Effects of a High-Protein Diet Replacement on Energy Homeostasis in Healthy Adults

by

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ABSTRACT

Lifestyle modifications that induce energy deficit, such as diet and physical activity are considered the cornerstone of weight management. The overall purpose of this research was to compare the effects of an acute nutritional intervention comprised of a high-protein (HP) diet replacement versus a standard North American dietary pattern on selected components of energy metabolism, metabolic blood markers, appetite sensations, and appetite-related hormones in healthy, normal-weight adults of both sexes.

Three studies are presented as part of two complementary randomized, controlled, cross-over clinical trials conducted separately in men and women. Two studies explored the impact individuals undergoing two isocaloric nutrition interventions: a) high-protein total diet replacement (HP-TDR): 35% carbohydrate, 40% protein, and 25% fat achieved through a nutritional supplement; b) control (CON): 55% carbohydrate, 15% protein, and 30% fat. Participants received the prescribed diets for 32 hours while inside a whole-body calorimetry unit (WBCU). The first dietary intervention randomly offered in the WBCU was designed to maintain energy balance and the second matched what was offered during the first stay.

The last study was a sub-analysis involving the isocaloric breakfasts during the WBCU stay: a) high-protein meal replacement (HP-MR): 30% carbohydrate, 43% protein, and 27% fat achieved through a nutritional supplement; b) CON: 55% carbohydrate, 15% protein, and 30% fat. Following the breakfast, participants performed a moderate-intensity aerobic exercise session. The following physiological changes were compared between groups: energy expenditure, energy balance, macronutrient oxidation rates and balances, metabolic blood markers, appetite sensations, and appetite-related hormones. Body composition was assessed at baseline using dual-energy X-ray absorptiometry.

In total, forty-three healthy, normal-weight adults (56% males) were included. Compared to the CON diet, the HP-TDR produced higher total energy expenditure (HP-TDR: 2143 ± 268 kcal/day; CON: 2061 ± 243 kcal/day; $p<0.001$), protein (HP-TDR: 91 ± 40 g/day; CON: 53 ± 20 g/day; $p<0.001$) and fat oxidation rates (HP-TDR: 79 ± 17 g/day; CON: 71 ± 16; $p=0.013$), and lower carbohydrate oxidation rate (HP-TDR: -48 ± 33 g/day; CON: 22 ± 26 g/day; $p<0.001$). Moreover, a HP-TDR led to a lower energy (-112 ± 85 kcal/day; $p<0.001$), fat (-22 ± 20 g/day; $p<0.001$), and carbohydrate balances (-69 ± 44 g/day; $p<0.001$), and higher protein balance (90 ± 32 g/day; $p<0.001$).

In the HP-TDR, only females experienced lower 24-h area under the curve for prospective food consumption, and higher composite satiety score after breakfast day 1, before lunch, and before dinner. Compared to the CON diet, the change in appetite-related hormones from fasting day 1 to fasting day 2 during the HP-TDR intervention was smaller for peptide tyrosine-tyrosine (PYY) and greater for leptin. Moreover, postprandial levels of glucagon-like peptide 1 (GLP-1) and PYY were higher in the HP-TDR.

In the last study, compared to the CON breakfast, the HP-MR produced higher fat oxidation (1.07 ± 0.33 g/session; $p=0.003$) and lower carbohydrate oxidation (-2.32 ± 0.98 g/session; $p=0.023$) and respiratory exchange ratio (-0.01 ± 0.00; $p=0.003$) during the exercise. After the exercise, increases in hunger were lower during the HP-MR condition. Changes in blood markers from the fasting state to post-exercise during the HP-MR condition were greater for insulin, PYY, and GLP-1, and lower for low-density lipoprotein cholesterol, triglyceride, and glycerol.

The major findings of this thesis were that, compared to a North American dietary pattern, a HP diet replacement improved selected components of energy metabolism favoring body weight and fat losses at rest and during exercise, partly improved individual's metabolic profile, and

elicited changes in appetite sensations and appetite-related hormones that reflected decrease hunger and increased satiety. Additionally, females and males responded differently to the dietary interventions with respect to appetite sensations and appetite-related hormones. Overall, females' response to the HP diet replacement was more pronounced in terms of appetite sensations, while in males this response was mostly related to appetite-related hormones. Findings from this research contribute to the body of literature pertaining to the effects of a HP diet replacement and physical activity. Collectively, they provide further insight into the potential role of these strategies for weight maintenance and prevention of obesity.

PREFACE

This thesis is an original work by Camila Lemos Pinto Oliveira. The research projects, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, "The impact of a high protein diet on substrate oxidation and energy metabolism", No. Pro00066006, October 5th, 2016, and "The impact of a high-protein diet on energy metabolism in healthy men", No. Pro00083005, July 9th, 2018.

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LIST OF ABBREVIATIONS

AE: Available energy.

AEBSF: 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride.

ALT: Alanine aminotransferase.

ANOVA: Analysis of variance.

AST: Aspartate aminotransferase.

AT: Activity thermogenesis.

ATP: Adenosine triphosphate.

AUC: Area under the curve.

%B: β-cell function.

BEE: Basal energy expenditure.

BMC: Bone mineral content.

BMI: Body mass index.

BMR: Basal metabolic rate.

CHOox: Carbohydrate oxidation.

CO_2: Carbon dioxide.

CON: Control.

CONSORT: Consolidated standards of reporting trials.

CSS: Composite satiety score.

CV: Coefficient of variation.

DXA: Dual-energy X-ray absorptiometry.

EAT: Exercise activity thermogenesis.

EDTA: K2-ethylenediaminetetraacetic acid.

EE: Energy expenditure.

EOSS: Edmonton Obesity Staging System.

F: Females.

FATox: Fat oxidation.

FFM: Fat-free mass.

FM: Fat mass.

GFR: Glomerular filtration rate.

GLP-1: Glucagon-like peptide 1.

^{2}H: Deuterium.

H_2O: Water.

HC: Heat of combustion.

HDL: High-density lipoprotein.

HOMA: Homeostatic model assessment.

HP: High-protein.

HP-MR: High-protein meal replacement.

HP-TDR: High-protein total diet replacement.

HNRU: Human Nutrition Research Unit.

IR: Insulin resistance.

KJ: Kilojoule.

LDL: Low-density lipoprotein.

LST: Lean soft tissue.

MR: Meal replacement.

N: Nitrogen.

N/A: Not applicable.

NEAT: Non-exercise activity thermogenesis.

NEFA: Non-esterified fatty acids.

O_2: Oxygen.

^{18}O: Oxygen-18.

Ox: Oxidation.

P: Protein oxidation.

PFC: Prospective food consumption.

PROox: Protein oxidation.

PYY: Peptide tyrosine-tyrosine.

REE: Resting energy expenditure.

RER: Respiratory exchange ratio.

RMR: Resting metabolic rate.

RQ: Respiratory quotient.

SD: Standard deviation.

SEE: Sleep energy expenditure.

SEM: Standard error of the mean difference.

SMR: Sleeping metabolic rate.

SPIRIT: Standard protocol items: recommendations for interventional trials.

T2D: Type 2 diabetes.

TDR: Total diet replacement.

TEE: Total energy expenditure.

TEF: Thermic effect of food.

TEI: Total energy intake.

TSH: Thyroid stimulating hormone.

VAS: Visual analogue scale.

VCO_2: Volume of carbon dioxide.

VO_2: Volume of oxygen.

WBCU: Whole-body calorimetry unit.

CHAPTER 1. INTRODUCTION

1.1 THESIS ORGANIZATION

This thesis has been prepared as a paper format according to specifications provided by the Faculty of Graduate Studies and Research at the University of Alberta. Following the Introduction is a Literature Review (Chapter 2), four individual manuscripts (Chapters 3 to 6), and a Discussion and Conclusions section (Chapter 7). A preface precedes Chapters 3, 4, 5, and 6 with a brief description of each study and collaborators' contributions. Related figures and tables are provided at the end of each chapter.

1.2 RATIONALE

Obesity has become an important public health problem worldwide (1). Its rising prevalence in both developing and developed countries has been described as a global pandemic (2). As a chronic disease, obesity is characterized by abnormal or excessive body fat accumulation that impairs health (3). This dysfunctional fat accumulation leads to a state of low-grade chronic inflammation, which has been associated with the onset and progression of several obesity-related comorbidities, such as type 2 diabetes, cardiovascular diseases, and cancer (4). Each year approximately 2.8 million adults die due to overweight and obesity worldwide (5).

Obesity is caused fundamentally by an energy imbalance between calories consumed and expended on a chronic basis (6). Although the balance concept seems uncomplicated, its regulation is highly complex and influenced by several factors, such as behavioural, environmental, physiological, genetic, social, and economic (7). Lifestyle modifications that induce energy deficit, such as diet and physical activity are considered the cornerstone of weight management, as they are key players in the "intake" and "expenditure" sides of the energy balance equation (8, 9).

Dietary intake is a modifiable factor that affect energy homeostasis and food intake and, hence, influence body weight control (10). Numerous dietary strategies exist and are continuously being developed in an attempt to induce a state of negative energy balance (11). Among those, weight loss strategies based on diet replacements and a higher protein intake have been a topic of investigation (12). Diet replacements can be subdivided into total diet replacements (TDR) and

meal replacements (MR), which are nutritionally complete formula foods designed to replace the whole diet for a specific period of time or one or more meals per day, respectively. These products are becoming increasingly popular as weight management strategies; however, research around this topic has not kept pace with its growth in popularity. To date, only a few studies have evaluated the effects of TDR in humans (13-20). Studies were mostly long-term intervention trials with all participants presenting with obesity (13-20) and sometimes with type 2 diabetes (13, 14, 16, 19, 20). Interventions consisted of calorie-restricted TDR and the primary outcome was mainly weight loss (13-20). Regarding MR, two meta-analyses have been published summarizing its effect on weight management (21, 22). Taken together, the results from these studies suggested both TDR and MR as potentially effective approaches for weight loss.

High-protein (HP) diets have also been growing in popularity, as evidence accumulates on its benefits for weight management. These diets are characterized by a protein content above recommended values (i.e., for healthy adults >19 years of age: 0.80 g/kg of body weight/day or 10 to 35% of total energy intake) (23) with varying levels of dietary carbohydrate and fat. High-protein diets appear to exert strong influence on the main factors involved in body weight regulation, which are energy metabolism, body composition, and appetite (24-29). This nutritional strategy appears to increase energy expenditure, fat oxidation, and spare lean mass during weight loss (30, 31). Moreover, it exerts a strong satiating effect and appears to decrease energy intake under *ad libitum* conditions (30).

In addition to the effects of dietary interventions on the energy balance equation, physical activity has also been described as an integral part of weight management, as it produces several health benefits, even in the absence of weight loss (32). Regular physical activity has been shown to contribute to modest body weight and fat losses and maintenance over time (33). Additionally, it has been associated with lower incidence of chronic diseases, cardiovascular risk factors, and all-cause mortality (34). Therefore, physical activity is an indispensable tool in weight management (33). The most recent Canadian Adult Obesity Clinical Practice Guidelines recommends that individuals aiming at losing body weight or maintaining the lost weight over time should perform 30 to 60 minutes of moderate- to vigorous-intensity aerobic physical activity most days of the week and that resistance exercises should be part of the training program (32).

Taken together, the benefits offered by diet replacements and physical activity seem to be an interesting combination for weight management. Not surprisingly, synergistic effects have been

noticed by industry and several HP diet replacements are widely available to consumers. Furthermore, these products are usually prescribed with a physical activity program for weight management. Although the effects of diet replacements and HP diets (alone) on components that regulate the homeostatic control of body weight have been partially investigated (13-22, 24-29), the synergistic effects of a combined high-protein total diet replacement (HP-TDR) or high-protein meal replacement (HP-MR) associated or not with physical activity have not yet been explored, despite their worldwide availability and high consumption by individuals with normal or excess adiposity. Of extreme importance is the study of these weight management interventions using state-of-the-art methodology in a controlled environment in healthy females and males with a normal body weight. These study design characteristics will eliminate the confounding effects of obesity and comorbidities on the results and allow a better understanding of the impact of these strategies in a normal physiological condition so that the results are better translated in individuals with obesity and its related comorbidities. Additionally, it will provide further insight into the potential role of these strategies for weight maintenance and prevention of obesity.

1.3 PURPOSE

The overall purpose of this research was to compare the effects of an acute nutritional intervention comprised of a HP diet replacement versus a standard North American dietary pattern on selected components of energy metabolism, metabolic blood markers, appetite sensations, and appetite-related hormones in healthy, normal-weight adults of both sexes.

1.4 RESEARCH QUESTIONS

The research questions for this thesis were:

1. Compared to an acute nutritional intervention comprised of a standard North American diet, what effects does a HP-TDR have on selected components of energy metabolism, metabolic blood markers, appetite sensations, and appetite-related hormones in healthy, normal-weight female and male adults?

2. Is there a sex difference in selected components of energy metabolism, metabolic blood markers, appetite sensations, and appetite-related hormones when consuming an acute nutritional intervention comprised of a North American dietary pattern versus a HP-TDR?
3. Compared to a standard North American breakfast, what effects does a breakfast comprised of a HP-MR have on selected components of energy metabolism during an exercise session, and on changes in metabolic blood markers, appetite sensations, and appetite-related hormones following it?
4. Is there a sex difference in selected components of energy metabolism during an exercise session, and on changes in metabolic blood markers, appetite sensations, and appetite-related hormones following it when consuming a North American breakfast versus a breakfast comprised of a HP-MR?

1.5 OBJECTIVES AND HYPOTHESES

1.5.1 The effects of a high-protein diet replacement on energy metabolism and metabolic blood markers (Chapter 4)

Objectives:

1.1 To compare the effects of an acute nutritional intervention comprised of a HP-TDR versus a control (CON) diet (North American) in healthy, normal-weight female and male adults on:

1.1a. total energy expenditure, energy balance, macronutrient oxidation rates, and macronutrient balances;

1.1b. metabolic blood markers (i.e., glucose, insulin, lipid panel, glycerol, and non-esterified fatty acids [NEFA]);

1.2. To explore differences between sexes on energy metabolism and metabolic blood markers during the HP-TDR and CON interventions.

Hypotheses:

1.1. Compared to the CON diet, participants consuming the HP-TDR will:

1.1a. expend more energy per day, be in negative energy balance, experience increased fat and protein oxidation rates, and decreased carbohydrate oxidation rate, and be in negative fat and carbohydrate balances, and in positive protein balance;

1.1b. present with an improved metabolic profile characterized by lower glucose, insulin, lipid panel, glycerol, and NEFA blood levels;

1.2. Females and males will respond similarly to the dietary interventions, despite known sex-related physiological differences.

1.5.2 The effects of a high-protein diet replacement on appetite sensations and appetite-related hormones (Chapter 5)

Objectives:

2.1. To compare the effects of an acute HP-TDR versus a CON diet (North American) in healthy, normal-weight female and male adults on:

2.1a. appetite sensations (i.e., hunger, satiety, fullness, and prospective food consumption [PFC]);

2.1b. appetite-related hormones (i.e., leptin, glucagon-like peptide 1 [GLP-1], peptide tyrosine-tyrosine [PYY], and ghrelin);

2.2. To correlate appetite sensations and appetite-related hormones with energy metabolism components of healthy, normal-weight female and male adults;

2.3. To explore differences between sexes on appetite sensations and appetite-related hormones during the HP-TDR and CON interventions.

Hypotheses:

2.1. Compared to the CON diet, participants consuming the HP-TDR will:

2.1a. experience increased satiety and fullness, and decreased hunger and PFC;

2.1b. have higher blood levels of GLP-1 and PYY, while no changes will be observed in leptin and ghrelin blood levels;

2.2. During the HP-TDR intervention, PYY will be positively correlated with postprandial energy expenditure, and during the CON intervention, hunger will be negatively correlated with energy balance. There will be no other correlations between appetite sensations and appetite-related hormones with energy metabolism components;

2.3. Females will experience increased satiety and increased blood levels of leptin during the CON and HP-TDR interventions. There will be no other differences in appetite sensations and appetite-related hormones between sexes.

1.5.3 The effects of a high-protein diet replacement on exercise metabolism (Chapter 6)

Objectives:

3.1. To compare the effects of the consumption of a breakfast comprised of a HP-MR versus a CON breakfast (North American) preceding an acute bout of moderate-intensity exercise in healthy normal-weight female and male adults on:

3.1a. energy expenditure and macronutrient oxidation rates during the exercise session;

3.1b. changes in appetite sensations (i.e., hunger, satiety, fullness, and PFC) following the exercise session;

3.1c. changes in appetite-related hormones (i.e., leptin, GLP-1, PYY, and ghrelin) following the exercise session;

3.1d. changes in metabolic blood markers (i.e., glucose, insulin, lipid panel, glycerol, and NEFA) following the exercise session;

3.2. To explore differences between sexes on exercise metabolism during the HP-MR and CON interventions.

Hypotheses:

3.1. Compared to the CON breakfast, participants consuming the HP-MR will:

3.1a. expend more energy and experience increased fat and protein oxidation rates, and decreased carbohydrate oxidation rate during the exercise session;

3.1b. experience increased satiety and fullness, and decreased hunger and PFC following the exercise session;

3.1c. have a greater increase in blood levels of GLP-1 and PYY, while no changes will be observed in leptin and ghrelin blood levels following the exercise session;

3.1d. present with an improved metabolic profile, characterized by lower glucose, insulin, lipid, glycerol, and NEFA blood levels following the exercise session;

3.2. Females will have increased satiety and increased blood levels of leptin during the CON and HP-MR interventions. There will be no other differences in appetite sensations and appetite-related hormones between sexes.

1.6 SIGNIFICANCE

Obesity has become an important public health problem worldwide (1) and substantial effort has been given to develop treatment strategies for this condition (35). In isolation, diet replacements, HP diets, and physical activity can induce energy deficit and, for this reason, are usually adopted as weight management strategies (13-22, 24-29, 32). Considering the possible synergistic effects of these approaches, this research focuses on comparing the effects of an acute nutritional intervention comprised of a HP diet replacement versus a standard North American dietary pattern on selected components of energy metabolism, metabolic blood markers, appetite sensations, and appetite-related hormones in healthy, normal-weight adults of both sexes.

The study of these interventions using state-of-the-art methodology in a controlled environment in healthy females and males with a normal body weight is of particular importance, as it will eliminate the confounding effects of obesity and comorbidities on the results and allow a better understanding of the impact of these strategies in a normal physiological condition so that the results are better translated in individuals with obesity and its related comorbidities. Additionally, it will provide further insight into the potential role of these strategies for weight maintenance and prevention of obesity. The data can then be used as reference for studies testing the effects of HP diet replacements combined with physical activity in individuals with obesity and comorbidities. Therefore, the results of this research can ultimately be used to develop future strategies aimed at optimizing diet quality and weight management. Strategies aimed at the prevention of overweight, obesity and related comorbidities are important for the improvement of health and quality of life, also ultimately addressing the growing financial burden of obesity and related diseases on the health care system.

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CHAPTER 2. LITERATURE REVIEW

2.1 ENERGY METABOLISM

Our cells need a constant supply of energy for survival and this energy comes from the combustion (i.e., oxidation) of macronutrients ingested through the diet or broken down from internal stores (1). These macronutrients are carbohydrate, protein, and fat (1). The oxidation reaction usually consumes oxygen (O_2) and produces carbon dioxide (CO_2), water, nitrogen (N, when protein is oxidized), energy, and heat; however, it is important to note that some energy-yielding pathways do not require O_2, such as the glycolysis (1). The energy generated from the combustion of macronutrients is salvaged in the form of high-energy bonds contained mainly in the molecule adenosine triphosphate (ATP) (1). This molecule is a storage form of energy in the cell and acts as the link between energy-releasing and energy-demanding reactions in the body (1). When the body needs to perform mechanical, chemical, and/or osmotic work ATP is hydrolyzed and energy is released (1). In human biology, energy is usually measured in kilocalories (kcal), which is the amount of energy necessary to raise the temperature of 1 kg of water by 1°C (2). Another commonly used unit is the kilojoule (kJ) (2). The conversion between kcal and kJ is 1 kcal=4.184 kJ (2).

All metabolic processes involving generation and consumption of energy via cellular metabolism and cell work, respectively, produce heat (1). Scientists and philosophers have been trying to understand and measure body heat since 300 BC (3). From ancient Greeks through Middle Ages, body heat and respiration were thought to be linked, but only in 1674 the English physician John Mayow discovered that combustion generated heat and that air was not a single element, but a mixture of gases (3). After a century of intense research, some major findings emerged: 1) scientists Regnault and Reiset demonstrated that the ratio of CO_2 produced to O_2 consumed varied according to the type of food ingested; 2) Antoine Lavoisier measured O_2 consumption in humans; and 3) Antoine Lavoisier and Pierre Simone Laplace developed the first calorimeter to measure body heat production (4, 5). These findings exponentially advanced knowledge in this field (3, 6). In 1862, the first human calorimeter to measure gas exchange was invented and, in 1905, the first device able to measure both heat and gas exchange was created, the Atwater-Rosa calorimeter (3). This calorimeter was the first to confirm that both heat and gas exchange measurements can

accurately quantify human energy expenditure (EE) (4). This finding marked the start of an era of intense research on energy metabolism in health and disease (3).

2.1.1 Techniques

Calorimetry is the measurement of heat energy and derives from the Latin word "*calor*" (heat) and the Greek word "*metrion*" (measure) (6). The study of energy metabolism relies mainly on the principle of measurement of heat transfer, which involve the direct measurement of heat production (i.e., direct calorimetry), or an estimation of it through the measurements of O_2 and CO_2 from respiration (i.e., indirect calorimetry) or by extrapolation from physiological measurements and observations (i.e., non-calorimetric methods) (7).

2.1.1.1 Direct Calorimetry

Direct calorimetry involves the measurement of total body heat production from both anaerobic and/or aerobic metabolic oxidation (6). The principle behind this technique is the law of conservation of energy, which states that energy can neither be created nor destroyed, it can only be converted to a different form. As energy from the food is converted to chemical energy stored in the body, metabolic processes transform this stored energy into work. All these processes produce heat that is ultimately captured by an enclosed, isolated system of known heat capacity known as direct calorimeter (6). The direct calorimeter is a very sophisticated system extremely costly and technically challenging (7). Modern systems can capture both evaporative heat loss and dry heat exchange (6). Because of the complexity of the technique and the costs involved in building and maintaining these devices, direct calorimeters are rarely available. In fact, there is only one operational unit of its kind in the world, which is located at the University of Ottawa (Ottawa, ON, Canada).

2.1.1.2 Indirect Calorimetry

Indirect calorimetry estimates the amount of energy released from the combustion of substrates from measurements of O_2 and CO_2 from respiration (7). It is also possible to correct the energy loss from urinary N excretion when protein is oxidized (7). This technique is based on the known amounts of heat produced per liter of O_2 and CO_2 consumed and produced when macronutrients are oxidized (**Tables 2.1 and 2.2**) (8). Because the oxidation of different macronutrients results in distinct volumes of O_2 and CO_2, indirect calorimetry estimates substrate oxidation rates and from caloric equivalents for macronutrients it estimates EE (8). Nowadays,

most commonly used indirect calorimeters are the metabolic cart and whole-body calorimetry unit (WBCU, also known as metabolic or respiration chamber).

Metabolic carts are portable devices used to assess energy metabolism during rest and exercise by continuously capturing O_2 and CO_2 from respiration during short periods of time (i.e., up to some hours) using a ventilated hood or canopy, a facemask, or a mouthpiece (8). These devices are inexpensive and easy to use. On the opposite side, the WBCU is a non-portable spacious airtight room that enables the continuous assessment of energy metabolism components for some hours up to several days by continuously capturing O_2 and CO_2 from respiration (4). Individuals can move freely while inside the chamber. With a controlled temperature, humidity, and air flow, these chambers usually look like a small hotel room and are typically equipped with a sink, toilet, armchair, bed, table, fridge, TV, intercom, phone, computer, windows, curtains, airlocks, and iris ports (**Figure 2.1**). Some of the chambers are also equipped with exercise equipment (e.g., treadmill or cycle ergometer, **Figure 2.1D**) for the assessment of exercise energy metabolism or to mimic individual's daily routine (9). To minimize the amount of O_2 and CO_2 that escapes from the chamber, food, beverages, and biological specimens (i.e., urine and feces) are passed through air lock systems (**Figure 2.2A**) and blood is collected through an iris port (**Figure 2.2B**). Because the measurements using the WBCU can be extended for up to several days, this device can assess several energy metabolism components, such as total EE (TEE) and its physiological compartments (i.e., resting EE [REE], thermic effect of food [TEF], and activity thermogenesis [AT]), substrate oxidation rates, and respiratory exchange rate (RER). Although the WBCU is expensive to build and maintain and requires high technical expertise, there are approximately 40 research laboratories with an operational unit of its kind around the world (i.e., North American, Europe, Asia, and Australia) (4). Because of its high accuracy and precision, this equipment is considered a gold-standard for the assessment of energy metabolism in humans (4).

2.1.1.3 Non-Calorimetric Methods

Non-calorimetric methods use physiological measurements and observations to estimate EE and are usually standardized against calorimetric methods (7). Among those, the doubly labeled water (DLW) is considered the most accurate and the gold-standard for measuring TEE in free-living conditions, with an estimated error of 1 to 3% (10). This method works by dosing an individual with water labeled with stable hydrogen (^2H, deuterium) and oxygen (^{18}O) isotopes (11). These tracers mix with body water and, as energy is expended, they are eliminated as water (H_2O)

in bodily fluids and as CO_2 via the breath in a rate of 5 to 20% per day (11, 12). Over the course of 4 to 20 days, the elimination rate of these isotopes is measured using mass spectrometry and the difference in disappearance is indicative of CO_2 production, which is used to calculate TEE through mathematical equations (11).

2.1.2 Energy Metabolism Components

Several energy metabolism components can be assessed using indirect calorimetry, such as TEE and its compartments, substrate oxidation rates, and RER. As mentioned in the previous subsection, measurement of EE and substrate oxidation rates by indirect calorimetry are based on the stoichiometric energy equivalents of O_2 and CO_2 of the macronutrients protein, carbohydrate, and fat (**Tables 2.1 and 2.2**). The coefficients for the oxidation of macronutrients are general or standard values; however, because the chemical composition of these molecules can change, depending on the substrate used in the experiments to establish them, these coefficients can differ (13). Coefficients need to be adjusted when an increasing amount of fuels are oxidized or end products of metabolism other than CO_2 and N are produced (e.g., ketone bodies, methane, and hydrogen) (13). Therefore, for an accurate assessment of EE and macronutrient oxidation rates, calorimetric coefficients and equations need to be carefully selected. Several scientists have dedicated their work to developing equations for the estimation of EE and macronutrient oxidation rates based on measurements of O_2, CO_2, and urinary N excretion (14, 15). The most commonly used equations to estimate these variables using indirect calorimetry are shown in **Table 2.3**. The only equation that does not have any variation is protein oxidation, as the amount of protein oxidized can be estimated from urinary N and that 6.25 g of protein are oxidized for every gram of urinary N (8). Although there is no scientific rationale for the choice of equations, taking into consideration the macronutrient distribution of the diet, its chemical composition, the ratio of fuels being oxidized, and the end products of metabolism can help improving the accuracy of the results.

2.1.2.1 Energy Expenditure

The major physiological compartments of TEE include REE, TEF, and AT (16, 17). Total energy expenditure comprises all energy expended throughout the day (i.e., 24 hours) (18). Resting energy expenditure is the largest component of TEE contributing to approximately 60 to 70%, but larger variations can happen depending on individual's physical activity levels (18). It represents the energy expended to maintain vital functions at rest, while awake, in a fasted state, and in a

thermoneutral environment (18). Thermic effect of food represents the energy expended for digestion, absorption, processing, and storage of ingested nutrients and accounts for approximately 10% of TEE when on energy balance (18). Activity thermogenesis is the most variable component of TEE and is divided into exercise AT (EAT) and non-exercise AT (NEAT) (18). Exercise AT is the energy expended in structured exercise, while NEAT accounts for energy expended in any type of physical activity, such as occupation, leisure, sitting, standing, and ambulation (18). Besides these major compartments, basal EE (BEE) and sleep EE (SEE) are usually assessed in studies with a duration ≥24 hours using the WBCU. The difference between REE and BEE relies mainly on how it is measured and, consequently, BEE is approximately 10% lower than REE (19). The main differences between REE and BEE measurement protocols are highlighted in **Table 2.4** and fully described elsewhere (20, 21). Although there is no consensus on how SEE should be assessed, this measurement is usually taken during the entire night or during a specific and continuous period of time when EE is the lowest (22). Depending on how it is measured, SEE can vary from 88 to 95% of BEE when assessed for 1 or 8.5 hours, respectively (22).

Several factors affect EE and its major physiological compartments such as genetics, age, sex, body composition, physical activity, diet composition, health status, and environmental stimuli (17). Total EE is generally higher in males than in females, mainly because of differences in body size, composition, and sex hormones (23, 24). Another important factor that affects TEE is the menstrual cycle. Webb (25) demonstrated that TEE is 8% higher in the luteal phase compared to the follicular phase. Resting EE is mostly affected by body size, body composition, and recent energy imbalance (16). In fact, fat-free mass (FFM) has been reported to be the main determinant of REE (26). Moreover, changes in body weight have been shown to affect REE. This energy metabolism compartment increases with weight gain and decrease with weight loss (27, 28). Most of this change in REE is attributed to a modification in metabolically active tissue (29); however, specifically in the context of weight loss, part of this change is not explained by body composition alone and this phenomenon has been referred to as metabolic adaptation or adaptive thermogenesis (28). Regarding TEF, the energy content of the meal and the macronutrients are its main determinants (23). Energy expended to digest, absorb, process, and store dietary protein is 25-30% of energy content of the meal, followed by carbohydrate (6-8%) and fat (2-3%) (30). The last major compartment of TEE and the most variable one is AT, which is mainly determined by physical activity levels, genetics, body size, and composition (23).

2.1.2.2 Macronutrient Oxidation

After digestion and absorption, macronutrients are either stored or oxidized. There seems to be a hierarchy that governs the order in which macronutrients are oxidized, which is an indicator of dominance within metabolic pathways (31, 32). This hierarchy is dominated by alcohol and followed by carbohydrate, protein, and fat (31, 32). Alcohol dominates the oxidative pathways because it is a toxin and cannot be stored in the body; therefore, it needs to be eliminated by oxidative disposal (31, 32). The rapid hepatic oxidation that happens when alcohol is ingested causes a suppression of the oxidation of carbohydrate, protein, and particularly fat (31, 32). Considering that carbohydrate and protein have small storage capacity (i.e., mostly as glycogen and amino acid pool, respectively) and that the body needs to maintain glucose homeostasis, these macronutrients have an excellent oxidative autoregulation (31, 32). The increase in the ingestion of these macronutrients induce an increase in its oxidation rate to the same extent and suppression of fat oxidation (31, 32). Fat is at the base of the hierarchy and has a very poor oxidative autoregulation, mainly because our body fat stores are abundant and there is almost an infinite fat storage capacity; therefore, this macronutrient's oxidation rate is mostly dictated by the presence or absence of alcohol, carbohydrate, and protein (31, 32). Sex hormones can influence nutrient partitioning (33, 34). At rest and in the postprandial state, males preferentially oxidize circulating free fatty acids, while females incorporate them into triglyceride to store fat (33, 34). On the other hand, when energy requirements increase, such as during an exercise session, females oxidize more fat relative to carbohydrate while males use mostly carbohydrate as a fuel (33, 34).

2.1.2.3 Energy and Macronutrient Balances

Body weight and composition are the result of the dynamic energy and macronutrient balances, which are defined as the difference between intake and expenditure/oxidation (35). In conditions of energy balance (i.e., energy intake = EE), macronutrient oxidation rates will closely match macronutrient intakes and, consequently, body weight and composition will be maintained (31). On the other hand, when there is energy imbalance (i.e., energy intake > or < EE), body fat stores will be predominantly affected, because carbohydrate and protein stores and oxidation rates are tightly regulated on a daily basis (31). Some years ago, the interconversion of macronutrients was the focus of debate, particularly *de novo* lipogenesis when excess carbohydrate is ingested and converted to fat. However, studies in humans demonstrated that this process is energetically

inefficient (20 to 30% of the energy is dissipated as heat) and very limited, only happening when large excesses of carbohydrate is consumed (31, 35).

Protein and carbohydrate balances are usually achieved on a day-to-day basis, considering their excellent oxidative autoregulation. Total body protein is usually constant and represent approximately one-third of the total stored energy in a 70 kg man (35). An increase in protein intake does not change body protein unless there is growth stimuli, such as hormones, physical activity or exercise, and weight gain (35). The same is true for carbohydrate stores, which are very limited as the body can only store 500 to 1000 g of glycogen (35). Therefore, excess protein and carbohydrate intakes is not the main cause of weight gain; however, these macronutrients can affect fat balance (35). Fat stores are considered an abundant energy buffer for the body. Any energy deficit or excess energy intake decrease or increase fat stores, respectively (35). This is particularly true for fat storage, which has been described as an extremely efficient process (32). Fat intake has been demonstrated to have no or very little influence on fat oxidation; however, the manipulation of carbohydrate intake seems to significantly affect fat oxidation in the opposite direction (32). Therefore, fat is the only nutrient able to directly affect adiposity; the other macronutrients only influence it indirectly.

2.1.2.4 Respiratory Exchange Ratio

Respiratory exchange ratio is calculated as the volume CO_2 produced divided by the volume of oxygen O_2 consumed from respiration. Although the terms RER and respiratory quotient (RQ) are used interchangeably, they are not measured the same way. Respiratory quotient is obtained directly from the tissues and reflect the exchange ratio of gases at the cellular level. Under steady state conditions, RER reflects RQ, which is an indicator of substrate oxidation. The assessment of RQ is invasive and therefore not very common; on the other hand, RER is easily assessed using indirect calorimetry and most commonly used in research and clinical settings.

Under steady state conditions, RER can vary from 0.7 to 1.0 (36). When it is 0.7, it means that fat is being oxidized to produce energy, at 1.0 is carbohydrate, and any number within this range indicates a mix of substrates being oxidized (36). Some changes in metabolism can lead to RER levels below 0.7 or above 1.0 and in these cases it does not reflect substrate utilization (36). Situations in which this variable can be below 0.7 includes oxidation of ethanol and ketones, lipolysis, underfeeding, diabetes, ketoacidosis, high rates of urinary glucose excretion, and hypoventilation (36). On the opposite side, when it is above 1.0 it indicates excessive CO_2

production, hydrogen-ion buffered by bicarbonate-generating CO_2, lipogenesis, overfeeding, hyperventilation, and metabolic alkalosis (36).

2.2 APPETITE

Appetite can influence energy intake and is affected by EE and substrate utilization, which makes it a key factor involved in the regulation of energy homeostasis and hence body weight (37). The control of appetite is regulated by a complex communication between organs and the brain through molecular and neural signals (38). Peripheral organs, including the liver, gut, muscles, pancreas, and adipose tissue send signals to the brain reflecting the body's energy status (38). After processing this information, the brain in turn controls organs and tissues responsible for the regulation of energy intake, EE, and substrate utilization (38, 39).

2.2.1 Appetite Sensations

The initiation and termination of a meal seems like a simple process, but involves a complex regulation of appetite sensations that are influenced by physiological, psychological, social, and cultural factors (40). The most commonly studied appetite sensations are hunger, satiation, and satiety. Hunger is defined as a conscious sensation reflecting a mental urge to eat, satiation is the sensation that leads to meal termination, and satiety is the postprandial process that inhibits further eating, suppresses hunger, and leads to a feeling of fullness (41). Satiation limits meal size and satiety regulates meal frequency (42). Several factors contribute to onset, timing, and duration of the appetite sensations, including sensory, cognitive, post-ingestive, and post-absorptive factors (42). The appetite sensations are also influenced by the characteristics of the diet ingested, such as macronutrient composition, energy density, physical structure, form of the food, and sensory qualities (41). In addition, there are differences in appetite sensations between sexes, which can be partly explained by the gonadal steroid hormones (43) and neuronal responses to food intake (44). Gonadal steroid hormones are able to influence neural processing of peripheral feedback signals that control eating, such as ghrelin, cholecystokinin, glucagon, insulin, and leptin (43). More specifically estrogen seems to have an inhibitory effect on food intake, which is reflected during the follicular phase of the menstrual cycle when this hormone's level is relatively high and female's energy intake is lower than the other phases of the cycle (45, 46).

2.2.2 Appetite-Related Hormones

Our body produces and responds to several hormones that, among several functions, influence appetite. There are mainly two types of control mechanisms that influence appetite and food intake: 1) tonic (long-term) and 2) episodic (short-term) (47). The tonic mechanisms do not fluctuate between or within a day and impose an enduring and stable influence over appetite and food intake (47). These tonic signals are related mainly to leptin, insulin, and EE of metabolically active tissues (47). On the other hand, the episodic signals respond to the presence or absence of nutrients in the gastrointestinal tract and varies considerable across the day (47). Some of the most studied episodic signals include the gut hormones glucagon-like peptide 1 (GLP-1) and peptide tyrosine-tyrosine (PYY), and the hunger hormone ghrelin (47, 48).

2.2.2.1 Leptin

Leptin is a hormone produced mainly by the white adipose tissue and released into the circulation (38). The concentration of circulating leptin is proportionate to body fat stores (38), which seems to be stimulated by estrogen in healthy females (49). The main function of this hormone is to communicate the brain on the availability of body's energy stores (50). The intensity of this signal is proportional to the concentration of circulating leptin (50). This hormone exerts a stronger effect on the preservation of body fat stores versus its reduction for reasons related to fertility and survival (50). After being produced by the adipose tissue and released into the circulation, leptin enters the brain through the blood-brain barrier via a saturable transporter and acts directly in the hypothalamus (38). The brain translates the leptin signal into neuroendocrine functions, autonomic efferent, and food-related behavior, which have been shown to increase EE and decrease energy intake (50). Individuals with excess adiposity usually present with elevated circulating leptin levels; however, in this population group this hormone does not affect food intake and EE as it should, which is explained by a state of leptin resistance (38). Although the causes underlining this resistance remains unclear, some mechanisms have been proposed, such as alterations in the operation of the leptin receptor, a defect in leptin signaling, a dysfunction in transporting leptin into the central nervous system, and endoplasmic reticulum stress (38). For this reason, exogenous administration of leptin is not the solution for excess adiposity; however, leptin sensitizing agents have been developed and may help with body weight regulation (50).

2.2.2.2 Glucagon-Like Peptide 1

Glucagon-like peptide 1 is considered a satiation hormone produced primarily by enteroendocrine L-cells in the proximal small intestine and distal colon in response to ingested nutrients, mainly monosaccharides, peptides and amino acids, monounsaturated and polyunsaturated fatty acids, and short-chain fatty acids (51). After food intake, GLP-1 blood levels increase two- to four-folds and reach a pick after approximately 60 minutes (51). In the gastrointestinal tract, this hormone slows gastric emptying and inhibits gastric acid secretion for a more efficient digestion and nutrient absorption (38). Glucagon-like peptide 1 acts mainly in the pancreas and central nervous system through endocrine and neural routes (51). In the pancreas, GLP-1 stimulates insulin secretion and inhibits glucagon release, positively influencing blood glucose homeostasis (51). In the brain, GLP-1 binds to its receptors and through peripheral and central actions, this hormone has been shown to reduce food intake and promote weight loss (38). The sex hormones estrogen and progesterone exert important effects on appetite-related hormones and have been shown to stimulate GLP-1 secretion (45, 52). In fact, blood levels of GLP-1 are lower during the follicular phase of the menstrual cycle due to the lower levels of estrogen and progesterone (45, 52). Individuals presenting with obesity have lower levels of GLP-1 than individuals with a normal body weight (38). Because of its effects on blood glucose control and food intake, exogenous administration of GLP-1 as well as dietary manipulation has been used as strategies to increase blood GLP-1 levels and serve as a treatment for type 2 diabetes (T2D) and obesity (48).

2.2.2.3 Peptide Tyrosine-Tyrosine

Similarly to GLP-1, PYY is considered a satiation hormone and is produced by the L-cells of the distal gastrointestinal tract in response to ingested nutrients, especially protein (53). This hormone's blood levels are usually lowest during fasting and increase after food ingestion, remaining elevated for several hours (53). In the gastrointestinal tract, PYY reduces gastric emptying, intestinal motility, pancreatic secretions, and absorption of fluids (38); however, its main action is in the central nervous system where it has been shown to reduce food intake through the stimulation of the Y2 receptor in the hypothalamus that inhibits the release of the appetite stimulant neuropeptide Y (54). Blood levels of PYY have been associated with an increase energy expenditure and a decrease in RQ; however, more research is needed to better understand these findings (55). Individuals with obesity present with lower fasting and postprandial levels of PYY

when compared to their normal-weight counterparts, a fact that has been proposed as a contributor to the onset and progression of obesity (54).

2.2.2.4 Ghrelin

Ghrelin is the only known circulating hunger hormone and is also considered a biomarker for satiety (53). It is produced primarily in the fundus of the stomach (54). This hormone's blood levels fall shortly after meal ingestion and return to pre-meal concentrations before the next eating episode (48). Ghrelin stimulates appetite and food intake via activation of hypothalamic neurocircuits (56). Additionally, this hormone activates gastric emptying, gastric motility, and gastric acid secretion (56). Ghrelin has been shown to act more as a meal initiator and control meal frequency rather than meal size (38). The magnitude of ghrelin suppression following a meal seems to be associated with the energy content and its macronutrient composition, with dietary fat having the smallest effect on the suppression of this hormone (57). In addition to the effects of ghrelin on appetite and food intake, this hormone seems to induce adipogenesis (57). This effect seems to happen through a switch from fat to carbohydrate as the major fuel source, increased lipogenesis in white adipose tissue through inhibition of the hypothalamic melanocortin system, and decreased EE and activity levels (57). Individuals with obesity have lower ghrelin levels than those with a normal body weight and this hormone's blood levels are higher in the context of weight loss (38).

2.3 OBESITY

2.3.1 Prevalence

Obesity has become an important public health problem worldwide (58). Its rising prevalence in both developing and developed countries has been described as a global pandemic (59). In 2016, more than 1.5 billion adults were classified as having overweight and 650 million having obesity, the latter representing 13% of the world's adult population (11% of men and 15% of women) (58). Taken together, overweight and obesity impose a global health cost equivalent to 2.8% of the world's gross domestic product, representing approximately US$ 2 trillion (60). More specifically in Canada, approximately 62.1% of the adult population have a body mass index (BMI) between 25.0 and 29.9 kg/m^2 and 24.3% have a BMI above 30.0 kg/m^2, which generated a cost to the health care system of, approximately, CAD$ 4.6 billion in 2008 (61).

2.3.2 Definition

Obesity is defined as a chronic disease, characterized by abnormal or excessive body fat accumulation that impairs health (62). Considering there is no established cut off points for the diagnosis of obesity based on the amount, location, and/or type of body fat, BMI is an uncomplicated and inexpensive anthropometric measurement used worldwide for this purpose. Body mass index is the weight divided by the square of the height and classified into ranges associated with health risk (63). Obesity is operationally defined when individual's BMI exceeds 30 kg/m^2 (58). This condition is further subclassified into obesity class I (BMI 30.0-34.9 kg/m^2), class II (BMI 35.0-39.9 kg/m^2), and class III (BMI >40.0 kg/m^2) (63). However, the most recent Canadian Adult Obesity Clinical Practice Guidelines states that obesity can only be defined when abnormal or excessive body fat accumulation impairs health; therefore, BMI is not an accurate tool for defining this condition (62). In addition to these definitions, obesity is usually subdivided into gynoid and android types, which are based on body fat distribution (60). The gynoid type of obesity is characterized by excess of subcutaneous fat, which is found mostly in the hip and thigh areas, giving the body the shape of a pear (60). On the other hand, the android type is characterized by excess of visceral fat concentrated in the abdominal region, giving the body the shape of an apple (60). The gynoid obesity is more common in women, while the android type is more common in men and postmenopausal women and tends to be more harmful to health (60).

All-cause mortality increases progressively when BMI exceeds 25.0 kg/m^2 (64); however, although helpful at a population level, the BMI-derived obesity classification system has several pitfalls and limits the ability of health practitioners to make clinical decisions at an individual level (65). Therefore, the Edmonton Obesity Staging System (EOSS) has been proposed as a risk-stratification system that classifies individuals diagnosed with obesity into five categories and identify the ones at greater mortality risk (65, 66). This clinical staging system is based on patient's medical history, clinical and functional assessments, and simple routine diagnostic investigations (65, 66). The results from this assessment provide a measure of presence and severity of risk factors, comorbidities, and functional limitations, helping health practitioners to better guide and evaluate treatment at an individual level (65, 66).

2.3.3 Causes and Consequences

Obesity is a complex and multifactorial disease fundamentally caused by an energy imbalance between calories consumed and expended on a chronic basis (67). Although the balance concept seems uncomplicated, its regulation is highly complex and influenced by several factors, such as behavioural, environmental, physiological, genetic, social, and economic (60). The genetic variability among individuals partly explains why some people will develop obesity when exposed to an obesogenic environment while others will not (67, 68); however, the percentage contribution is relatively low, which makes the interaction between genes and environment the biggest contributor (60). Among the behavioral, environmental, social, and economic factors known to influence the development and progression of obesity are poor diet quality (influenced by opportunities to eat, food availability, and affordability), physical inactivity, prolonged screen time, inadequate sleep, environmental exposures, and built environment (69). As fat accumulation progresses as a result of a chronic positive energy balance, adipocytes found in subcutaneous and visceral adipose tissues undergo hyperplasia (i.e., increase in cell number) and hypertrophy (i.e., enlargement of individual cells) (60, 70). This pathological tissue growth leads to a metabolically dysfunctional adipose tissue, characterized by increased secretion of pro-inflammatory adipokines and decreased secretion of anti-inflammatory adipokines (60). This dysfunctional metabolic milieu contributes to a state of low-grade chronic inflammation, which has been associated with the onset and progression of several obesity-related comorbidities, such as T2D, cardiovascular diseases, and cancer (70). It is estimated that each year 2.8 million adults die due to overweight and obesity worldwide (71).

2.3.4 Treatment Strategies

Considering the negative impact of excessive body fat accumulation on individual's health and on the public health system (72, 73), substantial effort has been given to develop guidelines for the treatment of overweight and obesity (74). Lifestyle modifications that induce an energy deficit, such as dietary strategies and physical activity are considered the cornerstone of weight management (75). These strategies are key players in the "intake" and "expenditure" sides of the energy balance equation (16).

2.3.4.1 Diet Replacements

Diet replacements are nutritional products adopted as a weight management strategy as they can help individuals reduce daily energy intake. This dietary strategy has been identified as a useful approach for weight management as it requires minimal support by health care professionals (76, 77), increases overall executive control over food intake (78), and has been proven as effective, safe, well-tolerated, and cost-effective (79, 80), when compared to usual medical care. This nutritional strategy can be subdivided into total diet replacements (TDR) and meal replacements (MR). Total diet replacements are nutritionally complete formula foods designed to replace the whole diet for a specific period of time, while MR are nutritionally fortified formula foods commonly used to replace one or more meals per day. Total diet replacements are commonly used to induce rapid weight loss over the short-term, while MR is a more flexible and convenient option to control portion sizes and energy intake while on a restricted diet.

The most recent Canadian Adult Obesity Clinical Practice Guidelines do not recommend the use of over-the-counter commercial TDR for obesity management due to lack of scientific evidence (62, 81). On the other hand, commercial programs that combines nutrition, physical activity, and behaviour change support, such as Weight Watchers, Optifast, Jenny Craig, and Nutrisystem were noted as potential strategies that can be used to achieve modest weight loss (62, 81). Additionally, the guidelines stated that MR can be used to reduce body weight, waist circumference, blood pressure, and improve glycemic control when used to replace one to two meals per day as part of a calorie-restricted intervention (62, 81).

2.3.4.1.1 Total Diet Replacements

Only a few studies have evaluated the effects of TDR in humans to date (78, 82-89). These were mostly long-term intervention trials published since 1986, the interventions consisted of calorie-restricted TDR, and the primary outcome was mainly weight loss (78, 82-89). Although obesity treatment is a long-term process, only one study had a duration >1 year (89), which hinders our ability to translate the findings to longer periods. Three of these studies included individuals diagnosed only with obesity (78, 84, 86). With the objective of testing the effectiveness and safety of a TDR program in n=278 individuals diagnosed with obesity, Astbury, Aveyard (86) assigned n=138 participants to a dietary intervention and n=140 to behavioral support. The dietary intervention consisted of TDR products (soups, shakes, and bars) that provided 810 kcal/day for 8 weeks followed by food reintroduction (86). After 1 year of intervention, the authors observed that

the TDR was tolerable and led to greater weight loss and improvements in the risk of cardiometabolic disease, when compared with the behavioral support (86). In a shorter intervention period, Ard, Lewis (84) examined the effectiveness of a low-calorie TDR (40% protein, 40% carbohydrate, and 20% fat) for 12 to 16 weeks followed by food reintroduction versus a food-based weight loss treatment in n=273 individuals diagnosed with obesity. After 26 and 52 weeks of intervention, participants were assessed and the ones receiving the TDR achieved greater weight loss and weight-loss maintenance when compared to the ones receiving the food-based intervention (84). An interesting study evaluated the effects of a 3-week liquid TDR intervention providing 1120 kcal/day versus an isocaloric portion-controlled intervention in n=32 individuals diagnosed with obesity using a functional magnetic resonance imaging food-cue reactivity (78). The authors observed that participants who had consumed the TDR experienced greater weight loss and reduced food cravings, suggested to relate to an increase in executive control showed in participant's brain images (78).

Most of the studies examining the effects of TDR in humans included individuals with obesity and sometimes with pre-diabetes (n=583) (87) or T2D (n=872) (82, 83, 85, 88, 89). The first experiment conducted with a TDR was an inpatient study, in which n=5 individuals diagnosed with obesity and n=10 diagnosed with obesity and T2D consumed a liquid diet of 300 kcal/day (30 g of protein, 40 g of carbohydrate, 2.5 g of fat,) for 40 days (88). The authors reported that this very-low calorie intervention was a safe and effective weight loss strategy that also improved participant's blood glucose and lipid levels (88). Several years later, Li, Tseng (87) analysed data retrospectively from n=2093 individuals with obesity (n=583 with pre-diabetes and n=367 with T2D) who were prescribed a very-low calorie diet (500-800 kcal/day) or a low-calorie diet (800-1200 kcal/day) based on a TDR and later transitioned to a MR products, while receiving recommendations about exercise practice and behavioral counselling sessions. After 1 year of intervention, participants had lost approximately 14% of their initial body weight, which was considered an effective result (87). An interesting study involving 49 primary care practices in Europe tested the effect of a low-calorie TDR (825-853 kcal/day, 61% carbohydrate, 13% fat, and 26% protein) for 3 to 5 months followed by food reintroduction versus best-practice care by guidelines in n=306 individuals diagnosed with obesity and T2D (85). One year later, participants were assessed and 24% of those in the intervention group had lost 15 kg, 46% achieved T2D remission, and their quality of life improved (85). A follow-up study reassessed these participants

2 years later and observed that more than 1/3 of participants were still on T2D remission and this remission rate was linked to the extent of sustained weight loss (89). It was shown that 11% of participants assigned to the intervention group and 2% of those in the control group lost at least 15 kg of body weight; the difference in body weight change between the control and intervention groups was -5.4 kg (89). More recently, McCombie, Brosnahan (83) reported the results of n=288 participants diagnosed with obesity (n=99 with T2D) who received the same nutritional intervention adopted by Lean, Leslie (85) for 12 weeks followed by food reintroduction. After 1 year of intervention, 22% of the participants had lost 15 kg and weight loss was equally effective in individuals presenting with T2D (83). The most recent study on the subject examined the effect of the same intervention used by Lean, Leslie (85) and McCombie, Brosnahan (83) for 12 weeks followed by food reintroduction versus standardized care in n=90 individuals diagnosed with obesity and T2D (82). At 1 year, participants receiving the TDR had experienced greater weight loss, reduction in insulin therapy, and improved quality of life (82).

2.3.4.1.2 Meal Replacements

To date, two meta-analyses summarizing the effects of MR on weight management have been published (76, 90). The first was published in 2003 and included six randomized controlled trials and data analysis from n=487 individuals with overweight and obesity (n=249 treated and n=238 controls), in which n=119 were diagnosed with T2D (90). This meta-analysis compared isocaloric dietary interventions of 3 to 51 months duration comprised of a liquid MR product or a conventional reduced calorie diet (90). Participants were mostly females (75%) and presented with a mean age of 46 years and a mean BMI of 31.0 kg/m^2 at baseline (90). After 3 months and 1 year of intervention, weight loss was significant in both intervention groups; however, the effect was greater in the group receiving the MR, but only at the 3-month time point (-2.54 kg, $p<0.01$) (90). Additionally, participants assigned to both groups experienced an improvement in risk factors for comorbidities associated with excess adiposity at both time points (90). None of the studies included in this meta-analysis reported adverse events from the dietary interventions (90).

The second meta-analysis was published in 2019 and included twenty-three randomized controlled trials that analysed data from n=8253 individuals with overweight or obesity (n=4411 treated and n=3852 controls) (76). The dietary intervention comprised of one or more MR daily, as part of an energy restricted diet intended for weight loss, while the comparator was minimal intervention without a MR or no intervention at all (76). Studies were only included if participant's

body weight was reported after 1 year of intervention (76). Similarly to the previous meta-analysis, participants were mostly females (79%) and presented with a mean age of 48 years and a mean BMI of 34.5 kg/m^2 at baseline (76). The meta-analysis revealed that mean weight change at 1 year favoured the MR group when a MR diet was compared with diet only (-1.44 kg, p=0.007), when a MR diet along with support was compared with a diet with support (-2.22 kg, p=0.01), when a MR diet along with support was compared with diet only (-3.87 kg, p=0.03), as well as when a MR diet with enhanced level of support was compared with diets with support (-6.13 kg, p<0.001) (76). Besides the weight loss effects, glycated haemoglobin was improved with the MR diet (76).

Although both meta-analyses reported findings 1 year post intervention, some included clinical trials which were conducted for longer periods of time (91-93). Rock, Flatt (93) examined the effects of a structured weight loss program comprised of MR, counselling sessions, and increased physical activity versus usual care over 2 years in n=407 females with overweight or obesity. After 2 years, the structured program resulted in greater weight loss (-7.4 kg and -6.2 kg for the center-based and telephone-based groups, respectively) compared to usual care (-2.0 kg). In a prospective single-arm 4-year clinical trial, Flechtner-Mors, Ditschuneit (91) evaluated n=75 individuals with overweight or obesity assigned to a control diet or an isoenergetic MR diet. After 4 years, weight loss was greater in the MR group (-8.4%) compared to the control diet (-3.2%). Moreover, only those who received the MR experienced improvement in triacylglycerol and systolic blood pressure (91). Lastly, Ptomey, Saunders (92) assessed the effects of a conventional diet or an intervention diet comprised of MR in n=149 individuals with overweight or obesity for 1.5 years. Although weight loss was greater in the intervention group (-7.0%) compared to the conventional diet (-3.8%) at 6 months, this difference disappeared 1.5 years post-study (92).

2.3.4.2 High-Protein Diets

According to the Institute of Medicine, the required amount of dietary protein for healthy adults aged >19 years is 0.80 g/kg of body weight/day or 10 to 35% of total energy intake (94). Therefore, diets with a protein content above recommended values can be considered high-protein (HP) diets; however, it is important to clarify that the literature lacks a clear definition, and the protein content of HP diets varies considerably among studies.

High-protein diets have been growing in popularity, as evidence accumulates on the benefits of this dietary strategy for weight management. High-protein diets appear to exert strong

influence on the main factors involved in body weight regulation, which are energy metabolism, body composition, and appetite (95-100).

High-protein diets have been shown to increase and sustain EE even when individuals are in a state of negative energy balance, which can be advantageous in the process of weight loss and its maintenance over time (96). The effect of HP diets on energy metabolism starts with their direct effect on TEF. As previously mentioned, protein requires 25 to 30% of the energy content of the meal to be digested, absorbed, processed, and stored, which is a significantly higher than the amount for the other macronutrients (i.e., carbohydrate: 6-8%, fat: 2-3%) (30). Because of the body's limited protein storage capacity, this macronutrient needs to be readily metabolized by costly mechanisms (95). This metabolic inefficiency is illustrated by the difference between the gross and metabolizable energy value of protein, which is estimated to be approximately 2.9 kcal/g but can vary depending on the amino acid composition of the protein (95). The effect of a HP diet on TEF was confirmed by two classic inpatient metabolic balance studies, in which healthy participants received a HP diet while inside a WBCU for 24 (101) and 36 consecutive hours (102). In both studies, TEF was shown to be increased with the HP diet (101, 102). In addition to its direct effects on TEF, HP diets have been shown to increase SEE and REE, which seems to be explained by stimulation of protein synthesis, protein turnover, and gluconeogenesis (95).

Wycherley, Moran (100) conducted a meta-analysis on the effects of energy-restricted HP diets and observed that REE was sustained during weight loss. This effect seemed to be related to a preservation of FFM (100), a body compartment that is the main determinant of REE and accounts for 50 to 70% of its variability (103). Considering weight loss leads to a decrease in EE, the preservation or even accretion of FFM and, consequently, REE is very important during this process (104). However, this is only possible with a state of neutral or positive protein balance, and HP diets have been shown to be a great allied in preserving or even increasing FFM during weight loss. Soenen, Martens (105) demonstrated that a protein intake of 1.2 g/kg body weight/day was able to preserve FFM and REE during weight loss. Another interesting study showed that participants consuming 1.6 and 2.4 g/kg body weight/day of dietary protein while in a 3-week energy restricted diet, lost more body fat and less FFM when compared to the ones receiving the recommended values (i.e., 0.8 g/kg body weight/day) (106). A meta-regression demonstrated that a daily protein intake >1.05 g/kg body weight during energy restriction was associated with greater FFM retention compared with diets with a protein content closer to recommended values (107).

Protein is the most satiating macronutrient, followed by carbohydrate, and fat (108). There also appears to be a dose-dependent satiating effect of protein, in which HP diets have a higher satiating effect than diets with a lower protein content (108). When consumed *ad libitum*, HP diets have been shown to reduce food intake (95). Moreover, a recent systematic review observed increased fullness and satiety with the HP diets (i.e., >1.2 g/kg body weight/day or >25% of total energy intake) compared with diets with a normal protein content (i.e., 0.8 to 1.2 g/kg body weight/day or 15 to 20% of total energy intake) in individuals with overweight and obesity (97). Several mechanisms and hypotheses have been proposed to understand the satiating effects of HP diets (95, 96, 99). These diets have been shown to stimulate the release of cholecystokinin, GLP-1, and PYY, and suppress postprandial ghrelin secretion, influencing appetite sensations (95). These effects seem to happen through a peripheral and central detection of amino acids that stimulate vagal activity in areas of the brain involved in the control of food intake (95). The elevated EE and consequently increased O_2 consumption and increased body temperature have also been postulated as possible mechanisms behind the HP-induced satiety (108). These consequences lead to a feeling of O_2 deprivation, which promotes satiety (108). Depending on the type of protein ingested, hunger can also be suppressed (95). Interestingly, the consumption of proteins deficient in one or more essential amino acids, such as gelatin, has been shown to provoke a signal to stop eating in humans (96). In animals, this is known as the indispensable amino acid deficiency theory, in which diets that lead to depletion of deficiency of essential amino acids are rejected and aversions to it are developed (96). Proteins from animal sources (e.g., meat, fish, poultry, eggs, and dairy) usually provide all indispensable amino acids, whereas plant-based proteins (e.g., beans, peas, lentils, nuts, and seeds) often lack one or more indispensable amino acid (109). An exception of plant-based protein that contain all indispensable amino acids is soy (109), which has been demonstrated to exert the same appetite response as whey protein (110). The protein leverage hypothesis has also been proposed as one of the mechanisms involved in the regulation of food intake with a HP diet (111, 112). This hypothesis suggests the existence of a protein-specific appetite, in which a low-protein intake would lead to a compensatory increase in energy intake from carbohydrate and fat in an attempt to achieve a target amount of protein, while a HP intake would lead to the opposite scenario (111, 112). This hypothesis have been considered a potential contributor to the obesity epidemic in the United States (113).

2.3.4.3 Physical Activity

Weight loss is usually the main outcome in the treatment of obesity. Considering the modest benefits of physical activity alone on weight and fat losses, its value is often underestimated by patients and health professionals (114). According to the most recent Canadian Adult Obesity Clinical Practice Guidelines, regular physical activity should be part of weight management interventions, as it produces several health benefits, even in the absence of weight loss (115). Regular physical activity has been shown to contribute to modest weight and fat losses, as well as weight loss maintenance over time (114). Additionally, it has been associated with lower incidence of chronic diseases, cardiovascular risk factors, and all-cause mortality (116). Therefore, physical activity is an indispensable tool in obesity management (114). Appropriate physical activity programs for weight management should combine negative energy balance, long-term adherence, and beneficial effects on health and well-being (114).

The Canadian Physical Activity Guidelines recommend adults aged 18 to 64 years to accumulate at least 150 minutes of moderate- to vigorous-intensity aerobic physical activity per week and add muscle- and bone-strengthening activities at least twice a week (117). In line with these recommendations, the Canadian Adult Obesity Clinical Practice Guidelines gives the following advices for weight management (115):

- 30 to 60 minutes of moderate- to vigorous-intensity aerobic physical activity performed most days of the week can help with weight management and produce several health benefits;
- Resistance training may promote benefits related to weight maintenance, body composition, and mobility;
- Higher-intensity exercises, such as high-intensity interval training, can improve cardiorespiratory fitness and reduce time spent on moderate-intensity aerobic physical activity;
- Regular physical activity produces several cardiometabolic benefits, improve quality of life, mood disorders, and body image, even in the absence of weight loss.

For weight and fat losses, most of the recommendations worldwide are similar; however, without dietary restriction, physical activity alone produces only modest weight reduction over the long-term (i.e., 2 to 3 kg), which is not considered clinically significant (i.e., ≥5% of initial body weight) (114). Petridou, Siopi (114) showed that 225 to 420 minutes of moderate- to vigorous-

intensity physical activity is the volume needed to achieve that. Moreover, the authors highlighted the importance of both aerobic and resistance exercises for weight and fat losses (114). High-intensity interval training has been associated with some benefits, but further research is needed to elucidate the optimal characteristics for individuals with overweight and obesity (114).

Weight regain following effective weight loss is often experienced by individuals with overweight and obesity, which seems to be caused by biological, behavioral, and environmental factors (118). Therefore, weight loss maintenance is the greatest challenge in the successful treatment of overweight and obesity (114, 118). Several observational and interventional studies reviewed by Petridou, Siopi (114) report that weight loss maintenance over the long-term can only be achieve with large increases in physical activity and more seems to be better than less. Studies included in the review reported successful weight loss maintenance with >60 to 80 minutes of moderate-intensity physical activity, >35 minutes of vigorous-intensity per day, or 200 to 300 minutes of moderate-intensity per week (114). However, it is important to highlight that even when successful weight loss maintenance is not achieved, regular physical activity after weight loss is associated with several health benefits (114).

Table 2.1. Standard volumes of oxygen consumed, carbon dioxide produced, and energy expended per gram of macronutrient oxidized

Substrate (1 gram)	O_2 (L)	CO_2 (L)	EE (kcal)
Carbohydrate	0.8288	0.8288	4.182
Protein	0.967	0.7752	4.316
Fat	2.0193	1.4311	9.461
Urinary N	N/A	N/A	-2.17

Abbreviations: CO_2: carbon dioxide; EE: energy expenditure; N: nitrogen; N/A: not applicable; O_2: oxygen.
Table adapted from Schoffelen and Plasqui (119).

Table 2.2 Standard energy equivalents of oxygen and carbon dioxide

Substrate	O_2 (kcal/L)	CO_2 (kcal/L)
Carbohydrate	5.04	5.04
Protein	4.47	5.59
Fat	4.68	6.64

Abbreviations: CO_2: carbon dioxide; O_2: oxygen.
Table adapted from Westerterp (23).

Table 2.3. Most commonly used equations to estimate energy expenditure and macronutrient oxidation rates by indirect calorimetry

Energy Metabolism Component	Author, Year	Equation
Energy Expenditure (kcal)	Zuntz, 1897	3.816 x VO_2 (L) + 1.230 x VCO_2 (L) - 1.916 x N (g)
	Magnus-Levy, 1907	3.816 x VO_2 (L) + 1.230 x VCO_2 (L) - 1.278 x N (g)
	Loewy, 1911	3.816 x VO_2 (L) + 1.230 x VCO_2 (L) - 2.026 x N (g)
	Lusk, 1928	3.816 x VO_2 (L) + 1.230 x VCO_2 (L) - 2.026 x N (g)
	Weir, 1949	3.941 x VO_2 (L) + 1.106 x VCO_2 (L) - 2.170 x N (g)
	Consolazio, 1963	3.781 x VO_2 (L) + 1.159 x VCO_2 (L) - 2.980 x N (g)
	Brouwer, 1965	3.866 x VO_2 (L) + 1.20 x VCO_2 (L) - 1.43 x N (g)
	Bursztein, 1977	3.788 x VO_2 (L) + 1.159 x VCO_2 (L) - 2.903 x N (g)
	Ben-Porat, 1983	3.912 x VO_2 (L) + 1.092 x VCO_2 (L) - 2.839 x N (g)
	Passmore and Eastwood, 1986	3.776 x VO_2 (L) + 1.161 x VCO_2 (L) - 2.868 x N (g)
	Brockway, 1987	3.962 x VO_2 (L) + 1.077 x VCO_2 (L) - 1.410 x N (g)
	Elia and Livesey, 1992	3.802 x VO_2 (L) + 1.245 x VCO_2 (L) – 1.111 x N (g)
Protein Oxidation (g)	N/A	6.25 x N (g)
Carbohydrate Oxidation (g)	Brouwer, 1957	4.170 x VCO_2 (L) - 2.965 x VO_2 (L) - 0.390 x P (g)
	Frayn, 1983	4.55 x VCO_2 (L) - 3.21 x VO_2 (L) - 2.87 x N (g)
	Jequier, 1987	4.113 x VCO_2 (L) - 2.907 x VO_2 (L) - 0.375 x P (g)
	Livesey and Elia, 1988	4.650 x VCO_2 (L) - 3.311 x VO_2 (L) - 3.518 x N (g)
	Ferrannini, 1988	4.55 x VCO_2 (L) - 3.21 x VO_2 (L) - 2.87 x N (g)
Fat Oxidation (g)	Brouwer, 1957	1.718 x VO_2 (L) - 1.718 x VCO_2 (L) - 0.315 x P (g)
	Frayn, 1983	1.67 x VO_2 (L) - 1.67 x VCO_2 (L) - 1.92 x N (g)

Jequier, 1987	1.689 x VO_2 (L) - 1.689 x VCO_2 (L) - 0.324 x P (g)
Livesey and Elia, 1988	1.720 x VO_2 (L) - 1.720 x VCO_2 (L) - 1.776 x N (g)
Ferrannini, 1988	1.67 x VO_2 (L) - 1.67 x VCO_2 (L) - 1.92 x N (g)

Abbreviations: EE: energy expenditure; N: urinary nitrogen; N/A: not applicable; P: protein oxidation; VCO_2: volume of carbon dioxide; VO_2: volume of oxygen.

Table 2.4. Resting and basal energy expenditure measurement protocols using indirect calorimetry

Criteria	Resting Energy Expenditure	Basal Energy Expenditure
Timing	After 10 to 20 minutes of rest	Measured taken in the morning (between 6 and 9 am) after sleep
Fasting	4 to 5 hours after meals or snacks	10 to 12 hours (overnight fast)
Physical activity	Moderate exercise: minimum abstention of 2 hours Vigorous exercise: minimum abstention of 14 hours	Overnight sleep at the site of measurement
Position	Reclined position and fully awake	Lying down and fully awake
Temperature	Thermoneutral (between 20°C and 25°C)	Thermoneutral (between 22°C and 26°C)

Adapted from Henry (20) and Compher, Frankenfield (21).

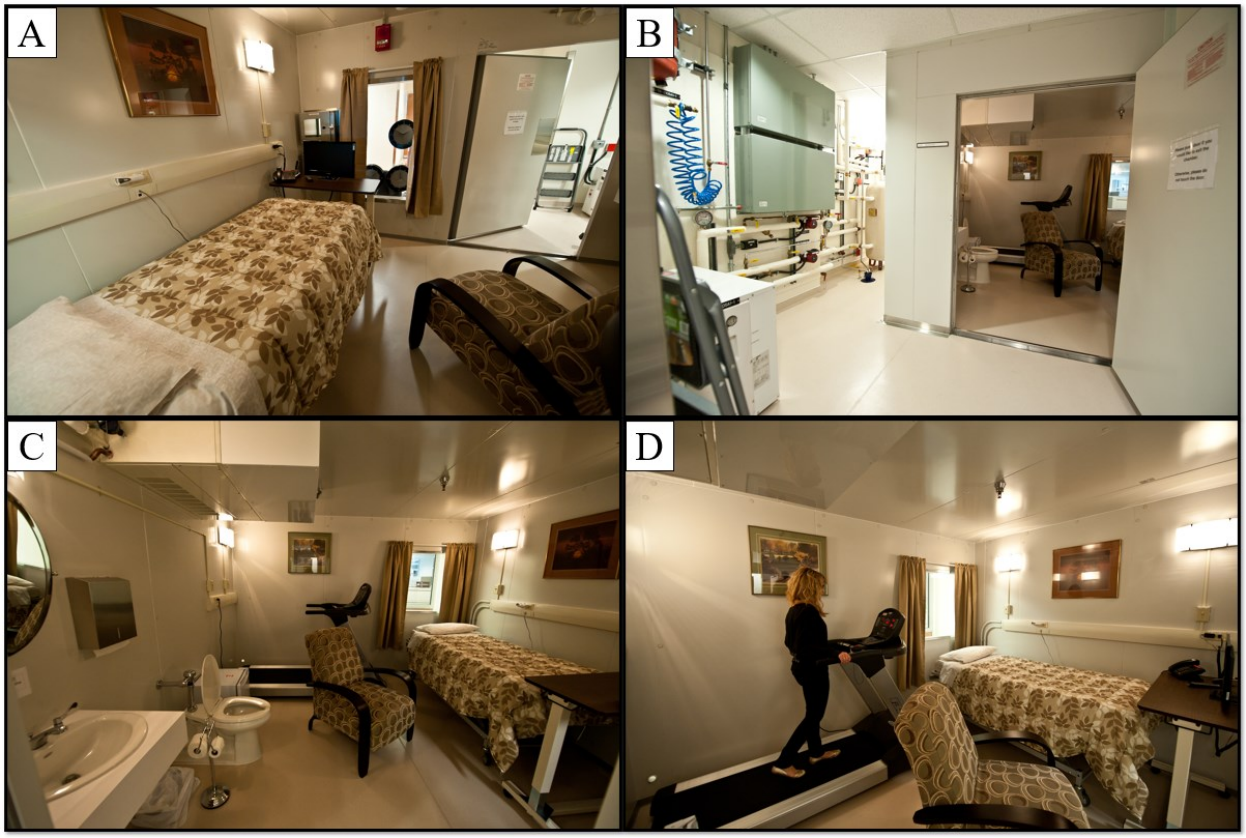

Figure 2.1. Pictures of the whole-body calorimetry unit located in the Human Nutrition Research Unit at the University of Alberta (Edmonton, AB, Canada).

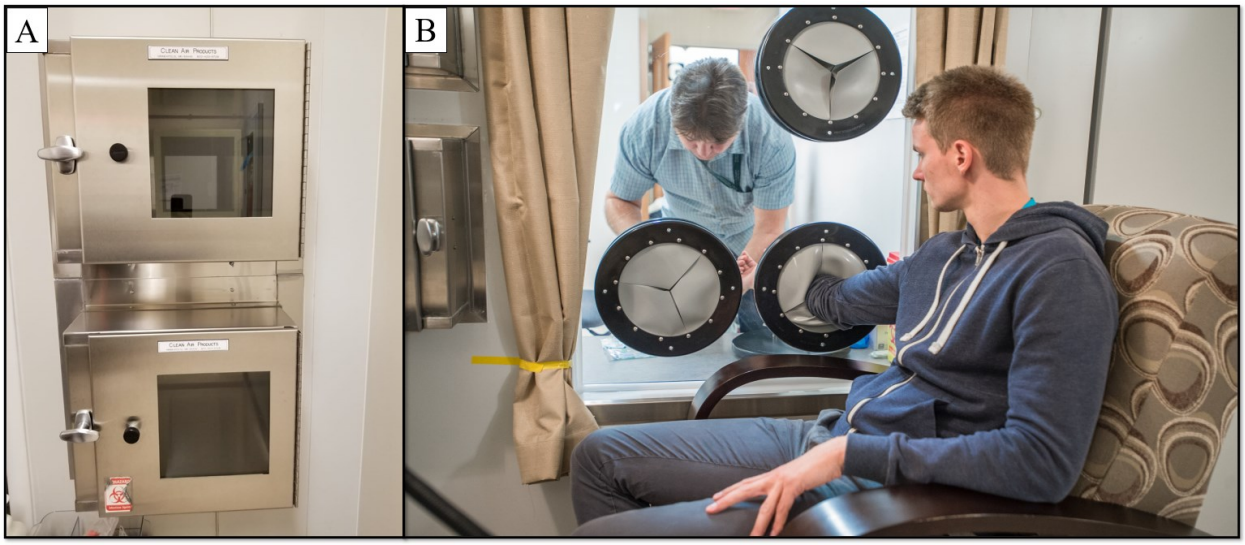

Figure 2.2. Pictures of the air lock systems (panel A) and iris port (panel B) of the whole-body calorimetry unit located in the Human Nutrition Research Unit at the University of Alberta (Edmonton, AB, Canada).

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CHAPTER 3. RESEARCH PROTOCOL

3.1 PREFACE

A version of this chapter has been published as: *Oliveira CLP, Boulé NG, Sharma AM, Elliott SA, Siervo M, Ghosh S, Prado CM. Examining the effects of a high-protein total diet replacement on energy metabolism, metabolic blood markers and appetite sensations in healthy adults: protocol for two complementary randomized, controlled, crossover trials. Trials. 2019; 20(1):787. doi: 10.1186/s13063-019-3950-y.* All authors and the external collaborator Aloys Berg were involved in the design of this research protocol. I was responsible for the manuscript composition. All authors contributed to manuscript edits. Carla M. Prado was the supervisory author and was involved with concept formation and manuscript composition.

3.2 ABSTRACT

Background: High-protein diets and total diet replacements are becoming increasingly popular for weight regulation; however, further research is needed to elucidate their impact on the physiology of body weight regulation. The aim of this inpatient metabolic balance study is to compare the impact of a high-protein total diet replacement versus a control diet (North American) on energy expenditure, macronutrient oxidation rates and balances, metabolic blood markers and appetite sensations in healthy adults.

Methods: Two randomized, controlled, cross-over clinical trials conducted separately in men and women will be conducted. In each trial, participants will be allocated to two isocaloric arms: a) Control diet: 55% carbohydrate, 15% protein, and 30% fat; b) High-protein total diet replacement: 35% of carbohydrate, 40% protein, and 25% fat. They will receive the prescribed diets for 32 h while inside the whole-body calorimetry unit. Diets will be designed to ensure participants are in energy balance. The following physiological changes will be compared between groups: energy expenditure, macronutrient oxidation rates and balances, metabolic blood markers, and appetite sensations. Body composition will be assessed at baseline using dual-energy X-ray absorptiometry.

Discussion: This will be the first inpatient metabolic balance study examining the impact of a high-protein total diet replacement on energy metabolism, metabolic blood markers and appetite sensations in healthy young adults (of both sexes) using a whole-body calorimetry unit. Results of this clinical trial can ultimately be used to develop strategies to optimize high-protein diet interventions and weight management.

3.3 BACKGROUND

Defining the ideal macronutrient composition to prevent and treat obesity and its related diseases has been the target of many studies dating back to the 1950s. At that time, Rubner and Atwater discovered that macronutrients (carbohydrate, protein, fat and alcohol) can elicit different responses in energy expenditure (1). Later on, it was demonstrated that the energy expended to absorb, process and store ingested nutrients is highest for protein (25-30% of energy content), followed by carbohydrate (6-8%) and fat (2-3%) (2), suggesting that a diet with a protein content above the recommended values (i.e. for healthy adults >19 years of age: 0.80 g/kg of body weight/day or 10 to 35% of total energy intake) (3) may be advantageous for weight management when compared to other diets with different proportions of macronutrients (4). Furthermore, protein appears to exert a stronger satiating effect and decrease energy intake under *ad libitum* conditions (5). Therefore, high-protein (HP) diets are becoming increasingly popular for weight loss; however, lack of dietary adherence due to behavior and/or environmental factors seems to impact long-term effectiveness of this type of diet on weight-loss maintenance (6).

Total diet replacements (TDR) are nutritionally complete formula foods designed to replace the whole diet for a specific period of time to facilitate weight loss. Insufficient evidence on the long-term effectiveness of this approach hinders its recommendation for the treatment of obesity (7). Astbury, Aveyard (8) showed that participants with obesity following a TDR weight loss program lost 7.2 kg more than individuals receiving usual care after one year of intervention and experienced greater improvements in biomarkers of cardiovascular and metabolic risk. In a similar study, Lean, Leslie (9) observed that half of participants on a one-year TDR weight management program reverted to a non-diabetic state and remission was shown to be closely related to the degree of weight loss.

Considering the benefits of a higher protein intake and TDR in isolation, the combination of these strategies might generate synergistic effects, promoting effective weight loss and its maintenance over time. However, to consider a HP-TDR as an option to prevent and treat obesity, it is imperative to first understand the physiological impact of this strategy in a healthy population group so that the effects are better translated in individuals with obesity and its related chronic diseases. Of particular importance is the study of these variables separately in men and women, as their metabolic regulation differs considerably (10-12). Therefore, inpatient controlled-feeding research using state-of-the-art technology, such as the whole-body calorimetry unit (WBCU), is needed to elucidate the exact role of HP diets comprised of a TDR on the regulation of energy expenditure, macronutrient oxidation rates and balances, appetite sensations and metabolic blood markers. To our knowledge, no research of this kind has been conducted separately in healthy men and women. Therefore, the aim of this inpatient metabolic balance study is to compare the impact of a HP-TDR versus a control (CON) diet (North American) on energy expenditure, macronutrient oxidation rates and balances, metabolic blood markers and appetite sensations in male and female healthy adults.

3.4 METHODS

3.4.1 Study Design and Ethical Procedures

These studies will be randomized, controlled, crossover design clinical trials conducted separately in women and men at the Human Nutrition Research Unit (HNRU), University of Alberta (Edmonton, AB, Canada). The corresponding research protocol fulfils the requirements of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist (13). The research protocols involving women and men were approved separately by the University of Alberta Ethics Board (Pro00066006 and Pro00083005) and complied with the standards as set out in the Canadian Tri-Council Policy statement on the use of human participants in research. If important protocol modifications are needed, the research team will contact the University of Alberta Ethics Board, trial registry (clinicaltrials.gov), and trial participants.

3.4.2 Outcome Measures

Primary outcome:

- Difference in fat balance (i.e., fat intake minus fat oxidation) between the HP-TDR and CON conditions.

Secondary outcome:

- Difference in 24-hour energy expenditure (EE) between the HP-TDR and CON conditions.

Exploratory outcomes:

- Difference in selected components of energy metabolism (resting metabolic rate [RMR], basal metabolic rate [BMR], sleeping metabolic rate [SMR], postprandial EE, exercise EE, 24-hour respiratory quotient [RQ], macronutrient oxidations rates, energy and macronutrient balances) between the HP-TDR and CON conditions;
- Change in concentrations of metabolic blood markers (glucose, insulin, cholesterol, high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol, triglyceride and non-HDL cholesterol, acyl-ghrelin, leptin, peptide YY [PYY], glucagon-like peptide 1 [GLP-1], free glycerol and non-esterified fatty acids [NEFA]) over time (within-groups) and between the HP-TDR and CON conditions;
- Difference in appetite sensations (hunger, satiety, fullness and prospective food consumption) between the HP-TDR and CON conditions.

The primary outcome of this study was initially planned to be RQ. However, it was changed to fat balance (i.e., fat intake minus fat oxidation) *before* any data analysis. The rational for the change was that it has been established that RQ is strongly associated to the dietary carbohydrate content (14) and it was felt that fat balance would be a more meaningful outcome.

3.4.3 Data Management

Before the commencement of the study, all participants will be informed of the procedures and potential risks involved in the investigation, and will be asked to provide written informed consent. Moreover, participants will be informed that all data collected during the study will be kept private and no personal identifiable information will be shared. All data will be securely stored for five years. Storage of research-related paper files will be in a locked cabinet at the HNRU, and all electronic files will be stored on a secured server. If participants decide to discontinue the study no other information will be collected; however, the data already collected will be kept, unless participants specifically request it to be destroyed. Only the study team will have access to all hard copy and electronic datasets.

3.4.4 Research Participants

Healthy women and men aged 18 to 35 years, with a body mass index (BMI) between 18.5 and 24.9 kg/m² will be recruited via advertisements placed on notice boards at the University of Alberta north campus. All participants will be nonsmokers and women will be required to have a regular menstrual cycle, lasting between 25 and 35 days. Exclusion criteria includes any diagnosed chronic disease; use of any medication able to impact energy metabolism or body composition; lactose, gluten and/or soy allergy/intolerance; adherence to a vegetarian, vegan or restrictive dietary pattern; pregnancy or lactation; use of nutritional supplements two months prior to study initiation; engagement of over an hour per day of leisure time physical activity or more than seven hours per week of strenuous activity three months prior to study initiation; exposure to a nuclear medicine scan or injection of an X-ray dye one week prior to study initiation, or a barium test/exam two weeks prior to study initiation; and/or diagnosis of claustrophobia.

Potential participants will be instructed to report to the HNRU for a screening visit that includes blood tests (albumin, creatinine, aspartate transaminase, alanine transaminase, sodium, potassium, chloride and thyroid-stimulating hormone), anthropometric measurements (height, weight and waist circumference), and the completion of questionnaires eliciting information about health, use of medications, caffeine consumption, physical activity (Godin-Shephard Leisure-Time Physical Activity Questionnaire), and palatability of the study foods. Participants will taste the HP-TDR and read the menus of the CON diet and only those confident of being able to consume the food items provided during the study period and fulfill the inclusion criteria requirements will be included in the study. Once deemed eligible, participants will be randomly assigned following simple randomization procedure separated by sex to begin with a HP-TDR or CON diet in a 1:1 allocation ratio that will be based on a computer-generated list of random numbers using Microsoft Office Excel 2010 (Microsoft, Redmond, WA, USA). The study team will enroll and assign participants to the interventions.

3.4.5 Experimental Protocol

An outline of the experimental protocol is given in **Figure 3.1** and the schedule of enrollment, interventions and assessments in **Figure 3.2**. Following the screening process, eligible participants will be invited to attend two study visits for body composition assessment, a 1-hour RMR test, and a fitness test. After these visits are complete, on separate intervention days,

participants will undergo two 32-hour whole-body calorimetry stays for the measurement of energy metabolism components, appetite sensations and metabolic blood markers while consuming HP-TDR and CON isocaloric diets in a random order. A 3-day run-in period with a controlled, energy-balanced diet will precede both intervention phases. For women, each intervention phase will be followed by a wash-out period of approximately one month to preclude influences of the menstrual cycle. For men, a two-week wash-out period will be required to eliminate any effect of the diet. Participants will be instructed to maintain a stable body weight and physical activity level throughout the study period. Since the length between the two 32-hour whole-body calorimetry stays was short, fluctuations in body weight are not expected in this cohort of healthy individuals.

3.4.6 Anthropometry and Body Composition

At baseline, height, weight, waist circumference and body composition will be assessed. All anthropometric measurements will be performed with participants' barefoot and wearing light clothing. Height will be measured using a 235 Heightronic Digital Stadiometer (Quick Medical, Issaquah, WA, USA) to the nearest 0.1 cm. Body weight will be measured using a calibrated digital scale (Health o meter® Professional Remote Display, Sunbeam Products Inc., FL, USA) to the nearest 0.1 kg. Waist circumference will be measured using a measuring tape.

Body composition will be assessed via dual-energy X-ray absorptiometry (DXA) using a GE Lunar iDXA (General Electric Company, Madison, USA; enCORE software 13.60 Lunar iDXA GE Health Care®). Participants will be instructed to remove all clothing (except underwear) and metal objectives (including underwire bras, piercings, etc.) and put on the hospital gown provided. Whole body and regional levels of fat mass (FM), lean soft tissue (LST) and bone mineral content (BMC) will be collected. A previous study (results not published) conducted with this device revealed coefficients of variation (CV) of 1.05% for FM (%), 0.99% for FM (g), 0.37% for LST (g), and 0.40% for BMC (g).

3.4.7 Fitness Test

Prior to the intervention phase, a fitness test will be conducted to personalize and standardize the speed and incline of the prescribed exercise session for each whole-body calorimetry condition. An 8- to 12-hour overnight fasting will be required. At the time of the test,

participants will be fed a breakfast with the same energy content and macronutrient composition of the CON condition (35% of total energy intake from carbohydrate, 40% from protein and 25% from fat). Breakfast and fitness test starting times will also be identical to the protocol of the whole-body calorimetry conditions (9:00am and 10:20am, respectively).

Participants will perform an incremental submaximal exercise test on a treadmill (Freemotion Incline Trainer, Freemotion Fitness, Logan, UT, USA). The fitness test will begin at an individualized walking speed characterized as comfortable and sustainable. After a 5-minute warm-up, the incline will be increased by 2% every 3 minutes and the speed will be maintained constant until a respiratory exchange ratio (RER) of 0.90 is achieved. During the fitness test, expired gases will be analyzed by a calibrated TrueMax® metabolic measurement system (Parvo Medics TrueOne® 2400 Metabolic Measurement System, Sandy, UT, USA). Heart rate will be monitored continuously with a Polar FT1 Heart Rate Monitor (Polar Electro Oy, Kempele, Finland). To determine the workload for the WBCU exercise session, RER of participants will be plotted against their fitness test workload. The speed and incline at which an RER of 0.85 occur will be selected as the intensity for the whole-body calorimetry exercise sessions.

3.4.8 Run-in Diet

A 3-day run-in period with a controlled, energy-balanced diet will precede both 32-hour whole-body calorimetry conditions to minimize the effects of any eating style or behavior on baseline data and standardize dietary intake among participants. Diets will be designed by a Registered Dietitian using the Food Processor Nutrition Analysis Software (version 11.0.124, ESHA Research, Salem, OR, USA). All food will be weighed and prepared at the metabolic kitchen of the HNRU by trained staff. Participants will receive three meals (breakfast, lunch and dinner) and two snacks (afternoon and evening snacks) per day for three days, and will be instructed to drink water *ad libitum.* They will be required to eat all the food provided and no additional food will be allowed. The run-in diet will provide 55% of total energy intake from carbohydrate, 15% from protein and 30% from fat, a macronutrient distribution within the Acceptable Macronutrient Distribution Range (3) and similar to a North American dietary pattern (15). To ensure participants are in energy balance, individual daily energy requirements will be calculated as RMR, determined by a 1-hour RMR test using indirect calorimetry, multiplied by a physical activity coefficient, according to the Dietary Reference Intakes (3), and a coefficient of

1.075 representing the metabolizable energy content of the diets (i.e., same coefficient will be applied for both HP-TDR and CON). (16). Strenuous physical activity and caffeinated food products will not be allowed during this 3-day run-in period.

3.4.9 Energy Metabolism

Energy expenditure, macronutrient oxidation rates and balances will be assessed by indirect calorimetry, by measuring the volume of oxygen (VO_2) and carbon dioxide (VCO_2), using an open-circuit WBCU. The ambient temperature will be maintained between 21 and 23°C. Mixed, expired air will be drawn out of the unit while fresh, conditioned air will be passively drawn into the unit at a pre-determined constant flow rate of 60 liters per minute. Differences in VCO_2 and VO_2 concentrations of expired and fresh air will be calculated minute-by-minute by the Advance Optima AO2000 Series CO_2 analyzer (ABB Automation GmbH, Frankfurt, Germany) and the Oxymat 6 O_2 analyzer (Siemens AG, Munich, Germany). Information will be translated from the gas analyzers to a computer (Acer Aspire AM3910-E3122, Acer Inc., New Taipei City, Taiwan) via the National Instruments NI USB-6221 device (National Instruments Corporation, Austin, TX, USA) using the PMCSS Software version 1.8 (Pennington Metabolic Chamber Software Suite, Pennington Biomedical Research Center, LA, USA). At baseline, participants will complete a 1-hour RMR indirect calorimetry test; then, two 32-hour tests will be conducted while participants consume the HP-TDR and CON diets.

3.4.9.1 RMR Test

At baseline, participants will be requested to attend the HNRU after fasting for 8 to 12 hours and refraining from exercise for 24 hours prior to the test. Only minimal physical activity will be allowed on the morning of the test (e.g.: getting dressed; driving from home to the research unit and short walk from the parking lot to the research unit). Participants will be instructed to rest in a supine position, being awake, but motionless. RMR will be measured for 60 minutes with the first 30 minutes excluded from analysis to account for acclimatization. Results from this test will be used to estimate energy requirements for the 3-day run-in diet and 32-hour whole-body calorimetry tests.

3.4.9.2 Whole-Body Calorimetry Tests

The day following the 3-day run-in periods, participants will return to the HNRU after fasting for 8 to 12 hours and will spend 32 consecutive hours in the WBCU (8:00am on day 1 until

4:00pm on day 2) while receiving the HP-TDR and CON diets in a random order. On the morning of the tests, only minimal physical activity and void bladder will be requested before entering the WBCU. Both 32-hour whole-body calorimetry tests will happen during the follicular phase of women's menstrual cycle (i.e.: between day 6th and 13th) and will be conducted, approximately, one month apart (women) and two weeks apart (men). The whole-body calorimetry conditions will follow a strict and standard schedule (**Table 3.1**). Throughout each test, participants will receive three meals (9:00am, 12:00pm and 6:00pm) and two snacks (3:00pm and 9:00pm). Appetite sensations will be rated immediately before and 30 minutes after each meal and snack. Blood will be drawn four times (~7:30am, 11:00am and ~2:30pm on day 1, and ~8:00am on day 2) and urine will be collected during the entire time. On the morning of the first day of test (10:20am), a 40-minute walking session on a treadmill (BH Fitness T8 SPORT, BH Fitness, Foothill Ranch, CA, USA) will be completed, at a personalized fixed pace. Sleep will only be allowed during the night (from 10:00pm to 6:00am).

3.4.9.3 24-hour Energy Expenditure and Substrate Oxidation

24-hour EE and macronutrient oxidation rates will be calculated from the measurements of VO_2, VCO_2 and urinary nitrogen (N) excreted by using the formula of Brouwer (17):

- $24h\ EE\ (kcal/day) = 3.866\ x\ VO2\ (L/day) + 1.20\ x\ VCO2\ (L/day) - 1.43\ x\ N\ (g/day)$

- $PROox\ (g/day) = 6.25\ x\ N\ (g/day)$

- $CHOox\ (g/day) = 4.170\ x\ VCO2\ (L/day) - 2.965\ x\ VO2\ (L/day) - 0.390\ x\ PROox\ (g/day)$

- $FATox\ (g/day) = 1.718\ x\ VO2\ (L/day) - 1.718\ x\ VCO2\ (L/day) - 0.315\ x\ PROox\ (g/day)$

where PROox, CHOox and FATox is protein, carbohydrate and fat oxidation rates, respectively, in grams per day. Energy and macronutrient balances will be calculated as the difference between intake and oxidation:

- $Energy\ Balance = Energy\ Intake\ (kcal/day) - Energy\ Expenditure(kcal/day)$

- $Macronutrient\ Balance = Intake\ (g/day) - Oxidized\ (g/day)$

Respiratory quotient will be calculated as the ratio of VCO_2 exhaled to VO_2 inhaled:

- $RQ = \frac{VCO2\ (L)}{VO2\ (L)}$

During each whole-body calorimetry test, the following components of participants' energy expenditure will be assessed: 24-hour EE, RMR, BMR (energy expended to maintain essential vital function), SMR, postprandial EE and exercise EE. RMR will be assessed as described above. BMR will be assessed in the morning of the second day of test. Participants will receive a wake-up call at 6:00am and will be instructed not to get up from bed and to rest in a supine position, being awake, but motionless for 60 minutes. SMR will be computed for a 3-hour period (2:00am to 5:00am), and sleep EE will be extrapolated to an 8-hour sleep night. Postprandial EE will be assessed on the second day of test after participants finish the breakfast for six consecutive hours. Exercise EE will be assessed in the morning of the first day of test for 40 minutes during the exercise session (10:20am to 11:00am).

3.4.10 Experimental Diets

The HP-TDR and CON diets that will be consumed during both 32-hour whole-body calorimetry conditions will be designed by a Registered Dietitian using the same approach as per the run-in diet. All food will be weighed and prepared by trained staff. Participants will receive three meals (breakfast, lunch and dinner) and two snacks (afternoon and evening snacks) throughout each whole-body calorimetry test (**Table 3.2**). Bottled water will be provided *ad libitum.* The CON diet will provide 55% of total energy intake from carbohydrate, 15% from protein and 30% from fat, and the HP-TDR diet will provide 35% of total energy intake from carbohydrate, 40% from protein and 25% from fat. **Table 3.3** illustrates the nutrient composition of a typical 2000 kcal diet. The CON diet will be comprised of food items that can be found at the local grocery stores without any specific choice of plant-based proteins and the HP-TDR diet will consist of a soy-protein nutritional supplement (Almased®, Almased USA, Inc., St. Petersburg,

FL, USA) mixed with olive oil and low-fat milk (1% fat) for the main meals, and with olive oil and apple juice for the snacks, per label instructions (18). The first dietary intervention randomly offered in the WBCU will be designed to maintain participants in energy balance and the energy content of each meal and snack will be identical for the HP-TDR and CON diets (eucaloric). Energy intake will be initially estimated using a 1-hour RMR test, and adjusted throughout the day on the basis of measured EE using WBCU data points. When the prescribed energy intake falls outside ±100 kcal of the predicted 24-hour EE, the amount of calories provided in the dinner and evening snack will be adjusted (added or subtracted) in units of 100 kcal in an attempt to achieve energy balance. Patients will be required to consume all the food provided and meal trays will be checked after consumption.

3.4.11 Appetite Sensations

During each whole-body calorimetry condition, participants will rate their appetite sensations (hunger, satiety, fullness, and prospective food consumption) using a validated anchored 100-mm visual analogue scale (VAS) (19). Questionnaires will be administered using the paper-and-pen method and participants will be instructed to make a single vertical mark at the appropriate point between the two anchors on each scale to indicate the intensity of their subjective states regarding each element. Participants will be asked to indicate, on a scale from 0 to 100 mm, how they feel at the moment they complete these questions: How hungry do you feel? (I am not hungry at all – I have never been more hungry); How satisfied do you feel? (I am completely empty – I cannot eat another bite); How full do you feel? (not at all full – total full); How much do you think you can eat? (nothing at all – a lot). Participants will complete the questionnaires immediately before and 30 minutes after each meal (breakfast, lunch and dinner) and snack (afternoon and evening). For each rating period, a new questionnaire will be provided to participants and they will not be allowed to consult their previous ratings.

3.4.12 Blood and Urine Analysis

Blood will be sampled by venipuncture from participants at four time points during each whole-body calorimetry condition: 1) the morning on the first day of test (Baseline); 2) immediately after the exercise session (4h Post); 3) two hours after lunch (7.5h Post); and 4) the morning on the second day of test (24h Post). Both morning blood draws will be sampled from

subjects after a 10-hour overnight fast. Blood will be drawn into BD Vacutainer® blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), spray-coated with silica and a polymer gel for serum separation or K_2-ethylenediaminetetraacetic acid (EDTA) for plasma separation. Before centrifugation, a protease inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) (Sigma-Aldrich, Oakville, ON, Canada) will be added to the K_2EDTA tubes. Samples will be centrifuged at 450 X g for 10 minutes and aliquoted for storage at -80°C for the subsequent measurement of biomarkers. Hydrochloric acid (1N, 100µL) will be added to the ghrelin aliquot prior to storage. Serum samples will be analyzed for glucose, insulin, cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, non-HDL cholesterol by DynaLIFE Medical Labs (Edmonton, AB, Canada). Plasma samples will be analyzed for ghrelin, PYY, GLP-1, leptin, free glycerol and non-esterified fatty acids (NEFA) at the HNRU (University of Alberta, Edmonton, AB, Canada). Leptin and GLP-1 (active) will be measured by electrochemiluminescence using the MULTI-ARRAY® Assay System (Meso Scale Discovery®, Gaithersburg, MD, USA) and V-PLEX® (Meso Scale Discovery®, Gaithersburg, MD, USA), respectively. Ghrelin (active) and PYY will be measured using enzyme-linked immunosorbent assay (ELISA) kits from EMD Millipore Co. (Billerica, MA, USA). Free glycerol and NEFA will be measured using enzyme immunoassay kits from Zen-Bio Inc. (Research Triangle Park, NC, USA).

Urine will be collected during the entire time participants are in the WBCU for the measurement of urinary N. Collections will be initiated after the first morning urinary void and collected into sterile urine jugs. During collection, urine will be stored at -1°C inside a fridge located in the WBCU and will be taken to the laboratory after each whole-body calorimetry test ends. Urine volume will be measured and aliquots (~1.5 mL each) will be placed into tubes for storage at -80°C before analysis. Total urinary N will be determined by chemiluminescence using a high temperature Shimadzu TOC-L CPH Model Total Organic Carbon Analyzer with an ASI-L autosampler and TNM-L unit (Shimadzu Corporation, October 2015. Suzhuo, Jiangsu, China).

3.4.13 Statistical Analysis

3.4.13.1 Sample Size Estimate

The sample size estimation was conducted separately for each sex. A total of 12 participants will be able to detect an effect size of 1.41, which was calculated based on differences

in RQ between dependent groups receiving a HP-TDR (0.85 ± 0.03) or maintaining usual dietary intake (0.90 ± 0.04) from a previously published study (20). A two-sided t-test achieves 88% power to infer that the mean difference is not 0.05 when the total sample size of a 2x2 crossover design is 12, the actual mean difference is 0.06, the standard deviation of the paired difference is 0.01, and the significance level is 0.05. Accounting for a 20% attrition rate, the total sample size of 14 participants of each sex would be needed to complete the study. The sample size calculation was done using PASS Power Analysis and Sample Size software version 19.0.1 (NCSS Statistical Software, Kaysville, Utah, USA).

3.4.13.2 Data Analysis

IBM® SPSS® Statistics version 24 (International Business Machines Corporation, New York, NJ, USA) will be used to perform statistical analyses. Differences will be regarded as statistically significant if $p<0.05$. Missing data will be omitted and the remaining data will be analyzed. To determine whether there is a statistically significant mean difference between the HP-TDR and CON groups, a paired-samples t-test or Wilcoxon signed rank test will be used, depending on data distribution. Shapiro Wilk's test will be used to check normality of the data. Inspection of a boxplot for values greater than 1.5 box-lengths from the edge of the box will be done to detect outliers. A two-way repeated measures analysis of variance (ANOVA) will be carried out to determine the effect of the dietary interventions on appetite sensations. Outliers will be detected by studentized residuals greater than ±3 standard deviations. Sphericity will be assessed for the interaction term by the Mauchly's test of sphericity. The relationships between variables will be determined by a Pearson's product-moment correlation. To determine whether the order of treatment has an effect on the results, a repeated measures ANOVA with the order of treatment as the between-subjects factor will be performed.

3.4.14 Data Monitoring

This will be an acute dietary intervention study and it is very unlikely that any adverse event and/or any unintended effect of trial intervention or trial conduct happen. It is possible that participants feel some discomfort in their muscles after walking on the treadmill; however the activity is moderate. Blood draws can cause injury and a small risk of infection, which are minimized with proper procedures. Participants may also feel uncomfortable being alone in the

WBCU; however there will always be research staff close by with an intercom system being available. The X-ray dose associated with DXA scan is very low and safe.

If abnormal results from questionnaires or blood tests are noticed, the study team will follow the University Emergency procedures, as required. If participant requires a medical referral, the physician involved in this clinical trial will assess the situation and refer the participant as required to the appropriate health care professional. If participants become ill or injured as a result of being in this study, they will receive any necessary medical treatment.

3.4.15 Dissemination Policy

The results of this study will be disseminated to researchers, health professionals and the general public by publication in peer-reviewed national and international journals, as well as by poster and oral presentations at conferences and meetings on nutrition and health. Authorship will follow the recommendations of the International Committee of Journal Editors.

3.5 DISCUSSION

Obesity is an important health care problem caused by an imbalance between calories consumed and expended over time (21, 22). To date, treatment strategies for obesity are lacking. Protein intake above the currently recommended amount (3) may confer a metabolic advantage by increasing energy expenditure and satiety; however, lack of dietary adherence to a HP diet seems to affect its long-term effectiveness (6). TDR have been pointed out as long-term therapeutic strategies to treat overweight and obesity (8, 9). Considering these strategies in combination, a HP TDR seems to be an interesting approach to treat overweight and obesity, promoting effective weight loss and its maintenance over the years. As mentioned previously, this research is novel. It is hypothesized that, when compared to individuals receiving a conventional diet of a typical North American macronutrient distribution with the same energy content, those receiving a HP diet comprised of a TDR will be in negative fat balance, have increased 24-hour EE, improved metabolic profile, and feel more satiated.

Investigating the impact of a HP diet comprised of a TDR in healthy individuals of both sexes will allow a better understanding of the effects of this strategy in a normal physiological condition. These data can then be used as reference for studies testing effects of HP TDR in

individuals with overweight and obesity associated or not with comorbidities. Therefore, the results of this clinical trial can ultimately be used to develop future strategies aimed at optimizing diet quality and weight management. Strategies aimed at the prevention of overweight, obesity and related chronic diseases are important for the improvement of health and quality of life, also ultimately addressing the growing financial burden of obesity and related diseases on the health care system.

Table 3.1. Schedule of the indirect calorimetry tests

TIME	ACTIVITIES
DAY 1	
8:00 a.m.	Test begins - 60 minutes of rest on bed
9:00 a.m.	Appetite sensation assessment
9:00 – 9:30 a.m.	Breakfast
9:30 a.m.	Appetite sensation assessment
9:30 – 10:20 a.m.	Leisure/work time (TV, computer, reading)
10:20 – 11:00 a.m.	Exercise session on treadmill
11:00 a.m.	Blood draw
11:00 – 12:00 p.m.	Leisure/work time (TV, computer, reading)
12:00 p.m.	Appetite sensation assessment
12:00 – 12:30 p.m.	Lunch
12:30 p.m.	Appetite sensation assessment
12:30 – 2:30 p.m.	Leisure/work time (TV, computer, reading)
2:30 p.m.	Blood draw
2:30 – 3:00 p.m.	Leisure/work time (TV, computer, reading)
3:00 p.m.	Appetite sensation assessment
3:00 – 3:30 p.m.	Afternoon snack
3:30 p.m.	Appetite sensation assessment
3:30 – 6:00 p.m.	Leisure/work time (TV, computer, reading)
6:00 p.m.	Appetite sensation assessment
6:00 - 6:30 p.m.	Dinner
6:30 p.m.	Appetite sensation assessment
6:30 – 9:00 p.m.	Leisure/work time (TV, computer, reading)
9:00 p.m.	Appetite sensation assessment
9:00 – 9:30 p.m.	Evening snack
9:30 p.m.	Appetite sensation assessment
9:45 p.m.	Get ready for bed
10:00 p.m. – 6:00 a.m.	Sleep

DAY 2	
6:00 am	Wake up call
6:00 – 7:00 a.m.	60 minutes of rest on bed
7:00 – 7:25 a.m.	Participant can use the washroom, brush teeth, change clothes, etc.
7:30 – 8:30 a.m.	60 minutes of rest on bed
8:30 – 8:35 a.m.	Blood draw
8:35 a.m.	Appetite sensation assessment
8:40 – 9:00 a.m.	Breakfast
9:30 a.m.	Appetite sensation assessment
9:00 – 3:00 p.m.	Participants seated or lying down without too much movement
3:00 p.m.	Participant get ready to leave the WBCU
3:10 p.m.	Participant leaves the WBCU

Abbreviations: WBCU: whole-body calorimetry unit.

Table 3.2. Composition of the CON and HP-TDR diets

Meals	CON Diet	HP-TDR
Breakfast	Whole Wheat Bread Peanut Butter Orange Juice Low-Fat (1%) Milk[1] Boiled Egg[1]	HP-TDR Powder Low-Fat (1%) Milk Olive Oil
Lunch	Turkey Wrap *Flour Tortilla* *Deli Style Turkey* *Cheese* *Lettuce* *Tomato* *Olive Oil* Tomato Soup Canned Peaches[1] Yogurt[1]	HP-TDR Powder Low-Fat (1%) Milk Olive Oil
Afternoon Snack	Apple Crackers Cheese Yogurt[1]	HP-TDR Powder Apple Juice Olive Oil
Dinner	Chicken Stir Fry *Chicken Breast* *Celery* *Carrot* *Onion* Brown Rice Yogurt[1]	HP-TDR Powder Low-Fat (1%) Milk Olive Oil
Evening Snack	Cereal Low-Fat (1%) Milk	HP-TDR Powder Apple Juice

	Almonds Canned Peaches[1]	Olive Oil

[1] Food items added or not, depending on the energy requirements of participants.
Abbreviations: CON: control; HP: high-protein.

Table 3.3. Nutrient composition of a typical study diet

	HP-TDR	**CON Diet**
Energy		
kcal/day	2000	2000
Protein		
g/day	200	76
% of TEI	40	15
Fat		
g/day	55	67
% of TEI	25	30
Carbohydrate		
g/day	175	280
% of TEI	35	55

Abbreviations: BW: body weight; CON: control; HP-TDR: high-protein total diet replacement; TEI: total energy intake.

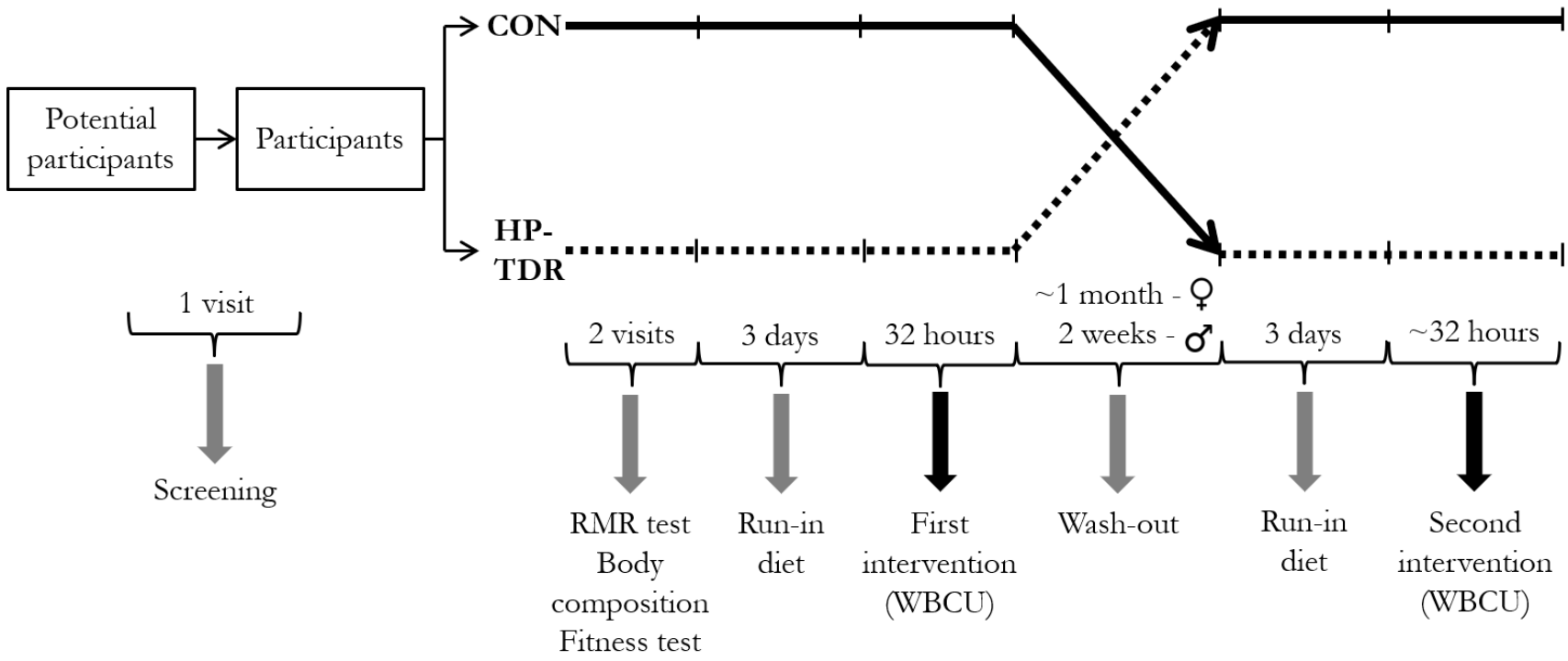

Figure 3.1. Experimental protocol.

Abbreviations: CON: control diet; HP-TDR: high-protein total diet replacement; RMR: resting metabolic rate; WBCU: whole-body calorimetry unit.

		STUDY PERIOD								
		Enrollment	Allocation	Post-Allocation						
TIMEPOINT		Screening visit	Post-screening	Visit 2	Visit 3	Run-in diet	Indirect calorimetry	Wash-out	Run-in diet	Indirect calorimetry
ENROLLMENT	Eligibility screen	X								
	Informed consent	X								
	Blood tests									
	Anthropometry									
	Questionnaires									
	RMR assessment			X						
	Body composition			X						
	Fitness test				X					
	Allocation		X							
INTERVENTIONS	HP-TDR → CON					X	X	X	X	X
	CON → HP-TDR					X	X	X	X	X
ASSESSMENTS	Energy metabolism						X			X
	Metabolic blood markers						X			X
	Appetite sensations						X			X

Figure 3.2. Schedule of enrollment, interventions, and assessments (SPIRIT figure).

Abbreviation: CON: control diet; HP-TDR: high-protein total diet replacement; RMR: resting metabolic rate.

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CHAPTER 4. THE EFFECTS OF A HIGH-PROTEIN DIET REPLACEMENT ON ENERGY METABOLISM AND METABOLIC BLOOD MARKERS

4.1 PREFACE

A version of this chapter has been published as: *Oliveira CLP, Boulé NG, Sharma AM, Elliott S, Siervo M, Ghosh S, Berg A, Prado CM. A High-Protein Total Diet Replacement Increases Energy Expenditure and Leads to Negative Fat Balance in Healthy Adults. Am J Clin Nutri. 2020. https://doi.org/10.1093/ajcn/nqaa283.* I was responsible for the data collection and analysis as well as the manuscript composition. All authors contributed to manuscript edits. Carla M. Prado was the supervisory author and was involved with concept formation and manuscript composition.

4.2 ABSTRACT

Background: High-protein diets and total diet replacements are becoming increasingly popular for weight loss; however, further research is needed to elucidate their impact on the mechanisms involved in weight regulation.

Objective: The aim of this inpatient metabolic balance study was to compare the impact of a high-protein total diet replacement (HP-TDR) versus a control diet (CON) on select components of energy metabolism in healthy adults of both sexes.

Methods: The acute intervention was a randomized, controlled, cross-over design with participants allocated to two isocaloric arms: a) HP-TDR: 35% carbohydrate, 40% protein, and 25% fat achieved through a nutritional supplement; b) CON: 55% carbohydrate, 15% protein, and 30% fat. Participants received the prescribed diets for 32 hours while inside a whole-body calorimetry unit (WBCU). The first dietary intervention randomly offered in the WBCU was designed to maintain energy balance and the second matched what was offered during the first stay. Energy expenditure, macronutrient oxidation rates and balances, and metabolic blood markers were assessed. Body composition was measured at baseline using dual-energy X-ray absorptiometry.

Results: Forty-three healthy, normal-weight adults (19 females and 24 males) were included. Compared to the CON diet, the HP-TDR produced higher total energy expenditure ([EE]

81 ± 82 kcal/day, p<0.001), protein and fat oxidation rates (38 ± 34 g/day, p<0.001; 8 ± 20 g/day, p=0.013, respectively), and lower carbohydrate oxidation rate (-38 ± 43 g/day, p<0.001). Moreover, a HP-TDR led to decreased energy (-112 ± 85 kcal/day; p<0.001), fat (-22 ± 20 g/day; p<0.001), and carbohydrate balances (-69 ± 44 g/day; p<0.001), and increased protein balance (90 ± 32 g/day; p<0.001).

Conclusions: Our primary findings were that a HP-TDR led to higher total EE, increased fat oxidation, and negative fat balance. These results suggest that a HP-TDR may promote fat loss compared to a conventional isocaloric diet.

4.3 BACKGROUND

Total diet replacements (TDR) are nutritionally complete formula foods designed to replace the whole diet for a specific period of time. In the context of obesity, they may facilitate weight loss. Considering the prevalence of obesity worldwide and its impact on population's health (1), TDR are becoming increasingly popular as a weight management strategy; however, research around this topic has not kept pace with its growth in popularity. To our knowledge, only a few studies have evaluated the effects of TDR in humans to date (2-9). Studies were mostly long-term intervention trials with all participants presenting with obesity (2-9) and sometimes with type 2 diabetes (2, 3, 5, 8, 9). Interventions consisted of calorie-restricted TDR and the primary outcome was mainly weight loss, which might have influenced all other variables assessed (2-9). None of these studies examined energy metabolism, only some measured metabolic blood markers (2, 5, 7-9), and no studies looked at potential sex-differences.

Another potential dietary strategy for body weight management is manipulation of macronutrient intake, particularly high-protein (HP) diets. These diets have gained popularity over the years and their main characteristic is a protein content above recommended values (i.e., for healthy adults > 19 years of age: 0.80 g/kg of body weight/day or 10 to 35% of total energy intake) (10) with varying levels of carbohydrate and fat intake. High-protein diets are known to increase satiety, energy expenditure (EE), and maintain or increase fat-free mass, which altogether have been shown to positively affect body weight loss and maintenance (11).

Taken together, the benefits offered by TDR and HP diets seem to be an interesting combination for weight management. Not surprisingly, these synergistic effects have been noticed

by industry and several high-protein total diet replacement (HP-TDR) products are widely available to consumers. Although some well-designed inpatient metabolic studies have already assessed the effects of HP diets on energy and substrate metabolism in healthy individuals (12-16), to our knowledge, no inpatient metabolic balance studies have evaluated the exact role of a liquid TDR with an increased protein content on EE, macronutrient oxidation rates and balances, and metabolic blood markers. Additionally, and of extreme importance is the study of this intervention using state-of-the-art methodology in a controlled environment in healthy females and males with a normal body weight to eliminate the confounding effects of obesity and comorbidities on the results. Therefore, the aim of this inpatient metabolic balance study was to compare the impact of a HP-TDR versus a control (CON) diet (North American) on EE, macronutrient oxidation rates and balances, and metabolic blood markers in healthy female and male adults. The primary outcome evaluated was the difference in fat balance between the HP-TDR and CON diets; secondary outcome was difference in the total EE, with remaining variables as exploratory. It was hypothesized that, compared to the CON diet, participants consuming the HP-TDR would be in negative fat balance, have increased energy expenditure, and improved metabolic profile. It was also hypothesized that females and males would respond similarly to the dietary interventions in spite of known sex-related physiological differences.

4.4 METHODS

4.4.1 Study Design and Ethical Procedures

Details of this study protocol have been previously described (17). Briefly, these were two complementary randomized, controlled, crossover inpatient studies conducted separately by sex between November 2016 and November 2019 at the Human Nutrition Research Unit (HNRU), University of Alberta (Edmonton, AB, Canada). Trial protocols were approved by the University of Alberta Ethics Board (Pro00066006 and Pro00083005) and registered as NCT02811276 (18) and NCT03565510 (19) on ClinicalTrials.gov. The studies complied with the standards as set out in the Canadian Tri-Council Policy statement on the use of human participants in research (20). Before study commencement, participants were informed of procedures and potential risks involved in the investigation and provided written informed consent.

4.4.2 Subjects

Healthy adults, 18 to 35 years of age, nonsmokers, with body mass index (BMI) between 18.5 and 24.9 kg/m^2 were recruited via advertisements placed on notice boards at the University of Alberta. Major exclusion criteria were the presence of any acute or chronic disease, the use of medications and/or nutritional supplements that affect energy metabolism or body composition (e.g., antidepressants, corticosteroids, thyroid disorder medications, creatine and protein supplements), dietary restrictions (e.g., food allergies and/or intolerances and vegetarianism), engagement in exercise practice >1 hour/day or >7 hours/week, recent exposure to tests involving radiation, claustrophobia, and specifically for females, pregnancy or lactation and irregular menstrual cycle.

4.4.3 Experimental Protocol

Potential participants were instructed to report to the HNRU for a screening visit and, once deemed eligible, were randomly assigned to a HP-TDR or CON diet (1:1) following a simple randomization procedure separated by sex. Following the screening process, eligible participants had their body composition and resting EE (REE) assessed. After these tests, participants underwent 32-hour whole-body calorimetry unit (WBCU) assessments for the measurement of energy metabolism components and metabolic blood markers while consuming an eucaloric diet, which was repeated at the second visit (when they crossed-over to the other diet). An eucaloric 3-day run-in diet preceded both intervention phases and was estimated as explained later in this section. Each intervention phase was followed by a wash-out period of approximately one month for females and two weeks for males. A brief description is presented below and illustrated in **Figure 4.1**, fully presented elsewhere (17).

4.4.4 Anthropometrics and Body Composition

At baseline, height, weight, waist circumference, and body composition were assessed. Body composition was assessed via dual-energy X-ray absorptiometry (DXA) using a GE Lunar iDXA (General Electric Company, Madison, USA; enCORE software 13.60 Lunar iDXA GE Health Care®). Whole-body and regional levels of fat mass (FM), lean soft tissue (LST) and bone mineral content (BMC) were assessed.

4.4.5 Study Diets

The 3-day individualized run-in diet offered prior to both 32-hour WBCU conditions included three meals (breakfast, lunch, and dinner) and two snacks (afternoon and evening snacks) per day. Participants were instructed to drink water *ad libitum*, not consume caffeinated food products or perform strenuous physical activity during this period. The run-in diet provided 55% of total energy intake from carbohydrate, 15% from protein and 30% from fat.

During both 32-hour WBCU stays, three meals (breakfast, lunch, and dinner) and two snacks (afternoon and evening snacks) were provided on day 1 and one meal (breakfast) on day 2 (food items fully described in our protocol paper) (17). Bottled water was provided *ad libitum.* The CON diet was comprised of standard food items and the HP-TDR diet consisted of a soy-protein nutritional supplement (Almased®, Almased USA, Inc., St. Petersburg, FL, USA) mixed with olive oil and low-fat milk (1% fat) for the main meals and with olive oil and apple juice for the snacks, per label instructions (21). The nutritional information and ingredient list of the nutritional supplement is described in **Supplementary Table 4.1**. The first dietary intervention randomly offered in the WBCU was designed to maintain participants in energy balance and the energy content of each meal and snack were similar for the HP-TDR and CON diets (isocaloric). The nutrient content of the dietary interventions is described in **Table 4.1**.

4.4.6 Energy Metabolism

Energy expenditure, macronutrient oxidation rates, and balances were assessed by indirect calorimetry measuring the volume of oxygen consumed (VO_2) and carbon dioxide produced (VCO_2), with the use of an open-circuit WBCU. This equipment had a geometric volume of 28.74 m^3 and was equipped with an oxygen (Oxymat, Siemens AG, Munich, Germany) and carbon dioxide (Advance Optima AO2000 Series, ABB Automation GmbH, Frankfurt, Germany) analyzers. The information on the volume of gases from the analyzers was then transmitted to a computer (Acer Aspire AM3910-E3122, Acer Inc., New Taipei City, Taiwan) via the National Instruments NI USB-6221 device (National Instruments Corporation, Austin, Tex., USA) using the PMCSS Software version 1.8 (Pennington Metabolic Chamber Software Suite, Pennington Biomedical Research Center, La., USA). A 1-hour REE indirect calorimetry test was performed at baseline; then, two 32-hour tests were conducted while participants consumed the HP-TDR and CON diets. The baseline REE test was used to estimate participant's energy requirements for the

3-day run-in diet and 32-h WBCU tests. To do that, REE was multiplied by a physical activity coefficient, according to the Dietary Reference Intakes (10), and a coefficient of 1.075 representing the metabolizable energy content of the diet (22). The morning following the 3-day run-in periods, participants returned to the HNRU after an 8 to 12 hours overnight fast and spent 32 consecutive hours in the WBCU while receiving the HP-TDR and CON diets in random order, **Figure 4.1A**. Both 32-hour WBCU tests happened during the follicular phase of women's menstrual cycle. Throughout each test, blood was drawn 3 times, and urine was collected for the entire time. On the morning of the first day of the test (10:20 am), a 40-min moderate walking session on a treadmill (BH Fitness T8 SPORT, BH Fitness, Foothill Ranch, Calif., USA) was completed, at a personalized fixed pace. Sleep was only allowed during the night.

Total EE and macronutrient oxidation rates were calculated from the measurements of VO_2, VCO_2 and urinary nitrogen (N) by using the formula of Brouwer (23). Energy and macronutrient balances were calculated as the difference between intake and oxidation. Respiratory exchange ratio (RER) was calculated as the average ratio of VCO_2 to VO_2 per minute during measurements of total EE, REE, basal EE, sleep EE, and postprandial EE. During each WBCU stay, the following EE components were assessed: total EE, REE, basal EE, sleep EE, and postprandial EE, **Figure 4.1B**. Diet-induced thermogenesis and arousal EE were not assessed in this study. An internally conducted reliability study for our WBCU (results not published) revealed coefficients of variation (CV) of 2.2% for total EE, 2.1% for basal EE, and 2.0% for 24-hour RER.

4.4.7 Blood and Urine Analysis

Blood was sampled by venipuncture at three time points during each WBCU stay through an iris port: 1) the morning on the first day of test (fasting day 1, ~7:30 am); 2) two hours after lunch (postprandial, ~ 2:30 pm); and 3) the morning on the second day of test (fasting day 2, ~8:00 am). Both morning blood draws were sampled from participants after a 10- to 12-hour overnight fast. Serum samples were analyzed for glucose, insulin, lipid panel (total cholesterol, high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol, triglyceride, and non-HDL cholesterol) by DynaLIFE Medical Labs (Edmonton, AB, Canada). Plasma samples of free and non-esterified fatty acids were analyzed in-house at the HNRU. The CV in females and males was 1.00% for glucose, 5.00% for insulin, 2.00% for total, HDL, LDL and non-HDL cholesterol, 3.00% for triglyceride, 7.44% for glycerol, and 6.26% and 9.18% for NEFA in females and males,

respectively. Homeostatic model assessment (HOMA) of β-cell function (%B) and insulin resistance (IR) were calculated using the HOMA2 Calculator (©Diabetes Trials Unit, University of Oxford, version 2.2.3). Urine was collected during the entire time participants were in the WBCU for the measurement of total urinary N, which was determined by chemiluminescence using a high temperature Shimadzu TOC-L CPH Model Total Organic Carbon Analyzer with an ASI-L autosampler and TNM-L unit (Shimadzu Corporation, October 2015. Suzhuo, Jiangsu, China).

4.4.8 Statistical Analysis

An *a priori* sample size estimation was conducted separately for each sex and was fully described elsewhere (17). Briefly, an effect size of 1.41 was detected with a total of 12 participants per sex based on differences in respiratory quotient from a previously published study (24). In this previous study, individuals receiving a HP-TDR presented a lower value (0.85 ± 0.03) compared with the ones maintaining their usual dietary intake (0.90 ± 0.03) (24). To account for possible dropouts, a total of 14 participants per sex would be required (88% power, α=0.05) to complete the study. The sample size calculation was done using PASS Power Analysis and Sample Size software version 19.0.1 (NCSS Statistical Software, Kaysville, Utah, USA). In addition to the *a priori* sample size estimation, a post hoc analysis was performed and the achieved power (1-β) calculated with the assistance of the G-Power® software (version 3.1.9.7). The power of this study was found to be 99.9% based on a difference in fat balance (i.e., primary outcome) of -22 ± 20 g/day between the HP-TDR and CON groups using a two-tailed test with an effect size of -1.10, a type I error probability of 0.05, and n=43.

Data were expressed as mean ± standard deviation (SD) for continuous variables and frequency and proportions for categorical variables. Mean ± standard error of the mean difference (SEM) was used to report differences between sexes. Independent t-tests were used to compare the mean differences of continuous variables between sexes at baseline. If the continuous variables were non-normally distributed Mann-Whitney U-tests were used to compare the means between the sexes. Chi-square tests were used to correlate two categorical variables and Fisher's exact test was used if the cell frequencies were less than a count of 5. Possible differences between the HP-TDR and CON diets were explored using a mixed analysis of variance (ANOVA) with within-subject factors (i.e., dietary interventions and/or time) and between subject factors (i.e., sex and/or

order of treatment). Post-hoc analyses were applied with all ANOVA tests using a Tukey test (equal variances assumed) or Games-Howell (equal variances not assumed). Diagnostics, such as assessing the normality of data, homogeneity of variances using the Box's test of equality of covariance matrices and Levene's test for equality of variances were used to check if the ANOVA assumptions were valid. If the ANOVA assumptions were not met, the corresponding variable was LOG transformed and the ANOVA analysis repeated. A Pearson's product-moment correlation was run to assess the relationship between continuous variables and Spearman's Rho was used for non-normally distributed data. Simple regression analysis was used to express total EE, sleep EE, and REE on day 2 as function of FFM. IBM® SPSS® Statistics version 24 (International Business Machines Corporation, New York, NJ, USA) was used to perform all statistical analyses. Differences were regarded as statistically significant if $p<0.05$.

4.5 RESULTS

4.5.1 Subjects

Of the 76 potential participants who were screened, 14 (18%) did not meet the eligibility criteria and 5 (6%) declined to participate. Fifty-seven participants were enrolled in the study; 13 (23%) dropped out before the first WBCU test, due to personal reasons. Forty-four participants completed the study (both WBCU tests, n=20 females; n=24 males). One female was excluded from analysis because she was not in the follicular phase of the menstrual cycle during the second WBCU test, **Figure 4.2**. No adverse events were reported during the study. Baseline characteristics of those who completed the study are summarized in **Table 4.2**. Compared to males, females were shorter (-8.6 ± 1.8 cm; $p<0.001$), had lower body weight (-5.9 ± 1.8 kg; $p=0.003$), smaller waist circumference (-5.4 ± 1.4 cm; $p=0.001$), higher FM (5.8 ± 1.3 kg; $p<0.001$), lower LST (-11.2 ± 1.5 kg; $p<0.001$), lower BMC (-0.4 ± 0.8 kg; $p<0.001$), and lower blood levels of albumin (-3 ± 1 g/L; $p<0.001$), creatinine (-17 ± 3 mmol/L; $p<0.001$), and sodium (-2 ± 1 mmol/L; $p<0.001$).

4.5.2 Energy Metabolism

Differences of selected energy metabolism components between the HP-TDR and CON diets are shown in **Table 4.3**. During the HP-TDR intervention, total and sleep EE were increased by 81 ± 82 kcal/day ($p<0.001$) and 17 ± 26 kcal/8-hour night ($p<0.001$), respectively. Resting EE

on day 1 (p=0.784), on day 2 (p=0.582), and basal EE (p=0.411) did not differ between diets. While consuming the HP-TDR, 24-hour RER was lower (-0.02 ± 0.01; p<0.001) compared to the CON diet. Respiratory exchange ratio during measurements of REE on day 2, basal EE, and sleep EE, were also lower during the HP-TDR diet, p<0.001. Carbohydrate oxidation rate was lower during the HP-TDR diet (-38 ± 43 g/day, p<0.001), and protein and fat oxidation rates were higher (38 ± 34 g/day, p<0.001; 8 ± 20 g/day, p=0.013, respectively). Compared to the CON diet, while consuming a HP-TDR, participants experienced lower carbohydrate (-69 ± 44 g/day; p<0.001), fat (-22 ± 20 g/day; p<0.001), and energy (-112 ± 85 kcal/day; p<0.001) imbalances, and greater protein imbalance (90 ± 32 g/day; p<0.001). Moreover, the HP-TDR led to an increased EE above resting (assessed on day 2) following the ingestion of isocaloric breakfasts (day 2 WBCU stay), **Figure 4.3**.

Although no diet *x* sex interaction was observed in any of the variables assessed (p>0.05), a main effect of sex on several energy metabolism variables was detected. Compared to males, females presented lower total EE (-303 ± 62 kcal/day, p<0.001), 24-hour RER (-0.008 ± 0.004, p=0.038), REE on day 1 (-284 ± 50 kcal/day, p<0.001) and on day 2 (-311 ± 48 kcal/day, p<0.001), basal EE (-291 ± 51, p<0.001), sleep EE (-53 ± 12 kcal/8-hour night, p<0.001), postprandial EE (-0.2 ± 0.04 kcal/min, p<0.001), RER during postprandial EE assessment (-0.01 ± 0.004, p=0.009), carbohydrate oxidation rate (-29 ± 10 g/day, p=0.007), protein oxidation rate (-36 ± 6 g/day, p<0.001), and greater protein (22 ± 6 g/day, p=0.001) and energy (109 ± 31 kcal/day, p=0.001) imbalances. More specifically, during each dietary intervention, energy balance was different between sexes (HP-TDR: females 32 ± 23 kcal/day, males -58 ± 22 kcal/day, p=0.008; CON: females 164 ± 23 kcal/day, males 35 ± 25 kcal/day, p=0.001).

Protein and fat balances were inversely correlated only in the HP-TDR diet (all: r = -0.57, p<0.001; females: r=-0.63, p=0.004; males: r=-0.55, p=0.005), **Figure 4.4**. Total EE and LST were positively correlated in both diets (HP-TDR: r=0.79, p<0.001; CON: r=0.79, p<0.001). In females and males, total EE, sleep EE and REE on day 2 were a function of FFM during the HP-TDR and CON conditions, except from sleep EE in females in the HP-TDR (**Supplementary Figures 4.1 and 4.2**). The order in which participants received the dietary interventions did not affect any of the energy metabolism variables analyzed (all p>0.05).

4.5.3 Metabolic Blood Markers

Metabolic blood markers assessed in a fasting state on days 1 and 2, and after lunch during the HP-TDR and CON diets are shown in **Table 4.4**. Glycerol (-4.2 ± 12.4 μM, p=0.031) and triglyceride (-0.07 ± 0.23 mmol/L, p=0.044) decreased more from fasting day 1 to fasting day 2 in the HP-TDR compared to the CON diet, and total, LDL, and non-HDL cholesterol blood levels increased more (0.10 ± 0.26 mmol/L, p=0.010; 0.12 ± 0.18 mmol/L, p<0.001; 0.09 ± 0.20 mmol/L, p=0.005; respectively). On the other hand, this change was not different between the dietary interventions for glucose, insulin, HOMA %B, HOMA IR, NEFA, and HDL cholesterol, p>0.05. There was a statistically significant interaction between diet and sex on the change in HDL cholesterol concentration (p=0.042). In the HP-TDR diet, HDL cholesterol concentration was greater in females compared to males (0.08 ± 0.03 mmol/L, p=0.007). Moreover, the change in HDL cholesterol from fasting day 1 to fasting day 2 was significantly different between interventions in females (HP-TDR: 0.03 ± 0.03 mmol/L; CON: -0.01 ± 0.02 mmol/L; p=0.043), but not in males (HP-TDR: -0.04 ± 0.02 mmol/L; CON: -0.05 ± 0.12 mmol/L; p=0.525). There was no difference between sexes in the CON diet (p=0.430).

Postprandially, glucose (-0.2 ± 0.5 mmol/L, p=0.044), insulin (-19.1 ± 44.6 pmol/L, p=0.007), and glycerol (-16.8 ± 25.9 μM, p<0.001) blood levels were lower in the HP-TDR diet compared to the CON, and total, LDL and HDL cholesterol levels were higher (0.12 ± 0.42 mmol/L, p=0.041; 0.12 ± 0.34 mmol/L, p=0.023; 0.06 ± 0.20 mmol/L, p=0.047, respectively). There was no diet *x* sex interaction in any of the variables analyzed postprandially (all p>0.05).

The order in which participants received the dietary interventions did not affect any of the metabolic blood markers analyzed, p>0.05. A diet *x* sex *x* time interaction was also explored, and no statistically significant three-way interaction was observed in any of the variables analyzed (**Supplementary Table 4.2**).

4.6 DISCUSSION

The present inpatient metabolic balance study compared the effects of an isocaloric HP-TDR versus a CON diet on energy expenditure, macronutrient oxidation rates and balances, and metabolic blood markers in female and male healthy adults. The primary findings of this study were that compared to a standard North American dietary pattern, a HP-TDR led to higher energy

expenditure, increased fat oxidation, and negative fat balance (likely implying body fat loss) (25). The only diet *x* sex interaction observed was on HDL cholesterol concentration in the HP-TDR diet. These results highlight the impact a HP-TDR consumption has on energy metabolism and metabolic blood markers of healthy adults and provides further insight into the potential role of this dietary strategy for weight management.

Regarding the components of participant's energy expenditure, this study showed that consumption of the HP-TDR led to higher daily, sleep, and postprandial EE. Collectively, these results add to the discussion that a calorie is *not* just a calorie (26) and that isocaloric diets with different proportion of macronutrients might offer a metabolic advantage (27-29), specifically an increase in EE and fat oxidation. There seems to be a consensus that the protein content of the diet can directly affect energy expenditure and substrate use (11, 30); however, the same is not true when it comes to the carbohydrate and fat contents (31-33). It is possible that energetic costs involved in the thermic effect of protein and the possible increase in protein turnover contributed to the observed increase in EE in this group (11, 30), which is concordant with the literature (13). On the other hand, 24 hours after the start of the interventions, participant's REE and basal EE did not differ between diets, contrasting previous findings (13). Interestingly, it seems that eucaloric HP diets are not able to change REE as it does with other components of EE, which can only be captured with sophisticated measurement of energy metabolism (i.e., using a WBCU). Previous studies showing an increase or decline in REE with HP diets were long-term interventions in which participants were in negative or positive energy balance. A meta-analysis of randomized controlled trials revealed that HP diets reduced the decline in REE during weight loss, which has been potentially attributed to a retention of lean mass, although this has not been determined (34). In addition to that, overfeeding a HP diet for 8 weeks has been shown to increase REE (227 kcal/day) and this result was associated with an accretion of 3.18 kg of lean mass (14). Due to our experimental design, lean mass and therefore REE was not expected to change, which is in line with current literature.

Over the years, experiments have shown that total body carbohydrate and protein content are tightly regulated by adjusting oxidation rates to intake levels, meaning that the manipulation of these macronutrients' intake affects their oxidation rates to the same direction and extent (25, 35, 36). In this study, a HP-TDR led to a decrease in carbohydrate oxidation rate and an increase in protein oxidation rate, which is in line with this rationale since the HP-TDR intervention has a

low-carbohydrate, HP content. Conversely, this autoregulatory process is inexistent for fat oxidation, which seems to be mostly driven by the presence or absence of other macronutrients, markedly carbohydrate (25). The dynamic interactions between carbohydrate and fat oxidations started to be described almost 60 years ago (37) and have been continuously explored as more research is made available (38). As comprehensively discussed by Hue and Taegtmeyer (38) and illustrated by Prentice (25), the low-carbohydrate characteristic of the HP-TDR seems to be responsible for the increased fat oxidation observed with this dietary intervention. This result is further demonstrated by the lower 24-hour RER observed in the HP-TDR diet. As a consequence of intake and oxidation rates in this study, participants consuming the HP-TDR experienced a decrease in energy and fat balances, likely implying body fat loss (25). In a classical inpatient experiment, Abbott, Howard (36) demonstrated that in conditions of energy imbalance, fat stores are mobilized to balance the body's energy budget, which is in agreement with results presented herein.

In this study, total, LDL, and non-HDL cholesterol blood levels increased more from fasting day 1 to fasting day 2 in the HP-TDR compared to the CON diet.. Although change in these markers was statistically significant, the absolute values remained within the reference ranges for this population group. Jones, Pappu (39) demonstrated that the ingestion of dietary cholesterol causes feedback inhibition of cholesterol biosynthesis in humans. Considering that the content of dietary cholesterol of the HP-TDR intervention was almost three times lower than the content of the CON diet, it might be possible that participant's biosynthesis was upregulated in the HP-TDR, causing an increase in blood lipid levels. On the contrary, blood triglyceride level was lower in the HP-TDR diet compared to the CON diet. This fact can be mainly attributed to the low carbohydrate content of this dietary intervention (40), also supported by previous studies (41). Additionally, blood glycerol decreased more from fasting day 1 to fasting day 2 in the HP-TDR compared to the CON diet. Circulating glycerol has been shown to result mainly from hydrolysis of triglyceride stored in adipose tissue, and constitutes a major substrate for glucose homeostasis (42). The increased fat oxidation and negative fat balance observed in the HP-TDR group are both indicative of increased hydrolysis of triglycerides in adipose tissue, which might have greatly contributed to the use of this substrate as an energy source, reducing its circulating levels. A significant interaction between diet and sex on the change in HDL cholesterol concentration was found in the HP-TDR diet, in which females presented greater values compared to males. An analysis of 1.3

million patients revealed that HDL cholesterol concentration is higher in females than males (43). This effect seems to be related to female's increased endogenous (44) and exogenous estrogens (e.g., estrogen-containing contraceptives) (45). Considering that the HP-TDR contained soy isoflavones (46), which are natural estrogen-like compounds, it might be possible that it could have elevated female's estrogen levels which accentuated the difference in HDL cholesterol concentration between sexes in the HP-TDR diet.

To date, studies investigating the effects of total diet replacements have been conducted in individuals with obesity and/or comorbidities in a state of negative energy balance with the main objective of weight loss (2, 4-9). The presence of several confounding variables in these studies, such as weight loss and comorbidities, hinders our understanding of the real physiological impact of total diet replacements. To our knowledge, this is the first study to compare a HP-TDR with a North American diet in healthy young adults of both sexes. In addition to being the first on the topic, this study has several strengths, including its crossover and rigorously controlled feeding design, allowing the detection of small diet effects on energy metabolism variables and metabolic blood markers. Moreover, the use of state-of-the-art technology, such as the WBCU, provide high accurate and precise results, reflecting the real effects of the dietary interventions. In addition to the design and technology used, the study of both females and males allowed us to explore how different sexes respond to these dietary interventions.

In this study, participants received isocaloric diets that were designed to mimic the North American dietary pattern and a nutritional product commercially available in many countries. For this reason, one or more macronutrients could not be kept constant in one dietary intervention while others were manipulated in the other intervention. When comparing the macronutrient distribution of the HP-TDR with the Acceptable Macronutrient Distribution Range (10), this dietary strategy can be characterized as a HP and low-carbohydrate. Therefore, it is not possible to attribute any of the results observed in this study to a single macronutrient. In addition, this study has other limitations, including the specificity of population being studied (i.e., healthy, young adults with a normal body weight) and the short-term intervention. These limitations restrict our ability to translate these results to other population groups and longer intervention periods. Therefore, future studies are needed to better understand the long-term effects of this dietary intervention on the physiology of healthy and diseased population groups.

Table 4.1. Nutrient content of the intervention diets

	HP-TDR	**CON**
Energy (kcal/day)	2129 ± 241	2128 ± 241
Protein		
% energy	39.9 ± 0.3	15.3 ± 0.3
g/day	211 ± 24	83 ± 9
Fat		
% energy	24.9 ± 0.3	30.2 ± 0.3
g/day	58 ± 6	72 ± 8
Carbohydrate		
% energy	35.2 ± 0.3	54.4 ± 0.4
g/day	186 ± 21	295 ± 34
Sugars (g/day)	179 ± 21	92 ± 12
Fiber (g/day)	4 ± 0	30 ± 3
Saturated Fat (g/day)	12 ± 1	17 ± 3
Monounsaturated Fat (g/day)	35 ± 3	31 ± 4
Polyunsaturated Fat (g/day)	5 ± 0	17 ± 2
Cholesterol (mg/day)	38 ± 9	107 ± 39

Data are expressed as mean ± standard deviation.
N=43 (N=19 females, N=24 males).
Abbreviations: CON: control; HP-TDR: high-protein total diet replacement.

Table 4.2. Baseline characteristics of the study participants

Characteristics	All (n=43)	Females (n=19)	Males (n=24)	Sex Difference[1]
Age (years)	24 ± 4	25 ± 3	23 ± 4	0.090
Height (cm)	171.1 ± 7.3	166.3 ± 5.7	174.9 ± 6.1	<0.001
Weight (kg)	64.4 ± 6.9	61.1 ± 4.8	67.0 ± 7.3	0.003
Waist Circumference (cm)	74.4 ±5.6	71.4 ± 2.8	76.9 ± 6.1	0.001
BMI (kg/m^2)	22.0 ± 1.4	22.2 ± 1.2	21.9 ± 1.6	0.522
FM (kg)	15.3 ± 5.1	18.6 ± 3.3	12.7 ± 4.9	<0.001
LST (kg)	46.4 ± 7.6	40.1 ± 4.4	51.4 ± 5.6	<0.001
BMC (kg)	2.7 ± 0.3	2.4 ± 0.2	2.9 ± 0.3	<0.001
Race				0.202
White	19 (44)	7 (37)	12 (50)	
Asian	14 (33)	5 (26)	9 (37)	
Hispanic	3 (7)	3 (16)	0 (0)	
Black	1 (2)	1 (5)	0 (0)	
Other	6 (14)	3 (16)	3 (13)	
Physical Activity Level[2]				0.270
Insufficiently Active	2 (5)	1 (6)	1 (4)	
Moderately Active	7 (16)	5 (26)	2 (8)	
Active	34 (79)	13 (68)	21 (88)	
Medication/Nutritional Supplement in Use				0.412
None	34 (79)	13 (68)	21 (88)	
Multivitamin/Mineral	5 (12)	3 (16)	2 (8)	
Antidepressant	3 (7)	2 (11)	1 (4)	
Antihistamine	1 (2)	1 (5)	0 (0)	
Birth Control Method in Use			N/A	N/A
None	9 (21)	9 (47)		
Birth Control Pills	9 (21)	9 (47)		
Non-Hormonal Intrauterine Device	1 (2)	1 (6)		

Blood Markers				
ALT (U/L)	22 ± 10	18 ± 5	24 ± 12	0.058
AST (U/L)	25 ± 21	21 ± 5	29 ± 27	0.233
Serum Albumin (g/L)	45 ± 2	43 ± 2	47 ± 2	<0.001
Creatinine (mmol/L)	80 ± 13	70 ± 10	88 ± 9	<0.001
Estimated GFR (mL/min/1.73m^2)	106 ± 14	105 ± 15	107 ± 13	0.572
Sodium (mmol/L)	141 ± 2	139 ± 2	142 ± 1	<0.001
Potassium (mmol/L)	4.3 ± 0.3	4.3 ± 0.3	4.4 ± 0.2	0.177
Chloride (mmol/L)	104 ± 2	104 ± 2	104 ± 2	0.742
TSH (mU/L)	1.74 ± 0.74	1.90 ± 0.75	1.61 ± 0.72	0.211

Data are expressed as mean ± standard deviation or n (%).

[1] P-values refer to differences between females and males. For continuous variables, p-values were detected with the use of an independent-samples t-test or Mann-Whitney U test, accordingly. For nominal variables, p-values were detected with the use of the Fisher's exact test.

[2] Physical activity levels were classified according to the Godin-Shephard Leisure-Time Physical Activity Questionnaire.

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMC: bone mineral content; FM: fat mass; GFR: glomerular filtration rate; LST: lean soft tissue; N/A: not applicable; TSH: thyroid stimulating hormone.

Table 4.3. Energy expenditure, respiratory exchange ratio, and macronutrient oxidation rates and balances during the HP-TDR and CON diets.

	HP-TDR			CON			Diet Effect[1]	Sex Effect[1]	Diet *x* Sex[1]
	All (n=43)	Female (n=19)	Male (n=24)	All (n=43)	Female (n=19)	Male (n=24)			
Total EE (kcal/day)	2143 ± 268	1967 ± 195	2283 ± 234	2061 ± 243	1899 ± 143	2189 ± 231	<0.001	<0.001	0.300
24-hour RER	0.85 ± 0.02	0.84 ± 0.01	0.86 ± 0.01	0.87 ± 0.01	0.87 ± 0.01	0.87 ± 0.02	<0.001	0.038	0.333
Resting									
REE Day 1 (kcal/day)	1620 ± 259	1432 ± 138	1768 ± 236	1621 ± 206	1491 ± 115	1724 ± 204	0.784	<0.001	0.067
RER - Day 1	0.81 ± 0.02	0.81 ± 0.03	0.81 ± 0.02	0.82 ± 0.04	0.81 ± 0.03	0.83 ± 0.03	0.213	0.063	0.066
REE Day 2 (kcal/day)	1612 ± 215	1462 ± 168	1731 ± 169	1605 ± 247	1408 ± 147	1761 ± 193	0.582	<0.001	0.064
RER - Day 2	0.82 ± 0.02	0.81 ± 0.02	0.82 ± 0.02	0.86 ± 0.03	0.85 ± 0.02	0.86 ± 0.03	<0.001	0.242	0.394
Basal									
Basal EE (kcal/day)	1584 ± 242	1409 ± 149	1723 ± 211	1600 ± 223	1450 ± 165	1719 ± 191	0.411	<0.001	0.315
RER	0.83 ± 0.02	0.83 ± 0.02	0.84 ± 0.02	0.87 ± 0.02	0.87 ± 0.02	0.87 ± 0.02	<0.001	0.518	0.689
Sleep									
Sleep EE (kcal/8-hour night)[3]	498 ± 49	469 ± 47	522 ± 37	481 ± 53	450 ± 44	505 ± 48	<0.001	<0.001	0.864
RER	0.82 ± 0.02	0.82 ± 0.02	0.82 ± 0.01	0.85 ± 0.02	0.85 ± 0.02	0.85 ± 0.02	<0.001	0.664	0.661
Carbohydrate Ox (g/day)	235 ± 43	219 ± 31	247 ± 47	273 ± 40	256 ± 27	286 ± 45	<0.001	0.007	0.867
Protein Ox (g/day)[2]	91 ± 40	67 ± 24	110 ± 40	53 ± 20	36 ± 8	67 ± 16	<0.001	<0.001	0.212
Fat Ox (g/day)	79 ± 17	78 ± 17	79 ± 16	71 ± 16	69 ± 9	72 ± 20	0.013	0.614	0.729
Carbohydrate Balance (g/day)	-48 ± 33	-43 ± 25	-51 ± 38	22 ± 26	21 ± 18	23 ± 32	<0.001	0.631	0.463

Protein Balance (g/day)	119 ± 34	132 ± 23	110 ± 39	29 ± 19	42 ± 6	19 ± 19	<0.001	0.001	0.959
Fat Balance (g/day)	-20 ± 17	-23 ± 17	-18 ± 16	1 ± 15	-0.6 ± 10	3 ± 18	<0.001	0.246	0.867
Energy Balance (kcal/day)	-18 ± 113	32 ± 101	-58 ± 109	92 ± 129	164 ± 100	35 ± 121	<0.001	0.001	0.143

Data are presented as mean ± standard deviation.
[1] P-values were detected with the use of a mixed analysis of variance.
[2] Data were not normally distributed and log-transformed for statistical analysis.
[3] Sleep EE reflects an 8-hour sleep period.
Abbreviations: CON: control; EE: energy expenditure; HP-TDR: high-protein total diet replacement; Ox: oxidation; REE: resting energy expenditure; RER: respiratory exchange ratio.

Table 4.4. Metabolic blood markers during the HP-TDR and CON diets.

	HP-TDR			CON			Δ[1]		Postprandial[2]	
	Fasting Day 1	**Postprandial**	**Fasting Day 2**	**Fasting Day 1**	**Postprandial**	**Fasting Day 2**	**Diet Effect**	**Diet *x* Sex**	**Diet Effect**	**Diet *x* Sex**
Glucose (mmol/L)[3]	4.8 ± 0.3	5.1 ± 0.4	4.7 ± 0.2	4.8 ± 0.3	4.9 ± 0.5	4.9 ± 0.3	0.126	0.502	0.044	0.765
Insulin (pmol/L)[3]	43.1 ± 15.4	62.8 ± 35.1	35.6 ± 13.8	44.8 ± 18.4	81.1 ± 50.2	37.1 ± 15.3	0.804	0.121	0.007	0.359
HOMA %B[3]	89.0 ± 20.5	-	78.7 ± 17.4	88.4 ± 21.7	-	76.2 ± 22.9	0.578	0.362	-	-
HOMA IR[3]	0.8 ± 0.3	-	0.6 ± 0.3	0.8 ± 0.3	-	0.7 ± 0.3	0.716	0.087	-	-
Glycerol (μM)[4]	27.5 ± 19.6	32.3 ± 23.0	19.3 ± 11.4	23.7 ± 14.4	47.6 ± 29.5	19.6 ± 12.0	0.031	0.684	<0.001	0.682
NEFA (μM)[4]	201.2 ± 191.6	115.2 ± 123.4	154.8 ± 139.5	182.1 ± 150.6	104.1 ± 88.4	145.7 ± 132.0	0.880	0.190	0.513	0.160
Lipid Panel[3]										
Total Cholesterol (mmol/L)	4.34 ± 0.73	4.33 ± 0.73	4.43 ± 0.78	4.30 ± 0.69	4.19 ± 0.72	4.28 ± 0.76	0.010	0.286	0.041	0.174
LDL Cholesterol (mmol/L)	2.41 ± 0.52	2.28 ± 0.49	2.54 ± 0.52	2.38 ± 0.49	2.13 ± 0.5	2.39 ± 0.5	<0.001	0.647	0.023	0.796

HDL Cholesterol (mmol/L)	1.45 ± 0.43	1.45 ± 0.46	1.44 ± 0.48	1.43 ± 0.43	1.40 ± 0.44	1.40 ± 0.43	0.214	0.042	0.047	0.436
Non-HDL Cholesterol (mmol/L)	2.89 ± 0.58	2.88 ± 0.52	2.99 ± 0.58	2.88 ± 0.5	2.79 ± 0.52	2.88 ± 0.56	0.005	0.645	0.082	0.258
Triglyceride (mmol/L)	1.06 ± 0.42	1.31 ± 0.63	0.98 ± 0.34	1.08 ± 0.41	1.45 ± 0.57	1.08 ± 0.42	0.044	0.958	0.128	0.143

Data are presented as mean ± standard deviation.

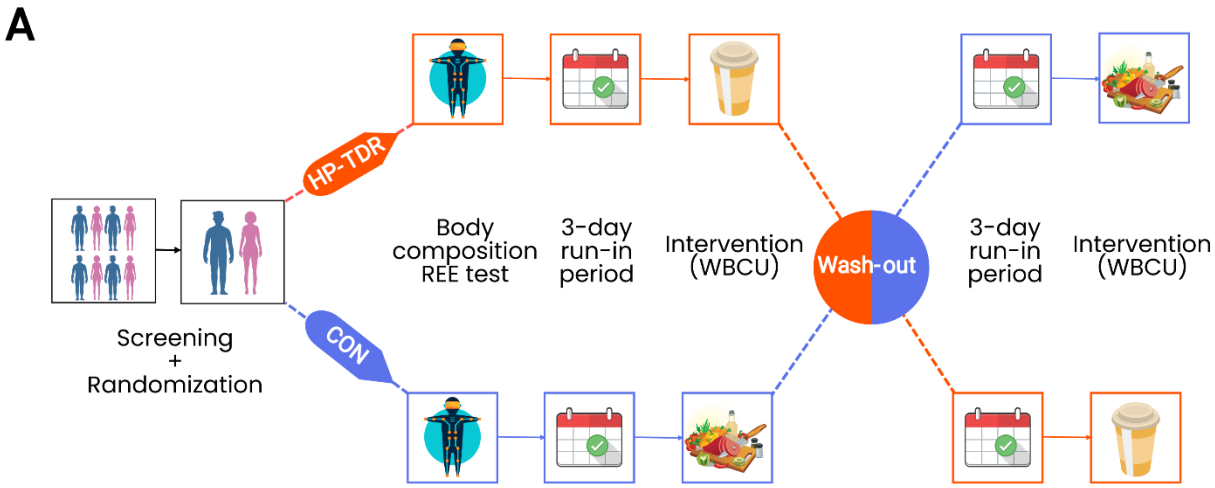
[1] P-values represent the main effect of diet on the change from fasting day 1 to fasting day 2 and were detected with the use of a mixed analysis of variance.

[2] P-values represent the main effect of diet on postprandial values and were detected with the use of a mixed analysis of variance.

[3] N=41 (N=17 females, N=24 males).

[4] N=42 (N=18 females, N=24 males).

Abbreviations: CON: control; HDL: high-density lipoprotein; HOMA %B: homeostatic model assessment of β-cell function; HOMA IR: homeostatic model assessment of insulin resistance; HP-TDR: high-protein total diet replacement; LDL: low-density lipoprotein.



B

	Day 1 (8:00 am to 11:59 pm)	Day 2 (12:00 am to ~3:00 pm)
Intervention (WBCU) →		
Blood draw →	~7:30 am and ~2:30 pm	~8:00 am
Meals →	9:00 am, 12:00 pm, 3:00 pm, 6:00 pm, and 9:00 pm	9:00 am
Exercise →	10:20 am to 11:00 am	N/A
24-h EE →	8:00 am to 11:59 pm	12:00 am to 8:00 am
REE →	8:00 am to 9:00 am	~7:30 am to ~8:30 am
Sleep EE →	N/A	2:00 am to 5:00 am
Basal EE →	N/A	6:00 am to 7:00 am
Postprandial EE →	N/A	~9:00 am to ~3:00 pm

Figure 4.1. Overview of the experimental protocol (A) and variables assessed during each 32-hour test (B).

Abbreviations: CON: control diet; EE: energy expenditure; HP-TDR: high-protein total diet replacement; N/A: not applicable; REE: resting energy expenditure; WBCU: whole-body calorimetry unit.

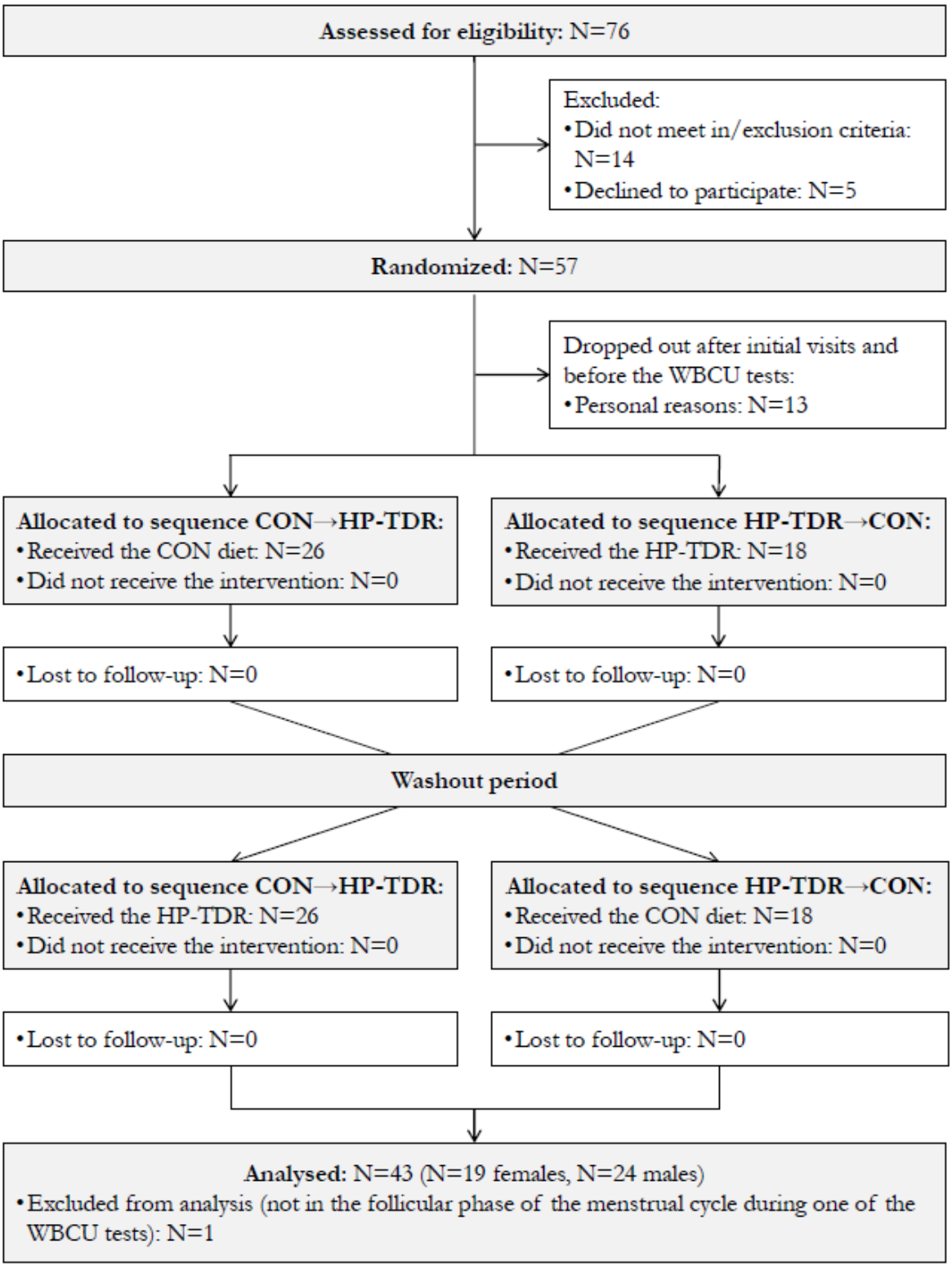


Figure 4.2. CONSORT flow diagram for crossover trials.

Abbreviations: CON: control diet; CONSORT: Consolidated Standards of Reporting Trials; HP-TDR: high-protein total diet replacement; WBCU: whole-body calorimetry unit.

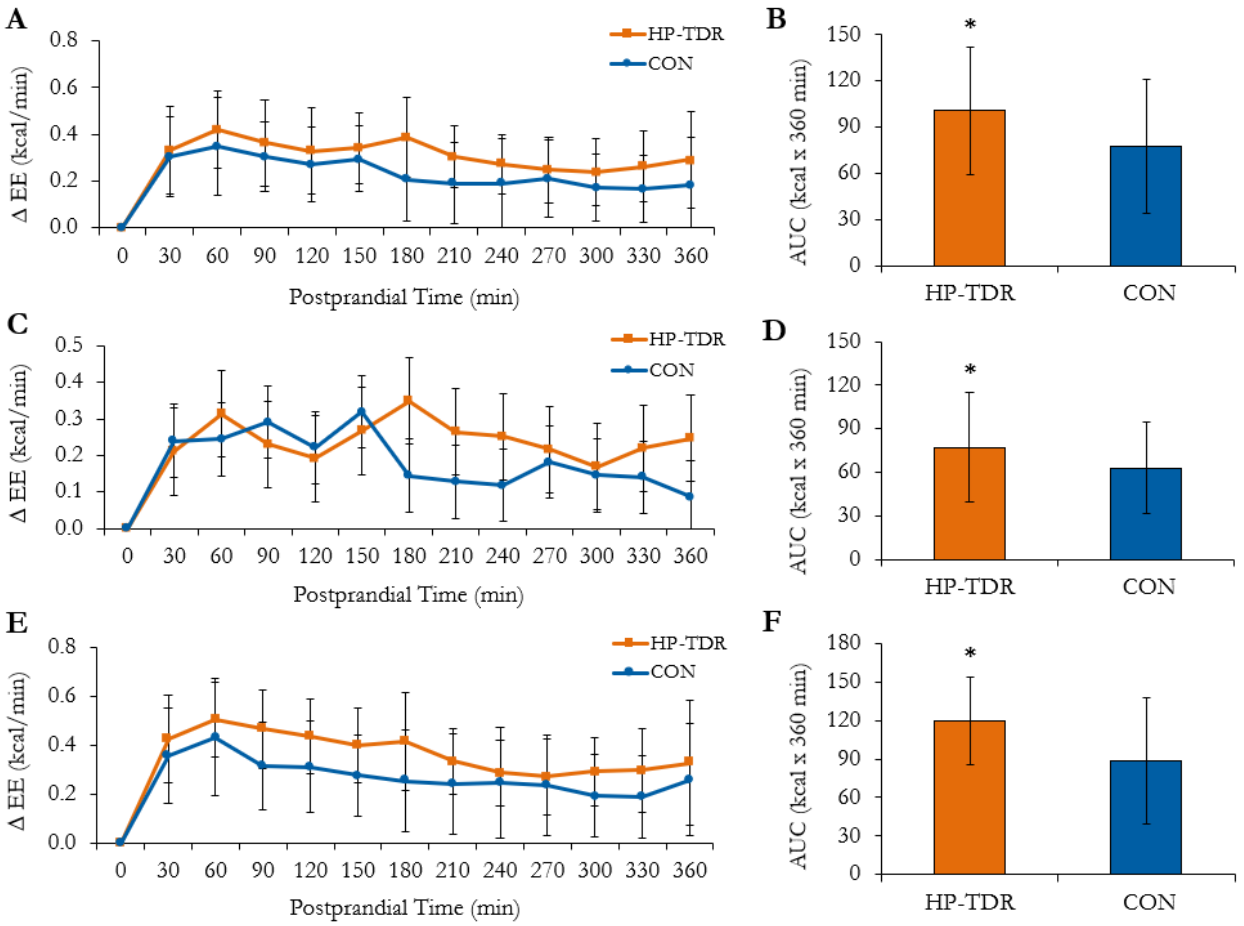


Figure 4.3. Change in resting energy expenditure (Δ EE) following ingestion of the isocaloric high-protein-total diet replacement (HP-TDR) and control (CON) breakfasts on the second day of intervention while participants were inside the whole-body calorimetry unit. Values are mean ± standard deviation. Left panels (A, C, and E) indicate 30-minute means; right panels (B, D, and F) indicate the total area-under-curve (AUC) over 360 minutes. Top panels (A and B) contain data from all participants (n=43); middle panels (C and D) contain data from females (n=19); and bottom panels (E and F) contain data from males (n=24). *Significant difference between the HP-TDR and CON conditions, $p<0.05$ as assessed by a mixed analysis of variance. Although there was no statistically significant interaction between the interventions and sex on AUC ($p=0.115$), the main effect of sex showed a significant difference in females and males ($p=0.003$), as assessed by a mixed analysis of variance.

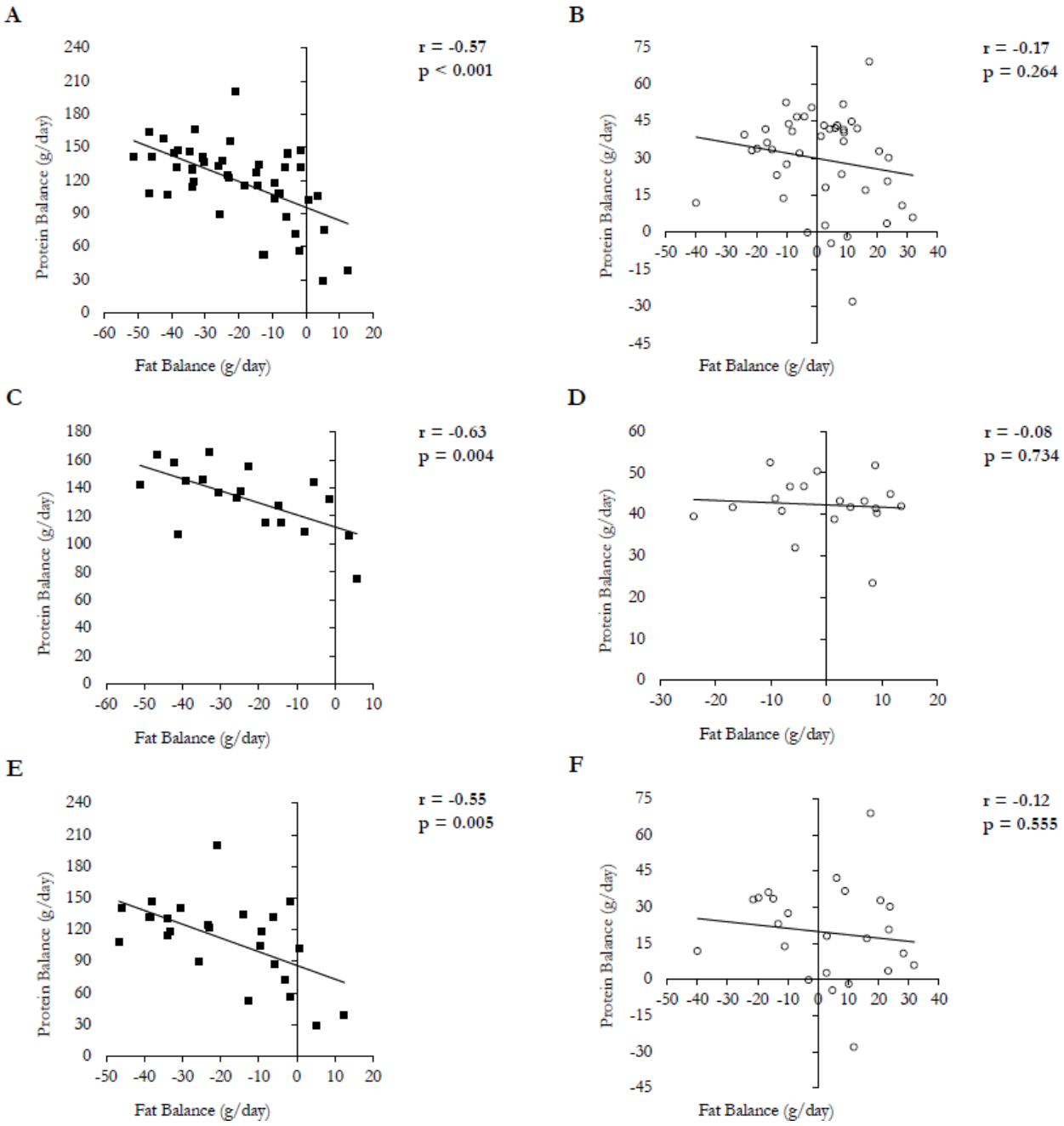


Figure 4.4. Correlation between protein and fat balances in all participants (n=43, panels A and B), females (n=19, panels C and D), and males (n=24, panels E and F). Black squares (■) represent the high-protein total diet replacement (HP-TDR) condition and empty circles (○) represent the control (CON) condition.

4.7 SUPPLEMENTARY MATERIAL

Supplementary Table 4.1. Nutrient content and ingredient list of the nutritional supplement used in the high-protein total diet replacement intervention.

Nutrients	Amount per 50 grams of powder
Energy (kcal)	180
Fat (g)	1.0
Saturated Fat (g)	0.5
Trans Fat (g)	0.0
Polyunsaturated Fat (g)	0.1
Monounsaturated Fat (g)	0.4
Cholesterol (mg)	3
Sodium (mg)	340
Potassium (mg)	500
Carbohydrate (g)	15
Fibre (g)	0.5
Sugars (g)	15
Protein (g)	27
Vitamin A (IU)	794
Vitamin C (mg)	16
Vitamin E (IU)	6
Vitamin B1 (mg)	5
Vitamin B2 (mg)	6
Vitamin B6 (mg)	7
Calcium (mg)	215
Iron (mg)	4.9
Ingredients: Soy protein isolate, honey, skim milk, yogurt powder, potassium chloride, magnesium carbonate, calcium citrate, vitamin C, niacin, color additive, riboflavin, vitamin E, zinc oxide, ferrous fumarate, manganese sulfate, calcium pantothenate, vitamin B2, vitamin B6, vitamin B1, vitamin A, folic acid, potassium iodide, sodium selenite, biotin, vitamin D3, and vitamin B12.	

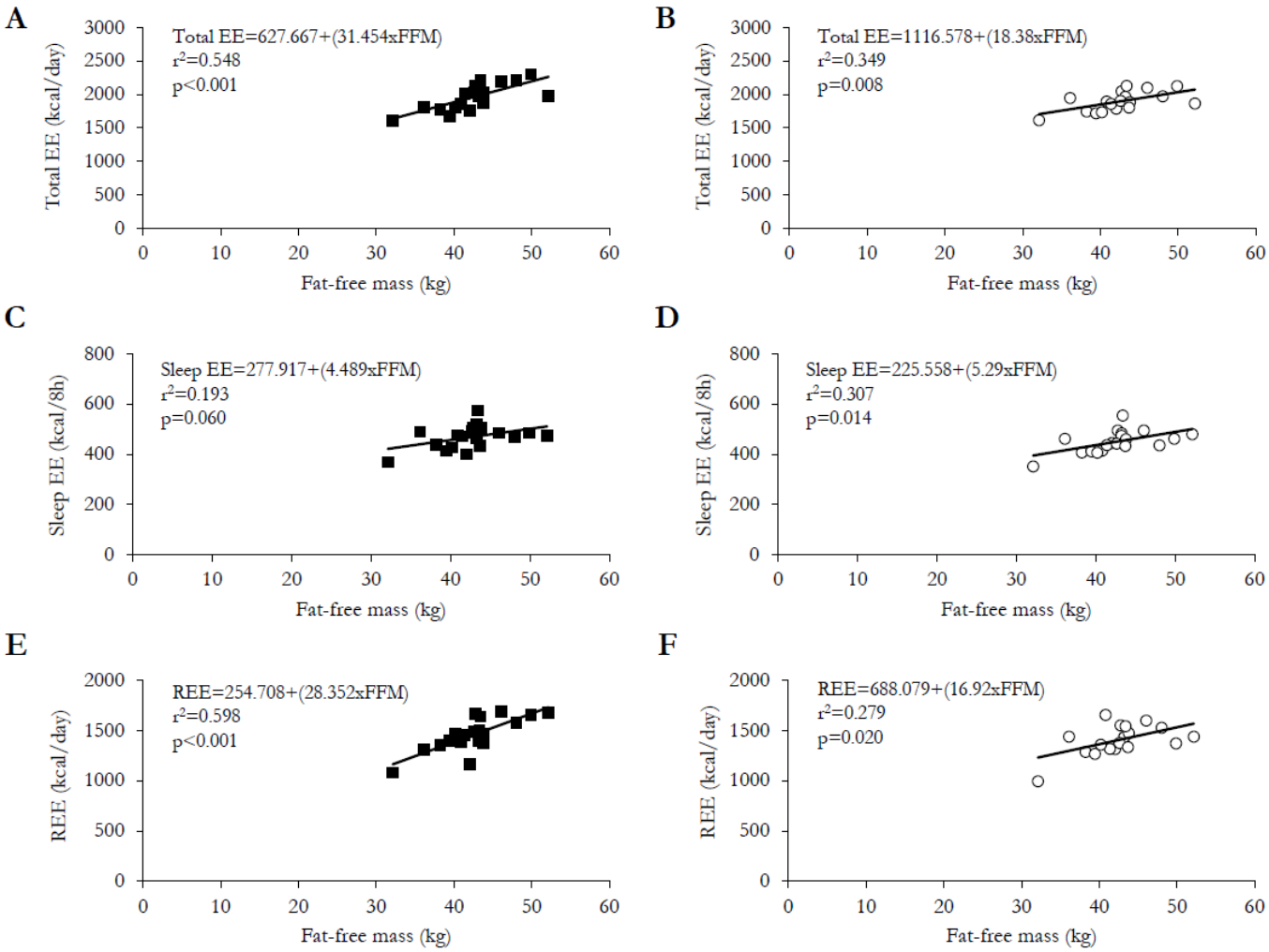
Supplementary Table 4.2. Secondary analysis of metabolic blood markers during the HP-TDR and CON diets.

	HP-TDR			CON			Diet *x* Sex *x* Time[1]	Diet *x* Sex[1]	Diet *x* Time[1]
	Fasting Day 1	**Postprandial**	**Fasting Day 2**	**Fasting Day 1**	**Postprandial**	**Fasting Day 2**			
Glucose (mmol/L)[3]	4.8 ± 0.3	4.7 ± 0.4	4.7 ± 0.2	4.8 ± 0.3	4.9 ± 0.5	4.9 ± 0.3	0.512	0.523	0.321
Insulin (pmol/L)[3]	43.1 ± 15.4	62.8 ± 35.1	35.6 ± 13.8	44.8 ± 18.4	81.1 ± 50.2	37.1 ± 15.3	0.474	0.194	0.018
HOMA %B[3]	89.0 ± 20.5	-	78.7 ± 17.4	88.4 ± 21.7	-	76.2 ± 22.9	0.362	0.236	0.578
HOMA IR[3]	0.8 ± 0.3	-	0.6 ± 0.3	0.8 ± 0.3	-	0.7 ± 0.3	0.087	0.377	0.716
Glycerol (μM)[4]	27.5 ± 19.6	32.3 ± 23.0	19.3 ± 11.4	23.7 ± 14.4	47.6 ± 29.5	19.6 ± 12.0	0.781	0.648	<0.001
NEFA (μM)[4]	201.2 ± 191.6	115.2 ± 123.4	154.8 ± 139.5	182.1 ± 150.6	104.1 ± 88.4	145.7 ± 132.0	0.164	0.758	0.870
Lipid Panel[3]									
Total Cholesterol (mmol/L)	4.34 ± 0.73	4.33 ± 0.73	4.43 ± 0.78	4.3 ± 0.69	4.19 ± 0.72	4.28 ± 0.76	0.510	0.256	0.047
LDL Cholesterol (mmol/L)	2.41 ± 0.52	2.28 ± 0.49	2.54 ± 0.52	2.38 ± 0.49	2.13 ± 0.5	2.39 ± 0.5	0.759	0.536	0.005
HDL Cholesterol (mmol/L)	1.45 ± 0.43	1.45 ± 0.46	1.44 ± 0.48	1.43 ± 0.43	1.4 ± 0.44	1.4 ± 0.43	0.193	0.358	0.095
Non-HDL Cholesterol (mmol/L)	2.89 ± 0.58	2.88 ± 0.52	2.99 ± 0.58	2.88 ± 0.5	2.79 ± 0.52	2.88 ± 0.56	0.808	0.371	0.046
Triglyceride (mmol/L)	1.06 ± 0.42	1.31 ± 0.63	0.98 ± 0.34	1.08 ± 0.41	1.45 ± 0.57	1.08 ± 0.42	0.142	0.394	0.263

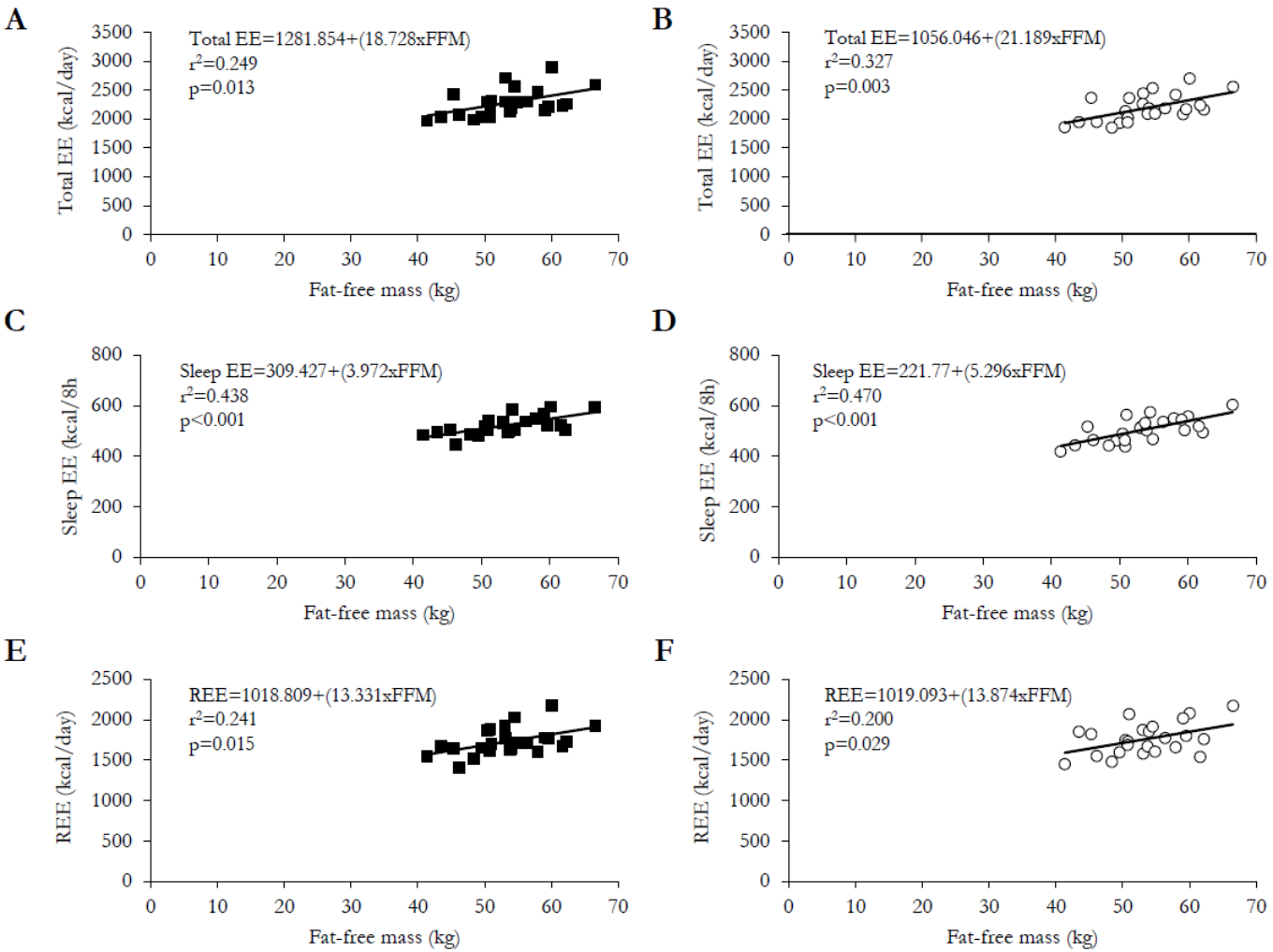
Data are presented as mean ± standard deviation.

[1] P-values represent diet *x* sex *x* time interaction and were detected with the use of a mixed analysis of variance.

[3] N=41 (N=17 females, N=24 males).
[4] N=42 (N=18 females, N=24 males).
Abbreviations: CON: control; HDL: high-density lipoprotein; HOMA %B: homeostatic model assessment of β-cell function; HOMA IR: homeostatic model assessment of insulin resistance; HP-TDR: high-protein total diet replacement; LDL: low-density lipoprotein.



Supplementary Figure 4.1. Correlation between fat-free mass (FFM) and total energy expenditure (total EE, panels A and B), sleep energy expenditure (sleep EE, panels C and D), and resting energy expenditure on day 2 (REE, panels E and F) in females (n=19). Black squares (■) represent the high-protein total diet replacement (HP-TDR) condition and empty circles (○) represent the control (CON) condition.



Supplementary Figure 4.2. Correlation between fat-free mass (FFM) and total energy expenditure (total EE, panels A and B), sleep energy expenditure (sleep EE, panels C and D), and resting energy expenditure on day 2 (REE, panels E and F) in males (n=24). Black squares (■) represent the high-protein total diet replacement (HP-TDR) condition and empty circles (○) represent the control (CON) condition.

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CHAPTER 5. THE EFFECTS OF A HIGH-PROTEIN DIET REPLACEMENT ON APPETITE SENSATIONS AND APPETITE-RELATED HORMONES

5.1 PREFACE

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5.2 ABSTRACT

Background: Dietary intake can affect energy homeostasis and influence body weight control.

Objective: The aim of this inpatient metabolic balance study was to compare the impact of high-protein total diet replacement (HP-TDR) versus a control (CON) diet on mechanisms involved in the regulation of food intake and energy homeostasis in healthy, normal-weight adults.

Methods: In this secondary analysis of an acute randomized, controlled, cross-over study, participants completed two isocaloric arms: a) HP-TDR: 35% carbohydrate, 40% protein, and 25% fat; b) CON: 55% carbohydrate, 15% protein, and 30% fat. The diets were offered for 32 hours while inside a whole-body calorimetry unit. Body composition was measured at baseline using dual-energy X-ray absorptiometry. Appetite sensations, appetite-related hormones, and energy metabolism were assessed.

Results: Forty-three healthy, normal-weight adults (19 females) participated. In the HP-TDR, only females experienced lower 24-h area under the curve for prospective food consumption (-6399.47 ± 2940.63 mm/24 hours; p=0.04). Compared to the CON diet, the change in blood markers from fasting day 1 to fasting day 2 during the HP-TDR intervention was smaller for peptide tyrosine-tyrosine (PYY; -18.03 ± 8.19 pg/mL, p=0.03) and greater for leptin (2061.71 ±

607.26 pg/mL, p=0.002). Compared to males, females presented with a smaller change in fasting ghrelin (-121.47 ± 38.96 pg/mL, p=0.003), greater change in fasting PYY (19.25 ± 8.3 pg/mL, p=0.02), and leptin (6300.93 ± 1009.34 pg/mL, p<0.001). Moreover, postprandial levels of glucagon-like peptide 1 (1.30 ± 0.34 pM, p=0.001) and PYY (28.92 ± 7.77 pg/mL, p=0.001) were higher in the HP-TDR. Compared to males, females presented higher postprandial ghrelin (162.92 ± 68.94 pg/mL, p=0.02) and leptin (16023.39 ± 1501.42 pg/mL, p<0.001), and lower postprandial GLP-1 (-4.21 ± 1.43 pM, p=0.006) and PYY (-77.88 ± 14.87 pg/mL, p<0.001).

Conclusions: In conclusion, compared to the CON diet, the HP-TDR increased blood levels of anorexigenic hormones and reduced the PFC in females. Moreover, females and males responded differently to the intervention in terms of appetite sensations and appetite-related hormones.

5.3 BACKGROUND

Food intake and energy homeostasis are key determinants of body weight control and regulated by several external and internal factors, such as the environment, individual's physiology, and genetics (1). Dysregulation in one or more of these factors can contribute to the development of obesity (2). More specifically, the interplay between appetite-related hormones and the central nervous system has recently received special attention (3). For instance, female's appetite sensations have been shown to be more sensitive and reactive to dietary manipulation than male's (4-7), which can be partly explained by the gonadal steroid hormones (8) and neuronal responses to food intake (6). In fact, the hormones estrogen seems to have an inhibitory effect on food intake (9, 10). It has been demonstrated that individuals with obesity present with a decreased response or resistance to peripheral and central regulators of food intake and energy homeostasis (11). In fact, an attenuated fall in postprandial ghrelin levels, leptin resistance, lower levels of peptide tyrosine-tyrosine (PYY), and glucagon-like peptide 1 (GLP-1) are usually present in obesity, as reviewed by Miller and Ullrey (12) and Perry and Wang (13). These abnormal hormonal responses can affect food intake and energy homeostasis, potentially contributing to a state of positive energy imbalance (i.e., energy intake > energy expenditure [EE]). Therefore, strategies able to normalize these responses can ultimately help with the prevention and treatment of obesity.

Dietary intake is a modifiable factor that can affect overall energy intake and energy homeostasis and, hence, influence body weight control (1). Total diet replacements (TDR) and high-protein (HP) diets are two very popular weight management strategies (14). Total diet replacements are nutritionally complete formula foods designed to replace the whole diet for a specific period of time to facilitate weight control. Nutritional strategies comprised of these products have been shown to induce a significant and sustained body weight reduction in individuals with obesity (15-22). High-protein diets are characterized by a protein content above recommended values (i.e., for healthy adults aged >19 y: 0.80g/kg of body weight/d or 10-35% of total energy intake) (23, 24) and have been demonstrated to facilitate weight management by increasing EE and satiety, and improving body composition (25). Although the effects of these dietary strategies in isolation on components related to food intake and energy homeostasis have been partially investigated (26, 27), the synergistic effects of a combined high-protein total diet replacement (HP-TDR) have not yet been explored, despite their worldwide availability and high consumption by individuals with normal or excess body weight.

Therefore, the assessment of the effects of a HP-TDR on components related to food intake and energy homeostasis is crucial. This is especially important in the context of a controlled environment using a state-of-the-art methodology in healthy individuals with a normal body weight. Such study design characteristics eliminate confounding effects of obesity and comorbidities, allowing a better understanding of the impact of this strategy in a normal physiological condition so that findings are better translated to individuals with obesity and its related comorbidities. Additionally, it will provide further insight into the potential role of a HP-TDR for weight maintenance and prevention of obesity.

Considering the aspects discussed above, the aim of this inpatient metabolic balance study was to compare the acute impact of HP-TDR versus a control (CON) diet (North American) on appetite sensations, appetite-related hormones, and its association with energy metabolism components in healthy, normal-weight adults of both sexes. We hypothesized that, compared to the CON diet, participants consuming the HP-TDR would feel more satiated, have higher blood levels of anorexigenic hormones (i.e., GLP-1, PYY, and leptin), lower levels of the orexigenic hormone ghrelin, and that appetite sensations and appetite-related hormones would correlate with energy metabolism components. Additionally, we hypothesized that females would experience increased satiety and increased blood levels of leptin during the HP-TDR and CON interventions,

and that there would be no other differences in appetite sensations and appetite-related hormones between sexes.

5.4 METHODS

5.4.1 Ethical Approval

This study was conducted at the Human Nutrition Research Unit (HNRU), University of Alberta (Edmonton, AB, Canada), from November 2016 to November 2019. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Alberta Ethics Board (Pro00066006 and Pro00083005) and registered as NCT02811276 and NCT03565510 on ClinicalTrials.gov. Written informed consent was obtained from all participants.

5.4.2 Study Design and Participants

This was a planned secondary analysis of a previously described randomized, controlled, crossover inpatient study conducted separately in females and males; full methodological details and findings on changes in energy metabolism have been published (28). Briefly, participants were invited to participate in this study by poster advertisements placed on notice boards at the University of Alberta north campus. Healthy adults aged 18 to 35 years were recruited, with a body mass index (BMI) ranging from 18.5 to 24.9 kg/m^2. Females were required to have a regular menstrual cycle. Potential participants were excluded if they had any diagnosed disease, were smokers, were taking any medication and/or nutritional supplement that could affect energy metabolism and/or body composition, had any dietary restrictions, were performing >1 hour/day or >7 hours/week of exercise, were recently exposed to tests involving radiation, had claustrophobia, and if females were pregnant or lactating.

5.4.3 Experimental Protocol

Eligible participants were enrolled in the study and randomly assigned to begin with a HP-TDR or CON diet. Participants consumed these isocaloric diets on separate intervention days for 32 consecutive hours while inside a whole-body calorimetry unit (WBCU) for the measurement of energy metabolism components, appetite sensations, and appetite-related hormones (**Figure**

5.1). Females were required to be in the follicular phase of their menstrual cycle during each 32-hour intervention phase. A wash-out period of approximately 1-month was required for females and 2 weeks for males. A 3-day run-in period with a controlled, energy-balanced diet preceded both intervention phases. At baseline, participant's height, weight, waist circumference, body composition (GE Lunar iDXA, General Electric Company, Madison, USA; enCORE software 13.60 Lunar iDXA GE Health Care®) and resting metabolic rate (RMR) were assessed.

5.4.4 Diets

Immediately before each 32-hour intervention period in the WBCU, participants received an eucaloric diet for three consecutive days and were instructed not to eat any other food item, except for calorie-free beverages, and not to consume any caffeinated food product. Moreover, participants were asked to maintain their physical activity levels as low as possible for the first two days and not to exercise on the third day. They received a breakfast, lunch, dinner, and two snacks per day and the macronutrient distribution was similar to the CON intervention (i.e., 55% carbohydrate, 15% protein, and 30% fat), which resembled the North American dietary pattern (29, 30). The energy content of the diet was calculated to maintain participants in energy balance and was based on the RMR test performed at baseline multiplied by a physical activity factor (23) and a coefficient representing the thermic effect of food (i.e., 1.075) (31).

Following this 3-day run-in period, participants entered the WBCU and stayed for 32 consecutive hours while consuming a HP-TDR and a CON diet in a random order. The first dietary intervention was designed to maintain participants in energy balance and the second matched its energy content (i.e., isocaloric). The energy content of the meals and snacks were also matched for the HP-TDR and CON diets. During each intervention period, a breakfast (9:00 am), lunch (12:00 pm), dinner (6:00 pm), and two snacks (3:00 pm and 9:00 pm) were provided on day 1 and a breakfast (~8:30 am) on day 2. Additionally, bottled water was provided ad libitum. The HP-TDR consisted of a soy-protein nutritional supplement (Almased®, Almased USA, Inc., St. Petersburg, FL, USA) mixed with olive oil and low-fat milk (1% fat) (for the main meals) or apple juice (for the snacks), per label instructions (32). The CON diet was comprised of regular food items (i.e., breakfast: bread, peanut butter, and orange juice; lunch: turkey wrap and tomato soup; afternoon snack: apple, crackers, and cheese; dinner: chicken stir fry and brown rice; evening snack: cereal, milk, and almonds), fully described elsewhere (28). Participants were required to

consume all the food provided and meal trays were checked after consumption. The nutrient content of the dietary interventions is described in **Table 5.1**. The run-in and the intervention diets were designed by a registered dietitian using the Food Processor Nutrition Analysis Software (version 11.0.124, ESHA Research, Salem, OR, USA) and prepared at the metabolic kitchen of the HNRU.

5.4.5 Energy Metabolism

During each dietary intervention period, participants stayed for 32 consecutive hours (8:00 am on day 1 until 4:00 pm on day 2) inside an open-circuit WBCU, where the volume of oxygen (VO_2) and carbon dioxide (VCO_2) were continuously measured. The whole-body calorimetry conditions included a strict and standard schedule fully described elsewhere (28). In short, participants were asked to keep a minimum physical activity level throughout the 32-hour tests, a 40-minute walking exercise session was performed on the morning of the first day of the test (10:20 am) on a treadmill (BH Fitness T8 SPORT, BH Fitness, Foothill Ranch, Calif., USA), and sleep was only allowed during the night. Additionally, participants were instructed to collect their urine the entire time for nitrogen excretion analysis. By using the formula of Brouwer (33), EE, macronutrient oxidation rates and balances were calculated, a method fully described elsewhere (28).

5.4.6 Appetite Sensations

During the HP-TDR and CON interventions, appetite sensations were rated (i.e., hunger, satiety, fullness, and prospective food consumption [PFC]) immediately before and 30 minutes after each meal and snack using a validated anchored 100-mm visual analogue scale (VAS) (34). A total of 12 assessments were performed throughout each intervention period using a paper-and-pen method and data have been double-entered to ensure quality. Questions were worded as follows: “How hungry do you feel”, “How satisfied do you feel”, “How full do you feel”, and “How much do you think you can eat?”. Answers for each of those questions were anchored as: “I am not hungry at all” to “I have never been more hungry”, “I am completely empty” to “I cannot eat another bite”, “Not at all full” to “Totally full”, and “Nothing at all” to “A lot”, respectively. Appetite sensation responses were evaluated by calculating the 24-hour area under the curve (AUC) using baseline values with the trapezoid method (35). Moreover, the composite satiety

score (CSS) was calculated at each time of measurement by using the following equation: CSS (mm) = (satiety + fullness + (100 – prospective food consumption) + (100 − hunger))/4 (36). A higher CSS is associated with a higher satiety sensation and a subsequent lower motivation to eat.

5.4.7 Blood Analyses

Blood was sampled by venipuncture at three time points during each intervention period 1) the morning on the first day of test (fasting day 1, 7:30 am); 2) two hours after lunch (postprandial, 2:30 pm); and 3) the morning on the second day of test (fasting day 2, 8:00 am). These timepoints were chosen to compare differences in blood markers in fasting and postprandial states. Assessing the effects of the dietary interventions on changes in fasting blood markers is especially important, considering that altered fasting levels of appetite-related hormones are usually common in metabolic disorders, such as obesity (11, 13).

Both morning blood draws were sampled from participants after a 10- to 12-hour overnight fast using BD Vacutainer® blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), spray-coated with silica and a polymer gel for serum separation or with K2-ethylenediaminetetraacetic acid (EDTA) for plasma separation. Serum samples were analyzed for leptin and plasma samples were analyzed for ghrelin (active), PYY, and GLP-1 (active). Before centrifugation, a protease inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) (Sigma-Aldrich, Oakville, ON, Canada) was added to the K2-EDTA tubes and after it, hydrochloric acid (1 N,100 µL) was added to the ghrelin aliquot. Leptin and GLP-1 were measured by electrochemiluminescence using the MULTI-ARRAY® Assay System (Meso Scale Discovery®, Gaithersburg, MD, USA) and V-PLEX® (Meso Scale Discovery®, Gaithersburg, MD, USA), respectively. Ghrelin and PYY were measured by enzyme-linked immunosorbent assay kits from EMD Millipore Co. (Billerica, MA, USA). All analyses were performed at the HNRU according to manufacturer's instructions, in duplicates, and were repeated when they had not fallen within the range of the standard curves. The coefficients of variation (CV) were 3.67% and 6.99% for leptin, 6.10% and 5.82% for ghrelin, 7.48% and 10.3% for PYY, and 5.34% and 5.24% for GLP-1 in females and males, respectively.

5.4.8 Statistical Analysis

Sample size calculation for the primary study has been described elsewhere (28). An additional sample size calculation was conducted for this planned, secondary analysis based on differences in satiety between dependent groups receiving a HP diet (973 ± 178 mm/24 hours) or an adequate protein diet (765 ± 304 mm/24 hours) from a previously published study (26). Group sample sizes of 14 in each arm would achieve 80% power to detect a difference of 208 mm/24 hours with a two-sided significance level of 0.05. Assuming a 20% attrition rate, a total 17 participants would be needed in each group. The sample size calculation was done using a software developed by David Schoenfeld:

(http://hedwig.mgh.harvard.edu/sample_size/js/js_crossover_quant.html).

Data were expressed as mean ± standard deviation (SD) for continuous variables and mean ± standard error of the mean difference (SEM) to report differences between sexes and groups. Shapiro Wilk's test was used to check the normality of the data. Independent samples t-test or Mann-Whitney U tests were used as appropriate to compare nutrient intake and baseline appetite-related hormones between sexes. Possible differences between the HP-TDR and CON diets were explored using a mixed analysis of variance (ANOVA) with within-subject factors (i.e., dietary interventions and/or time) and a between subject factor (i.e., sex). Post-hoc analyses were applied with all ANOVA tests using a Tukey test (equal variances assumed) or Games-Howell (equal variances not assumed). Diagnostics, such as the assessment of normality, homogeneity of variances using the Box's test of equality of covariance matrices and Levene's test for equality of variances were used to check if the ANOVA assumptions were valid. Estimates of effect size were described as partial eta squared (partial η^2) and were defined as small (η^2=0.01), medium (η^2=0.06), and large (η^2=0.14) effects (37). Partial correlation analyses controlling for sex were performed between energy metabolism components, appetite sensations, and appetite-related hormones. IBM® SPSS® Statistics version 24 (International Business Machines Corporation, New York, NJ, USA) was used to perform all statistical analyses. A $p<0.05$ was used for most of the comparisons and test specific p-values were used for multiple comparisons.

5.5 RESULTS

5.5.1 Participants

A total of 43 healthy adults (n=19 females and n=24 males) completed the study and were included in the analyses. Characteristics of study participants are shown in **Table 5.2**, with a more extensive description presented previously (30). Briefly, they were primarily a group of white healthy adults in their 20s with a normal BMI, and active lifestyle. Most of them (79%) were not taking any medication or nutritional supplement, 12% were taking multivitamin/mineral, 7% antidepressant, and 2% antihistamine. Compared to males, females were shorter, had lower body weight, smaller waist circumference, higher fat mass (FM), lower lean soft tissue (LST) and bone mineral content (BMC), all $p<0.05$. There was no difference in the volume of liquid consumed during the CON and HP-TDR diets ($p=0.651$, data not shown).

5.5.2 Energy Metabolism

Energy metabolism components assessed during the HP-TDR and CON diets are illustrated in **Figure 5.2** and reported in detail elsewhere (30). While consuming the HP-TDR, participants presented higher 24-hour EE ($p<0.001$), protein and fat oxidation rates ($p=0.013$ and $p<0.001$, respectively), and lower carbohydrate oxidation rate ($p<0.001$). Additionally, the HP-TDR led to lower energy ($p<0.001$), fat ($p<0.001$), and carbohydrate ($p<0.001$) imbalances, and greater protein imbalance ($p<0.001$) compared to the CON diet. The order in which participants received the dietary interventions did not affect any of the energy metabolism variables analyzed (all $p>0.05$).

5.5.3 Appetite Sensations

The 24-hour AUC for hunger, satiety, fullness, and PFC are shown in **Figure 5.3**. There was a significant interaction between diet and sex on 24-hour AUC for PFC ($p=0.04$, partial $\eta^2=0.092$). In the HP-TDR diet, the 24-hour AUC for PFC was lower in females compared to males (-6908.16 ± 3424.67 mm/24 hours; $p=0.05$, partial $\eta^2=0.090$), while no significant difference between sexes was observed in the CON diet (253.18 ± 2659.62 mm/24 hours; $p=0.92$). In females, the 24-hour AUC for PFC was lower with the HP-TDR compared to the CON diet (-6399.47 ±2940.63 mm/24 hours; $p=0.04$, partial $\eta^2=0.208$), but no significant difference between the diets

was observed in males (761.87 ± 2101.70 mm/24 hours; p=0.72). No significant diet x sex interaction was observed in 24-hour AUC for hunger (p=0.49), satiety (p=0.06), and fullness (p=0.13). Regardless of sex, there was no significant difference in 24-hour AUC for hunger (p=0.10), satiety (p=0.43), fullness (p=0.09), and PFC (p=0.11) between the HP-TDR and CON diets. Significant correlations were noted between energy balance and satiety, as well as between energy balance and PFC only when participants were fed the HP-TDR, **Table 5.3**. The CSS throughout the HP-TDR and CON interventions is illustrated in **Figure 5.4**. There was no significant interaction between diet, sex, and time (p=0.45). The order in which participants received the dietary interventions did not affect any of the appetite sensations analyzed (all p>0.05).

5.5.4 Appetite-Related Hormones

Appetite-related hormones assessed in a fasting state on days 1 and 2, and after lunch during the HP-TDR and CON diets are illustrated in **Figure 5.5** and shown in **Supplementary Table 5.1**. Before the dietary interventions (i.e., fasting day 1), blood levels of GLP-1, PYY, and leptin were different between sexes. Compared to males, females presented lower levels of GLP-1 (HP-TDR: -2.15 ± 0.82 pM, p=0.015; CON: -2.22 ± 0.96 pM, p=0.02) and PYY (HP-TDR: -85.71 ± 13.99 pg/mL, p<0.001; CON: -94.52 ± 12.63 pg/mL, p<0.001), and higher levels of leptin (HP-TDR: 17,068.28 ± 1,594.92 pg/mL, p<0.001; CON: 18,562.50 ± 2,134.02 pg/mL, p<0.001).

There was a significant interaction between diet and sex on the change from fasting day 1 to fasting day 2 in leptin concentration (p=0.006, partial η^2=0.167). In both sexes, this change was higher with the HP-TDR intervention (females: 3803.29 ± 1361.79 pg/mL, p=0.01, partial η^2=0.302; males: 320.13 ± 113.51 pg/mL, p=0.01, partial η^2=0.257). In both intervention groups, the change in this biomarker was greater in females compared to males (HP-TDR: 8042.51 ± 1214.59 pg/mL, p<0.001, partial η^2=0.517; CON: 4559.36 ± 1140.11 pg/mL, p<0.001, partial η^2=0.281). Regardless of sex, compared to the CON diet, the change in fasting blood markers with the HP-TDR was smaller for PYY (-18.03 ± 8.19 pg/mL, p=0.03, partial η^2=0.113) and greater for leptin (2061.71 ± 607.26 pg/mL, p=0.002, partial η^2=0.219). On the other hand, this change was not different between the dietary interventions for ghrelin (p=0.08) and GLP-1 (p=0.10). A main effect of sex was detected on the change in fasting ghrelin concentration, PYY, and leptin. Compared to males, females presented with a smaller change in fasting ghrelin (-121.47 ± 38.96

pg/mL, p=0.003, partial η^2=0.192), greater change in fasting PYY (19.25 ± 8.3 pg/mL, p=0.02, partial η^2=0.124), and leptin (6300.93 ± 1009.34 pg/mL, p<0.001, partial η^2=0.487).

Postprandially, there was a significant interaction between diet and sex on ghrelin (p=0.048, partial η^2=0.094), GLP-1 (p=0.003, partial η^2=0.225), and PYY (p=0.03, partial η^2=0.108). In the HP-TDR diet, postprandial ghrelin concentration was higher in females compared to males (197.33 ± 74.36 pg/mL, p=0.01, partial η^2=0.147). There was no significant difference between sexes in the CON diet (p=0.08). Postprandial GLP-1 (HP-TDR: -5.35 ± 1.46 pM, p=0.001, partial η^2=0.264; CON: -3.19 ± 1.22 pM, p=0.01, partial η^2=0.152) and PYY (HP-TDR: -97.15 ± 17.95 pg/mL, p<0.001, partial η^2=0.417; CON: -60.75 ± 15.04 pg/mL, p<0.001, partial η^2=0.289) were lower in females compared to males in both diets. In males, postprandial levels of GLP-1 and PYY were higher in the HP-TDR compared to the CON diet (GLP-1: 2.38 ± 0.49 pM, p<0.001, partial η^2=0.505; PYY: 46.05 ± 7.40 pg/mL, p<0.001, partial η^2=0.627), while in females these markers were not different between dietary interventions (GLP-1: 0.21 ± 0.12 pM, p=0.10, partial η^2=0.201; PYY: 11.78 ± 15.04 pg/mL, p=0.44, partial η^2=0.035). Regardless of sex, postprandial levels of GLP-1 (1.30 ± 0.34 pM, p=0.001, partial η^2=0.295) and PYY (28.92 ± 7.77 pg/mL, p=0.001, partial η^2=0.257) were higher in the HP-TDR compared to the CON diet, while no significant differences were observed for ghrelin (p=0.47) and leptin (p=0.18). A main effect of sex was detected on the postprandial ghrelin concentration, GLP-1, PYY, and leptin. Compared to males, females presented higher postprandial ghrelin (162.92 ± 68.94 pg/mL, p=0.02, partial η^2=0.123) and leptin (16023.39 ± 1501.42 pg/mL, p<0.001, partial η^2=0.740), and lower postprandial GLP-1 (-4.21 ± 1.43 pM, p=0.006, partial η^2=0.197) and PYY (-77.88 ± 14.87 pg/mL, p<0.001). The order in which participants received the dietary interventions did not affect any of the appetite-related hormones analyzed (all p>0.05).

5.6 DISCUSSION

The primary findings of our study were that compared to a standard North American diet (CON), the HP-TDR increased blood levels of anorexigenic hormones and reduced the PFC in females. Moreover, correlations were observed between energy balance and satiety, as well as between energy balance and PFC. Interestingly, females and males responded differently to the dietary intervention. While females presented with a response to the HP-TDR in terms of appetite

sensations, males' response to this intervention was mostly on appetite-related hormones. These results highlight the impact a HP-TDR consumption has on appetite sensations, appetite-related hormones, and energy metabolism components of healthy, normal-weight adults and provides further insight into the impact of this strategy on the mechanisms involved in the regulation of food intake and energy homeostasis. Additionally, the different findings between males and females may potentially impact the prescription of future nutritional weight management strategies that are sex-specific.

In our study, only females experienced a decrease in appetite with the HP-TDR characterized by a reduced 24-hour AUC for PFC. These results suggest that female's appetite sensations are more sensitive and reactive to dietary manipulation than male's, which is in line with previous nutritional intervention studies (4-7). In a similar design, Westerterp-Plantenga, Lejeune (5) observed a more pronounced decrease in hunger and increase in satiety in females in response to an acute dietary intervention comprised of 30% of protein. The differences observed in appetite sensations between sexes can be partly explained by the gonadal steroid hormones (8) and neuronal responses to food intake (6). Gonadal steroid hormones are able to influence neural processing of peripheral feedback signals that control eating, such as ghrelin, cholecystokinin, glucagon, insulin, and leptin (8). More specifically estrogen seems to have an inhibitory effect on food intake, which is reflected during the follicular phase of the menstrual cycle when this hormone's level is relatively high and female's energy intake is lower than the other phases of the cycle (9, 10). In fact, in our study, females were evaluated during the follicular phase, which could have impacted their response to the dietary intervention, accentuating the decrease in appetite. In addition to the physiological sex differences in appetite sensations, gender can also play a role (7). Social and cultural aspects have been shown to influence appetite sensations differently in females and males (38, 39). Although, to our knowledge, there is no evidence of gender's effect on PFC, Zylan (39) demonstrated that factors that led to meal termination in females were related to the culinary qualities of the food eaten, whereas in males it was related to the amount of food left. For this reason, further research is needed to examine if the decrease in appetite persists during other phases of the menstrual cycle with this nutritional intervention. Additionally, studies should also explore the effects of gender on other aspects of appetite sensations.

Notwithstanding the influences of the follicular phase, a higher protein intake has been demonstrated to improve satiety, being the most satiating macronutrient, followed by

carbohydrate, and fat (40). Therefore, irrespective of the menstrual cycle, a higher protein intake is likely the biggest contributor to the decrease in appetite observed in our study. In addition to the macronutrient composition of the intervention diets, the physical state of the food has been shown to impact appetite sensations (41). In fact, Martens, Lemmens (42) showed that a high-protein solid meal evoked stronger suppression of hunger and desire to eat than a liquid meal of identical nutrient profile in healthy, normal-weight, young adults. Considering that in this study females presented with a reduced PFC with a liquid diet, it is possible that other factors such as the diet's macronutrient composition might have had a stronger effect on appetite suppression than its physical state. In addition, taste, smell, texture, appearance (43), and energy density (44) have been shown to influence appetite sensations, and this study did not assess any of these variables. Therefore, future studies should consider assessing these variables when studying the effects of the nutritional interventions evaluated herein.

Although males did not experience a direct change in appetite sensations with the dietary intervention, a partial correlation analysis controlling for sex revealed a negative correlation between energy balance and satiety, and a positive correlation between energy balance and PFC only in the HP-TDR arm. These findings are very interesting, considering that participants were in negative energy imbalance during the HP-TDR intervention. A state of negative energy imbalance has been shown to provoke a compensation in energy intake in the short term and a metabolic adaptation over the long term, contributing to the onset and progression of obesity (45). This compensation is partly driven by an increase in hunger (46). Therefore, strategies able to control one or more factors leading to an energy compensation in response to a state of negative energy imbalance are crucial in the prevention and treatment of obesity. Interestingly, even though participants were in a state of positive energy balance with the CON intervention, no significant correlations were found between energy balance and appetite sensations in this group. Hence, results from our study suggest that a HP-TDR might be an interesting strategy to counteract the increased hunger and, consequently, an energy compensation in the presence of a state of negative energy imbalance.

Regarding the change from fasting day 1 to fasting day 2 in appetite-related hormones, our study showed an interaction between diet and sex in leptin levels. In both intervention groups, the change in this biomarker was greater in females. Additionally, in both sexes, this change was higher with the HP-TDR intervention. Leptin is a hormone produced primarily by white adipose

tissue and its plasma levels are positively correlated with total body fat (2). Although in this study females presented with higher FM than males, their leptin levels were higher at baseline (i.e., fasting day 1) even after controlling for FM (results not shown, $p<0.001$). This difference at baseline partly explains the greater change observed in this biomarker in females, which is in line with previous studies (47, 48). Additionally, estrogen seems to stimulate leptin secretion in healthy females (49), further explaining the results presented herein. It has been demonstrated that leptin decreases food intake and increases EE by acting directly in the hypothalamus and controlling regions that regulate food intake and energy homeostasis (2, 3). Although promising, increased leptin levels does not lead to decreased food intake and increased EE in individuals presenting with obesity, which is explained by a state of leptin resistance (2). Therefore, increasing leptin levels via medications and/or nutritional strategies is not an effective strategy for treating obesity, but it can be a promising strategy for preventing it. In our study, the change from fasting day 1 to fasting day 2 in leptin was higher with the HP-TDR intervention. Although we are unable to quantify the variation in food intake and EE resulted from this change, this contributes to the prevention of obesity; since it is a multifactorial disease, every contribution counts (50). For instance, an intervention able to reduce energy intake by as little as 50 kcal/day could offset weight gain in 90% of the population (51). Therefore, the observed increase in fasting leptin levels in this study is promising, considering the physiological effects of this hormone on the control of food intake and energy homeostasis in individuals not diagnosed with obesity.

Regardless of sex, our study showed an increase in postprandial levels of GLP-1 and PYY with the HP-TDR. Glucagon-like peptide 1 is considered a biomarker of satiation and is produced primarily in the ileum in response to the presence of nutrients (52). Its main functions involve the stimulation of insulin release and adjustments of stomach and gut motility, which contribute to its role as an appetite-suppressing peptide (53). Peptide tyrosine-tyrosine is another gut-derived peptide that is released from the colon and has been shown to suppress food intake in individuals with normal weight and obesity (53). The production of both gut-derived peptides has been shown to increase with HP meals (26, 54, 55). Considering the macronutrient composition of the HP-TDR, it is very likely that its protein content was the main contributor to the increased levels of GLP-1 and PYY observed in our study. Interestingly, only males experienced an increase in postprandial levels of GLP-1 and PYY, and a decrease in postprandial ghrelin with the HP-TDR intervention. Dietary protein has been shown to suppress postprandial ghrelin production (56) and,

as mentioned above, increases GLP-1 and PYY (26, 54, 55), partially explaining the result presented herein. In addition to that, males presented with higher baseline blood levels of GLP-1 and PYY (i.e., fasting day 1), also contributing to the observed results. Although females did not experience a similar response with the HP-TDR, it is possible that the phase of the menstrual cycle could have contributed to the lack of effect observed. Estrogen and progesterone have important effects on appetite-related hormones (57) and, considering females were tested during the follicular phase of the menstrual cycle when these hormones are at their lowest concentration, it is possible that the stimuli was not enough to affect these gut-derived peptides.

This study has several strengths, including the well-controlled design, the dietary intervention, and the use of state-of-the-art techniques for the assessment of energy metabolism. Some potential limitations are the acute dietary intervention, the specificity of the population group (i.e., healthy, normal-weight young adults), a selective menstrual cycle phase, a limited number of appetite sensation assessments throughout each intervention period, the lack of assessment on diet liking, the standardized meal portions, the physical state of the HP-TDR (i.e., liquid), and the single protein source of the HP-TDR (i.e., soy protein). Considering that the length of the washout period was different between females and males, it is possible that the memory effects of the interventions on participant's behavior differed by sex. Moreover, some of the analyses might be underpowered as this is a secondary analysis of a previously described clinical trial (28). These limitations restrict our ability to translate the results presented herein to longer intervention periods, other population groups, to other menstrual cycle phases, and to other types of HP-TDR. Therefore, future studies are needed to better understand the long-term effects of this type of intervention on the mechanisms involved in the regulation of food intake and energy homeostasis of healthy and diseased population groups in more than one menstrual cycle phase.

In conclusion, this study showed that compared to a standard North American diet, an isocaloric HP-TDR increased blood levels of anorexigenic hormones and reduced the PFC in females. Moreover, correlations were observed between energy balance and satiety, as well as between energy balance and PFC. Females' response to the HP-TDR was related to appetite sensations, while males' was mostly related to appetite hormones. These results provide further insight into the impact of this dietary strategy on the mechanisms involved in the regulation of food intake and energy homeostasis.

Table 5.1. Nutrient content of the intervention diets.

	HP-TDR					CON				
	Females		Males		Sex Difference[a]	Females		Males		Sex Difference[a]
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Energy										
kcal/day	2008	167	2225	250	0.002	2007	167	2224	250	0.002
Kcal/kg/day	33.0	2.5	33.4	3.9	0.67	32.9	2.5	33.4	3.9	0.67
Protein										
% energy	39.9	0.2	39.8	0.3	0.35	15.4	0.3	15.2	0.2	0.02
g/day	199	16	220	24	0.002	78	6	86	9	0.007
g/kg/day	3.3	0.2	3.3	0.4	0.72	1.3	0.1	1.3	0.1	0.978
Fat										
% energy	25.0	0.3	24.9	0.1	0.85	30.3	0.5	30.2	0.0	0.29
g/day	55	4	61	6	0.004	68	5	76	8	0.001
g/kg/day	0.9	0.7	0.9	0.1	0.71	1.1	0.1	1.1	0.1	0.72
Carbohydrate										
% energy	35.1	0.3	35.2	0.3	0.30	54.2	0.6	54.6	0.2	0.04
g/day	175	14	195	22	0.001	277	24	309	34	0.001
g/kg/day	2.9	0.2	2.9	0.3	0.62	4.5	0.4	4.6	0.5	0.62
Sugars (g/day)	169	13	188	22	0.002	86	9	97	12	0.002
Fiber (g/day)	4	0	4	0	0.003	27	2	31	3	<0.001
Saturated Fat (g/day)	11	1	12	1	0.001	16	2	17	2	0.23

Monounsaturated Fat (g/day)	34	2	37	3	0.003	28	3	33	3	<0.001
Polyunsaturated Fat (g/day)	5	0	5	0	0.004	16	1	17	1	0.001
Cholesterol (mg/day)	34	10	42	7	0.005	103	55	110	16	0.007

[a] P-values represent the difference between females and males and were detected with the use of an independent-samples t-test or a Mann-Whitney U test, as appropriate.
n=43 (females: n=19; males: n=24).
Abbreviations: CON: control; HP-TDR: high-protein total diet replacement; SD: standard deviation.
A version of this table has been published elsewhere (30).

Table 5.2. Baseline characteristics of the study participants.

Characteristics	Females			Males		
	Mean	**SD**	**n (%)**	**Mean**	**SD**	**n (%)**
Age (years)	25	3		23	4	
Height (cm)	166.3	5.7		174.9	6.1	
Weight (kg)	61.1	4.8		67.0	7.3	
Waist Circumference (cm)	71.4	2.8		76.9	6.1	
BMI (kg/m^2)	22.2	1.2		21.9	1.6	
FM (kg)	18.6	3.3		12.7	4.9	
LST (kg)	40.1	4.4		51.4	5.6	
BMC (kg)	2.4	0.2		2.9	0.3	
Race						
White	7		37	12		50
Asian	5		26	9		37
Hispanic	3		16	0		0
Black	1		5	0		0
Other	3		16	3		13
Physical Activity Level[a]						
Insufficiently Active	1		6	1		4
Moderately Active	5		26	2		8
Active	13		68	21		88

[a] Physical activity levels were classified according to the Godin-Shephard Leisure-Time Physical Activity Questionnaire.
n=43 (females: n=19; males: n=24).
Abbreviations: BMC: bone mineral content; BMI: body mass index; FM: fat mass; LST: lean soft tissue; SD: standard deviation.
A version of this table has been published elsewhere (30).

Table 5.3. Partial correlation analyses (controlling for sex) between energy metabolism components and 24-hour area under the curve for each appetite sensation.

		Hunger	Satiety	Fullness	PFC
Energy Expenditure (kcal/day)	HP-TDR	-0.07	0.12	0.04	0.01
	CON	0.06	-0.11	-0.06	0.07
Fat Oxidation (g/day)	HP-TDR	-0.21	0.23	0.1	-0.16
	CON	0.04	0.10	0.09	-0.02
Protein Oxidation (g/day)	HP-TDR	0.10	-0.15	-0.16	0.05
	CON	-0.12	0.08	0.08	-0.30
Carbohydrate Oxidation (g/day)	HP-TDR	0.03	0.06	0.09	0.12
	CON	0.09	-0.25	-0.19	0.22
Energy Balance (kcal/day)	HP-TDR	0.28	-0.41[a]	-0.25	0.33[b]
	CON	-0.03	0.16	0.15	-0.10
Fat Balance (g/day)	HP-TDR	0.24	-0.26	-0.13	0.22
	CON	-0.02	-0.11	-0.09	0.03
Protein Balance (g/day)	HP-TDR	-0.06	0.11	0.11	0.06
	CON	0.10	-0.04	-0.02	0.25
Carbohydrate Balance (g/day)	HP-TDR	0.01	-0.12	-0.16	-0.05
	CON	-0.05	0.32[b]	0.27	-0.27

[a] $p<0.01$;
[b] $p<0.05$;
n=43 (females n=19; males n=24).
Abbreviations: CON: control; HP-TDR: high-protein total diet replacement; PFC: prospective food consumption.

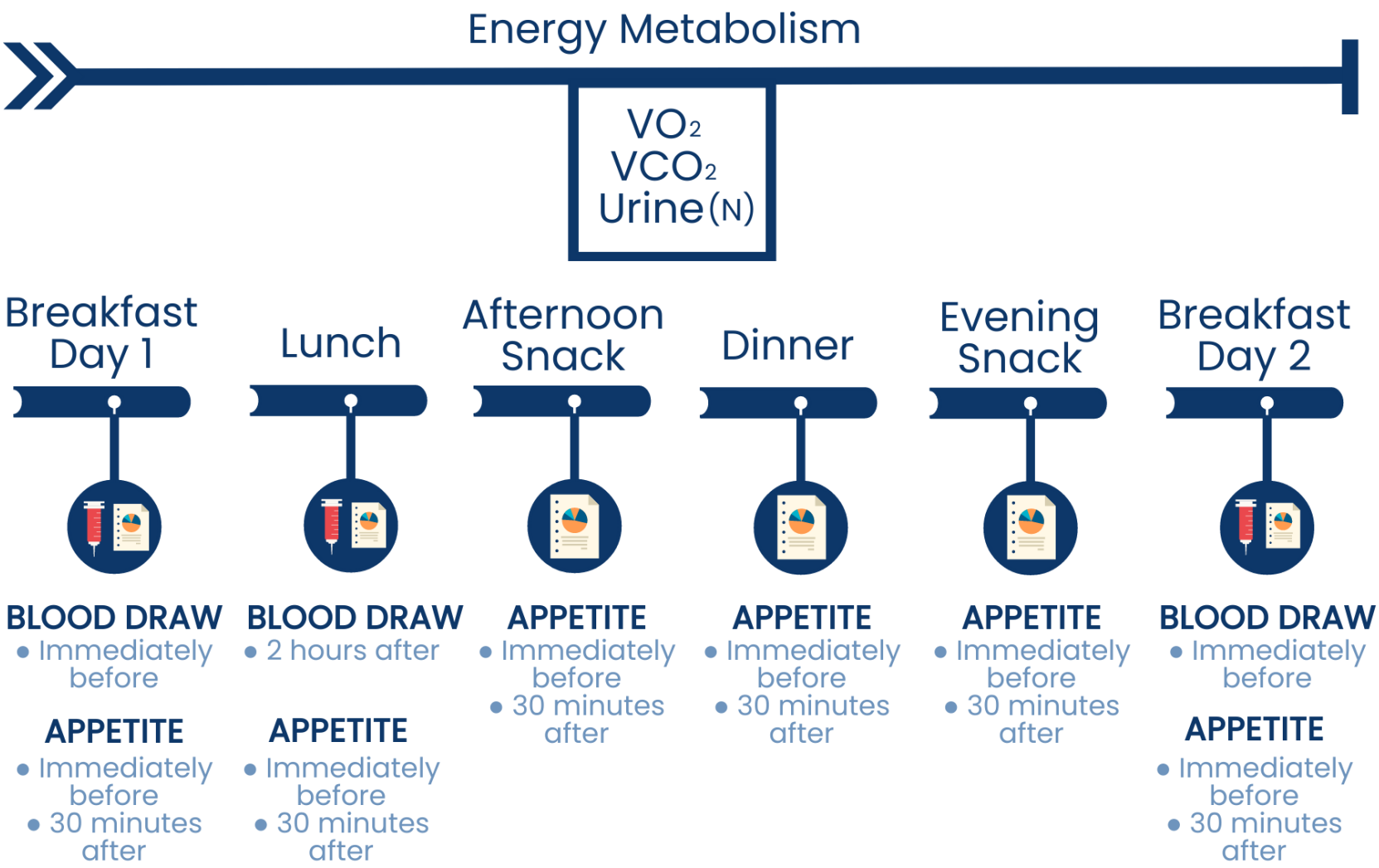


Figure 5.1. Overview of the experimental protocol.

Abbreviations: N: nitrogen; VCO_2: volume of carbon dioxide; VO_2: volume of oxygen.

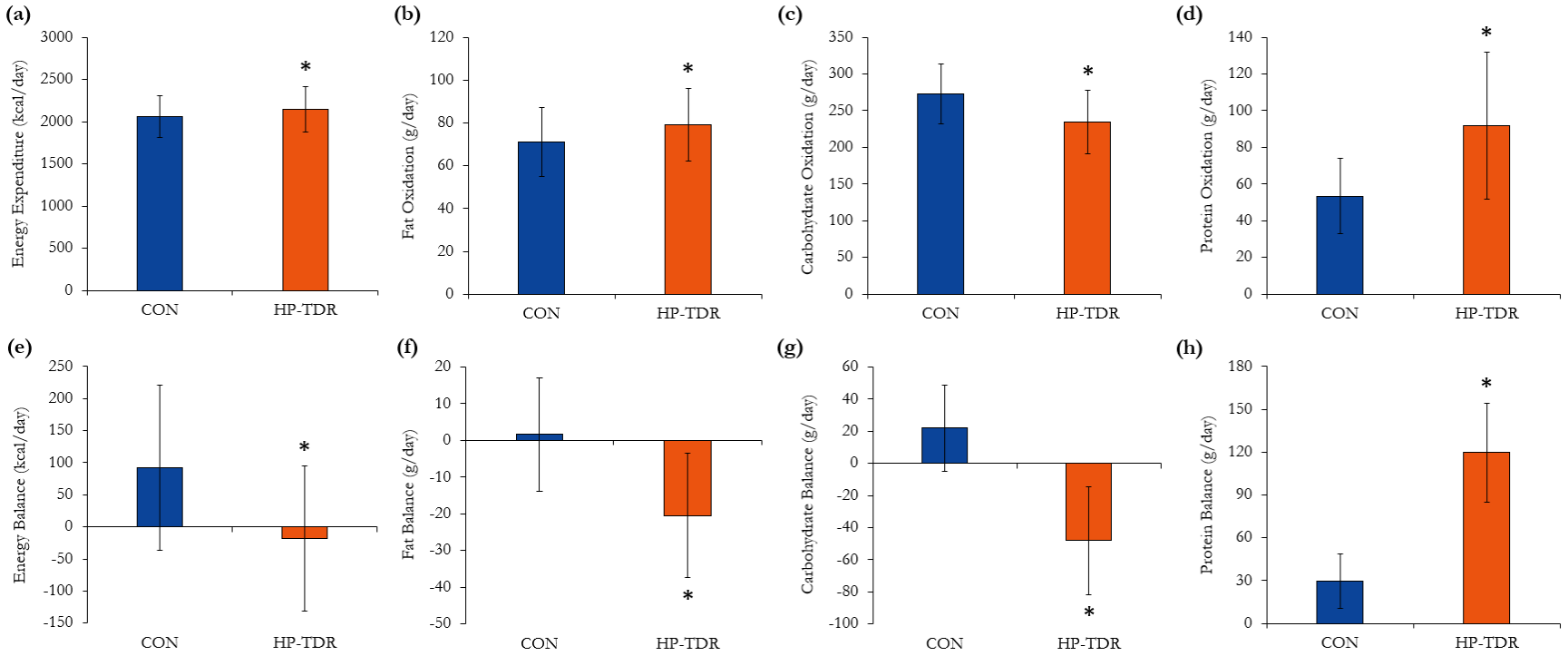


Figure 5.2. Energy expenditure (panel a), macronutrient oxidation rates (panels b, c, and d), energy balance (panel e), and macronutrient balances (panels f, g, and h) during the CON and HP-TDR interventions. Values are mean (standard deviation). N=43 (females N=19; males N=24). *Significant difference between the HP-TDR and CON diets, $p<0.01$ as assessed by a mixed analysis of variance. CON: control; HP-TDR: high-protein total diet replacement.

Figure 5.3. 24-hour AUC for hunger, satiety, fullness, and PFC in females and males during the HP-TDR and CON diets. Data are mean (standard deviation). N=43 (N=19 females; N=24 males). There was a statistically significant interaction between diet and sex on 24-hour AUC for PFC, p=0.04 as assessed by a mixed analysis of variance. In females, the 24-hour AUC for PFC was lower with the HP-TDR compared to the CON diet, p=0.04 as assessed by a post hoc test for a mixed analysis of variance. In the HP-TDR diet, the 24-hour AUC for PFC was lower in females compared to males, p=0.05 as assessed by a post hoc test for a mixed analysis of variance. AUC: area under the curve; CON: control; HP-TDR: high-protein total diet replacement; PFC: prospective food consumption.

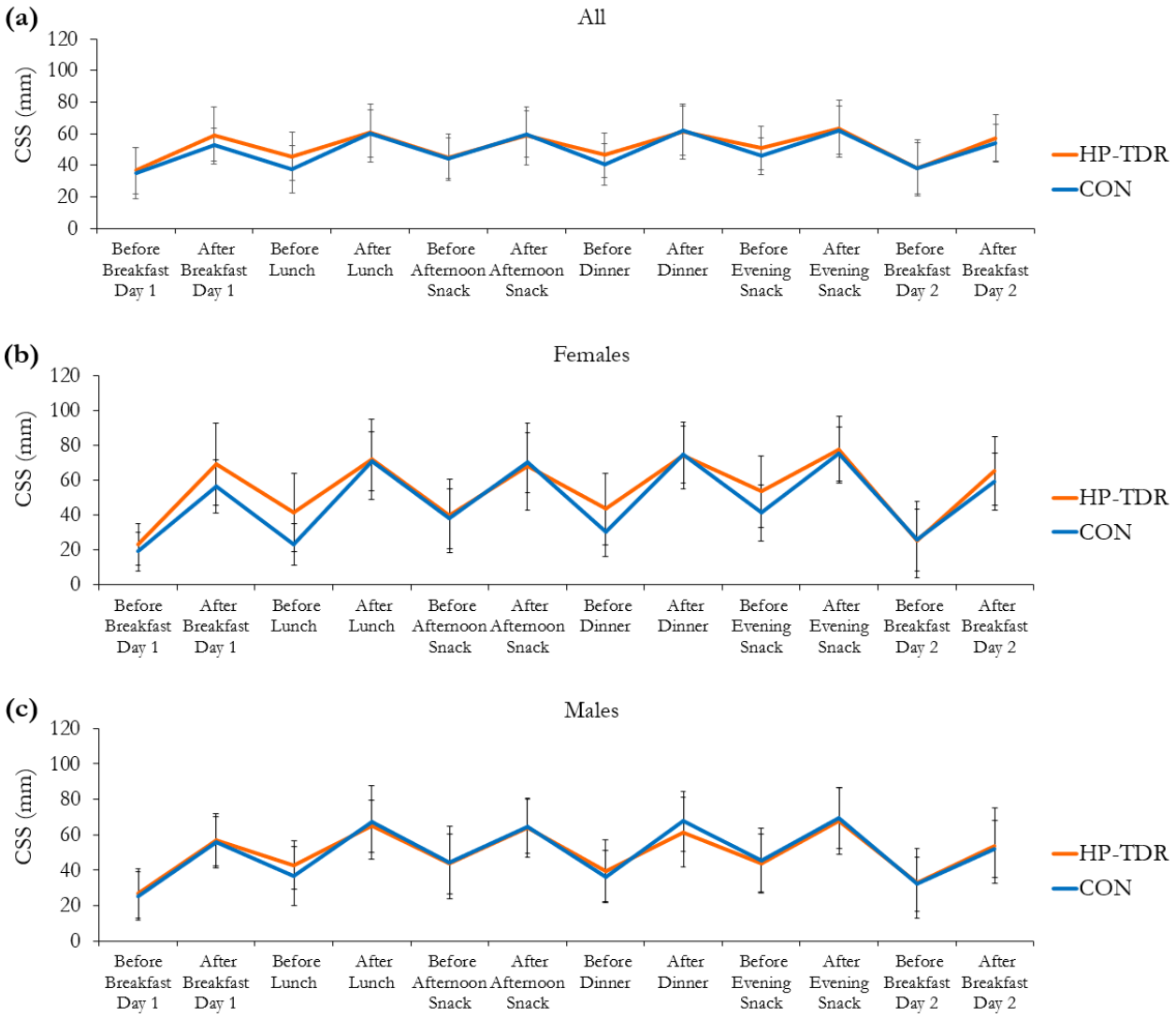


Figure 5.4. Composite satiety score during the HP-TDR and CON diets in all participants (N=43, panel a), females (N=19, panel b), and males (N=24, panel c). Data are mean (standard deviation). CON: control; CSS: composite satiety score; HP-TDR: high-protein total diet replacement.

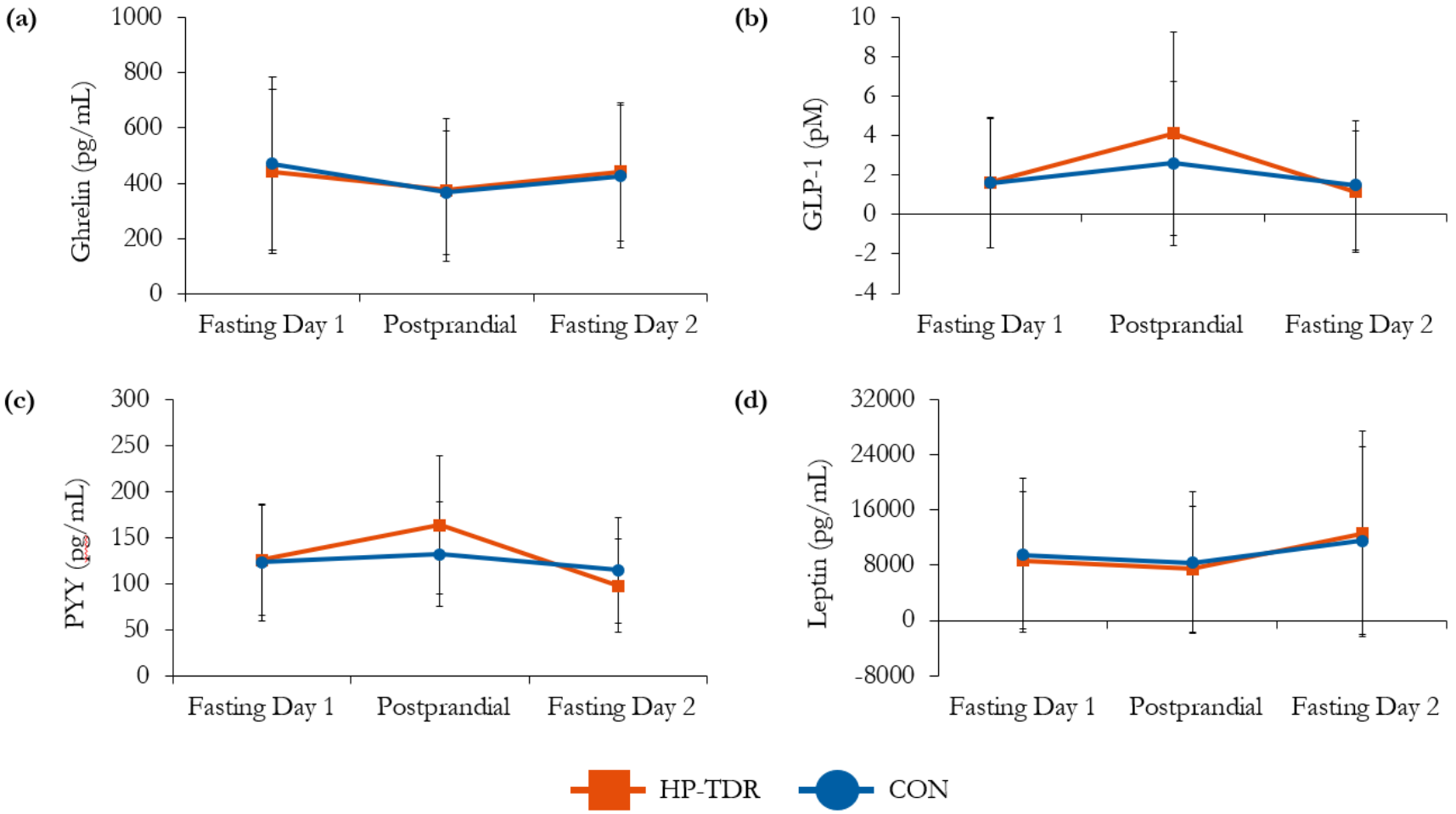


Figure 5.5. Ghrelin (panel a), glucagon-like peptide 1 (panel b), peptide tyrosine-tyrosine (panel c), and leptin (panel d) during the HP-TDR and CON diets. Data are mean (standard deviation). CON, control; GLP-1, glucagon-like peptide 1; HP-TDR, high-protein total diet replacement; PYY, peptide tyrosine-tyrosine.

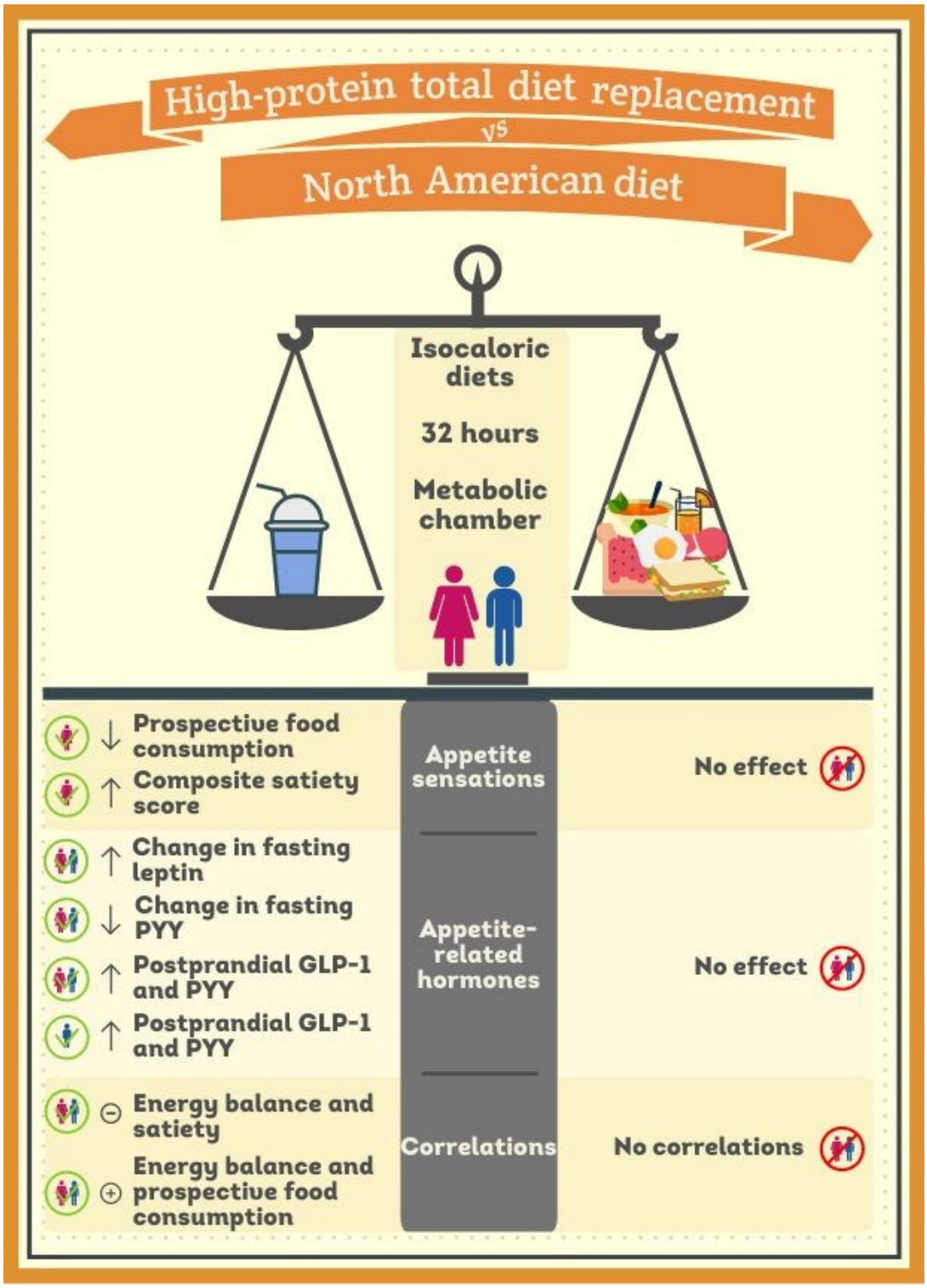
5.7 SUPPLEMENTARY MATERIAL

Supplementary Table 5.1. Appetite-related hormones during the HP-TDR and CON diets.

	HP-TDR						CON						Δ[a]			Postprandial[b]		
	Fasting Day 1		Postprandial		Fasting Day 2		Fasting Day 1		Postprandial		Fasting Day 2		Diet *x* Sex	Diet Effect	Sex Effect	Diet *x* Sex	Diet Effect	Sex Effect
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD						
Ghrelin (pg/mL)[c]	442.76	296.12	374.06	258.99	441.91	251.69	470.97	313.84	365.90	222.92	424.88	258.08	0.96	0.08	0.003	0.04	0.47	0.02
GLP-1 (pM)[d]	1.62	3.32	4.11	5.15	1.15	3.09	1.6	3.28	2.59	4.18	1.48	3.26	0.39	0.10	0.16	0.003	0.001	0.006
PYY (pg/mL)[e]	125.44	60.27	163.41	75.14	97.88	50.90	123.11	63.10	132.05	56.55	114.52	56.95	0.57	0.03	0.02	0.03	0.001	<0.001
Leptin (pg/mL)[c]	8702.85	9994.54	7422.73	9075.59	12591.94	14951.60	9522.76	11155.41	8367.14	1024 7.52	11552.64	13591.56	0.006	0.002	<0.001	0.25	0.18	<0.001

[a] P-values represent the effect of the interventions on the change from fasting day 1 to fasting day 2 and were detected with the use of a mixed analysis of variance.
[b] P-values represent the effect of the interventions on postprandial values and were detected with the use of a mixed analysis of variance.
[c] Δ: N=43 (N=19 females, N=24 males); Postprandial: N=42 (N=18 females, N=24 males).
[d] Δ: N=38 (N=14 females, N=24 males); Postprandial: N=37 (N=13 females, N=24 males).
[e] Δ: N=40 (N=16 females, N=24 males); Postprandial: N=42 (N=18 females, N=24 males).
Abbreviations: CON: control; GLP-1: glucagon-like peptide 1; HP-TDR: high-protein total diet replacement; PYY: peptide tyrosine tyrosine.

5.8 GRAPHICAL ABSTRACT



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CHAPTER 6. THE EFFECTS OF A HIGH-PROTEIN DIET REPLACEMENT ON EXERCISE METABOLISM

6.1 PREFACE

A version of this chapter has been published as**:** *Oliveira CLP, Boulé NG, Berg A, Sharma AM, Elliott SA, Siervo M, Ghosh S, Prado CM. Consumption of a High-Protein Meal Replacement Leads to Higher Fat Oxidation, Suppression of Hunger, and Improved Metabolic Profile after an Exercise Session. Nutrients. 2021; 13(1):155. https://doi.org/10.3390/nu13010155*. I was responsible for the data collection and analysis as well as the manuscript composition. All authors contributed to manuscript edits. Carla M. Prado was the supervisory author and was involved with concept formation and manuscript composition.

6.2 ABSTRACT

Objective: The aim of this study was to compare the impact of a high-protein meal replacement (HP-MR) versus a control (CON) breakfast on exercise metabolism.

Methods: In this acute, randomized controlled, cross-over study, participants were allocated into two isocaloric arms: (a) HP-MR: 30% carbohydrate, 43% protein, and 27% fat; (b) CON: 55% carbohydrate, 15% protein, and 30% fat. Following breakfast, participants performed a moderate-intensity aerobic exercise while inside a whole-body calorimetry unit. Energy expenditure, macronutrient oxidation, appetite sensations, and metabolic blood markers were assessed.

Results: Forty-three healthy, normal-weight adults (24 males) participated. Compared to the CON break-fast, the HP-MR produced higher fat oxidation (1.07 ± 0.33 g/session; p=0.003) and lower carbo-hydrate oxidation (-2.32 ± 0.98 g/session; p=0.023) and respiratory exchange ratio (-0.01 ± 0.00; p=0.003) during exercise. After exercise, increases in hunger were lower during the HP-MR condition. Changes in blood markers from the fasting state to post-exercise during the HP-MR condition were greater for insulin, low-density lipoprotein cholesterol, peptide tyrosine-tyrosine, and glucagon-like peptide 1, and lower for triglyceride and glycerol.

Conclusions: Our primary findings were that a HP-MR produced higher fat oxidation during the exercise session, suppression of hunger, and improved metabolic profile after it.

6.3 BACKGROUND

Maintenance of a healthy body weight is essential to decrease morbidity and mortality associated with excess body weight (1, 2). Considering the negative impact of excessive body fat accumulation on individual's health and on the public health system (2, 3), substantial effort has been given to develop guidelines for its prevention and treatment (4). Some of the factors influencing weight management include the food environment, physical activity, and environmental/behavioral factors (5). Lifestyle modifications that induce an energy deficit, such as diet and physical activity, are considered the cornerstone of weight management (6).

Diet and physical activity are key players in the "intake" and "expenditure" sides of the energy balance equation (7). Although the balance concept seems uncomplicated, its regulation is highly complex and influenced not only by energy intake and energy expenditure (EE), but also by physiologic and behavioral factors, such as age, hormones, and appetite sensations (8). In fact, there is a 1-2% decline in basal metabolic rate per decade of life (9). Therefore, the "eat less" and "exercise more" solution for weight management is not a simple one. Although caloric restriction and exercise can help with weight loss (10), the long-term weight maintenance can be challenging, and most individuals usually regain their body weight (11). Part of this response seems to be associated with compensatory adjustments to diet- and exercise-induced perturbations in the energy balance equation, including a decrease in EE, upregulation in appetite and orexigenic hormones (e.g., ghrelin), and a decrease in anorexigenic hormones (e.g., leptin, peptide tyrosine tyrosine [PYY], and glucagon-like peptide 1 [GLP-1]) (12, 13), which seem to be regulated differently in females and males (14). Interestingly, males appear to experience greater exercise-induced weight loss than females (15), who seem to demonstrate higher compensatory responses to exercise in order to preserve body fat stores and reproductive function (16).

Numerous dietary strategies exist and are continuously being developed in an at-tempt to induce a state of negative energy balance (17). Among those, weight loss strategies based on meal replacements (18-20) and a higher protein intake (21) have been a topic of investigation. A recent systematic review and meta-analysis showed that weight loss was greater after one year of

intervention when meal replacements were incorporated in the diets, compared to dietary advice or diet plans (19). A higher protein intake appears to increase fat oxidation, EE, and spare lean mass during weight loss (22, 23). Moreover, it exerts a stronger satiating effect and appears to decrease energy intake under ad libitum conditions (22). Although these dietary strategies are gaining popularity worldwide (17), little is known about the effects on the mechanisms involved in body weight regulation of females and males, especially when associated with exercise. It is important to study the physiological impact of these strategies in a healthy, normal-weight population without the confounding effects of obesity and other comorbidities.

We investigated the effects of the consumption of a high-protein meal replacement (HP-MR) versus a control (CON) breakfast (North American) preceding an acute bout of moderate-intensity exercise on selected components of exercise energy metabolism, appetite sensations, and metabolic blood markers in healthy, normal-weight young adults of both sexes. We hypothesized that compared to a typical North American breakfast, participants consuming an isocaloric HP-MR prior to a moderate-intensity aerobic exercise session would present with an energy metabolism profile favoring increased EE and fat oxidation during exercise. Moreover, they would present with an improved metabolic profile and decreased appetite that would reflect an in-crease in blood levels of anorexigenic hormones and decreased levels of the orexigenic hormone ghrelin following the exercise session.

6.4 METHODS

6.4.1 Study Design and Participant Details

This randomized, controlled, cross-over study was a planned secondary analysis of a 32-h intervention trial and conducted separately in females and males at the University of Alberta (Edmonton, AB, Canada), fully described elsewhere (24). The results from the primary analysis have also been published (25). Study protocols were approved by the University of Alberta Ethics Board (Pro00066006 and Pro00083005 approved on 5 October 2016 and on 9 July 2018, respectively) and registered in ClinicalTrials.gov (NCT02811276 and NCT03565510). Both protocols complied with the standards as set out in the Canadian Tri-Council Policy statement on the use of human participants in research. Before study commencement, all participants provided written informed consent.

Eligible individuals were healthy females and males between 18 and 35 years old with a body mass index (BMI) of 18.5 to 24.9 kg/m^2. Potential participants were excluded if they had any diagnosed acute and/or chronic disease, claustrophobia, dietary restrictions, recent exposure to tests involving radiation, were using medications and/or nutritional supplements that could affect energy metabolism or body composition, and were performing >1 h/day or >7 h/week of exercise. Females with an irregular menstrual cycle, pregnant, or lactating were also excluded.

6.4.2 Experimental Protocol

The experimental protocol is illustrated in **Figure 6.1**. Potential participants attended a screening visit at the Human Nutrition Research Unit (HNRU) that included the assessment of their height, weight, waist circumference, blood tests (albumin, creatinine, aspartate transaminase, alanine transaminase, sodium, potassium, chloride, and thyroid-stimulating hormone), and the completion of questionnaires eliciting information about health, use of medications, caffeine consumption, physical activity levels (26), and palatability of the study foods. Once deemed eligible, participants were randomly assigned to start with a HP-MR or CON breakfast.

Following a simple randomization procedure separated by sex, participants at-tended two study visits for the assessment of body composition (GE Lunar iDXA, General Electric Company, Madison, USA; enCORE software 13.60 Lunar iDXA GE Health Care®) and resting energy expenditure (REE). Participants also performed a standardized fitness test. Subsequently, participants underwent a 3-day run-in period consuming a eucaloric diet, which preceded both conditions. The day after the run-in period, they received the intervention breakfasts and performed the exercise session while inside the whole-body calorimetry unit (WBCU). Both conditions happened during the follicular phase of women's menstrual cycle. Each condition was followed by a washout period of approximately one month for females and two weeks for males.

6.4.3 Resting Energy Expenditure

At baseline, participants completed a 1-h REE indirect calorimetry test, where the volume of oxygen (VO_2) and carbon dioxide (VCO_2) were continuously measured by an open-circuit WBCU, a method fully described elsewhere (24). Results from this test were used to estimate participant's total energy expenditure (TEE) for the 3-day run-in diet and intervention breakfasts by using the following formula:

- $Estimated\ TEE\ (kcal/day)\ =\ REE\ (kcal/day)\ x\ PA\ x\ 1.075$

where REE was multiplied by a physical activity coefficient (PA), according to the Dietary Reference Intakes (27), and a coefficient of 1.075 representing the metabolizable energy content of the diet (28).

6.4.4 Standardized Fitness Test

At baseline, a fitness test was performed to personalize and standardize the intensity of the prescribed exercise session for each condition. After an 8- to 12-hour overnight fast, participants attended the HNRU and were fed a breakfast at 9:00 am with the same energy content and macronutrient composition of the CON breakfast (55% of total energy intake from carbohydrate, 15% from protein, and 30% from fat). At 10:20 am, they performed an incremental submaximal exercise test on a treadmill (Freemotion Incline Trainer, Freemotion Fitness, Logan, UT, USA). Breakfast and fitness test starting times were identical to the protocol of the WBCU conditions (9:00 am and 10:20 am, respectively). The fitness test started with a 5-min warm-up phase in which participants chose a walking speed characterized as comfortable and sustainable. After the warm-up, the incline was increased by 2% every 3 min, and a constant speed was maintained until a respiratory exchange ratio (RER) of 0.90 was achieved. During the test, participant's heart rate was continuously monitored with a Polar FT1 Heart Rate Monitor (Polar Electro Oy, Kempele, Finland), and expired gases were analyzed by a calibrated TrueMax® metabolic measurement system (Parvo Medics TrueOne® 2400 Metabolic Measurement System, Sandy, UT, USA). The workload for the WBCU exercise session was determined by plotting participant's RER against their fitness test workload. The speed and incline at which an RER of 0.85 occurred were selected as the intensity for the WBCU exercise sessions. The RER of 0.85 was chosen because it reflects fuel oxidation, which is the closest variable to the primary outcome of this study (i.e., fat balance) (24). Moreover, 0.85 is in the middle of the possible steady state range (i.e., 0.70 to 1.00), leaving participant's with enough room for variation depending on the nutritional intervention.

6.4.5 Run-in Period

Prior to the intervention visits, participants received a 3-day eucaloric diet and were instructed not to eat any other food item, not to consume any caffeinated food products, and to abstain from strenuous exercise. Participants received three meals (breakfast, lunch, and dinner) and two snacks (afternoon and evening snacks) per day that provided 55% of carbohydrate, 15% of protein, and 30% of fat, a macronutrient distribution similar to the CON condition, which resembled the North American dietary pattern (29).

6.4.6 Energy Metabolism

The morning following the 3-day run-in periods, participants returned to the HNRU after an 8- to 12-hour overnight fast, entered the WBCU at 8:00 am, received the HP-MR or CON breakfasts at 9:00 am, and performed the exercise session from 10:20 am to 11:00 am. Energy expenditure, carbohydrate, and fat oxidation rates during the exercise session were calculated from the measurements of VO_2 and VCO_2 by using the formula of Brouwer (30):

- $EE\ (kcal)\ =\ 3.866\ x\ VO2\ (L)\ +\ 1.20\ x\ VCO2\ (L)$
- $Carbohydrate\ Oxidation\ (g)\ =\ 4.170\ x\ VCO2\ (L)\ -\ 2.965\ x\ VO2\ (L)$
- $Fat\ Oxidation\ (g)\ =\ 1.718\ x\ VO2\ (L)\ -\ 1.718\ x\ VCO2\ (L)$

Respiratory exchange ratio was calculated as the average ratio of VCO_2 to VO_2 per minute during the exercise session:

- $RER\ =\ (VCO2\ (L))/(VO2\ (L))$

6.4.7 Interventions

While inside the WBCU, participants received the HP-MR or CON breakfasts at 9:00 am in a random order. The CON breakfast was comprised of whole wheat bread, peanut butter, and orange juice, mimicking the food items and macronutrient distribution of a typical North American breakfast (29). As an exception, low-fat mozzarella and boiled egg were added to the diets of n=2

participants (females) to increase the energy content of their meals, as their energy requirements were elevated compared to other participants. The HP-MR breakfast consisted of a soy-protein nutritional supplement (Almased®, Almased USA, Inc., St. Petersburg, FL, USA) mixed with olive oil and low-fat milk (1% fat), per label instructions (31). The energy content of the HP-MR and CON breakfasts were similar and represented approximately 20% of participant's estimated TEE. The nutrient content of the isocaloric breakfasts is described in **Table 6.1**. At 10:20 am, a 40-min moderate exercise session on a treadmill (BH Fitness T8 SPORT, BH Fitness, Foothill Ranch, Calif., USA) was completed, at a personalized fixed pace based on the fitness test's results.

6.4.8 Appetite Sensations

Throughout each condition, participants rated their appetite sensations (i.e., hunger, satiety, fullness, and prospective food consumption (PFC)) a total of three times using a validated anchored 100-mm visual analogue scale (VAS) (32): (1) immediately before breakfast (~9:00 am); (2) 30 min after breakfast was finished (~9:40 am); and (3) 1 h after the exercise session (~12:00 pm), **Figure 6.1b**. The composite satiety score (CSS) was calculated at each time of measurement by using the following equation: CSS (mm) = (satiety + fullness + (100 − prospective food consumption) + (100 − hunger))/4 (33). A higher CSS is associated with a higher satiety sensation and a sub-sequent lower motivation to eat.

6.4.9 Metabolic Blood Markers

Blood was sampled by venipuncture at two time points during each condition: 1) in the morning before breakfast (fasting, ~7:30 am); and 2) immediately after the exercise session (post-exercise, ~11:00 am), **Figure 6.1b**. The first blood draw was sampled from participants after a 10- to 12-hour overnight fast.

Serum samples were analyzed for glucose, insulin, and lipid panel (total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, and non-HDL cholesterol) by DynaLIFE Medical Labs (Edmonton, AB, Canada) and leptin in-house at the HNRU. Plasma samples of free fatty acids, non-esterified fatty acids (NEFA), ghrelin (active), PYY, and GLP-1 (active) were analyzed at the HNRU. Leptin and GLP-1 were measured by electrochemiluminescence using the MULTI-ARRAY® Assay System (Meso Scale Discovery®, Gaithersburg, MD, USA) and V-PLEX® (Meso Scale Discovery®, Gaithersburg,

MD, USA), respectively. Ghrelin and PYY were measured by enzyme-linked immunosorbent assay kits from EMD Millipore Co. (Billerica, MA, USA). These analyses were performed according to manufacturer's instructions, in duplicates, and were repeated when they had not fallen within the range of the standard curves. In females (HP-MR: n=4; CON: n=2), GLP-1 was not detectable in any of the time points because of a technical problem and assigned a value of 0.01 pM, defined as the lower limit of detection of the kit. The coefficients of variation (CV) from the serum samples analyzed by DynaLIFE Medical Labs were 1% for glucose, 5% for insulin, 2% for total, HDL, LDL, and non-HDL cholesterol, and 3% for triglyceride. The CVs from the samples analyzed at the HNRU in females and males were 6.26% and 9.18% for NEFA, 7.44% and 7.44% for glycerol, 3.67% and 6.99% for leptin, 6.10% and 5.82% for ghrelin, 7.48% and 10.30% for PYY, and 5.34% and 5.24% for GLP-1, respectively.

6.4.10 Statistical Analysis

Sample size calculation for the primary study has been described elsewhere (24). Data were expressed as mean ± standard deviation (SD) for continuous variables and frequency and proportions for categorical variables. Paired-samples t-tests were used to compare the mean differences of dietary intake between groups. If dietary intake was nonnormally distributed, Wilcoxon signed-rank tests were used to compare the means between groups. Possible differences between the HP-MR and CON conditions were explored using a mixed analysis of variance (ANOVA) with within-subject factors (i.e., dietary interventions and/or time) and a between-subject factor (i.e., sex). Post-hoc analyses were applied with all ANOVA tests using a Tukey test (equal variances assumed) or Games-Howell (equal variances not assumed). Mean ± standard error of the mean difference (SEM) was used to report the main effect of diet and sex. Partial correlation analyses controlling for sex were performed between continuous variables. IBM® SPSS® Statistics version 24 (International Business Machines Corporation, New York, NJ, USA) was used to perform all statistical analyses. Differences were regarded as statistically significant if $p<0.05$.

6.5 RESULTS

6.5.1 Participants

A total of 76 potential participants were initially screened. After completion of the screening visit, 57 were deemed eligible and randomly assigned to begin with the HP-MR or CON condition. Of these, 13 dropped out before the beginning of the dietary interventions due to personal reasons. Forty-four participants completed the study (n=20 females and n=24 males). One participant (n=1 female) was excluded from data analysis because she was not in the follicular phase of the menstrual cycle during one of the conditions, **Figure 6.2**. Baseline characteristics of participants are described in **Table 6.2**. Seventy-nine percent of participants were classified as active, 16% as moderately active, and 5% as insufficiently active.

6.5.2 Energy Metabolism

Differences of selected energy metabolism components between the HP-MR and CON conditions are shown in **Figure 6.3**. Compared to the CON breakfast, after receiving the HP-MR participants experienced higher fat oxidation rate during the exercise session (1.07 ± 0.33 g/session; p=0.003) and lower carbohydrate oxidation rate (-2.32 ± 0.98 g/session; p=0.023) and RER (-0.01 ± 0.00; p=0.003). Energy expenditure during the exercise did not differ between conditions (p=0.833). Although no diet *x* sex interactions were observed in any of the variables assessed (p>0.262), a borderline main effect of sex on RER was detected, in which females presented lower RER than males during the exercise session (-0.01 ± 0.01, p=0.050).

6.5.3 Appetite Sensations

Participant's appetite sensations before breakfast were not different between the HP-MR and CON conditions, except from PFC, which was lower in the HP-MR compared to the CON (-5 ± 15 mm; p=0.045). Changes in appetite sensations from after breakfast to after the exercise session are illustrated in **Figure 6.4**. Compared to the CON condition, hunger increased less in the HP-MR condition (-10 ± 4 mm; p=0.014), **Figure 6.4a**. The change in satiety (p=0.229), fullness (p=0.955), and PFC (p=0.218) was not different between conditions. No interaction between dietary interventions *x* sex was observed in any of the appetite sensations (p≥0.484).

No significant three-way interaction between diet, sex and time for CSS (p=0.322) was observed; however, there was a significant two-way interaction between diet and sex (p=0.037), and diet and time for females (p=0.036), but not for males (p=0.489), **Figure 6.5**. In females, CSS was higher after breakfast and after the exercise session in the HP-MR compared to the CON condition (13 ± 5 mm; p=0.034; 18 ± 5 mm; p=0.003, respectively), while no difference was observed in males in any of the time points (p>0.05). In the HP-MR condition, CSS after the exercise session was higher in females compared to males (12 ± 6 mm; p=0.048), while in the CON group, CSS after the exercise session was significantly lower in females compared to males (-14 ± 4 mm; p=0.005).

6.5.4 Metabolic Blood Markers

Metabolic blood markers assessed in a fasting state and after the exercise session during the HP-MR and CON conditions are shown in **Table 6.3**. Compared to the CON condition, the change in blood markers from the fasting state to post-exercise in the HP-MR was greater for insulin (19.2 ± 9.1 pmol/L; p=0.042), LDL cholesterol (0.08 ± 0.02 mmol/L; p=0.003), PYY (22.78 ± 10.19 pg/mL; p=0.031), and GLP-1 (1.45 ± 0.40 pM; p=0.001), and lesser for triglyceride (-0.14 ± 0.04 mmol/L; p=0.002) and glycerol (-6.4 ± 2.5 μM; p=0.015). On the other hand, this change was not different between the conditions for glucose, total cholesterol, HDL cholesterol, non-HDL cholesterol, NEFA, leptin, and ghrelin, p>0.05.

There was a significant interaction between diet and sex on the change from the fasting state to post-exercise in GLP-1 concentration (p=0.009). In males, the change in GLP-1 was greater in the HP-MR compared to the CON condition (2.55 ± 0.65 pM; p=0.001), while in females, this change was not different between conditions (0.35 ± 0.30 pM; p=0.252). In both conditions, the change in GLP-1 was greater in males compared to females (HP-MR: 5.04 ± 0.64 pM; p<0.001; CON: 2.80 ± 0.74 pM; p=0.001).

6.6 DISCUSSION

The primary findings of our study were that, compared to a standard North American meal (CON), the HP-MR led to higher fat oxidation during the exercise session, and a suppression of hunger and improved metabolic profile after exercise. Females and males responded differently to

the conditions. Females presented a stronger response in appetite sensations, while in males, this response was related to the appetite-related hormone GLP-1. Interestingly, all these effects were produced with an acute nutritional intervention and in the absence of a difference in exercise EE between groups. These results highlight the impact a HP-MR has during and after an exercise session on energy metabolism, appetite sensations, and metabolic blood markers of healthy adults, and provides further insight into the potential role of these combined strategies for weight management.

This study showed that consumption of the HP-MR led to higher fat oxidation and lower carbohydrate oxidation during the exercise session, which is reflected by lower RER levels observed following the consumption of this dietary intervention. It is well known that substrate oxidation during exercise is highly influenced by substrate availability (34), meaning that dietary intake is an important determinant of nutrient partitioning during exercise. The HP-MR breakfast had carbohydrate levels below the Acceptable Macronutrient Distribution Range (i.e., ~30% of total energy intake), which characterizes this dietary intervention as low-carbohydrate (27). It has been demonstrated that carbohydrate consumption directly regulates fat oxidation at rest (35) and during exercise (34), although the exact mechanisms behind this regulation remain to be fully understood (36). These observations are in agreement with the results presented herein and are further supported by another study that observed increased fat oxidation during and after a moderate-intensity aerobic exercise following the consumption of an acute low-carbohydrate diet in healthy, normal-weight women (37). Although the difference in substrate oxidation rates and RER values between conditions was statistically significant, the clinical meaningfulness of the relatively small numbers is unknown. However, even small changes in nutrient partitioning towards increased fat oxidation from dietary manipulation and physical activity result in significant changes in body weight and composition over the long term (8, 38, 39). In fact, low RER values, and hence low rates of fat oxidation, have been shown to predict long-term weight gain (40, 41).

The potential compensatory increases in energy intake in response to an exercise-induced energy deficit have been discussed since the 1950s (42, 43). Although the exact mechanisms underlying the causes of this compensation are still poorly understood, more recent evidence supports the hypothesis that an increase in hunger and energy intake is due to an increase in EE resultant from exercise practice (44, 45). This compensation can undermine the exercise-induced

weight-loss, which partially explains why some individuals do not lose or even gain body weight after starting an exercise training program (44). Therefore, dietary interventions able to minimize this compensation have the potential to improve an individual's response to exercise-induced energy deficit and, consequently, weight loss. In this study, the increase in hunger was lower after the exercise session when individuals consumed the HP-MR compared to the CON breakfast. This effect might be related to the higher protein content of the HP-MR. Protein is the most satiating macronutrient, followed by carbohydrate and fat (22). Different mechanisms and pathways seem to be involved in the appetite responses to dietary protein, such as secretion of gut hormones, effects on digestion, blood concentrations of amino acids, and EE (46). In a randomized, crossover trial, Dougkas and Östman (47) fed young adults isovolumetric and isoenergetic liquid meals matched for energy density and sensory properties with increasing amounts of dietary protein (i.e., 9%, 24%, and 40% of total energy intake). They reported that most appetite ratings were suppressed with increasing protein content of the test meals (47), which is in line with our study's findings. Interestingly, even though the HP-MR was in liquid form and the CON breakfast was solid, the HP-MR was still able to suppress hunger to a greater extent after the exercise session. Research has shown that the physical state of the food can affect appetite sensations (48). When comparing a high-protein solid meal versus a liquid one of identical nutrient profile, researchers observed that the solid version was able to evoke stronger suppression of hunger and desire to eat than the liquid meal in healthy, normal-weight, young adults (49). Therefore, according to our study's results, it seems that the macronutrient distribution of the meal might have a stronger effect on hunger suppression than the physical state of it.

After the exercise session, the increase in GLP-1 and PYY was higher with the consumption of the HP-MR compared to the CON breakfast, confirming the findings discussed above on hunger suppression. These two anorexigenic hormones are synthesized and released from the L-cells of the gastrointestinal tract and have been shown to modulate functional brain activation after food intake decreasing hunger and promoting meal cessation (50, 51). Both gut-derived peptides are secreted in response to nutrient intake, particularly dietary protein (52). In a similar study design, Lejeune, Westerterp (53) fed healthy, normal-weight women an energy-balanced diet comprised of 10% or 40% of protein for 36 h while participants stayed inside a WBCU. The authors demonstrated that blood levels of GLP-1 were significantly higher after dinner when participants were fed the diet comprised of 40% protein (53). Similar to the effects on GLP-1,

research has shown that a high-protein meal increases PYY in normal-weight individuals to a greater extent than a high-carbohydrate or high-fat meal (54). Therefore, the higher protein content of the HP-MR breakfast seems to be an important contributor to the greater increase in GLP-1 and PYY observed in this study. In addition to the effects observed on the secretion of gut-derived peptides, the HP-MR breakfast also increased blood insulin levels to a greater extent after the exercise session than the CON breakfast. This effect seems to be related to the type of dietary protein found in the HP-MR (i.e., soy). It has been shown that the consumption of a high-protein meal containing soy increased insulin secretion in both primates (55) and humans (56, 57). This effect seems to be related to the protein content of the meal (57) and the isoflavone genistein contained in the soy, which seems to exert an insulinotropic effect by acting directly on pancreatic β-cells (58).

In our study, triglyceride blood concentration increased less from the fasting state to post-exercise after participants ingested the HP-MR compared to the CON breakfast. The low carbohydrate content of this dietary intervention might have been responsible for this effect (59). This was demonstrated by Wolfe and Piche (60), who observed a reduction in blood triglyceride levels of healthy individuals after replacing dietary carbohydrate with protein in a diet with fixed fat content. On the other hand, blood concentration of LDL cholesterol decreased less from the fasting state to post-exercise after participants ingested the HP-MR compared to the CON breakfast. This effect might be partially related to the lower dietary fibre of the HP-MR. Dietary fibre is known for its cholesterol-lowering effects (61), as it binds to bile acids in the intestinal lumen decreasing cholesterol reabsorption during the enterohepatic cycle (62). Additionally, blood glycerol concentration increased less from the fasting state to post-exercise after participants ingested the HP-MR compared to the CON breakfast. Circulating glycerol results mainly from hydrolysis of triglyceride stored in adipose tissue and constitutes a major substrate for glucose homeostasis (63). The increased fat oxidation observed during exercise after the consumption of the HP-MR suggests an increased hydrolysis of triglyceride in adipose tissue and subsequent use of circulating glycerol as an energy source, which might have contributed to its reduced circulating levels.

Interestingly, the improved metabolic profile associated with the ingestion of the HP-MR occurred in the context of no differences in exercise EE between groups. Manore, Larson-Meyer (8) discuss that exercise affects energy balance beyond simply expending energy. In fact, the most

recent Canadian Adult Obesity Clinical Practice Guidelines states that regular physical activity should be part of weight management interventions, as it produces several health benefits, even in the absence of weight loss (64). For instance, Barwell, Malkova (39) reported that the shift in fasting RER resulted from exercise training was independently associated with the change in fat mass in adult females. Moreover, increased fat oxidation has been shown to result in metabolic benefits beyond the regulation of body weight, such as improvement in insulin sensitivity (65, 66). In addition, the HP-MR decreased hunger and increased blood levels of the anorexigenic hormones GLP-1 and PYY after the exercise session, which can ultimately affect energy intake and hence body weight regulation. Altogether, the improved metabolic profile associated with the ingestion of the HP-MR prior to the exercise session can ultimately potentially affect the energy balance equation and produce several health-related benefits, even in the absence of an increase in exercise EE.

In this study, the HP-MR breakfast elicited different responses in females and males in respect to appetite sensations and its related hormones. In females, CSS was affected, while in males, the effect was only on GLP-1 blood levels, their appetite sensations were not impacted. These results suggest that female's appetite sensations were more sensitive and reactive to dietary manipulation than male's. Our results are in agreement with a previously conducted randomized, cross-over study testing an acute dietary intervention comprised of 10% or 30% protein in healthy adults of both sexes (67). Differences in hunger and satiety between the diets were more pronounced in females than in males, while the increase in GLP-1 was greater in males with the higher protein diet (67), which is in agreement with our study results. The authors discussed that weight-loss strategies should be different by sex and that diets able to stimulate satiety should be preferred as a weight loss strategy for females. Differences between sexes in gonadal steroid hormones (68) and neuronal responses to food intake (69) partly explain the discrepancy in appetite sensations observed herein. Estrogen has been shown to inhibit food intake, and this hormone is higher during the follicular phase of the menstrual cycle (70, 71), the cycle phase of females tested in our study. Although GLP-1 blood levels increased more in males than in females, it has been demonstrated that its release is lower during the follicular phase of the menstrual cycle compared to the luteal phase due to the lower levels of estrogen and progesterone, two hormones that exert important effects on appetite-related hormones (70, 72). Considering that females were

on the follicular phase of their menstrual cycle, the effect of these conditions in the luteal phase remains to be elucidated.

Strengths of this study include a well-controlled study design and dietary intervention. Moreover, state-of-the-art techniques for the assessment of exercise energy metabolism were used. However, some limitations must be acknowledged, such as the acute intervention, specificity of the population group (i.e., healthy, normal-weight young adults), a selective menstrual cycle phase, and the fact that the HP-MR and CON conditions were not performed without exercise. Moreover, this study had a limited number of assessments of appetite sensations and appetite-related hormones. These limitations restrict our ability to translate these results to longer intervention periods, other population groups, to other phases of the female menstrual cycle and hamper our ability to conclude the exact role of exercise on the findings. Therefore, future studies are needed to better understand the long-term effects of these conditions on the physiology of healthy and diseased population groups in more than one phase of female's menstrual cycle.

6.7 CONCLUSION

In conclusion, this study showed that, compared to a standard North American breakfast (CON), an isocaloric HP-MR led to higher fat oxidation during exercise, suppression of hunger, and improved metabolic profile after exercise. Females and males responded differently to the dietary interventions. Females presented a stronger response in appetite sensations, while in males, this response was related to the appetite-related hormone GLP-1. These results highlight the impact HP-MR consumption has during and after an exercise session on energy metabolism, appetite sensations, and metabolic blood markers of healthy, normal-weight adults of both sexes, and provides further insight into the potential role of these combined strategies for weight management.

Table 6.1. Nutrient content of the intervention breakfasts.

	HP-MR			CON			Diet Difference[a]
	All (n=43)	**Females (n=19)**	**Males (n=24)**	**All (n=43)**	**Females (n=19)**	**Males (n=24)**	
Energy							
Kcal/meal	413 ± 74	366 ± 59	450 ± 63	409 ± 72	360 ± 51	448 ± 63	<0.001
kcal/kg body weight	6 ± 1	6 ± 1	7 ± 1	6 ± 1	6 ± 1	7 ± 1	<0.001
Protein							
% energy	42.6 ± 0.8	43.0 ± 0.9	42.3 ± 0.4	14.7 ± 0.8	14.3 ± 1.1	15.1 ± 0.2	<0.001
g/meal	44 ± 7	39 ± 6	47 ± 6	16 ± 3	14 ± 3	17 ± 2	<0.001
g/kg body weight	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	<0.001
Fat							
% energy	26.6 ± 0.6	26.4 ± 0.4	26.7 ± 0.6	30.2 ± 1.8	30.5 ± 2.8	29.9 ± 0.3	<0.001
g/meal	12 ± 2	11 ± 2	13 ± 2	14 ± 3	12 ± 2	15 ± 2	<0.001
g/kg body weight	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	<0.001
Carbohydrate							
% energy	30.8 ± 0.6	30.6 ± 0.8	31.0 ± 0.4	55.0 ± 1.9	55.2 ± 2.8	54.9 ± 0.3	<0.001
g/meal	32 ± 6	28 ± 5	35 ± 5	58 ± 10	52 ± 8	63 ± 9	<0.001
g/kg body weight	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	<0.001
Sugars (g/meal)	32 ± 6	28 ± 5	35 ± 5	27 ± 4	25 ± 3	29 ± 4	<0.001
Fiber (g/meal)	1 ± 0	1 ± 0	1 ± 0	7 ± 1	6 ± 1	8 ± 1	<0.001
Saturated Fat (g/meal)	3 ± 0	2 ± 0	3 ± 0	3 ± 1	3 ± 0	3 ± 0	<0.001

Monounsaturated Fat (g/meal)	7 ± 1	6 ± 1	8 ± 1	6 ± 1	6 ± 1	7 ± 1	<0.001
Polyunsaturated Fat (g/meal)	1 ± 0	1 ± 0	1 ± 0	4 ± 1	3 ± 1	4 ± 1	<0.001
Cholesterol (mg/meal)	12 ± 3	10 ± 3	13 ± 2	4 ± 28	10 ± 42	0 ± 0	<0.001

Data are expressed as mean ± standard deviation.
[a] P-values represent the difference between groups in all participants (n=43) and were detected with the use of paired-samples t-test or Wilcoxon signed-rank test, accordingly.
Abbreviations: CON: control, standard North American diet; HP-MR: high-protein meal replacement.

Table 6.2. Baseline characteristics of participants.

Characteristics	**All (n=43)**
Age (years)	24 ± 4
Height (cm)	171.1 ± 7.3
Weight (kg)	64.4 ± 6.9
Waist Circumference (cm)	74.4 ±5.6
BMI (kg/m^2)	22.0 ± 1.4
FM (kg) (F/M)	18.6 ± 3.3 / 12.7 ± 4.9
LST (kg) (F/M)	40.1 ± 4.4 / 51.4 ± 5.6
Ethnicity	
White	19 (44)
Asian	14 (33)
Hispanic	3 (7)
Black	1 (2)
Other	6 (14)

Data are expressed as mean ± standard deviation or n (%).
Abbreviations: BMI: body mass index; F: females; FM: fat mass; LST: lean soft tissue; M: males.

Table 6.3. Metabolic blood markers before and after the exercise session.

	HP-MR					CON					Δ[a]	
	Fasting	**Post-Exercise**	**Δ[a]**	**Time Effect[b]**	**Time x Sex[b]**	**Fasting**	**Post-Exercise**	**Δ[a]**	**Time Effect[b]**	**Time x Sex[b]**	**Diet Effect[c]**	**Diet x Sex[c]**
Glucose (mmol/L)	4.8 ± 0.3	5.1 ± 0.4	0.3 ± 0.4	<0.001	0.371	4.8 ± 0.3	5.1 ± 0.6	0.2 ± 0.6	0.008	0.366	0.662	0.800
Insulin (pmol/L)[d]	43.1 ± 15.4	95.8 ± 59.7	52.9 ± 52.2	<0.001	0.123	44.3 ± 18.4	78.5 ± 40.0	34.1 ± 33.2	<0.001	0.057	0.042	0.744
Lipid Panel[d]												
Total Cholesterol (mmol/L)	4.34 ± 0.73	4.37 ± 0.73	0.02 ± 0.18	0.196	0.017	4.28 ± 0.68	4.29 ± 0.69	0.01 ± 0.17	0.643	0.294	0.528	0.266
HDL Cholesterol (mmol/L)	1.45 ± 0.43	1.46 ± 0.45	0.01 ± 0.06	0.125	0.056	1.43 ± 0.43	1.44 ± 0.43	0.01 ± 0.07	0.214	0.795	0.987	0.251
Non-HDL Cholesterol (mmol/L)	2.89 ± 0.57	2.90 ± 0.55	0.01 ± 0.13	0.287	0.020	2.85 ± 0.49	2.85 ± 0.49	-0.01 ± 0.11	0.909	0.146	0.323	0.376
LDL Cholesterol (mmol/L)	2.40 ± 0.52	2.38 ± 0.48	-0.02 ± 0.13	0.314	0.010	2.36 ± 0.48	2.26 ± 0.47	-0.11 ± 0.14	<0.001	0.014	0.003	0.978

Triglyceride (mmol/L)	1.06 ± 0.42	1.15 ± 0.50	0.08 ± 0.19	0.008	0.829	1.07 ± 0.42	1.30 ± 0.51	0.22 ± 0.27	<0.001	0.194	0.002	0.337
Glycerol (μM)[e]	27.5 ± 19.6	36.3 ± 28.8	7.8 ± 15.1	<0.001	0.001	23.1 ± 14.1	37.3 ± 22.3	14.3 ± 14.8	<0.001	0.001	0.015	0.975
NEFA (μM)[e]	201.2 ± 191.6	188.5 ± 222.9	-13.9 ± 204.4	0.774	0.296	176.5 ± 147.9	183.6 ± 173.4	7.1 ± 114.9	0.607	0.391	0.514	0.551
Leptin (pg/mL)[d]	8702.86 ± 9994.55	7418.60 ± 8780.62	-1241.71 ± 1903.88	<0.001	<0.001	9026.12 ± 10798.76	7300.37 ± 8360.41	-1725.74 ± 3147.62	<0.001	<0.001	0.154	0.153
PYY (pg/mL)[f]	123.34 ± 61.70	196.76 ± 79.42	73.90 ± 50.70	<0.001	0.265	124.89 ± 64.05	168.55 ± 79.87	47.65 ± 61.48	<0.001	0.229	0.031	0.052
GLP-1 (pM)[e]	1.44 ± 3.17	5.03 ± 5.59	3.68 ± 3.25	<0.001	<0.001	1.47 ± 3.16	3.54 ± 4.47	2.07 ± 2.74	<0.001	0.001	0.001	0.009
Ghrelin (pg/mL)[e]	442.76 ± 292.54	292.54 ± 190.23	-149.38 ± 157.07	<0.001	0.023	474.33 ± 316.86	288.58 ± 183.35	-185.75 ± 199.17	<0.001	0.009	0.128	0.335

Data are presented as mean ± standard deviation. N=43, unless otherwise stated.
[a] Δ: Post-exercise minus baseline values.
[b] P-values represent the effect of time within groups and were detected with the use of a mixed analysis of variance.
[c] P-values represent the effect of the dietary interventions on the Δ (i.e., change from baseline to post-exercise) between groups and were detected with the use of a mixed analysis of variance.
[d] HP-MR: N=42; CON: N=43.
[e] HP-MR: N=43; CON: N=42.
[f] HP-MR: N=42; CON: N=41.

Abbreviations: CON: control, standard North American diet; GLP-1: Glucagon-like peptide; HDL: high-density lipoprotein; HP-MR: high-protein meal replacement; LDL: low-density lipoprotein; NEFA: non-esterified free fatty acids; PYY: Peptide tyrosine-tyrosine.

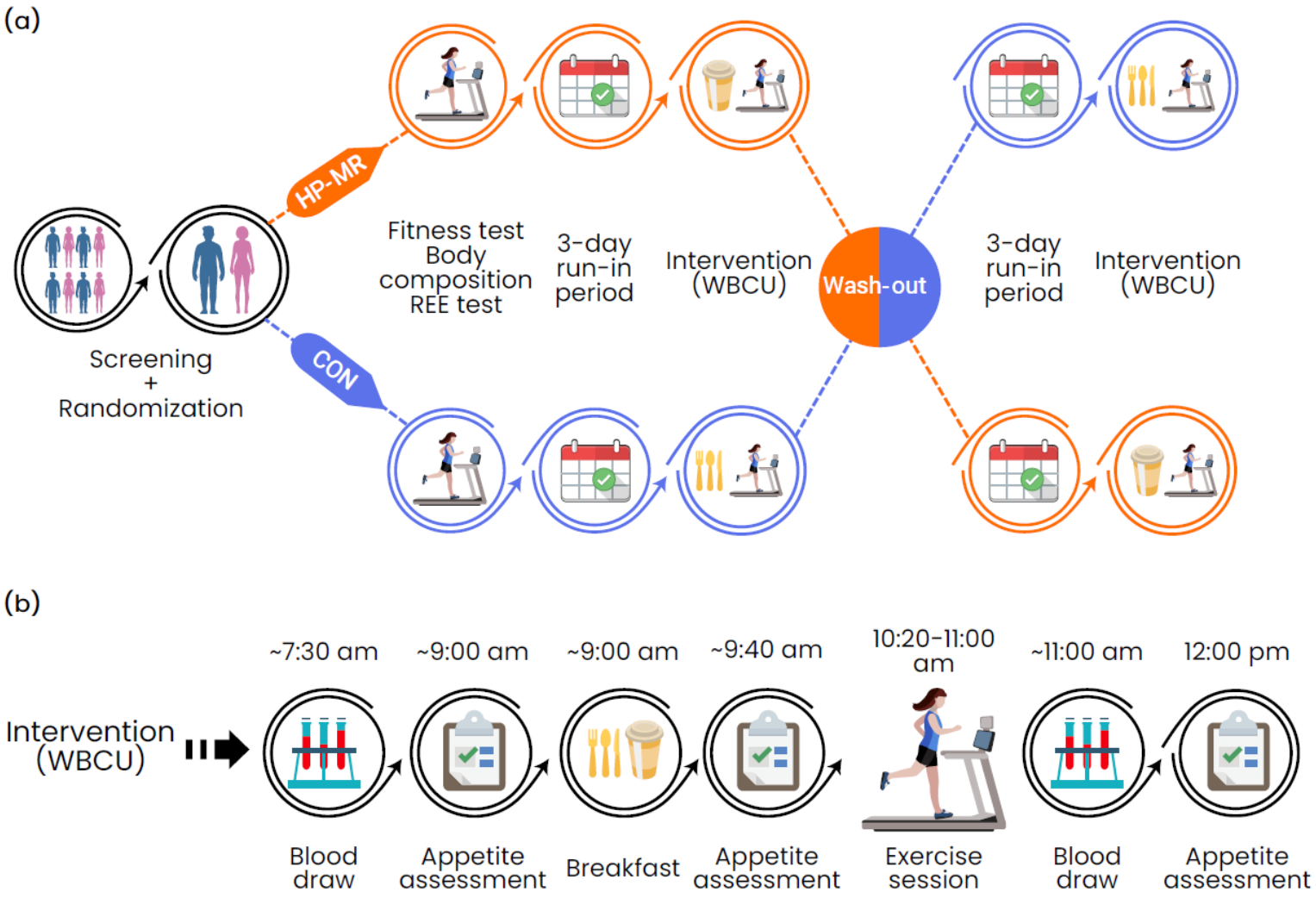


Figure 6.1. Overview of the experimental protocol.

Abbreviations: CON: control; HP-MR: high-protein meal replacement; REE: resting energy expenditure; WBCU: whole-body calorimetry unit.

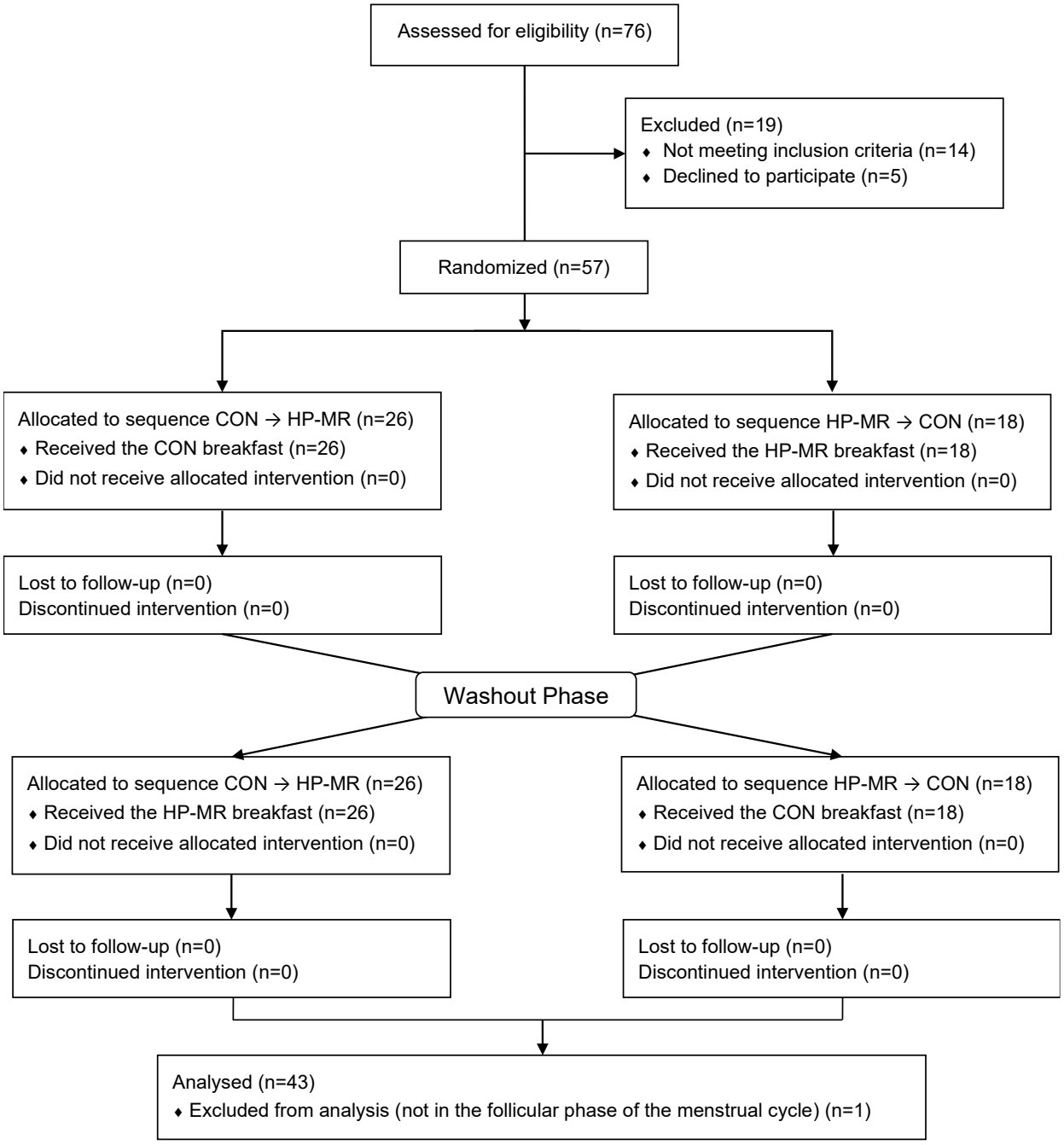


Figure 6.2. CONSORT flow diagram. CON: control diet; CONSORT: Consolidated Standards of Reporting Trials; HP-MR: high-protein meal replacement. Adapted from Oliveira, Boulé (25).

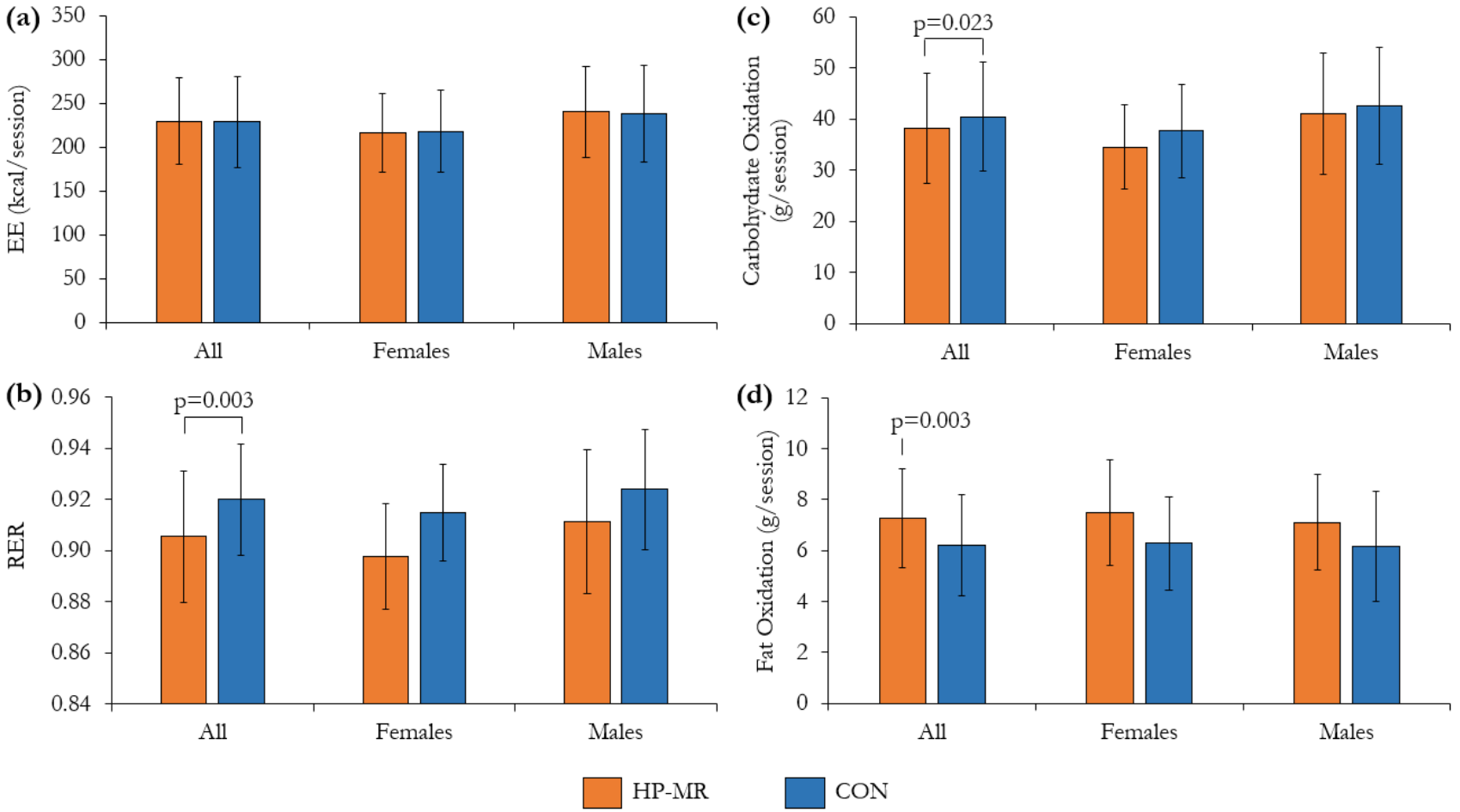


Figure 6.3. Energy expenditure (EE, panel a), respiratory exchange ratio (RER, panel b), carbohydrate oxidation (panel c), and fat oxidation (panel d) during the exercise session following the consumption of the isocaloric high-protein meal replacement (HP-MR) and control (CON) breakfasts while participants were inside the whole-body calorimetry unit. Values are mean ± standard deviation. n=43 (females n=19; males n=24). P-values represent significant difference between the HP-MR and CON groups, as assessed by mixed analysis of variance**.**

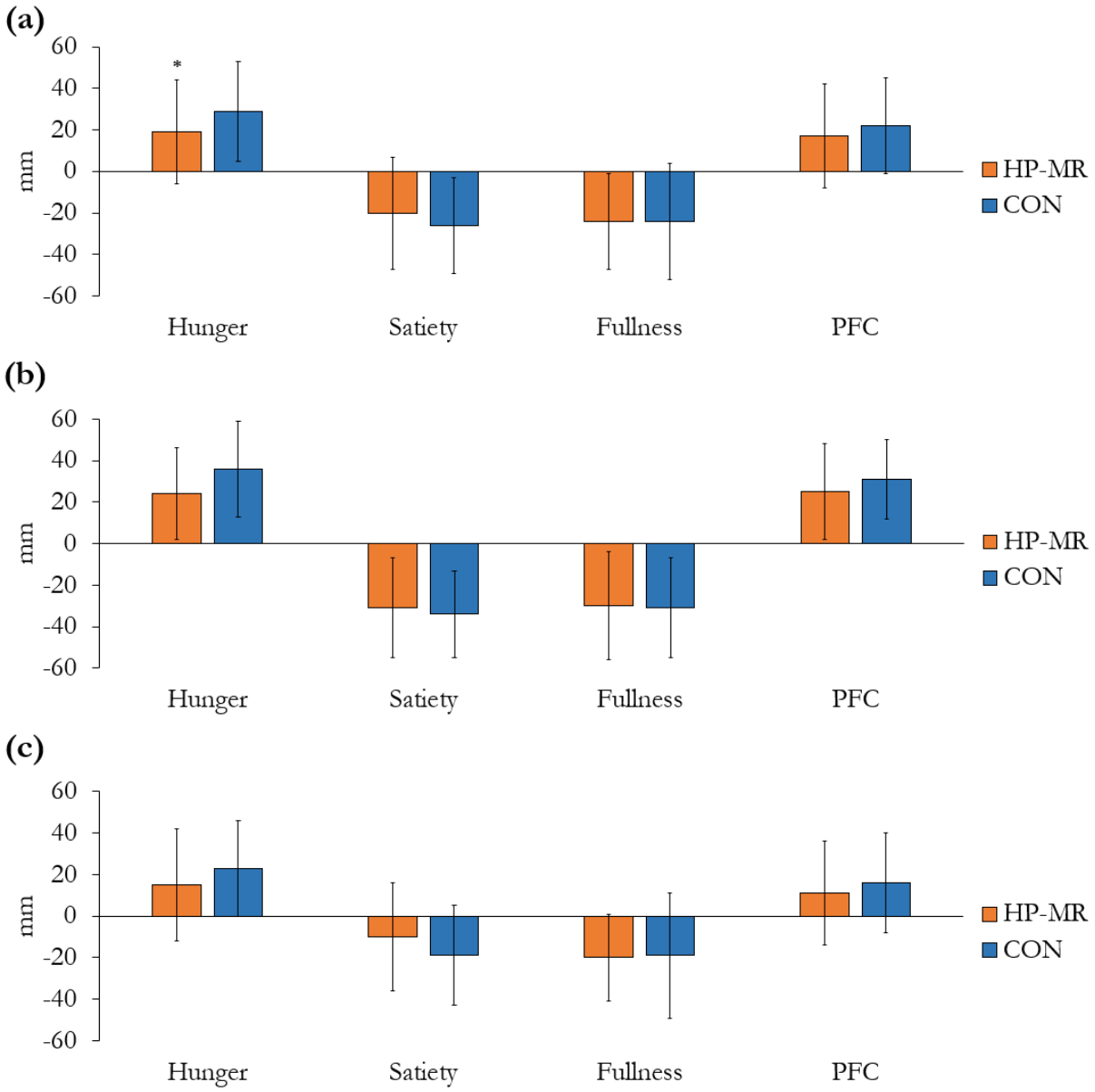


Figure 6.4. Changes in each appetite sensations from after the breakfast meal to after the exercise session during the HP-MR and CON intervention phases in all participants (n=43, panel a), females (n=19, panel b), and males (n=24, panel c). Values are mean ± standard deviation. *Significant difference (p=0.014) in all participants (n=43) between the HP-MR and CON interventions, as assessed by mixed analysis of variance. Abbreviations: CON: control, standard North American diet; HP-MR: high-protein meal replacement; PFC: prospective food consumption.

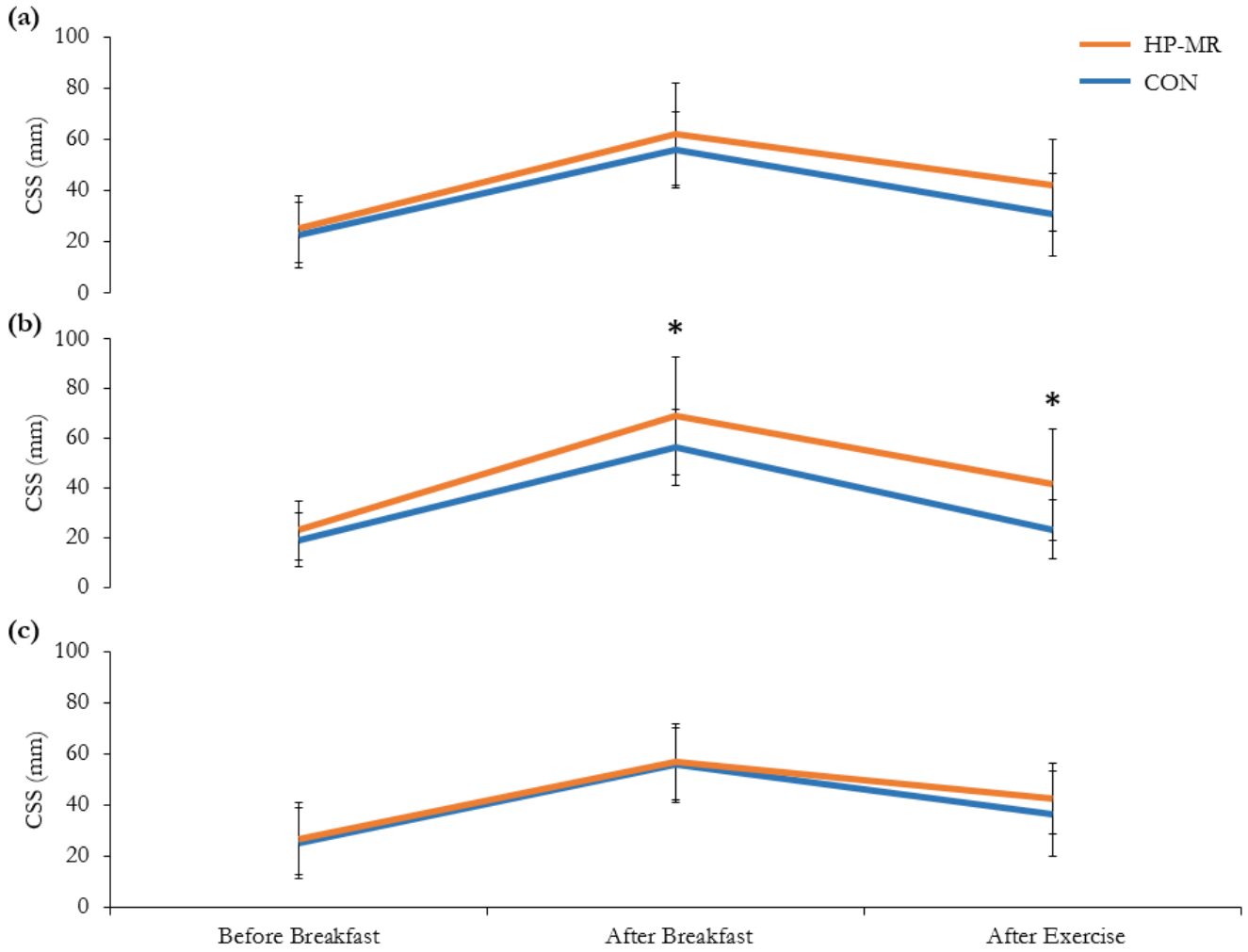
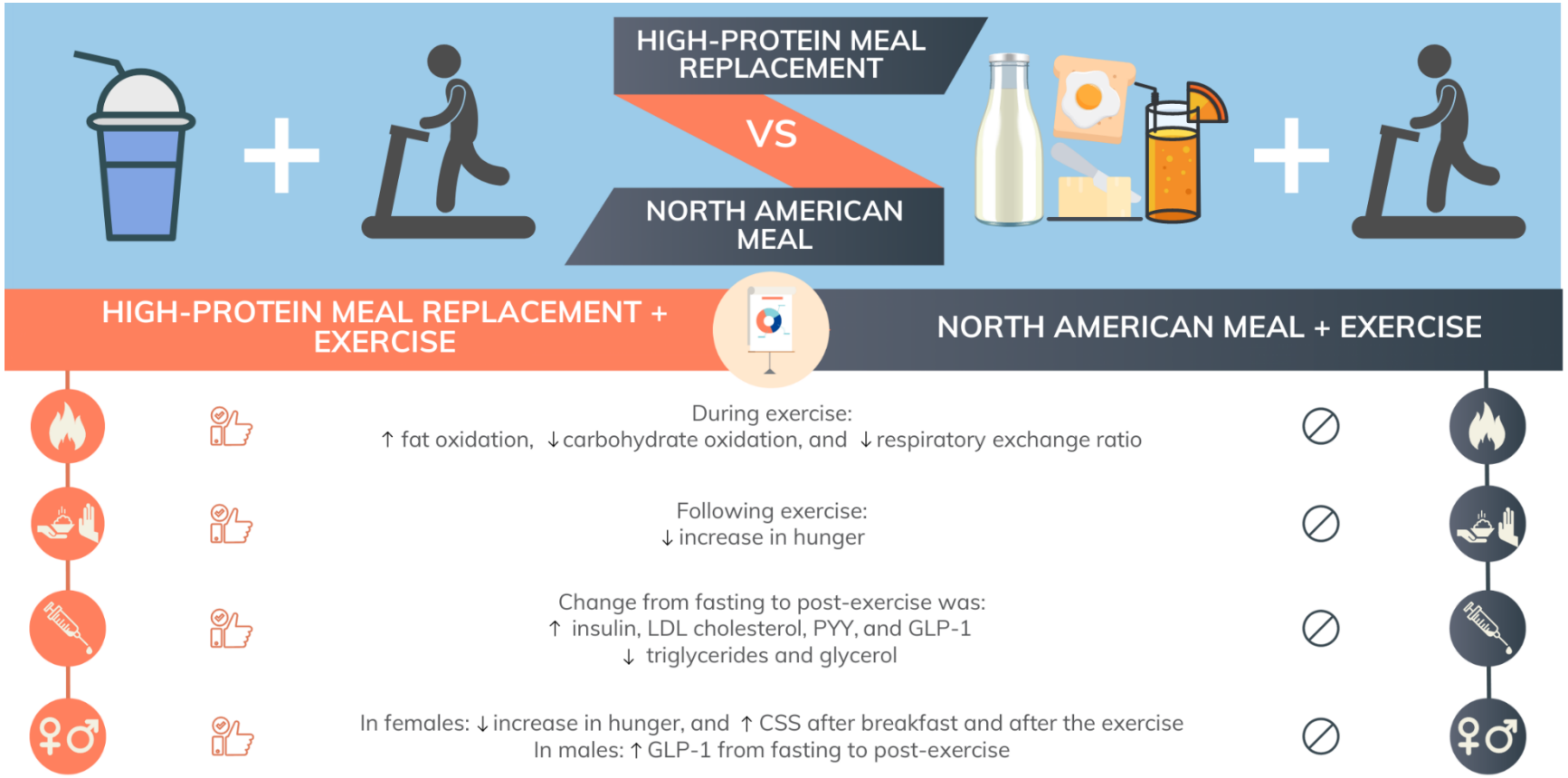


Figure 6.5. Composite satiety score during the HP-MR and CON interventions in all participants (N=43, panel a), females (N=19, panel b), and males (N=24, panel c). Data are mean ± standard deviation. *Significant difference ($p<0.03$) between the HP-MR and CON diets, as assessed by a mixed analysis of variance. Abbreviations: CON: control, standard North American diet; CSS: composite satiety score; HP-MR: high-protein meal replacement.

6.8 GRAPHICAL ABSTRACT



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CHAPTER 7. DISCUSSION AND CONCLUSION

Obesity has become an important public health problem worldwide and substantial effort has been given to develop treatment strategies for this condition (1, 2). Dietary intake and physical activity are modifiable factors that affect energy homeostasis and food intake and, hence, influence body weight control (3, 4). This thesis compared the effects of an acute nutritional intervention comprised of a high-protein (HP) diet replacement versus a standard North American dietary pattern on selected components of energy metabolism, metabolic blood markers, appetite sensations, and appetite-related hormones in healthy, normal-weight adults of both sexes (n=19 females and n=24 males). Although the studies reported in this thesis consisted of acute nutritional interventions, they were extremely complex and involved a considerable number of hours of preparation and data collection. They involved 43 participants (excluding dropouts and ineligible), who attended a total of 6 study visits, in which two of them consisted of a 32-hour assessment inside the whole-body calorimetry unit (WBCU). In total, this study involved 4,472 hours of direct assessment (i.e., 38% in study visits and 62% in the WBCU).

This research was an important step prior to investigating the effects of this dietary intervention in individuals with obesity and its related comorbidities as it eliminated the confounding effects of these diseases on the results and allowed a better understanding of the impact of a HP diet replacement in a normal physiological condition. A summary of the main thesis findings is presented in **Figure 7.1**.

In **Chapter 4**, it was hypothesized that compared to the control (CON, North American) diet, participants consuming an isocaloric high-protein total diet replacement (HP-TDR) would present with an energy metabolism profile favouring increased total energy expenditure (TEE) and negative fat balance and have an improved metabolic profile. Moreover, it was hypothesized that females and males would respond similarly to the dietary interventions. We demonstrated that compared to a standard North American dietary pattern, a HP-TDR led to higher TEE and negative fat balance, likely implying body fat loss, and a partial improvement in participant's metabolic profile. Regarding sex differences, we observed a different response in high-density lipoprotein (HDL) cholesterol with the HP-TDR intervention, with females presenting greater values compared with males.

In **Chapter 5**, it was hypothesized that compared to the North American dietary pattern, the HP-TDR would lead to a decrease in appetite that would be reflected in increased plasma concentrations of anorexigenic hormones and decreased levels of the orexigenic hormone ghrelin. It was also hypothesized that that appetite sensations and appetite-related hormones would significantly correlate with selected energy metabolism components. In addition, compared with males, females would experience increased satiety and blood levels of appetite-related hormones reflective of this sensation, regardless of dietary intervention. The results showed that, regardless of sex, appetite sensations were not different between intervention arms; however, females receiving the HP-TDR presented with a decrease in prospective food consumption (PFC). Regarding appetite-related hormones, compared to the CON diet, the HP-TDR led to a partial increase in anorexigenic hormones and no response in blood levels of the orexigenic hormone ghrelin. Significant correlations were noted between energy balance and appetite sensations reflective of satiety. Interestingly, females and males responded differently to the dietary intervention. While females presented with a response to the HP-TDR in terms of appetite sensations, males' response to this intervention was mostly on appetite-related hormones.

In **Chapter 6**, it was hypothesized that compared to a typical North American breakfast, participants consuming an isocaloric high-protein meal replacement (HP-MR) prior to a moderate-intensity aerobic exercise session would present with an energy metabolism profile favouring increased energy expenditure and fat oxidation during the exercise. Moreover, they would present with an improved metabolic profile and decreased appetite that would be reflected in increased blood levels of anorexigenic hormones and decreased levels of the orexigenic hormone ghrelin following the exercise session. It was demonstrated that compared to the CON breakfast, the HP-MR led to higher fat oxidation during the exercise session, and a suppression of hunger and improved metabolic profile after the exercise. Females and males responded differently to the dietary interventions. Females presented a stronger response in composite satiety score (CSS), while in males this response was related to the appetite-related hormone glucagon-like peptide 1 (GLP-1).

Altogether, these findings contribute to the body of literature pertaining to the effects of a HP diet replacement and physical activity in healthy, normal-weight adults, and they collectively provide further insight into the potential role of these strategies for weight management. Little is understood about the physiological effects of HP diet replacements combined or not with physical

activity in a normal physiological condition, as most studies examining the effects of these interventions have been usually restricted in calories and conducted in individuals with obesity and comorbidities. The studies presented in this thesis advanced the knowledge on the potential role of HP diet replacements combined or not with physical activity for the prevention and treatment of obesity. The following sections discuss the implications and limitations of key findings in Chapters 4, 5, and 6. Furthermore, suggestions for future research are provided.

7.1 THE EFFECTS OF A HIGH-PROTEIN DIET REPLACEMENT ON SELECTED COMPONENTS OF ENERGY METABOLISM

Chapters 4 and 6 investigated the effects of a HP diet replacement versus a North American dietary pattern on key components of energy metabolism (i.e., TEE and its main physiological compartments, energy balance, respiratory exchange ratio [RER], macronutrient oxidation rates and balances) while participants stayed inside a WBCU. As illustrated in **Figure 7.2**, compared to the North American diet, the HP diet replacement increased participant's TEE, sleep EE, and postprandial EE; however, there was no difference between the interventions in resting, basal, and exercise EE. It is interesting to note that TEE was 81 kcal/day higher with the HP diet replacement. Adding up the significant changes in sleep and postprandial EE they represent only 44 kcal/day. If the other compartments that were not statistically significant are included in the calculation, a total of 68 kcal were increased per day. However, it is important to note that we did not measure the non-exercise activity thermogenesis (NEAT), which might have contributed to the 14 kcal/day gap left between the increase in TEE and the sum of its isolated compartments. Considering that our study was not powered to detect differences in the isolated compartments of TEE, it is possible that our sample size and length of intervention were not big and long enough.

It is important to note that the postprandial EE measured in this study does not represent solely the thermic effect of food (TEF) as our participants were not completely still for 6 consecutive hours after receiving the meal (5, 6). They could get up, use the washroom, read, watch television, and work. For this reason, it is possible that some of the postprandial EE was part of other TEE compartments, such as the NEAT.

Considering the macronutrient distribution of the HP diet replacement, according to the existing literature, most of the effects of this type of dietary intervention on EE can be attributed

to its elevated protein content. Evidence suggests that the energetic costs involved in the thermic effect of protein and the possible increase in protein turnover are the main factors responsible for this increase (7, 8). Considering the standard heat of combustion of macronutrients in a bomb calorimeter as 5.65 kcal/g, 9.40 kcal/g, and 4.10 kcal/g for protein, fat, and carbohydrate, respectively, the TEF of the CON diet can be roughly estimated at 11% and the HP diet replacement 17%, **Appendix 1** (9). Comparing the postprandial EE between the diets we observed a difference of ~5%, which is very similar to the estimated difference in TEF. Although our results are close to standard values, more research is needed to clarify the contribution of the other compartments to the TEE. It might be possible that we did not detect significant differences in other compartments due to the length of our intervention and our sample size. Therefore, further research with a longer intervention period and enough power to detect differences in isolated compartments of TEE is needed.

Although an increase in TEE of 81 kcal/day with the HP diet replacement seems small, this difference can be a great ally in preventing rising rates of obesity. Applying a mathematical model to a US population database, Hall, Sacks (10) concluded that an energy imbalance gap of only 10 kcal/day sustained over several years can explain the obesity epidemic. Compared to this number, 81 kcal/day seems more than enough to prevent weight gain over time. Although very optimistic, it is important to note once again that a 32-hour intervention limits the extrapolation of the findings to longer periods of time. The human body adapts to perturbations in components of the energy balance equation to maintain EE within a physiological range over time in an attempt to resist weight loss (11). Therefore, it is possible that physiological adaptations may occur over time with the negative energy balance caused by our dietary intervention. However, Hill, Wyatt (12) highlight that preventing weight gain is a lot easier to achieve than weight loss, because only smaller changes in energy intake and expenditure are needed and they produce less compensation by the energy balance regulatory system.

Another important factor to examine when considering a HP diet replacement as a potential strategy for obesity prevention is the sustainability of a protein intake of ~3.3 g/kg/day in a liquid form. Over time, behavior modifications are very likely to occur, jeopardizing individual's adherence to the dietary intervention. In the context of weight loss, a very interesting and well conducted study showed that reduced-calorie diets emphasizing different contents of carbohydrates, fat, and protein led to similar weight loss in 811 adults with overweight over two

years (13). One explanation for this observation could be due to participants being unable to adhere to the macronutrient goals initially suggested, subsequently reverting to their usual macronutrient intakes soon after trial initiation (13). Once gain, it is important to highlight the need for more studies with a similar dietary intervention for longer periods of time.

Regarding other components of energy metabolism, the HP diet replacement produced higher fat oxidation at rest and during exercise, which led to negative fat balance (i.e., -22 g/day). As discussed in previous chapters, fat oxidation is mostly driven by the presence or absence of other macronutrients, markedly carbohydrate (14). As comprehensively discussed by Hue and Taegtmeyer (15) and illustrated by Prentice (14), the low-carbohydrate characteristic of the HP diet replacement seems to be responsible for the increased fat oxidation observed with this dietary intervention. This result is further demonstrated by the lower RER observed with this intervention at rest and during exercise. A classical experiment conducted by Abbott, Howard (16) showed that fluctuations in energy balance are mainly regulated by fat usage or storage. Considering that our participants were in negative energy and fat balances when receiving the HP diet replacement, it is very likely that this dietary intervention led to body fat loss.

Regarding sex differences, females and males responded similarly to the dietary interventions. To our knowledge, only one study assessing the effects of a 4-day higher protein intake (i.e., 10% versus 30% protein) in healthy adults of both sexes has been published to date (17). Interestingly, the authors did not run a mixed analysis of variance (ANOVA) followed by post-hoc tests to establish if a diet *x* sex interaction exists as we did, but preferred to directly compare the individual changes in energy metabolism variables between the sexes using multiple Mann-Whitney U tests (17). Each time the authors ran a Mann-Whitney U test, the chances of making a type I error (i.e., false-positive) increased above the usual 5% (e.g., two tests 10%, three tests 14.3%, and so on). On the other hand, if they had used a mixed ANOVA, the chances of getting a false-positive would have been controlled for and remained at 5%. In addition, the mixed ANOVA could have provided a two-way interaction effect, which was not reported by the authors. According to the results reported by Westerterp-Plantenga, Lejeune (17), differences in TEE and RER between the two protein arms were more substantial in males compared to females. They reported that the difference between diets (i.e., 30% protein minus 10% protein) in TEE was 95 ± 7 kcal/day and 76 ± 7 kcal/day and in RER was 0.05 ± 0.001 and 0.04 ± 0.001 in males and females, respectively, concluding that males presented with a more substantial response to the higher

protein intake than females (17). Running the same type of statistical analysis with our results, the difference between diets in TEE was 94 ± 76 kcal/day and 68 ± 89 kcal/day and in RER was 0.02 ± 0.03 and 0.03 ± 0.02 in males and females, respectively. However, although larger, the differences between sexes were not statistically significant (TEE: p=0.448; RER: p=0.400). Our results are not in agreement; however, considering the averages were close, it is possible that our higher standard deviations were one of the causes of a lack of significance.

Our findings indicated that a HP diet replacement can provide a metabolic advantage when compared to an isocaloric North American diet in the short-term in healthy adults with a normal body weight. Although we cannot extrapolate our results to longer periods of time or other population groups, this nutritional strategy appears to be a great ally in the maintenance of weight and prevention of obesity.

7.2 THE EFFECTS OF A HIGH-PROTEIN DIET REPLACEMENT ON METABOLIC BLOOD MARKERS

Participant's blood levels of glucose, insulin, lipid panel, glycerol, and non-esterified fatty acids were assessed in a fasting state on days 1 and 2, after lunch, and after an exercise session. Studies investigating the physiological effects of HP diets and diet replacements have been conducted mainly in individuals with obesity and comorbidities, such as type 2 diabetes, and weight loss was typically the main objective (18-29). Considering that individuals with obesity and comorbidities usually present with altered levels of metabolic blood markers and that weight loss is an effective strategy to improve it (30), isolating the effects of the dietary intervention from other confounding variables in these studies is challenging. Therefore, examining the effects of a HP diet replacement on metabolic blood markers in healthy adults was fundamental to understand the isolated impact of this nutritional strategy in a normal physiological condition. Consequently, the results can be better translated in individuals with obesity and its related comorbidities.

Individuals with obesity often present with a metabolic profile characterized by high blood levels of glycerol (31), an atherogenic lipid profile (i.e., increased triglyceride, total cholesterol, LDL cholesterol, and decreased HDL cholesterol), and high blood levels of insulin and glucose (32). The studies reported in Chapters 4 and 6 demonstrated that a HP diet replacement decreased

participant's blood glycerol concentration, partially improved their lipid profile and glucose control.

As discussed in previous chapters, the increased fat oxidation and negative fat balance observed in the HP diet replacement group are both indicative of increased hydrolysis of triglycerides in adipose tissue (33), which might have greatly contributed to the use of blood glycerol as an energy source, reducing its circulating levels. Participant's lipid profile partially improved, as noticed by decreased concentration of blood triglycerides and increased total and LDL cholesterol. The observed decrease in triglycerides can be mainly attributed to the low carbohydrate content of the HP diet replacement (34), as demonstrated by Wolfe and Piche (35) and further supported by several studies included in a meta-analysis comparing diets with a normal and elevated protein content (36). Regarding blood cholesterol and its fractions, although the increase was statistically significant, the absolute values remained within reference ranges for healthy adults. As previously discussed in Chapter 4, the low content of dietary cholesterol in the HP diet replacement might have upregulated participant's cholesterol biosynthesis and its low dietary fibre might have contributed to the increase in blood LDL cholesterol. Dietary fibre is known for its cholesterol-lowering effects (37), as it binds to bile acids in the intestinal lumen decreasing cholesterol reabsorption during the enterohepatic cycle (38).

Postprandially, blood levels of glucose and insulin were lower in the HP diet replacement. This observation likely resulted from the low-carbohydrate characteristic of this intervention (i.e., 35% of total energy intake), as postprandial glucose and insulin responses are determined by the amount of dietary carbohydrate (39, 40). A lower carbohydrate intake results in lower glucose blood levels, which in turn decreases endogenous insulin requirements, resulting in lower postprandial blood levels of glucose and insulin (40). However, we also observed an increase in blood insulin after the exercise, which might be related to the soy protein contained in the HP diet replacement (41, 42). It is worth mentioning that the role of HP diets on insulin sensitivity is controversial (43). Although Johnston, Tjonn (44) demonstrated a decrease in blood insulin concentration in healthy adults consuming a 30% protein diet for 6 weeks, a literature review showed that HP diets can lead to hyperinsulinemia and in the long-term cause insulin resistance (45). This effect seems to be related to the increase in plasma amino acid levels, especially branched-chain amino acids, resulting from a higher protein intake (46, 47). Although the exact mechanisms remain to be elucidated, the elevated amino acid blood levels seem to hyperactivate

the mammalian target of rapamycin complex 1, which degrades the insulin receptor substrate, resulting in reduced glucose uptake (43, 45).

Considering the effects of the HP diet replacement on metabolic blood markers of healthy, normal-weight adults, this nutritional strategy can be a promising option to improve the metabolic profile of individuals with obesity and comorbidities. However, caution is needed when studying the effects of dietary strategies with a higher protein content in the context of insulin resistance and type 2 diabetes.

7.3 THE EFFECTS OF A HIGH-PROTEIN DIET REPLACEMENT ON APPETITE SENSATIONS AND APPETITE-RELATED HORMONES

Chapters 5 and 6 investigated the effects of a HP diet replacement versus a North American dietary pattern on appetite sensations (i.e., hunger, satiety, fullness, and PFC) and appetite-related hormones (i.e., leptin, GLP-1, peptide tyrosine-tyrosine [PYY], and ghrelin). In both studies, participants experienced a decrease in appetite with the HP diet replacement which might be explained due to its higher protein content. Protein is the most satiating macronutrient, followed by carbohydrate, and fat (28). Different mechanisms and pathways seem to be involved in appetite responses to dietary protein, such as secretion of gut hormones, effects on digestion, blood concentrations of amino acids, and energy expenditure (7). In a randomized, crossover trial, Dougkas and Östman (48) fed young adults isovolumetric and isoenergetic liquid meals matched for energy density and sensory properties with increasing amounts of dietary protein (i.e., 9%, 24%, and 40% of total energy intake). They reported that most appetite ratings were suppressed with increasing protein content of the test meals (48), which is in line with our study's findings.

Results from both studies suggest that female's appetite sensations are more sensitive and reactive to dietary manipulation than male's, which is in line with previous nutritional intervention studies (17, 49-51). In a similar design, Westerterp-Plantenga, Lejeune (17) observed a more pronounced decrease in hunger and increase in satiety in females than in males in response to an acute dietary intervention comprised of 30% of protein. However, this is the same study criticized in subsection 7.1 due to authors' statistical approach and the results might have suffered from a higher type I error. Notwithstanding this study's results, the literature shows that the differences in appetite sensations between sexes can be partly explained by the gonadal steroid hormones (52)

and neuronal responses to food intake (50). Gonadal steroid hormones are able to influence neural processing of peripheral feedback signals that control eating, such as appetite-related hormones (52). More specifically estrogen seems to have an inhibitory effect on food intake, which is reflected during the follicular phase of the menstrual cycle when this hormone's level is relatively high and female's energy intake is lower than the other phases of the cycle (53, 54). In fact, females were evaluated during the follicular phase in our studies, which could have impacted their response to the dietary intervention, accentuating the decrease in appetite. In addition to the physiological differences between sexes in appetite sensations, gender can also influence these variables (51). Social and cultural aspects have been shown to influence appetite sensations differently in females and males (55, 56). For instance, Zylan (56) demonstrated that factors that led to meal termination in females were related to the culinary qualities of the food eaten, whereas in males it was related to the amount of food left.

Regardless of sex, both studies showed an increase in GLP-1 and PYY with the HP diet replacement interventions. High protein meals have been shown to produce an increase in both gut-derived peptides (48, 57, 58). In a similar study design, Lejeune, Westerterp (58) fed healthy, normal-weight women an energy balanced diet comprised of 10% or 40% of protein for 36 hours while participants stayed inside a WBCU. The authors demonstrated that blood levels of GLP-1 were significantly higher after dinner when participants were fed the diet comprised of 40% protein (58). Similar to the effects on GLP-1, research has shown that a high-protein meal increases PYY in normal-weight individuals to a greater extent than a high-carbohydrate or high-fat meal (57). Therefore, considering the macronutrient composition of the intervention diets, it is very likely that its protein content was the main contributor to the increased levels of GLP-1 and PYY observed in our studies.

Interestingly, there were some differences in appetite-related hormones between sexes. In Chapter 5, we observed that the HP-TDR led to an increase in postprandial levels of GLP-1 and PYY, and a decrease in postprandial ghrelin only in males. Similarly, in Chapter 6, only males experienced increased levels of GLP-1 with the HP-MR breakfast. Baseline blood levels of GLP-1 and PYY (i.e., fasting day 1) were higher in males compared to females, partially explaining our results. Moreover, although females did not experience a similar response with the HP diet replacement, it is possible that the phase of the menstrual cycle could have contributed to the observed lack of effect. Estrogen and progesterone have important effects on appetite-related

hormones (59) and, considering females were tested during the follicular phase of the menstrual cycle when these hormones are at their lowest concentration, it is possible that the stimuli was not enough to affect these gut-derived peptides. Moreover, an interaction between diet and sex in leptin levels was observed in Chapter 5. Change in this biomarker was greater in females in both intervention groups. Additionally, this change was higher with the HP-TDR intervention in both sexes. Leptin is a hormone produced primarily by white adipose tissue and its plasma levels are positively correlated with total body fat (60). Although females presented with higher FM than males in this study, their leptin levels were higher at baseline (i.e., fasting day 1) even after controlling for FM (results not shown, $p<0.001$). This difference at baseline partly explains the greater change observed in females, which is in line with previous studies (61, 62). Additionally, estrogen seems to stimulate leptin secretion in healthy females (63), further explaining our results.

The differences in appetite sensations and appetite-related hormones observed between females and males with the HP diet replacement suggest that dietary interventions for weight management should consider sex differences. Such dietary interventions could involve the manipulation of the macronutrient distribution of the diet, its energy content, food form/texture, comparison of different types of diets, and eating habits, among others. As more research comparing the responses of females and males to weight-loss strategies are made available, the interest in tailoring weight management strategies by sex has increased exponentially over the years (64, 65). Although our studies were very short in duration, we detected significant differences in these variables. It was interesting to note that, although males experienced a higher increase in GLP-1 and PYY with the HP diet replacement compared to females, these changes were not translated into changes in appetite sensations. On the other hand, although females presented with decreased PFC, this sensation was not caused by a greater change in appetite-related hormones when compared to males. It is possible that the effects of the HP diet replacement on the regulation of and integration between appetite sensations and appetite-related hormones might differ from the ones explored in this thesis. Moreover, longer interventions are needed to elucidate whether these effects will last or disappear over time, especially in individuals with excessive adiposity.

7.4 STRENGTHS AND LIMITATIONS

The findings of this thesis should be assessed considering its strengths and limitations. The research reported in Chapters 4, 5, and 6 have several strengths, including its crossover and rigorously controlled feeding design, including the run-in diet, which allowed the detection of small diet effects on energy metabolism variables, metabolic blood markers, appetite sensations, and appetite-related hormones. Moreover, the use of state-of-the-art technology, such as the WBCU, provide high accurate (2-3%) and precise (1-2%) results (66), reflecting the real effects of the dietary interventions. In addition to the design and technology used, the study of both females and males allowed us to explore how different sexes respond to the dietary interventions.

Although the research reported in this thesis has several strengths, some limitations must be considered. First, participants received isocaloric diets that were designed to mimic the North American dietary pattern and a nutritional product commercially available in many countries. For this reason, one or more macronutrients could not be kept constant in the diets while others were manipulated. When comparing the macronutrient distribution of the HP diet replacement with the Acceptable Macronutrient Distribution Range (67), this dietary strategy can be characterized as a HP and low carbohydrate. Therefore, it is not possible to attribute any of the results observed in the studies to a single macronutrient. Moreover, we compared solid food (i.e., CON) versus food in a liquid form (i.e., HP diet replacement) and we did not match the energy density of the diets. Even when controlling for the same food item and macronutrient composition, Martens, Lemmens (68) found differences in appetite sensations of healthy participants. Therefore, some of the differences in appetite sensations between diets might have been influenced by the form of food offered to participants and the energy density of the diets.

Although the use of the WBCU has several advantages, participants were confined to a chamber of limited size for 32 hours, which might have underestimated the non-resting compartments of their TEE compared to free-living conditions (66). Additionally, the specificity of the cohort studied (i.e., healthy, young adults with a normal body weight) can also be considered a limitation, as it hinders our ability to translate our findings to other population groups, such as individuals with excess adiposity combined or not with comorbidities, older adults, and athletes. Notably, our cohort are potential consumers of HP diet replacements.

Another limitation is the length of the intervention. The research reported in Chapters 4 and 5 tested the HP-TDR for 32 hours and the research in Chapter 6 tested only one meal. As a result of modifications in energy intake and expenditure, several of the variables assessed in our studies can change over time. Human's physiology is in constant adaptation to resist weight loss (11). Therefore, considering the dynamic physiological adaptation to perturbations in energy balance, our findings cannot be extrapolated to longer intervention in a linear way, because adaptations to these dietary interventions are very likely to occur over time.

As discussed above, females were tested during the follicular phase of the menstrual cycle. Variations in sex hormones result in several changes in female's metabolism throughout the menstrual cycle (69). Estrogen and progesterone have been shown to exert a powerful influence on energy metabolism (70, 71), appetite sensations (53, 54), and appetite-related hormones (59). For instance, during the luteal phase, when these hormones are higher, TEE and energy intake have been reported to be significantly higher than the other phases of the menstrual cycle (53, 54, 70, 71). Lastly, an important limitation involving the research reported in Chapter 6 is that the HP-MR and CON conditions were not performed without exercise. For this reason, it is not possible to isolate the effects observed in energy metabolism, metabolic blood markers, appetite sensations, and appetite-related hormones to the dietary intervention or exercise alone, but a combination of both.

7.5 DIRECTIONS FOR FUTURE RESEARCH

The limitations discussed above hinders our ability to translate our results to other population groups, longer intervention periods, and other phases of female's menstrual cycle. Therefore, future studies are needed to fill these gaps. In fact, we have already started a follow-up study, the Premium Study (https://premium.ualberta.ca/) (72). This is a randomized, controlled clinical trial investigating the impact of a 12-week HP-MR on inflammation, gut microbiota diversity and composition, metabolic blood markers, energy metabolism, body composition, and appetite sensations in individuals with excessive adiposity (HP-MR n=39; CON n=39). This study will allow us to better understand the longer-term effects of this dietary intervention in a larger population group that is more likely to benefit from this type of nutritional product. Individuals eligible to this study cannot have any type of acute or chronic diseases and must maintain a stable

body weight over the study period. Therefore, the level of control in this study is still high but will help us answer two important questions emerging from the studies reported in this thesis: the effects of a HP diet replacement 1) on another population group and 2) over longer periods of time. Unfortunately, the Premium study will not be able to address the effects of a HP diet replacement in more than one phase of female's menstrual cycle. Ideally, additional acute studies (similar to those reported in Chapters 4 to 6) are needed to directly compare the phases of the menstrual cycle (i.e., follicular versus luteal).

7.6 CONCLUSION

The major findings of this thesis were that, compared to a North American dietary pattern, a HP diet replacement improved selected components of energy metabolism favoring body weight and fat losses at rest and during exercise, partly improved individual's metabolic profile, and elicited changes in appetite sensations and appetite-related hormones that reflected a decrease hunger and increase in satiety. Additionally, females and males responded differently to the dietary interventions with respect to appetite sensations and appetite-related hormones. Overall, females' response to the HP diet replacement was more pronounced in terms of appetite sensations, while in males this response was mostly related to appetite-related hormones. Findings from this research contribute to the body of literature pertaining to the effects of a HP diet replacement and physical activity. Collectively, they provide further insight into the potential role of these strategies for weight maintenance and prevention of obesity, with the ultimate goal of developing future strategies aimed at optimizing diet quality and weight management based on sex.

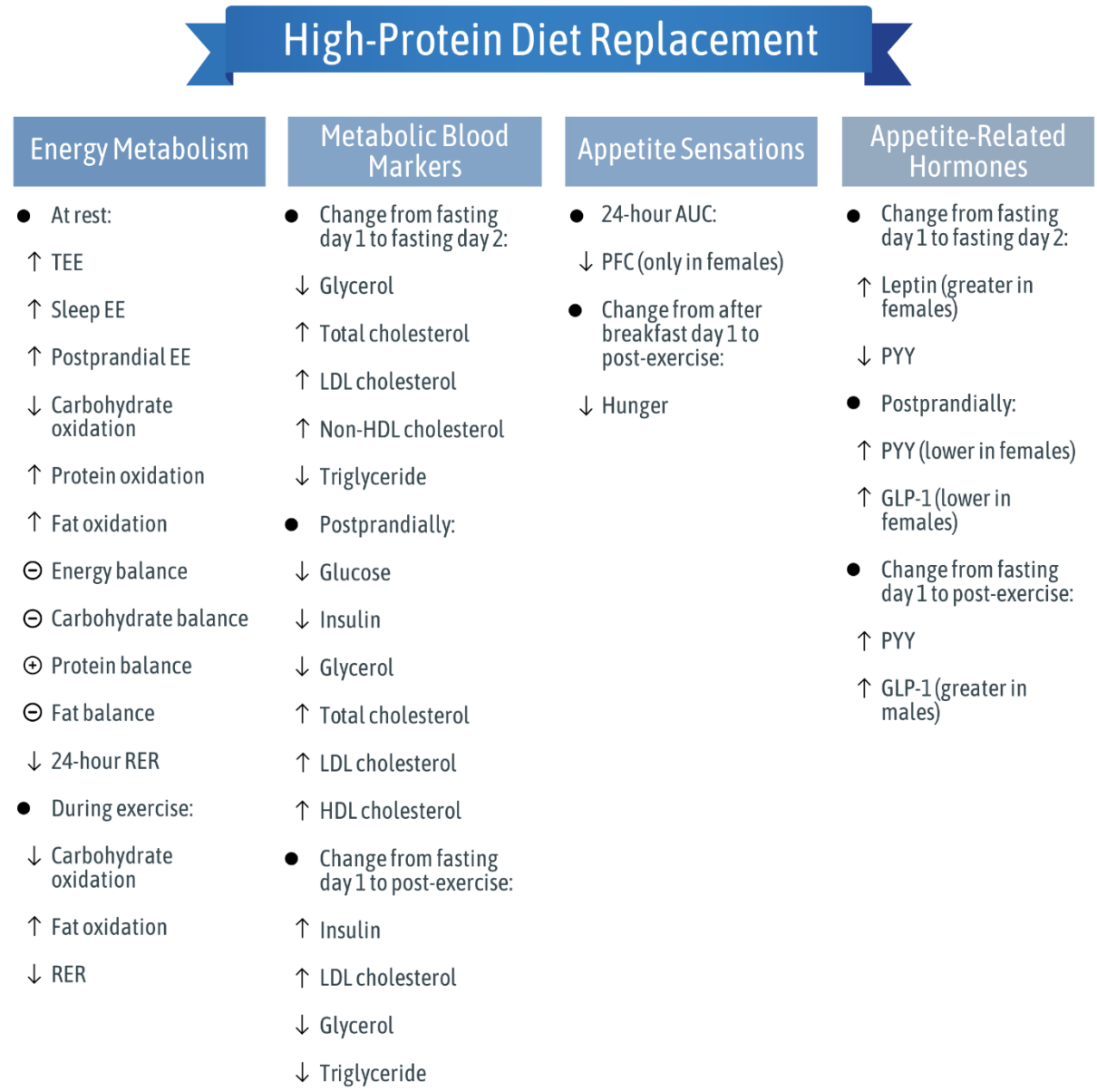

Figure 7.1. Summary of the statistically significant effects of a high-protein diet replacement versus a North American dietary pattern on selected components of energy metabolism, metabolic blood markers, appetite sensations, and appetite-related hormones in healthy, normal-weight adults.

Abbreviations: AUC: area under the curve; CSS: composite satiety score; EE: energy expenditure; GLP-1: glucagon-like peptide 1; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PFC: prospective food consumption; PYY: peptide tyrosine-tyrosine; RER: respiratory exchange ratio; TEE: total energy expenditure.

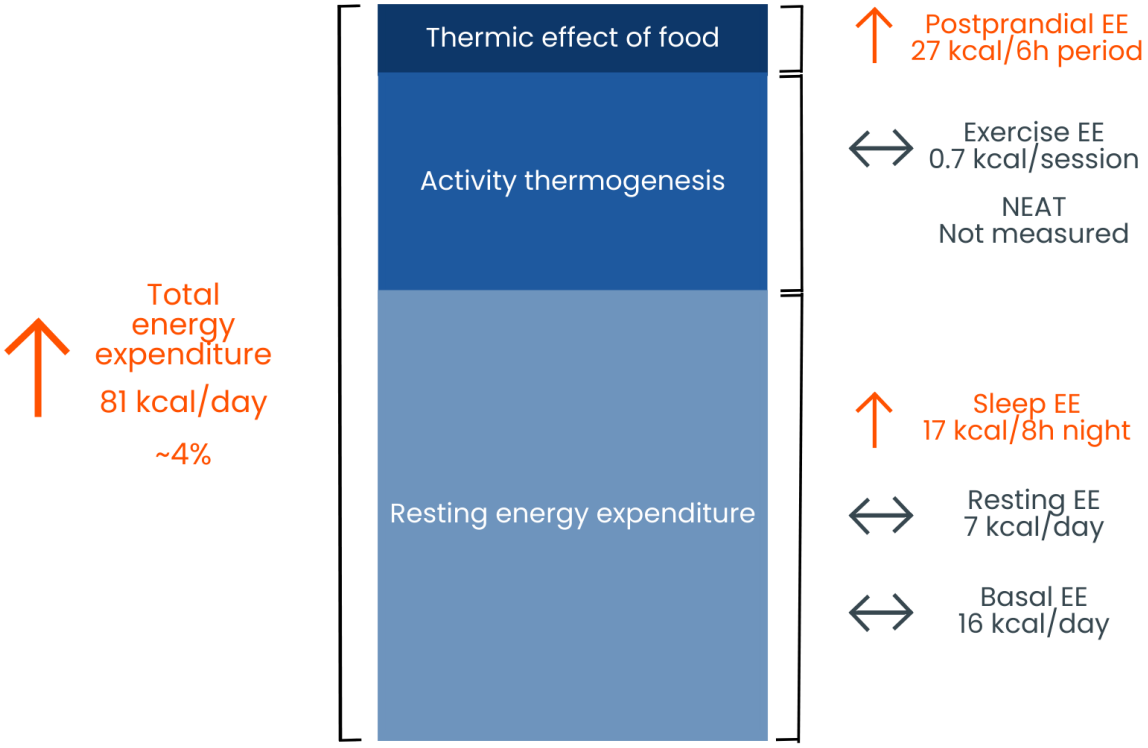

Figure 7.2. The effect of the high-protein total diet replacement compared to the North American diet on changes in total energy expenditure and its main physiological compartments. Statistically significant changes indicated in orange.

Abbreviations: EE: energy expenditure; NEAT: non-exercise activity thermogenesis.

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APPENDICES

APPENDIX 1. ESTIMATION OF THE THERMIC EFFECT OF FOOD OF THE HIGH-PROTEIN DIET REPLACEMENT AND NORTH AMERICAN DIETARY INTERVENTIONS

	TEF (%) - CON	TEF (%) - HP-TDR	Heat of combustion (kcal/g)	Cost of TEF (kcal/g) - CON	Cost of TEF (kcal/g) - HP-TDR	Available energy after average TEF - CON	Available energy after average TEF - HP-TDR
Protein	30.57	28.74	5.65	1.73	1.62	3.92	4.03
Carbohydrate	4.29	1.73	4.1	0.18	0.07	3.92	4.03
Fat	5.04	2.77	9.4	0.47	0.26	8.93	9.14

	CON		HP-TDR	
	Intake (g)	Intake (kcal) - Heat of combustion	Intake (g)	Intake (kcal) - Heat of combustion
Protein	83	469	211	1192
Carbohydrate	295	1210	186	763
Fat	72	677	58	545
Total	450	2355	455	2500

Real values

Standard values

	CON		HP-TDR	
	Intake (kcal) - Available energy	Diff between HC and AE (kcal)	Intake (kcal) - Available energy	Diff between HC and AE (kcal)
Protein	326	143	849	343
Carbohydrate	1158	52	749	13
Fat	643	34	530	15
Total	2126	229	2129	371
TEF (%)	10.79		17.42	6.63

	CON			HP-TDR		
	% energy	Available energy from diet (kcal)	Available energy (kcal)	% energy	Available energy from diet (kcal)	Available energy (kcal)
Protein	15.3	2128	326	39.9	2129	849
Carbohydrate	54.4		1158	35.2		749
Fat	30.2		643	24.9		530

Abbreviations: AE: available energy; CON: control; HC: heat of combustion; HP-TDR: high-protein total diet replacement; TEF: thermic effect of food.