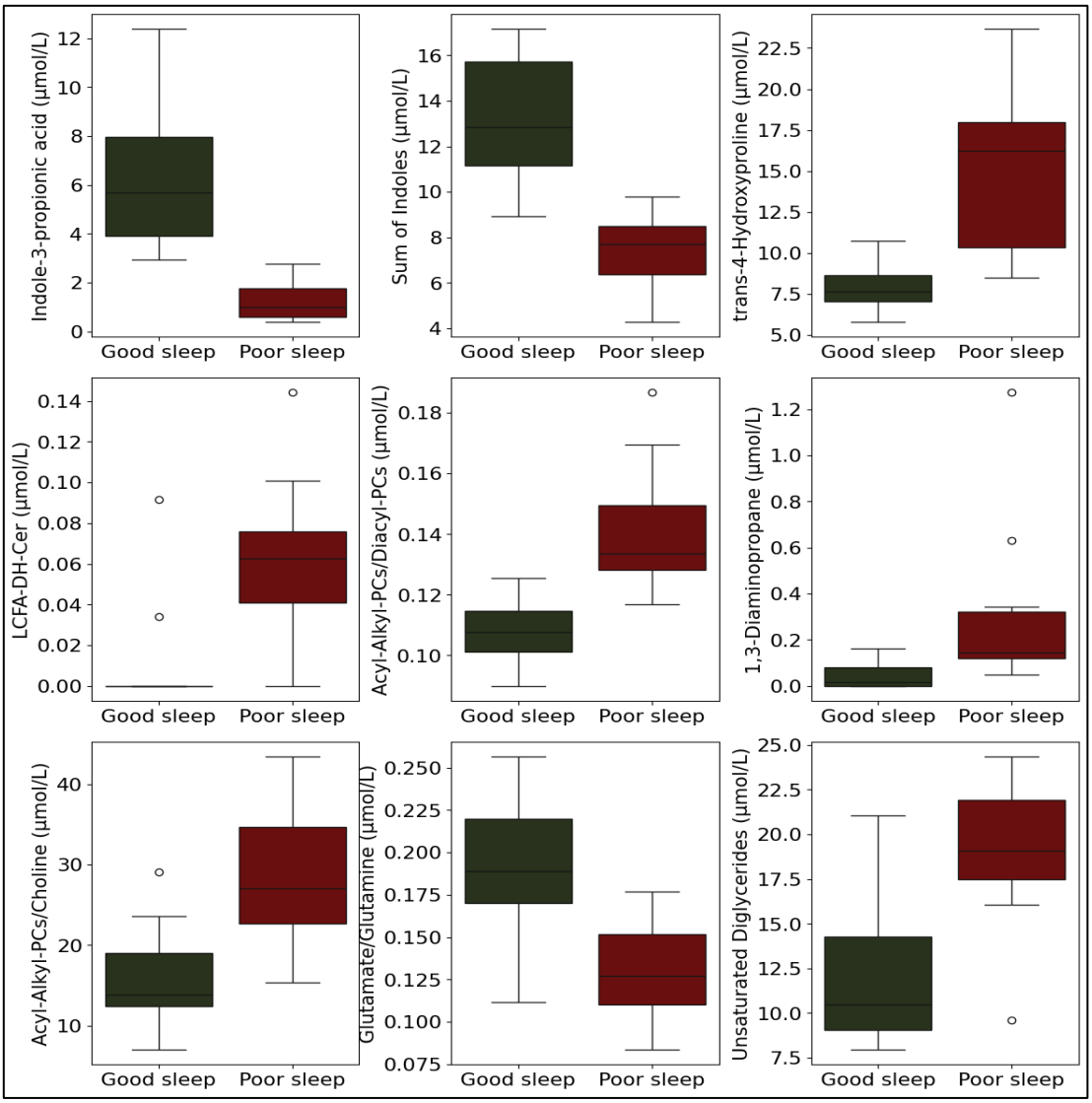

Figure 4.42 - Boxplots of the top 9 significantly different plasma metabolites between poor and good sleep quality days; LCFA-DH-Cer (long-chain fatty acid dihydroceramides); Acyl-Alkyl-PCs/Diacyl-PCs (ratio of acyl-alkyl-phosphatidylcholines to diacyl-phosphatidylcholines)

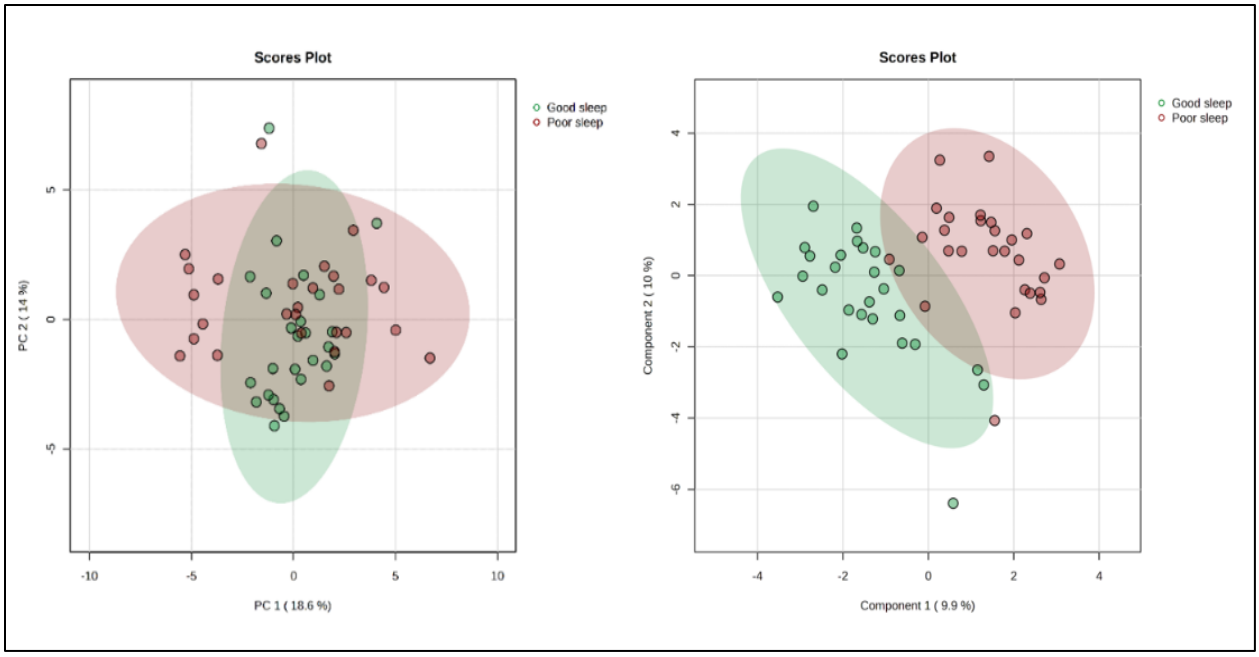

Figure 4.43 - PCA (left and PLS-DA (right) of urine samples of good vs poor sleep

Plasma and urine concentrations of 5-hydroxyindoleacetic acid, a serotonin metabolite, were significantly higher in the plasma samples from the good-quality sleep nights in comparison to the poor-quality of sleep nights (p = 0.0017, and p = 0.0048, respectively). Serotonin is a key neurotransmitter in the regulation of sleep and wakefulness, primarily by encouraging wakefulness and suppressing rapid eye movement (REM) sleep [427]. It is also known as the "happy hormone" [428]. When serotonin levels are higher (i.e. when an individual is in a good mood), its breakdown into 5-hydroxyindoleacetic acid increases, resulting in elevated concentrations of this metabolite in both plasma and urine. Additionally, good sleep reduces stress and anxiety. Stress and anxiety are known to negatively affect serotonin production [427,429]. Stress reduction enhances serotonin synthesis and its subsequent metabolism into 5-hydroxyindoleacetic acid, further contributing to the higher levels observed during nights of good sleep. Moreover, good quality sleep helps maintain stable circadian rhythms, which are essential for efficient metabolic processes, including the metabolism of serotonin into 5-hydroxyindoleacetic acid. Stable circadian rhythms ensure that neurotransmitter production and breakdown occur at consistent rates. Interestingly, the results from my POMS analysis (see next section) align with the sleep data as the nights with lower-quality sleep resulted in a worse score of total mood disturbance the next day. Lack of sleep, limited

opportunities for sleep, and disruptions in the body's internal clock can lead to adverse impacts on mental well-being and metabolic health issues [423].

Taurine levels (although not quite statistically significant between the two groups) were found to be higher in the urine samples of the good sleep quality nights. Taurine is an amino acid that occurs naturally within the body and plays a crucial role in cardiovascular function, the development of the nervous system, and the regulation of antioxidants and electrolytes [430,431]. Its impact on sleep can be understood through its actions on the central nervous system and its influence on neurotransmitters that affect sleep. Taurine acts as a modulator of neurotransmitters in the brain. It can exert a calming effect by activating GABA receptors, the primary inhibitory

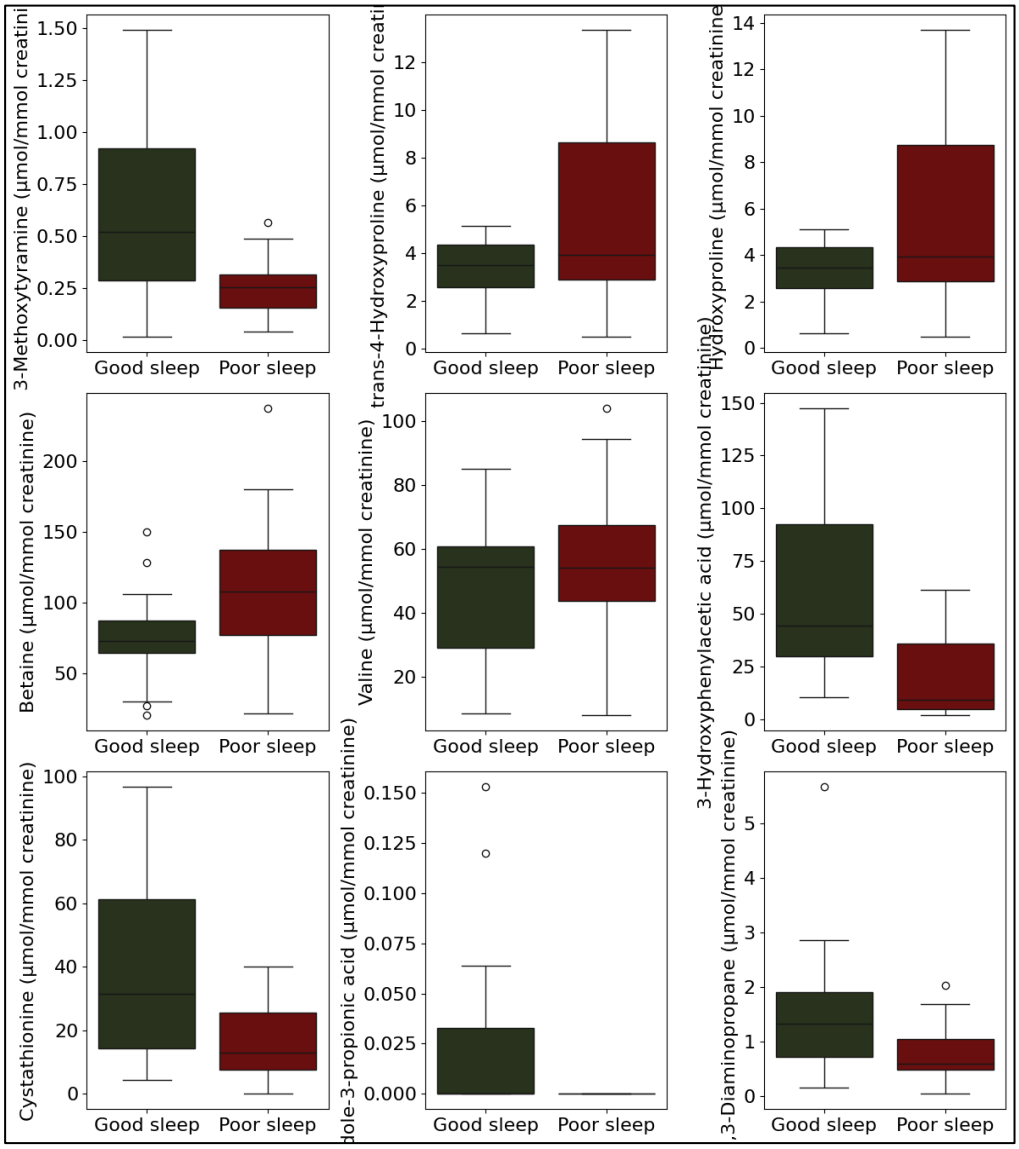

Figure 4.44 - Top 9 significantly different urine metabolites in good vs poor sleep groups

neurotransmitter in the brain [431,432]. By enhancing GABAergic activity, taurine can promote relaxation and potentially aid in the initiation and quality of sleep. Due to its role in enhancing GABAergic activity, taurine may also help reduce feelings of anxiety and stress [433], which are common culprits behind sleep disturbances. By mitigating these negative emotional states, taurine supplementation might contribute to better sleep patterns.

Urinary levels of 3-hydroxyisobutyric acid (p = 0.032) and valine (p = 0.00014) were higher after my poor-quality sleep nights. It is important to remember that individuals suffering from long periods of poor-quality sleep or poor sleep hygiene tend to gain excess weight and are more prone to developing diabetes [434]. 3-hydroxyisobutyric acid, a catabolic intermediate of the BCAA valine, is associated with insulin resistance, T2D, and obesity [435]. Elevated levels of circulating 3-hydroxyisobutyric acid have been observed in conditions of hyperglycemia, insulin resistance and established T2D, linking it to metabolic health [435]. Furthermore, 3-hydroxyisobutyric acid influences white and brown adipocyte metabolism, modulating fatty acid uptake and insulin-stimulated glucose uptake, which suggests its involvement in metabolic functions closely linked to obesity, insulin resistance, and T2D [435]. Given the connection between metabolic health and sleep quality, with poor sleep contributing to metabolic dysregulation, it is plausible that increased levels of metabolites like 3-hydroxyisobutyric acid and valine could reflect broader metabolic shifts that occur with sleep deprivation. Metabolic dysregulation, in turn, may influence sleep quality, forming a cycle where each condition potentially exacerbates the other.

The concentration of creatine in the urine and plasma samples measured after my poor sleep quality nights was found to be higher than in the good sleep quality samples. This increase in creatine levels in response to sleep deprivation highlights a complex interplay between sleep and energy metabolism, however, there are no studies highlighting the direct impact of creatine on sleep. Several studies have shown that creatine can improve cognitive performance during periods of sleep deprivation [436,437]. For instance, a high dose of creatine (0.35 g/kg body weight) has been found to enhance cognitive abilities and processing speed in sleep-deprived individuals. This suggests that creatine could partially reverse metabolic alterations and cognitive deterioration caused by sleep deprivation [436]. Additionally, cortisol, a primary stress hormone, is known to increase during periods of sleep deprivation or disturbed sleep [438]. Elevated cortisol levels promote the breakdown of muscle proteins to release amino acids, which are then used for

gluconeogenesis and energy production, and this catabolic state results in the increased production of creatine.

Overall, the findings highlighted here underscore the significance of integrating sleep monitoring with metabolomic analysis. The significant correlations observed between sleep quality and various metabolites, such as IPA, serotonin, taurine, and 3-hydroxyisobutyric acid, emphasize the complex interactions between metabolic processes, oxidative stress, and neurotransmitter regulation. These findings emphasize the value of using metabolomics to identify biomarkers that can predict sleep quality and provide insights into individual metabolic health.

4.4.7. The Metabolome versus Profile of Mood States (POMS) – Dietary Effects

It has been shown that fluctuations in specific metabolites are linked to changes in mood states, suggesting that metabolic pathways play a significant role in emotional regulation [439–441]. For instance, alterations in amino acid metabolism, neurotransmitter synthesis, and energy production are all critical factors that can influence mood and are reflected in metabolomic data [441,442]. To further investigate these diet-induced changes in mood, a correlation analysis was performed to understand the possible connections and correlations between blood and urine metabolites and the monitored mood changes. The comparison was conducted using the total mood disturbance score. Positive correlations suggest a possible connection between increased metabolite levels and increased disturbed mood levels. The Pearson correlation coefficients and p-values are presented in Appendix Table 4-K (for plasma and POMS) and Appendix Table 4-L (for urine and POMS).

As seen in Appendix Table 4-K, the total plasma concentration of intermediates of the TCA cycle ($r = 0.688$, $p = 0.0008$), including citrate, cis-aconitate, α-ketoglutarate, succinate, fumarate, and malate, were positively correlated with mood disturbance scores [439]**.** Citrate is the first intermediate in the citric acid cycle, formed by the condensation of acetyl-CoA and oxaloacetate. Citrate has been shown to modulate brain energy metabolism and neurotransmitter synthesis, which can influence mood and behavior. Disruptions in citrate levels have been associated with mood disorders, such as depression [439,443]. Cis-aconitate is an intermediate formed during the

isomerization of citrate to isocitrate, catalyzed by the enzyme aconitase. Aconitase deficiency (leading to very high levels of citrate and low levels of cis-aconitate), which can arise from genetic mutations or oxidative stress, has been linked to mood disorders and neurological conditions [443]. Reduced levels of cis-aconitate have been observed in individuals with aconitase deficiency, potentially contributing to mood dysregulation [443]. Another TCA metabolite, α-ketoglutarate is a key intermediate in the citric acid cycle and plays an important role in energy metabolism and neurotransmitter synthesis. Alterations in α-ketoglutarate levels have also been associated with mood disorders, such as depression [443]. Additionally, α-ketoglutarate has been studied for its potential therapeutic effects in treating mood disorders due to its involvement in glutamate metabolism and its well-known antioxidant properties. While the direct links between succinate, fumarate, and malate and mood regulation are less well-established, these intermediates are essential for the proper functioning of the citric acid cycle and energy production [439]. Disruptions in their levels or the enzymes involved in their metabolism can potentially impact mood and mental health through their effects on cellular energy homeostasis and oxidative stress.

The concentration of proteinogenic BCAAs such as leucine (r = -0.625, p = 3.42E-07), isoleucine (r = -0.572, p = 4.97E-06), and valine (r = -0.641, p = 1.33E-07) in urine was negatively correlated with mood disturbance scores. In other words, high urinary levels of BCAAs corresponded to better mood states. However, no significant associations were detected with plasma concentrations of these metabolites. Previous studies have confirmed that these metabolites have a negative association with the development of depression [444–446] and that higher dietary intake of BCAAs has shown an inverse association with the state of depression and anxiety. The mechanism behind BCAAs and mood states is complex. It is thought that BCAAs can serve as an energy source for the brain under certain conditions. Improved energy availability in the brain may enhance cognitive function and mood, helping to ward off depressive symptoms. BCAAs also appear to have anti-inflammatory properties and contribute to neuroprotection. Chronic inflammation is linked to depression, so the anti-inflammatory effects of BCAAs could be one way they help improve mood and prevent depressive symptoms. Another mechanism may be through the mTOR (Mechanistic Target of Rapamycin) pathway. The mTOR pathway is a critical regulator of cellular growth, protein synthesis, and synaptic plasticity. BCAAs, particularly leucine, activate the mTOR pathway, which in turn influences several cellular processes vital for brain function [444]. Activation of the mTOR pathway by BCAAs supports neuroplasticity, the brain's ability to

adapt and reorganize itself. In depression, neuroplasticity is often impaired, leading to reduced synaptic connections and brain atrophy in regions critical for mood regulation, such as the hippocampus and prefrontal cortex. By promoting protein synthesis and neuronal growth, BCAAs can enhance neuroplasticity and potentially alleviate depressive symptoms. Additionally, the mTOR pathway plays a role in modulating the synthesis of neurotransmitters and maintaining energy balance within the brain, further linking BCAAs to good mood regulation.

One aspect to consider when discussing the KD and mood is the phenomenon known as "keto flu". Keto flu refers to a set of symptoms that some individuals experience when transitioning to a KD [442]. These symptoms can include headache, fatigue, irritability, dizziness, nausea, difficulty sleeping, and mood swings [442,447], which was similar to my experience while being on the KD. The keto flu is believed to result from the body adapting to a new source of energy and shifting from glucose to ketone bodies, along with changes in electrolyte balance and hydration levels [442]. While these symptoms are usually temporary and resolve within a few days to a week, they can impact an individual's mood and overall well-being during the initial phase of the KD.

Urine levels of glycine showed a positive correlation with mood disturbance scores (r = 0.659, p = 4.55E-08), meaning that high levels of urinary glycine led to a worse mood state. Elevated urinary levels of glycine are indicative of plasma levels of glycine being modestly reduced or glycine being transported away from key tissues and organs. Glycine's role in neurotransmission, particularly its involvement with NMDA receptors, means that reduced levels in the brain could disrupt brain function, leading to mood disturbances [448]. Additionally, high urinary glycine might reflect increased oxidative stress or inflammation, both linked to depression. Thus, elevated urinary glycine could signal underlying issues that negatively impact mood. Glycine is a major neurotransmitter involved in various neuronal processes, including modulating mood and affective states. Increased levels of glycine have been found to stimulate the production of serotonin, the "feel-good" hormone, which helps elevate mood, improve sleep, and enhance cognitive functions [448,449]. It stands to reason that reduced plasma levels of glycine (due to its removal into the urine) would lead to reduced levels of serotonin and a less pleasant mood state.

Overall, although this section has discussed only a selection of the relationships between mood and metabolites, the findings highlight the significance of integrating mood monitoring with

metabolomic analysis. These findings also emphasize the value of using metabolomics to identify biomarkers that can predict mood changes and provide insights into individual mental health.

4.4.8. The Metabolome versus Mental Cognitive Assessment tests (MCA) – Dietary Effects

Previous studies have demonstrated that changes in metabolites involved in energy metabolism, neurotransmitter synthesis, and amino acid processing can significantly impact cognitive abilities. For example, alterations in metabolites such as glucose and ATP can affect brain energy levels and, consequently, cognitive performance [450]. Similarly, variations in neurotransmitter precursors and breakdown products, such as glutamate, GABA, and dopamine, are closely linked to learning, memory, and executive functions [451]. To further investigate these associations, a correlation analysis was performed to understand the possible connections and correlations between the blood and urine metabolites and mental cognitive assessment tests. This correlation analysis was performed using the total mental cognitive assessment scores, meaning a positive correlation indicates a positive association of a given metabolite with improved mental performance. The Pearson correlation coefficients and p-values are presented in Appendix Table 4-M (for plasma and MCA) and Appendix Table 4-N (for urine and MCA). Of the plasma metabolites, only malonic acid (r = 0.613, p = 0.0018) and argininic acid (r = 0.586, p = 0.0033) showed a significant positive correlation with mental performance test scores, while several other metabolites showed a negative correlation.

The direct cognition-promoting association of malonic acid and argininic acid has not been investigated and I can provide no rationale as to why these two compounds would lead to enhanced cognition.

The total concentration of urea cycle amino acids (ornithine, arginine, citrulline, and aspartic acid) in plasma showed a significantly negative correlation with mental performance (r = -0.569, p = 0.005). This negative correlation was also observed with urinary levels of these metabolites, but it was weaker (r = -0.291, p = 0.016). As mentioned earlier, the urea cycle is a metabolic pathway that plays a crucial role in regulating amino acid levels and detoxifying ammonia by converting it to urea, which can be safely excreted by the body [452,453]. This cycle is

particularly important for brain function and mental performance due to its involvement in maintaining neurotransmitter balance and preventing ammonia toxicity [452]. The urea cycle is responsible for detoxifying ammonia, a neurotoxin byproduct of amino acid catabolism, which can cross the blood-brain barrier, causing cerebral edema, oxidative stress, and neurotransmitter imbalances if allowed to accumulate. This disrupts neurotransmitter balance and energy metabolism in the brain, contributing to cognitive impairment [452,453]. Arginine and citrulline are intermediates in the urea cycle that help in the detoxification of ammonia. Events such as deficiencies in enzymes that process these amino acids can lead to their accumulation and subsequent hyperammonemia. For instance, deficiencies in ornithine transcarbamylase (OTC) prevent the conversion of carbamoyl phosphate to citrulline, leading to ammonia build-up [454]. Aspartate is another amino acid involved in the urea cycle, acting as a nitrogen donor. Its role is crucial for the synthesis of argininosuccinate from citrulline. Disruptions in this process can also lead to the accumulation of ammonia and other toxic intermediates, affecting cognitive function [454].

It is worth noting that, studies investigating the association between diet and cognition in older people have shown that adherence to the MD is associated with reduced risk of mild cognitive impairment and dementia [455]. This association is believed to be due to the diet's positive impact on cerebrovascular health and its prevention of neurodegeneration [54,456]. The significant correlations observed between cognitive performance and various metabolites, such as urea cycle amino acids, highlight the intricate connections between metabolic processes, neurotransmitter regulation, and cognitive function. These findings emphasize the value of using metabolomics to identify biomarkers that can predict cognitive performance and provide insights into individual mental health. Overall, although this section has discussed only a selection of the relationships between MCA scores and metabolites, the findings emphasize the significance of integrating cognitive performance monitoring with metabolomic analysis.

4.4.9. The Metabolome versus Cytokine Proteomic Responses – Dietary Effects

The integration of metabolomic and proteomic analyses has shown that there are significant connections between metabolic processes and protein expression, particularly with regard to

cytokines which are involved in immune responses [457–459]. Studies have demonstrated that metabolic alterations, such as those involving glucose and certain amino acids, can modulate cytokine production and secretion. For instance, elevated glucose levels have been linked to increased production of pro-inflammatory cytokines such as IL-8, highlighting the interplay between metabolic status and immune function [457,458]. Similarly, amino acids such as glutamine reduce the production of pro-inflammatory cytokines by the human intestinal mucosa, likely through a post-transcriptional pathway [460,461]. This modulation can be beneficial in managing inflammatory conditions such as inflammatory bowel disease (IBD), where there is an imbalance in cytokine production[461]. To investigate these connections further, a correlation analysis was performed to understand the possible connections and correlations between the blood and urine metabolites and the cytokines that were quantified through my proteomics analysis. The Pearson correlation coefficients and p-values are presented in Appendix Table 4-P (for plasma and cytokines) and Appendix Table 4-Q (for urine and cytokines). Only one cytokine exhibited any clear metabolite associations. Specifically, IL-8 concentrations were significantly and positively correlated to plasma glucose levels, as measured through LC/MS testing (r = 0.9627, p =3.18E-05). This result is consistent with other studies as it has been shown that high glucose concentrations can directly stimulate the production and secretion of IL-8 from various cell types [457]. *In vitro* studies on cultured human endothelial cells showed that high plasma glucose levels induced significant increases in IL-8 mRNA expression and protein release compared to normal glucose conditions [457]. These findings suggest that acute exposure to elevated glucose levels can directly trigger IL-8 secretion, even in normoglycemic individuals. Moreover, several studies have shown that individuals with obesity and impaired glucose tolerance exhibit an exaggerated IL-8 response to an oral glucose load compared to those with normal glucose tolerance [458]. More specifically, Straczkowski et al. [458] found that in subjects with obesity and impaired glucose tolerance, post-load IL-8 levels correlated positively with post-load glucose concentrations, independent of insulin sensitivity. It is worth noting that this correlation was not observed with the daily average BG levels that were monitored through a continuous glucose monitor.

Cytokines like IL-8 are small signaling proteins that mediate and regulate immunity, inflammation, and hematopoiesis. They are produced in response to various stimuli, including metabolic changes [459]. High levels of glucose can influence cytokine production by activating various signaling pathways such as the NF-κB pathway [459]. When cells are exposed to high

glucose concentrations, reactive oxygen species (ROS) are generated as by-products of glucose metabolism. ROS can activate the NF-κB pathway, leading to the transcription of pro-inflammatory cytokines, including IL-8 [459]. The integration of metabolomic and proteomic analyses provides a comprehensive view of the biochemical processes that influence health and disease. The significant correlations between glucose levels and IL-8 concentrations underscore the connections between metabolism and immune function. These findings highlight the potential of using combined metabolomic and proteomic data to identify biomarkers that can predict inflammatory responses and metabolic health.

4.4.10. The Metabolome versus The Gut Microbiome – Dietary Effects

The gut microbiome, which is composed of trillions of microorganisms, produces a vast array of metabolites that can impact various physiological processes, including nutrient absorption, energy balance, and immune function [333,334]. Microbial metabolites, such as SCFAs, bile acids, and certain amino acids, interact with the host's metabolic pathways, thereby influencing overall health and disease states [337,462]. To understand the connections between diet-induced gut microbiome changes along with blood and urine metabolome changes, correlation analyses were conducted. Several significant correlations were observed between gut microbiome plasma metabolites and a few of those correlations are discussed in this section.

While each individual has a unique microbiome, researchers have discovered that as a general rule, microbiomes can be categorized into one of three enterotypes. Enterotypes are defined as a signature composition of microbes that is similar to communities in other individuals based on the presence and relative abundance of certain detectable microbial species. These three enterotypes are distinguished by three dominant bacterial species: *Bacteroides*, *Prevotella*, or *Ruminococcus*. The prevailing species, and consequently the enterotype, is influenced by a person's diet [18,463]. A *Prevotella*-dominant enterotype is associated with diets rich in carbohydrates, while individuals consuming high amounts of protein are more likely to have a *Bacteroides*-dominant enterotype [463]. My gut microbiome results aligned with this finding with the MD, where I had the highest carbohydrate intake it was found that *Prevotella* was significantly enhanced in abundance, while the KD diet, which had the highest protein intake led to a

Bacteroides-dominant enterotype. Additionally, dietary patterns can significantly affect the *Firmicutes* to *Bacteroidetes* ratio. High-fiber diets have been associated with higher proportions of *Bacteroidetes*, while diets high in protein and fat tend to favor *Firmicutes* [464]. This trend is also evident in my dietary and gut microbiome results. The FFD, which had the least fiber intake compared to the other interventions, showed the lowest percentage of *Bacteroidetes* in the gut microbiome. Additionally, the KD, with the highest intake of fat and protein, had the highest percentage of *Firmicutes* in comparison to the other interventions. It is worth noting that the higher this *Firmicutes* to *Bacteroidetes* ratio there is greater propensity towards weight gain and obesity [20]. Although my weight changes in this study were minimal and not close to obese levels, it is notable that the highest *Firmicutes* to *Bacteroidetes* ratio was observed with the FFD, where I also had my highest body weight compared to the other interventions. *Proteobacteria* concentrations in the gut microbiome have shown a positive correlation with fat intake in human [465] and animal [466] studies. *Proteobacteria* were not detected (or the abundance was less than 2%) after the FFD, MD, and RD, but they were detected in higher levels (3.36%) after the KD.

Akkermansia muciniphila is a gut bacterium important for degrading mucins, which make up the protective mucus layer of the intestinal epithelium [467]. By breaking down mucin, it stimulates the production of more mucus, helping to maintain a healthy, thick mucus layer that serves as a protective barrier between the gut lining and potential pathogens or harmful substances A sufficient level of *A. muciniphila* ensures protection against intestinal leakage and is linked to lower risks of obesity, diabetes, and inflammation. *A. muciniphila* concentrations were the lowest after the FFD, while highest concentrations were detected in my microbiome after the KD. Increasing the abundance of *A. muciniphila* can be achieved by consuming fructooligosaccharides (FOS) and polyphenol-rich foods such as leafy greens while reducing sugar intake, similar to what is typical of a KD [467]. Fiber-degrading bacteria such as *Prevotella* and *Bifidobacterium* break down complex carbohydrates that are indigestible to humans, producing beneficial compounds and contributing to a healthy digestive tract [468]. The concentration of these bacteria was in parallel with the dietary fiber intake with the highest being with the MD and the lowest being with the FFD (MD>RD>KD>FFD).

Several microbiome species are known to interact with the host's metabolism through the production of SCFAs via the fermentation of dietary fibers [469]. SCFAs, particularly acetate,

propionate, and butyrate, are involved in numerous physiological processes, including the modulation of gut barrier integrity, energy homeostasis and anti-inflammatory effects [334,338]. These bacterial metabolites can influence systemic metabolism by modulating insulin sensitivity and reducing inflammation, which are critical factors in glucose homeostasis and overall metabolic health [469]. The correlation analysis between the metabolome and microbiome results did not return significant correlations between these metabolites and SCFA-producing bacteria concentrations. However, the concentration of SCFAs (including butyrate and propionate) in both plasma and urine were significantly higher while on the KD. Additionally, a higher abundance of butyrate-producing bacteria such as *Bacteroides, Ruminococcus, Odoribacter, Faecalibacterium, and Clostridium XIVa,* and propionate-producing bacteria, *Phascolarctobacterium,* were detected in gut microbiome after the KD intervention. The SCFAs produced by *Bacteroides* are absorbed by the host and can significantly influence liver function and overall metabolic processes [470,471].

Faecalibacterium, known for its anti-inflammatory properties and butyrate production capabilities, showed a strong positive correlation with alpha-aminobutyric acid, an intermediate in methionine and cysteine metabolism which has known antioxidant properties (r = 0.956, p = 0.044) [337]. This relationship suggests that *Faecalibacterium*'s production of butyric acid may enhance the synthesis of alpha-aminobutyric acid, supporting the host's antioxidative capacity. Butyric acid acts as an energy source for colonocytes and modulates gene expression related to oxidative stress [337,338]. *Bacteroides* levels also showed a significant correlation with alpha-aminobutyric acid (r = 0.970, p = 0.030). Similar to *Faecalibacterium*, *Bacteroides* can influence host metabolism through SCFA production and bile acid modulation [338]. The strong positive correlation with alpha-aminobutyric acid levels suggests that *Bacteroides*' metabolic by-products may enhance the levels of this antioxidant amino acid, contributing to more robust antioxidative mechanisms and stress responses. SCFAs from *Bacteroides* can enter the circulation and influence amino acid metabolism, including pathways leading to alpha-aminobutyric acid production [338,472].

Enterobacter was significantly positively correlated with the plasma levels of 1-methylnicotinamide (r = 0.99, p = 1.11E-16). 1-methylnicotinamide is a primary metabolite of nicotinamide, a derivative of niacin (Vitamin B3) involved in NAD+ metabolism crucial for cellular energy production correlation. It is produced via the enzymatic methylation of nicotinamide-by-nicotinamide N-methyltransferase (NNMT) [473]. It is worth noting that I had

higher levels of niacin intake while on the MD, while the higher *Enterobacter* levels were seen after or during the KD, showing that the observed elevation of *Enterobacter* and 1-methylnicotinamide is not due to the consumption of niacin-rich foods. The rationale for the increased levels of 1-methylnicotinamide may be due to the fact that NNMT plays a crucial role in cellular metabolism, particularly in the regulation of energy balance and detoxification processes [473]. The presence of *Enterobacter* may influence the expression and activity of NNMT, leading to higher production of 1-methylnicotinamide from nicotinamide. NNMT expression is known to be upregulated in conditions of metabolic stress and inflammation, which can be exacerbated by *Enterobacter*-induced dysbiosis [474]. Furthermore, *Enterobacter* species, through the production of LPS, can induce systemic inflammation. Inflammatory cytokines can upregulate NNMT expression as part of the cellular response to inflammation, resulting in increased conversion of nicotinamide to 1-methylnicotinamide [475].

4.5. General Metabolome Changes:

One of the primary objectives of this study was to understand the changes induced in my metabolome (urine and plasma) due to different dietary interventions (FFD, MD, KD, and RD). This was elaborated in Chapter Three, where I showed (through multivariate statistics) a clear distinction between the diet interventions and the corresponding plasma and urine metabolomics profiles. To gain a better understanding of how the diets affected my metabolome I explored two facets: 1) the effect of specific foods on my metabolome in terms of specific metabolites being biomarkers of food intake (BFI) [476] and 2) the effects of specific diets on my metabolome in terms of specific metabolites being biomarkers of health or health status. These distinctions are important. Some foods are rich (or poor) in specific compounds and these compounds will increase (or decrease) in their plasma or urine concentration, within 1-10 hours after the food has been ingested. The amount detected will correlate closely to how much of that specific food has been consumed. These compounds are called biomarkers of food intake or BFIs [476] and their concentrations in plasma or urine have little to do with any kind of physiological state or as an indicator of a metabolic response. Many of these compounds are non-nutrients or are products of the rapid metabolism of the food of interest into non-usable metabolites. Examples of BFIs are proline-betaine for citrus consumption, 3-methylhistidine for chicken and fish consumption, caffeine for coffee consumption, genistein for soy consumption as well as, hippuric acid for fruit, vegetable and tea consumption [477,478]. While BFIs are typically detectable within a few hours after food consumption, many metabolites are known to change independently of what specific food is consumed (1-10 hours beforehand). These metabolites are changing in response to the physiological "stress" that a long-term diet (such as KD, MD or FFD intervention over two weeks), a long-term activity or an existing medical condition may induce. These compounds are produced by the body, often over the course of days, weeks, or months, to respond to or to defend itself against some kind of metabolic or physiological stressor. Identifying and analyzing these compounds is useful for uncovering specific metabolic responses and specific metabolite biomarkers of health status (BHS) associated with different dietary interventions or dietary patterns. Knowing which metabolites are changed purely due to food consumption (i.e., BFIs) and which metabolites are changed due to health status (i.e., BHS) is important for interpreting metabolomic data from a physiological response perspective. I will discuss the measurement and

identification of these BFIs first and then discuss the identification of longer-term, diet-induced metabolic responses (BHSs) in the second part of this section.

4.5.1. Biomarkers of Food Intake – Dietary Effects

BFIs are measurable indicators (i.e., metabolites) that reflect the consumption of specific foods or nutrients [478]. These chemical biomarkers can be found in blood, urine, or saliva and they provide objective and quantifiable data about what food item has been consumed over the previous 1-10 hours. BFIs aid in some aspects of dietary assessments and nutritional research and can be helpful for understanding the relationship between diet and health [478]. This is because they offer more accurate dietary intake data compared to self-reported methods [479]. Their use can help identify links between dietary patterns and disease risk, enabling personalized nutrition and improving public health strategies. To identify BFIs, I analyzed my dietary and metabolomic data in two ways. One was a categorical method where I scanned my metabolomic data to look for a unique and consistent set of one or metabolites that were increased in plasma or urine within 1-10 hours after consuming a specific food. This approach is called categorical BFI analysis. The second was a correlational analysis where I compared the amount of a specific food item consumed (1-10 hours previously) to the concentration of a specific compound measured in my plasma or urine. This approach is called correlational BFI analysis. Both approaches are useful for identifying BFIs as some BFIs respond linearly to dose, while others do not exhibit a linear response. Several BFIs were identified in both plasma and urine, some of which appeared to be novel, others which were previously known [480].

4.5.1.1. Meat Consumption

Several metabolites detected in both urine and plasma indicated a direct connection to dietary meat intake. As meat (in the form of red or white meat) was consumed during all dietary interventions, a comparison of “High” (>200 g/day) and “Low” (<200 g/day) meat intake was performed. Plasma methylhistidine levels were significantly higher on days with higher meat intake ($p = 0.0063$). Methylhistidine is a known biomarker for the consumption of meat [481]. There are two types of methylhistidine: 1-methylhistidine and 3-methylhistidine. White meat (chicken and fish) is particularly rich in anserine, a dipeptide consisting of beta-alanine coupled with 3-methylhistidine. Anserine is primarily degraded and excreted as beta-alanine and 3-

methylhistidine, with the latter compound serving as an indicator of white meat consumption [481]. Red meat (beef and lamb) and human skeletal muscle are particularly rich in carnosine, a dipeptide consisting of beta-alanine coupled with histidine. The histidine residue in carnosine may be post-translationally methylated to become 1-methylhistidine. Because the endogenous formation of 3-methylhistidine in humans is minimal, its plasma and urinary levels mainly reflect dietary intake from poultry and fish. However, the endogenous formation of 1-methylhistidine is continuous as it reflects both muscle breakdown and red meat consumption. Previously published studies have shown a very good dose-response relationship between urinary 3-methylhistidine levels and chicken and/or fish (especially salmon and cod) protein intake [482]. Generally, urinary 3-methylhistidine is linked to the consumption of white meat, while urinary 1-methylhistidine is more associated with red meat intake [483]. The MEGA metabolomic assay used for my metabolomic studies is not capable of distinguishing between 3-methylhistidine and 1-methylhistidine, so only total methylhistidine is reported.

Regardless, it is still possible to interpret type-of-meat consumption with total methylhistidine levels. For instance, the total plasma methylhistidine was higher in the MD group (22.66 ± 16.17) compared to the FFD group (7.47 ± 1.24), which likely indicates increased chicken and fish consumption while on the MD. In contrast to the plasma levels, urinary levels of methylhistidine were higher while on the FFD (17.78 ± 9.85) and RD (17.71 ± 7.80) and they were generally within the normal range or lower while on the MD (13.88 ± 8.57) and KD (14.04 ± 6.45). Diets high in animal proteins, such as those from beef, pork, poultry, and fish, contribute to higher levels of total methylhistidine due to their muscle protein content. Conversely, reducing meat consumption or adopting a vegetarian or plant-based diet, which usually includes lower levels of total methylhistidine, leads to decreased concentrations of this amino acid in the blood and urine [481].

Another marker, notably tiglylglycine was detected in significantly higher concentrations in plasma (p = 8.47E-08) and urine (p = 0.0012) during the days with higher meat consumption. This increase in plasma tiglylglycine levels with meat consumption can be primarily attributed to the metabolic processing of BCAAs, such as isoleucine, leucine, and valine, which are abundant in meat. When meat is consumed, isoleucine intake increases, necessitating its catabolism [484]. The metabolic breakdown of isoleucine follows a specific pathway: it first undergoes transamination

to alpha-keto-beta-methylvalerate, which is then decarboxylated to form tiglyl-CoA. Tiglyl-CoA is further processed, and one of its metabolic byproducts is tiglylglycine [484]. However, tiglylglycine itself is not commonly highlighted as a direct biomarker for meat intake in the literature. Additionally, significantly higher levels of 3-aminoisobutyric acid were detected in urine after higher meat consumption (p = 0.0019). Meat, being rich in proteins, including BCAAs, provides the substrates for 3-aminoisobutyric acid production. The process involves the transamination of valine, followed by decarboxylation to produce 3-aminoisobutyric acid [485]. Additionally, 3-aminoisobutyric acid has been identified as a myokine (a signaling molecule that is produced and released by muscle cells during muscle contraction). It is released during physical activity and influences fat oxidation and glucose regulation, which suggests its production can also be stimulated by dietary protein intake influencing muscle metabolism [485].

Several triglycerides (TG (22:6_34:2), TG (22:6_32:0), and TG (18:1_38:6)) were significantly higher in plasma of the days with higher meat consumption (p = 3.46E-08, 8.64E-08, 1.07E-07, respectively). Studies have shown that meat consumption, particularly red and processed meat, is associated with higher plasma triglyceride levels [486,487]. When dietary fats from meat are ingested, they are broken down into free fatty acids and monoglycerides in the intestines. These are then re-esterified into triglycerides within the enterocytes and packaged into chylomicrons, which are lipoproteins responsible for transporting dietary lipids through the lymphatic system and into the bloodstream [487]. Elevated levels of chylomicrons in the blood increase the overall TG concentration. Additionally, saturated fats commonly found in red and processed meats are known to reduce the activity of lipoprotein lipase (LPL), an enzyme crucial for the hydrolysis of triglycerides in chylomicrons and very-low-density lipoproteins (VLDL) into free fatty acids that can be used by tissues. This reduced activity of LPL leads to decreased clearance of triglycerides from the blood, contributing to higher plasma TG levels [488]. Moreover, high intake of these fats can upregulate the hepatic synthesis of VLDL, which is another significant carrier of triglycerides in the blood [488].

Plasma levels of urea were also significantly higher in days with higher meat intake (p = 6.70E-06). Consumption of meat, particularly cooked meat, has been shown to increase plasma urea levels. This is primarily due to the high protein content in meat, which leads to increased production of urea as a byproduct of protein metabolism [489]. When proteins are digested, they are

broken down into amino acids, which are further deaminated in the liver, producing ammonia as a byproduct. The liver then converts this toxic ammonia into urea through the urea cycle, a process crucial for detoxification. Urea is subsequently released into the bloodstream and filtered out by the kidneys for excretion [490]. This physiological response ensures that excess nitrogen from protein metabolism is safely managed and excreted, leading to higher plasma urea levels after consuming protein-rich foods like meat.

Nudifloramide levels in plasma (p = 6.67E-05) and urine (p = 0.0016) were significantly higher on days with higher meat consumption levels. Nudifloramide is one of a number of metabolic products of nicotinamide adenine dinucleotide (NAD) degradation. Because both red and white meat are particularly high in levels of NAD, it stands to reason that this metabolite of NAD would be elevated [491].

p-Cresol sulfate levels were significantly higher in plasma on days with higher meat consumption (p = 2.33E-05). p-Cresol sulfate is produced by the gut microbiota from the microbial metabolism of dietary amino acids of tyrosine and phenylalanine, which are amino acids commonly found in protein-rich foods, including meat. Upon ingestion, these amino acids are metabolized by specific gut bacteria, producing p-cresol. This compound is then absorbed into the bloodstream and transported to the liver, where it undergoes sulfation to form p-cresol sulfate. The mechanism involves the fermentation of tyrosine and phenylalanine by gut microbiota, absorption of p-cresol, and subsequent sulfation in the liver. This process leads to elevated levels of p-cresol sulfate in the bloodstream following meat consumption. A study conducted by Patel et al. [24], investigated the difference in the production of p-cresol sulfate and indoxyl sulfate between vegetarians and non-vegetarians. Their results showed a significant decrease in the concentration of both metabolites in vegetarians. They suggested that a higher fiber intake in a vegetarian diet offers more substrate for microbial fermentation, which leads to amino acids being used for microbial growth instead of being broken down into waste solutes. Moreover, a reduction in protein intake can lower the amount of amino acids reaching the colon, thereby decreasing their availability for conversion into p-cresol sulfate and indoxyl sulfate [24].

Significantly higher levels of uric acid were detected in plasma (p = 5.7078E-4) samples of the days with high meat intake. Overall, plasma levels of uric acid were higher during the KD

and RD (p = 3.07E-15), while the urinary levels of uric acid were significantly higher during the FFD and KD (p = 0.007). Serum uric acid levels are influenced by dietary purine intake, the rate of uric acid production, and its excretion. High-purine foods include certain meats (such as bacon, beef, pork, and lamb) and seafood (salmon, tuna, Basa, shrimp, crabs, etc.). Medium-purine foods, which also contribute to uric acid levels, include some vegetables (such as legumes, spinach, cauliflower, and green peas) and grains (such as oatmeal and wheat) [492].

Several metabolites, such as creatine (p = 0.0044 in plasma; p = 0.00011 in urine), and beta-alanine (p = 0.0005 in plasma; p = 0.0065 in urine), were significantly higher in days with higher meat consumption. As explained earlier, both of these metabolites are found in high concentration in meat and are known biomarkers of meat consumption [493,494]. However, to investigate the effect of the amount of meat intake on the concentration of the metabolites discussed in this section, including the known biomarkers of meat intake, and BCAAs, a correlational analysis was performed between the amount of meat (g/day) consumption and the concentration of various metabolites in both plasma (Figures 4.45-4.47) and urine (Figure 4.48).

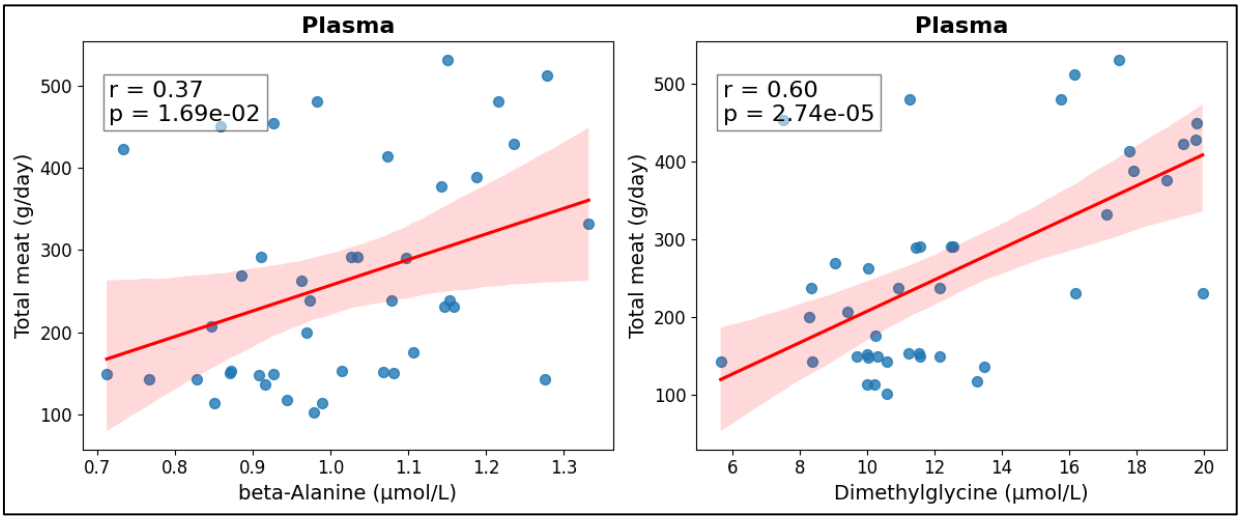

Figure 4.45 - Correlation analysis between meat consumption (g/day) and several plasma metabolites (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

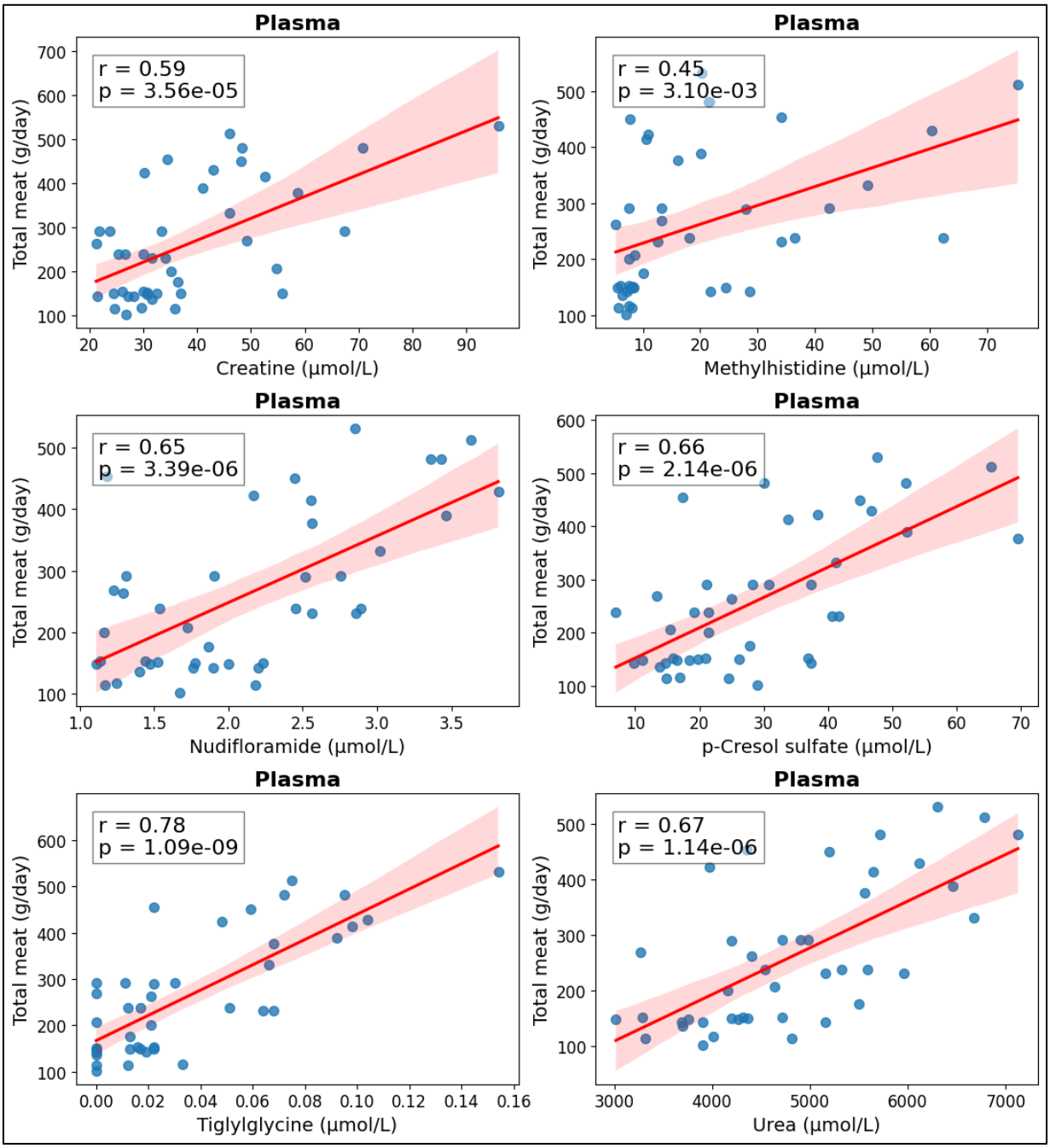

Figure 4.46 - Correlation analysis between meat consumption (g/day) and several plasma metabolites (µmol/L). Each subplot the correlation between the two variables with a regression line, and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot

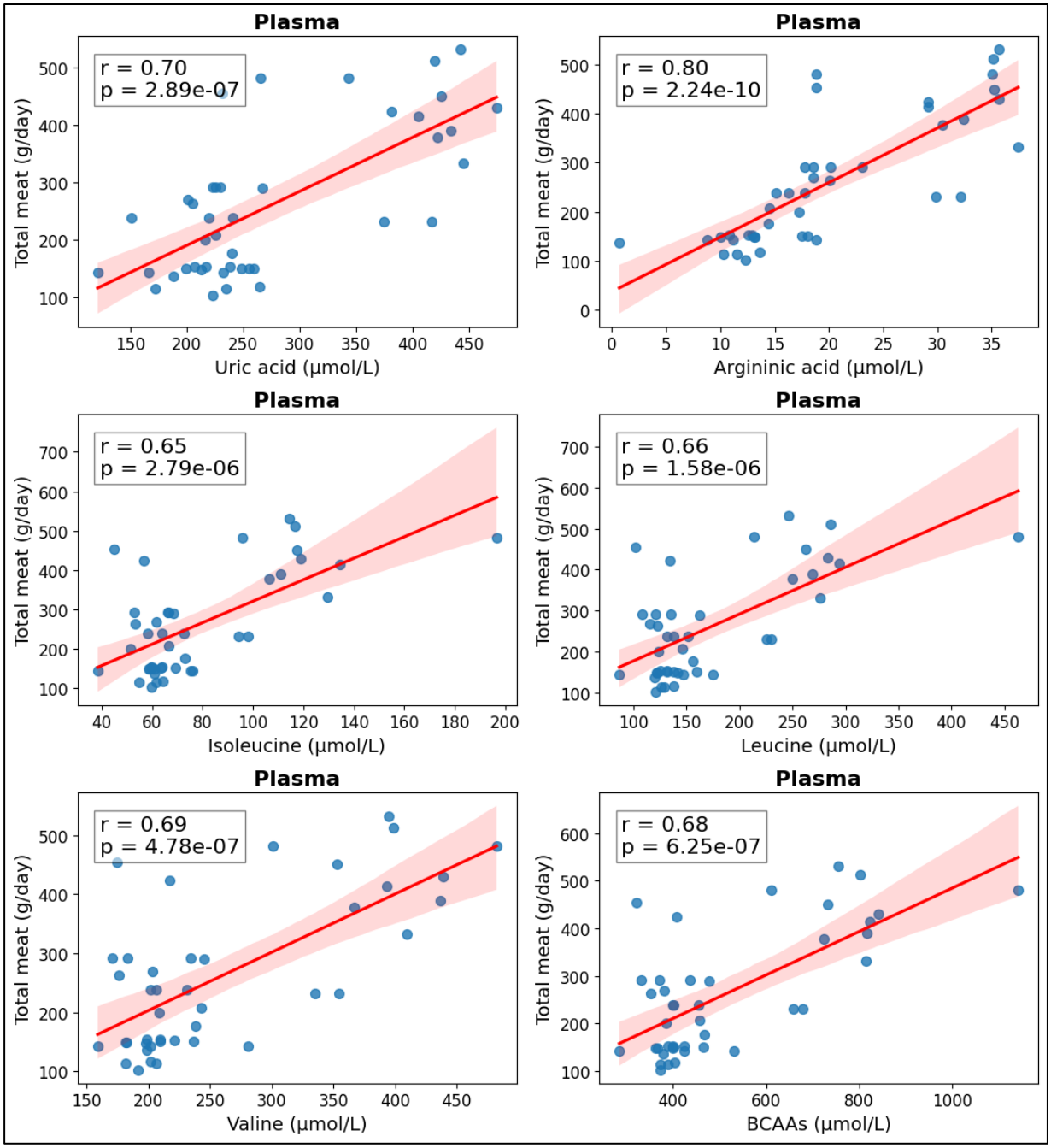

Figure 4.47 - Correlation analysis between meat consumption (g/day) and several plasma metabolites (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

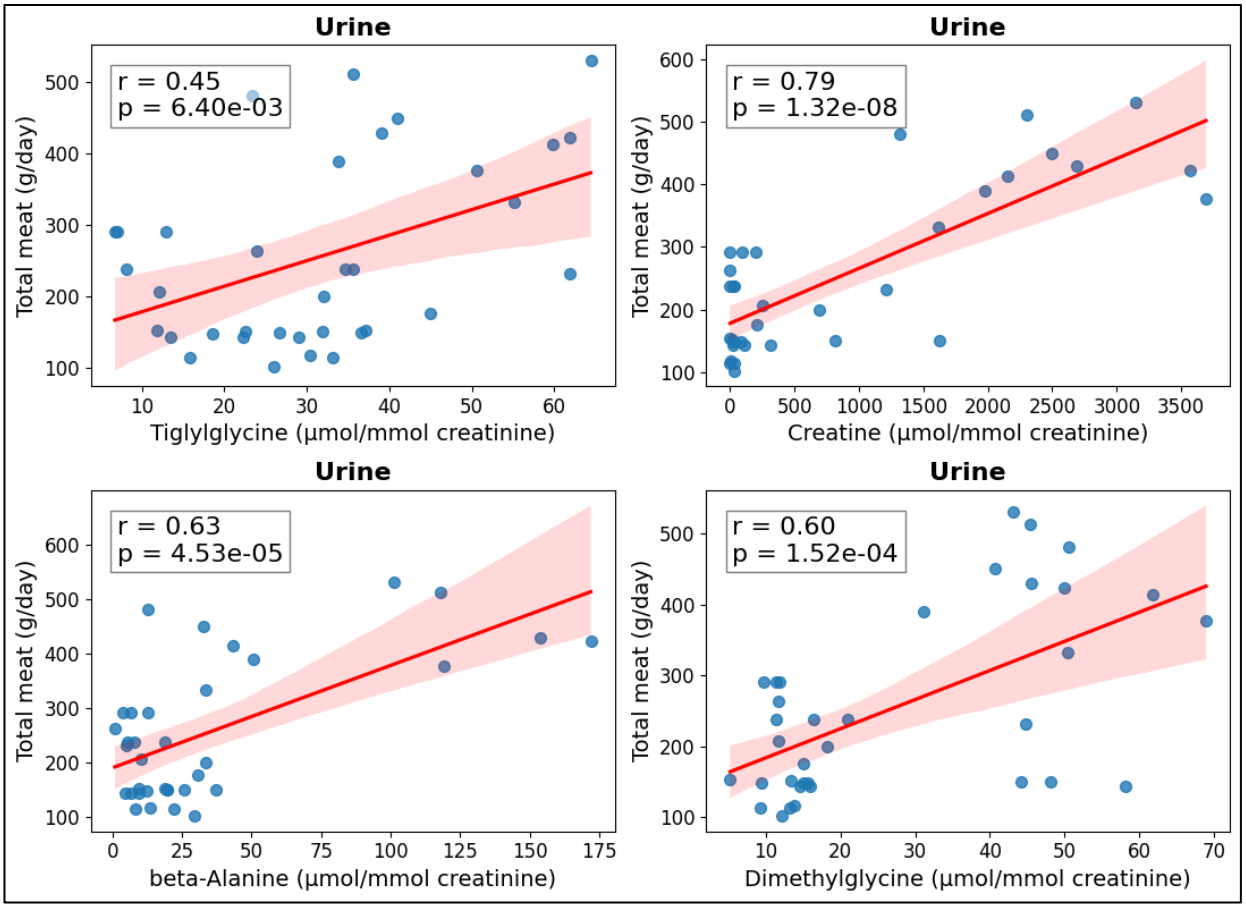

Figure 4.48 - Correlation analysis between meat consumption (g/day) and several urinary metabolites (µmol/mmol creatinine). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

4.5.1.2. Red Meat Consumption

Several metabolites detected in both urine and plasma indicated a direct connection to dietary red meat (including beef and pork) intake. Trans-4-hydroxyproline in plasma was identified as the most significantly different (p = 1.2713E-11) metabolite between the FFD (characterized by very high red meat consumption but limited to low quality cuts) and the MD (characterized by lower red meat consumption but high-quality cuts). Additionally, the overall concentration of hydroxyproline was elevated in both urine and plasma collected while on the FFD dietary intervention. This pattern was also observed when comparing plasma and urine samples that were collected following red meat consumption to the days with no red meat consumption (p = 1.29E-12 for plasma, and p = 9.07E-06 for urine). It is worth noting that the KD had a higher level of red meat consumption (141.2 g/day ± 82.3), compared to the FFD (116.9 g/day ± 38.4). However, trans-4-hydroxyproline levels were significantly higher while on the FFD in both plasma (p = 8.1717E-4), and urine (p = 0.0039) compared to the KD. Hydroxyproline is an essential component of collagen, a fundamental protein found in connective tissue, organs, and muscle in all mammals (such as cows). It arises from the hydroxylation of proline after the synthesis of the collagen protein, significantly enhancing the stability of collagen. The richest sources of proline and hydroxyproline are found in collagen, along with proteins in milk and mammalian organ and muscle and tendons, especially in beef [482,495,496]. This highlights the fact that lower quality of meat (primarily hamburger meat consisting of primarily low-quality ground organ meat or ground beef with lots of connective tissue, such as tendons, ligaments, silverskin and fascia) was consumed while on the FFD. This suggests that hydroxyproline serves as a measure of the quality of the beef rather than just the measure of the amount of beef consumed.

Trimethylamine N-Oxide (TMAO) was significantly higher in plasma of the days with higher red meat consumption (p = 0.0027). TMAO is produced in the body from nutrients like carnitine and choline, which are abundant in red meat, through the action of gut bacteria and subsequent liver oxidation [497]. Choline-rich foods are commonly found in omnivorous diets, especially those that include a lot of red meat. The bacterial metabolism of choline produces TMAO through an intermediate called trimethylamine [497]. Trimethylamine is transported to the liver via the portal circulation, where it undergoes hepatic oxidation facilitated by the enzyme flavin monooxygenase 3 (FMO3). Although mammals lack the enzyme to produce trimethylamine,

gut microbes possess trimethylamine lyases that can cleave the C-N bond in these nutrients, ultimately generating TMAO [497]. *Enterobacter* and *Bacteroides* are two types of bacteria associated with the production of trimethylamine (TMA), a precursor to TMAO. TMA is formed during the metabolism of dietary components (choline and L-carnitine), which are abundant in red meat. The gut microbiota, including bacteria from the *Enterobacter*iaceae family, plays a crucial role in this process [498]. The abundance of *Enterobacter* and *Bacteroides* were higher while on the KD compared to the other interventions which aligns with the higher consumption of red meat while on this diet. Indeed, *Enterobacter* comprised 1.03% of the microbiome for the KD, while it was not detected in the microbiome after the other dietary interventions. *Bacteroides* comprised 7.42% of the gut microbiome after the KD, while they comprised 19.26% after the FFD, 5.87% after the MD, and 7.85% after the RD.

Another metabolite that was significantly elevated after days where red meat was consumed was creatine (p = 0.00063 in plasma and p = 0.0015 in urine). Interestingly, urinary levels of creatine were at the highest level while on the KD, and lowest while on the FFD (1753.86 ± 1136.04, and 121.26 ± 139.45, respectively; p = 6.0953E-9). Red meat is a rich source of creatine, a compound stored in muscle tissues [499]. When consumed, creatine from red meat is absorbed into the bloodstream, contributing to higher levels in blood and urine. Creatine is synthesized in the body from amino acids such as arginine, glycine, and methionine, but dietary intake from meat significantly boosts its levels. A study conducted by Playdon et al. [500], investigated the diet-related metabolome changes in postmenopausal females from the Women's Health Initiative during a 14-days controlled feeding period. Their results indicated that serum creatine levels were positively correlated with red meat consumption. Creatine is an important compound found in high amounts in red meat, particularly in skeletal muscle tissue. On average, red meat contains about 3 to 5 grams of creatine per kilogram of fresh meat, which translates to roughly 300–500 milligrams per 100 grams of meat [501]. Creatine, especially phosphocreatine, in red meat plays a vital role in energy metabolism during high-intensity physical activities. It helps regenerate ATP, which is crucial for muscle contractions and short bursts of energy [502]. However, the creatine content can vary depending on factors such as the specific cut of meat, the animal's diet, and cooking methods. For instance, high-temperature cooking can cause some degradation of creatine, reducing its content by about 25% compared to control meals with lower temperature cooking [501]. The correlational analysis of the amount of red meat consumed (g/day) to the

concentration of creatine measured in my plasma and urine return significant positive correlations (Figures 4.49 and 4.51).

Meat is a rich source of various dipeptides, including carnosine and anserine, which are composed of beta-alanine and histidine (carnosine) or methylated histidine (anserine) [493]. Following meat consumption, these dipeptides are digested and broken down, releasing beta-alanine into the bloodstream and later into the urine. Beta-alanine is a non-essential, non-proteogenic amino acid, but it can be metabolized into aspartic acid. However, the process is relatively slow, leading to a greater secretion of beta-alanine into the urine than other amino acids. The comparison of plasma samples following red meat consumption to samples following no red meat consumption, did not return significant differences in the concentration of beta-alanine and carnosine. However, the urinary concentrations of carnosine (p = 2.82E-06) and beta-alanine (p = 0.0046) were significantly higher in samples collected after red meat consumption.

The concentration of hydroxylated carnitines was significantly higher in urine after red meat consumption (p = 0.00054). Carnitine is indeed abundant in red meat, with beef steak containing between 64-88 mg per 100 g serving [500,503]. As a result of this high carnitine content, studies have observed an increase in carnitine-related metabolites in urine following meat consumption. When consumed, carnitine undergoes extensive metabolism in the liver, leading to the production of various metabolites, including hydroxylated carnitine. This hydroxylation process involves specific enzymes called hydroxylases, which incorporate a hydroxyl group into the carnitine molecule. The liver's role in metabolizing the substantial carnitine intake from red meat results in elevated levels of hydroxylated carnitine, subsequently excreted in the urine.

IPA levels in urine and plasma were significantly lower after red meat consumption (p = 1.46E-16 for plasma and p = 0.0021444 for urine). IPA is both a plant-derived hormone and a gut-derived. In mammals IPA plays a protective role at the cellular and tissue levels by reducing inflammation, lipid peroxidation, and free radical formation [425]. It impacts the immune, nervous, gastrointestinal, and cardiovascular systems. IPA levels are decreased in several diseases, including colitis, diabetes, and obesity [425]. Diets with high red meat consumption, specifically FFDs is known to decrease the plasma concentration of IPA [504], while diets high in fibre [505], and plant matter, such as the MD [504] are known to significantly increase the plasma concentration of IPA

(although the positive correlations to the concentration of IPA and other foods such as banana is discussed in later sections).

The correlational analysis of the amount of red meat consumed (g/day) to the concentration of plasma metabolites (including trans-4-hydroxy proline, TMAO, and beta-alanine) did not return strong correlations, suggesting that these metabolites may not be good dose-dependant markers for red meat consumption. Urinary carnosine levels showed weak associations, while the urinary concentration of hydroxylated carnitines, and beta-alanine were more strongly associated with the amount of red meat consumption (Figure 4.51). Additionally, strong positive correlations were observed between plasma concentrations of 2-hydroxybutyric acid, 3-hydroxyisobutyric acid, 2-hydroxyisovaleric acid, 3-hydroxyisovaleric acid, and 2-hydroxy-3-methylvaleric acid and the amount of red meat consumed (g/day). These correlations can be attributed to the metabolic processing of BCAAs and other amino acids prevalent in red meat. As mentioned earlier, red meat is a rich source of BCAAs, including leucine, isoleucine, and valine, as well as other amino acids such as threonine. These amino acids undergo catabolism in the body, leading to the production of various metabolites, including the ones just mentioned. 2-hydroxybutyric acid is formed from the metabolism of threonine and from glutathione synthesis, particularly under conditions of oxidative stress and increased fatty acid oxidation, both of which can be triggered by a high-protein diet that includes lots of red meat [506]. Elevated levels of 2-hydroxybutyric acid are often seen as an early marker of insulin resistance, reflecting disruptions in glucose and lipid metabolism that can occur with excessive intake of saturated fats from red meat [506]. Similarly, 3-hydroxyisobutyric acid is a product of valine catabolism, 2-hydroxyisovaleric acid and 3-hydroxyisovaleric acid are intermediates in the catabolism of leucine, valine and isoleucine, and 2-hydroxy-3-methylvaleric acid is a product of isoleucine metabolism [409]. These three BCAAs are all abundant in red meat. The increase in red meat intake may therefore elevate the plasma levels of these amino acids, first, followed by their metabolites, potentially contributing to metabolic alterations associated with excessive consumption of red meat (Figures 4.50 and 4.52). Additionally, a positive correlation between red meat consumption and the levels of certain organic acids in urine was observed which reflects the increased metabolism of amino acids found in meat. Interestingly, a positive correlation was observed between the amount of red meat consumed and malonic acid and indolelactic acid in plasma (Figure 4.49).

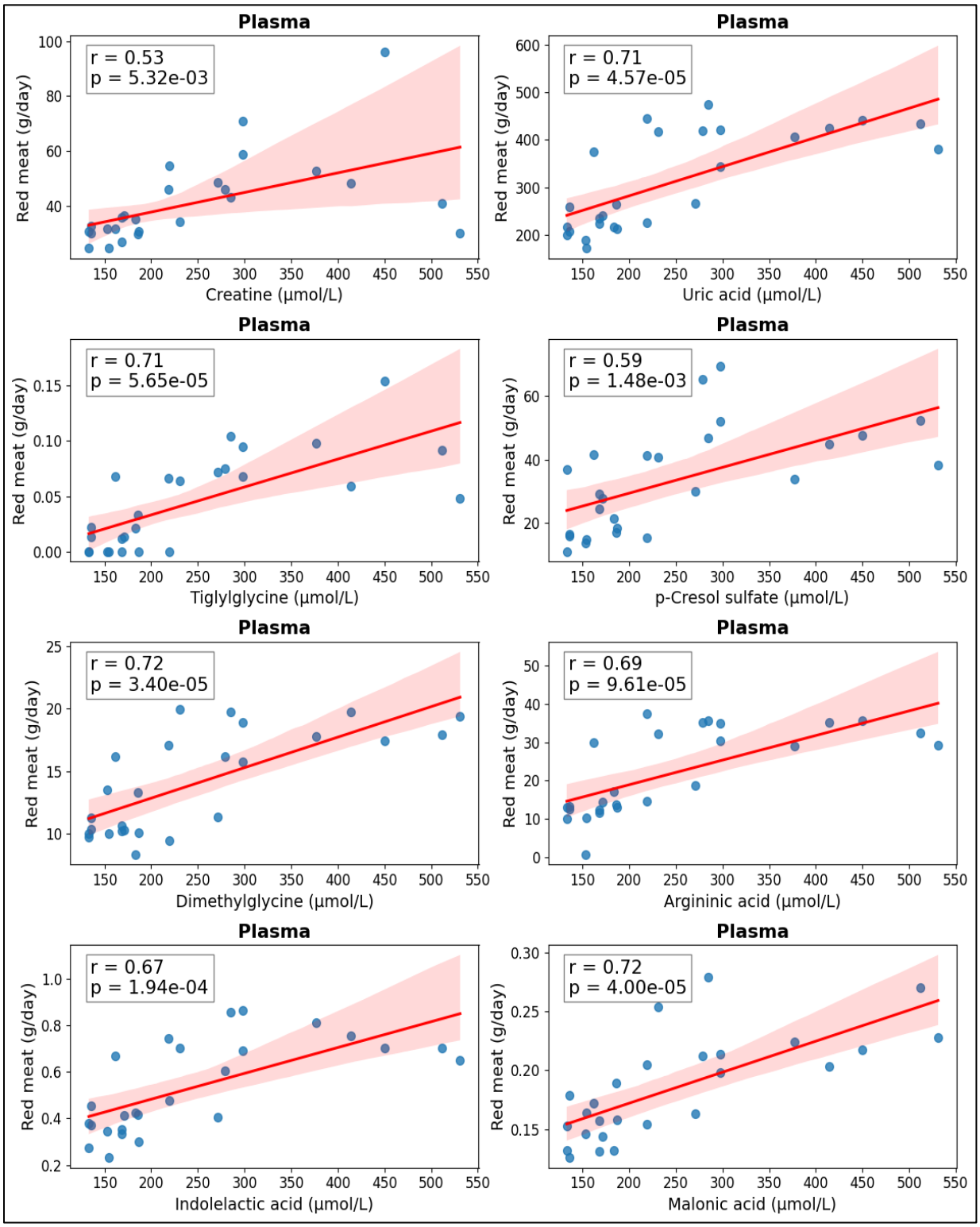

Figure 4.49 - Correlation analysis between red meat consumption (g/day) and several plasma metabolites (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

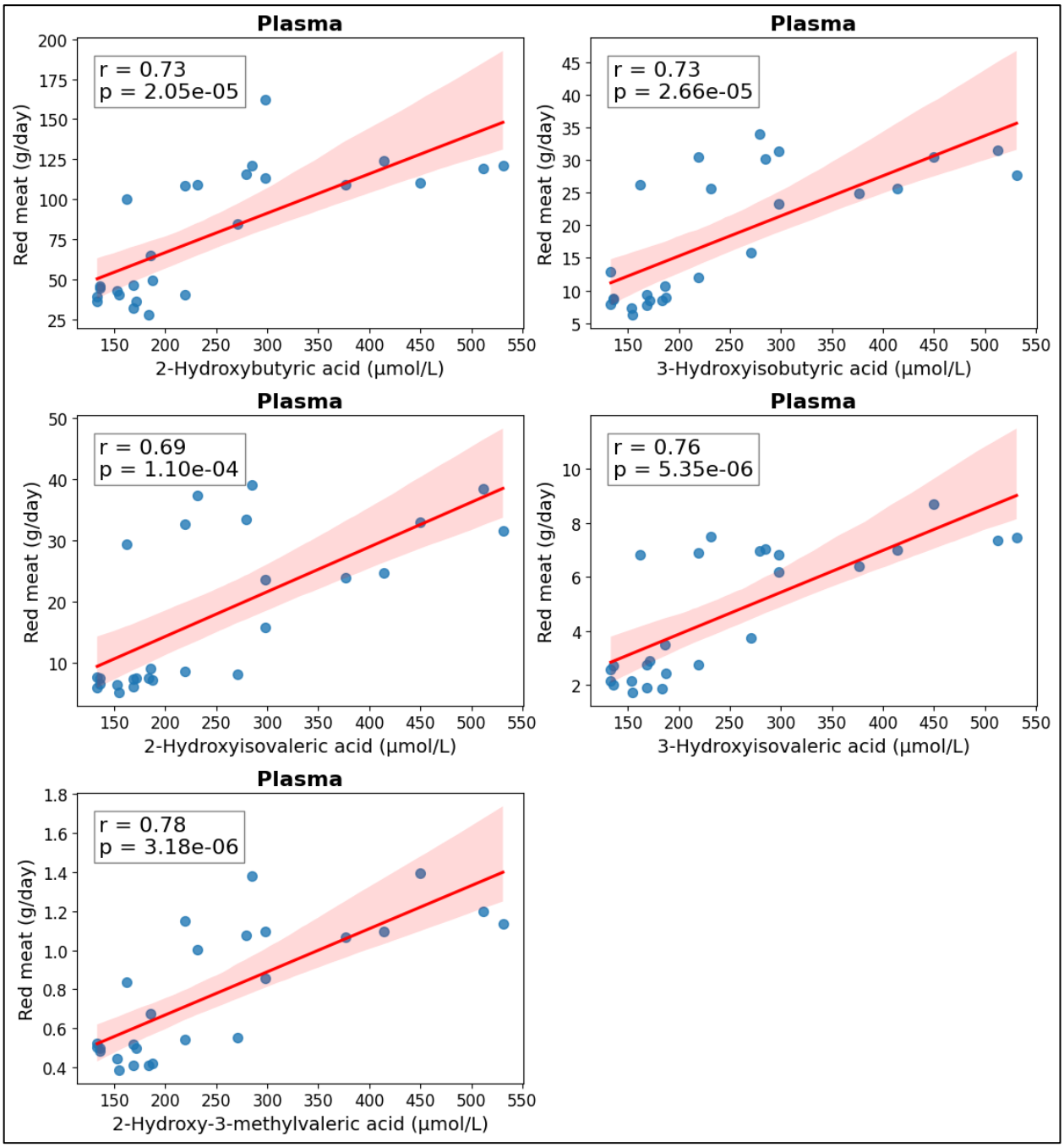

Figure 4.50 - Correlation analysis between red meat consumption (g/day) and several plasma metabolites (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

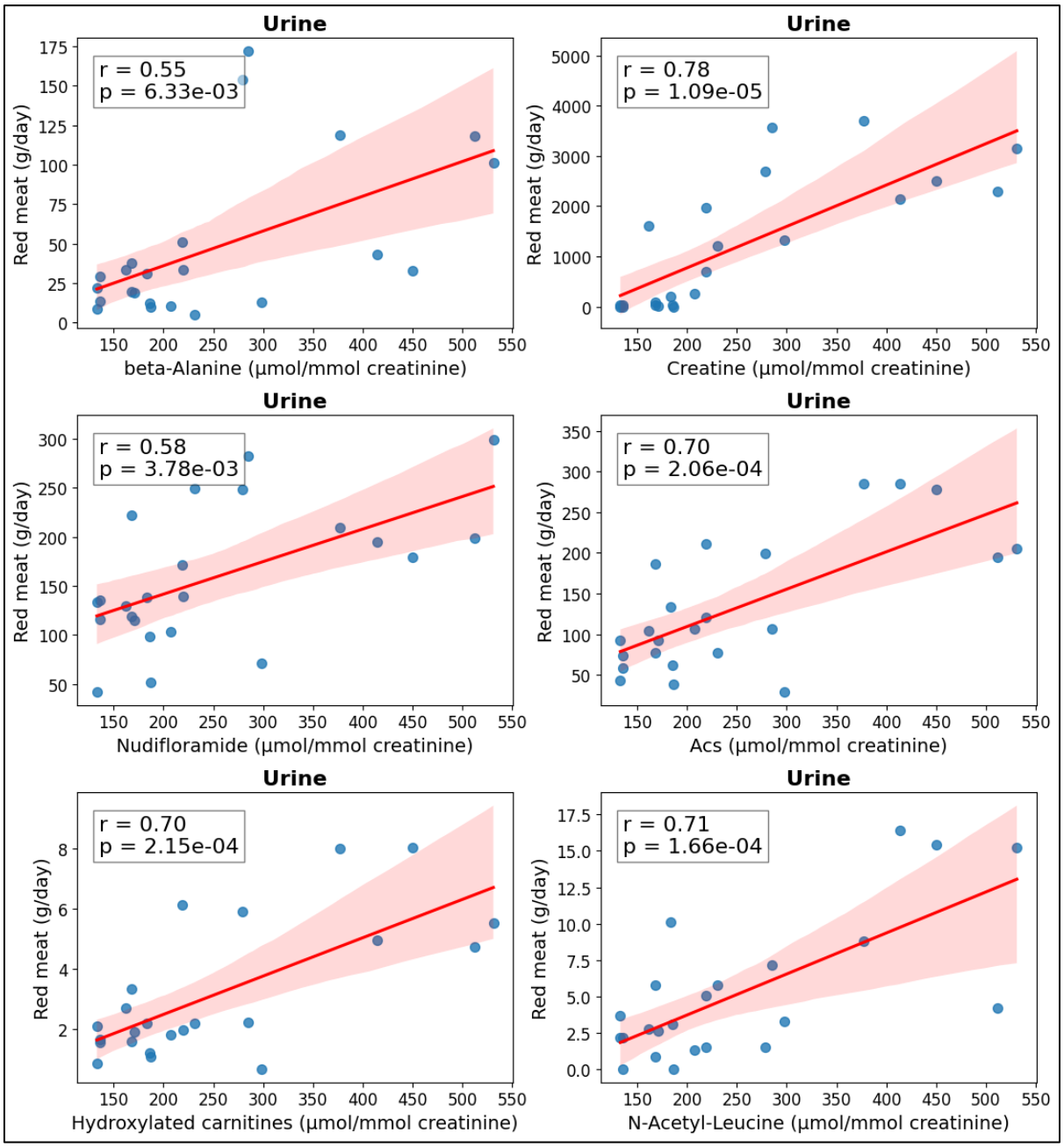

Figure 4.51 - Correlation analysis between red meat consumption (g/day) and several urinary metabolites (µmol/ mmol creatinine). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

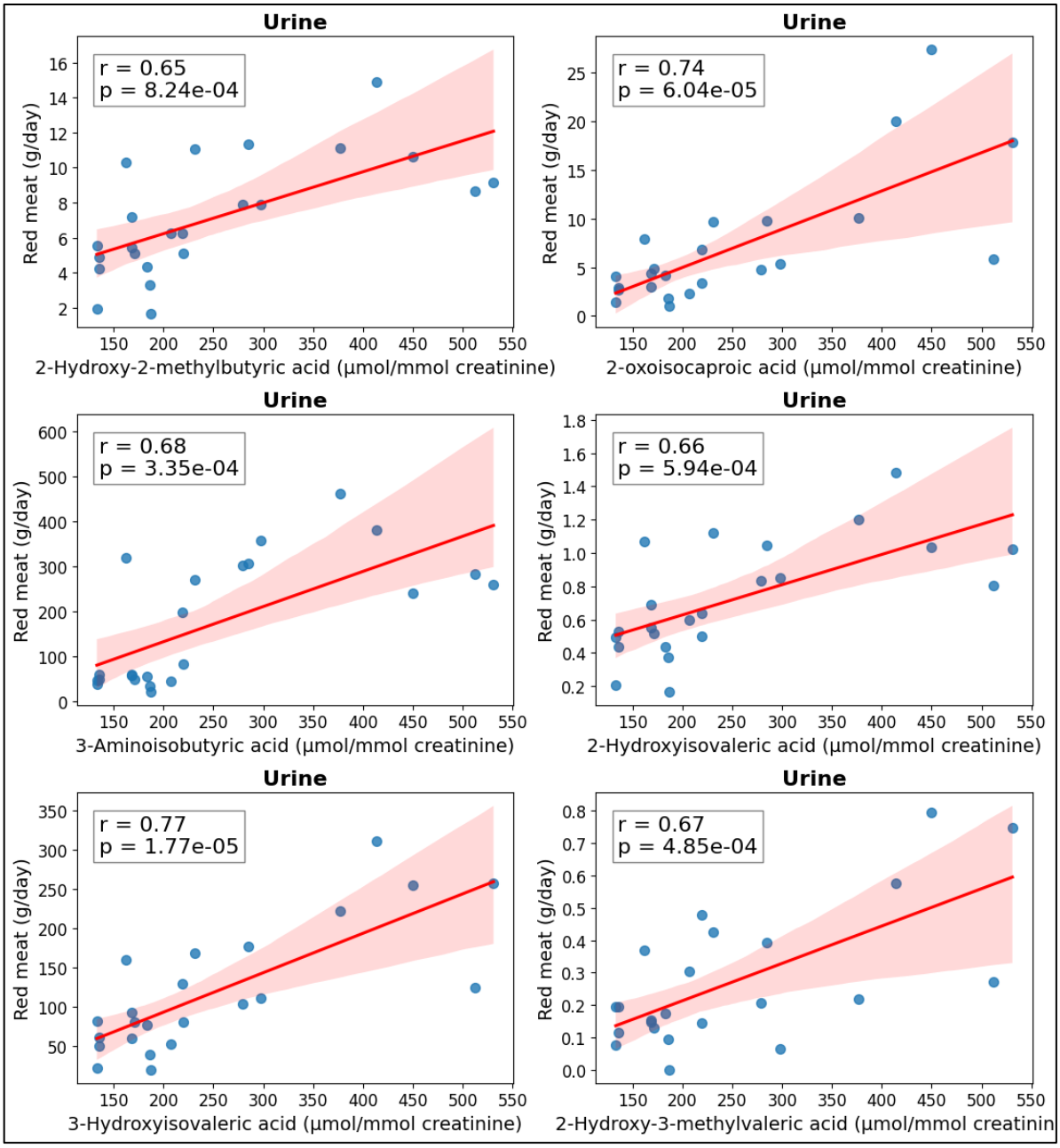

Figure 4.52 - Correlation analysis between red meat consumption (g/day) and several urinary metabolites (µmol/ mmol creatinine). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

4.5.1.3. Poultry Consumption

Significantly higher levels of methylhistidine (p = 9.4701E-8), uridine (p = 6.3672E-5), and nudifloramide (p = 1.706E-4) were detected in plasma after poultry (chicken or turkey) consumption. As explained in the earlier section on meat consumption, higher levels of methylhistidine and nudifloramide were detected in days with higher levels of meat consumption (>200 g/day). These connections show that the increase in the abundance of these metabolites was associated with poultry intake as this increase was absent in the samples following red meat consumption. Additionally, significantly lower levels of trans-4-hydroxyproline were detected in plasma samples with poultry consumption (p = 1.5514E-4), further confirming trans-4-hydroxyproline's association with red meat and red meat quality. Interestingly, urinary methylhistidine levels were significantly lower on days with poultry consumption (p = 9.4701E-8).

Urinary levels of taurine were found in higher levels in samples collected following poultry consumption (p = 3.9808E-4). Taurine is an amino acid found in high concentrations in animal tissues, particularly in muscle and viscera [507]. It is not incorporated into proteins or degraded by mammalian tissues, and its primary dietary sources are foods of animal origin, such as poultry and other meats. Research indicates that taurine intake from animal sources leads to increased urinary excretion of taurine. For instance, a study observed higher urinary taurine excretion when individuals consumed a high-meat diet compared to a low-meat or vegetarian diet [508]. However, the specific impact of poultry consumption on taurine levels in urine is not directly detailed in the available studies. The taurine content in chicken, for example, varies between different parts of the bird, with chicken legs containing higher taurine concentrations than chicken breast [507]. A t-test was conducted between the urine and plasma samples of taurine levels collected after eating chicken breast and chicken thigh. However, no significant differences were found between the two groups.

Dimethylglycine (DMG) was found in significantly higher concentrations in urine samples collected on days with poultry consumption (p = 0.0036). Dimethylglycine is a dietary supplement that is frequently added to the diet of poultry to enhance dietary fat utilization [509]. The higher levels of DMG found in my urine may reflect the higher levels of DMG found in chicken that has

been supplemented with DMG. Additionally, urinary levels of tryptophan were significantly higher on days with poultry consumption (p = 8.7453E-4). Tryptophan is an essential amino acid that must be obtained from the diet, with poultry being a rich source [510]. In the body, tryptophan serves as a precursor for several critical biomolecules, including serotonin, melatonin, and niacin. The high content of tryptophan in chicken and other poultry products makes it a useful marker for dietary intake. When poultry is consumed, tryptophan is absorbed in the small intestine and enters systemic circulation, where it is utilized for protein synthesis or converted into various metabolites. The relationship between poultry intake and increased levels of tryptophan or its metabolites (such as serotonin or kynurenine) in biological fluids has been documented in nutritional studies, suggesting that elevated tryptophan levels can be indicative of poultry consumption.

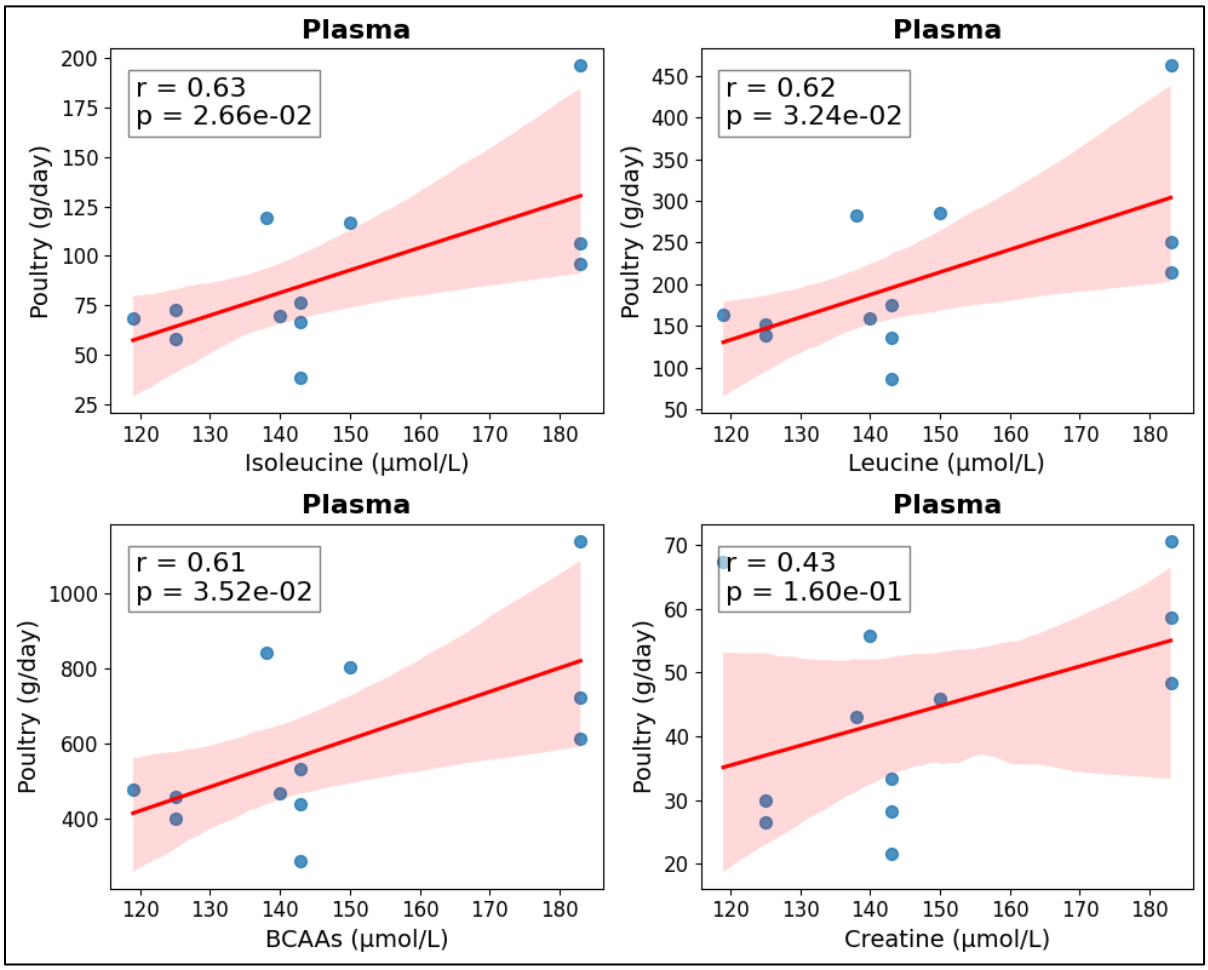

Figure 4.53 - Correlation analysis between poultry consumption (g/day) and several plasma metabolites (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

A positive correlation was observed between the amount of poultry consumed and the urinary concentration of SCFAs, butyric acid and isobutyric acid, and valeric acid combined with isovaleric acid (Figure 4.54). It has been shown that diets rich in animal protein, such as meat, are associated with lower SCFA levels compared to diets higher in fiber and lower in animal protein [511]. This is because SCFAs are primarily produced by the fermentation of dietary fibers by gut bacteria, and meat-based diets typically contain less fiber [512]. The diet with the most poultry consumption was the MD. Although the MD had a high protein intake, it also had the highest levels of fibre intake (24.9 g/day ± 12.6 on MD, compared to 7.3 g/day ± 1.8 while on the FFD, 8.6 g/day ± 6.4 while on the KD, and 19.7 g/day ± 2.8 while on the RD). Butyric acid is mainly produced by bacteria from the *Firmicutes* phylum, particularly members of the *Ruminococcaceae* and *Lachnospiraceae* families. Key butyrate-producing bacteria include *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Eubacterium hallii*, *Roseburia*, and *Coprococcus* [170,513]. However, the results from the gut microbiome testing only showed higher levels of *Eubacterium*, and *Roseburia* after being on the MD (Table 3.18). Additionally, valeric acid is produced by specific bacteria in the gut such as *Clostridium*, *Oscillibacter*, and *Eubacterium* [514]. Interestingly, higher concentrations of *Eubacterium* were detected in the gut after the MD, and *Oscillibacter* was only detected after the MD (Table 3.18).

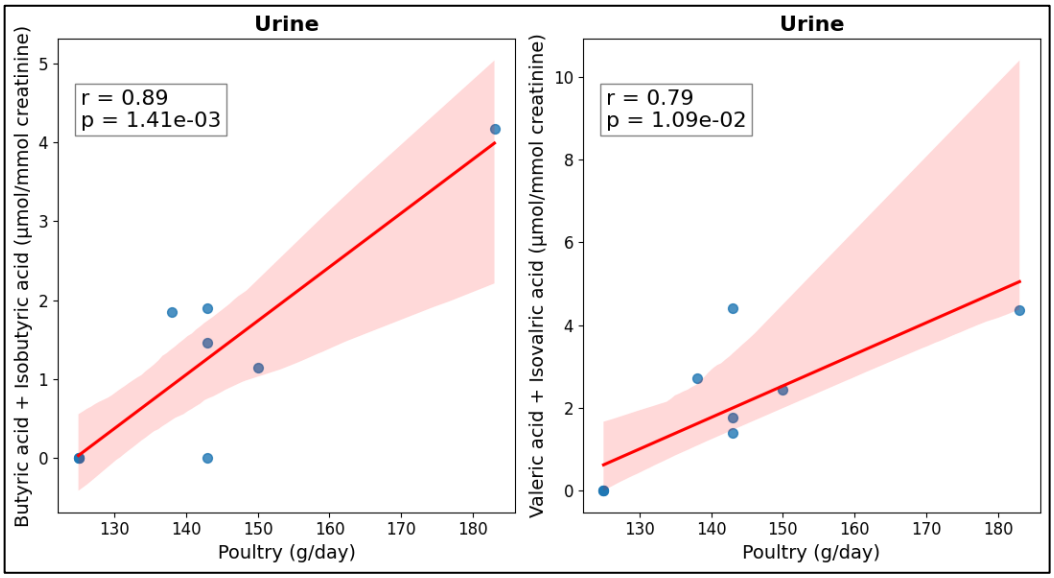

Figure 4.54 - Correlation analysis between poultry consumption (g/day) and several urinary metabolites (µmol/ mmol creatinine). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

4.5.1.4. Fish Consumption

By comparing the plasma samples collected after fish consumption (including salmon, basa, and tuna), significantly higher levels of N2-acetyl-ornithine (p = 5.4331E-5), and PC aa C42:6 (p = 1.1356E-4) were detected. However, no significant differences in urinary metabolites were detected. N2-acetyl-ornithine levels may also be associated with banana consumption which will be discussed later. Interestingly, by only comparing the samples collected after salmon consumption, significantly higher levels of betaine were detected in plasma (p = 7.9772E-4). Betaine levels in plasma were higher during the KD, RD, and MD (where some amounts of fish (especially salmon) were consumed), and lowest during the FFD (where no seafood or fish was consumed). Several studies indicate that consuming salmon can lead to increased plasma betaine levels. One study noted that dietary betaine from various sources, including salmon, is rapidly absorbed and leads to significant increases in plasma concentrations. Another study noted that foods rich in choline, such as salmon average, contribute to elevated plasma betaine levels due to the metabolic conversion of choline to betaine in the liver [515,516]. Urinary betaine levels did not show the same pattern as the highest levels of urinary betaine were detected while I was on the FFD and KD.

Moreover, significantly higher levels of xanthosine (p = 7.58E-05) were detected in urine after consumption of salmon. Fish, including salmon, like other meat sources, contains purines, which are metabolized in the body into intermediates such as xanthosine. Xanthosine is a purine nucleoside composed of xanthine and ribose. It is involved in various enzymatic reactions within humans and is a precursor in the biosynthesis of compounds like caffeine. Xanthosine can be converted into xanthine and ribose 1-phosphate by the enzyme purine nucleoside phosphorylase, and it is an intermediate in purine metabolism [517]. The level of xanthosine in the urine can be indicative of purine metabolism, which is influenced by dietary intake. Thus, increased consumption of purine-rich foods, including salmon, can lead to higher concentrations of xanthosine in the urine [482]. Additionally, there was an increase in urinary N-acetyl-methionine levels following salmon intake (p = 0.0006), which may be linked to the metabolism of methionine, an essential amino acid that is quite abundant in salmon. Methionine plays a crucial role in various biological processes, including the synthesis of S-adenosylmethionine (SAM), a key methyl donor in the body [518]. When methionine intake is high, as with salmon consumption,

the body may convert some excess methionine to N-acetyl-methionine as part of its regulatory mechanisms, possibly to prevent the accumulation of methionine or its byproducts [518]. The correlational analysis performed on the amount of salmon consumed (g/day) and the concentration of various metabolites in urine, did not return significant correlations. However, the correlational analysis of the plasma metabolites returned significant correlations with metabolites such as beta-alanine, creatine, and urea which as discussed earlier, are known biomarkers of meat intake [493,494]. Betaine levels did not show a strong correlation with the amount of salmon consumed. However, significantly strong correlations were observed between the amount of salmon consumed (g/day) and PC aa C40:4, SM C26:1, CE (14:0), and TG (18:1_38:6) (Figures 4.55 and 4.56).

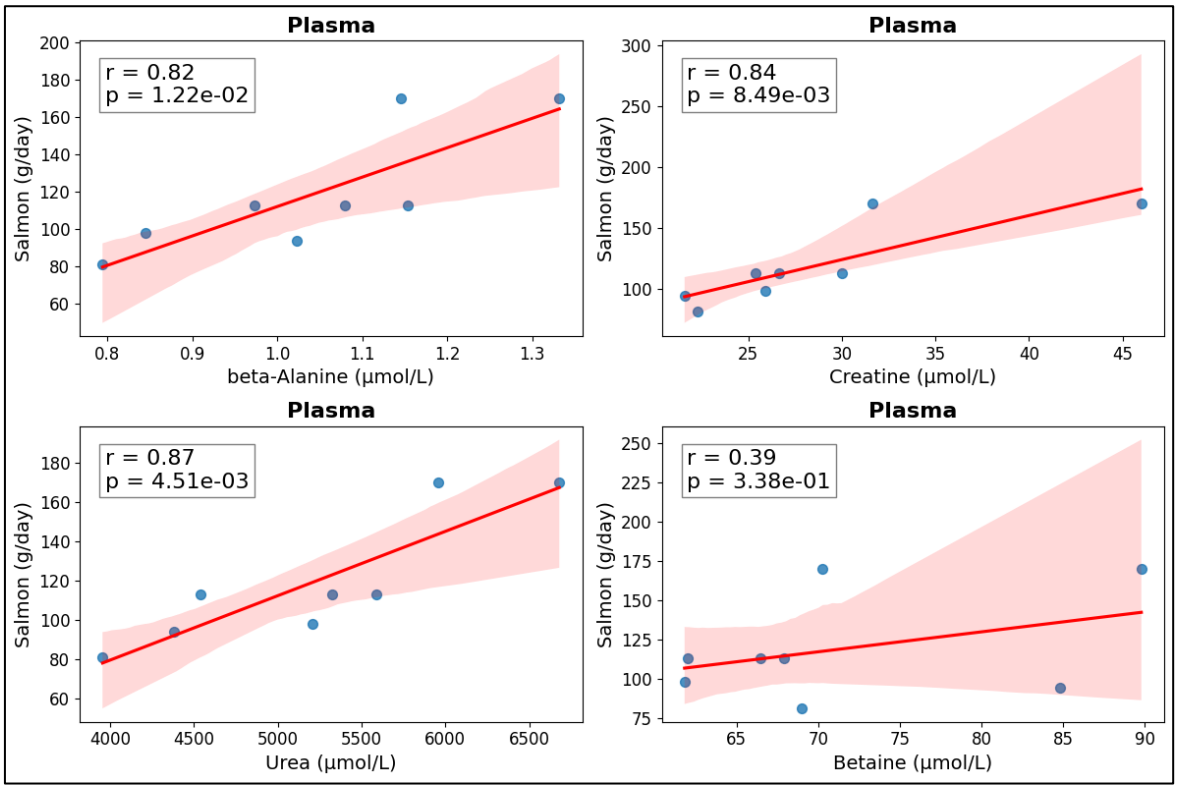

Figure 4.55 - Correlation analysis between salmon consumption (g/day) and several plasma metabolites (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

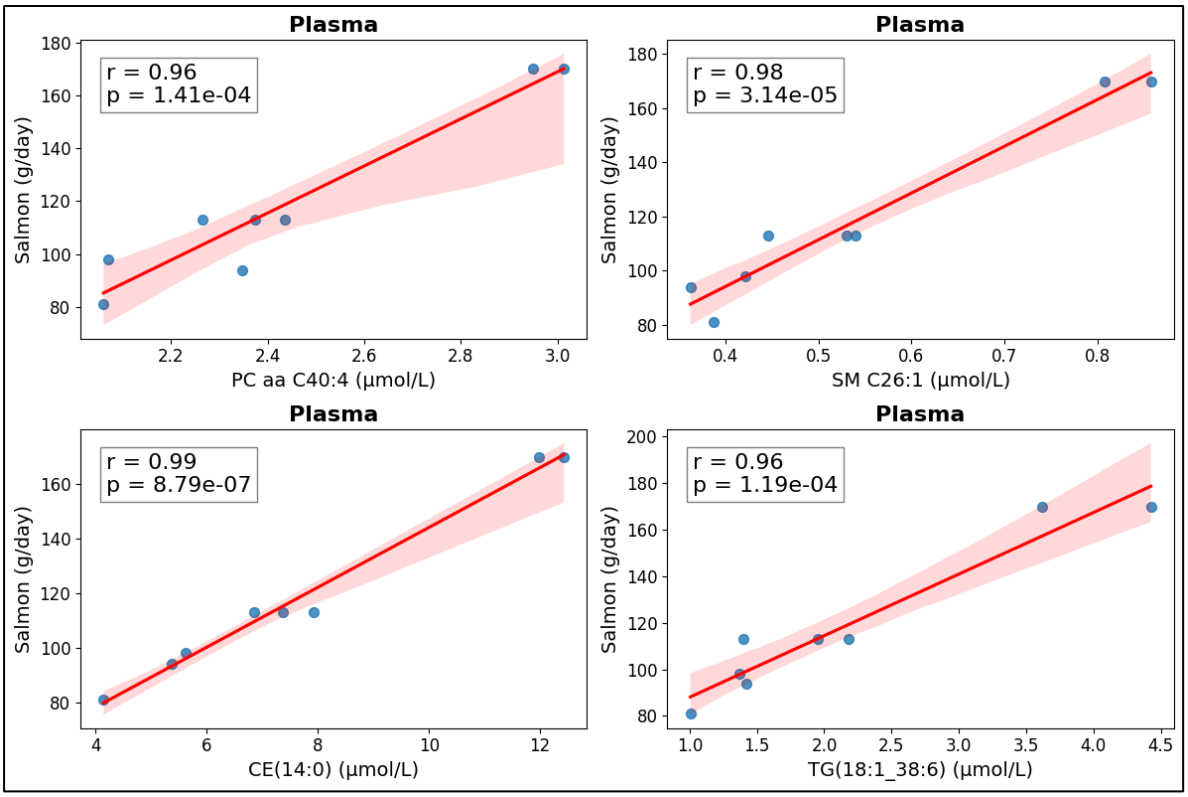

Figure 4.56 - Correlation analysis between salmon consumption (g/day) and several plasma metabolites (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

4.5.1.5. Banana

Indole-3-propionic acid (IPA) was detected at higher levels in plasma on days following banana consumption (p = 6.71E-8). IPA is a metabolite derived from the amino acid tryptophan. There are two sources of IPA for humans, one is from gut microbial production (via tryptophan metabolism) and the other is from consumption of plants and plant foods. IPA is a plant hormone found in almost all plants, and it exhibits several effects on plant physiology. IPA influences plant growth by acting similarly to auxins, a class of plant hormones that regulate various aspects of plant development, including cell division, elongation, and differentiation. IPA can promote root development, enhance stress tolerance, and influence seed germination and fruiting. Bananas and plantains are known to contain IPA [519]. The other source of IPA is through the metabolism of tryptophan by the gut microbiota. The process begins when dietary tryptophan is broken down by bacteria into indole and other compounds, and further transformed into IPA. Common natural food sources of tryptophan include turkey, chicken, oats, bananas, dried prunes, milk, tuna, and salmon. However, no increase in IPA was found when I consumed poultry, salmon or other tryptophan-rich foods, so it appears that the source of the IPA is not from gut bacterial metabolism, but rather from the bananas themselves.

Another amino acid metabolite, N2-acetyl-ornithine (p = 4.76E-6) was also detected in higher abundance in plasma samples collected after banana consumption. N2-acetyl-ornithine is formed through the acetylation of ornithine, a process that can regulate the levels of ornithine available for further metabolic pathways, including the production of polyamines, which are crucial for cell growth and differentiation [520]. N-acetyl-ornithine appears to be a common component of plant protein [521] and because bananas are particularly protein-rich, they may contain higher levels of N2-acetyl-ornithine, thereby leading to the higher levels of this amino acid seen in my blood plasma. Further analysis will be performed using the TMIC MEGA assay in the future to investigate if bananas have high levels of free N-acetyl ornithine and IPA.

Glyceric acid (p = 0.0004) was also detected in higher concentrations in plasma after banana consumption. Glyceric acid is one of the organic acids found in both banana leaves and banana fruit pulp [522]. In banana leaves, it is present along with other amino acids such as glutamic acid and aspartic acid as well as organic acids such as glutaric, glycolic, glyoxylic, shikimic,

succinic, pyruvic, malonic, and α-ketoglutaric acids, although these are typically in trace amounts [522]. In the ripening banana fruit, glyceric acid is also present among a variety of other compounds, including glutamic acid and aspartic acid as well as glutaric, quinic, glycolic, and succinic acids, as well as several keto acids [523]. In a study conducted by Wyman and Palmer [523] to investigate the changes in the levels of organic acids throughout the ripening process of banana, it was observed that as banana ripens, its acidity increases and results in higher levels of several organic acids including glyceric acid. This shows that the increase in the abundance of this metabolite in plasma, can be associated with consumption of ripened bananas on those days.

The correlational analysis of the amount of banana consumed and the plasma concentration of these metabolites did now return strong correlations. However, a positive correlation was observed with plasma concentration of serotonin (Figure 4.57). Bananas contain serotonin, but it is important to note that the serotonin found in bananas does not cross the blood-brain barrier, meaning it does not directly affect brain serotonin levels or mood [524]. However, bananas are rich in tryptophan. When consumed, tryptophan enters the bloodstream and can cross the blood-brain barrier, although only a small fraction of dietary tryptophan makes it to the brain because it competes with other amino acids for transport. Once in the brain, tryptophan is hydroxylated by the enzyme tryptophan hydroxylase to form 5-hydroxytryptophan (5-HTP). This is the rate-limiting step in serotonin synthesis. 5-HTP is then decarboxylated by aromatic L-amino acid decarboxylase to produce serotonin (5-hydroxytryptamine, 5-HT). This serotonin can be used in various brain functions, including mood regulation, sleep, and appetite [525]. Therefore, I calculated the correlation between mood scores and the amount of banana consumption. Several mood metrics, including the total mood score, anger score, and fatigue did not return significant or strong correlations. However, the strongest correlation with the mood metrics was observed between the amount of banana consumed (g/day) and reduced depression scores (Figure 4.57).

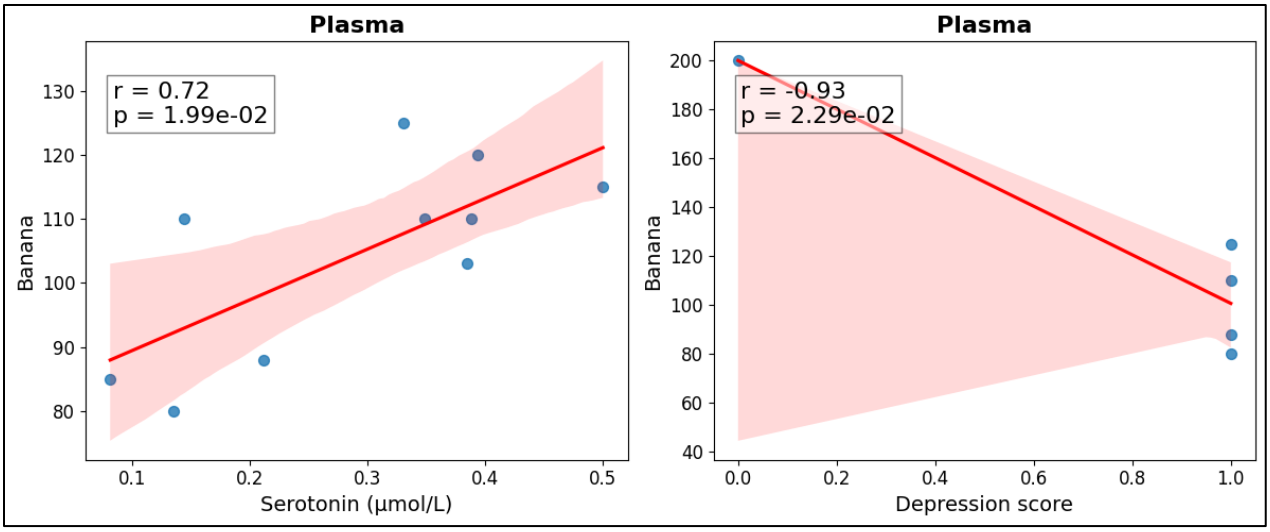

Figure 4.57 - Correlation analysis between banana consumption (g/day) and serotonin levels plasma (µmol/L) (left). Correlation analysis between banana consumption (g/day) and depression scores from Profile of Mood State questionnaires (right). Each plot shows the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

As bananas are rich in protein [526], a general increase in the abundance of urinary amino acids was observed after banana consumption. The most significant increase was observed in the total concentration of aromatic amino acids (p = 1.27E-05), which corresponded to a significant increase in the urinary concentration of phenylalanine (p = 4.39E-06), tryptophan (p = 5.05E-05), and tyrosine (p = 0.0002). Bananas contain a variety of aromatic amino acids, including tryptophan, phenylalanine, and tyrosine, which are essential or conditionally essential in the human diet. These amino acids serve as precursors to important neurotransmitters, such as serotonin (from tryptophan) and dopamine (from tyrosine). Bananas are relatively rich in these amino acids, though in modest amounts compared to other high-protein foods such as meat [526]. Additionally, the urinary concentration of tyramine (p = 0.0012) was significantly higher after banana consumption. Tyramine is a naturally occurring monoamine derived from the amino acid tyrosine, which is abundant in bananas. Tyramine is also present in bananas. In fact, tyramine levels increase as the fruit ripens, which is why overripe bananas tend to have higher tyramine content. The process of tyrosine decarboxylation, catalyzed by the enzyme tyrosine decarboxylase, leads to the formation of tyramine, both in bananas and humans [527]. Tyramine plays a significant role in the regulation of BP and functions as a neurotransmitter within the central nervous system. It is classified as an indirectly acting sympathomimetic agent, meaning it can stimulate the release of norepinephrine,

a neurotransmitter that increases heart rate and blood pressure. This mechanism involves the uptake of tyramine by norepinephrine reuptake transporters, leading to the release of norepinephrine from presynaptic storage [527]. However, the correlational analysis between the urinary levels of tyramine and BP levels did not return any strong results (Figure 4.58). In addition to bananas, tyramine is commonly found in fermented, aged, or spoiled foods. Foods high in tyramine include aged cheeses, pickled vegetables, and certain alcoholic beverages [527]. Interestingly, comparing the amount of cheese consumed during the dietary interventions, with the urinary concentration of tyramine returned a significant correlation (Figure 4.59). However, correlational analysis of urinary concentrations of aromatic amino acids and the amount of banana consumed did not return strong correlations.

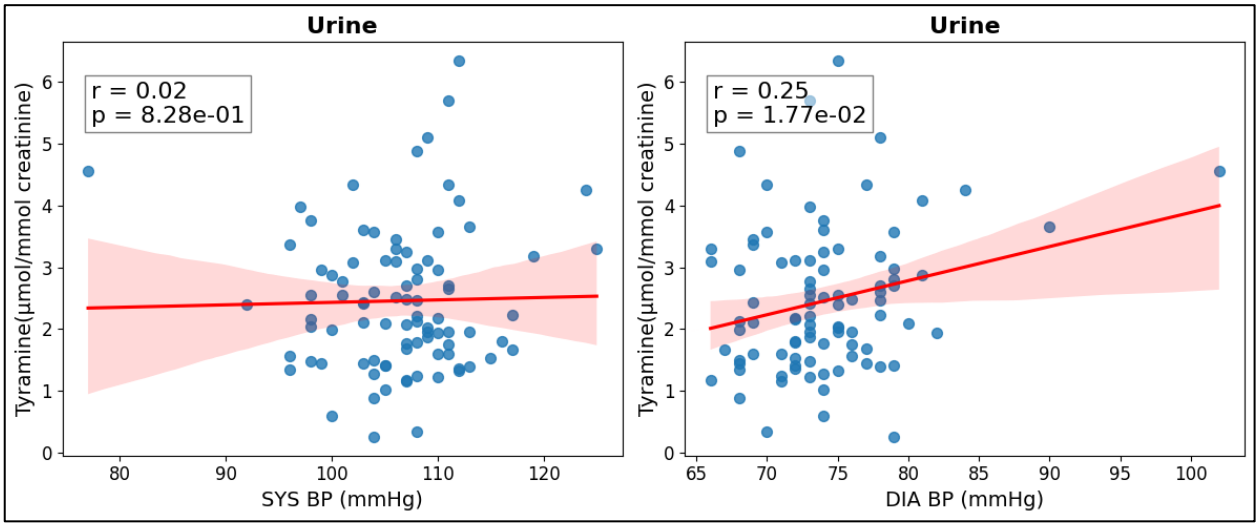

Figure 4.58 - Correlation analysis between systolic blood pressure (SYS BP), diastolic blood pressure (DIA BP) and urinary concertation of tyramine (µmol/ mmol creatinine). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

Glucose levels in urine were significantly higher after banana consumption (p = 0.00095). Bananas contain simple carbohydrates, including approximately 15 grams of sugar in a medium-sized banana, which can cause a rise in blood sugar levels more significantly than other nutrients [528]. The glycemic index (GI) of bananas varies depending on their ripeness, with ripe bananas having a higher GI and causing a more significant increase in blood sugar levels compared to unripe bananas [529]. Upon consumption, glucose is rapidly absorbed into the bloodstream through

the intestinal epithelium via the sodium-glucose linked transporter (SGLT1) and facilitated diffusion via GLUT2, leading to an increase in BG levels [530] (Figure 4.60).

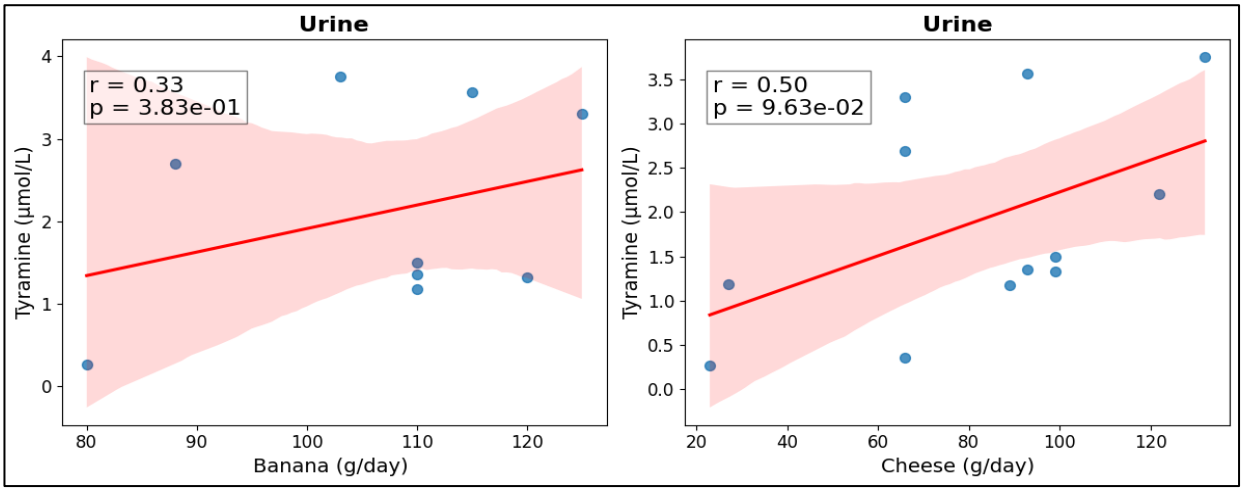

Figure 4.59 - Correlation analysis between banana consumption (g/day), cheese consumption (g/day) and the urinary concertation of tyramine (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

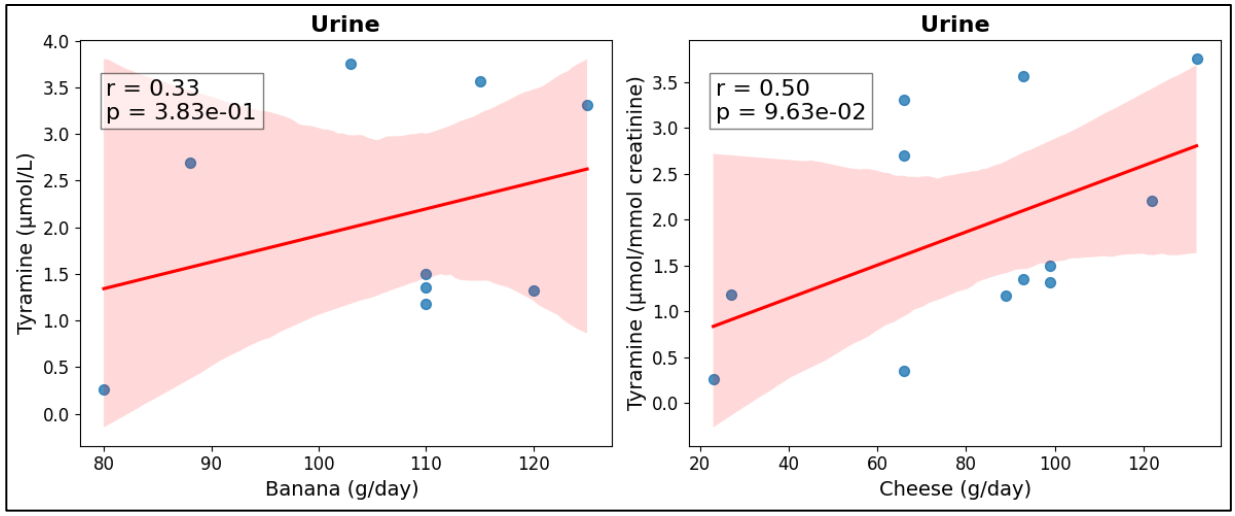

Figure 4.60 - Correlation analysis between banana consumption (g/day), cheese consumption (g/day) and the urinary concertation of tyramine (µmol/mmol creatinine). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot.

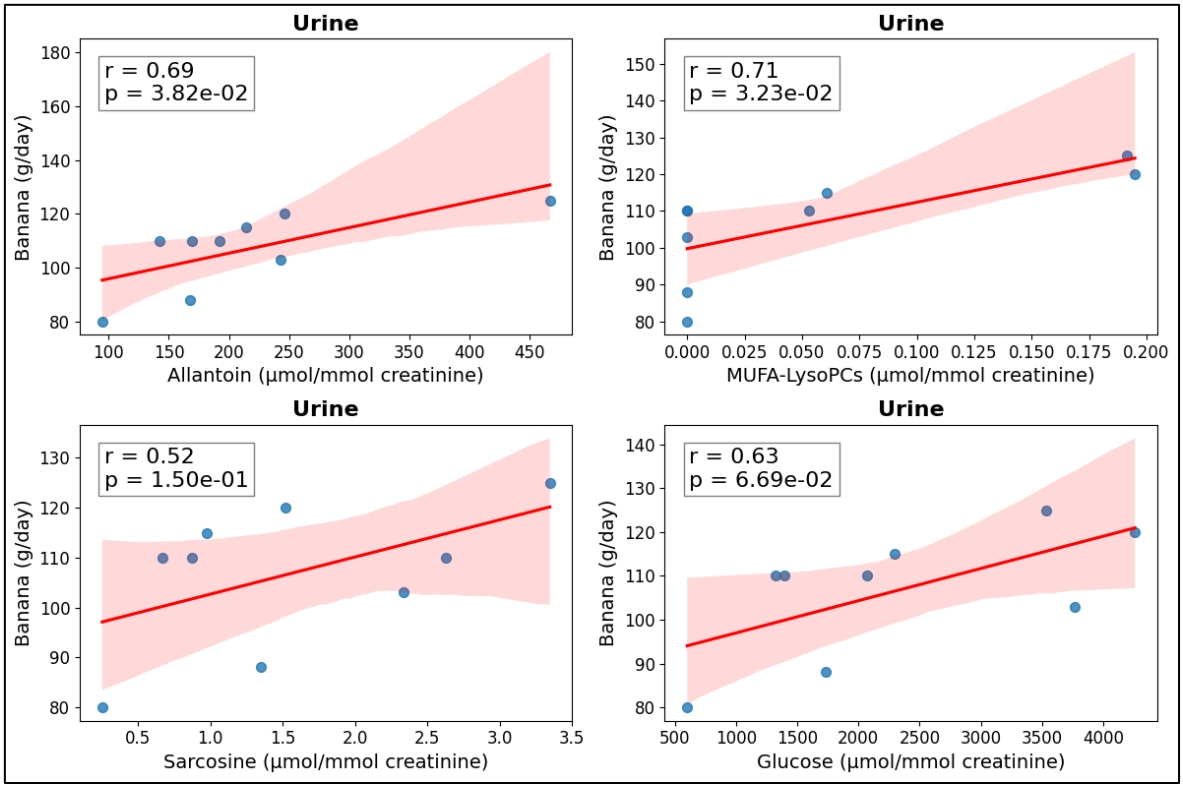

Figure 4.61 - Correlation analysis between banana consumption (g/day), and several plasma metabolites (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot; MUFA-LysoPCs: monounsaturated fatty acid lyso-phosphatidylcholines

4.5.1.6. Zucchini

No differences were detected in plasma metabolites after zucchini consumption. However, significantly higher levels of caffeic acid (p = 0.00052) and hippuric acid (p = 0.0006) were detected in the urine following zucchini consumption. Caffeic acid is a type of hydroxycinnamic acid found in high concentrations in several fruits and vegetables, including zucchini [531]. Caffeic acid concentrations in zucchini range from 0.43 to 0.51 mg per100 g [532]. When zucchini is consumed, the caffeic acid it contains is absorbed in the small intestine and metabolized in the liver. During this process, caffeic acid is conjugated with glucuronic acid or sulfate, making it more water-soluble and facilitating its excretion through urine. This direct excretion leads to higher levels of caffeic acid in the urine. Additionally, some of the caffeic acid and other phenolic compounds may undergo further metabolism, including degradation by gut microbiota, resulting in the formation of benzoic acid. This benzoic acid is then conjugated with glycine in the liver to produce hippuric acid, which is also excreted in the urine. In addition, zucchini contains small amounts of benzoic acid, ranging from 0.099 to 0.11 mg per 100 g [532]. However, neither of these metabolites showed a dose-response effect to the urinary metabolites in the correlational analysis.

4.5.1.7. Coffee

As coffee was consumed daily a comparison between days with and without coffee consumption was not possible. The comparison of the days with high (>400 g/day) and low (<400g/day) coffee consumption in both urine and plasma, did not return any significant differences. However, the correlational analysis of plasma metabolites and the amount of coffee consumed (g/day) returned significant correlations with a number of plasma lipids (Figure 4.61). Coffee consumption, particularly unfiltered coffee, has been shown to increase blood lipid levels, specifically total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) [533]. This effect is primarily attributed to the natural oils found in coffee, such as cafestol and kahweol, which are more prevalent in unfiltered coffee methods. Studies indicate a dose-response relationship, where higher coffee consumption is associated with greater increases in serum lipid levels [533]. This effect is more pronounced in individuals with hyperlipidemia or those consuming large quantities of unfiltered coffee.

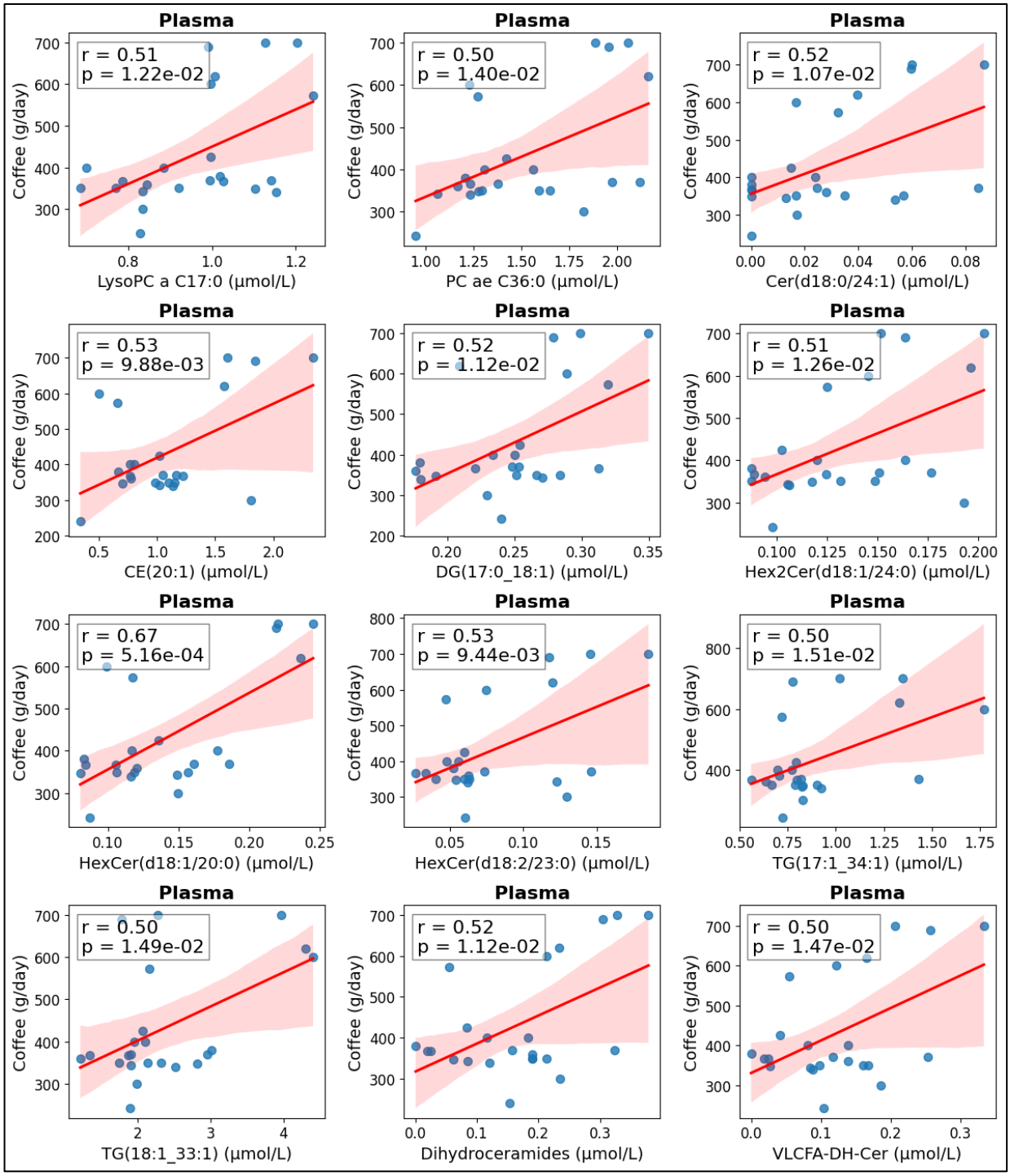

Figure 4.62 - Correlation analysis between coffee consumption (g/day), and several plasma lipids (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot; LysoPC: Lysophosphatidylcholine acyl; PC ae: Phosphatidylcholine acyl-alkyl; Cer: Ceramides; CE: Cholesteryl Esters; DG: Diglycerides, Hex2Cer: Dihexosylceramides; HexCer: Hexosylceramides; TG: Triglycerides; VLCFA-DH-Cer: Very Long Chain Fatty Acid Dihydroceramides

4.5.1.8. Apple

The comparison of plasma and urine samples of the days with apple consumption to the days without any apple consumption (i.e., categorical analysis) did not return any significant differences. However, a significantly positive correlation was observed between the amount of apple consumed (g/day) and the plasma concentration of polyamines, putrescine, spermidine and spermine (Figure 4.62). Various concentrations of these metabolites have been identified in apple [534]. Polyamines can be metabolized into 1,3-diaminopropane, which was also positively

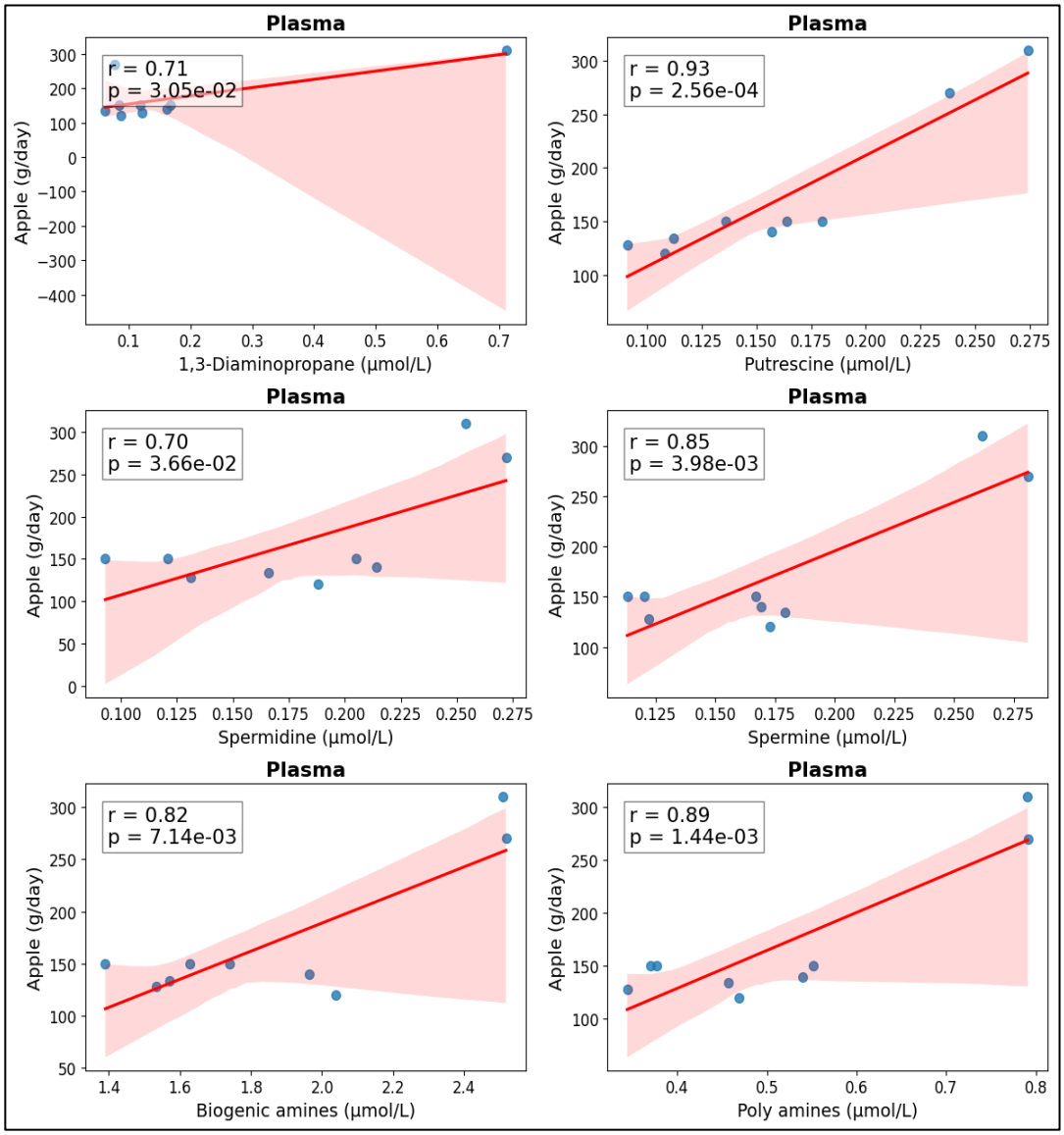

Figure 4.63 - Correlation analysis between apple consumption (g/day), and several plasma metabolites (µmol/L). Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot

correlated with the amount of apple consumed. The correlational analysis of the urinary metabolites did not return any significant correlations.

4.5.2. Metabolites and Long-term Health/Diet Effects

Several metabolites were detected that have been proven to be correlated with health outcomes such as HR, BP, and cardiovascular health. As an example, homoarginine levels in plasma were significantly lowered while I was on the FFD and KD, but much higher while on the RD and MD. Specifically, they were 0.34 ± 0.08 µmol/L while on the FFD, 0.84 ± 1.00 µmol/L while on the MD, 0.42 ± 0.14 µmol/L while on the KD, and 0.90 ± 0.13 µmol/L while on the RD. Homoarginine is a naturally occurring amino acid in the body, produced in the liver by an enzymatic reaction facilitated by L-arginine: glycine amidinotransferase (AGAT). This enzymatic process involves the transfer of an amidino group from arginine to lysine, allowing homoarginine to act as an alternative substrate for NO synthase. The availability of homoarginine enhances NO production, influencing endothelial function crucial for maintaining cardiovascular health [535]. Higher concentrations of L-homoarginine are linked to positive health outcomes, including enhanced endothelial function, decreased platelet aggregation, and increased insulin secretion. Conversely, research indicates a strong association between low levels of homoarginine in the blood and an increased risk of death from cardiovascular diseases [536,537]. Additionally, homoarginine supplementation has demonstrated protective impacts in certain animal studies of cardiovascular disease [538]. Therefore, homoarginine could serve as a potential biomarker and influence the progression of cardiometabolic diseases, as well as act as a protective element in cardiovascular health. The higher average (daily) HR levels that were noted while I was on the FFD and KD align with the decreased homoarginine levels while I was on the FFD and KD, especially in comparison to the MD and RD. This decrease in homoarginine levels and its possible connection to the increased HR observed during the FFD and KD was further highlighted by the calculated ratio of homoarginine to ADMA (asymmetric dimethyl arginine) (0.63 µmol/L ± 0.17 while on the FFD and 0.87 µmol/L ± 0.25 while on the KD, 1.47 µmol/L ±1.92 while on the MD, and 2.08 µmol/L ±1.10 while on the RD; p = 1.79E-5), which is associated with an increased risk of endothelial dysfunction and overall cardiovascular health [539]. In contrast to the FFD and KD, the RD and MD had a higher ratio of homoarginine to arginine plus lysine (0.0032 µmol/L ± 0.0040 while on the MD, 0.0044 µmol/L ± 0.0016 while on the RD, and 0.0013 µmol/L ± 0.0004 while

on the FFD, and 0.0020 µmol/L ± 0.0006 while on the KD; p = 3.66E-6) a known indicator of L-arginine: glycine amidinotransferase activity, which is the enzyme that converts arginine and lysine to homoarginine [536]. Homoarginine levels were also significantly higher while I was on the RD compared to the FFD or KD (0.34 ± µmol/L 0.08 while on the FFD, 0.84 ± µmol/L 1.00 while on the MD, 0.42 µmol/L ± 0.14 while on the KD, and 0.90 µmol/L ± 0.13 while on the RD; p = 7.896E-4).

An overall increase in plasma levels of various forms of ceramides, including those with very long-chain fatty acids, glycosylceramides, monohexosylated ceramides, and dihexosylceramides, was observed while I was on the FFD and the KD (Table 4.1). On the other hand, when I was on the MD, these ceramides were at significantly lower levels (Table 4.1). High plasma ceramide levels have been linked to the onset of metabolic disorders or influence the risk of cardiovascular diseases [540]. Ceramides play a key role in the progression of diabetes, highlighting the connection between excessive caloric intake, inflammation, and elevated ceramide levels, which may lead to insulin resistance, T2D, and heart disease [541]. This was noted in several studies that show how a high-fat diet correlates with increased lipid storage in muscles, obesity, insulin resistance, T2D, and metabolic syndrome [540,541].

Table 4.1 – The averages and p values of plasma very long-chain fatty acid ceramides (VLCFA-Cer), glycosylceramides (Glycosyl-Cer), monohexosylated ceramides (Hex Cer), and dihexosylceramides (hex 2 Cer) while on FFD, MD and KD

	VLCFA-Cer	**Glycosyl-Cer**	**Hex Cer**	**Hex 2 Cer**
FFD	4.85 µmol/L ± 0.73	9.57 µmol/L ±1.11	6.48 µmol/L ± 0.72	2.87 µmol/L ± 0.47
MD	3.23 µmol/L ± 0.39	6.95 µmol/L ± 0.75	4.49 µmol/L ± 0.51	2.27 µmol/L ± 0.28
KD	4.86 µmol/L ± 0.83	11.12 µmol/L ± 1.92	7.43 µmol/L ± 1.30	3.45 µmol/L ± 0.61
p-value	1.42E-06	5.80E-10	8.39E-11	6.58E-07

FFDs are typically rich in saturated fats and sugars. The high intake of saturated fats and refined carbohydrates in fast food can disrupt normal fatty acid metabolism [542]. These dietary components can significantly alter lipid metabolism, leading to increased synthesis of ceramides and their derivatives. High levels of saturated fatty acids are known to upregulate ceramide synthesis, contributing to the observed peak in plasma ceramide concentrations seen on day 4 of the FFD as the body adjusts to the influx of these dietary fats [540]. FFDs are known to trigger an acute inflammatory response due to their high content of saturated fats, trans fats, and sugars.

These dietary components can induce inflammation through various mechanisms, including the activation of toll-like receptors (TLRs) on immune cells, leading to the release of pro-inflammatory cytokines [540,543]. Interestingly, the inflammatory biomarkers IFN- γ, IL-6, IL-8, IL-10, IL-13, and IL-1β were higher on day 5 of the FFD (5.77 pg/mL, 1.83 pg/mL, 49.99 pg/mL, 0.28 pg/mL, 2.85 pg/mL, and 0.50 respectively– which likely reflects the body's adjustment to the dietary change. It is worth noting that the concentration of acylcarnitines (including short-chain, medium-chain, and long-chain acylcarnitines) peaked on day 5 of the FFD (16.47 μmol/L). Acylcarnitines are metabolites that play a significant role in inflammation, which is a key factor in many diseases such as insulin resistance, cardiomyopathy, and central nervous system disorders [372]. The accumulation of long-chain acylcarnitines has been associated with the induction of inflammation and oxidative stress in various cell types, including myotubes and monocytes [372]. Acylcarnitines, particularly those with chain lengths between C10 and C18, can activate proinflammatory signaling pathways in a concentration-dependent manner. This activation is not mediated through Toll-like receptors but rather involves downstream components like the myeloid differentiation factor 88 (MyD88) [372]. Elevated levels of long-chain acylcarnitines have been linked to increased plasma concentrations of proinflammatory cytokines such as IFN-γ and IL-8 while reducing anti-inflammatory cytokines like IL-10 [372].

Kynurenine levels while on the FFD, also increased on day 5 (from 1.23 μmol/L on day 4 to 1.78 μmol/L on day 5). However, another increasing pattern in plasma kynurenine levels was observed on day 11 of the FFD which continued until the end of the dietary intervention period and kynurenine levels peaked on day 14 (1.87 μmol/L). Kynurenine is a metabolite derived from the amino acid tryptophan and plays a significant role in the body's inflammatory processes. It is produced through the activation of the enzyme indoleamine 2,3-dioxygenase (IDO), which is stimulated by pro-inflammatory cytokines such as IFN-γ and TNF-α [544]. Kynurenine acts as a high-affinity ligand for the aryl hydrocarbon receptor (AHR), which has immunosuppressive properties. This binding can downregulate inflammatory responses and promote endotoxin tolerance [544]. Additionally, kynurenine is metabolized into several other compounds, including kynurenic acid and quinolinic acid, which have opposing effects on inflammation and neurotoxicity. Kynurenic acid is neuroprotective, whereas quinolinic acid is neurotoxic and can contribute to neurodegenerative diseases [545]. Interestingly, plasma concentrations of kynurenic acid and quinolinic acid during the FFD, peaked on day 8 of the intervention (0.018 μmol/L, and

0.292 µmol/L, respectively) which aligned with the increase observed in concentration of TNF-α on day 7 (1.90 pg/mL) and day 9 (1.90 pg/mL) of the FFD. However, the overall average of kynurenine was the highest on the MD (1.73 µmol/L ± 0.37), kynurenic acid was highest on the KD (0.015 µmol/L ± 0.007), and quinolinic acid levels were similar between all dietary groups.

Uremic toxins such as indoxyl sulfate and p-cresol sulfate are associated with endothelial dysfunction and immune dysregulation. They activate pathways such as MAPK/NF-κB, which are involved in inflammatory responses, leading to increased expression of pro-inflammatory cytokines [546]. The concentration of indoxyl sulfate and p-cresol sulfate in plasma peaked on day 11 of the FFD (7.37 µmol/L and 36.92 µmol/L). Although the concentrations of most pro-inflammatory cytokines were not at their highest levels on day 11 of the FFD, but increased levels of IFN-γ, IL-1β, and TNF-α were detected on day 13 of the FFD. Other uremic toxins such as ADMA slightly increased to 0.63 µmol/L on day 5 and 0.66 µmol/L on day 6 (versus 0.37 µmol/L on day 3) and peaked to 0.71 µmol/L on day 9. Uric acid peaked to 264.6 µmol/L on day 8 and 259.9 µmol/L on day 9 (while it was 213.2 µmol/L on day 7). Hippuric acid peaked on day 9 at 12.96 µmol/L (6.87 µmol/L on day 8 and 0.27 µmol/L on day 7). This pattern, similar to kynurenine and kynurenic acid, also aligned with the increase observed in the concentration of TNF-α on days 7-9 of the FFD.

The overall concentration of uremic toxins varied across all dietary groups. The highest levels of indoxyl sulfate in plasma were detected while on the RD (6.17 µmol/L ± 2.19), and the lowest levels were detected while on the MD (4.04 µmol/L ± 1.96; p = 2.2532E-4). On the other hand, the highest levels of p-cresol sulfate were observed while on the KD (44.42 µmol/L ± 13.37), while the lowest concentrations were observed while on the RD (13.28 µmol/L ± 2.65; p = 6.03E-13). Plasma ADMA levels were highest while on the FFD (0.55 µmol/L ± 0.10) and the lowest ADMA levels were observed while on the RD (0.43 µmol/L ± 0.13; p = 0.012). Uric acid levels were highest on the KD (391.5 µmol/L ± 68.96) and lowest while on the MD (213.4 µmol/L ± 41.34; p = 1.32E-9). Hippuric acid levels were highest on the RD (9.05 µmol/L ± 5.08) and lowest while on the FFD (2.75 µmol/L ± 3.53; p = 5.5079E-4), likely reflecting different vegetable and fruit content.

In addition to cytokines, ceramides play a role in mediating inflammatory responses [547]. Ceramides can activate signaling pathways that lead to the production of pro-inflammatory cytokines [547]. The peak levels of plasma ceramides by day 4 of the FFD might have contributed to the heightened inflammatory response observed on day 5 [540,543]. The reduction in plasma ceramide levels by day 14 of the FFD shows a possible correlation with the decrease in inflammatory protein biomarkers by days 11 and 13, indicating that as the body adapts to an inflammatory diet, the inflammatory response diminishes. The body's inflammatory response to a high-fat diet might initially be robust but tends to decrease as the body adapts. This adaptation phase involves the downregulation of inflammatory pathways and the activation of anti-inflammatory mechanisms to restore homeostasis. This might include changes in immune cell function, metabolic adjustments, and alterations in lipid metabolism that reduce the production of pro-inflammatory mediators over time [540].

Recent studies have provided mixed results on the link between saturated fat and health [548,549]. However, saturated fatty acids (SFA) have long been associated with negative health effects, particularly concerning heart disease [548]. Saturated fats can raise LDL (low-density lipoprotein) cholesterol levels in the blood, which is considered "bad" cholesterol. High levels of LDL cholesterol can lead to the buildup of cholesterol in arteries, increasing the risk of heart disease and stroke [548,549]. Therefore, I investigated the differences in saturated and unsaturated lipid concentrations across all dietary interventions. SFA acylcarnitines were significantly higher in plasma while on the KD (p = 5.1072E-11) compared to other interventions. Similarly, SFA sphingomyelins were highest on the KD, while the lowest levels were detected on the MD (p = 1.0135E-9). SFA concentrations, including cholesteryl esters, phosphatidylcholines, and lysophosphatidylcholines, followed a similar trend, being notably higher on the KD and FFD, with significantly lower levels on the MD. SFA diglycerides and triglycerides also showed the highest concentrations on the FFD and KD, with the lowest on the MD and RD (p = 6.0425E-8). These elevated SFA levels on the KD and FFD could be associated with the higher heart rates (HRs) observed on these diets, while the lower SFA levels on the MD may relate to the lower HR seen during this diet.

In plasma, unsaturated fatty acids (UFA) showed distinct patterns across the diets. Elevated levels of unsaturated fatty acids can have both beneficial and potentially harmful effects depending

on the type, balance (particularly between omega-3 and omega-6), and overall dietary context. Generally, a balanced intake that favors omega-3s and includes adequate UFAs is associated with positive health outcomes. The highest levels of unsaturated carnitines were observed on the RD and FFD, with the lowest levels on the MD. FFD and KD had elevated levels of monounsaturated fatty acid (MUFA) acylcarnitines, while the FFD and RD were associated with higher levels of polyunsaturated fatty acid (PUFA) acylcarnitines (Table 4.2). MUFA and PUFA cholesteryl esters were most abundant on the RD and KD, with significantly lower levels on the FFD and MD. Similarly, MUFA (p = 1.1006E-8) and PUFA (p = 3.3384E-11) phosphatidylcholines peaked while on the KD and RD, while the lowest levels were found on the FFD and MD. UFA diglycerides were highest on the FFD and lowest on the RD (p = 1.0027E-12). Unsaturated triglycerides were most abundant while on the KD and MD, while the lowest levels were seen on the FFD and RD. UFA sphingomyelins were significantly higher on the KD and RD compared to the FFD and MD (p = 3.9751E-7). Lastly, UFA lysophosphatidylcholines were elevated on the RD and lowest on the MD (p = 5.2265E-6).

Table 4.2 – The average and standard deviation of saturated and unsaturated fatty acids across all dietary interventions

	Fatty acids	FFD	MD	KD	RD
Saturated	SFA acylcarnitines	8.48 ± 2.27	8.57 ± 2.48	16.92 ± 5.35	7.62 ± 1.82
	SFA cholesteryl esters	137.49 ± 29.67	121.44 ± 32.53	193.63 ± 51.00	195.66 ± 37.72
	SFA diglycerides	14.17 ± 3.16	9.95 ± 4.09	11.25 ± 2.43	6.73 ± 2.76
	SFA triglycerides	9.23 ± 9.02	7.02 ± 3.56	8.48 ± 3.72	5.04 ± 5.17
	SFA phosphatidylcholines	20.38 ± 4.68	18.65 ± 1.67	24.39 ± 5.15	22.89 ± 3.08
	SFA sphingomyelins	130.29 ± 35.53	121.48 ± 11.93	203.36 ± 55.69	161.47 ± 14.14
	SFA lyso-phosphatidylcholines	80.55 ± 23.29	74.74 ± 9.66	81.62 ± 13.00	104.43 ± 13.02
Unsaturated	UFA carnitines	0.65 ± 0.45	0.45 ± 0.17	0.56 ± 0.31	0.72 ± 0.23
	MUFA acylcarnitines	0.77 ± 0.30	0.61 ± 0.11	0.79 ± 0.24	0.77 ± 0.23
	PUFA acylcarnitines	0.13 ± 0.19	0.05 ± 0.05	0.08 ± 0.08	0.13 ± 0.07

	MUFA cholesteryl esters	359.24 ± 51.27	350.02 ± 65.70	513.70 ± 124.12	541.52 ± 113.41
	PUFA cholesteryl esters	1391.34 ± 227.63	1317.40 ± 274.18	2279.54 ± 668.63	2280.50 ± 440.82
	UFA diglycerides	21.70 ± 2.99	16.85 ± 4.87	17.97 ± 2.70	11.22 ± 3.42
	UFA triglycerides	929.04 ± 486.06	1030.68 ± 266.52	1082.39 ± 287.75	812.23 ± 301.12
	MUFA phosphatidylcholines	301.57 ± 77.89	241.02 ± 21.38	318.46 ± 49.66	334.12 ± 45.90
	PUFA phosphatidylcholines	1269.57 ± 335.52	1012.52 ± 101.90	1509.88 ± 281.84	1467.03 ± 134.91
	UFA sphingomyelins	98.81 ± 25.56	94.08 ± 9.54	137.06 ± 31.93	121.22 ± 6.66
	UFA lyso-phosphatidylcholines	66.73 ± 21.57	51.34 ± 9.87	56.32 ± 14.42	84.73 ± 18.57

Higher levels of short-chain acylcarnitines were observed in urine and plasma of the KD samples (p = 3.1355E-11 in plasma; and p = 1.1853E-15 in urine). Similarly, the calculated fraction of short-chain acylcarnitines was higher in both plasma and urine of the KD compared to all other diets (p = 1.1716E-6 in plasma; and p = 7.65E-4 in urine). Short-chain acylcarnitines are intermediates in the metabolism of fatty acids. When the body is in ketosis, it breaks down lipids into fatty acids and then into ketone bodies to be used for energy [550]. This process increases the demand for carnitine as carnitine is essential for the transport of fatty acids into the mitochondria for oxidation and energy production [550]. As a result, the concentration of acylcarnitine derivatives in the urine can increase because these compounds are part of the mechanism that helps shuttle fatty acids into the mitochondria where they can be burned for energy [550]. An increase in urinary short-chain acylcarnitines while on the KD indicates the expected increase in fat metabolism. It is a sign that the body is effectively breaking down fats into energy, leading to the production of these metabolites as part of the process. This is generally considered a normal and expected biochemical response to a KD, reflecting the body's adaptation to a different energy source than glucose [384,550]. In addition, the ratio between short-chain acylcarnitines and total acylcarnitines can provide insights into the relative abundance and utilization of short-chain fatty acids as an energy source.

Changes in this fraction indicate alterations in fatty acid metabolism, mitochondrial function, or specific metabolic disorders [371,542,551].

The total concentration of acylcarnitines (including short, medium, and long-chain acylcarnitines) was significantly higher while on the KD in both plasma (p = 2.172E-10) and urine (p = 7.87E-15). Acylcarnitines are derivatives of carnitine that play a critical role in the transport of fatty acids into the mitochondria for β-oxidation, where they undergo metabolism to produce energy. However, under conditions of metabolic stress or mitochondrial dysfunction, such as during inflammation, the fatty acid oxidation process becomes less efficient [552]. This inefficiency leads to an accumulation of acylcarnitines in both cells and the bloodstream. Accumulation of specific acylcarnitines, particularly medium- and long-chain species, can activate pro-inflammatory pathways. For instance, acylcarnitines can interact with tTLRs on immune cells, leading to the activation of NF-κB and other transcription factors that promote the production of inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 [552]. Acylcarnitines have also been shown to impair insulin signaling in muscle and adipose tissues, contributing to the inflammation seen in metabolic disorders such as obesity and T2D [552]. In order to investigate this, I looked at the correlation between the concentration of medium- and long-chain acylcarnitines in both urine and plasma and the concentration of cytokines detected in plasma. Interestingly, only concentrations of IL-8 were significantly correlation to the medium- and long-chain acylcarnitines in plasma (Figure 4.63). Additionally, the total concentration of acylcarnitines in urine and plasma was negatively correlated with IL-12p70 (Figure 4.63).

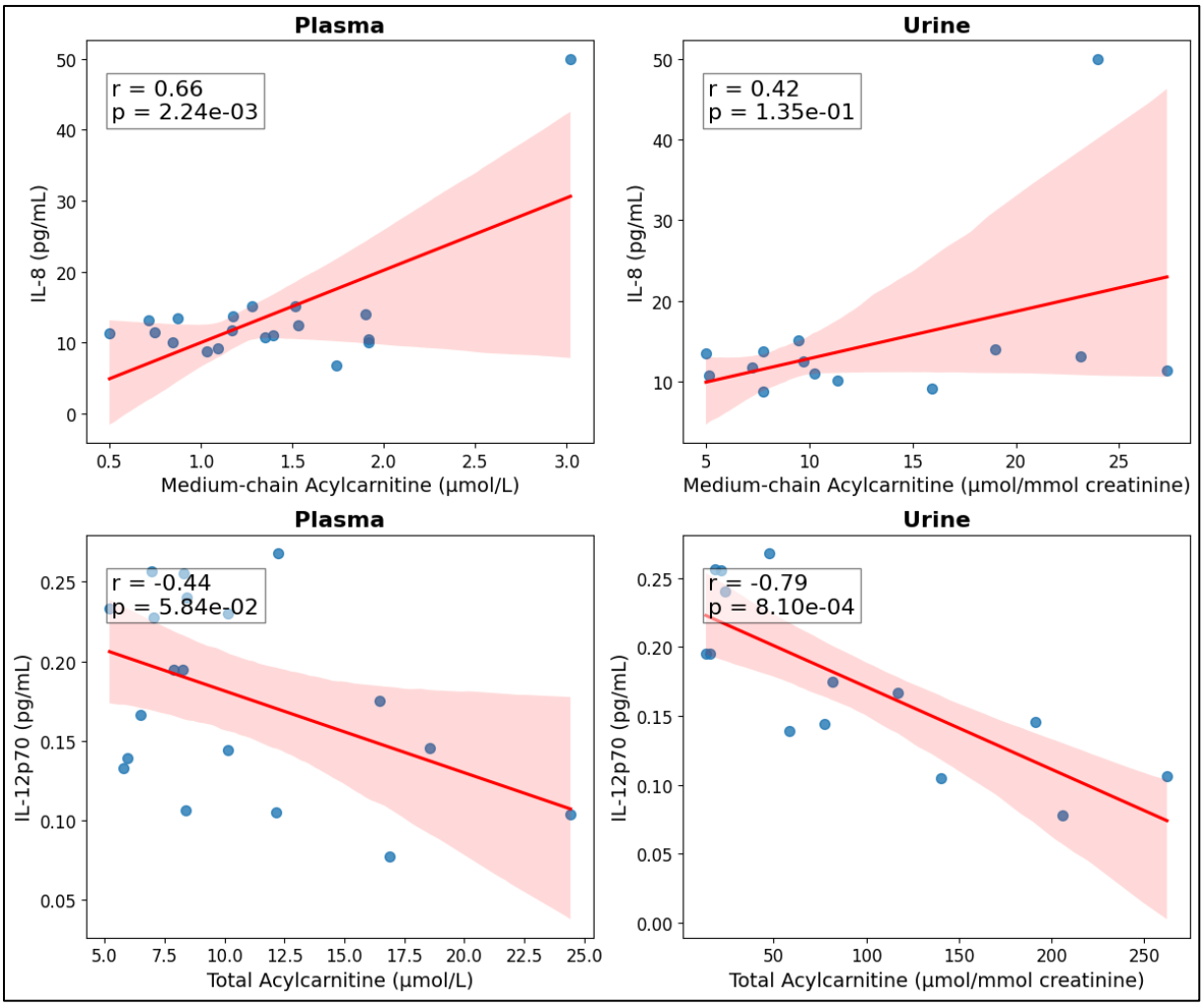

Figure 4.64 - Correlation analysis between plasma and urine concentration of medium-chain acylcarnitine (µmol/ mmol creatinine) and IL-8 concentrations (pg/mL) on the top. Correlation analysis between plasma (µmol/L) and urine (µmol/mmol creatinine) concentration of acylcarnitine and IL-12p70 concentrations (pg/mL) on the bottom. Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot

In addition to acylcarnitines impacting health outcomes and inflammation, there are other lipid-like molecules that play an equally important role, namely triglycerides (TGs). TGs are esters derived from glycerol and three fatty acids and are the main constituents of body fat in humans. High levels of TGs in the bloodstream have been linked to atherosclerosis and an elevated risk of heart disease, stroke [248,249], and obesity [553]. The total concentration of TGs was quantified and

calculated across all plasma samples. Although the differences in the overall plasma concentration of TGs were not statistically significant between diets, the KD did have higher plasma levels of TGs in comparison to the other diet groups (1111.31 μmol/L ± 308.22), while the RD had the lowest levels of TGs in the plasma samples (856.12 μmol/L ± 308.47). Chronically high levels of TGs are a known risk factor for a number of diseases and can lead to atherosclerosis, heart attack and stroke. They are also associated with metabolic syndrome, which includes conditions like hypertension, high BG, and abdominal obesity, further elevating the risk of diabetes and heart disease [554].

In addition to the effects of lipids and acylcarnitines on health, I also explored the effects of several amino acids on longer term diet/health effects. The body naturally produces several non-essential amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine), eliminating the need for their dietary intake. The total amount of these non-essential amino acids serves as a measure of protein turnover [555]. Total levels of non-essential amino acids reached their highest in plasma and urine while I was on the FFD, and they were at their lowest while on the KD. More specifically, alanine levels were reduced while I was on the KD (159.77 μmol/L ± 47.48 in plasma, and 146.19 μmol/mmol creatinine ± 24.63 in urine), and elevated while I was on the FFD (238.65 μmol/L ± 36.77 in plasma and 342.04 μmol/mmol creatinine ± 176.39 in urine; p = 7.6995E-7 for plasma, and 6.7557E-8 for urine) in both urine and plasma. Alanine levels are closely linked with glucose concentrations. This means that when plasma BG levels rise, as seen in diabetes, prediabetes or hyperglycemia, alanine levels tend to increase accordingly, and vice versa. It is worth noting that the concentration of glucogenic amino acids was significantly lower in both urine and plasma during the KD compared to the FFD. Specifically, during the KD, the glucogenic amino acids levels were 1741.07 μmol/L ± 233.92 in plasma and 2483.73 μmol/ mmol creatinine ± 1095.73 in urine. In contrast, during the FFD, the levels were 2038.44 μmol/L ± 152.74 in plasma and 4992.80 μmol/mmol creatinine ± 2054.59 in urine (p = 0.0039 in plasma, and 7.8147E-6 in urine). This reduction is due to the depletion of these amino acids as they were being converted to glucose, compensating for the low BG levels during the KD. While on the FFD, where glucose levels were high, these amino acids were not being utilized for glucose production. Glucogenic amino acids play a crucial role in maintaining BG levels, especially during periods of low BG, such as fasting or intense exercise. These amino acids can be converted into glucose via gluconeogenesis, which primarily occurs in the liver [556].

When BG levels fall, muscle protein is broken down, releasing glucogenic amino acids such as alanine into the bloodstream. Alanine is then transported to the liver, where it undergoes transamination to become pyruvate. Pyruvate can be further converted into oxaloacetate and eventually into glucose, which helps maintain BG levels [556]. During low BG episodes, such as fasting, the body initially relies on glycogen stores for glucose. Once these stores are depleted, gluconeogenesis becomes the primary source of glucose production, utilizing substrates such as lactate, glycerol, and glucogenic amino acids [557]. This metabolic shift is crucial for sustaining energy levels and preventing hypoglycemia during prolonged periods without food intake.

It has been found that a FFD can lead to elevated levels of alanine aminotransferase (ALT), an enzyme that is used by the liver to convert alanine to pyruvate which is subsequently used for glucose production. High levels of ALT indicate impaired liver function but are also likely a result of high levels of alanine being present in the bloodstream (which is a characteristic of the FFD). This elevation is also associated with non-alcoholic fatty liver disease (NAFLD), commonly induced by high-fat, high-sugar diets typical of fast-food consumption. The FFD induces significant metabolic stress, contributing to altered amino acid levels, including alanine [558]. A recent study conducted on mice fed a FFD showed significant increases in plasma ALT levels, indicating liver stress and changes in alanine metabolism. The FFD led to obesity, higher plasma glucose, cholesterol, and insulin levels, along with increased liver inflammation and steatosis [559]. Overall, elevated serum levels of aminotransferases, including ALT, have been linked to the consumption of unhealthy foods such as fast food, soft drinks, and sweet snacks. Increased dietary intake from these sources correlates with higher levels of liver enzymes, reflecting changes in amino acid metabolism and potential liver damage [558]. Additionally, alanine plays a crucial role in glucose metabolism and the citric acid cycle, and variations in dietary carbohydrate and protein intake can influence alanine levels.

Another non-essential amino acid of interest is arginine. Arginine is a precursor for NO and is involved in protein synthesis and immune function. NO is synthesized from arginine by nitric oxide synthase (NOS) and is essential for vascular regulation, immune response, and neurotransmission [560]. Arginine is also involved in the production of creatine, polyamines, proline, and agmatine, all of which are important for cell function and metabolism [561]. Dietary intake and the overall amino acid composition of the diet can influence arginine levels. Adequate

protein intake ensures a sufficient supply of arginine, which is necessary for maintaining its physiological functions [560]. The regulation of arginine levels and its conversion to NO are critical for maintaining vascular health and proper immune responses [561]. On average, the highest levels of arginine in plasma were observed while I was on the FFD (85.72 µmol/L ± 17.83), while the lowest levels of plasma arginine were observed while I was on the KD (64.77 µmol/L ±16.08; p = 0.0088).

Plasma levels of tryptophan, tyrosine, and phenylalanine were notably higher in both urine and plasma of the FFD. Tryptophan was measured at 43.61 µmol/L ± 5.04 in plasma and 71.10 µmol/mmol creatinine ±24.84 in urine (p = 0.026 for plasma and 6.8878E-12 in urine). Tyrosine was measured at 44.07 µmol/L ± 6.42 in plasma and 31.34 µmol/mmol creatinine ± 11.53 in urine ($p > 0.05$ in plasma, and p = 2.3849E-7 in urine). Phenylalanine was measured at 64.14 µmol/L ± 4.36 in plasma and 84.08 µmol/mmol creatinine ± 29.54 in urine (p = 0.030 in plasma, and 4.7707E-14 in urine). Increased plasma levels of tryptophan, tyrosine, and phenylalanine, known as aromatic amino acids, have been linked to the severity of insulin resistance, elevated BG, and diabetes [562,563]. These levels can also serve as markers of nutritional health. It is notable that consumption of bananas (which are high in these aromatic amino acids) led to high levels of urinary aromatic amino acids, but not high levels in plasma. This highlights how the type of "matrix" in which a given nutrient is found can affect the levels of amino acids found in different biofluids. Aromatic amino acids are also precursors for several biogenic amines and neurotransmitters such as dopamine and serotonin, which affect mood and cognition.

Levels of dopamine, histamine, and serotonin were compared in plasma samples for the different diets. These compounds are classified as biogenic amines that act as neurotransmitters. They are pivotal in managing mood, motivation, cognition, sleep, and various other bodily functions. Serotonin is often referred to as the "feel-good" or "happy hormone" neurotransmitter because it contributes to feelings of well-being and happiness. It plays a crucial role in regulating mood, anxiety, and happiness. Low levels of serotonin are linked to depression and anxiety disorders. Serotonin also influences other functions such as appetite, sleep, and memory [429]. Dopamine is known as the "reward" neurotransmitter. It plays a key role in the brain's reward system and is associated with pleasure, motivation, and reinforcement of rewarding behaviors. Dopamine levels influence mood, motivation, and feelings of euphoria [564]. Histamine plays a

significant role in regulating the sleep-wake cycle and cognitive functions [565]. While dopamine and histamine were not detected in plasma using the MEGA kit, serotonin was. I found that serotonin levels peaked while I was on the MD. Serotonin showed the lowest levels while I was on the KD. These levels correlated (as highlighted in Chapter 3) to my mood and feeling of well-being when on these two diets.

On the other hand, dopamine, histamine, and serotonin could be readily detected in urine. The presence of these compounds in urine is likely due to their production by gut microbiota [566]. However, it is also believed that urinary levels of these neurotransmitters partially reflect their levels in the blood and brain (blood-brain barrier) [567]. In my urine samples, the total concentration of dopamine, histamine and serotonin was the lowest while on the KD and highest while on the FFD. These data for both plasma and urine help explain some of the mood testing results obtained from the POMS test, where the KD led to the highest scores for depression, and worst in terms of overall mood level. The higher levels of serotonin (especially) as seen in the FFD may be a reason why many people use fast foods as "comfort" foods and why there seems to be a strong addiction to FFD by so many individuals across the developed world [568]. On the other hand, I felt best while on the MD and this is reflected in the higher plasma levels of serotonin while I was on the MD. Monitoring the levels of these neurotransmitters can offer valuable information about their balance and activity, essential for sustaining both mental and physical health [429,564,565]. Additionally, increased plasma levels of monoamine neurotransmitters [569,570], and decreased levels of citrulline and arginine [571] in serum have shown associations with major depression. The sum of citrulline and arginine and their individual abundances were significantly lowered while I was on the KD, which aligns with the higher scores of depression obtained from the POMS test results.

Ketogenic amino acids such as leucine and lysine can be converted into acetyl-CoA and can contribute to energy production through the citric acid cycle or be used in the synthesis of ketone bodies (acetoacetate, 3-hydroxybutyrate, and acetone) in a fasted or ketogenic state [556]. Additionally, ketogenic amino acids, when not converted into ketone bodies, can be rerouted towards fatty acid synthesis and storage, thereby contributing to an increase in triglycerides and the accumulation of fat in the liver, contributing to the development of NAFLD [572]. The plasma concentration of these ketogenic amino acids was highest while I was on the KD, and the second highest levels in plasma were observed while I was on the FFD. On the other hand, when measured

in urine the highest levels of ketogenic amino acids were observed for the FFD and the second highest levels were seen for the KD. Ketogenic acids are increased when one is in a state of ketosis, so the high levels of these amino acids while on the KD likely reflect their role in generating ketone bodies. Indeed, while I was on the KD, the plasma levels of acetoacetate (333.96 μmol/L ±296.00), and 3-hydroxybutyrate (1266.59 μmol/L ± 438.90) were highest. However, while I was on the FFD, these ketone bodies were very low (acetoacetate: 8.43 μmol/L ±7.43, 3-hydroxy butyrate: 127.81 μmol/L ± 82.74) (p = 2.3683E-11 for acetoacetate, and 9.8536E-12 for 3-hydroxy butyrate in plasma; p = 9.4823E-12 for acetoacetate, and 1.3137E-10 for 3-hydroxy butyrate in urine). This suggests that ketogenic amino acids were being converted to fatty acids for fat storage – in the liver. This was confirmed by the higher levels of lipids and fatty acids appearing in my plasma (mentioned earlier) while I was on the FFD. High levels of ketogenic amino acids, without concomitant conversion to ketone bodies, is another way in which the FFD can lead to fat accumulation and NAFLD [572]. The opposing fates of high levels of ketogenic acids, with the FFD leading to fat production and the KD leading to ketone body production, is intriguing. This result suggests that because the FFD contains both high levels of carbohydrates and high levels of protein, while the KD has low levels of carbohydrates and high levels of protein, this difference in carbohydrate abundance is what shifts metabolism towards an unhealthy outcome with the FFD.

The concentration of BCAAs (leucine, isoleucine, and valine) was significantly higher in plasma (p = 3.63E-12) and in urine (p = 1.147E-13) while on the KD. BCAAs have been discussed in previous sections in my comparison with various physiological responses. However, in this section, I focus more on the differences between the diets in terms of BCAA concentrations. BCAAs have a branched molecular structure, and are considered essential because the body cannot produce them - they must be obtained from dietary intake or supplements [414]. BCAAs are generally considered beneficial, especially for muscle growth, recovery, and energy production, particularly in physically active individuals [410]. However, excessive intake or imbalanced consumption of BCAAs could have potential negative effects on health. Studies suggest that chronic high intake of BCAAs may be linked to insulin resistance, particularly in individuals with pre-existing metabolic conditions [410,414]. Elevated BCAA levels have been associated with the development of T2D in certain populations, though this link is still being investigated [573]. Elevated plasma BCAA levels are often observed in individuals with obesity, insulin resistance, and T2D [573]. While it is unclear whether elevated BCAA levels are a cause or consequence of

metabolic dysfunction, these amino acids are linked to impaired insulin sensitivity [573]. Leucine, a potent activator of the mTOR (mammalian target of rapamycin) pathway, plays a crucial role in muscle protein synthesis. However, chronic activation of mTOR, particularly when coupled with a high-calorie diet, may lead to disruptions in insulin signaling. mTOR activation inhibits insulin receptor substrate (IRS)-mediated signaling, which is essential for glucose uptake. This can contribute to insulin resistance over time [573]. Additionally, Gut bacteria metabolize BCAAs into various byproducts, some of which can be beneficial (e.g., SCFAs), while others may be harmful (e.g., BCFA derivatives) [574]. An excessive intake of BCAAs may lead to an overproduction of branched-chain fatty acids, which are linked to increased inflammation in the gut and other tissues. This chronic low-grade inflammation is a well-known risk factor for insulin resistance and metabolic disorders [574]. Additionally, emerging evidence suggests that high levels of BCAAs may impair gut-barrier function, leading to increased intestinal permeability ("leaky gut") [575]. This condition allows endotoxins, such as lipopolysaccharides (LPS), to enter the bloodstream. LPS triggers systemic inflammation by activating the immune system, contributing to insulin resistance and other metabolic disturbances [575].

One metabolite of interest which appears to have positive health effects is 1-methylnicotinamide. For many years, 1-methylnicotinamide was considered a biologically inactive metabolite of nicotinamide. However, several studies have shown antithrombotic, anti-inflammatory, gastroprotective and vasoprotective properties of this compound [576,577]. Research has also shown that 1-methylnicotinamide can also play a role in protecting against heart damage caused by high-fat diets and high levels of palmitic acid by influencing the Nrf2 and NF-κB pathways [578]. Interestingly, levels of 1-methylnicotinamide were slightly elevated in my plasma (0.03 µmol/L ± 0.07; p = 0.022425) while I was on the KD, and significantly higher in urine when I was on the KD (41.85 µmol/mmol creatinine ±21.25; p = 1.0802E-14). These data suggest that the KD was leading to a “protective” physiological response by synthesizing 1-methylnicotinamide to protect my body against some of the (normally) adverse responses to high levels of amino acids and fat in the KD. Furthermore, the KD is particularly rich in foods (such as fish, beef, poultry, avocado, and nuts) that are high in levels of NAD (the precursor to 1-methylnicotinamide). On the other hand, no 1-methylnicotinamide was detected in plasma samples of the other dietary interventions, and the diet that had the lowest urinary levels of 1-methylnicotinamide was the FFD (23.18 µmol/mmol creatinine ±15.85), which again highlights

the fact that this diet does not lead to the production of beneficial metabolites or metabolites that are protective against high-fat consumption.

3-aminoisobutyric acid (or beta- aminoisobutyric acid or BAIBA), as highlighted earlier, is a myokine [485]. It is known to exert anti-inflammatory and antioxidant effects and to help convert nutrients into muscle. The highest plasma levels of 3-aminoisobutyric acid were found while I was on the KD (1.33 µmol/L ± 0.51), and MD (1.15 µmol/L ±0.43), while the lowest levels in plasma were with the FFD (0.91 µmol/L ±0.51) and the RD (0.83 µmol/L ± 0.27) (p = 0.0092). On the other hand, the highest urinary levels of 3-aminoisobutyric acid were detected while on the KD (233.38 µmol/mmol creatinine ±102.24), and the lowest urine levels were detected while on the FFD (88.93 µmol/mmol creatinine ± 69.36) (p = 1.9256E-7). KD is characterized by high fat, moderate protein, and very low carbohydrate intake. As mentioned earlier, this diet induces a metabolic state known as ketosis, in which the body primarily burns fats rather than carbohydrates for energy. Muscle cells, under such conditions, increase fatty acid oxidation and reduce glucose metabolism [579]. BAIBA is a myokine, meaning it is a signaling molecule released by muscle cells during exercise and muscle contraction. It plays a role in promoting fatty acid oxidation and has been linked to increased mitochondrial function and improved energy metabolism [579]. The higher plasma and urinary levels of BAIBA while on the KD could be due to increased muscle adaptation to fat metabolism. Since the body relies on fat for energy in the absence of carbohydrates, there is greater muscle contraction and possibly increased BAIBA production to promote energy efficiency and fat utilization. Additionally, BAIBA has been linked to anti-inflammatory and antioxidant effects, which are crucial during periods of metabolic stress like ketosis [579]. The elevated BAIBA levels on the KD may reflect the muscle's enhanced response to these metabolic shifts, aiding in the maintenance of muscle integrity and reducing inflammation. On the other hand, FFDs are typically high in unhealthy fats and refined carbohydrates, and low in essential nutrients such as vitamins, minerals, and antioxidants. It has been shown that fast food consumption can impair muscle function and metabolic health [210], potentially reducing the ability of muscle cells to release myokines like BAIBA. The lower BAIBA levels in both plasma and urine while on the FFD could be a result of impaired fatty acid metabolism and increased inflammation, which hampers the normal muscle signaling processes that promote BAIBA release. The diet's inflammatory nature might also interfere with antioxidant defense mechanisms, further reducing BAIBA's beneficial effects on muscle tissue.

The plasma levels of several key uremic toxins (ADMA, uric acid, TMAO), which have shown associations with kidney failure and cardiovascular diseases, [580,581] were at their highest while I was on the KD and lowest while I was on the MD. This is generally in line with the observation that the MD is known to be beneficial to cardiovascular and kidney health [248], while the KD is known to have negative effects on kidney and heart health (increased risk for kidney stones, electrolyte imbalance, general inflammation, and greater oxidative stress) [582]. These three uremic toxins were also present in urine and were at their highest while I was on the FFD and lowest while I was on the RD. The presence of uremic toxins in urine is less of a concern as it simply indicates that the kidneys were effectively clearing these compounds.

Plasma levels of argininic acid were significantly higher during the KD. Argininic acid has been identified as a uremic toxin according to the European Uremic Toxin Working Group [583]. In addition, significantly higher levels of urinary and plasma 3-hydroxyisobutyric acid and 2-hydroxybutyric acid were detected in the samples collected during the KD compared to the other three dietary interventions. The high protein consumption in KD can lead to the production of more uremic toxins as protein metabolism generates these compounds. Uremic toxins accumulate because the kidneys are less able to excrete them effectively [584]. Additionally, the high-protein diet associated with the KD can also alter the gut microbiota, favoring the growth of bacteria that produce uremic toxins. Bacteria such as *Clostridia*, *Bacteroides* and *Faecalibacterium* were detected in higher abundance in my gut microbiome after the KD. *Clostridium XIVa* had the highest abundance after the KD (1.21%) and the lowest in the FFD (0.57%). *Clostridium IV* was present only in the KD (0.71%) and was absent in the gut following the FFD, MD, and RD. *Clostridium XVIII* showed relatively consistent abundance across all diets, with 1.75% in the FFD, 1.63% in the MD, 1.81% in the KD, and 1.20% in the RD. *Bacteroides* were significantly more abundant in the KD (19.26%) compared to the other diets, with 7.42% in the FFD, 5.87% in the MD, and 7.58% in the RD.

I also looked at the correlation between the BG levels detected through the CGM and glucose levels detected in plasma using the TMIC MEGA assay. As the plasma samples were fasting plasma, I looked at the correlation of TMIC MEGA BG and daily average CGM BG, in addition to the correlation of TMIC MEGA BG and fasting CGM BG. Fasting CGM BG was calculated by averaging the BG values between 2 to 5 am of each day, as it was at least 4 hours

after the last meal consumption across all interventions (Figure 4.64). Although there was a positive correlation between the values, however, the correlations were not strong. The difference between plasma glucose and BG detected through CGM primarily lies in their measurement locations and the physiological processes involved. Plasma glucose was measured in the blood, specifically in the capillary blood, however, CGM devices typically measure glucose levels in the interstitial fluid, which is the fluid surrounding the cells in the tissues beneath the skin [585]. In addition, there is a time lag between changes in BG levels and the corresponding changes in interstitial glucose levels [586]. This lag is due to the time it takes for glucose to diffuse from the capillaries into the interstitial space, leading to a lag in the rise of interstitial fluid glucose compared to plasma glucose. The lag can range from 5 to 25 minutes, depending on the metabolic rate, the type of carbohydrates consumed, and insulin levels [585]. This time lag means that interstitial glucose levels may not immediately reflect rapid changes in BG, such as those occurring after meals or during physical activity. However, I tried to overcome this issue by averaging the BG levels from the CGM. Additionally, the concentration of glucose in the interstitial fluid is generally lower than in plasma. This is because glucose is continuously being taken up by cells from the interstitial space for energy production. The difference in concentration is also because glucose must first pass through the capillary walls before entering the interstitial fluid [8]. Interstitial glucose levels tend to be lower than blood glucose levels when glucose is rising, and they may be similar or slightly higher when glucose levels are declining. This is due to the physiological processes of glucose uptake, utilization, and elimination [586].

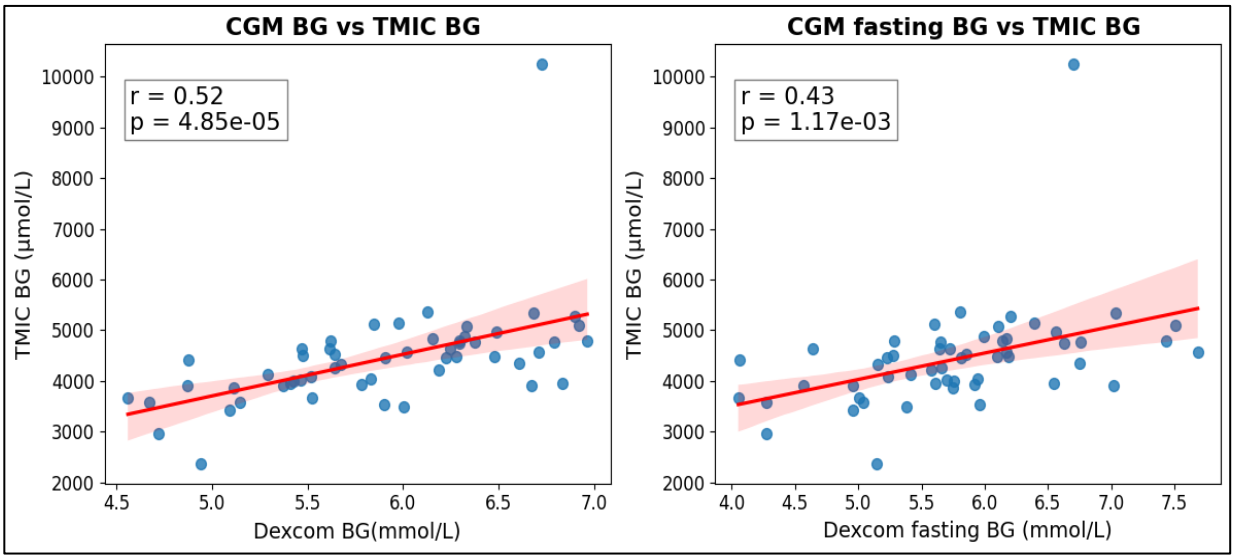

Figure 4.65 - Correlation analysis between BG levels detected through TMIC MEGA assay in plasma and daily average (left plot) and average fasting BG levels (right plot) detected through CGM. Each subplot the correlation between the two variables with a regression line and annotating the Pearson correlation coefficient (r) and p-value (p) within the plot. CGM: continuous glucose monitor; BG: blood glucose

In addition to the metabolites discussed in this section, several other metabolites showed significant differences across the dietary interventions and within each dietary period, emphasizing the complex metabolic responses to various food patterns. These findings reveal not only the expected shifts in nutrient metabolism but also provide insight into the nuanced ways each diet interacts with the body's biochemistry. Overall, these data highlight how the metabolome can reflect both short-term (food intake) and long-term (diet, lifestyle, physiological) effects. It is important to be able to distinguish between these as if one misinterprets food intake effects as physiological responses, this can lead to incorrect scientific conclusions. In this section, we were able to identify the key metabolites that are affected by food intake. Some of these were well-known (beta-alanine, methylhistidine, creatine, etc.) while others were novel or not previously identified. These BFIs represent <5% of the metabolites measured by the MEGA assay. Nevertheless, this N-of-1 study helps define which metabolites should be excluded from consideration when trying to assess physiological effects that may be altered by long-term diet, long-term lifestyle choices or general, long-term physiological/health responses. I believe this is a

very important contribution of this study to the field of PN study as very few, if any, human metabolomic studies have been able to identify (or exclude) these BFIs from consideration. In other words, this work provides a list of key ("no-go") compounds that should be removed from consideration when performing metabolomic biomarker identification for health or disease.

By excluding this set of BFIs, it becomes possible to more accurately interpret the metabolites that were altered due to diet-induced effects on physiology rather than simply reflecting recent food consumption. This approach allows for the identification of longer-term metabolic changes that are directly influenced by dietary interventions, providing deeper insights into how these diets impact health and metabolic processes over time. This was done in the second half of this section. Several metabolites that are known to be connected to health were quantified and several meaningful connections were identified to the physiological measurements obtained from wearable devices. For example, elevated levels of ceramides were observed during the FFD and KD, which aligned with increased HR and markers of inflammation detected by wearable devices. Ceramides, which are sphingolipids implicated in insulin resistance and cardiovascular diseases, were notably lower during the MD, which correlates with the improved cardiovascular health metrics seen in this diet, such as lower HR and more stable BP. Additionally, acylcarnitines, which play a role in fatty acid metabolism, were significantly elevated during the KD, reflecting the body's shift towards fat oxidation in the absence of carbohydrates. Wearable devices revealed that during this period, HR variability and BT fluctuations were more pronounced, suggesting that the body was undergoing significant metabolic adaptation to the KD, and shifting to a higher metabolic rate. These changes in energy utilization and metabolic stress were also reflected in the increased production of ketone bodies such as acetoacetate and 3-hydroxybutyrate, which are hallmarks of ketosis.

While I was on the MD, SCFAs such as butyrate were more abundant, indicating enhanced gut health and better microbial fermentation of dietary fibers. This aligns with improved sleep quality and lower levels of restlessness, as captured by wearable devices. The connection between SCFAs and improved gut-brain signaling may have contributed to these positive effects on sleep and mood regulation. Furthermore, homoarginine, a metabolite involved in nitric oxide synthesis and cardiovascular health, was found to be elevated while I was on the RD and MD, while lower levels of this amino acid were detected while on the FFD and KD. This finding suggests a link

between the dietary patterns that promote better vascular function and the improved heart rate metrics recorded by wearable devices. The presence of higher homoarginine levels also indicates enhanced endothelial function and potential protective effects against cardiovascular disease.

The use of wearable devices in this study provided a continuous stream of physiological data, which allowed for real-time monitoring of the body's responses to different diets. These measurements revealed correlations between diet-induced changes in metabolites and physiological markers such as HR, BT, and sleep patterns. For example, higher urea and creatine levels, indicative of protein metabolism, were observed in diets with higher protein intake, such as the KD. Correspondingly, wearable data showed an increase in BT and greater variability in HR, suggesting that the body was under metabolic stress from processing higher amounts of protein. In addition, serotonin levels, which influence mood and sleep, were more stable during the MD. This diet was associated with improved sleep quality and lower depression scores, as measured by wearable devices and POMS questionnaires. The connection between serotonin and dietary patterns points to the broader effects that nutrition can have on mental and emotional well-being, which are often reflected in sleep patterns and mood metrics tracked by wearables.

Overall, the integration of metabolomic data with physiological measurements obtained from wearable devices highlighted the potential to extract expected as well as new and unexpected relationships between diet, metabolism, molecular measurements, and physiological consequences. By quantifying key metabolites such as ceramides, acylcarnitines, SCFAs, and homoarginine, and linking them to physiological responses such as HR, BT, and sleep data, this study provided a comprehensive view of how different diets affect both metabolic and physiological health. These findings underscore the potential of combining molecular scale measurements with physiological and psychological assessment to move toward PN and optimize health outcomes.

I believe these data also demonstrate the potential of targeted, fully quantitative metabolomics to help identify biomarkers that can predict health outcomes and monitor the efficacy of dietary interventions. This work emphasizes the importance of using a comprehensive, quantitative approach that employs quantitative microbial, proteomic and metabolomic profiling to gain a holistic understanding of how diet affects health. Such insights can be used to develop

personalized dietary recommendations that are more effective in preventing and managing metabolic disorders or chronic diseases. Ultimately, the findings from this study demonstrate the value of combining cheap, low-tech, wearable health monitors with more expensive, advanced omics technologies to capture a complete picture of an individual's health. By continuously and longitudinally monitoring physiological parameters and integrating these data with molecular-scale (metabolomic, proteomics, microbiomics) analyses, we can achieve a level of precision in nutrition and health management that was previously unattainable. I believe this approach not only enhances our understanding of the complex interactions between diet, and metabolism but also paves the way for more personalized and effective healthcare solutions.

Chapter 5
Conclusion

Precision nutrition (PN) is a branch of nutrition science which aims to provide precise, customized dietary guidance based on an individual's needs, physiology, and molecular characteristics. PN needs precise tools to measure both diet and dietary consequences. The central objective of this thesis was to determine whether integrating wearable devices, quantitative omics techniques, and mental/physical performance testing could provide sufficient information to identify specific foods, diets or habits that impact physiology and improve individual well-being. To address this objective, I investigated the feasibility of using comprehensive, weighed dietary records (for precise diet monitoring), wearable devices (for precise physiological monitoring), objective psychological testing (for precise psychological monitoring) and advanced, quantitative omics methods (for precise molecular monitoring) to precisely characterize the molecular, psychological, and physiological consequences of different diets. In addition to addressing this primary goal, there were five other secondary questions that I attempted to answer:

1) Is it possible to conduct N-of-1 self-monitoring and biosample self-collection for PN studies?
2) Can self-monitoring be carried out cost-effectively?
3) Can monitoring mental and physical performance offer valuable insights in an N-of-1 PN study?
4) Can quantitative omics data enhance the analysis and interpretation of N-of-1 nutrition studies?
5) Which data analysis techniques are most effective for interpreting dietary N-of-1 studies?

With regard to the primary objective of this thesis, I believe that I have demonstrated that by integrating wearable devices, quantitative omics techniques, and mental/physical performance testing, with precise dietary monitoring it is possible to identify optimal diets, foods, and lifestyle activities at an individual level. The data is quite clear for me that the MD was the most optimal diet for my body and my overall well-being. This diet was associated with improved cardiovascular

health, and improved the "quality" of gut microbiome, cognition, mood, and mental state. This study also proved that the FFD, even over a period of time as short as two weeks drove my body towards a chronic inflammatory state that was characterized by poor sleep, poor mental performance, and poor overall health. A similar conclusion would be obtained for the KD, which also led to a number of negative health, physiological and psychological impacts. My regular diet (RD), which was already rated quite highly in the healthy eating index seems to provide me with almost the same benefits. More detailed studies highlighted my low susceptibility to high glycemic index foods but also pointed out my heightened glycemic susceptibility to orange juice. The molecular studies also highlighted the significant benefits of banana consumption (with high levels of IPA) and chicken consumption and suggested that I should either supplement my RD or MD with more of these foods. Similarly, the consumption of green, leafy vegetables (mainly learned from the KD) seemed to provide some additional health benefits. Detailed analysis of the dietary components showed a general underconsumption of fibre and several other essential nutrients (Mg, Ca, K, and several vitamins) for all the diets I investigated. These findings indicate that both my RD and MD diets could be improved even further, perhaps with the inclusion of more supplements, more milk products, more olive oil and more nuts or grains in my diet. It is important to note that these findings are unique to me, my genetics, my historical diet, my physiology, my microbiome, and my lifestyle. Different individuals would likely find different results if they conducted the same study using the same diet and measurement tools.

One of the more surprising results from this study was the relatively short timeframe in which significant and measurable changes occurred. The dietary interventions were all two weeks long, but substantial changes in my metabolome, my proteome as well as my physiology and emotional state were often evident within 4-5 days. Changes in my microbiome seemed to require about two weeks to settle in, although the washout period of just one week seemed to be sufficient to allow my microbiome to be mostly restored. The plasticity of the microbiome was quite unexpected as were the range of physiological and metabolomic changes. The fact that I am a young, healthy female may have made my physiology and biology much more plastic, but also more resilient to adverse change than someone who is older or less healthy. Again, this emphasizes that different results will arise from different individuals and circumstances.

Overall, this work highlights the importance of frequent testing and frequent sample collection for analysis in a PN setting rather than six-month or yearly check-ups. However, the data collection burden in an N-of-1 setting can be time-consuming and tedious, leading to participant fatigue, decreased adherence, and potential dropout, impacting the study's validity.

With regard to the secondary objective, the results from this study showed that self-sample collection and longitudinal monitoring is feasible using a combination of wearable devices and lower-cost omics measurements, particularly metabolomics. Recent advances in at-home testing kits have enabled more frequent and longitudinal tracking without the need for in-person testing or specialized phlebotomists and clinical nurses in clinical or research labs.

N-of-1 studies often require extensive resources, including wearable devices, biosample collection kits, and laboratory analyses. The high costs can limit large-scale implementation. For this reason, I investigated the cost-effectiveness of this N-of-1 physiological response monitoring. Table 5.1 breaks down the total costs for omics testing and wearable devices used in this study over 56 days. Approximately 170 urine and plasma samples were collected for metabolomics analysis and about 50 plasma samples for proteomics and cytokine quantification. Genomics testing was conducted once, and gut microbiome testing was performed after each dietary intervention (for a total of four times). Blood glucose monitoring used one transmitter and six sensors over 56 days, with blood collection devices being used daily. The highest cost item for the entire study (see Table 5.1) was for the metabolomics testing, which was run on 168 samples at CAD 40 per sample. While the overall cost of these metabolomics tests may seem high, testing for 40-50 amino acids and fatty acids through a private clinical lab in Alberta, Canada, would have cost over CAD 1500 per sample. Overall, comprehensive blood panel testing in Canada ranges from CAD 150 for standard tests to as high as CAD 3,000 for extensive and specialized testing, which might measure up to 100 compounds. So, the cost-benefit of using a metabolomics assay that can measure >700 metabolites for $40 a sample is clear.

Table 5.1 – The cost breakdown of omics measurements and wearable devices across all dietary interventions for 56 days. All expenses are reported in Canadian Dollars (CAD)

	Test	Cost (CAD)	Samples/data points
Omics measurements	Proteomics	$2211	52 samples
	Gut microbiome	$608	4 tests
	Genomics	$124	1 test
	Metabolomics	$6720	168 samples, 29,893 data points for plasma and 20,506 data points for urine
Wearable devices	Blood glucose monitor	$689	15,552 data points
	Body temperature monitor	$372.45	12,782 data points
	Blood pressure monitor	$417	560 data points
	Blood collection device	$1400	56 collection days
	Smartwatch	$400	73,342 data points
	Total	$ 10,951.45	

Implementing continuous monitoring and data collection technologies poses technical and logistical challenges. Ensuring data accuracy, managing large datasets, and maintaining participant compliance require robust infrastructure and support. This highlights the need for identifying the most optimal methods for collecting meaningful and useful information while avoiding over-testing and wasting time and resources. Overall, the genomics data proved to be the least informative and I would not recommend it for pursuing PN studies. We also determined that microbiome data only needed to be collected at two-week intervals as it takes time for the microbiome to change due to the diet. Proteomic and metabolomic changes on the other hand appeared to be sensitive at the hour-to-day time scale. The cost per sample for proteomic and metabolomic analysis was approximately the same. However, the number of metabolites that were measured (500+) versus the number of proteins measured (10), made the metabolomic assay more cost-effective. Because our physiology, physiological responses, diet, and lifestyle choices are reflected in our metabolome, I believe metabolomics is currently the most cost-effective and optimal tool for longitudinal and frequent monitoring of physiological responses.

Additionally, I evaluated the effectiveness of mental and physical performance testing in providing meaningful information at an individual level. As discussed in previous chapters, several significant correlations were observed between mental and physical performance results, the data collected from wearable monitors, and the multi-omics measurements. Some of the variables measured for this study might not need to be measured for everyone in the same way or to the same extent. Certain variables might be more useful for specific individuals based on their health goals and objectives. For example, physical testing might be more beneficial for someone looking

to enhance their overall athletic performance, whereas metabolic health measurements might be more critical for someone focused on managing chronic conditions or optimizing their metabolic health. However, the results from this study show that by continuously tracking these parameters, we can gather valuable data on how an individual responds to a specific intervention over time. This approach not only provides insights into the physiological impacts of a treatment or intervention but also allows for real-time feedback. As a result, treatments can be fine-tuned and adjusted to better align with the individual's ultimate health goals. This iterative, dynamic process ensures that interventions remain personalized and effective, adapting them appropriately to the individual's unique physiological and psychological responses.

The quantity and diversity of data collected for this N-of-1 study required advanced statistical methods along with detailed personal knowledge which necessitates specialized skills and resources. I made use of several statistical and machine-learning tools that were available through MetaboAnalyst and other software in my supervisor’s laboratory. I also wrote several programs to facilitate other kinds of correlation analyses. Obviously, several other techniques could have been used. For instance, time series analysis could have been used for tracking changes in physiological and metabolic parameters over time, allowing a better approach to identify trends and patterns that correspond to dietary interventions. Mixed-effects models could have also been used as these can account for both fixed and random effects, providing a robust framework for analyzing individual variations while considering the impact of different factors. Machine learning algorithms can also be employed to uncover complex relationships between variables and predict outcomes based on individual data points. These methods help in understanding how specific dietary changes affect an individual over time, providing tailored insights for PN. Another approach that could have been employed was network analysis and pathway enrichment analysis. These techniques can help identify key molecular pathways and key molecular interactions affected by dietary changes, offering deeper insights into the mechanisms underlying individual responses. I believe that implementing these more advanced data analysis techniques would not only improve the accuracy and depth of my own N-of-1 dietary study but would also improve other N-of-1 studies so that dietary recommendations could become truly personalized and effective for meeting an individual’s specific health goals.

Challenges and Future Directions

There are several challenges associated with PN approaches. While N-of-1 trials offer significant potential in the context of precision health and nutrition they only serve a single individual. By aggregating results from multiple N-of-1 trials, researchers could gain valuable insights that would allow the identification of groups, individuals, or subpopulations that share common genetic and phenotypic factors and common responses to certain foods or diets. Expanding these kinds of trials to include more individuals would also contribute to implementing broader population health strategies that incorporate personalized responses to therapies. As discussed in earlier chapters, I believe the best go-forward strategy is to cluster individuals with similar traits and dietary responses into groups (metabotypes) and provide tailored modifications to their PN strategy based on modest distinctive characteristics. However, the identification of the optimal separating factors for defining metabotypes will require further study and evaluation.

Another challenge facing PN approaches involves translating and interpreting "big data" into personalized, clinically relevant solutions. The complexity of multi-dimensional omics data requires specialized computational tools for analysis, which are still evolving, and further research is needed to enhance their effectiveness. Translating omics-generated data into practical clinical applications faces validation and standardization problems. Given the sensitive nature of genetic and health-related data, ensuring data security and confidentiality is crucial. Establishing clear consent processes, robust data anonymization techniques, and strict data governance policies is essential to protect individuals' privacy while enabling valuable data use for research and clinical purposes. Nevertheless, with the decreasing costs of omics analysis, the availability of powerful computational tools for “big data” handling, the integration of data informatics into healthcare systems, and improved training for clinicians – I believe these changes will pave the way for the routine implementation of multi-omics precision health monitoring. We are clearly at the early stages of this process, but some of the tools are almost at hand and these integrated approaches will revolutionize clinical nutrition and clinical medicine.

The results from this study demonstrate that a uniform methodology is not necessary across all PN interventions, particularly when considering varied health objectives. This particular project showed that when comparing metabolomic data from the first and second weeks of each dietary intervention, there were no significant differences in most metabolite levels (except for betaine where its concentration was significantly higher during the second week of the MD diet compared to the first (p=9.2683E-5), suggesting its role as a biomarker of dietary intake of seafood, which was not consumed in the first week of the MD). Additionally, my analysis of just the first three days of each dietary intervention revealed that most of the significantly different metabolites could be detected, indicating that early-stage monitoring or even short-term monitoring (of just 3-4 days) can effectively capture metabolic changes. This finding would potentially reduce the required frequency and burden of data collection (Figure 5.1).

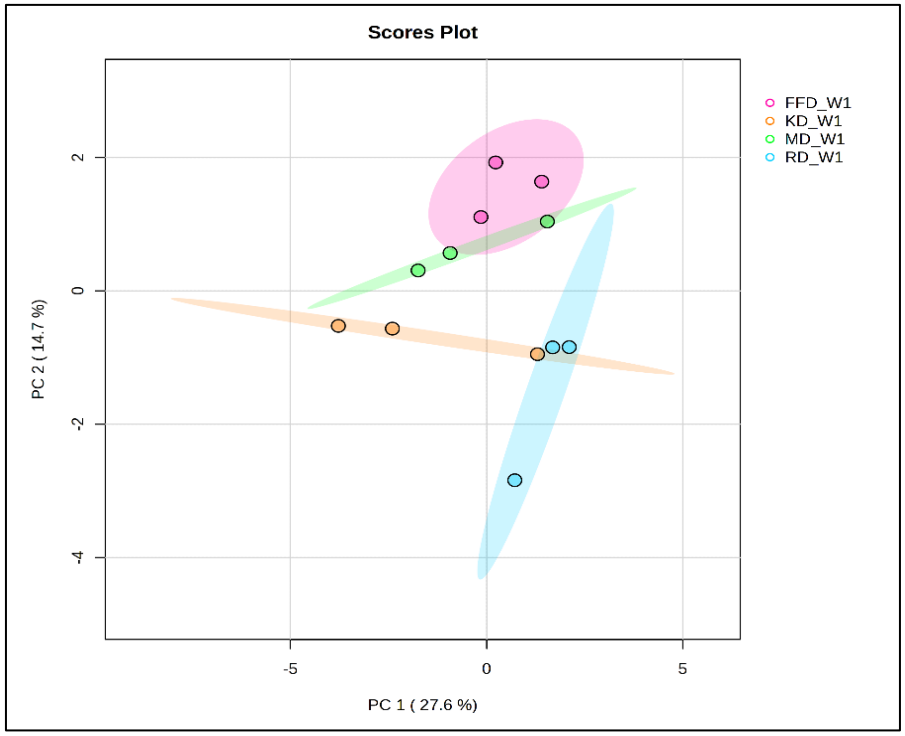

Figure 5.1 – PCA plot of the plasma samples of the initial 3 days of each dietary intervention.

Overall, these findings emphasize the need for PN projects to be tailored to the individual's needs, their health status, and their health objectives, which means there is no one-size-fits-all approach. The methodology I used was tailored to specific research goals and might not be directly applicable to others with different conditions or objectives. Each PN project should be designed to

consider not only the individual but also their unique metabolic responses, dietary preferences, and health goals. Furthermore, logistical considerations such as available technology, budget constraints, and participants' ability to adhere to the chosen monitoring processes can significantly influence how a PN project is structured. For instance, the intensity and frequency of data collection might need adjustment based on these factors. Therefore, while the principles of the integrated PN approach I have developed here are broadly applicable, the exact methods and tools used can vary greatly to best meet the diverse needs of different individuals. This flexibility ensures that future PN projects could be more practical and effective, maximizing benefits for individuals while considering their specific circumstances.

While my focus in this project was on the metabolomic characterization of biofluids, it would have certainly helped to apply the same TMIC MEGA assay to the characterization of the specific foods I consumed. Doing so would help ensure the validity of our findings by enabling more precise tracking of specific food compounds before and after food consumption. This information would facilitate a better understanding of the metabolic shifts associated with specific dietary intakes. This approach will also allow for the validation of the observed correlations and contribute to more accurate assessments in future PN studies. Further testing is essential to precisely and accurately “metabotype” individuals. This will require refining methods for detecting the key metabolic features that are most indicative of an individual's unique responses to dietary (and other lifestyle) interventions. Simultaneously, it is critical to identify and eliminate uninformative or irrelevant metabolic responses or metabolites (i.e., the “no-go” metabolites) that may confound accurate analysis and accurate metabotype classification.

To conclude, I have described, developed, and implemented a practical, low-cost, comprehensive, and quantitative approach to performing PN studies. This N-of-1 approach integrates the large amount of data that can be obtained from weighed food records with physiological data that can be easily generated by wearable monitoring devices with objective, low-cost psychological testing and molecular data measured via multi-omics technologies. I have demonstrated that the technologies I have chosen are compatible with low-cost, DIY, at-home collection, and preparation. Through this work, I have developed a number of useful analytical procedures and through these analyses found that there was a specific diet and specific foods that provided substantial physiological, molecular, and mental health benefits to me. I also identified

other foods and diets that had highly negative consequences. In other words, my findings were sufficiently robust and sufficiently well rationalized that I could develop my own PN plan. While not quite practical for widespread implementation, I believe this approach could be easily refined, shortened, and made far more cost-effective with only a few modest adjustments (some of which were already mentioned). Furthermore, the unique and innovative nature of this concept has led to the submission of a US patent application, and through this patent, I hope to establish a commercial entity that makes use of these (and other emerging) concepts to bring the benefits of PN to everyone.

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Appendices

Chapter 1 Appendices

Appendix 1

Appendix 1 Table 1 - An example of a typical day during the intervention *

Activity	Time of day	Duration
Wake up	8:00 AM	
Morning blood pressure	8:05 AM	5 minutes
Morning sample collection	8:15 AM	10 minutes for collection and 45 minutes for sample preparation
Body measurements	8:30	15 minutes
Blood samples centrifuge and sample aliquoting	8:45	
Breakfast	9:00 AM	
After breakfast blood pressure	9:35 AM	5 minutes
Breakfast log in food diaries	9:45 AM	10 minutes
Mental cognitive assessment tests (30 minutes)	10:15 AM	30 minutes
Lunch	12:30 PM	
After lunch blood pressure	13:05	5 minutes
Lunch log in food diaries	13:15	10 minutes
Physical activity	15:00	45 minutes
Cooking, measuring and preparing foods for the following day	18:00	1 hour
Dinner	19:00	
After dinner blood pressure	19:35	5 minutes
Dinner log in food diaries	19:45	10 minutes
Nighttime urine sample collection	22:00	5 minutes
Nighttime blood pressure measurement	22:05	5 minutes
Sleep	23:00	

* The times are approximate and may not reflect exact schedules. This table serves to illustrate a daily routine while self-monitoring.

Appendix 1 Table 2 - Daily calorie intakes (kcal) on each day, across all interventions

Day	FFD (kcal)	MD (kcal)	KD (kcal)	RD (kcal)
1	1929.8	2182.8	2186.5	1867.1
2	1822.6	1809.1	1934.4	1900.3
3	1865.2	1855.9	1979.7	1915.4
4	1898.0	1920.8	1888.4	1938.4
5	1987.3	1933.1	2154.6	1916.1
6	2163.0	1806.4	1970.0	1957.6
7	1920.1	2064.7	2043.8	1847.3
8	1895.5	1838.9	2155.3	1880.8
9	1982.3	2044.3	1915.9	1926.9
10	1978.4	1931.6	1913.4	1947.3
11	2160.4	1953.5	1665.0	2047.3
12	2037.9	1896.8	1900.6	2002.3
13	2059.6	1957.5	1873.0	1954.4
14	2096.9	1969.6	1970.3	2086.3

Appendix 1 Table 3 – Menu items of a day on each intervention

	Meal	Food	Amount
FFD	Breakfast	Sausage McMuffin	1 sandwich
	Lunch	Big Mac	1 sandwich
	Dinner	Cheeseburger	1 sandwich
		Fries	100g
		Coke	450 g
	Snack	Hamburger	1 sandwich
		Coffee	440 g
		Coffee creamer	2 tbsp
MD	Breakfast	Bread	2 slices
		Cheese	60 g
		Avocado	80 g
		Tomatoes	110 g
	Lunch	Olive oil	5 tbsp
		Orzo	85 g
		Feta cheese	26 g
		Chicken breast	150g
	Dinner	Olive oil	5 tbsp
		Basa fillet	150 g
		Yello potato	70 g
		Red onion	50 g
		Red bell pepper	90 g
	Snack	Apple	150 g
		Banana	85 g
		Coffee	500 g
		Milk	100 g
KD	Breakfast	Eggs	2 eggs
		Bacon	4 slices
	Luncg	Zucchini	120 g
		Chicken thighs	180 g
		Spinach	60 g
		Mayonnaise	30 g
	Dinner	Pork chop	190 g
		Shallots	22 g
		Kale	83 g
	Snack	Coffee cream (half and half)	1tbsp
		Coffee	360 g
		Coconut oil	1 tbsp

Appendix 1_A

Serial subtraction test

Serial Subtraction Test
#Dorsa Yahya Rayat
#2022

Introduction
This Python script implements a Serial Subtraction Test where the user is prompted to subtract a random number from a starting point within a specified duration. The script calculates the number of correct and incorrect answers given by the user during the test.

Prerequisites
- Python 3.x

Installation
Ensure you have Python 3.x installed on your machine. You can download it from [Python's official website](https://www.python.org/downloads/).

Usage Instructions
1. **Download or Clone the Repository**:
  ```sh
  git clone <repository_url>
  cd <repository_directory>
  ```

2. **Run the Script**:
  ```sh
  python serial_subtraction_test.py
  ```

Code Explanation
The script includes functions to:
- Generate a random starting point and a random number to subtract.
- Collect user inputs within a specified duration.
- Calculate and display the number of correct and incorrect answers.

```python
import random
import time

def generate_starting_point():
  return random.randint(100, 999)

def choose_subtraction_value():
```

```
  num_list = [3, 4, 6, 7, 8]
  return random.choice(num_list)

def get_user_inputs(duration):
  start = time.time()
  inputs = []
  while time.time() - start < duration:
    try:
      user_input = int(input("Enter the result of subtraction: "))
      inputs.append(user_input)
    except ValueError:
      print("Please enter a valid integer.")
  return inputs

def check_answers(starting_point, subtraction_value, user_inputs):
  correct_count = 0
  incorrect_count = 0
  current_value = starting_point

  for user_input in user_inputs:
    expected_value = current_value - subtraction_value
    if user_input == expected_value:
      correct_count += 1
      print(f"Correct: {user_input}")
    else:
      incorrect_count += 1
      print (f"Not correct: {user_input}")
    current_value -= subtraction_value

  return correct_count, incorrect_count

def main():
  duration = 60 # Time in seconds for each test
  num_tests = 5 # Number of tests to perform
  total_correct = 0
  total_questions = 0

  for i in range(num_tests):
    print(f"Test {i + 1}/{num_tests}")
    starting_point = generate_starting_point()
    print(f"Your starting point is {starting_point}")

    subtraction_value = choose_subtraction_value()
    print(f"Your subtraction value is: {subtraction_value}")

    print(f"You have {duration} seconds. Start subtracting!")
```

```
    user_inputs = get_user_inputs(duration)

    correct_count, incorrect_count = check_answers(starting_point, subtraction_value, user_inputs)
    total_correct += correct_count
    total_questions += (correct_count + incorrect_count)
    print(f"Test {i + 1} - Correct: {correct_count}, Incorrect: {incorrect_count}\n")

  average_percentage = (total_correct / total_questions) * 100 if total_questions > 0 else 0
  print(f"Average percentage of correct answers: {average_percentage:.2f}%")

if __name__ == "__main__":
  main()
```

Appendix 1_B

Reaction time test

```
# Reaction Time Test
#Dorsa Yahya Rayat
#2022

## Introduction
This Python script implements a Reaction Time Test where the user is prompted to press Enter as soon as they see a message. The script measures and displays the user's reaction time for each test and calculates the average reaction time after multiple tests.

## Prerequisites
- Python 3.x

### Installation
Ensure you have Python 3.x installed on your machine. You can download it from [Python's official website](https://www.python.org/downloads/).

## Usage Instructions
1. **Download or Clone the Repository**:
  ```sh
  git clone <repository_url>
  cd <repository_directory>
  ```

2. **Run the Script**:
  ```sh
  python reaction_time_test.py
  ```

Code Explanation
The script includes functions to:
- Prompt the user to get ready and wait for a random duration.
- Measure the user's reaction time when they press Enter.
- Calculate and display the average reaction time over multiple tests.

```python
import time
import random

def reaction_time_test():
  print("Get ready to test your reaction time...")
  time.sleep(2) # Give the user some time to get ready
```
```

```
  # Wait for a random time between 2 to 5 seconds before prompting the user
  wait_time = random.uniform(2, 5)
  time.sleep(wait_time)

  print("Press Enter as soon as you see this message!")
  start_time = time.time()
  input() # Wait for the user to press Enter
  end_time = time.time()

  reaction_time = end_time - start_time
  print(f"Your reaction time is {reaction_time:.3f} seconds")

  return reaction_time

def main():
  num_tests = 5 # Number of tests to perform
  total_reaction_time = 0

  for i in range(num_tests):
    print(f"Test {i + 1}/{num_tests}")
    reaction_time = reaction_time_test()
    total_reaction_time += reaction_time
    print()

  average_reaction_time = total_reaction_time / num_tests
  print(f"Average reaction time: {average_reaction_time:.3f} seconds")

if __name__ == "__main__":
  main()
```

Appendix 1_C

Trail-making test

Trail Making Test
#Dorsa Yahya Rayat
#2022

Introduction
This Python script generates an image for the Trail Making Test (TMT), which includes alternating numbers and letters arranged in a randomized pattern.

Prerequisites
- Python 3.x
- numpy
- PIL (Pillow)

Installation
You can install the required libraries using pip:
```sh
pip install numpy pillow
```

Usage Instructions
1. **Download or Clone the Repository**:
  ```sh
  git clone <repository_url>
  cd <repository_directory>
  ```

2. **Run the Script**:
  ```sh
  python trail_making_test.py
  ```

Code Explanation
The script includes functions to:
- Generate random positions for nodes.
- Ensure no circles (nodes) overlap.
- Draw the nodes and corresponding text on an image.

```python
import numpy as np
from PIL import Image, ImageDraw, ImageFont

def length_AB(A, B):
```

```
  return np.sqrt((A[0] - B[0])**2 + (A[1] - B[1])**2)

def check_circles_cross(A, ra, B, rb):
  return length_AB(A, B) <= ra + rb

def gradient_2_dots(A, B):
  return np.inf if A[0] == B[0] else (B[1] - A[1]) / (B[0] - A[0])

def calc_pq(A, B, C, D):
  q = ((D[1] - B[1]) * (A[0] - B[0]) - (D[0] - B[0]) * (A[1] - B[1])) / ((C[0] - D[0]) * (A[1] - B[1])
- (C[1] - D[1]) * (A[0] - B[0]))
  p = (q * (C[0] - D[0]) + D[0] - B[0]) / (A[0] - B[0])
  return p, q

def check_AB_CD_cross(A, B, C, D):
  k1 = gradient_2_dots(A, B)
  k2 = gradient_2_dots(C, D)

  if k1 == k2:
    return False

  p, q = calc_pq(A, B, C, D) if k1 != np.inf else calc_pq(C, D, A, B)
  return p * (1 - p) >= 0 and q * (1 - q) >= 0

def get_nodes(n_nodes, space, white, diameter):
  cross = True
  while cross:
    nodes = np.c_[np.random.rand(n_nodes) * (space[0] - 2 * white[0]) + white[0],
          np.random.rand(n_nodes) * (space[1] - 2 * white[1]) + white[1]]
    cross = check_all_circles_cross(nodes, diameter / 2)
  return nodes

def check_all_circles_cross(nodes, r):
  return any(check_circles_cross(nodes[x], r, nodes[y], r)
        for x in range(nodes.shape[0] - 1)
        for y in range(x + 1, nodes.shape[0]))

def uncrosser(nodes, space, white, diameter):
  cross = True
  while cross:
    nodes, cross = line_uncrosser(nodes)
  return nodes

def line_uncrosser(nodes):
  for x in range(nodes.shape[0] - 3):
    for y in range(x + 2, nodes.shape[0] - 1):
```

```
      if check_AB_CD_cross(nodes[x], nodes[x + 1], nodes[y], nodes[y + 1]):
        nodes = np.r_[nodes[:x + 1], nodes[y:x:-1], nodes[(y + 1):]]
        return nodes, True
  return nodes, False

# Parameters
n_nodes = 52 # Number of numbers and letters (26 numbers + 26 letters)
space_w, space_h = 1050, 1485
white_w, white_h = 50, 50
diameter = 50

space = np.array([space_w, space_h])
white = np.array([white_w, white_h])

nodes = get_nodes(n_nodes, space, white, diameter)
nodes = uncrosser(nodes, space, white, diameter)

# Create Image
im = Image.new('RGB', (space[0], space[1]), (240, 240, 240))
draw = ImageDraw.Draw(im)

# Use a larger font size
try:
  font = ImageFont.truetype("DejaVuSans-Bold.ttf", 24)
except IOError:
  font = ImageFont.load_default()

characters_num = [str(i) for i in range(1, 27)] # Numbers 1-26
characters_jpn = [chr(i) for i in range(65, 91)] # Letters A-Z
characters = [None] * n_nodes
characters[::2] = characters_num
characters[1::2] = characters_jpn

for i in range(nodes.shape[0]):
  temp = nodes[i]
  draw.ellipse((temp[0] - diameter / 2, temp[1] - diameter / 2, temp[0] + diameter / 2, temp[1] +
diameter / 2),
        fill=(255, 255, 255), outline=(0, 0, 0))
  text = characters[i]
  text_size = draw.textsize(text, font=font)
  text_x = temp[0] - text_size[0] / 2
  text_y = temp[1] - text_size[1] / 2
  draw.text((text_x, text_y), text, (0, 0, 0), font)

im.save('Trail_Making_Test.png')
```

Appendix 1_D

Stroop effect test

Stroop Effect Test
#Dorsa Yahya Rayat
#2022

Introduction
This Python script generates a list of 100 color names, with the words written in various colors. The participant's task is to say aloud the color in which each word is written, not the actual color names. The test records the time taken to complete the task (in seconds) and the list of correct answers as the final result.

Prerequisites
- Python 3.x
- IPython

Installation
Ensure you have Python 3.x and IPython installed on your machine. You can install the required packages using pip:
```sh
pip install ipython
```

Usage Instructions
1. **Download or Clone the Repository**:
  ```sh
  git clone <repository_url>
  cd <repository_directory>
  ```

2. **Run the Script**:
  ```sh
  python stroop_effect_test.py
  ```

Code Explanation
The script includes functions to:
- Generate a list of 100 color names with random colors.
- Display the words in various colors every 2 seconds.
- Collect and display the list of correct answers at the end.

```python
import random
import time
```

```
from IPython.display import clear_output, display, HTML

# Define colors and their corresponding HTML styles
COLORS = ["Red", "Blue", "Green", "Yellow", "Purple", "Orange", "Pink", "Black", "Brown",
"Gray"]
COLOR_CODES = {
  "Red": "red",
  "Blue": "blue",
  "Green": "green",
  "Yellow": "yellow",
  "Purple": "purple",
  "Orange": "orange",
  "Pink": "pink",
  "Black": "black",
  "Brown": "brown",
  "Gray": "gray"
}

# Function to generate the Stroop effect test
def generate_stroop_test():
  test_items = []
  for _ in range(100):
    word = random.choice(COLORS)
    color = random.choice(COLORS)
    test_items.append((word, color))
  return test_items

# Function to display colored text and generate correct answers
def stroop_test():
  test_items = generate_stroop_test()
  correct_answers = []

  start_time = time.time()

  for index, (word, color) in enumerate(test_items):
    clear_output(wait=True)
    display(HTML(f"<h2                style='color:{COLOR_CODES[color]};                font-size:
48px;'>{word}</h2>"))
    correct_answers.append(color)
    time.sleep(2) # Display each word for 2 seconds

  end_time = time.time()
  total_time = end_time - start_time

  clear_output(wait=True)
  print(f"\nTime taken: {total_time:.2f} seconds")
```

```
  print("List of correct answers:")
  print(correct_answers)

# Run the Stroop test
print("Stroop Effect Test")
print("You will see color names displayed in various colors.")
print("Your task is to say the color in which the word is printed, not the word itself.")
input("Press Enter to start the test...")

stroop_test()
```

Appendix 1_E

Digit span test

Digit Span Test
#Dorsa Yahya Rayat
#2022

Introduction
This Python script implements a Digit Span Test where the user is prompted to remember and enter a sequence of digits. The script measures and displays the number of correct digits recalled by the user for each test and calculates the average accuracy after multiple tests.

Prerequisites
- Python 3.x

Installation
Ensure you have Python 3.x installed on your machine. You can download it from [Python's official website](https://www.python.org/downloads/).

Usage Instructions
1. **Download or Clone the Repository**:
  ```sh
  git clone <repository_url>
  cd <repository_directory>
  ```

2. **Run the Script**:
  ```sh
  python digit_span_test.py
  ```

Code Explanation
The script includes functions to:
- Generate a random sequence of digits and display them one by one.
- Collect the user's input of the digit sequence.
- Check the user's input against the generated sequence and count the number of correct digits.
- Calculate and display the average accuracy over multiple tests.

```python
import secrets
import time
from google.colab import output

# Generate a random sequence of digits
```

```
def generate_digit_sequence(length):
  sequence = []
  for _ in range(length):
    num = secrets.choice(range(10))
    sequence.append(num)
    print(num)
    time.sleep(2) # Display each number for 2 seconds
    output.clear() # Clear the output
    time.sleep(0.5) # Brief pause before showing the next number
  return sequence

# Collect user answers
def collect_user_answers(length):
  user_input = input(f"Enter the {length} numbers you saw, separated by spaces: ")
  user_sequence = [int(num) for num in user_input.split()]
  return user_sequence

# Check the user answers
def check_answers(sequence, user_sequence):
  correct_count = sum(1 for s, u in zip(sequence, user_sequence) if s == u)
  return correct_count

def main():
  num_tests = 5 # Number of tests to perform
  sequence_length = 10 # Length of the digit sequence
  total_correct = 0
  total_digits = num_tests * sequence_length

  for i in range(num_tests):
    print(f"Test {i + 1}/{num_tests}")
    sequence = generate_digit_sequence(sequence_length)

    print("Now, try to recall the numbers.")
    user_sequence = collect_user_answers(sequence_length)

    correct_count = check_answers(sequence, user_sequence)
    total_correct += correct_count
    print(f"You got {correct_count}/{sequence_length} correct in this test.")
    print(f"The correct sequence was: {sequence}\n")

  average_accuracy = (total_correct / total_digits) * 100
  print(f"Average accuracy over {num_tests} tests: {average_accuracy:.2f}%")

if __name__ == "__main__":
  main()
```

Chapter 3 Appendices

Appendix 3_A

Appendix Table 3-A - The mean 14-d intake of consumed foods, beverages, and dietary supplements with breakfasts across all dietary interventions

Food Group	FFD	MD	KD	RD
Energy (kcal/day)	431.6 ± 140.7	439.2 ± 241.0	304.4 ± 40.6	349.2 ± 129.9
Protein (g/day)	19.1 ± 9.0	18.7 ± 8.4	20.4 ± 2.6	12.1 ± 3.6
Total Fat (g/day)	25.2 ± 8.9	13.4 ± 10.9	23.5 ± 3.2	17.2 ± 9.4
Carbohydrate (g/day)	32.4 ± 8.2	61.8 ± 32.7	1.4 ± 0.2	40.1 ± 16.7
Alcohol (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Caffeine (mg/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Theobromine (mg)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sugars, total (g/day)	3.5 ± 2.2	15.8 ± 11.6	0.8 ± 0.2	12.5 ± 12.1
Fiber, total dietary (g/day)	0.9 ± 0.4	7.0 ± 6.2	0.0 ± 0.0	6.3 ± 4.5
Calcium (mg/day)	262.7 ± 86.5	322.4 ± 140.7	49.2 ± 9.5	112.5 ± 91.7
Iron (mg/day)	3.9 ± 1.2	2.8 ± 1.5	1.7 ± 0.3	2.1 ± 0.8
Magnesium (mg/day)	29.0 ± 10.9	93.1 ± 57.2	16.6 ± 2.0	72.5 ± 22.3
Phosphorus (mg/day)	240.9 ± 111.9	419.0 ± 212.8	224.5 ± 28.6	214.5 ± 90.9
Potassium (mg/day)	265.3 ± 140.9	701.9 ± 454.5	241.5 ± 28.9	435.6 ± 340.7
Sodium (mg/day)	906.0 ± 304.7	1245.0 ± 579.9	732.3 ± 85.4	537.8 ± 270.1
Zinc (mg/day)	2.2 ± 1.9	2.6 ± 1.4	2.0 ± 0.3	1.6 ± 0.6
Copper (mg/day)	0.3 ± 0.1	0.3 ± 0.2	0.1 ± 0.0	0.3 ± 0.1
Selenium (mcg/day)	33.3 ± 10.2	34.4 ± 17.7	47.7 ± 7.2	18.6 ± 6.5
Vitamin C (mg/day)	0.4 ± 0.7	48.1 ± 45.9	0.0 ± 0.0	4.5 ± 7.3
Thiamin (mg/day)	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.1
Riboflavin (mg/day)	0.3 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.3 ± 0.2
Niacin (mg/day)	5.4 ± 2.0	4.6 ± 2.4	2.7 ± 0.4	5.2 ± 1.6
Vitamin B-6 (mg/day)	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.0	0.2 ± 0.2
Folate, total (mcg/day)	69.6 ± 20.2	103.9 ± 56.5	55.4 ± 11.3	91.9 ± 65.5
Folic acid (mcg/day)	43.3 ± 10.6	35.4 ± 26.4	0.0 ± 0.0	31.2 ± 17.5

Folate, food (mcg/day)	26.3 ± 12.1	68.6 ± 55.6	55.4 ± 11.3	60.9 ± 51.9
Folate, DFE (mcg_DFE/day)	99.9 ± 26.9	128.7 ± 64.0	55.4 ± 11.3	113.9 ± 76.3
Vitamin B-12 (mcg/day)	1.3 ± 0.9	0.6 ± 0.3	1.9 ± 0.3	0.1 ± 0.2
Vitamin A, RAE (mcg_RAE/day)	74.2 ± 31.7	112.6 ± 50.3	221.5 ± 44.8	26.5 ± 41.9
Retinol (mcg/day)	71.5 ± 30.3	96.6 ± 48.6	217.7 ± 44.0	22.1 ± 35.1
Carotene, beta (mcg/day)	43.0 ± 50.1	156.0 ± 157.1	43.8 ± 9.0	42.5 ± 67.6
Carotene, alpha (mcg/day)	0.2 ± 0.7	31.3 ± 36.4	0.0 ± 0.0	10.7 ± 17.5
Cryptoxanthin, beta (mcg/day)	2.4 ± 4.7	42.8 ± 41.6	8.6 ± 1.8	12.5 ± 20.4
Lycopene (mcg/day)	523.5 ± 1689.1	360.0 ± 918.6	0.0 ± 0.0	0.0 ± 0.0
Lutein + zeaxanthin (mcg/day)	90.2 ± 150.9	212.6 ± 192.1	504.3 ± 103.3	151.9 ± 213.8
Vitamin E, alpha-tocopherol (mg/day)	1.0 ± 0.8	2.2 ± 2.7	3.5 ± 0.7	2.6 ± 1.2
Vitamin K, phylloquinone (mcg/day)	3.8 ± 2.2	13.3 ± 14.7	5.9 ± 1.2	11.6 ± 15.2
Cholesterol (mg/day)	81.3 ± 62.9	24.8 ± 12.5	398.0 ± 76.1	5.7 ± 9.0
Fatty acids, total saturated (g/day)	9.9 ± 3.9	5.4 ± 3.1	6.9 ± 0.9	3.8 ± 2.2
4:0, Butanoic acid (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
6:0, Hexanoic acid (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
8:0, Octanoic acid (g/day)	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
10:0, Decanoic acid (g/day)	0.1 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.1
12:0, Dodecanoic acid (g/day)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
14:0, Tetradecanoic acid (g/day)	0.8 ± 0.4	0.7 ± 0.4	0.2 ± 0.0	0.2 ± 0.3
16:0, Hexadecanoic acid (g/day)	5.7 ± 2.1	3.1 ± 2.0	4.7 ± 0.6	2.3 ± 1.8
18:0, Octadecanoic acid (g/day)	2.8 ± 1.1	0.9 ± 0.5	1.8 ± 0.2	0.6 ± 0.3
Fatty acids, total monounsaturated (g/day)	9.6 ± 3.4	5.5 ± 5.9	9.9 ± 1.3	9.0 ± 5.7

16:1, Hexadecenoic acid, undifferentiated(g/day)	0.6 ± 0.2	0.4 ± 0.4	0.5 ± 0.1	0.4 ± 0.5
18:1, Octadecenoic acid, undifferentiated (g/day)	8.7 ± 3.4	5.1 ± 5.5	9.2 ± 1.2	8.5 ± 5.3
20:1, Eicosenoic acid, undifferentiated (g/day)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
22:1, Docosenoic acid, undifferentiated (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fatty acids, total polyunsaturated (g/day)	3.6 ± 1.3	1.8 ± 1.7	4.5 ± 0.6	3.3 ± 1.5
18:2, Octadecadienoic acid (g/day)	2.9 ± 1.0	1.6 ± 1.5	3.6 ± 0.5	3.2 ± 1.4
18:3, Octadecatrienoic acid (g/day)	0.2 ± 0.1	0.2 ± 0.2	0.4 ± 0.1	0.1 ± 0.1
18:4, Octadecatetraenoic acid (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:4, Eicosatetraenoic acid (g/day)	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.0 ± 0.0
20:5 n-3, Eicosapentaenoic acid [EPA] (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:5 n-3, Docosapentaenoic acid [DPA] (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:6 n-3, Docosahexaenoic acid [DHA] (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Vitamin D (D2 + D3) (mcg/day)	0.7 ± 0.6	0.3 ± 0.2	2.6 ± 0.5	0.2 ± 0.1
Choline, total (mg/day)	60.0 ± 48.9	45.6 ± 26.9	273.3 ± 51.1	29.1 ± 11.7
Added Vitamin E (mg/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Added Vitamin B-12 (mcg/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Intact fruits of citrus, melons, and berries (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Intact fruits; excluding citrus, melons, and berries (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fruit juices (g/day)	0.0 ± 0.0	123.7 ± 133.5	0.0 ± 0.0	0.0 ± 0.0

Dark green vegetables (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Tomatoes and tomato products (g/day)	4.2 ± 14.3	19.8 ± 50.6	0.0 ± 0.0	0.0 ± 0.0
Other red and orange vegetables, excluding tomatoes and tomato products (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
White potatoes (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Other starchy vegetables, excluding white potatoes (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Other vegetables not in the vegetable components listed above (g/day)	4.2 ± 12.7	47.5 ± 85.2	0.0 ± 0.0	74.0 ± 120.9
Beans and peas (legumes) computed as vegetables (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Whole grains (g/day)	0.0 ± 0.0	43.2 ± 59.2	0.0 ± 0.0	11.0 ± 7.3
Refined grains (g/day)	62.4 ± 16.0	44.3 ± 33.2	0.0 ± 0.0	40.3 ± 15.6
Beef, veal, pork, lamb; excludes organ meat and cured meat (g/day)	8.7 ± 24.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cured meat (g/day)	26.6 ± 15.4	0.0 ± 0.0	17.6 ± 2.7	0.0 ± 0.0
Poultry (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Seafood (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eggs (g/day)	4.4 ± 11.3	0.0 ± 0.0	50.7 ± 10.4	0.0 ± 0.0
Soy products, excluding calcium fortified soy milk and immature soybeans (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Peanuts, tree nuts, and seeds; excludes coconut (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	28.4 ± 28.4
Beans and Peas (legumes) computed as protein foods (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Milk (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Yogurt (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Cheese (g/day)	109.9 ± 67.4	339.5 ± 170.9	0.0 ± 0.0	77.7 ± 123.3
Added sugars(g/day)	3.0 ± 2.4	4.0 ± 1.9	0.0 ± 0.0	11.5 ± 12.2

Appendix 3_B

Appendix Table 3-B - The mean 14-d intake of consumed foods, beverages, and dietary supplements with lunches across all dietary interventions

Food Group	FFD	MD	KD	RD
Energy (kcal/day)	688.4 ± 194.5	575.3 ± 159.2	747.4 ± 296.6	531.5 ± 176.7
Protein (g/day)	35.0 ± 10.3	39.4 ± 8.3	55.9 ± 25.3	33.2 ± 10.0
Total Fat (g/day)	37.5 ± 10.0	32.1 ± 14.6	53.3 ± 21.8	23.6 ± 14.5
Carbohydrate (g/day)	51.4 ± 21.6	34.2 ± 13.0	10.9 ± 5.4	49.2 ± 19.6
Alcohol (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Caffeine (mg/day)	7.4 ± 11.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Theobromine (mg)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sugars, total (g/day)	15.7 ± 13.3	7.2 ± 3.6	5.3 ± 3.7	5.0 ± 3.0
Fiber, total dietary (g/day)	2.3 ± 1.0	3.9 ± 1.8	2.3 ± 1.4	5.9 ± 4.2
Calcium (mg/day)	303.5 ± 91.2	159.1 ± 68.0	208.9 ± 116.5	126.5 ± 121.2
Iron (mg/day)	4.8 ± 1.2	2.7 ± 0.8	4.2 ± 3.1	2.8 ± 0.7
Magnesium (mg/day)	51.1 ± 18.9	89.3 ± 20.9	89.5 ± 25.7	87.3 ± 26.8
Phosphorus (mg/day)	436.2 ± 150.2	691.9 ± 302.9	694.6 ± 331.4	680.2 ± 420.4
Potassium (mg/day)	634.5 ± 313.2	958.1 ± 320.4	1003.1 ± 275.0	763.1 ± 326.3
Sodium (mg/day)	1350.7 ± 336.2	1034.1 ± 247.7	1618.9 ± 789.5	1076.0 ± 233.9
Zinc (mg/day)	6.7 ± 1.6	2.0 ± 0.6	8.3 ± 7.9	2.5 ± 0.7
Copper (mg/day)	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Selenium (mcg/day)	38.4 ± 10.5	65.3 ± 11.3	67.7 ± 23.3	54.9 ± 18.9
Vitamin C (mg/day)	6.1 ± 7.6	32.3 ± 28.6	19.9 ± 21.9	28.9 ± 36.7
Thiamin (mg/day)	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.5	0.4 ± 0.2
Riboflavin (mg/day)	0.4 ± 0.1	0.4 ± 0.2	0.6 ± 0.2	0.4 ± 0.2
Niacin (mg/day)	8.3 ± 2.8	14.2 ± 6.7	14.9 ± 6.6	15.4 ± 7.4
Vitamin B-6 (mg/day)	0.5 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	0.8 ± 0.4

Folate, total (mcg/day)	96.0 ± 25.3	84.6 ± 34.5	62.0 ± 35.8	113.4 ± 114.6
Folic acid (mcg/day)	49.2 ± 5.8	34.3 ± 31.1	0.0 ± 0.0	34.0 ± 42.0
Folate, food (mcg/day)	46.8 ± 21.4	50.4 ± 19.5	62.0 ± 35.8	79.4 ± 78.4
Folate, DFE (mcg_DFE/day)	130.5 ± 28.4	108.6 ± 53.9	62.0 ± 35.8	137.2 ± 141.9
Vitamin B-12 (mcg/day)	3.1 ± 1.1	3.1 ± 1.4	3.0 ± 2.6	1.9 ± 1.3
Vitamin A, RAE (mcg_RAE/day)	71.2 ± 33.9	172.5 ± 245.2	186.6 ± 128.5	208.2 ± 86.1
Retinol (mcg/day)	59.0 ± 29.8	55.1 ± 14.9	77.5 ± 35.7	62.9 ± 41.6
Carotene, beta (mcg/day)	149.5 ± 55.6	1410.0 ± 2943.2	1262.9 ± 1554.1	1485.0 ± 581.7
Carotene, alpha (mcg/day)	2.3 ± 0.4	9.3 ± 25.5	81.1 ± 163.7	502.9 ± 444.6
Cryptoxanthin, beta (mcg/day)	6.4 ± 3.6	3.0 ± 7.0	5.7 ± 9.4	13.9 ± 38.8
Lycopene (mcg/day)	1976.6 ± 3085.5	2858.9 ± 1724.2	427.3 ± 1070.9	3339.9 ± 9248.4
Lutein + zeaxanthin (mcg/day)	97.8 ± 23.0	877.7 ± 1390.2	2798.5 ± 3910.6	783.3 ± 472.0
Vitamin E, alpha-tocopherol (mg/day)	0.9 ± 0.6	4.8 ± 2.0	2.9 ± 1.9	3.7 ± 1.6
Vitamin K, phylloquinone (mcg/day)	31.7 ± 18.2	44.9 ± 50.6	132.2 ± 139.9	85.1 ± 73.6
Cholesterol (mg/day)	104.5 ± 32.4	104.6 ± 40.0	194.5 ± 89.8	70.6 ± 45.7
Fatty acids, total saturated (g/day)	12.7 ± 4.7	6.8 ± 3.4	16.1 ± 11.5	5.2 ± 2.9
4:0, Butanoic acid (g/day)	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.2
6:0, Hexanoic acid (g/day)	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1
8:0, Octanoic acid (g/day)	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.0
10:0, Decanoic acid (g/day)	0.1 ± 0.1	0.3 ± 0.4	0.1 ± 0.2	0.1 ± 0.1
12:0, Dodecanoic acid (g/day)	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.1 ± 0.1

14:0, Tetradecanoic acid (g/day)	1.1 ± 0.5	0.6 ± 0.4	1.1 ± 1.1	0.4 ± 0.4
16:0, Hexadecanoic acid (g/day)	7.1 ± 2.5	4.2 ± 1.9	9.8 ± 5.9	3.4 ± 1.9
18:0, Octadecanoic acid (g/day)	3.5 ± 1.3	1.0 ± 0.5	4.6 ± 3.6	0.8 ± 0.5
Fatty acids, total monounsaturated (g/day)	13.7 ± 5.6	17.6 ± 9.6	21.4 ± 9.7	10.3 ± 8.0
16:1, Hexadecenoic acid, undifferentiated(g/day)	0.7 ± 0.3	0.5 ± 0.2	1.5 ± 1.5	0.5 ± 0.4
18:1, Octadecenoic acid, undifferentiated (g/day)	9.4 ± 7.1	16.4 ± 9.3	19.7 ± 7.7	9.2 ± 8.0
20:1, Eicosenoic acid, undifferentiated (g/day)	0.1 ± 0.0	0.4 ± 0.2	0.3 ± 0.3	0.3 ± 0.3
22:1, Docosenoic acid, undifferentiated (g/day)	0.0 ± 0.0	0.2 ± 0.3	0.1 ± 0.3	0.3 ± 0.3
Fatty acids, total polyunsaturated (g/day)	7.8 ± 3.8	5.6 ± 2.4	10.4 ± 9.2	6.2 ± 4.4
18:2, Octadecadienoic acid (g/day)	6.8 ± 3.3	3.8 ± 1.7	8.7 ± 7.7	4.4 ± 4.2
18:3, Octadecatrienoic acid (g/day)	1.0 ± 0.5	0.6 ± 0.3	1.2 ± 1.1	0.7 ± 0.5
18:4, Octadecatetraenoic acid (g/day)	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1
20:4, Eicosatetraenoic acid (g/day)	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1

20:5 n-3, Eicosapentaenoic acid [EPA] (g/day)	0.0 ± 0.0	0.3 ± 0.3	0.1 ± 0.3	0.3 ± 0.4
22:5 n-3, Docosapentaenoic acid [DPA] (g/day)	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.1
22:6 n-3, Docosahexaenoic acid [DHA] (g/day)	0.0 ± 0.0	0.5 ± 0.4	0.2 ± 0.5	0.4 ± 0.6
Vitamin D (D2 + D3) (mcg/day)	0.1 ± 0.1	7.2 ± 5.9	3.5 ± 6.3	6.1 ± 8.2
Choline, total (mg/day)	87.1 ± 29.7	138.0 ± 24.5	184.3 ± 66.1	114.9 ± 56.5
Added Vitamin E (mg/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Added Vitamin B-12 (mcg/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Intact fruits of citrus, melons, and berries (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Intact fruits; excluding citrus, melons, and berries (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fruit juices (g/day)	0.0 ± 0.0	12.7 ± 17.0	0.0 ± 0.0	3.8 ± 9.8
Dark green vegetables (g/day)	0.0 ± 0.0	7.6 ± 18.6	27.4 ± 45.1	48.8 ± 98.2
Tomatoes and tomato products (g/day)	16.7 ± 26.2	80.6 ± 55.4	23.5 ± 58.9	35.8 ± 70.4
Other red and orange vegetables, excluding tomatoes and tomato products (g/day)	0.0 ± 0.0	7.8 ± 25.6	3.2 ± 8.6	23.3 ± 22.1
White potatoes (g/day)	28.4 ± 56.5	11.3 ± 40.7	0.0 ± 0.0	0.0 ± 0.0

Other starchy vegetables, excluding white potatoes (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	45.7 ± 34.5
Other vegetables not in the vegetable components listed above (g/day)	68.8 ± 21.2	82.5 ± 83.6	166.8 ± 93.5	58.9 ± 121.3
Beans and peas (legumes) computed as vegetables (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Whole grains (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	12.7 ± 19.7
Refined grains (g/day)	70.5 ± 5.0	24.4 ± 14.8	0.0 ± 0.0	20.6 ± 16.4
Beef, veal, pork, lamb; excludes organ meat and cured meat (g/day)	64.9 ± 18.2	1.2 ± 2.8	134.4 ± 128.7	1.8 ± 5.4
Cured meat (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Poultry (g/day)	0.0 ± 0.0	38.2 ± 59.9	40.8 ± 81.2	23.9 ± 44.5
Seafood (g/day)	0.0 ± 0.0	92.1 ± 83.1	18.3 ± 46.6	57.7 ± 82.0
Eggs (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.8	1.2 ± 2.0
Soy products, excluding calcium fortified soy milk and immature soybeans (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Peanuts, tree nuts, and seeds; excludes coconut (g/day)	0.0 ± 0.0	1.4 ± 2.3	0.0 ± 0.0	0.0 ± 0.0
Beans and Peas (legumes) computed as protein foods (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Milk (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Yogurt (g/day)	0.0 ± 0.0	18.9 ± 29.5	0.0 ± 0.0	0.0 ± 0.0
Cheese (g/day)	207.0 ± 87.7	50.0 ± 75.3	160.6 ± 173.7	51.5 ± 108.1

Added sugars(g/day)	15.7 ± 12.8	0.1 ± 0.1	1.0 ± 2.1	0.4 ± 0.8

Appendix 3_C

Appendix Table 3-C - The mean 14-d intake of consumed foods, beverages, and dietary supplements with dinners across all dietary interventions

Food Group	FFD	MD	KD	RD
Energy (kcal/day)	614.8 ± 329.8	639.8 ± 159.2	641.9 ± 172.9	484.4 ± 113.8
Protein (g/day)	26.6 ± 12.9	34.1 ± 12.4	46.0 ± 19.8	33.4 ± 6.5
Total Fat (g/day)	24.6 ± 15.9	30.6 ± 8.7	45.5 ± 19.0	26.3 ± 12.0
Carbohydrate (g/day)	72.2 ± 37.9	60.2 ± 20.6	11.8 ± 6.0	27.8 ± 23.3
Alcohol (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Caffeine (mg/day)	15.1 ± 16.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Theobromine (mg)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sugars, total (g/day)	25.1 ± 20.9	15.2 ± 5.6	5.7 ± 4.0	5.8 ± 4.9
Fiber, total dietary (g/day)	2.9 ± 1.2	8.1 ± 5.0	3.0 ± 1.5	4.1 ± 2.6
Calcium (mg/day)	264.6 ± 175.5	214.0 ± 125.3	177.8 ± 126.3	100.0 ± 44.3
Iron (mg/day)	4.9 ± 2.5	4.8 ± 2.1	3.7 ± 2.8	2.8 ± 1.3
Magnesium (mg/day)	49.6 ± 19.9	127.8 ± 41.8	78.6 ± 28.9	68.9 ± 14.6
Phosphorus (mg/day)	345.9 ± 178.8	562.4 ± 161.5	469.2 ± 149.8	336.1 ± 54.0
Potassium (mg/day)	567.9 ± 292.8	1291.5 ± 684.7	988.5 ± 338.6	842.0 ± 224.4

Sodium (mg/day)	1262.9 ± 599.3	3084.7 ± 2358.7	1099.0 ± 498.7	1146.0 ± 367.2
Zinc (mg/day)	4.6 ± 2.1	3.4 ± 1.4	5.3 ± 3.4	2.2 ± 0.8
Copper (mg/day)	0.3 ± 0.2	0.6 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
Selenium (mcg/day)	35.9 ± 20.3	44.4 ± 23.8	60.9 ± 25.6	43.9 ± 22.3
Vitamin C (mg/day)	8.0 ± 9.8	136.9 ± 95.5	25.0 ± 13.7	30.2 ± 22.7
Thiamin (mg/day)	0.6 ± 0.3	0.6 ± 0.4	0.6 ± 0.4	0.3 ± 0.2
Riboflavin (mg/day)	0.4 ± 0.2	0.4 ± 0.2	0.6 ± 0.2	0.4 ± 0.1
Niacin (mg/day)	7.7 ± 3.2	7.6 ± 3.0	9.7 ± 3.4	11.6 ± 2.9
Vitamin B-6 (mg/day)	0.4 ± 0.1	0.9 ± 0.4	0.9 ± 0.4	0.8 ± 0.4
Folate, total (mcg/day)	103.0 ± 39.7	151.7 ± 64.8	78.9 ± 44.1	125.0 ± 71.7
Folic acid (mcg/day)	61.2 ± 28.8	35.5 ± 41.1	0.0 ± 0.0	20.7 ± 33.4
Folate, food (mcg/day)	41.8 ± 20.0	116.2 ± 72.5	78.9 ± 44.1	104.4 ± 63.5
Folate, DFE (mcg_DFE/day)	145.9 ± 58.1	176.6 ± 74.0	78.9 ± 44.1	139.5 ± 85.1
Vitamin B-12 (mcg/day)	1.9 ± 1.0	2.2 ± 1.7	2.3 ± 2.5	0.9 ± 1.1
Vitamin A, RAE (mcg_RAE/day)	69.2 ± 61.3	469.7 ± 456.6	333.8 ± 176.0	350.9 ± 234.1

Retinol (mcg/day)	62.0 ± 58.0	115.5 ± 50.5	75.6 ± 66.5	22.3 ± 13.4
Carotene, beta (mcg/day)	82.9 ± 50.4	4145.3 ± 5875.6	3033.4 ± 2486.1	3689.1 ± 2620.2
Carotene, alpha (mcg/day)	1.1 ± 1.1	19.4 ± 13.5	112.9 ± 151.8	496.8 ± 499.2
Cryptoxanthin, beta (mcg/day)	2.3 ± 2.3	203.7 ± 233.9	12.4 ± 11.1	1.1 ± 1.5
Lycopene (mcg/day)	1463.7 ± 330.2	2543.9 ± 5063.0	1647.8 ± 1668.8	6272.9 ± 11480.6
Lutein + zeaxanthin (mcg/day)	54.9 ± 31.1	1568.5 ± 1278.2	4860.7 ± 6308.3	2625.2 ± 1993.7
Vitamin E, alpha-tocopherol (mg/day)	1.0 ± 0.8	5.8 ± 2.7	3.9 ± 1.7	4.5 ± 2.6
Vitamin K, phylloquinone (mcg/day)	10.2 ± 11.5	45.3 ± 41.0	257.0 ± 256.6	184.6 ± 115.8
Cholesterol (mg/day)	64.9 ± 40.8	135.0 ± 92.5	183.1 ± 121.7	88.2 ± 42.5
Fatty acids, total saturated (g/day)	8.3 ± 6.0	6.4 ± 3.1	11.5 ± 4.0	5.0 ± 1.8
4:0, Butanoic acid (g/day)	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0

6:0, Hexanoic acid (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
8:0, Octanoic acid (g/day)	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
10:0, Decanoic acid (g/day)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
12:0, Dodecanoic acid (g/day)	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0
14:0, Tetradecanoic acid (g/day)	0.6 ± 0.5	0.3 ± 0.4	0.7 ± 0.4	0.1 ± 0.1
16:0, Hexadecanoic acid (g/day)	4.7 ± 3.4	4.0 ± 1.7	7.3 ± 2.6	3.5 ± 1.4
18:0, Octadecanoic acid (g/day)	2.4 ± 1.7	1.5 ± 0.6	3.2 ± 1.3	1.1 ± 0.4
Fatty acids, total monounsaturated (g/day)	10.5 ± 6.4	13.5 ± 5.7	15.6 ± 7.0	8.1 ± 3.3
16:1, Hexadecenoic acid, undifferentiated(g/day)	0.5 ± 0.3	0.3 ± 0.3	0.6 ± 0.6	0.2 ± 0.2

18:1, Octadecenoic acid, undifferentiated (g/day)	6.4 ± 5.8	13.0 ± 5.5	15.3 ± 6.9	7.7 ± 3.2
20:1, Eicosenoic acid, undifferentiated (g/day)	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
22:1, Docosenoic acid, undifferentiated (g/day)	0.1 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Fatty acids, total polyunsaturated (g/day)	3.6 ± 3.1	8.2 ± 2.1	14.2 ± 10.8	10.9 ± 7.1
18:2, Octadecadienoic acid (g/day)	3.0 ± 2.6	7.0 ± 1.8	12.6 ± 9.7	10.4 ± 7.0
18:3, Octadecatrienoic acid (g/day)	0.4 ± 0.4	0.9 ± 0.4	1.5 ± 1.4	0.4 ± 0.2
18:4, Octadecatetraenoic acid (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:4, Eicosatetraenoic acid (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0

20:5 n-3, Eicosapentaenoic acid [EPA] (g/day)	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
22:5 n-3, Docosapentaenoic acid [DPA] (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:6 n-3, Docosahexaenoic acid [DHA] (g/day)	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Vitamin D (D2 + D3) (mcg/day)	0.1 ± 0.3	2.3 ± 1.8	1.1 ± 1.1	0.3 ± 0.4
Choline, total (mg/day)	71.1 ± 31.1	141.0 ± 47.9	188.4 ± 90.6	124.6 ± 28.5
Added Vitamin E (mg/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Added Vitamin B-12 (mcg/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Intact fruits of citrus, melons, and berries (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Intact fruits; excluding citrus, melons, and berries (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fruit juices (g/day)	0.0 ± 0.0	8.4 ± 18.2	0.5 ± 1.6	0.0 ± 0.0
Dark green vegetables (g/day)	0.0 ± 0.0	0.0 ± 0.0	110.8 ± 105.1	185.9 ± 155.7
Tomatoes and tomato products (g/day)	11.2 ± 4.6	62.3 ± 140.2	90.7 ± 91.8	65.6 ± 95.7
Other red and orange vegetables, excluding tomatoes and tomato products (g/day)	0.0 ± 0.0	104.9 ± 94.9	2.3 ± 8.2	26.4 ± 28.2
White potatoes (g/day)	44.8 ± 74.9	40.3 ± 60.3	0.0 ± 0.0	0.0 ± 0.0
Other starchy vegetables, excluding white potatoes (g/day)	0.0 ± 0.0	26.5 ± 52.7	0.0 ± 0.0	0.0 ± 0.0

Other vegetables not in the vegetable components listed above (g/day)	19.3 ± 21.9	237.9 ± 125.0	157.8 ± 106.1	54.2 ± 46.4
Beans and peas (legumes) computed as vegetables (g/day)	0.0 ± 0.0	32.3 ± 65.3	0.0 ± 0.0	0.0 ± 0.0
Whole grains (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 5.2
Refined grains (g/day)	77.4 ± 42.4	24.5 ± 39.0	0.0 ± 0.0	22.0 ± 24.4
Beef, veal, pork, lamb; excludes organ meat and cured meat (g/day)	48.8 ± 19.0	0.0 ± 0.0	109.8 ± 97.3	4.1 ± 8.1
Cured meat (g/day)	6.6 ± 18.0	0.0 ± 0.0	5.7 ± 9.3	6.1 ± 17.3
Poultry (g/day)	0.0 ± 0.0	26.6 ± 52.9	20.0 ± 48.7	92.7 ± 58.6
Seafood (g/day)	0.0 ± 0.0	67.6 ± 83.1	0.0 ± 0.0	13.3 ± 37.5
Eggs (g/day)	0.0 ± 0.0	0.0 ± 0.0	7.3 ± 18.0	0.4 ± 0.4

Soy products, excluding calcium fortified soy milk and immature soybeans (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Peanuts, tree nuts, and seeds; excludes coconut (g/day)	0.0 ± 0.0	1.0 ± 1.3	6.7 ± 12.8	0.0 ± 0.0
Beans and Peas (legumes) computed as protein foods (g/day)	0.0 ± 0.0	15.3 ± 31.0	0.0 ± 0.0	0.0 ± 0.0
Milk (g/day)	0.0 ± 0.0	5.1 ± 10.2	0.0 ± 0.0	0.0 ± 0.0
Yogurt (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cheese (g/day)	122.3 ± 113.7	34.0 ± 126.6	67.4 ± 116.6	0.6 ± 1.3
Added sugars(g/day)	22.4 ± 19.9	3.3 ± 4.4	0.9 ± 2.0	1.7 ± 1.4

Appendix 3_D

Appendix Table 3-D - The mean 14-d intake of consumed foods, beverages, and dietary supplements with snacks across all dietary interventions

Food Group	FFD	MD	KD	RD
Energy (kcal/day)	504.1 ± 313.1	354.9 ± 223.7	177.8 ± 95.5	254.7 ± 183.5
Protein (g/day)	12.1 ± 9.6	9.8 ± 8.2	2.8 ± 3.2	6.9 ± 9.0
Total Fat (g/day)	22.5 ± 13.0	5.7 ± 8.9	18.0 ± 9.3	6.3 ± 7.4
Carbohydrate (g/day)	67.5 ± 44.8	72.8 ± 39.4	2.9 ± 2.6	46.7 ± 27.6
Alcohol (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Caffeine (mg/day)	164.2 ± 73.5	145.2 ± 16.2	163.1 ± 50.7	75.6 ± 66.1
Theobromine (mg)	76.3 ± 46.4	0.0 ± 0.0	0.0 ± 0.0	16.4 ± 33.6
Sugars, total (g/day)	46.0 ± 23.9	45.5 ± 24.6	0.3 ± 0.7	27.5 ± 13.7
Fiber, total dietary (g/day)	2.5 ± 2.0	9.1 ± 5.4	0.8 ± 2.0	4.3 ± 2.0
Calcium (mg/day)	255.6 ± 238.6	214.9 ± 186.8	58.0 ± 31.6	117.7 ± 148.5
Iron (mg/day)	1.9 ± 1.4	1.2 ± 1.0	0.2 ± 0.3	1.4 ± 1.4
Magnesium (mg/day)	66.1 ± 33.4	85.8 ± 45.2	20.6 ± 10.7	54.8 ± 25.7
Phosphorus (mg/day)	298.2 ± 238.0	238.5 ± 211.5	67.7 ± 46.4	153.6 ± 177.4
Potassium (mg/day)	847.4 ± 540.8	1137.0 ± 503.8	320.8 ± 174.3	743.0 ± 322.5
Sodium (mg/day)	446.9 ± 423.4	327.1 ± 518.1	85.8 ± 133.7	191.1 ± 322.7
Zinc (mg/day)	2.0 ± 1.7	1.4 ± 1.3	0.5 ± 0.4	0.9 ± 1.0
Copper (mg/day)	0.2 ± 0.1	0.3 ± 0.2	0.0 ± 0.1	0.2 ± 0.1
Selenium (mcg/day)	10.4 ± 10.4	11.7 ± 13.7	2.6 ± 6.2	12.2 ± 21.0
Vitamin C (mg/day)	8.2 ± 13.5	67.7 ± 57.1	2.0 ± 3.7	29.0 ± 25.0
Thiamin (mg/day)	0.3 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
Riboflavin (mg/day)	0.7 ± 0.4	0.7 ± 0.3	0.4 ± 0.2	0.5 ± 0.4
Niacin (mg/day)	3.1 ± 2.7	2.9 ± 1.7	1.2 ± 0.8	1.9 ± 1.2
Vitamin B-6 (mg/day)	0.2 ± 0.2	0.6 ± 0.3	0.1 ± 0.1	0.5 ± 0.3
Folate, total (mcg/day)	51.4 ± 37.3	68.2 ± 40.2	20.6 ± 24.9	59.7 ± 48.2
Folic acid (mcg/day)	9.0 ± 17.8	5.4 ± 11.2	0.0 ± 0.0	8.7 ± 26.2
Folate, food (mcg/day)	42.4 ± 29.9	62.7 ± 36.8	20.6 ± 24.9	50.9 ± 37.8
Folate, DFE (mcg_DFE/day)	57.7 ± 46.0	72.0 ± 44.1	20.6 ± 24.9	65.8 ± 61.4
Vitamin B-12 (mcg/day)	1.1 ± 1.0	0.6 ± 0.5	0.2 ± 0.3	0.5 ± 1.1
Vitamin A, RAE (mcg_RAE/day)	155.2 ± 162.6	147.3 ± 100.4	47.0 ± 34.7	347.6 ± 376.8
Retinol (mcg/day)	153.0 ± 161.7	77.4 ± 78.6	43.2 ± 33.2	56.2 ± 119.5

Carotene, beta (mcg/day)	22.0 ± 18.5	809.5 ± 891.8	40.6 ± 80.3	2926.9 ± 3192.8
Carotene, alpha (mcg/day)	0.1 ± 0.5	30.7 ± 15.8	7.4 ± 18.9	1144.0 ± 1353.5
Cryptoxanthin, beta (mcg/day)	0.2 ± 0.9	35.0 ± 26.7	3.3 ± 8.4	7.6 ± 9.0
Lycopene (mcg/day)	226.3 ± 450.7	1468.4 ± 1848.5	124.1 ± 464.2	323.2 ± 968.4
Lutein + zeaxanthin (mcg/day)	16.4 ± 21.0	128.9 ± 127.9	56.0 ± 93.9	286.4 ± 386.4
Vitamin E, alpha-tocopherol (mg/day)	1.7 ± 1.1	1.3 ± 1.4	2.4 ± 1.4	1.1 ± 1.3
Vitamin K, phylloquinone (mcg/day)	5.8 ± 4.9	8.4 ± 9.3	13.1 ± 9.4	10.6 ± 8.9
Cholesterol (mg/day)	37.9 ± 32.2	12.4 ± 15.8	25.1 ± 48.3	67.4 ± 165.5
Fatty acids, total saturated (g/day)	8.0 ± 5.0	2.5 ± 3.4	4.2 ± 1.9	2.6 ± 2.8
4:0, Butanoic acid (g/day)	0.3 ± 0.3	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.1
6:0, Hexanoic acid (g/day)	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
8:0, Octanoic acid (g/day)	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
10:0, Decanoic acid (g/day)	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
12:0, Dodecanoic acid (g/day)	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.3
14:0, Tetradecanoic acid (g/day)	0.9 ± 0.8	0.3 ± 0.4	0.4 ± 0.2	0.2 ± 0.2
16:0, Hexadecanoic acid (g/day)	4.1 ± 2.2	1.4 ± 2.0	2.3 ± 1.2	1.3 ± 1.5
18:0, Octadecanoic acid (g/day)	1.9 ± 1.1	0.4 ± 0.5	0.8 ± 0.4	0.8 ± 0.9
Fatty acids, total monounsaturated (g/day)	8.9 ± 7.0	2.0 ± 4.2	9.6 ± 5.5	2.1 ± 3.0
16:1, Hexadecenoic acid, undifferentiated(g/day)	0.3 ± 0.2	0.1 ± 0.3	0.2 ± 0.2	0.1 ± 0.2
18:1, Octadecenoic acid, undifferentiated (g/day)	7.8 ± 6.9	1.7 ± 3.8	9.1 ± 5.2	2.0 ± 2.8

20:1, Eicosenoic acid, undifferentiated (g/day)	0.0 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
22:1, Docosenoic acid, undifferentiated (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Fatty acids, total polyunsaturated (g/day)	4.4 ± 2.7	0.7 ± 1.0	3.4 ± 2.0	0.9 ± 1.2
18:2, Octadecadienoic acid (g/day)	3.9 ± 2.5	0.6 ± 0.9	2.8 ± 1.6	0.7 ± 1.0
18:3, Octadecatrienoic acid (g/day)	0.5 ± 0.3	0.1 ± 0.1	0.7 ± 0.3	0.1 ± 0.2
18:4, Octadecatetraenoic acid (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
20:4, Eicosatetraenoic acid (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1
20:5 n-3, Eicosapentaenoic acid [EPA] (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:5 n-3, Docosapentaenoic acid [DPA] (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:6 n-3, Docosahexaenoic acid [DHA] (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Vitamin D (D2 + D3) (mcg/day)	1.4 ± 1.6	1.0 ± 0.6	0.2 ± 0.3	0.7 ± 1.4
Choline, total (mg/day)	65.3 ± 48.1	59.4 ± 27.9	28.3 ± 35.5	81.1 ± 123.1
Added Vitamin E (mg/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Added Vitamin B-12 (mcg/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Intact fruits of citrus, melons, and berries (g/day)	0.0 ± 0.0	149.0 ± 187.6	0.0 ± 0.0	35.6 ± 97.6
Intact fruits; excluding citrus, melons, and berries (g/day)	0.0 ± 0.0	531.1 ± 339.2	0.0 ± 0.0	235.9 ± 234.7
Fruit juices (g/day)	0.0 ± 0.0	17.9 ± 62.1	0.0 ± 0.0	0.0 ± 0.0

Dark green vegetables (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Tomatoes and tomato products (g/day)	1.3 ± 2.7	0.0 ± 0.0	6.8 ± 25.5	0.0 ± 0.0
Other red and orange vegetables, excluding tomatoes and tomato products (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	61.5 ± 75.0
White potatoes (g/day)	54.9 ± 98.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Other starchy vegetables, excluding white potatoes (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Other vegetables not in the vegetable components listed above (g/day)	3.2 ± 6.6	15.1 ± 52.3	19.6 ± 49.8	29.4 ± 45.4
Beans and peas (legumes) computed as vegetables (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Whole grains (g/day)	0.0 ± 0.0	14.8 ± 28.4	0.0 ± 0.0	0.0 ± 0.0
Refined grains (g/day)	10.8 ± 21.8	6.3 ± 16.7	0.0 ± 0.0	7.4 ± 22.3
Beef, veal, pork, lamb; excludes organ meat and cured meat (g/day)	10.1 ± 21.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cured meat (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 3.1	0.0 ± 0.0
Poultry (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Seafood (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eggs (g/day)	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 5.7	10.0 ± 24.8
Soy products, excluding calcium fortified soy milk and immature soybeans (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Peanuts, tree nuts, and seeds; excludes coconut (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Beans and Peas (legumes) computed as protein foods (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Milk (g/day)	141.1 ± 165.5	90.0 ± 58.7	17.6 ± 6.8	49.5 ± 83.8
Yogurt (g/day)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Cheese (g/day)	0.0 ± 0.0	69.9 ± 163.2	5.7 ± 21.2	0.0 ± 0.0
Added sugars(g/day)	35.9 ± 17.2	0.9 ± 1.5	0.0 ± 0.0	8.0 ± 13.7

Appendix 3_E

```
#Dorsa Yahya Rayat
#2023

# This code is to calculate the 2019 HEI calculation - Canada version
# Obtained from the SAS code from the NIH website (ASA24)
# Canada HEI guidelines: PMID: 35030038
# Note: reference amounts (RAs) calculated using: https://www.canada.ca/en/health-canada/services/technical-documents-labelling-requirements/table-reference-amounts-food/nutrition-labelling.html#v

# Import libraries
import pandas as pd
import numpy as np

# Data set import
df = pd.read_csv("ASA24_totals with RAs - aves.csv", encoding='latin1')

# Calculate total foods
df["total_foods"] = df["PROT_RA"] + df["G_TOTAL_RA"] + df["F_TOTAL_RA"] + df["V_TOTAL_RA"]

# Component 1: Vegetables and Fruits
df["VegFru_to_TotFood"] = (df["F_TOTAL_RA"] + df["V_TOTAL_RA"]) / df["total_foods"]
df["comp1_veg"] = df["VegFru_to_TotFood"].apply(lambda x: 20 if x >= 0.5 else (0 if x == 0 else 10))

# Component 2: Whole-Grain Foods
df["wholeGr_to_TotFood"] = df["G_WHOLE_RA"] / df["total_foods"]
df["comp2_whGr"] = df["wholeGr_to_TotFood"].apply(lambda x: 5 if x >= 0.25 else 0)

# Component 3: Grain Foods Ratio
df["wholGr_to_totGr"] = df["G_WHOLE_RA"] / df["G_TOTAL_RA"]
df["comp3_GrFoods"] = df["wholGr_to_totGr"].apply(lambda x: 5 if x == 1 else 0)

# Component 4: Protein Foods
df["prot_to_TotFood"] = df["PROT_RA"] / df["total_foods"]
df["comp4_prot"] = df["prot_to_TotFood"].apply(lambda x: 5 if x >= 0.25 else 0)
```

```
# Component 5: Plant-Based Protein Foods
df["plaPro_to_totPro"] = df["PF_LEGUMES_RA"] / df["PROT_RA"]
df["comp5_planPro"] = df["plaPro_to_totPro"].apply(lambda x: 5 if x > 0.5 else 0)

# Component 6: Beverages
df["all_bevs"] = df["ADD_SUGARS"] + df["D_MILK_RA"] + df["MOIS"] + df["F_JUICE_RA"]
df["w_to_all"] = (df["MOIS"] + df["D_MILK_RA"] + df["F_JUICE_RA"]) / df["all_bevs"]
df["comp6_bevs"] = df["w_to_all"].apply(lambda x: 10 if x == 1 else 0)

# Component 7: Fatty Acids Ratio
df["UNSF"] = df["TFAT"] - df["SFAT"] # Assuming TFAT includes all types of fats
df["unsat_to_sat"] = df["UNSF"] / df["SFAT"]
df["comp7_FA"] = df["unsat_to_sat"].apply(lambda x: 5 if x >= 2.6 else (0 if x <= 1.1 else 2.5))

# Component 8: Saturated Fats
df["SFAT_%E"] = (df["SFAT"] * 9 / df["KCAL"]) * 100
df["comp8_FAE"] = df["SFAT_%E"].apply(lambda x: 5 if x <= 10 else 0)

# Component 9: Free Sugars
df["SUGR%E"] = (df["ADD_SUGARS"] * 16 / df["KCAL"]) * 100
df["comp9_sugars"] = df["SUGR%E"].apply(lambda x: 10 if x < 10 else 0)

# Component 10: Sodium
df["sod_to_cal"] = df["SODI"] / df["KCAL"]
df["comp10_sod"] = df["sod_to_cal"].apply(lambda x: 10 if x < 0.9 else (0 if x >= 2 else 5))

# HEI calculation
df["Total_HEI"] = df["comp1_veg"] + df["comp2_whGr"] + df["comp3_GrFoods"] + df["comp4_prot"] + df["comp5_planPro"] + df["comp6_bevs"] + df["comp7_FA"] + df["comp8_FAE"] + df["comp9_sugars"] + df["comp10_sod"]

# Rename columns for final output
df.rename(
  columns={
    "Total_HEI": "Total HEI-2019 Score",
    "comp1_veg": "C1 - Vegetables and Fruits",
    "comp2_whGr": "C2 - Whole-Grain Foods",
    "comp3_GrFoods": "C3 - Grain Foods Ratio",
    "comp4_prot": "C4 - Protein Foods",
    "comp5_planPro": "C5 - Plant-Based Protein Foods",
    "comp6_bevs": "C6 - Beverages",
    "comp7_FA": "C7 - Fatty Acids Ratio",
    "comp8_FAE": "C8 - Saturated Fats",
    "comp9_sugars": "C9 - Free Sugars",
    "comp10_sod": "C10 - Sodium"
```

```
  },
  inplace=True)

# Export the dataframe to a CSV file
df.to_csv("HEI_2019_Scores.csv", index=False)
```

Appendix 3_F

Appendix Table 3-F – ANOVA table of significantly different plasma metabolites across all dietary interventions.

	f.value	p.value	-LOG10(p)	FDR	Fisher's LSD
2-Hydroxyisovaleric acid	101.84	2.65E-19	18.578	6.75E-17	KD - FFD; KD - MD; KD - RD; RD - MD
Acetoacetic acid	97.625	5.74E-19	18.241	7.32E-17	KD - FFD; MD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD
Ratio of carnitine to total acylcarnitines	90.761	2.17E-18	17.664	1.84E-16	FFD - KD; FFD - MD; MD - KD; RD - KD
Ratio of Isoleucine, Leucine and Valine to Phenylalanine, Tryptophan, and Tyrosine	80.163	2.02E-17	16.695	1.29E-15	KD - FFD; MD - FFD; RD - FFD; KD - MD; KD - RD
3-Hydroxyisobutyric acid	77.573	3.62E-17	16.442	1.85E-15	KD - FFD; KD - MD; KD - RD
Ratio of Acetylcarnitine to Carnitine	75.862	5.37E-17	16.27	2.28E-15	KD - FFD; MD - FFD; KD - MD; KD - RD; MD - RD
Ratio of Acyl-Alkyl-Phosphatidylcholines to Diacyl-Phosphatidylcholines	69.386	2.56E-16	15.591	9.34E-15	KD - FFD; FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
3-Hydroxyisovaleric acid	68.316	3.36E-16	15.474	1.07E-14	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
Indole-3-propionic acid	65.635	6.71E-16	15.173	1.90E-14	FFD - KD; MD - FFD; RD - FFD; MD - KD; RD - KD
2-Hydroxy-3-methylvaleric acid	65.105	7.72E-16	15.113	1.97E-14	KD - FFD; KD - MD; KD - RD
CMPF	62.707	1.47E-15	14.833	3.40E-14	KD - FFD; RD - FFD; KD - MD; RD - KD; RD - MD
Uric acid	57.13	7.10E-15	14.148	1.51E-13	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
Total concentration of uremic toxins	56.746	7.96E-15	14.099	1.56E-13	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
3-Hydroxybutyric acid	55.798	1.05E-14	13.977	1.92E-13	KD - FFD; MD - FFD; RD - FFD; KD - MD; KD - RD
Argininic acid	54.228	1.70E-14	13.771	2.88E-13	KD - FFD; MD - FFD; KD - MD; KD - RD
2-Hydroxybutyric acid	51.706	3.72E-14	13.429	5.93E-13	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
trans-4-Hydroxyproline	50.385	5.69E-14	13.245	8.53E-13	FFD - KD; FFD - MD; FFD - RD; KD - MD; KD - RD
Ratio of Leucine and Isoleucine to PC aa(34:3)	50.079	6.28E-14	13.202	8.90E-13	KD - FFD; MD - FFD; KD - MD; KD - RD; MD - RD
Valine	43.718	5.56E-13	12.255	7.46E-12	KD - FFD; KD - MD; KD - RD

Benzoic acid	42.307	9.32E-13	12.031	1.19E-11	KD - FFD; KD - MD; KD - RD
Dimethylglycine	39.976	2.25E-12	11.648	2.73E-11	KD - FFD; KD - MD; KD - RD
Indolelactic acid	39.839	2.37E-12	11.625	2.75E-11	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
Tiglylglycine	38.629	3.81E-12	11.419	4.22E-11	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD
Sum of Unsaturated Diglycerides	37.46	6.08E-12	11.216	6.46E-11	FFD - KD; FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
Sum of Branched-Chain Amino Acids	37.01	7.30E-12	11.137	7.44E-11	KD - FFD; KD - MD; KD - RD
Ratio of Acyl-Alkyl-Phosphatidylcholines to Choline	34.635	1.97E-11	10.706	1.93E-10	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD
Ratio of Betaine to Choline	33.25	3.59E-11	10.445	3.39E-10	KD - FFD; MD - FFD; FFD - RD; KD - RD; MD - RD
N2-Acetyl-Ornithine	32.164	5.82E-11	10.235	5.17E-10	MD - FFD; RD - FFD; MD - KD; MD - RD
Sum of Acyl-Alkyl-Phosphatidylcholines	32.141	5.88E-11	10.23	5.17E-10	KD - FFD; FFD - MD; KD - MD; KD - RD; RD - MD
p-Cresol sulfate	32.04	6.16E-11	10.21	5.24E-10	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD
Short-chain acylcarnitine concentration	31.735	7.07E-11	10.15	5.82E-10	KD - FFD; KD - MD; KD - RD
Sum of Polyunsaturated Fatty Acid Cholesteryl Esters	31.629	7.42E-11	10.13	5.91E-10	KD - FFD; RD - FFD; KD - MD; RD - MD
Leucine	31.102	9.45E-11	10.025	7.30E-10	KD - FFD; KD - MD; KD - RD
Ratio of phenylalanine to PC aa(34:3)	30.871	1.05E-10	9.9782	7.85E-10	KD - FFD; MD - FFD; FFD - RD; KD - RD; MD - RD
Sum of Saturated Fatty Acid Acylcarnitines	30.818	1.08E-10	9.9675	7.85E-10	KD - FFD; KD - MD; KD - RD
Sum of HexCer	30.198	1.44E-10	9.8417	1.02E-09	KD - FFD; FFD - MD; KD - MD; KD - RD; RD - MD
2-oxoisocaproic acid	30.077	1.52E-10	9.8171	1.05E-09	KD - FFD; FFD - MD; KD - MD; KD - RD; RD - MD
Sum of Long-Chain Fatty Acid Cholesteryl Esters	29.35	2.15E-10	9.6669	1.44E-09	KD - FFD; RD - FFD; KD - MD; RD - MD
Sum of Cholesteryl Esters	29.292	2.21E-10	9.6548	1.45E-09	KD - FFD; RD - FFD; KD - MD; RD - MD
Concentration of hydrophobic Amino Acids	28.794	2.82E-10	9.5505	1.79E-09	KD - FFD; KD - MD; KD - RD
Sum of Diglycerides	28.547	3.18E-10	9.4982	1.98E-09	FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
Ratio of Sphingomyelins to Phosphatidylcholines	28.208	3.75E-10	9.426	2.28E-09	KD - FFD; MD - FFD; RD - FFD; KD - MD; KD - RD; MD - RD

Choline	28.095	3.96E-10	9.402	2.28E-09	FFD - MD; RD - FFD; KD - MD; RD - KD; RD - MD
Ratio of esterified carnitine to free carnitine	28.094	3.97E-10	9.4017	2.28E-09	KD - FFD; KD - MD; KD - RD
Sum of VLCFA-Glycosyl-Cer	28.033	4.09E-10	9.3886	2.28E-09	KD - FFD; FFD - MD; KD - MD; KD - RD; RD - MD
Total acylcarnitine concentration	28.023	4.11E-10	9.3866	2.28E-09	KD - FFD; KD - MD; KD - RD
Sum of Glutamic acid and Glycine	27.713	4.79E-10	9.3196	2.60E-09	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD
Fraction of medium-chain acylcarnitines	27.314	5.85E-10	9.233	3.11E-09	FFD - KD; RD - FFD; MD - KD; RD - KD; RD - MD
1,3-Diaminopropane	26.909	7.18E-10	9.1442	3.73E-09	FFD - RD; KD - RD; MD - RD
Sum of Glycosyl-Cer	26.33	9.64E-10	9.0157	4.92E-09	KD - FFD; FFD - MD; KD - MD; KD - RD; RD - MD
Ratio of carnitine to medium-chain acylcarnitines	25.579	1.42E-09	8.8463	7.08E-09	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD
Lysine	25.553	1.44E-09	8.8403	7.08E-09	FFD - KD; FFD - MD; FFD - RD; KD - RD; MD - RD
Total concentration of saturated Sphingomyelins	25.274	1.67E-09	8.7768	7.95E-09	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
Orotic acid	25.262	1.68E-09	8.774	7.95E-09	RD - FFD; RD - KD; RD - MD
Sum of Very Long-Chain Fatty Acid Cholesteryl Esters	24.733	2.23E-09	8.6518	1.03E-08	KD - FFD; RD - FFD; KD - MD; RD - MD
alpha-Aminobutyric acid	24.641	2.34E-09	8.6302	1.07E-08	KD - FFD; KD - MD; KD - RD
Glycine	24.448	2.60E-09	8.5853	1.16E-08	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD
Serine	24.272	2.86E-09	8.5439	1.26E-08	FFD - KD; FFD - MD; FFD - RD; MD - KD; MD - RD
Kynurenic acid	23.61	4.10E-09	8.3868	1.77E-08	KD - FFD; RD - FFD; KD - MD; RD - MD
Oxalic acid	23.338	4.77E-09	8.3214	2.03E-08	KD - FFD; MD - FFD; MD - KD; KD - RD; MD - RD
5-Hydroxyindoleacetic acid	23.169	5.24E-09	8.2807	2.19E-08	RD - FFD; RD - KD; RD - MD
Sum of Long-Chain Fatty Acid Sphingomyelins	22.572	7.33E-09	8.135	3.01E-08	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
Isoleucine	21.905	1.07E-08	7.9694	4.34E-08	KD - FFD; KD - MD; KD - RD
Sum of Monounsaturated Fatty Acid Cholesteryl Esters	21.368	1.47E-08	7.8341	5.84E-08	KD - FFD; RD - FFD; KD - MD; RD - MD
Total concentration of non-hydroxylated Sphingomyelins	21.256	1.56E-08	7.8056	6.14E-08	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD

Sum of Sphingomyelins	21.125	1.69E-08	7.772	6.53E-08	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
Sum of Saturated Cholesteryl Esters	20.941	1.88E-08	7.7248	7.17E-08	KD - FFD; RD - FFD; KD - MD; RD - MD
Sum of Indoles	20.869	1.97E-08	7.7063	7.37E-08	RD - FFD; RD - KD; RD - MD
Methionine sulfoxide	20.614	2.29E-08	7.6405	8.46E-08	MD - FFD; RD - FFD; RD - KD; RD - MD
Total concentration of hydroxylated Sphingomyelins	19.657	4.09E-08	7.3887	1.49E-07	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
Ratio of Valine to PCae 32:2	19.303	5.09E-08	7.2936	1.83E-07	KD - FFD; MD - FFD; KD - MD; KD - RD; MD - RD
Alanine	19.123	5.69E-08	7.2452	2.01E-07	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD
Nudifloramide	18.678	7.52E-08	7.1236	2.63E-07	KD - FFD; MD - FFD; KD - MD; KD - RD; MD - RD
Ratio of Proline to Citrulline	18.586	7.97E-08	7.0986	2.75E-07	FFD - KD; FFD - RD; MD - KD; MD - RD
Sum of Very Long-Chain Fatty Acid Sphingomyelins	18.564	8.09E-08	7.0922	2.75E-07	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
N-Acetyl-Histidine	18.304	9.54E-08	7.0206	3.20E-07	FFD - KD; FFD - MD; FFD - RD; RD - KD; RD - MD
Ratio of carnitine to long-chain acylcarnitines	18.092	1.09E-07	6.9615	3.55E-07	FFD - KD; MD - KD; RD - KD
Glutaric acid	18.09	1.09E-07	6.9608	3.55E-07	KD - FFD; FFD - MD; FFD - RD; KD - MD; KD - RD
Total PC aa concentration	18.084	1.10E-07	6.9593	3.55E-07	KD - FFD; RD - FFD; KD - MD; RD - MD
Sum of LCFA-DH-Cer	17.779	1.34E-07	6.8736	4.26E-07	FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
Glutamic acid	17.548	1.56E-07	6.8081	4.90E-07	FFD - KD; RD - FFD; MD - KD; RD - KD; RD - MD
Sum of Saturated Diglycerides	17.368	1.75E-07	6.757	5.44E-07	FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
N-Acetyl-Aspartic acid	17.047	2.16E-07	6.6649	6.65E-07	FFD - MD; FFD - RD; KD - MD; KD - RD; RD - MD
Sum of ceramides	16.989	2.25E-07	6.648	6.83E-07	FFD - MD; KD - MD; RD - MD
Ratio of Sphingomyelins to Ceramides	16.735	2.66E-07	6.5744	7.99E-07	KD - FFD; MD - FFD; RD - FFD
Sum of Non-Essential Amino Acids	16.552	3.01E-07	6.5209	8.94E-07	FFD - KD; FFD - RD; MD - KD; RD - KD; MD - RD
Ratio of Glutamate to Glutamine	16.404	3.33E-07	6.4774	9.76E-07	RD - FFD; RD - KD; RD - MD

Proline	16.102	4.09E-07	6.3882	1.19E-06	FFD - KD; FFD - RD; MD - KD; RD - KD; MD - RD
Sum of Polyunsaturated Fatty Acid Phosphatidylcholines	16.015	4.34E-07	6.3622	1.24E-06	KD - FFD; FFD - MD; RD - FFD; KD - MD; RD - MD
Total concentration of unsaturated Sphingomyelins	15.6	5.78E-07	6.2377	1.64E-06	KD - FFD; RD - FFD; KD - MD; RD - MD
Concentration of ketogenic Amino Acids	15.549	6.00E-07	6.2222	1.68E-06	KD - FFD; FFD - RD; KD - MD; KD - RD
Fraction of short-chain acylcarnitines	15.393	6.69E-07	6.1748	1.85E-06	KD - FFD; KD - MD; KD - RD; MD - RD
Sum of LCFA-Glycosyl-Cer	15.137	8.00E-07	6.0968	2.18E-06	KD - FFD; FFD - MD; KD - MD; KD - RD; RD - MD
Sum of Phosphatidylcholines	15.13	8.04E-07	6.0947	2.18E-06	KD - FFD; FFD - MD; RD - FFD; KD - MD; RD - MD
Sum of Hex2Cer	14.944	9.17E-07	6.0375	2.46E-06	KD - FFD; FFD - MD; KD - MD; KD - RD; RD - MD
Sum of VLCFA-Cer	14.701	1.09E-06	5.9622	2.90E-06	FFD - MD; KD - MD; RD - MD
Creatine	14.607	1.17E-06	5.933	3.04E-06	KD - FFD; FFD - RD; KD - MD; KD - RD
Ratio of Cx-DC to total acylcarnitines concentration	14.606	1.17E-06	5.9325	3.04E-06	FFD - KD; MD - KD; RD - KD; RD - MD
Sum of Choline and Choline-Based Lipids	14.491	1.27E-06	5.8966	3.26E-06	KD - FFD; FFD - MD; RD - FFD; KD - MD; RD - MD
Sum of MUFA-LysoPCs	14.479	1.28E-06	5.8927	3.26E-06	FFD - KD; FFD - MD; RD - FFD; RD - KD; RD - MD
Asparagine	14.039	1.76E-06	5.7537	4.45E-06	FFD - KD; FFD - RD; MD - KD; KD - RD; MD - RD
Sum of Diacyl-Phosphatidylcholines	13.929	1.91E-06	5.7188	4.78E-06	KD - FFD; FFD - MD; RD - FFD; KD - MD; RD - MD
N-Acetyl-Glutamic acid	13.811	2.08E-06	5.6809	5.16E-06	KD - FFD; FFD - MD; KD - MD; RD - MD
Urea	13.798	2.10E-06	5.6768	5.16E-06	KD - FFD; KD - MD; KD - RD
Caprylic acid	13.508	2.61E-06	5.5833	6.34E-06	KD - FFD; FFD - MD; FFD - RD; KD - MD; KD - RD
Total PC ae concentration	13.424	2.78E-06	5.556	6.69E-06	KD - FFD; FFD - MD; KD - MD; KD - RD; RD - MD
Sum of Methionine, Glycine, Serine, Choline, Betaine, Sarcosine, Dimethylglycine	13.045	3.70E-06	5.432	8.77E-06	FFD - KD; FFD - MD; FFD - RD; MD - KD
N1-Acetyl-Lysine	13.039	3.71E-06	5.4301	8.77E-06	FFD - MD; RD - FFD; KD - MD; RD - KD; RD - MD
Ratio of Diacyl-Phosphatidylcholines to Choline	12.878	4.20E-06	5.3767	9.83E-06	KD - FFD; FFD - RD; KD - RD; MD - RD

Sum of Essential Amino Acids	12.775	4.54E-06	5.3427	1.05E-05	KD - FFD; KD - MD; KD - RD
alpha-Ketoglutaric acid	12.608	5.17E-06	5.2869	1.19E-05	KD - FFD; FFD - MD; FFD - RD; KD - MD; KD - RD
Concentration of polar Amino Acids	12.583	5.27E-06	5.2786	1.20E-05	FFD - KD; FFD - RD; MD - KD; MD - RD
N-Acetyl-Valine	12.324	6.44E-06	5.1913	1.45E-05	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD
Ratio of LysoPC(20:4) to PC aa(32:0)	12.286	6.63E-06	5.1787	1.48E-05	FFD - KD; RD - FFD; MD - KD; RD - KD; RD - MD
Ratio of Homoarginine to Arginine and Lysine	12.166	7.28E-06	5.138	1.61E-05	MD - FFD; RD - FFD; RD - KD; RD - MD
Sum of Long Chain Fatty Acid-LysoPCs	12.052	7.96E-06	5.0991	1.75E-05	RD - FFD; RD - KD; RD - MD
Sum of basic amino acids	12.013	8.21E-06	5.0857	1.79E-05	FFD - KD; FFD - RD; MD - RD
Total concentration of LysoPCs	11.988	8.37E-06	5.0771	1.80E-05	RD - FFD; RD - KD; RD - MD
Threonine	11.982	8.41E-06	5.0752	1.80E-05	FFD - KD; FFD - MD; FFD - RD; MD - KD
Total concentration of unsaturated LysoPCs	11.916	8.86E-06	5.0524	1.88E-05	FFD - MD; RD - FFD; RD - KD; RD - MD
4-Hydroxyphenylpyruvic acid	11.694	1.06E-05	4.9762	2.23E-05	FFD - MD; KD - MD; RD - MD
Glutamine	11.251	1.51E-05	4.8217	3.15E-05	FFD - KD; FFD - RD; MD - KD; MD - RD
Concentration of charged Amino Acids	10.968	1.90E-05	4.7216	3.94E-05	FFD - KD; FFD - MD; FFD - RD; MD - KD
Sum of PUFA-LysoPCs	10.845	2.10E-05	4.6778	4.32E-05	FFD - MD; RD - FFD; RD - KD; RD - MD
Sum of Monounsaturated Fatty Acid Phosphatidylcholines	10.767	2.24E-05	4.6501	4.57E-05	FFD - MD; RD - FFD; KD - MD; RD - MD
Indoxyl sulfate	10.348	3.17E-05	4.4989	6.42E-05	FFD - MD; KD - MD; RD - MD
Ratio of Homoarginine to Asymmetric Dimethylarginine	10.248	3.45E-05	4.4625	6.92E-05	RD - FFD; RD - KD; RD - MD
3-Methyladipic acid	10.226	3.51E-05	4.4545	7.00E-05	KD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
Total concentration of saturated LysoPCs	10.104	3.89E-05	4.4095	7.64E-05	RD - FFD; RD - KD; RD - MD
Sum of SFA-LysoPCs	10.104	3.89E-05	4.4095	7.64E-05	RD - FFD; RD - KD; RD - MD
Butyric acid + Isobutyric acid	9.9968	4.26E-05	4.3703	8.30E-05	KD - FFD; MD - FFD; KD - MD; KD - RD
Aspartic acid	9.832	4.91E-05	4.3093	9.48E-05	FFD - KD; RD - FFD; MD - KD; RD - KD; RD - MD
2,5-Furandicarboxylic acid	9.7553	5.24E-05	4.2808	0.0001	FFD - KD; FFD - MD; MD - KD; RD - KD; RD - MD
Trimethylamine N-Oxide	9.7135	5.43E-05	4.2652	0.000103	FFD - MD; FFD - RD; KD - MD; KD - RD

Malonic acid	9.1247	9.05E-05	4.0432	0.000171	KD - FFD; RD - FFD; KD - MD; RD - MD
Ethanolamine	8.9087	0.00011	3.9604	0.000205	FFD - RD; KD - RD; MD - RD
Betaine	8.7338	0.000128	3.8929	0.000238	KD - FFD; MD - FFD; RD - FFD; KD - MD
Hippuric acid	8.7218	0.000129	3.8883	0.000239	KD - FFD; MD - FFD; RD - FFD
3-Indoleacetic acid	8.6198	0.000142	3.8486	0.00026	FFD - MD; RD - FFD; KD - MD; RD - MD
Fraction of long-chain acylcarnitines	8.5764	0.000147	3.8317	0.000268	FFD - KD; MD - KD; RD - KD
Creatinine	8.5505	0.000151	3.8216	0.000273	FFD - RD; KD - RD; MD - RD
Sum of dihydroceramides	8.5255	0.000154	3.8118	0.000277	FFD - MD; FFD - RD; KD - MD; KD - RD
Sum of Hex3Cer	8.3625	0.000179	3.7479	0.000319	RD - FFD; KD - MD; RD - MD
Glyceric acid	8.2099	0.000205	3.6877	0.000363	MD - FFD; MD - KD; MD - RD
N1-Acetylspermidine	7.8897	0.000275	3.5602	0.000484	MD - FFD; FFD - RD; MD - KD; KD - RD; MD - RD
Pyruvic acid	7.6539	0.000343	3.4651	0.000598	FFD - KD; FFD - MD; FFD - RD
N-Acetyl-Glycine	7.4392	0.000419	3.3778	0.000727	KD - FFD; KD - MD; KD - RD
Sarcosine	7.196	0.000527	3.2779	0.000909	KD - FFD; MD - FFD; RD - FFD
Sum of VLCFA-DH-Cer	7.1616	0.000545	3.2637	0.000932	FFD - MD; KD - MD; KD - RD
Ratio of Citrulline to Ornithine	6.8811	0.000713	3.1471	0.001204	RD - FFD; RD - KD; RD - MD
Ratio of Ornithine to Citrulline	6.8811	0.000713	3.1471	0.001204	FFD - RD; KD - RD; MD - RD
Sum of Saturated Fatty Acid Phosphatidylcholines	6.7708	0.000793	3.1009	0.00133	KD - FFD; RD - FFD; KD - MD; RD - MD
N1-Acetyl-Lysine + N6-Acetyl-Lysine	6.6668	0.000877	3.0571	0.001461	FFD - MD; KD - MD; RD - MD
Uridine	6.6079	0.000929	3.0322	0.001538	FFD - RD; KD - RD; MD - RD
Homoarginine	6.372	0.00117	2.9319	0.001924	RD - FFD; RD - KD; RD - MD
beta-Alanine	6.2707	0.001293	2.8885	0.002113	KD - FFD; KD - RD; MD - RD
Ratio of Cer to DH-Cer	6.218	0.001362	2.8659	0.00221	RD - FFD; RD - KD; RD - MD
Concentration of glucogenic Amino Acids	6.2126	0.001369	2.8636	0.00221	FFD - KD; FFD - RD
Guanidoacetic acid	6.1861	0.001406	2.8522	0.002254	FFD - KD; FFD - MD; RD - KD
Glucose	6.1725	0.001425	2.8463	0.00227	FFD - KD; MD - KD; RD - KD
Sum of Butyric acid, Isobutyric acid, Propionic acid, Valeric acid, and Isovaleric acid	6.1604	0.001442	2.8411	0.002278	KD - FFD; RD - FFD; KD - MD

Methylhistidine	6.1568	0.001447	2.8396	0.002278	KD - FFD; MD - FFD; RD - FFD
Homovanillic acid	5.9928	0.001704	2.7686	0.002665	FFD - RD; KD - MD; KD - RD
alpha-Aminoadipic acid	5.9096	0.001852	2.7324	0.002879	KD - FFD; KD - MD; KD - RD
Caproic acid	5.7114	0.002261	2.6457	0.003495	FFD - MD; KD - MD; RD - MD
Ethylmalonic acid	5.4342	0.002998	2.5232	0.004605	FFD - MD; KD - MD; RD - MD
2-hydroxyglutaric acid	5.3039	0.003427	2.4651	0.005233	FFD - MD; RD - KD; RD - MD
Sum of LCFA-Cer	5.1597	0.003977	2.4004	0.006037	FFD - MD; KD - MD; RD - MD
Concentration of hydroxylated carnitines	5.0439	0.004485	2.3483	0.006767	KD - FFD; KD - MD; KD - RD
Total concentration of biogenic amines	5.0315	0.004543	2.3427	0.006814	KD - FFD; MD - FFD; RD - FFD
Ratio of C5 to C3	4.9641	0.004874	2.3121	0.007268	KD - FFD; RD - FFD; KD - MD; RD - MD
5-Oxoproline	4.948	0.004957	2.3048	0.007348	FFD - KD; FFD - MD; RD - KD; RD - MD
Valeric acid + Isovaleric acid	4.9235	0.005085	2.2937	0.007495	KD - FFD; KD - MD
Malic acid	4.8445	0.005523	2.2578	0.008094	FFD - MD; KD - MD; RD - MD
Phenylacetylglutamine	4.8392	0.005555	2.2553	0.008094	KD - FFD; KD - MD; RD - MD
N-Acetyl-Asparagine	4.8315	0.0056	2.2518	0.008113	FFD - KD; FFD - RD
Ornithine	4.5228	0.007759	2.1102	0.011178	FFD - KD; FFD - RD; MD - RD
Salicylic acid	4.4389	0.008484	2.0714	0.012146	RD - FFD; RD - KD; RD - MD
Spermidine	4.4343	0.008526	2.0693	0.012146	KD - MD; RD - MD
5-Hydroxylysine	4.3895	0.008944	2.0485	0.01267	FFD - RD; KD - RD; MD - RD
Asymmetric dimethylarginine	4.3815	0.009021	2.0448	0.012709	FFD - RD; MD - RD
Ratio of free carnitine to C16 and C18	4.3655	0.009176	2.0374	0.012856	FFD - KD; FFD - RD; MD - KD; MD - RD
Concentration of ketogenic and glucogenic Amino Acids	4.3403	0.009427	2.0256	0.013136	FFD - KD; FFD - RD
Quinolinic acid	4.2981	0.009863	2.006	0.013655	RD - FFD; KD - MD; RD - MD
Pipecolic acid	4.294	0.009907	2.0041	0.013655	FFD - KD; FFD - MD; RD - KD; RD - MD
Urea cycle Amino Acids	4.2859	0.009994	2.0003	0.013701	FFD - KD; FFD - RD
3-Aminoisobutyric acid	4.2689	0.010178	1.9923	0.013879	KD - FFD; MD - FFD; KD - RD
Histidine	4.1337	0.011772	1.9291	0.015968	FFD - RD; MD - KD; MD - RD
Sum of citrate, cis-aconitate, alpha-ketoglutarate, succinate, fumarate, malate	4.1226	0.011915	1.9239	0.016075	FFD - MD; KD - MD; RD - MD
Citric acid	4.0298	0.013174	1.8803	0.01768	FFD - MD; KD - MD; RD - MD
Succinic acid	3.9832	0.013857	1.8583	0.018501	FFD - MD; KD - MD; RD - MD

Medium-chain acylcarnitine concentration	3.9639	0.014151	1.8492	0.018794	RD - FFD; RD - MD
Taurine	3.9326	0.014641	1.8344	0.019345	FFD - MD; FFD - RD
Ratio of Phenylalanine to Tyrosine	3.9256	0.014754	1.8311	0.019393	FFD - RD; KD - RD; MD - RD
Methionine	3.8172	0.016606	1.7797	0.021715	FFD - KD; FFD - MD; FFD - RD
Propionic acid	3.7412	0.018048	1.7436	0.02348	KD - FFD; RD - FFD; KD - MD
Methylmalonic acid	3.7357	0.018157	1.7409	0.023503	FFD - KD; MD - KD; RD - KD
Inosine	3.7109	0.018658	1.7291	0.024029	RD - FFD; RD - KD; RD - MD
p-Hydroxyhippuric acid	3.6798	0.019306	1.7143	0.024738	MD - FFD; MD - KD
N-Acetyl-Serine	3.6692	0.019532	1.7093	0.024903	RD - MD
Sum of Citrulline and Arginine	3.6302	0.020388	1.6906	0.025866	FFD - KD; FFD - MD; FFD - RD
Sum of Amino Acids	3.5783	0.02159	1.6658	0.027254	FFD - RD
1-Methylnicotinamide	3.4273	0.025514	1.5932	0.03205	KD - FFD; KD - MD; KD - RD
cis-Aconitic acid	3.4102	0.026003	1.585	0.032504	KD - RD
Arginine	3.3632	0.027399	1.5623	0.034082	FFD - KD; FFD - MD; FFD - RD
Concentration of very long-chain LysoPCs	3.3201	0.028744	1.5415	0.035581	MD - KD
Tryptophan	3.2736	0.030274	1.5189	0.037294	FFD - KD; RD - KD
2-Hydroxy-2-methylbutyric acid	3.254	0.030941	1.5095	0.037932	KD - FFD; KD - MD
N-Acetyl-Alanine	3.0881	0.037256	1.4288	0.045456	FFD - MD; KD - MD; RD - MD
Phenylacetic acid	3.0757	0.037779	1.4228	0.045874	KD - FFD; KD - MD

Appendix 3_G

Appendix Table 3-G - ANOVA table of significantly different urine metabolites across all dietary interventions.

	f.value	p.value	-LOG10(p)	FDR	Fisher's LSD
Acetoacetic acid	106.24	5.44E-29	28.265	1.23E-26	KD - FFD; KD - MD; KD - RD
Guanidinopropionic acid	75.715	3.55E-24	23.449	4.02E-22	KD - FFD; KD - MD; KD - RD
3-Hydroxybutyric acid	63.231	8.67E-22	21.062	6.53E-20	KD - FFD; KD - MD; KD - RD
3-Hydroxyisobutyric acid	59.103	6.28E-21	20.202	3.55E-19	KD - FFD; KD - MD; KD - RD
3-Methoxytyramine	52.586	1.75E-19	18.758	7.89E-18	MD - FFD; RD - FFD; MD - KD; RD - KD; RD - MD
3-Hydroxyphenylacetic acid	51.47	3.17E-19	18.5	1.19E-17	KD - FFD; MD - FFD; RD - FFD; MD - RD
Ethanolamine	49.721	8.18E-19	18.087	2.64E-17	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD; MD - RD
Allantoin	48.455	1.65E-18	17.783	4.66E-17	FFD - KD; FFD - RD; MD - KD; RD - KD; MD - RD
2-Hydroxybutyric acid	46.526	4.90E-18	17.31	1.23E-16	KD - FFD; KD - MD; KD - RD; RD - MD
Threonine	45.941	6.86E-18	17.164	1.55E-16	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD; MD - RD
Short-chain acylcarnitine concentration	45.614	8.29E-18	17.082	1.70E-16	KD - FFD; FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
Glutamine	43.309	3.21E-17	16.493	6.05E-16	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD; MD - RD
Valine	42.876	4.16E-17	16.381	7.24E-16	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD
Phenylalanine	41.183	1.16E-16	15.934	1.88E-15	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD; MD - RD
Sum of Saturated Fatty Acid Acylcarnitines	41.022	1.29E-16	15.891	1.94E-15	KD - FFD; FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
Nudifloramide	40.549	1.72E-16	15.764	2.43E-15	KD - FFD; MD - FFD; KD - MD; KD - RD; MD - RD
Total acylcarnitine concentration	40.27	2.05E-16	15.688	2.73E-15	KD - FFD; FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD

N-Acetyl-Histidine	39.938	2.52E-16	15.598	3.17E-15	FFD - KD; FFD - MD; FFD - RD; MD - KD; MD - RD
Concentration of polar Amino Acids	39.766	2.81E-16	15.552	3.34E-15	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD; MD - RD
Proline	38.706	5.49E-16	15.26	6.20E-15	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD; MD - RD
Citric acid	38.459	6.43E-16	15.192	6.92E-15	FFD - KD; MD - KD; RD - KD
Creatine	33.786	1.42E-14	13.848	1.46E-13	KD - FFD; KD - MD; KD - RD
Sum of aromatic amino acids	33.543	1.68E-14	13.775	1.65E-13	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD; MD - RD
3-Hydroxyisovaleric acid	33.291	2.00E-14	13.7	1.88E-13	KD - FFD; KD - MD; KD - RD
Sum of Branched-Chain Amino Acids	32.934	2.56E-14	13.592	2.31E-13	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD
Concentration of hydroxylated carnitines	32.793	2.82E-14	13.549	2.45E-13	KD - FFD; FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
Urea cycle Amino Acids	31.336	7.89E-14	13.103	6.60E-13	FFD - KD; MD - FFD; MD - KD; RD - KD; MD - RD
trans-4-Hydroxyproline	31.164	8.92E-14	13.05	7.20E-13	FFD - KD; FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
Total concentration of hydroxyproline	30.858	1.11E-13	12.955	8.65E-13	FFD - KD; FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
1-Methylnicotinamide	29.794	2.40E-13	12.619	1.81E-12	KD - FFD; MD - FFD; KD - MD; KD - RD; MD - RD
Caproic acid	28.884	4.70E-13	12.328	3.43E-12	KD - FFD; MD - FFD; RD - FFD; KD - MD; KD - RD; RD - MD
2-Hydroxy-3-methylvaleric acid	28.695	5.41E-13	12.267	3.82E-12	KD - FFD; FFD - MD; FFD - RD; KD - MD; KD - RD; MD - RD
2-oxoisocaproic acid	28.652	5.59E-13	12.253	3.83E-12	KD - FFD; KD - MD; KD - RD
Tryptophan	28.605	5.79E-13	12.237	3.85E-12	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD; MD - RD
Ratio of Kynurenine to Tryptophan	28.172	8.01E-13	12.097	5.17E-12	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD

Medium-chain acylcarnitine concentration	27.139	1.75E-12	11.756	1.10E-11	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD
Alanine	26.223	3.56E-12	11.449	2.17E-11	FFD - KD; FFD - RD; MD - KD; MD - RD
Cytidine	25.151	8.26E-12	11.083	4.91E-11	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD
Sum of Non-Essential Amino Acids	24.844	1.05E-11	10.977	6.11E-11	FFD - KD; FFD - MD; FFD - RD; MD - KD; MD - RD
N-Acetyl-Glycine	24.023	2.04E-11	10.691	1.15E-10	KD - FFD; KD - MD; KD - RD
Sum of citrate, cis-aconitate, alpha-ketoglutarate, succinate, fumarate, malate	23.645	2.77E-11	10.557	1.53E-10	FFD - KD; MD - KD; RD - KD
Aspartic acid	23.297	3.69E-11	10.434	1.98E-10	FFD - KD; MD - FFD; MD - KD; RD - KD; MD - RD
alpha-Ketoisovaleric acid	23.187	4.03E-11	10.394	2.12E-10	KD - FFD; KD - MD; KD - RD
Asparagine	22.933	4.97E-11	10.304	2.55E-10	FFD - KD; FFD - RD; MD - KD; RD - KD; MD - RD
Tyrosine	22.374	7.91E-11	10.102	3.97E-10	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD
Serine	20.154	5.25E-10	9.2798	2.58E-09	FFD - KD; FFD - MD; FFD - RD; MD - KD; MD - RD
Orotic acid	19.524	9.12E-10	9.0399	4.39E-09	FFD - KD; FFD - RD; MD - KD; MD - RD
Leucine	19.389	1.03E-09	8.9884	4.84E-09	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD
Sum of Polyunsaturated Fatty Acid Acylcarnitines	18.493	2.29E-09	8.6411	1.05E-08	FFD - KD; MD - KD; RD - KD; MD - RD
Arginine	17.558	5.34E-09	8.2721	2.42E-08	FFD - KD; MD - FFD; MD - KD; RD - KD; MD - RD
Tryptamine	17.515	5.56E-09	8.2547	2.46E-08	KD - FFD; KD - MD; KD - RD
Sum of Glutamic acid and Glycine	16.962	9.27E-09	8.0331	4.03E-08	FFD - KD; FFD - MD; FFD - RD; MD - KD; MD - RD
Glycine	16.9	9.82E-09	8.008	4.19E-08	FFD - KD; FFD - MD; FFD - RD; MD - KD; MD - RD
Butyric acid + Isobutyric acid	16.749	1.13E-08	7.9469	4.73E-08	KD - FFD; KD - MD; KD - RD
7-Methylguanine	15.965	2.36E-08	7.6264	9.71E-08	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD
Isoleucine	15.931	2.44E-08	7.6126	9.85E-08	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD

Concentration of hydrophobic Amino Acids	15.282	4.54E-08	7.3432	1.80E-07	FFD - KD; FFD - MD; FFD - RD; MD - KD; MD - RD
Betaine	14.756	7.55E-08	7.1221	2.94E-07	FFD - RD; KD - RD; MD - RD
Carnosine	14.629	8.55E-08	7.0682	3.27E-07	FFD - MD; FFD - RD; KD - MD; KD - RD
Concentration of glucogenic Amino Acids	14.274	1.21E-07	6.9174	4.56E-07	FFD - KD; FFD - MD; FFD - RD; MD - KD; MD - RD
Sum of Amino Acids	13.835	1.87E-07	6.729	6.90E-07	FFD - KD; FFD - MD; FFD - RD; MD - KD; MD - RD
Methylamine	13.821	1.89E-07	6.7229	6.90E-07	KD - FFD; MD - FFD; RD - FFD; KD - MD
Sum of Sphingomyelins	13.61	2.33E-07	6.632	8.37E-07	FFD - KD; FFD - RD; MD - KD; MD - RD
Concentration of ketogenic and glucogenic Amino Acids	13.488	2.64E-07	6.5788	9.31E-07	FFD - KD; FFD - MD; FFD - RD; MD - KD; MD - RD
Norepinephrine	13.466	2.70E-07	6.5692	9.38E-07	FFD - KD; RD - FFD; MD - KD; RD - KD; RD - MD
Fumaric acid	12.864	4.94E-07	6.3058	1.69E-06	KD - FFD; KD - MD; KD - RD
Dimethylglycine	12.777	5.40E-07	6.2675	1.82E-06	KD - FFD; KD - MD; KD - RD
Sum of Long-Chain Fatty Acid Sphingomyelins	12.709	5.78E-07	6.2377	1.92E-06	FFD - KD; FFD - RD; MD - KD; MD - RD
Total concentration of hydroxylated Sphingomyelins	12.508	7.10E-07	6.1485	2.33E-06	FFD - KD; FFD - RD; MD - KD; RD - KD
Total concentration of non-hydroxylated Sphingomyelins	12.312	8.68E-07	6.0616	2.80E-06	FFD - KD; FFD - RD; MD - KD; MD - RD
2,5-Furandicarboxylic acid	12.052	1.13E-06	5.945	3.59E-06	FFD - KD; FFD - MD; MD - KD; RD - KD
Ornithine	12.045	1.14E-06	5.942	3.59E-06	FFD - KD; FFD - RD; MD - KD; MD - RD
Total concentration of unsaturated Sphingomyelins	12.021	1.17E-06	5.9312	3.63E-06	FFD - KD; FFD - RD; MD - KD; RD - KD
Total concentration of saturated Sphingomyelins	11.993	1.21E-06	5.9188	3.68E-06	FFD - KD; FFD - RD; MD - KD; MD - RD
Sum of Monounsaturated Fatty Acid Acylcarnitines	11.938	1.28E-06	5.8941	3.85E-06	KD - FFD; FFD - MD; FFD - RD; KD - MD; KD - RD
Malonic acid	11.827	1.43E-06	5.8441	4.26E-06	KD - FFD; KD - MD; KD - RD
Sum of Very Long-Chain Fatty Acid Sphingomyelins	11.81	1.46E-06	5.8365	4.28E-06	FFD - KD; FFD - MD; FFD - RD; MD - KD; RD - KD
3-Aminoisobutyric acid	11.793	1.48E-06	5.8288	4.30E-06	KD - FFD; KD - MD; KD - RD

Uric acid	11.512	1.99E-06	5.7014	5.69E-06	FFD - RD; KD - RD; MD - RD
Sum of ADMA, Uric acid, TMAO	10.404	6.42E-06	5.1926	1.81E-05	FFD - RD; KD - RD; MD - RD
Quinoline-4-carboxylic acid	9.6602	1.43E-05	4.8434	3.98E-05	KD - FFD; MD - FFD; KD - MD; KD - RD
Spermidine	9.6536	1.44E-05	4.8403	3.98E-05	FFD - KD; FFD - MD; FFD - RD
Glucose	9.413	1.88E-05	4.7261	5.12E-05	FFD - KD; FFD - RD; MD - KD; MD - RD
Total Dimethylarginine	9.3366	2.04E-05	4.6897	5.50E-05	FFD - KD; MD - FFD; MD - KD; RD - KD; MD - RD
Propionic acid	8.8832	3.37E-05	4.4725	8.96E-05	KD - FFD; KD - MD; KD - RD
N2-Acetyl-Ornithine	8.7802	3.78E-05	4.4228	9.93E-05	KD - FFD; MD - FFD; RD - FFD; KD - MD
Taurine	8.7488	3.91E-05	4.4077	0.000102	FFD - KD; FFD - MD; RD - KD; RD - MD
alpha-Aminoadipic acid	8.6271	4.48E-05	4.3488	0.000115	KD - FFD; KD - MD; KD - RD
Tiglylglycine	8.6091	4.57E-05	4.3401	0.000116	KD - FFD; FFD - RD; KD - MD; KD - RD; MD - RD
Kynurenine	8.5299	4.99E-05	4.3016	0.000125	FFD - RD; KD - RD; MD - RD
Succinic acid	8.3327	6.23E-05	4.2057	0.000154	MD - FFD; MD - KD; RD - KD
Uridine	8.3283	6.26E-05	4.2036	0.000154	FFD - KD; FFD - RD; MD - KD; MD - RD
Sum of Saturated Fatty Acid Phosphatidylcholines	8.2604	6.75E-05	4.1704	0.000164	FFD - KD; FFD - MD; FFD - RD; MD - RD
Epinephrine	8.2472	6.86E-05	4.164	0.000165	RD - FFD; RD - KD; RD - MD
Adenine	7.9182	9.94E-05	4.0027	0.000236	FFD - KD; RD - FFD; MD - KD; RD - KD; RD - MD
alpha-Aminobutyric acid	7.8028	0.000113	3.9458	0.000267	FFD - MD; FFD - RD; KD - RD; MD - RD
Glyceric acid	7.621	0.000139	3.8559	0.000325	KD - FFD; KD - MD; KD - RD; MD - RD
Hippuric acid	7.4981	0.00016	3.7949	0.000369	KD - FFD; MD - FFD; RD - FFD
Total concentration of monamine neurotransmitters	7.4914	0.000162	3.7916	0.000369	MD - FFD; MD - KD; MD - RD
Asymmetric dimethylarginine	7.4055	0.000178	3.7488	0.000403	FFD - KD; MD - KD; MD - RD
Ratio of TMAO to Choline	7.3063	0.0002	3.6994	0.000447	KD - FFD; KD - MD; KD - RD
N-Acetyl-Valine	7.2563	0.000212	3.6745	0.000469	KD - FFD; KD - MD; KD - RD
Concentration of ketogenic Amino Acids	7.2404	0.000216	3.6665	0.000473	FFD - MD; FFD - RD; KD - RD; MD - RD

Homocitrulline	7.1255	0.000246	3.609	0.000535	RD - FFD; KD - MD; RD - MD
Ratio of Non-Essential to Essential Amino Acids	7.1104	0.00025	3.6014	0.000539	FFD - KD; FFD - RD; MD - KD; MD - RD
Caffeic acid	7.0739	0.000261	3.5832	0.000557	KD - FFD; MD - FFD; RD - FFD
Sum of Butyric acid, Isobutyric acid, Propionic acid, Valeric acid, and Isovaleric acid	6.8484	0.000339	3.4698	0.000716	KD - FFD; KD - MD; KD - RD
Sum of Choline and Choline-Based Lipids	6.4948	0.000512	3.2909	0.001071	FFD - KD; FFD - MD; FFD - RD
N-Acetyl-Leucine	6.4367	0.000548	3.2613	0.001136	KD - FFD; KD - MD; KD - RD
Dopamine	6.2496	0.000682	3.166	0.00139	MD - FFD; MD - KD; MD - RD
Lysine	6.2494	0.000682	3.1659	0.00139	FFD - KD; FFD - MD; FFD - RD; MD - RD
Histamine	6.0068	0.000908	3.0418	0.001833	FFD - RD; MD - KD; MD - RD
Glutamic acid	5.9377	0.000986	3.0063	0.001971	FFD - RD; MD - KD; MD - RD
Trimethylamine N-oxide	5.8865	0.001047	2.98	0.002076	KD - FFD; KD - MD; KD - RD
Tartaric acid	5.8547	0.001088	2.9636	0.002137	RD - FFD; RD - KD; RD - MD
Malic acid	5.7848	0.001182	2.9276	0.002302	KD - FFD; KD - MD; KD - RD
N-Acetyl-Methionine	5.6996	0.001307	2.8836	0.002525	KD - FFD; MD - FFD; RD - FFD
Choline	5.6712	0.001352	2.869	0.00259	FFD - KD; FFD - MD; FFD - RD
Indole-3-propionic acid	5.6135	0.001448	2.8391	0.002751	MD - FFD; RD - FFD; MD - KD; RD - KD
Trimethylamine	5.4303	0.001802	2.7442	0.003394	FFD - MD; KD - MD; RD - MD
Salicylic acid	5.4123	0.001841	2.7349	0.003439	MD - FFD; KD - RD; MD - RD
beta-Alanine	5.4047	0.001858	2.7309	0.003442	KD - MD; KD - RD
Uracil	5.065	0.002793	2.5539	0.005133	MD - FFD; RD - FFD; RD - KD
Sum of Phosphatidylcholines	5.0202	0.002948	2.5304	0.005373	FFD - KD; FFD - MD; FFD - RD
Deoxyguanosine	5.0114	0.00298	2.5258	0.005387	FFD - RD; KD - RD; MD - RD
Nicotinamide ribotide	4.9751	0.003113	2.5068	0.005584	KD - FFD; KD - RD; MD - RD
Sum of Diacyl-Phosphatidylcholines	4.9379	0.003256	2.4874	0.005794	FFD - KD; FFD - MD; FFD - RD
Indolelactic acid	4.7684	0.003996	2.3984	0.007055	KD - FFD; KD - RD; MD - RD
Caprylic acid	4.7497	0.004087	2.3886	0.00716	KD - FFD; KD - RD
N-Acetyl-Proline	4.6385	0.004677	2.3301	0.00813	RD - FFD; RD - KD; RD - MD
Sum of Essential Amino Acids	4.5815	0.005012	2.3	0.008646	FFD - MD; FFD - RD

p-Hydroxyhippuric acid	4.4336	0.005998	2.222	0.01027	MD - FFD; MD - KD
N6-Acetyl-Lysine	4.388	0.006341	2.1979	0.010772	FFD - MD; KD - MD; KD - RD
Pyruvic acid	4.3821	0.006387	2.1947	0.010772	FFD - KD; FFD - MD; RD - KD
Lactic acid	4.3509	0.006635	2.1782	0.011107	KD - FFD; KD - MD; KD - RD
4-Hydroxybenzoic acid	4.3448	0.006684	2.175	0.011107	FFD - RD; MD - KD; MD - RD
5-Hydroxyindoleacetic acid	4.3236	0.006859	2.1637	0.011315	KD - FFD; MD - FFD; RD - FFD
Benzoic acid	4.2641	0.007375	2.1322	0.012078	MD - FFD; MD - KD; MD - RD
Guanosine	4.2192	0.007791	2.1084	0.012667	FFD - KD; FFD - MD; RD - KD; RD - MD
Spermine	4.1616	0.008358	2.0779	0.013492	KD - RD; MD - RD
3-Deoxyglucosone	4.0276	0.009847	2.0067	0.015783	FFD - KD; FFD - MD; FFD - RD
alpha-Ketoglutaric acid	4.0037	0.010139	1.994	0.016137	FFD - KD; RD - KD
Maleic acid	3.937	0.011003	1.9585	0.01739	KD - FFD; KD - MD; KD - RD
N-Acetyl-Aspartic acid	3.8995	0.011521	1.9385	0.018081	FFD - KD; MD - KD; RD - KD
Total concentration of biogenic amines	3.8553	0.012163	1.9149	0.018958	FFD - RD; MD - KD; MD - RD
Shikimic acid	3.8166	0.012755	1.8943	0.019744	MD - KD; MD - RD
Serotonin	3.7826	0.013299	1.8762	0.020445	MD - FFD; MD - KD; MD - RD
1,3-Diaminopropane	3.7761	0.013405	1.8727	0.02047	RD - FFD; RD - MD
N-Acetyl-Asparagine	3.7247	0.01428	1.8453	0.02166	FFD - KD; FFD - RD; MD - KD; MD - RD
Sum of Acyl-Alkyl-Phosphatidylcholines	3.5766	0.017134	1.7661	0.025816	FFD - RD; MD - RD
Xanthine	3.2895	0.024411	1.6124	0.036425	FFD - RD; MD - RD
Sum of PUFA-LysoPCs	3.2866	0.024498	1.6109	0.036425	FFD - RD; KD - RD; MD - RD
2-Hydroxyisovaleric acid	3.2009	0.027232	1.5649	0.040225	KD - FFD; RD - FFD
Ratio of Acyl-Alkyl-Phosphatidylcholines to Choline	3.1756	0.028096	1.5514	0.041012	FFD - RD; MD - RD
Methionine	3.1747	0.028128	1.5509	0.041012	FFD - KD; FFD - MD
Tyramine	3.1436	0.02923	1.5342	0.042274	FFD - RD; MD - RD
Valeric acid + Isovalric acid	3.1398	0.029367	1.5321	0.042274	KD - FFD; KD - RD
N-Acetyl-Tyrosine	3.1192	0.030123	1.5211	0.042945	MD - KD; MD - RD
3-Methyladipic acid	3.1168	0.030214	1.5198	0.042945	KD - FFD
Dimethylamine	3.0703	0.032002	1.4948	0.045203	KD - FFD; RD - FFD
Phenylethylamine	2.9945	0.035143	1.4542	0.049331	RD - FFD; RD - MD

Chapter 4 Appendices

Appendix 4_A

Appendix Table 4-A - The correlation table of BG and plasma most correlated metabolites including the Pearson r correlation coefficient and p-value

Metabolites	r	P
Ratio of carnitine to total acylcarnitines	0.68354	8.00E-05
Sum of glutamic acid and glycine	0.66489	3.09E-08
Glycine	0.65881	4.55E-08
Fraction of long chain acylcarnitines to total acylcarnitines	0.63185	2.30E-07
Sum of methionine, glycine, serine, choline, betaine, sarcosine, dimethylglycine	0.62868	2.75E-07
Sum of non-essential amino acids	0.62783	2.89E-07
Serine	0.62135	4.15E-07
Alanine	0.60574	9.58E-07
Glucose	0.60417	1.04E-06
Proline	0.58719	2.45E-06
2,5-Furandicarboxylic acid	0.56082	8.45E-06
N-Acetyl-Histidine	0.55994	8.79E-06
Fraction of medium chain acylcarnitines to total acylcarnitines	0.55649	1.03E-05
Concentration of polar amino acids	0.53133	3.00E-05
Guanidoacetic acid	0.52271	4.24E-05
Ratio of carnitine to medium chain acylcarnitines	0.50638	8.87E-09
Glutamine	0.49143	0.000139
Concentration of charged amino acids	0.49015	0.000146
Arginine	0.46264	0.000376
Ratio of Cx-DC to total acylcarnitines concentration	0.46253	0.000377
Sum of citrulline and arginine	0.46119	0.000394
Urea cycle amino acids	0.45504	0.000482
Concentration of glucogenic amino acids	0.44134	0.000744
Threonine	0.41759	0.001513
Sum of essential amino acids	-0.40057	0.002441
Butyric acid + isobutyric acid	-0.41363	0.001695
Ratio of sphingomyelins to ceramides	-0.41394	0.00168
Concentration of ketogenic amino acids	-0.41649	0.001562
Glutaric acid	-0.42748	0.001133
alpha-aminoadipic acid	-0.44293	0.000708
3-Methyladipic acid	-0.45673	0.000456
N-acetyl-glycine	-0.45847	0.000431

Tiglylglycine	-0.48947	0.000149
p-Cresol sulfate	-0.49237	0.000134
2-oxoisocaproic acid	-0.49409	0.000126
Argininic acid	-0.49521	0.000121
Dimethylglycine	-0.49569	0.000119
Ratio of sphingomyelins to phosphatidylcholines	-0.49687	0.000114
alpha-aminobutyric acid	-0.50945	7.12E-05
Kynurenic acid	-0.51041	6.86E-05
Urea	-0.54671	1.57E-05
Nudifloramide	-0.54775	1.50E-05
Isoleucine	-0.57243	4.97E-06
Fraction of short chain acylcarnitines to total acylcarnitines	-0.57399	4.61E-06
Total concentration of acylcarnitines	-0.58159	3.22E-06
Acetoacetic acid	-0.58236	3.10E-06
The ratio of esterified carnitine to free carnitine	-0.58271	3.05E-06
Total concentration of saturated fatty acids acylcarnitines	-0.59807	1.42E-06
Ratio of valine to PC ae 32:2	-0.60298	1.11E-06
Uric acid	-0.60703	8.96E-07
Short chain acylcarnitines	-0.61327	6.43E-07
Leucine	-0.62483	3.42E-07
Ratio of isoleucine, leucine and valine to tyrosine	-0.62799	2.86E-07
Indolelactic acid	-0.63194	2.29E-07
Sum of branched-chain amino acids	-0.63358	2.08E-07
Ratio of leucine and isoleucine to PC aa (34:3)	-0.63714	1.69E-07
Valine	-0.64124	1.33E-07
2-Hydroxy-3-methylvaleric acid	-0.65434	6.02E-08
3-Hydroxyisobutyric acid	-0.66368	3.34E-08
2-Hydroxybutyric acid	-0.66454	3.16E-08
2-Hydroxyisovaleric acid	-0.66652	2.78E-08
3-Hydroxybutyric acid	-0.67596	1.49E-08
The ratio of total acylcarnitines to carnitine	-0.68771	6.63E-09
Fischer Ratio	-0.70074	2.58E-09
3-Hydroxyisovaleric acid	-0.72731	3.20E-10

Appendix 4_B

Appendix Table 4-B - The correlation table of BG and most correlated urinary metabolites including the Pearson r correlation coefficient and p.value

Metabolites	r	p
N1-Acetyl-Lysine	0.73041	2.22E-16
Isocitric acid	0.72918	2.22E-16
N-Acetyl-Glutamic acid	0.72362	4.44E-16
4-Hydroxybenzoic acid	0.71485	1.78E-15
N-Acetyl-Asparagine	0.70599	5.33E-15
2-Hydroxyisovaleric acid	0.70518	6.22E-15
Indoxyl glucuronide	0.69953	1.22E-14
Argininic acid	0.69784	1.51E-14
2-Hydroxyisobutyric acid	0.69747	1.58E-14
Tiglylglycine	0.69668	1.73E-14
3-Methyladipic acid	0.69168	3.18E-14
N-Acetyl-Valine	0.68494	7.02E-14
3,4-Dihydroxybutyric acid	0.67483	2.22E-13
alpha-Ketoglutaric acid	0.67452	2.30E-13
2-Hydroxy-2-methylbutyric acid	0.67308	2.70E-13
cis-Aconitic acid	0.66428	7.05E-13
N-Acetyl-Serine	0.65919	1.21E-12
3-Deoxyglucosone	0.65878	1.26E-12
Allantoin	0.65875	1.27E-12
Phenylacetylglutamine	0.64829	3.72E-12
N-Acetyl-Tryptophan	0.64715	4.18E-12
Uric acid	0.63867	9.67E-12
4-Hydroxyphenylacetic acid	0.6348	1.41E-11
3-Hydroxyphenylacetic acid	0.63172	1.89E-11
Succinic acid	0.63033	2.16E-11
Glutaric acid	0.62789	2.71E-11
Total concentration of TCA acids	0.62365	4.02E-11
Total abundance of uremic toxins	0.62278	4.36E-11
N-Acetyl-Proline	0.60445	2.23E-10
Quinoline-4-carboxylic acid	0.60355	2.42E-10
N-Acetyl-Tyrosine	0.58303	1.33E-09
Citric acid	0.58001	1.69E-09
Guanidoacetic acid	0.57928	1.79E-09
p-Hydroxyhippuric acid	0.57912	1.81E-09
Hippuric acid	0.55568	1.08E-08

Caffeic acid	0.55396	1.23E-08
2-Hydroxyphenylace	0.53534	4.57E-08
Glucose	0.5172	1.53E-07
Dimethylglycine	0.48918	8.64E-07
Malonic acid	0.48436	1.15E-06
Pyruvic acid	0.47005	2.59E-06
Glutamic acid	0.46819	2.87E-06
5-Hydroxyindoleacetic acid	0.44285	1.10E-05
N-Acetyl-Methionine	0.42903	2.20E-05
Maleic acid	0.42826	2.29E-05
4-Hydroxyphenylpyruvic acid	0.42249	3.02E-05
3-Aminoisobutyric acid	0.41774	3.79E-05
Valeric acid + Isovaleric acid	0.41059	5.29E-05
Xanthosine	0.40941	5.59E-05
Methylhistidine	-0.41479	4.35E-05
N1-Acetylspermidin	-0.41479	4.35E-05
Serotonin	-0.42473	2.71E-05
Dimethylamine	-0.44159	1.18E-05
Diacetylspermine	-0.45198	6.88E-06
Uracil	-0.46881	2.77E-06
Inosine	-0.50777	2.79E-07
N-Acetylputrescine	-0.52269	1.07E-07
Guanosine	-0.65104	2.82E-12
Adenosine	-0.69935	1.24E-14

Appendix 4_C

Appendix Table 4-C - The correlation table of HR and most correlated plasma metabolites including the Pearson r correlation coefficient and p.value

Metabolites	r	p
Sum of ceramides	0.5027	9.17E-05
Sum of VLCFA-Cer	0.5008	9.86E-05
Sum of Hex2Cer	0.4808	0.0002026
Sum of LCFA-Glycosyl-Cer	0.4729	0.00026667
Caprylic acid	0.4644	0.00035455
Short-chain acylcarnitine concentration	0.4573	0.00044752
alpha-Aminobutyric acid	0.4572	0.00044966
Sum of Saturated Fatty Acid Acylcarnitines	0.4553	0.00047754
Isoleucine	0.4538	0.00050122
Sum of Glycosyl-Cer	0.4496	0.00057366
Sum of essential amino acids	0.4489	0.00058754
Total acylcarnitine concentration	0.4469	0.0006256
Concentration of hydroxylated carnitines	0.4463	0.00063819
Ratio of esterified carnitine to free carnitine	0.445	0.00066459
Concentration of ketogenic amino acids	0.4409	0.00075491
Sum of HexCer	0.4311	0.001017
Sum of Branched-Chain Amino Acids	0.4298	0.0010569
trans-4-Hydroxyproline	0.428	0.0011162
Valine	0.4184	0.0014769
Leucine	0.4168	0.0015486
Sum of Acyl-Alkyl-Phosphatidylcholines	0.408	0.001987
2-Hydroxy-3-methylvaleric acid	0.4052	0.0021498
Fraction of medium-chain acylcarnitines	-0.4539	0.00049991
Indole-3-propionic acid	-0.4876	0.00015963

Appendix 4_D

Appendix Table 4-D - The correlation table of HR and most correlated urine metabolites including the Pearson r correlation coefficient and p.value

Metabolites	r	P
Ethylmalonic acid	0.73094	2.22E-16
2-Hydroxyisobutyric acid	0.72386	4.44E-16
N1-Acetyl-Lysine	0.72327	4.44E-16
N-Acetyl-Glutamic acid	0.72286	4.44E-16
Quinoline-4-carboxylic acid	0.7215	6.66E-16
3-Methyladipic acid	0.71475	1.78E-15
Threonic acid	0.71265	2.22E-15
N-Acetyl-Aspartic acid	0.71264	2.22E-15
Phenylacetylglutamine	0.71194	2.66E-15
3-Hydroxyphenylacetic acid	0.70856	4.00E-15
Xanthine	0.70661	5.11E-15
N-Acetyl-Glutamine	0.70428	6.66E-15
N-Acetyl-Tryptophan	0.69252	2.89E-14
2-hydroxyglutaric acid	0.67892	1.40E-13
4-Hydroxyphenylacetic acid	0.67037	3.64E-13
3,4-Dihydroxybutyric acid	0.66556	6.15E-13
Dimethylglycine	0.66173	9.26E-13
Methylmalonic acid	0.65612	1.67E-12
4-Hydroxybenzoic acid	0.64721	4.15E-12
Orotic acid	0.64367	5.92E-12
N-Acetyl-Serine	0.63466	1.43E-11
Glutaric acid	0.62212	4.63E-11
N-Acetyl-Asparagine	0.61745	7.09E-11
Malonic acid	0.61297	1.06E-10
3-Aminoisobutyric acid	0.6028	2.58E-10
3-Deoxyglucosone	0.59572	4.69E-10
Uric acid	0.58095	1.57E-09
alpha-Ketoglutaric acid	0.57782	2.01E-09
Sum of ADMA, Uric acid, TMAO	0.57261	3.02E-09
N-Acetyl-Proline	0.57194	3.18E-09
Succinic acid	0.56905	3.98E-09
2-Hydroxyphenylacetic acid	0.55821	8.98E-09
3-Hydroxyisovaleric acid	0.55413	1.21E-08
Guanidoacetic acid	0.55334	1.28E-08
Hippuric acid	0.53695	4.09E-08

Caffeic acid	0.53647	4.23E-08
Valeric acid + Isovaleric acid	0.53106	6.12E-08
p-Hydroxyhippuric acid	0.52755	7.74E-08
Allantoin	0.52148	1.16E-07
N-Acetyl-Tyrosine	0.51397	1.88E-07
Sum of citrate, cis-aconitate, alpha-ketoglutarate, succinate, fumarate, malate	0.50638	3.04E-07
Sum of Butyric acid, Isobutyric acid, Propionic acid, Valeric acid, and Isovaleric acid	0.48974	8.36E-07
Sum of Monounsaturated Fatty Acid Acylcarnitines	0.488	9.26E-07
Glucose	0.48523	1.09E-06
Caproic acid	0.46842	2.83E-06
Citric acid	0.45208	6.85E-06
5-Hydroxyindoleacetic acid	0.44566	9.56E-06
N-Acetyl-Methionine	0.44047	1.25E-05
Maleic acid	0.43276	1.83E-05
Butyric acid + Isobutyric acid	0.4316	1.94E-05
Malic acid	0.42098	3.25E-05
Glutamic acid	0.41865	3.63E-05
Ratio of TMAO to Choline	0.41673	3.98E-05
Leucine	0.41278	4.78E-05
Pyruvic acid	0.41051	5.31E-05
Xanthosine	0.39743	9.59E-05
Dimethylamine	-0.43085	2.02E-05
Methylhistidine	-0.44189	1.16E-05
N1-Acetylspermidine	-0.44189	1.16E-05
7-Methylguanine	-0.4549	5.90E-06
Serotonin	-0.45967	4.57E-06
Uracil	-0.48826	9.12E-07
Diacetylspermine	-0.49128	7.63E-07
N-Acetylputrescine	-0.51479	1.79E-07
Inosine	-0.51762	1.49E-07
Guanosine	-0.70297	7.99E-15
Adenosine	-0.71787	1.33E-15

Appendix 4_E

Appendix Table 4-E - The correlation table of average SYS BP and most correlated plasma metabolites including the Pearson r correlation coefficient and p.value

Metabolites	r	p
Ratio of Ornithine to Citrulline	0.38586	0.00362
Lysine	0.37167	0.005207
1,3-Diaminopropane	0.37128	0.005258
Threonine	0.36702	0.005847
trans-4-Hydroxyproline	0.3555	0.007733
Ornithine	0.35518	0.007792
Sum of Saturated Trigylcerides	0.34054	0.010957
Asparagine	0.32521	0.015405
Sum of basic amino acids	0.32077	0.016955
Serine	0.31783	0.018049
Ratio of Phe to PC aa (34:3)	0.30364	0.024218
Total concentration of LysoPCs	-0.30161	0.025234
Sum of Sphingomyelins	-0.30191	0.025081
Sum of Diacyl-Phosphatidylcholines	-0.30507	0.023526
Sum of Long Chain Fatty Acid-LysoPCs	-0.30664	0.022786
Betaine	-0.3091	0.021661
Sarcosine	-0.30973	0.021384
Total concentration of saturated LysoPCs	-0.30999	0.021269
Sum of SFA-LysoPCs	-0.30999	0.021269
Total PC aa concentration	-0.31676	0.018461
Hippuric acid	-0.31917	0.01754
Hydroxylated Sphingomyelins	-0.33266	0.013082
unsaturated Sphingomyelins	-0.33336	0.01288
3-Hydroxyphenylacetic acid	-0.33664	0.011969
Total concentration of biogenic amines	-0.34169	0.010675
Sum of Very Long-Chain Fatty Acid Sphingomyelins	-0.36524	0.006109
Ratio of Citrulline to Ornithine	-0.38586	0.00362
CMPF	-0.39874	0.002567

Appendix 4_F

Appendix Table 4-F - The correlation table of average SYS BP and urine metabolites including the Pearson r correlation coefficient and p.value

Metabolites	r	p
2-Hydroxyisobutyric acid	0.72797	4.44E-16
Orotic acid	0.72346	4.44E-16
3-Methyladipic acid	0.71995	8.88E-16
cis-Aconitic acid	0.71941	8.88E-16
Methylmalonic acid	0.71879	8.88E-16
Phenylacetylglutamine	0.70074	1.07E-14
4-Hydroxybenzoic acid	0.68877	4.49E-14
3-Hydroxyphenylacetic acid	0.68827	4.75E-14
N-Acetyl-Tryptophan	0.68814	4.84E-14
3,4-Dihydroxybutyric acid	0.68552	6.57E-14
N-Acetyl-Asparagine	0.68218	9.68E-14
Quinoline-4-carboxylic acid	0.68159	1.03E-13
N-Acetyl-Serine	0.66676	5.40E-13
4-Hydroxyphenylacetic acid	0.66246	8.57E-13
alpha-Ketoglutaric acid	0.64855	3.63E-12
Glutaric acid	0.64372	5.88E-12
3-Deoxyglucosone	0.62972	2.28E-11
Uric acid	0.62299	4.28E-11
Sum of ADMA, Uric acid, TMAO	0.61133	1.22E-10
Succinic acid	0.60994	1.39E-10
N-Acetyl-Proline	0.60681	1.82E-10
Allantoin	0.59883	3.61E-10
Dimethylglycine	0.5915	6.67E-10
Guanidoacetic acid	0.58151	1.50E-09
Sum of citrate, cis-aconitate, alpha-ketoglutarate, succinate, fumarate, malate	0.57608	2.30E-09
2-Hydroxyphenylacetic acid	0.57124	3.36E-09
N-Acetyl-Tyrosine	0.5689	4.02E-09
p-Hydroxyhippuric acid	0.56727	4.55E-09
Malonic acid	0.56311	6.23E-09
Hippuric acid	0.56245	6.55E-09
Caffeic acid	0.56056	7.54E-09
Citric acid	0.52515	9.08E-08
3-Aminoisobutyric acid	0.5246	9.42E-08
Glucose	0.51445	1.82E-07

3-Hydroxyisovaleric acid	0.48806	9.23E-07
Valeric acid + Isovalric acid	0.47212	2.31E-06
5-Hydroxyindoleacetic acid	0.46128	4.19E-06
Glutamic acid	0.4573	5.19E-06
N-Acetyl-Methionine	0.4516	7.02E-06
Pyruvic acid	0.45151	7.05E-06
Sum of Monounsaturated Fatty Acid Acylcarnitines	0.44598	9.40E-06
Maleic acid	0.43661	1.51E-05
Sum of Butyric acid, Isobutyric acid, Propionic acid, Valeric acid, and Isovaleric acid	0.41996	3.41E-05
4-Hydroxyphenylpyruvic acid	0.41928	3.52E-05
Xanthosine	0.41545	4.22E-05
Malic acid	0.41015	5.40E-05
Caproic acid	0.40662	6.35E-05
Methylhistidine	-0.42353	2.88E-05
N1-Acetylspermidine	-0.42353	2.88E-05
7-Methylguanine	-0.42563	2.60E-05
Dimethylamine	-0.43202	1.90E-05
Serotonin	-0.44122	1.20E-05
Diacetylspermine	-0.48297	1.24E-06
Uracil	-0.48331	1.22E-06
N-Acetylputrescine	-0.51976	1.29E-07
Inosine	-0.52278	1.06E-07
Guanosine	-0.69711	1.64E-14
Adenosine	-0.71036	3.11E-15

Appendix 4_G

Appendix Table 4-G - The correlation table of BT and most correlated plasma metabolites including the Pearson r correlation coefficient and p.value

Metabolites	r	p
Glutamic acid	0.47132	0.000281
Ratio of glutamate to glutamine	0.42093	0.001374
Total concentration of non-hydroxylated Sphingomyelins	-0.40144	0.002384
Sum of long-chain fatty acid sphingomyelins	-0.40412	0.002214
Total concentration of saturated sphingomyelins	-0.40925	0.001919
2-hydroxyisovaleric acid	-0.41291	0.001731
Acetoacetic acid	-0.43312	0.000957

Appendix 4_H

Appendix Table 4-H - The correlation table of BT and urine metabolites including the Pearson r correlation coefficient and p.value

Metabolites	r	p
2-hydroxyglutaric acid	0.73196	2.22E-16
Xanthine	0.73057	2.22E-16
2-Hydroxy-2-methylbutyric acid	0.7273	4.44E-16
2-Hydroxyisobutyric acid	0.72508	4.44E-16
3-Methyladipic acid	0.71851	1.11E-15
N-Acetyl-Valine	0.71565	1.55E-15
Methylmalonic acid	0.71429	1.78E-15
cis-Aconitic acid	0.70932	3.55E-15
Tiglylglycine	0.70879	3.77E-15
Orotic acid	0.7031	7.99E-15
3,4-Dihydroxybutyric acid	0.6999	1.15E-14
Phenylacetylglutamine	0.69387	2.44E-14
3-Hydroxyphenylacetic acid	0.68755	5.17E-14
N-Acetyl-Tyrosine	0.68262	9.19E-14
Quinoline-4-carboxylic acid	0.67244	2.90E-13
N-Acetyl-Asparagine	0.6716	3.18E-13
4-Hydroxybenzoic acid	0.66786	4.79E-13
4-Hydroxyphenylacetic acid	0.66121	9.79E-13
N-Acetyl-Serine	0.65413	2.05E-12
alpha-Ketoglutaric acid	0.64089	7.78E-12
Glutaric acid	0.64002	8.48E-12
3-Deoxyglucosone	0.62323	4.18E-11
N-Acetyl-Proline	0.61563	8.35E-11

Allantoin	0.61355	1.01E-10
Succinic acid	0.61311	1.05E-10
Uric acid	0.59021	7.42E-10
2-Hydroxyphenylacetic acid	0.58509	1.13E-09
Dimethylglycine	0.58395	1.23E-09
Sum of citrate, cis-aconitate, alpha-ketoglutarate, succinate, fumarate, malate	0.57859	1.89E-09
Guanidoacetic acid	0.57136	3.33E-09
Sum of ADMA, Uric acid, TMAO	0.56926	3.91E-09
N-Acetyl-Tyrosine	0.56837	4.19E-09
p-Hydroxyhippuric acid	0.56493	5.43E-09
Hippuric acid	0.55779	9.26E-09
Caffeic acid	0.55686	9.92E-09
Malonic acid	0.54614	2.15E-08
Citric acid	0.52919	6.94E-08
3-Aminoisobutyric acid	0.52171	1.14E-07
Glucose	0.5201	1.27E-07
5-Hydroxyindoleacetic acid	0.4766	1.79E-06
N-Acetyl-Methionine	0.47021	2.57E-06
Valeric acid + Isovalric acid	0.45855	4.85E-06
Pyruvic acid	0.45566	5.67E-06
3-Hydroxyisovaleric acid	0.45557	5.69E-06
Glutamic acid	0.45263	6.65E-06
Xanthosine	0.425	2.68E-05
Sum of Monounsaturated Fatty Acid Acylcarnitines	0.41747	3.84E-05
Maleic acid	0.41692	3.94E-05
4-Hydroxyphenylpyruvic acid	0.4087	5.77E-05
Sum of Butyric acid, Isobutyric acid, Propionic acid, Valeric acid, and Isovaleric acid	0.40184	7.88E-05
Malic acid	0.39696	9.79E-05
Methylhistidine	-0.41553	4.20E-05
N1-Acetylspermidine	-0.41553	4.20E-05
Dimethylamine	-0.41969	3.46E-05
Diacetylspermine	-0.44653	9.14E-06
Uracil	-0.44727	8.80E-06
Inosine	-0.47799	1.66E-06
N-Acetylputrescine	-0.49236	7.15E-07
Adenosine	-0.64652	4.45E-12
Guanosine	-0.65953	1.17E-12

Appendix 4_I

Appendix Table 4-I - The correlation table of weight and most correlated plasma metabolites including the Pearson r correlation coefficient and p.value

Metabolites	r	p
Long-chain Acylcarnitines	0.54849	1.46E-05
Sum of Unsaturated Diglycerides	0.52277	4.23E-05
Sum of Diglyceride	0.48483	0.000176
Fraction of long-chain Acylcarnitines	0.42927	0.001074
Sum of Saturated Diglycerides	0.40744	0.002019
Ratio of Homoarginine to ADMA	-0.40394	0.002226
Ratio of carnitine to long-chain Acylcarnitines	-0.42208	0.001328
CMPF	-0.46661	0.00033
Orotic acid	-0.47809	0.000223
Cystathionine	-0.48572	0.000171
Deoxyuridine	-0.48572	0.000171
Salicylic acid	-0.57173	5.13E-06

Appendix 4_J

Appendix Table 4-J - The correlation table of weight and urine metabolites including the Pearson r correlation coefficient and p.value

Metabolites	r	p
2-Hydroxy-2-methylbutyric acid	0.73203	2.22E-16
2-Hydroxyisobutyric acid	0.72782	4.44E-16
Tiglylglycine	0.72728	4.44E-16
Orotic acid	0.71641	1.33E-15
cis-Aconitic acid	0.71427	1.78E-15
3-Methyladipic acid	0.71286	2.22E-15
Methylmalonic acid	0.71041	3.11E-15
Phenylacetylglutamine	0.70114	9.99E-15
3-Hydroxyphenylacetic acid	0.69473	2.20E-14
N-Acetyl-Tryptophan	0.68895	4.40E-14
Quinoline-4-carboxylic acid	0.68415	7.68E-14
3,4-Dihydroxybutyric acid	0.68293	8.86E-14
4-Hydroxybenzoic acid	0.68269	9.10E-14
N-Acetyl-Asparagine	0.67052	3.58E-13
4-Hydroxyphenylacetic acid	0.66432	7.03E-13
N-Acetyl-Serine	0.65175	2.62E-12
alpha-Ketoglutaric acid	0.64096	7.73E-12

Glutaric acid	0.6397	8.75E-12
3-Deoxyglucosone	0.62512	3.51E-11
Uric acid	0.613	1.06E-10
Succinic acid	0.61223	1.13E-10
N-Acetyl-Proline	0.60437	2.25E-10
Sum of ADMA, Uric acid, TMAO	0.60085	3.04E-10
Allantoin	0.59723	4.14E-10
Dimethylglycine	0.59134	6.75E-10
Guanidoacetic acid	0.5725	3.05E-09
p-Hydroxyhippuric acid	0.56989	3.73E-09
2-Hydroxyphenylacetic acid	0.56858	4.12E-09
Sum of citrate, cis-aconitate, alpha-ketoglutarate, succinate, fumarate, malate	0.56857	4.12E-09
N-Acetyl-Tyrosine	0.56618	4.95E-09
Hippuric acid	0.5654	5.25E-09
Caffeic acid	0.56352	6.05E-09
Malonic acid	0.55883	8.58E-09
3-Aminoisobutyric acid	0.52568	8.77E-08
Citric acid	0.51763	1.49E-07
Glucose	0.51263	2.05E-07
3-Hydroxyisovaleric acid	0.48253	1.27E-06
Valeric acid + Isovalric acid	0.48094	1.40E-06
5-Hydroxyindoleacetic acid	0.46031	4.42E-06
N-Acetyl-Methionine	0.45191	6.91E-06
Glutamic acid	0.44772	8.59E-06
Sum of Monounsaturated Fatty Acid Acylcarnitines	0.44566	9.56E-06
Pyruvic acid	0.44416	1.03E-05
Maleic acid	0.43358	1.76E-05
Sum of Butyric acid	0.42837	2.28E-05
Caproic acid	0.42037	3.35E-05
Xanthosine	0.41291	4.75E-05
4-Hydroxyphenylpyruvic acid	0.40729	6.15E-05
Malic acid	0.40541	6.71E-05
Dimethylamine	-0.4313	1.97E-05
Methylhistidine	-0.4314	1.96E-05
N1-Acetylspermidine	-0.4314	1.96E-05
7-Methylguanine	-0.4346	1.67E-05
Serotonin	-0.4458	9.50E-06
Uracil	-0.4832	1.23E-06

Diacetylspermine	-0.4884	9.07E-07
N-Acetylputrescine	-0.5186	1.39E-07
Inosine	-0.5274	7.82E-08
Guanosine	-0.7031	7.99E-15
Adenosine	-0.7165	1.33E-15

Appendix 4_K

Appendix Table 4-K - The correlation table of total mood disturbance scores and plasma metabolites including the Pearson r correlation coefficient and p.value.

Metabolites	r	p
trans-4-Hydroxyproline	0.83517	4.61E-06
Ratio of Acyl-Alkyl-PCs to Diacyl-PCs	0.8066	1.74E-05
N-Acetyl-Aspartic	0.796	2.70E-05
Sum of citrate, cis-aconitate, alpha-ketoglutarate, succinate, fumarate, malate	0.68807	0.000798
Citric acid	0.67048	0.001216
Concentration of ketogenic Amino Acids	0.60492	0.004717
Trimethylamine N-Oxide	0.59324	0.005831
Sum of Acyl-Alkyl-Phosphatidylcholines	0.58344	0.006925
Glutaric acid	0.5677	0.00903
Sum of HexCer	0.552	0.011621
Ratio of Acyl-Alkyl-PCs to Diacyl-PCs	0.54828	0.012314
alpha-Ketoglutaric acid	0.54516	0.012922
Homovanillic acid	0.54302	0.013354
Sum of Glycosyl-Cer	0.53931	0.014129
Sum of LCFA-Glycosyl-Cer	0.53504	0.015065
Sum of LCFA-Cer	0.52463	0.017555
Long-chain Acs	0.52245	0.018115
alpha-Aminobutyric acid	0.50208	0.024082
Sum of Essential Amino acids	0.50117	0.024381
Lysine	0.49436	0.026713
Sum of Hex2Cer	0.48408	0.030562
Sum of ceramides	0.47615	0.033816
Ethylmalonic acid	0.47395	0.034765
Ornithine	0.45394	0.04438
2-oxoisocaproic acid	0.45028	0.046345
3-Hydroxyisobutyric acid	0.44821	0.047482
VLCFA-Glycosyl-Cer	0.43316	0.056418
Malic acid	0.43257	0.056788
Succinic acid	0.4297	0.058642
Kynurenine	-0.4286	0.059362
Methionine sulfoxide	-0.51423	0.020362
N2-Acetyl-Ornithine	-0.54556	0.012843
Hippuric acid	-0.54733	0.012498
Sum of Indoles	-0.66443	0.001396

Indole-3-propionic acid	-0.78284	4.50E-05

Appendix 4_L

Appendix Table 4-L - The correlation table of POMS and urine metabolites including the Pearson r correlation coefficient and p.value

Metabolites	r	P
Ratio of carnitine to total acylcarnitines	0.68354	8.00E-05
Sum of glutamic acid and glycine	0.66489	3.09E-08
Glycine	0.65881	4.55E-08
Fraction of long chain acylcarnitines to total acylcarnitines	0.63185	2.30E-07
Sum of methionine, glycine, serine, choline, betaine, sarcosine, dimethylglycine	0.62868	2.75E-07
Sum of non-essential amino acids	0.62783	2.89E-07
Serine	0.62135	4.15E-07
Alanine	0.60574	9.58E-07
Glucose	0.60417	1.04E-06
Proline	0.58719	2.45E-06
2,5-Furandicarboxylic acid	0.56082	8.45E-06
N-Acetyl-Histidine	0.55994	8.79E-06
Fraction of medium chain acylcarnitines to total acylcarnitines	0.55649	1.03E-05
Ratio of proline to citrulline	0.55495	1.10E-05
Concentration of polar amino acids	0.53133	3.00E-05
Guanidoacetic acid	0.52271	4.24E-05
Ratio of carnitine to medium chain acylcarnitines	0.50638	8.87E-09
Glutamine	0.49143	0.000139
Concentration of charged amino acids	0.49015	0.000146
Arginine	0.46264	0.000376
Ratio of Cx-DC to total acylcarnitines concentration	0.46253	0.000377
Sum of citrulline and arginine	0.46119	0.000394
Urea cycle amino acids	0.45504	0.000482
Concentration of glucogenic amino acids	0.44134	0.000744
Threonine	0.41759	0.001513
Sum of essential amino acids	-0.40057	0.002441
Butyric acid + isobutyric acid	-0.41363	0.001695
Ratio of sphingomyelins to ceramides	-0.41394	0.00168
Concentration of ketogenic amino acids	-0.41649	0.001562
Glutaric acid	-0.42748	0.001133
alpha-aminoadipic acid	-0.44293	0.000708
3-Methyladipic acid	-0.45673	0.000456
N-acetyl-glycine	-0.45847	0.000431

Tiglylglycine	-0.48947	0.000149
p-Cresol sulfate	-0.49237	0.000134
2-oxoisocaproic acid	-0.49409	0.000126
Argininic acid	-0.49521	0.000121
Dimethylglycine	-0.49569	0.000119
Ratio of sphingomyelins to phosphatidylcholines	-0.49687	0.000114
alpha-aminobutyric acid	-0.50945	7.12E-05
Kynurenic acid	-0.51041	6.86E-05
Urea	-0.54671	1.57E-05
Nudifloramide	-0.54775	1.50E-05
Isoleucine	-0.57243	4.97E-06
Fraction of short chain acylcarnitines to total acylcarnitines	-0.57399	4.61E-06
Total concentration of acylcarnitines	-0.58159	3.22E-06
Acetoacetic acid	-0.58236	3.10E-06
The ratio of esterified carnitine to free carnitine	-0.58271	3.05E-06
Total concentration of saturated fatty acids acylcarnitines	-0.59807	1.42E-06
Ratio of valine to PC ae 32:2	-0.60298	1.11E-06
Uric acid	-0.60703	8.96E-07
Short chain acylcarnitines	-0.61327	6.43E-07
Leucine	-0.62483	3.42E-07
Ratio of isoleucine, leucine, and valine to tyrosine	-0.62799	2.86E-07
Indolelactic acid	-0.63194	2.29E-07
Sum of branched-chain amino acids	-0.63358	2.08E-07
Ratio of leucine a isoleucine to PC aa (34:3)	-0.63714	1.69E-07
Valine	-0.64124	1.33E-07
2-Hydroxy-3-methylvaleric acid	-0.65434	6.02E-08
3-Hydroxyisobutyric acid	-0.66368	3.34E-08
2-Hydroxybutyric acid	-0.66454	3.16E-08
2-Hydroxyisovaleric acid	-0.66652	2.78E-08
3-Hydroxybutyric acid	-0.67596	1.49E-08
The ratio of total acylcarnitines to carnitine	-0.68771	6.63E-09
Fischer Ratio	-0.70074	2.58E-09
3-Hydroxyisovaleric acid	-0.72731	3.20E-10

Appendix 4_M

Appendix Table 4-M - The correlation table of total MCA and plasma metabolites including the Pearson r correlation coefficient and p.value.

Metabolites	r	P
Malonic acid	0.6133866	0.0018547
Argininic acid	0.5856964	0.0033202
Uric acid	0.5605843	0.0053957
Indolelactic acid	0.5153993	0.0118362
Hippuric acid	0.4737422	0.0223993
Dimethylglycine	0.4676176	0.0244494
Kynurenic acid	0.4396563	0.0357994
2-oxoisocaproic acid	0.4370464	0.0370414
Sum of LCFA-DH-Cer	-0.414035	0.0495211
Glucose	-0.417237	0.0476122
trans-4-Hydroxyproline	-0.430423	0.0403464
Concentration of polar Amino Acids	-0.435503	0.0377918
Arginine	-0.436954	0.0370858
Lysine	-0.444636	0.0335209
Ratio of Proline to Citrulline	-0.447343	0.0323314
Concentration of glucogenic Amino Acids	-0.460514	0.0270129
Sum of Citrulline and Arginine	-0.461971	0.0264701
Ornithine	-0.46311	0.0260523
Sum of Glutamic acid and Glycine	-0.480753	0.0202247
Sum of Non-Essential Amino Acids	-0.494586	0.0164342
Sum of basic amino acids	-0.496295	0.0160091
Concentration of charged Amino Acids	-0.503488	0.0143163
Sum of Methionine, Glycine, Serine, Choline, Betaine, Sarcosine, Dimethylglycine	-0.513482	0.0122098
Proline	-0.528346	0.0095521
Glycine	-0.555148	0.0059647
Urea cycle Amino Acids	-0.569254	0.0045824
Serine	-0.583623	0.003461

Appendix 4_N

Appendix Table 4-N - The correlation table of total MCA and urine metabolites including the Pearson r correlation coefficient and p.value.

Metabolites	r	P
Caproic acid	0.6090457	3.57E-08
3-Hydroxybutyric acid	0.5101451	8.83E-06
Acetoacetic acid	0.5025829	1.26E-05
Quinoline-4-carboxylic acid	0.5005487	1.38E-05
Creatine	0.4989964	1.48E-05
Guanidinopropionic acid	0.4777835	3.78E-05
3-Hydroxyisobutyric acid	0.4762406	4.04E-05
2-oxoisocaproic acid	0.4572554	8.85E-05
3-Hydroxyisovaleric acid	0.408641	0.0005409
alpha-Ketoisovaleric acid	0.3906504	0.0009892
3-Hydroxyphenylacetic acid	0.390387	0.0009977
Malonic acid	0.3850068	0.0011872
Methylamine	0.3733444	0.0017137
2-Hydroxybutyric acid	0.3721969	0.0017755
N-Acetyl-Glycine	0.3649453	0.0022141
beta-Alanine	0.3482569	0.0036113
N-Acetyl-Leucine	0.3446248	0.0040035
Fumaric acid	0.3390924	0.0046735
2-Hydroxyphenylacetic acid	0.3242	0.0069941
2-Hydroxy-3-methylvaleric acid	0.3231458	0.0071914
N2-Acetyl-Ornithine	0.3104993	0.009966
N-Acetyl-Valine	0.3083635	0.0105167
Butyric acid + Isobutyric acid	0.3055289	0.0112883
Uridine	-0.300973	0.0126314
Aspartic acid	-0.304883	0.0114709
Glutamic acid	-0.313596	0.0092122
4-Hydroxybenzoic acid	-0.317362	0.0083626
7-Methylguanine	-0.32272	0.0072725
Sum of Long-Chain Fatty Acid Sphingomyelins	-0.329958	0.0059983
6Total concentration of saturated Sphingomyelins	-0.331575	0.0057421
8Total concentration of non-hydroxylated Sphingomyelins	-0.332836	0.0055491
1Total concentration of hydroxyproline	-0.333742	0.005414
trans-4-Hydroxyproline	-0.335016	0.0052289
Ornithine	-0.336198	0.0050622
Sum of Sphingomyelins	-0.338947	0.0046925

Kynurenine	-0.341688	0.0043477
Sum of Saturated Fatty Acid Phosphatidylcholines	-0.342534	0.0042459
5Total concentration of unsaturated Sphingomyelins	-0.344981	0.0039634
7Total concentration of hydroxylated Sphingomyelins	-0.355787	0.0029052
Glucose	-0.35822	0.002705
Xanthine	-0.360261	0.0025466
Sum of Very Long-Chain Fatty Acid Sphingomyelins	-0.375028	0.0016266
Sum of citrate, cis-aconitate, alpha-ketoglutarate, succinate, fumarate, malate	-0.378079	0.0014789
Choline	-0.381836	0.0013136
Spermidine	-0.386428	0.0011342
Citric acid	-0.411378	0.000492
2,5-Furandicarboxylic acid	-0.411583	0.0004885
Sum of Choline and Choline-Based Lipids	-0.424861	0.0003047
Serine	-0.429787	0.0002545
Concentration of ketogenic and glucogenic Amino Acids	-0.430102	0.0002516
Sum of Amino Acids	-0.432992	0.0002261
Ethanolamine	-0.436109	0.0002012
Concentration of glucogenic Amino Acids	-0.438155	0.0001863
Tyrosine	-0.452873	0.0001054
Proline	-0.457627	8.72E-05
Tryptophan	-0.46879	5.53E-05
1Concentration of hydrophobic Amino Acids	-0.475538	4.16E-05
Asparagine	-0.483866	2.91E-05
Alanine	-0.485892	2.66E-05
Sum of aromatic amino acids	-0.486996	2.54E-05
Phenylalanine	-0.487239	2.51E-05
Glycine	-0.487363	2.50E-05
Sum of Glutamic acid and Glycine	-0.488858	2.34E-05
Ratio of Non-Essential to Essential Amino Acids	-0.489736	2.25E-05
N-Acetyl-Histidine	-0.518648	5.88E-06
Allantoin	-0.535516	2.53E-06
Sum of Non-Essential Amino Acids	-0.537204	2.32E-06
Medium-chain acylcarnitine concentration	-0.542443	1.77E-06
Concentration of polar Amino Acids	-0.573081	3.27E-07
Glutamine	-0.600582	6.16E-08
Orotic acid	-0.624884	1.23E-08
Threonine	-0.642873	3.40E-09

Appendix 4_O

Appendix Table 4-O – The correlation analysis of microbiome and other physiological measures.

Bacteria		BG	BT	HR	BP SYS	BP DIA	Sleep quality score
Prevotella	r	0.849	-0.991	-0.915	0.278	-0.045	0.611
	p	0.151	0.009	0.085	0.722	0.955	0.389
Bacteroides	r	-0.946	0.972	0.808	-0.381	0.126	-0.596
	p	0.054	0.028	0.192	0.619	0.874	0.404
Faecalibacterium	r	-0.968	0.951	0.764	-0.460	0.064	-0.527
	p	0.032	0.049	0.236	0.540	0.936	0.473
Holdemanella	r	-0.141	0.629	0.874	0.358	0.037	-0.582
	p	0.859	0.371	0.126	0.642	0.963	0.418
Bifidobacterium	r	0.944	-0.922	-0.749	0.558	0.137	0.385
	p	0.056	0.078	0.251	0.442	0.863	0.615
Ruminococcus	r	-0.899	0.611	0.316	-0.892	-0.394	0.060
	p	0.101	0.389	0.684	0.108	0.606	0.940
Blautia	r	0.175	-0.559	-0.765	-0.053	0.348	0.261
	p	0.825	0.441	0.235	0.947	0.652	0.739
Collinsella	r	0.646	-0.190	0.190	0.611	-0.171	-0.013
	p	0.354	0.810	0.810	0.389	0.829	0.987
Romboutsia	r	0.737	-0.537	-0.254	0.224	-0.588	0.545
	p	0.263	0.463	0.746	0.776	0.412	0.455
Coprococcus	r	-0.679	0.256	-0.118	-0.565	0.240	-0.079
	p	0.321	0.744	0.882	0.435	0.760	0.921
Phascolarctobacterium	r	-0.998	0.837	0.572	-0.653	-0.046	-0.331
	p	0.002	0.163	0.428	0.347	0.954	0.669
Ruminococcus2	r	0.310	0.226	0.544	0.882	0.535	-0.700
	p	0.690	0.774	0.456	0.118	0.465	0.300
Dorea	r	0.782	-0.531	-0.293	0.916	0.610	-0.215
	p	0.218	0.469	0.707	0.084	0.390	0.785
Gemmiger	r	0.793	-0.343	0.040	0.837	0.131	-0.115
	p	0.207	0.657	0.960	0.163	0.869	0.885
Lachnospiracea incertae sedis	r	-0.264	-0.017	-0.157	-0.861	-0.946	0.721
	p	0.736	0.983	0.843	0.139	0.054	0.279
Fusicatenibacter	r	-0.958	0.655	0.318	-0.749	-0.041	-0.172
	p	0.042	0.345	0.682	0.251	0.959	0.828
Clostridium XVIII	r	-0.265	0.569	0.632	0.586	0.884	-0.982
	p	0.735	0.431	0.368	0.414	0.116	0.018
Roseburia	r	0.526	-0.698	-0.734	0.389	0.520	0.127
	p	0.474	0.302	0.266	0.611	0.480	0.873
	r	0.738	-0.416	-0.077	0.414	-0.420	0.321

Clostridium sensu stricto	p	0.262	0.584	0.923	0.586	0.580	0.679
Eubacterium	r	-0.114	-0.229	-0.477	-0.045	0.567	-0.066
	p	0.886	0.771	0.523	0.955	0.433	0.934
Intestinibacter	r	0.851	-0.437	-0.066	0.859	0.175	-0.076
	p	0.149	0.563	0.934	0.141	0.825	0.924
Clostridium XIVa	r	-0.948	0.715	0.401	-0.544	0.229	-0.402
	p	0.052	0.285	0.599	0.456	0.771	0.598
Parabacteroides	r	-0.391	0.494	0.530	-0.488	-0.727	0.139
	p	0.609	0.506	0.470	0.512	0.273	0.861
Anaerostipes	r	0.313	-0.758	-0.917	-0.482	-0.504	0.915
	p	0.687	0.242	0.083	0.518	0.496	0.085
Parasutterella	r	-0.792	0.948	0.901	-0.331	-0.132	-0.487
	p	0.208	0.052	0.099	0.669	0.868	0.513
Murimonas	r	-0.914	0.574	0.238	-0.873	-0.260	0.021
	p	0.086	0.426	0.762	0.127	0.740	0.979
Turicibacter	r	0.343	-0.755	-0.927	-0.135	0.074	0.543
	p	0.657	0.245	0.073	0.865	0.926	0.457
Senegalimassilia	r	-0.823	0.994	0.937	-0.218	0.087	-0.653
	p	0.177	0.006	0.063	0.782	0.913	0.347
Eisenbergiella	r	-0.885	0.700	0.411	-0.371	0.422	-0.536
	p	0.115	0.300	0.589	0.629	0.578	0.464
Lactobacillus	r	0.100	-0.345	-0.398	-0.666	-0.977	0.894
	p	0.900	0.655	0.602	0.334	0.023	0.106
Other	r	-0.962	0.865	0.651	-0.662	-0.197	-0.283
	p	0.038	0.135	0.349	0.338	0.803	0.717
Streptococcus	r	0.446	-0.382	-0.209	-0.177	-0.862	0.696
	p	0.554	0.618	0.791	0.823	0.138	0.304
Intestinimonas	r	0.100	-0.345	-0.398	-0.666	-0.977	0.894
	p	0.900	0.655	0.602	0.334	0.023	0.106
Alistipes	r	-0.492	0.539	0.526	-0.581	-0.721	0.138
	p	0.508	0.461	0.474	0.419	0.279	0.862
clostridium IV	r	-0.956	0.950	0.760	-0.366	0.214	-0.623
	p	0.044	0.050	0.240	0.634	0.786	0.377
Insolitispirillum	r	0.576	-0.047	0.334	0.850	0.235	-0.370
	p	0.424	0.953	0.666	0.150	0.765	0.630
Barnesiella	r	0.576	-0.047	0.334	0.850	0.235	-0.370
	p	0.424	0.953	0.666	0.150	0.765	0.630
Oscillibacter	r	0.280	-0.557	-0.697	0.183	0.527	0.099
	p	0.720	0.443	0.303	0.817	0.473	0.901
Enterobacter	r	-0.956	0.950	0.760	-0.366	0.214	-0.623
	p	0.044	0.050	0.240	0.634	0.786	0.377
Haemophilus	r	-0.956	0.950	0.760	-0.366	0.214	-0.623

	p	0.044	0.050	0.240	0.634	0.786	0.377
Odoribacter	r	-0.956	0.950	0.760	-0.366	0.214	-0.623
	p	0.044	0.050	0.240	0.634	0.786	0.377

Appendix 4_P

Appendix Table 4-P - The correlation table of the top 50 most significant correlations between cytokines and plasma metabolites including the Pearson r correlation coefficient and p.value

Cytokine	Metabolite	r	p
IL-8	Glucose	0.962722	3.18E-05
IL-8	Concentration of hydroxylated carnitines	0.952506	7.34E-05
IL-8	PUSFA Acs	0.926508	0.00033
IL-1β	Salicylic acid	0.911194	0.000629
IL-13	Concentration of unsaturated carnitines	0.90602	0.000763
IL-6	Concentration of hydroxylated carnitines	0.891474	0.001245
IL-1β	Ratio of LysoPC (20:4) to PC aa (32:0)	0.88706	0.001425
IL-13	Medium-chain Acs	0.87162	0.002197
IL-13	PUSFA Acs	0.871305	0.002215
IL-6	PUSFA Acs	0.864037	0.002665
IL-8	Medium-chain Acs	0.863517	0.002699
IL-10	Uric acid	0.8621	0.002794
IL-8	Sum of PCs	0.861669	0.002824
IL-8	Sum of Choline and Choline-Based Lipids	0.861079	0.002865
IL-13	Long-chain Acs	0.860292	0.002919
IL-8	Sum of Polyunsaturated FA PCs	0.858622	0.003038
IL-8	Sum of Diacyl-Phosphatidylcholines	0.850833	0.003636
IL-8	Sum of Monounsaturated Fatty Acid Phosphatidylcholines	0.849109	0.003779
IL-13	5Total concentration of saturated LysoPCs	0.848448	0.003835
IL-13	Sum of SFA-LysoPCs	0.848448	0.003835
IL-8	Total PC ae concentration	0.842805	0.004333
IFN-γ	Dimethylglycine	0.841777	0.004428
IL-4	6Total concentration of unsaturated LysoPCs	0.8401	0.004587
IL-4	Sum of PUFA-LysoPCs	0.837161	0.004874
IL-13	Sum of MUFA-LysoPCs	0.836901	0.0049
IL-13	4Total concentration of LysoPCs	0.831437	0.005468
IL-10	Ratio of Proline to Citrulline	-0.835795	0.005011
TNF-α	Sum of basic amino acids	-0.845566	0.004084
IL-2	2-oxoadipic acid	-0.847564	0.00391
IFN-γ	C0 to C5OH	-0.848253	0.003851
IL-10	Histidine	-0.850571	0.003658
IL-12p70	Xanthine	-0.854139	0.003373

IL-12p70	Ratio of Acyl-Alkyl-PCs to Diacyl-PCs	-0.864322	0.002646
TNF-α	Sum of Amino Acids	-0.866174	0.002527
IL-10	Sum of Non-Essential Amino Acids	-0.86689	0.002481
TNF-α	Ratio of Diacyl-PCs to Choline	-0.86706	0.002471
IL-10	2-oxoadipic acid	-0.873067	0.002114
IL-12p70	p-Cresol sulfate	-0.875131	0.002001
IL-10	Proline	-0.877694	0.001866
IL-10	Concentration of glucogenic Amino Acids	-0.878024	0.001849
IL-10	Threonine	-0.880174	0.001741
IL-4	5-Methyluridine	-0.88784	0.001392
IL-10	Glycine	-0.888632	0.001359
IL-10	Concentration of charged Amino Acids	-0.902033	0.000879
IL-10	very long-chain LysoPCs	-0.906258	0.000757
IL-2	Lysine	-0.908595	0.000695
IL-10	Sum of Glutamic acid and Glycine	-0.910094	0.000656
IL-2	Pyruvic acid	-0.91898	0.00046
TNF-α	1,3-Diaminopropane	-0.943344	0.000135
IL-2	N-Acetyl-Alanine	-0.981522	2.77E-06

Appendix 4_Q

Appendix Table 4-Q - The correlation table of the top 50 most significant correlations between cytokines and urine metabolites including the Pearson r correlation coefficient and p.value.

Cytokine	Metabolite	R Value	P Value
IL-8	Spermidine	0.900129808	4.99E-09
IL-1	p-Hydroxyhippuric acid	0.868983405	7.49E-08
IL-1β	Caffeic acid	0.855342276	1.99E-07
IL-1β	Hippuric acid	0.846885872	3.47E-07
IL-1β	Indole-3-propionic acid	0.826337508	1.18E-06
TNF-α	Caproic acid	0.815558419	2.12E-06
IFN-γ	Acetoacetic acid	0.801221729	4.35E-06
IL-1β	4-Hydroxyphenylacetic acid	0.7947224	5.91E-06
IFN-γ	3-Hydroxyisobutyric acid	0.793832149	6.16E-06
IFN-γ	3-Hydroxybutyric acid	0.792232191	6.63E-06
IL-12p70	3-Methoxytyramine	0.775842397	1.36E-05
IFN-γ	Caproic acid	0.772067233	1.59E-05
IFN-γ	Guanidinopropionic acid	0.769253564	1.79E-05
IL-1β	2-hydroxyglutaric acid	0.763131514	2.28E-05

IFN-γ	2-oxoisocaproic acid	0.755252353	3.10E-05
IL-1β	N-Acetyl-Tyrosine	0.752027077	3.50E-05
IFN-γ	Sum of Butyric acid, Isobutyric acid, Propionic acid, Valeric acid, and Isovaleric acid	0.748960985	3.93E-05
IL-12p70	Succinic acid	0.747439357	4.15E-05
IFN-γ	3-Hydroxyisovaleric acid	0.737863871	5.86E-05
IL-1β	Succinic acid	0.734903836	6.50E-05
IL-10	Quinaldic acid	0.732540638	7.05E-05
IL-8	2Total concentration of biogenic amines	0.729787247	7.74E-05
IFN-γ	Creatine	0.72566899	8.90E-05
IL-1β	Threonic acid	0.720815777	0.000104427
IFN-γ	alpha-Ketoisovaleric acid	0.720521984	0.000105434
IFN-γ	Butyric acid + Isobutyric acid	0.719320996	0.000109641
IFN-γ	N-Acetyl-Leucine	0.703720096	0.000179148
IL-10	3-Hydroxyphenylacetic acid	0.7004808	0.000197618
IL-10	Caproic acid	0.699667653	0.000202506
IFN-γ	2-Hydroxybutyric acid	0.698128156	0.000212049
IL-1β	alpha-Ketoglutaric acid	0.692587179	0.000249698
IFN-γ	Valeric acid + Isovalric acid	0.690143933	0.000268059
IL-8	3Total concentration of polyamines	0.689031314	0.0002768
IL-1β	3-Methoxytyramine	0.686562026	0.000297087
IFN-γ	Propionic acid	0.676473274	0.000393935
IL-1β	Uracil	0.671207321	0.000454533
IL-1β	Pyruvic acid	0.665881825	0.00052383
IL-2	N-Acetyl-Proline	0.664391128	0.000544786
IL-8	4Total concentration of monamine neurotransmitters	0.663850657	0.00055256
IL-8	Histamine	0.662655982	0.000570084
IL-12p70	N-Acetyl-Proline	0.658376811	0.000636836
IFN-γ	Glyceric acid	0.657253862	0.000655428
IL-1β	Inosine	0.65624336	0.000672555
IL-1β	N-Acetyl-Proline	0.653384646	0.000723106
IFN-γ	2-Hydroxy-3-methylvaleric acid	0.649454215	0.000797915
IL-10	Alanine	-0.649443227	0.000798133
IL-10	Ratio of Non-Essential to Essential Amino Acids	-0.656369841	0.00067039
IFN-γ	Orotic acid	-0.668493283	0.00048879
IFN-γ	Ratio of Non-Essential to Essential Amino Acids	-0.691304843	0.000259194
IL-10	N-Acetyl-Histidine	-0.726067295	8.78E-05