The Role of Dietary Fatty Acids on Immune Function in the Context of Obesity

by

Carolina Gonzalez Hernandez

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Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Obesity is a major public health problem since it is associated with different complications, including cardiovascular diseases, cancer, type 2 diabetes (T2D), and a higher risk of infections. This is due in part to the fact that obesity is characterized by the presence of chronic low-grade systemic inflammation, which alongside the consumption of a high-fat diet (HFD) and the development of insulin resistance may lead to immune dysfunction.

Fatty acids (FAs) are known for having immunomodulatory properties. Saturated fatty acids (SFAs) are considered pro-inflammatory since they can directly bind to the Toll-like receptor 4 (TLR4). In the case of polyunsaturated fatty acids (PUFAs), omega 6 (n-6) are recognized to contribute to inflammation, whereas omega 3 (n-3) facilitate the resolution of inflammation. Conversely, monounsaturated fatty acids (MUFAs) are considered more neutral regarding their immunomodulatory effects. Therefore, the objective of this thesis is to investigate the role that dietary FAs have on immune function in the context of obesity.

In our first study, we evaluated the effects of a high MUFA diet on peripheral immune function (spleen) in the context of a diet-induced obesity (DIO) rat model. Male Sprague Dawley rats 6-weeks-old were consuming either a low-fat high carbohydrate diet (HC) (24% calories from fat), a standard high-fat diet (HF) (providing 35% calories from fat), or a high-fat diet with olive oil (HFOO) high in MUFA (providing 35% calories from fat where 33% of the fat mixture was replaced by olive oil). During the first 3 weeks, HC and HF diets were used to induce obesity. After that time, animals in the HC group continued receiving the same diet, and those fed the HF diet were randomized to either continue on the same HF control diet or to receive the HFOO for an additional 4 weeks. We observed that MUFA reduced the

production of pro-inflammatory cytokines after T cell stimulation, specifically by reducing Tumor Necrosis Factor Alpha (TNF-α), and interleukin (IL)-6, with a trend towards a reduction in Interferon-gamma (IFN-γ). However, there was no effect on antigen-presenting cells (APC) function. In addition to that, HFOO significantly diminished the secretion of IL-2 after concanavalin A (ConA) stimulation compared to HF, suggesting a reduction in T cell proliferation.

In our second study, we explored the relationship between dietary FAs and immune function in people living with obesity and with and without metabolic complications. Data from the Nutrition and Immunity (NutrIMM) study was used. In this study, participants were allocated into one of four groups depending on their metabolic phenotype: Lean-normoglycemic (Lean), Obese-normoglycemic (Obese-NG), Obese-glucose intolerant (Obese-GI), Obese with type 2 diabetes (Obese-T2D), and subsequently were fed a North American diet for 4 weeks. At baseline, our results found that the proportions of SFAs and MUFAs in plasma increased across groups, while PUFAs decreased. Additionally, SFAs in plasma, particularly palmitic acid, were positively associated with the production of IL-1B from APCs after stimulation. Plasma MUFAs were not associated with changes in the production of pro-inflammatory cytokines following stimulation. No significant association was found with n-3; however, PUFAs, especially linoleic acid, were negatively associated with the secretion of IL-1B by APC after LPS stimulation.

In this research we concluded that SFAs exhibited pro-inflammatory effects, MUFAs did not show a direct relationship with the production of pro-inflammatory cytokines after immune cell stimulation on their own; however, when replacing SFAs, MUFAs displayed anti-

inflammatory properties by decreasing T helper (Th) 1 response and diminishing IL-2 secretion after ConA stimulation. PUFAs, particularly linoleic acid, were found to also exert anti-inflammatory properties. Therefore, it is recommended to avoid the consumption of high SFAs such as those high in the North American diet, which may contribute to chronic low-grade systemic inflammation and immune dysfunction, and to prefer the consumption of diets high in MUFAs and PUFAs such as the Mediterranean diet.

Preface

This thesis is an original work by Carolina González Hernández. The research projects, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, under Project Name "The Alberta nutrimm study – nutrition and immunity", (Pro00085839) on February 8, 2019, and "Feeding High Oleic Acid Canola Oil or Olive Oil Alters Inflammation, Insulin Resistance and Lipid Metabolism in a Rodent Model of Diet Induced Obesity", (ACUCL092) on May 10, 1992.

The studies presented in this thesis were conducted collaboratively with various collaborators. Chapter 3 describes a study designed by Chan, C. and Field, C.J., with animal handling done by Coursen, N., data collection conducted by Ruby, K. as part of her MSc. Research with technical help from Goruk, S., and Iglinski. P. I was responsible for the data analysis related to immune function and chapter preparation. CJF obtained funding for this study from Alberta Innovates.

Similarly, Chapter 4 outlines a study designed by Richard, C., with data collection carried out mostly by Braga Tibaes, J.R., Blanco Cervantes, P. and Barreto Silva, M.I., Makarowski, A. Mereu, L. I assumed responsibility for the data analysis related to plasma lipids and manuscript preparation for this chapter. The overall discussion and conclusion presented in Chapter 5, as well as the literature review presented in Chapter 1, are my original work. Richard, C., contributed to the concept of the thesis and manuscripts preparation and obtained the funding for this study.

The tool Grammarly IA was used to enhance the grammar and style of this document.

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I would also like to express my gratitude to Dr. Catherine Field, my co-supervisor, and member of my supervisory committee, for her guidance and mentorship throughout my studies. I extend my thanks to Dr. Rhonda Bell and Dr. Ben Willing for acting as my external examiner and chair for the defense, respectively. Additionally, I am grateful to Alexander Makarowski for imparting the essential lab techniques necessary for my research.

To my labmates, your friendship and the unforgettable moments we shared over the years have been a source of joy and happiness. I am truly grateful for your support and encouragement.

I extend special thanks to COLFUTURO for their financial support, which has made it possible for me to pursue my academic aspirations.

Last but not least, sincere gratitude to my family for their persistent support throughout this journey. Your love and encouragement have been my greatest source of strength and motivation.

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List of Abbreviations

AA - arachidonic acid

APC - antigen-presenting cell

BMI - body mass index

ConA- concanavalin A

CRP - c-reactive Protein

CVD - cardiovascular disease

DC - dendritic cell

DHA - docosahexaenoic acid

DHQ III- Diet History Questionnaire III

DNA - deoxyribonucleic acid

DIO - diet-induced obesity

ELISA- enzyme-linked immunosorbent assay

EPA - eicosapentaenoic acid

FA - fatty acid

FCS - fetal bovine serum

HbA1c - glycated hemoglobin

HDL - high-density lipoprotein

HFD - high-fat diet

HOMA-IR - homeostatic model assessment for insulin resistance

iAUC - incremental area under the curve

IFN-γ - interferon-gamma

Ig - Immunoglobulin

IKK - IκB kinase

IL- interleukin

InsR - insulin receptor

LDL - low-density lipoprotein

LPS - lipopolysaccharide

MCP-1 - monocyte chemoattractant protein-1

MedDiet - mediterranean diet

MHC - major histocompatibility complex

MUFA - monounsaturated fatty acids

N-3 - Omega 3

N-6 - Omega 6

NK - natural killer

NutrIMM - nutrition and immunity study

OGTT - oral glucose tolerance test

PBMC - peripheral blood mononuclear cell

PHA - phytohaemagglutinin

PI3K - phosphoinositide 3-kinase

PKC - protein kinase C

PUFA - polyunsaturated fatty acids

RBC - red blood cell

SEM - standard error of the mean

SFA - saturated fatty acids

T2D - Type 2 Diabetes

TG - triglycerides

TGF-β - transforming growth factor beta

TLR - toll-like receptor

Th - T helper

TLR4 - toll-like receptor - 4

TNF-α - tumor necrosis factor-alpha

Treg - regulatory T cell

VLDL - very low-density lipoprotein

Chapter 1: Introduction and Literature Review

1.1 Overview of the immune system

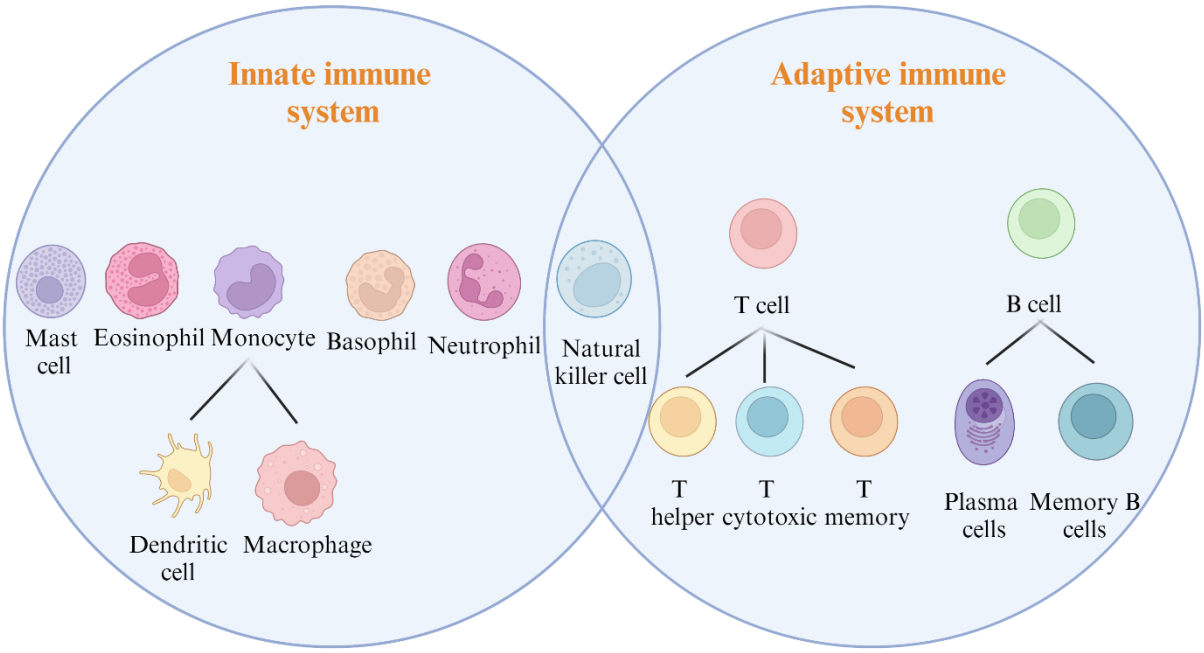
The main purpose of the immune system is to defend the body against pathogens by utilizing diverse organs and cells. Immune cells are produced in the bone marrow by hematopoiesis, where stem cells differentiate into different types of blood cells. Some of them mature in the bone marrow, whereas others mature in the thymus (Kuby et al., 2007; Mountain, 1955), where they are trained to properly recognize "self" and "non-self" antigens to start an immune response or lead to tolerance when necessary (P. C. Calder, 2023). After the maturation, they circulate in the bloodstream or lymphatic system, being prepared to respond against infections (Janeway et al., 2001b).

The immune system can be divided into peripheral and gut-associated immune systems. The peripheral immune system is involved in the overall immune function of the organism, playing an important role in the interaction of immune cells with antigens and mounting a proper immune response. The main tissues involved are the spleen, which filters the blood, and lymph nodes, which filter lymphatic fluid. In both cases, immune cells monitor if there is the presence of a pathogen to initiate an immune response (Kuby et al., 2007).

On the other hand, the gut-associated immune system plays an important role in regulating immune responses within the gastrointestinal tract and promotes tolerance against commensal bacteria and dietary antigens. It can be categorized into inductive sites, including Peyer's patches, isolated lymphoid follicles, and mesenteric lymph nodes, where the activation, differentiation, and proliferation of immune cells occurs. Effector sites, on their part, comprise lamina propria and epithelium, where immune cells carry out their effector or suppressor functions (Mörbe et al., 2021).

The immune system is also divided into two lines of defense: the innate and adaptive immune systems, which work together to fight against infections effectively. (P. Calder & Kulkarni, 2018). These lines of defense are summarized in **Figure 1-1.**

Figure 1-1. Overview of innate and adaptive immune systems.



Adapted from Torang 2019. Created with BioRender.com

1.1.1 Innate immune system

The innate immune system is considered the first line of defense of the body. It is characterized by being rapid and nonspecific since the cells that comprise this arm are not able to recognize specific antigens and therefore do not produce memory cells. (Aristizábal & González, 2013). However, they can recognize pathogens through pattern recognition receptors that identify pathogen-associated molecular patterns (Innes & Calder, 2018). Upon recognition, these immune cells start producing cytokines to induce inflammation, chemoattractants, and adhesion molecules such as Monocyte Chemoattractant Protein-1 (MCP-1), Vascular Cell Adhesion Molecule-1, and Intercellular Adhesion Molecule-1 involved in the migration of other immune cells to the site of infection (Ren et al., 2011). All this together helps start mounting a proper immune response. The main components of the innate immune system include:

Physical barriers: Skin and mucus impede the entry of pathogens into the organism. Skin acts as a physical barrier and its pH inhibits the growth of microorganisms. Mucus entraps

foreign microbes and produces antimicrobial peptides, enzymes, and glycoproteins that help eliminate pathogens (Kuby et al., 2007).

Granulocytes: Within this group, neutrophils are the first responders against bacterial infections; they destroy pathogens through phagocytosis and the production of enzymes. Eosinophils defend the organism against parasites and play a role in allergic reactions by releasing cytotoxic granules and cytokines that contribute to allergic responses, respectively. Basophils and mast cells have a similar role as eosinophils but mast cells also secrete histamine that contributes to inflammation by leading to vasodilation and increasing vascular permeability to facilitate the recruitment of other immune cells to the site of infection (Kuby et al., 2007).

Natural killer: Natural killer cells (NKs) possess a cytotoxic activity that leads them to eliminate tumors or infected cells by releasing perforin and granzymes that induce apoptosis. They also produce cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), therefore modulating the immune response (Paul & Lal, 2017; Vivier et al., 2008).

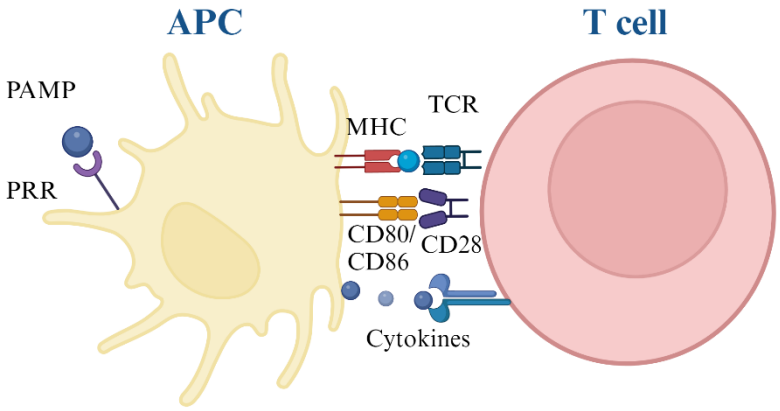
Monocytes and dendritic cells: Monocytes circulate in the bloodstream, and once they migrate into tissues, they differentiate into dendritic cells (DCs) or macrophages. Both are considered antigen-presenting cells (APCs) since they capture pathogens and present their antigens through the major histocompatibility complex (MHC) to activate cells of the adaptive immune system, followed by the secretion of cytokines and chemokines that regulate the immune response. In addition to that, both types of cells contribute to phagocytosis; however, macrophages are considered specialized phagocytic cells, helping with the elimination of pathogens (Théry & Amigorena, 2001; Weisheit et al., 2015). They are both major producer of IL-1B that can induce a fever.

1.1.2 Adaptive immune system

The adaptive immune system, in contrast, has a slower response when compared to the innate immune system but is able to identify specific antigens, produce memory cells, and generate

stronger responses in subsequent encounters with the same antigen. The activation of adaptive immune cells is done by a process called antigen presentation, depicted in **Figure 1-2.** This process is enabled by APCs, which engulf pathogens and exhibit small peptides to MHC molecules that are recognized by T cells, leading to their activation and differentiation (P. Calder & Kulkarni, 2018). The two main cell lines of the adaptive immune system are T and B lymphocytes.

Figure 1-2. Antigen presentation process of T cells. [1]



[1] Abbreviations: APC, antigen-presenting cell; MHC, major histocompatibility complex; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor. Adapted from Lee 2020. Created with Biorender.com

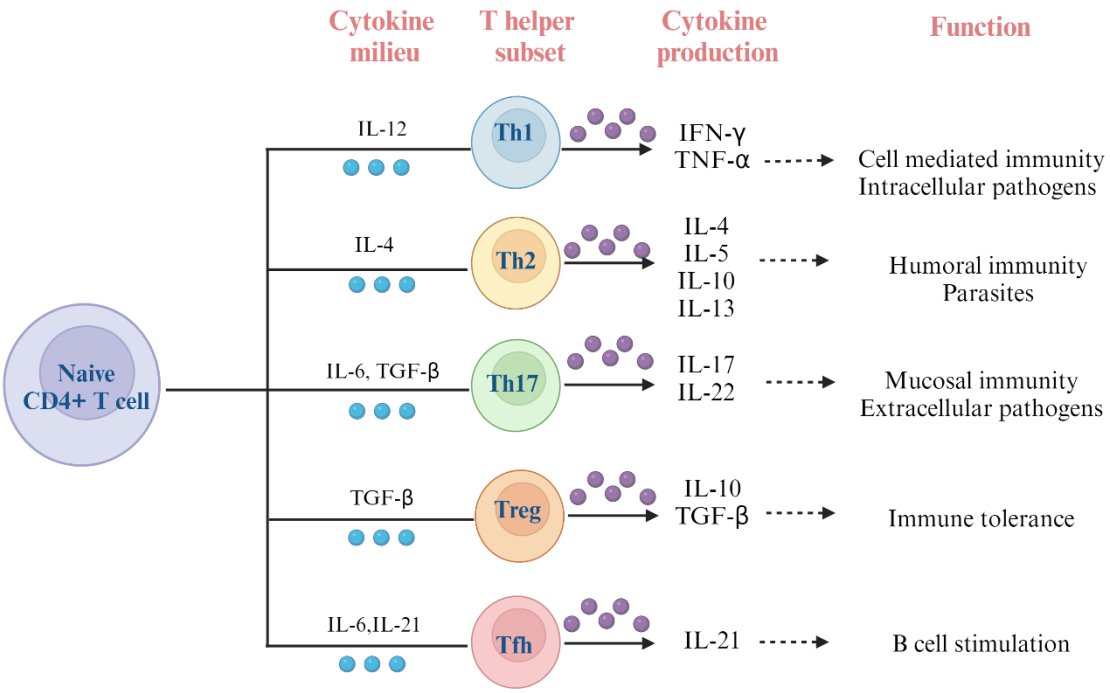
T lymphocytes

T cells provide cell-mediated immunity. They originate in the bone marrow and then mature in the thymus, where they acquire the T cell receptor and express either CD4+ or CD8+ markers. After maturation, they migrate to the peripheral circulation, where they encounter antigens presented by APCs. Once T cells get activated, they start secreting IL-2, which promotes their proliferation and differentiation (Bachmann & Oxenius, 2007; Kuby et al., 2007).

T cells are classified into two different groups. T cytotoxic and T helper cells, each of these types of T cells expresses different markers on their surface and have particular functions. T cytotoxic cells express CD8+ and recognize antigens via MHC class I. Their function is to eliminate cells that have been infected by programmed cell death, also known as apoptosis.

Conversely, Th cells express the CD4+ marker and recognize antigens by MHC class II. Their main function is to modulate the immune response, by assisting immune cells with the production of cytokines. Once they are activated, Th cells differentiate into various subsets depending on the cytokine milieu; each subset produces different cytokines, leading to diverse functions (**Figure 1-3)** (P. Calder & Kulkarni, 2018; Corripio-Miyar et al., 2022).

Figure 1-3. T helper cell differentiation. [1]



[1] Abbreviations: IFN-γ, interferon-gamma; IL, interleukin; TGF-β, transforming growth factor-beta, TNF-α, tumor necrosis factor-alpha; Treg, T regulatory cell; Tfh, follicular helper T cell. Adapted from Zhang 2013. Created with Biorender.com

B lymphocytes

B cells are another essential component of the immune system. These immune cells are part of humoral immunity since they are able to produce antibodies that help in the neutralization and elimination of extracellular pathogens. B cells are also considered APCs as they have the ability to present antigens through MHC class II. The maturation of B cells occurs in the bone marrow, where they start expressing B-cell receptors, to subsequently migrate to lymphoid organs. Once in there, they can be activated by the recognition of antigens mediated by the B-cell receptor, or by the help of T cells. After their activation, they start proliferating and

differentiating into either plasma or memory B cells. (P. Calder & Kulkarni, 2018; Kuby et al., 2007).

The main function of plasma cells is the production of antibodies, which are also called immunoglobulins (Igs). Their main role is to eliminate pathogens through different steps that involve neutralization, precipitation, agglutination, opsonization, and complement fixation (P. Calder & Kulkarni, 2018).

These Igs are comprised of five main classes. The first is IgM. It is the first antibody to be produced and its function is to neutralize pathogens. The second is IgG, which is the most prevalent antibody. It is involved with neutralizing toxins, facilitating opsonization, and mediating the body's reaction to allergens. The third class is IgE which has similar functions as IgG. The fourth class is IgA, which is involved with mucosal immune responses. The exact function of the fifth class - IgD - is currently unknown (Aziz et al., 2023).

In contrast to plasma cells, memory B cells produce a more robust response after re-exposure to antigens, similar to the response provided by memory T cells (Ratajczak et al., 2018).

To summarize, both the innate and adaptive immune systems play an important role in the immune system. They must work together to mount an effective response against pathogens and also resolve inflammation to maintain homeostasis in the body.

Energy metabolism as it relate to immune cells

Immune cells require energy to perform their vital functions. However, their metabolism may change across their lifecycle. Inactive immune cells have lower requirements for energy and therefore usually use fatty acid oxidation to obtain energy (Pearce & Pearce, 2013). Nevertheless, once they are activated there is an increased demand for energy to proliferate and differentiate, leading to a shift towards aerobic glycolysis, also known as the Warburg effect. This effect involves the utilization of glucose using aerobic glycolysis instead of oxidative phosphorylation. This shift might be explained by the fact that this type of metabolism reserves amino acids and fatty acids for the synthesis of cellular components, in

addition to the production of lactate, which facilitates rapid energy production and expansion of immune cells, supporting their growth, proliferation, and effector functions (Pearce, 2010). The Warburg effect is usually found in some immune cells such as effector T cells, M1 macrophages, DC, neutrophils, and activated NK cells (Kornberg, 2020).

1.2 Assessing immune function in animal and human studies

It is well known that nutritional status, specific nutrients, and dietary patterns have an impact on immune function (see section 1.3). Therefore, in order to evaluate all these properties, different methodologies have arisen, such as studies performed in humans, animals, or in vitro. Each of them provides different information and it is important to consider their own strengths and limitations to select the proper one to answer the specific research question. For the context of this thesis, we are going to focus on animal and human studies.

1.2.1 Animal models and their importance in the field of nutritional immunology

Animal studies are commonly used in nutritional immunology research. They provide many benefits since the majority of the animal models are easy to handle, and investigators are able to control different variables, such as the environment, the diet composition and food intake, which allows them to evaluate the effect of specific nutrients and diets (Mukherjee et al., 2022). In addition to that, it is possible to collect certain tissues that are not able, or especially difficult to obtain in humans. This leads to the analysis of different components of the immune system. For instance, the spleen can be used to assess the peripheral immune system, while Peyer´s patches and mesenteric lymph nodes evaluate the gut-associated immune system. Moreover, some animal models have shorter lifespans compared to humans, enabling to study long-term effects in a reasonable time frame (Folch et al., 2018).

The most important limitation of animal models is the fact that they do not completely replicate human physiology (Mestas & Hughes, 2004; Payne & Crooks, 2007), and therefore cannot be extrapolated directly to humans. Yet, they provide preliminary evidence for future potential interventions and a mechanistic understanding of action before moving to human studies. However, different species exist that can also be genetically manipulated to select a model that more closely fits the research question. Different types of animals may be utilized, including small models such as rodents, rabbits, and ferrets, or larger ones like nonhuman

primates, pigs, cows, and sheep, among others (Wagar et al., 2018). For this thesis, we will focus on rodents.

Rodents are one of the most common models in research fields since they are small, easy to handle, and have a low maintenance cost; however, they only share a 10% similarity of the immune system with humans (Pabst, 2020). Regarding rats, diverse strains are used to evaluate different outcomes of immune function. For instance, Lewis rats are an inbred line that offers genetic homogeneity, characterized by having a strong Th1 response, making them susceptible to autoimmune diseases (Pastoret et al., 1998). However, we can also study very specific perturbations, such as the immune dysfunction related to obesity, using strains like Sprague-Dawley and Wistar rats, which even though have a robust immune response are susceptible to developing obesity, by using diet-induced obesity (DIO) models (i.e. consuming a high-fat diet (HFD)) (Boi et al., 2016; Levin et al., 1997). This model leads to the assessment of the interplay between obesity, metabolism, and the immune system, being useful for mimicking the development of obesity through excessive caloric intake (Boi et al., 2016; Lamas et al., 2002; Luck et al., 2015; Richard et al., 2017). Although rats are generally less used than mice in research settings, they are bigger and therefore are better suited to study the gut-associated immune system whereas in mice tissues have to be pooled from many mice to have enough immune cells to assess their function.

When looking at different strains of mice, some of the most used are C57BL/6 which is an inbred strain, characterized by being genetically similar, this allows higher reproducibility, in addition to being relatively resistant to infections (Adam et al., 2018; Song & Hwang, 2017; Talmadge et al., 1980). Conversely, BALB/c mice are usually utilized to investigate infectious diseases due to their Th2-biased immune response, being a good model to study diseases when Th2 responses are dominant, such as in allergies (Adam et al., 2018; Song & Hwang, 2017; Talmadge et al., 1980). Moreover, more specialized strains like ob/ob and db/db mice are leptin-deficient, affecting T cell proliferation and the expression of markers on Th and T cytotoxic cells and how metabolic disorders such as obesity affect the immune response (Hsu et al., 2007; She et al., 2022).

1.2.2 Human studies and their importance in the field of nutritional immunology

Human studies, on the contrary, provide direct clinical relevance, giving information about real-life conditions, and serving as a validation to animal studies. However, human studies have limitations, due to the fact that humans have many variables difficult to control, making it complex to evaluate the effects of specific nutrients. In addition, it is hard to obtain specific samples, thus, limiting the amount of analysis that can be performed. The most frequent samples collected to assess the immune system is blood due to its easy accessibility, which provides useful information regarding the systemic immune system, although, it may not reflect local immune functions (Cunningham-Rundles, 1994).

Some of the most common study designs that explore the associations between nutrition and immunology include observational studies which evaluate dietary intake and immune markers or the prevalence of infection/allergies at a single point in time. These studies do not establish causality, only associations to generate hypotheses. Conversely, clinical trials are prospective interventions in which a potential treatment is given to an intervention and a control group to compare the results. These studies are expensive and time-consuming but establish causality. When there is additional randomization, as in the case of randomized control trials, they are considered the gold standard for evaluating interventions (Chidambaram & Josephson, 2019).

In clinical research, there are different ways to control dietary conditions. For instance, when it is necessary to exert a high degree of control over food intake, it may be useful to design a control feeding trial, in which the diet is provided by the researchers in a supervised facility. This ensures standardization and control of external variables, giving highly precise results; however, such trials are expensive and may lead to lower compliance since participants might find it challenging to adhere to such a strict diet. On the other hand, studies in free-living conditions are designed for participants to consume their habitual diets. This leads to more variation in food intake, and it is difficult to isolate the effects of specific nutrients, but they might be useful for evaluating long-term dietary patterns.

Techniques to assess immune function

Nowadays, researchers have at their disposal various techniques that can be used to evaluate immune function. Leukocyte count, for example, gives the number of white blood cells found in a sample, being useful to have an overall overview of the immune system (Blumenreich, 1990). The lymphocyte proliferation assay uses a fluorescent dye to identify the ability that lymphocytes have to proliferate after a specific stimulus (Bercovici et al., 2000; Lindemann, 2014). The chromium release assay measures the cytotoxic activity that possesses T cells and NK cells (Lisby et al., 2022). Oxidative burst evaluates the response that neutrophils have against antigens by measuring the generation of reactive oxygen species (ROS) (Chen & Junger, 2012). Western blotting identifies specific proteins within a sample and can be used to study the signaling pathways in immune cells (P.-C. Yang & Mahmood, 2012). Polymerase chain reaction amplifies deoxyribonucleic acid (DNA) sequences, being useful for studying genes involved in immune responses (Wages, 2005). Flow cytometry can have many uses, one of the most important is to identify cells within a complex mixture by their surface antigens (Lindemann, 2014; Maruyama, 2018). In clinical settings, immune function may also be assessed by indirect indicators, including morbidity, mortality (Resnick et al., 2012), hospitalization rates (Government of Canada, 2016), incidence of allergies (Long et al., 2023), infection rates (Yang et al., 2021) as well as vaccination effectiveness (Zimmermann & Curtis, 2019).

For this thesis, we are going to focus on Enzyme-linked immunosorbent assay (ELISA), a technique used to measure the concentration of soluble antigens such as chemokines, inflammatory mediators, immunoglobulins, and cytokines present in biological samples such as organ tissues or blood samples. For blood, peripheral blood mononuclear cells (PMBCs) are usually isolated since they contain lymphocytes, monocytes, NKs, and dendritic cells and are then stimulated to assess the cytokines produced. The most frequent method is Sandwich ELISA. It involves a capture antibody that binds to an analyte from the sample. A second analyte-specific antibody is then added, and optionally a third "detection" antibody with an enzyme that produces a fluorescence, which is then measured by a spectrophotometer (BD biosciences, 2003; Lindemann, 2014).

This method can be used to assess immune function by measuring the number of cytokines produced by immune cells after the stimulation with a mitogen, which are substances that stimulate immune cells and lead to an immune response. There are different types of mitogens, each with its own characteristics, as mentioned in **Table 1-1** (Mak & Saunders, 2006).

Table 1-1. Comparison of different types of mitogens.

Mitogen	Description	Primary target
Phytohemagglutinin (PHA)	Lectin derived from kidney beans	T cells
Concanavalin A (ConA)	Lectin derived from the jack-bean plant.	T cells
Lipopolysaccharide (LPS)	Component of the outer membrane of Gram-negative bacteria.	APCs
Pokeweed Mitogen (PWM)	Lectin derived from the pokeweed plant.	T and B cells.
Phorbol myristate acetate + ionomycin (PMAi)	Synthetic compound	Non-specific lymphocyte stimulant

Even though ELISA provides high sensitivity and specificity to quantify the production of cytokines, it does not indicate which specific cell types produce them. We also only analyze the production of cytokines at a single time point, not being able to evaluate changes over time in the immune response (i.e. initial inflammatory response vs. resolving phase of inflammation).

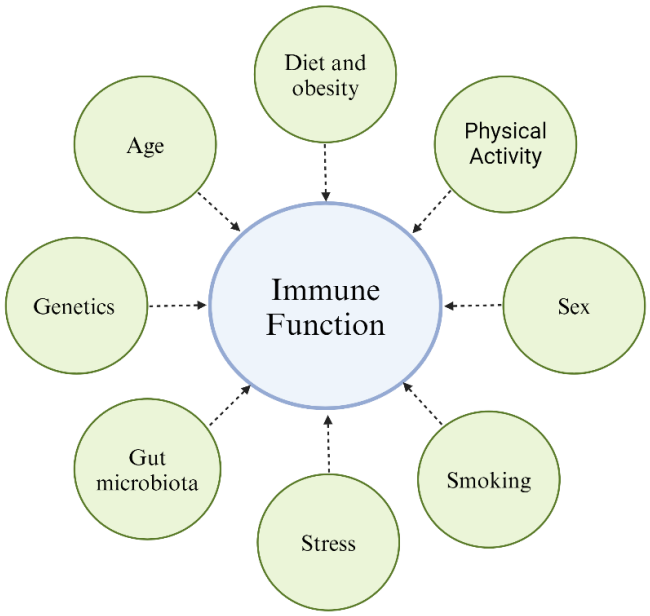
To overcome these limitations, adjustments in the methodology might be made in order to complement the results. Intracellular staining using flow cytometry, for example, allows the detection of the cell types producing the cytokines by staining the cells and the intracellular cytokines (Jung et al., 1993; S. G. Smith et al., 2015). Enzyme-linked Immunospot can also be useful since it determines cytokine production at the single-cell level, providing information about the frequency of the secretion of cytokines (Tanguay & Killion, 1994). In addition, if necessary, the collection of multiple time points might be useful to assess the profile of cytokine secretion over time. Therefore, complementing different assays and

indicators may be useful to control their limitations, giving a better understanding of the immune response.

1.2.3 Factors affecting the immune system

A diversity of factors has been found to affect the immune system, they are summarized in **Figure 1-4**. Ideally one would like to consider each of them and understand the way they impact immune function since it will help create strategies to optimize immune responses and reduce susceptibility to diseases.

Figure 1-4. Factors affecting the immune function.



Adapted from Calder 2021. Created with Biorender.com

Age: It has been found that elderly people have a higher risk of infections. Immunosenescence is the gradual deterioration of the immune system that occurs most often with age. (Agarwal & Busse, 2010; Bartoszko & Loeb, 2021; Bektas et al., 2017; Watson & Wilkinson, 2021). This condition involves an accumulation of abnormalities in the immune system that include the reduction in the production of immune cells in the bone marrow and lower diversity in the T cell receptors, leading to lower T cell counts in the blood and an accumulation of memory T cells (Palmer, 2013). In addition, the elderly population also has low-grade chronic inflammation, characterized by high levels of pro-inflammatory cytokines

in the bloodstream, overall leading to an impaired immune function over time (Krabbe et al., 2004).

Physical activity: It has been demonstrated that physical activity prevents systemic inflammation (King et al., 2003) by lowering IL-6 concentration (Hamer, 2007), and C-reactive protein (CRP) levels. Whereas the absence of physical activity has shown an increased amount of visceral adipose tissue, which attracts more immune cells, leading to a higher production of pro-inflammatory cytokines (P. C. Calder et al., 2013).

Sex: Studies have found that sex is a factor that affects immune function. Males have a higher expression of toll-like receptor 4 (TLR4), leading to a higher production of pro-inflammatory cytokines (Rettew et al., 2008). While females have concentrations of B and Th1 cells (Furman et al., 2014; B.-W. Lee et al., 1996; Uppal et al., 2003). Additionally, regarding inflammation, males have increased levels of IL-6, TNF-α, and CRP, which may contribute to their increased disease risk (Klein & Flanagan, 2016; Marques-Vidal et al., 2011).

Smoking: Smoking induces oxidative stress, which leads to an inflammatory response characterized by an increased concentration of circulating IL-6, TNF-α, and CRP, having a negative impact on immune function (Bermudez et al., 2002; P. C. Calder et al., 2013; Frohlich, 2003).

Stress: Stress influences the immune response through the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Acute stress has been found to induce the production of pro-inflammatory cytokines and chronic stress can diminish T cell proliferation and the cytotoxic activity of NKs (Claus et al., 2016).

Gut microbiota: The gut microbiota plays an important role in the immune function in different ways. It produces short-chain fatty acids that have anti-inflammatory effects (Fattahi et al., 2020). Gut microbiota also establishes a protective barrier in which microbiota compete with pathogens for nutrients. Additionally, it produces antimicrobial proteins and lactic acid, which inhibit the growth of pathogens (P. C. Calder et al., 2022). It has also been

found that in the absence of gut microbiota, there is a decrease in the development of lymphoid organs, and the production of immunoglobulins and lymphocytes (Round & Mazmanian, 2009). Whereas changes in the composition of gut microbiota such as a reduction in Firmicutes phylum result in increased inflammation (Sokol et al., 2008).

Genetics: Genes impact the immune system since genetic variations and epigenetics can influence the expression of inflammatory genes, regulate the production of cytokines and immune mediators, and the ability of immune cells to respond to antigens (Dahmer et al., 2016).

Diet and obesity: The influence that diet and obesity have on immune function will be further discussed in sections 1.2 and 1.3.

In conclusion, immune function is affected by different factors that influence the ability of the body to respond against pathogens. Understanding how these factors are related is important to develop strategies that enhance immune health.

1.2 Obesity

Obesity is a chronic disease that has become a public health problem worldwide, affecting both developed and developing countries since it is related to various comorbidities that increase mortality (Abdelaal et al., 2017; Yach et al., 2006). Obesity is characterized by an excess accumulation of fat, determined by a body mass index (BMI) of ≥ 30 kg/m². In addition, a waist circumference greater than 102 cm in men and 88 cm in women (Purnell, 2000) helps to identify central obesity, which is related to having a higher risk of comorbidities (Janssen et al., 2004).

This disease is multifactorial, resulting from genetic, environmental, behavioral, and metabolic factors; the primary cause being a prolonged positive energy balance. Behavioral factors, such as the consumption of unhealthy diets like the North American diet and insufficient physical activity, are also considered significant factors in the development of this disease (Vandevijvere et al., 2015).

1.2.1 Prevalence

In 2022, 16% of the global population was living with obesity, which corresponds to 890 million people. International rates of obesity have increased over the past decades, rising from 100 million in 1975 to 671 million in 2016 (WHO, 2024). Two-thirds of this population live in developing countries (Rogero & Calder, 2018a), with black women and Hispanic people having higher rates (Purnell, 2000). In 2018, in Canada, 26.8% of adults were living with obesity, with males having a higher prevalence. Newfoundland and Labrador and Prince Edward Island are the provinces with the highest obesity level. Moreover, adults with lower education levels experience higher rates (Statistics Canada, 2019).

1.2.2 Obesity-related metabolic complications

Obesity has been related to the development of metabolic complications associated with the excess accumulation of body fat. Some of the most common complications include insulin resistance, type 2 diabetes (T2D), dyslipidemia, hypertension, and cardiovascular disease.

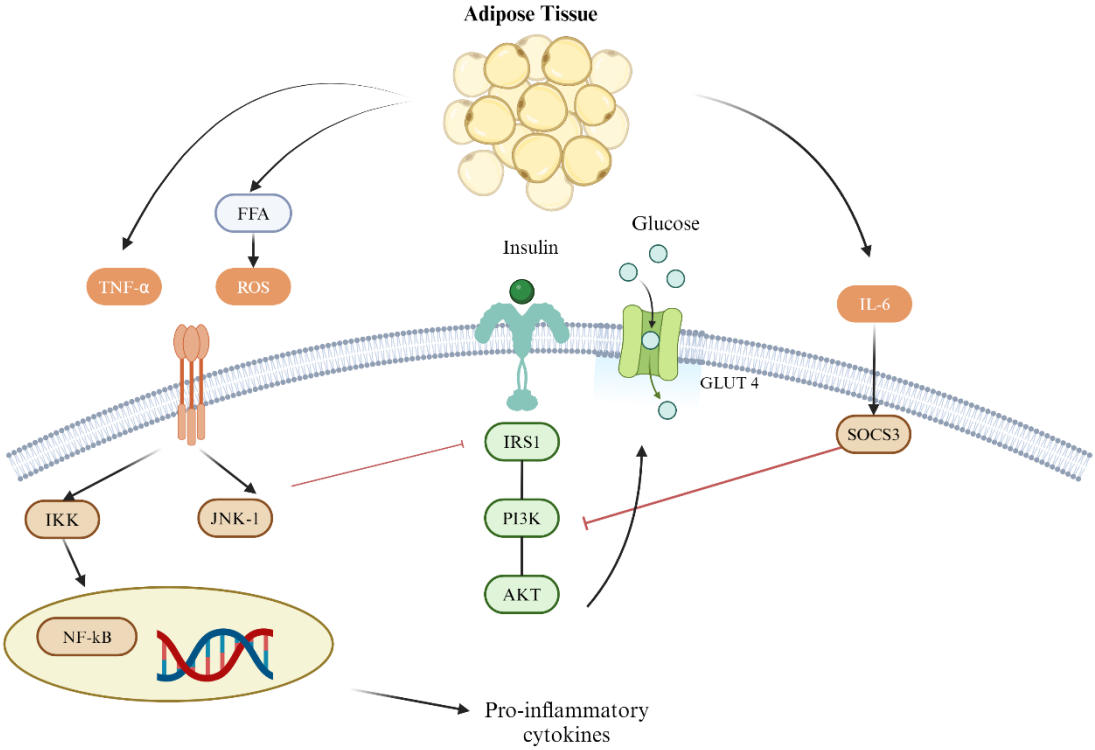
Insulin resistance

Insulin is a hormone that plays an important role in the metabolism. It increases glucose uptake in immune cells through the mediation of glucose transporters, especially when cells are activated (Rahman et al., 2021). It also promotes the uptake of amino acids and the synthesis of proteins by the activation of the mTOR pathway (Prod´homme et al., 2004), and decreases proteolysis (Everman et al., 2016). Additionally, insulin influences fat metabolism by promoting lipogenesis, enhancing the uptake of FA into adipocytes, and inhibiting lipolysis. This reduces fat oxidation and promotes the use of carbohydrates as an energy source (Carpentier, 2021; Kersten, 2001).

On the other hand, insulin resistance is a condition, in which the cells of the body become less responsive to the insulin, leading to a higher amount of glucose in the bloodstream since it cannot be taken up as efficiently by the cells. It is also characterized by hyperinsulinemia as a compensation mechanism to maintain normal glucose levels in the blood (S. H. Kim & Reaven, 2008; Reaven, 1988).

Obesity is related to the development of insulin resistance in different ways, summarized in **Figure 1-5**. People living with obesity have chronic systemic inflammation, characterized by high levels of pro-inflammatory cytokines such as IL-6 and TNF-α. In addition, the expansion of adipose tissue causes elevated amounts of free fatty acids, that produce reactive oxygen species. All of this together activates certain protein kinases like Inhibitor of kappa B kinase, c-Jun N-terminal kinase -1, and Suppressor of cytokine signaling 3, which impair the insulin signaling pathway, leading to insulin resistance and afterward, hyperinsulinemia (Aguirre et al., 2000; Blair et al., 1999; S. Guo, 2014; Han et al., 2013; Hirosumi et al., 2002; Kievit et al., 2006; Kyriakis & Avruch, 1996; Lauterbach & Wunderlich, 2017; Qatanani & Lazar, 2007; Rogero & Calder, 2018a; Shulman, 2000; Yuan et al., 2001).

Figure 1-5. Pathways through which obesity leads to insulin resistance.[1]



[1] Abbreviations: AKT, Protein Kinase B; FFA, Free Fatty Acids; GLUT 4, Glucose Transporter Type 4; IKK, Inhibitor of kappa B kinase; IL, Interleukin; IRS1, Insulin Receptor Substrate 1; JNK-1, c-Jun N-terminal kinase 1; NF-Kb, Nuclear Factor kappa B ; PI3K, Phosphatidylinositol-4,5-bisphosphate 3-kinase; ROS, Reactive Oxygen Species; SOCS3, Suppressor of Cytokine Signaling 3; TNF-α, Tumor Necrosis Factor-alpha. Adapted from Thorn 2013. Created with Biorender.com.

Type 2 diabetes

T2D is a metabolic disease distinguished by chronic hyperglycemia, resulting from insulin resistance. It is closely related to obesity since 50% of people having T2D are also living with obesity (Hossain et al., 2007). In addition, studies have found that the risk of developing T2D increases by 20% for each 1 kg/m² increment in BMI (Hartemink et al., 2006). Diabetes is a complex disease as it has the potential to cause diverse complications including cardiovascular diseases (CVD), stroke, peripheral vascular diseases, retinopathy, nephropathy, and neuropathy (Abdelaal et al., 2017).

The mechanism by which obesity contributes to the apparition of T2D is mainly the development of insulin resistance, causing elevated blood glucose levels and subsequent hyperinsulinemia. Initially, hyperinsulinemia maintains normal blood glucose levels,

however over time, the pancreas becomes exhausted, resulting in the dysfunction of beta-cells, this reduces the production of insulin, leading to chronic hyperglycemia. This persistent hyperglycemia, along with elevated levels of free fatty acids, contributes to glucotoxicity and lipotoxicity, damaging the beta-cells, and affecting even more the course of the disease (Abdelaal et al., 2017).

Dyslipidemia

Dyslipidemia is characterized by high levels of triglycerides (TG) and low-density lipoprotein (LDL) cholesterol, alongside reduced levels of high-density lipoprotein (HDL) cholesterol in the bloodstream (Mosca et al., 2022). Its appearance is influenced by the presence of obesity and insulin resistance. Collectively these risk factors refer to the metabolic syndrome that increases the risk of CVD.

Under normal conditions, insulin inhibits hormone-sensitive lipase, which has the function of hydrolyzing intracellular lipids into free fatty acids. However, in the context of insulin resistance, there is a reduction in the inhibitory effect that insulin has on this enzyme, leading to increased lipolysis and therefore, a higher release of free fatty acids into circulation. They are then taken up by the liver and converted into TGs, which are then packaged into very low-density lipoproteins (VLDLs) that are released into the bloodstream. This increased production of VLDLs competitively inhibits the hydrolysis of chylomicrons by lipoprotein lipase, resulting in increased triglycerides (TG) remnants. Additionally, obesity reduces the expression and activity of lipoprotein lipase, further contributing to hypertriglyceridemia (Karpe et al., 2011; Klop et al., 2013; McQuaid et al., 2011).

The decreased levels of HDL cholesterol appear since there is a lower clearance of lipoproteins rich in TG, increasing the clearance rate of HDL particles (Ashen & Blumenthal, 2005; Stadler & Marsche, 2020). Furthermore, the presence of pro-inflammatory cytokines in obesity reduces the production of lecithin-cholesterol acyltransferase, limiting the conversion of cholesterol esters, and diminishing the maturation of HDL (Feingold & Grunfeld, 2000). For those reasons, the presence of obesity and insulin resistance disrupt normal lipid metabolism, contributing to dyslipidemia.

Hypertension

Hypertension is a condition characterized by having constant high blood pressure levels, defined as a pressure equal to or higher than 140/90 mmHg (WHO, 2023b). The apparition of this disease may occur due to different mechanisms related to obesity. One of them is through the adipose tissue that produces angiotensinogen, a precursor of Angiotensin II, which promotes sodium and water retention, vasoconstriction, and the production of aldosterone, leading to elevated blood pressure (Yiannikouris et al., 2012). A high-calorie diet also increases norepinephrine levels, which affects the stimulation of peripheral α1 and β-adrenergic receptors, increasing sympathetic activity and contributing to hypertension (Jiang et al., 2016). Additionally, free fatty acids can bind to sodium-potassium adenosine, resulting in increased reactive oxygen species. Pro-inflammatory cytokines and oxidative stress subsequently decrease nitric oxide levels, leading to vasoconstriction and increased vascular resistance, further contributing to hypertension (Jiang et al., 2016).

Therefore, conditions related to obesity such as the increment of adipose tissue, the consumption of high-calorie diets, and the state of chronic inflammation have an impact on the development of hypertension.

Cardiovascular disease

Obesity increases the risk of cardiovascular diseases. However, obesity itself can also affect cardiovascular health, since it has been found that people living with obesity have higher blood volume and cardiac output, producing changes in the heart like left ventricular hypertrophy, which is a predisposition for having heart failure (Lopez-Jimenez et al., 2022; Wilding & Jacob, 2021). Higher levels of leptin, resistin, IL-6, VLDL, and remnants of chylomicrons contribute to the development of atherosclerosis. In addition, aldosterone, a hormone elevated in obesity, produces platelet aggregation, and vascular endothelial dysfunction (Klop et al., 2013; Lopez-Jimenez et al., 2022).

1.2.3 Obesity and Immune Dysfunction

One key element of the immune response is the inflammation. It is important to create an appropriate environment to defend the host against infections however when there is a poor resolution it can lead to chronic-low grade systemic inflammation. This is often found in people living with obesity, and is characterized by an increase in pro-inflammatory cytokines

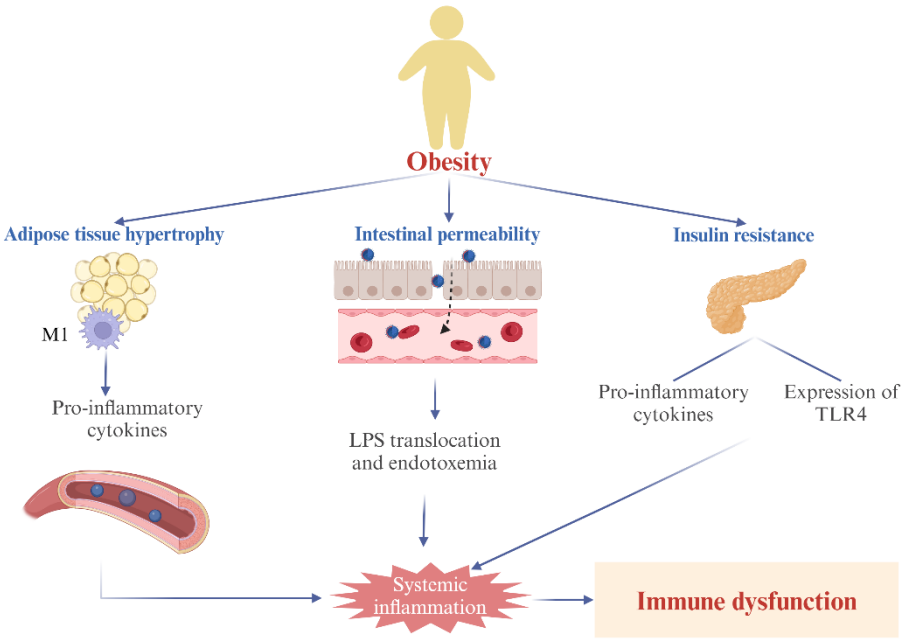
in the bloodstream such as IL-6, CRP, and TNF-α, which can lead to complications and tissue damage over time (P. C. Calder, 2022; de Heredia et al., 2012; Innes & Calder, 2018; H. S. Park et al., 2005). **Figure 1-6**. Show the different mechanisms by which obesity leads to immune dysfunction.

One major pathway is through adipose tissue hypertrophy, which leads to local infiltration of inflammatory cells. It has been found that people living with obesity have increased infiltration of macrophages in their adipose tissue, around 40% compared to 18% in lean controls (Lumeng et al., 2007; Weisberg et al., 2003a). These macrophages shift from an anti-inflammatory phenotype (M2) to a pro-inflammatory phenotype (M1) (de Heredia et al., 2012). M1 macrophages produce high amounts of TNF-α, IL-6, IL-1B and MCP-1, which recruits more monocytes, promoting inflammation (C.-S. Kim et al., 2006; Makki et al., 2013; Milner & Beck, 2012; Surmi & Hasty, 2008). The infiltration of other immune cells such as T and B cells, and dendritic cells also contributes to the inflammation (Kintscher et al., 2008; Srikakulapu & McNamara, 2020). Obesity complications like hyperglycemia, dyslipidemia, and insulin resistance increase the ratio of Th1 to Th2 cells (Matia-Garcia et al., 2021), and lower levels of Treg cells leading to a more pro-inflammatory state and a reduction in the resolution of inflammation (C. Li et al., 2021; Wagner et al., 2013). The production of these pro-inflammatory cytokines primarily occurs in adipose tissue; however, they can spread to different organs and travel in the bloodstream, leading to systemic inflammation (Innes & Calder, 2018; She et al., 2022).

The gut microbiota and diet also play a role in systemic inflammation. It has been found that the consumption of a HFD produces dysbiosis and increases intestinal permeability, resulting in the translocation of LPS into the bloodstream, a condition also known as metabolic endotoxemia (Luck et al., 2019a). LPS is a component of the cell membrane of gram-negative bacteria. It directly binds to the TLR4, initiating an immune response characterized by the secretion of pro-inflammatory cytokines such as IL-1B, TNF-α, IL-6, IL-8, and IL-12 (Dobrovolskaia & Vogel, 2002a; Rogero & Calder, 2018a). Additionally, in response to LPS, macrophages release prostaglandins, enzymes, and reactive oxygen species, further increasing the production of pro-inflammatory mediators and promoting macrophage infiltration (Cani et al., 2008).

We previously mentioned that obesity and chronic inflammation induce insulin resistance. However, insulin resistance also plays a role in inducing inflammation. Insulin resistance may contribute to a Th1 pro-inflammatory response, and the production of MCP-1 which recruits monocytes that get activated as M1 macrophages (Shimobayashi et al., 2018). In addition, insulin resistance favors the expression of TLR4, therefore promoting an immune response (Rogero & Calder, 2018a).

Figure 1-6. Mechanisms by which obesity leads to immune dysfunction.[1]



[1] Abbreviations: M, M1 macrophage; LPS, Lipopolysaccharide; TLR4, Toll-Like Receptor 4. Adapted from She 2022. Created with Biorender.com

To recapitulate, we already presented diverse mechanisms that lead to systemic inflammation, which include adipose tissue hypertrophy, intestinal permeability, and insulin resistance. The presence of systemic inflammation has been associated with a higher risk of infections and impaired immune responses (Furman et al., 2019; Mcdade et al., 2011; Sheridan et al., 2012); however, the exact mechanism is still unknown. Some hypotheses have pointed out that pro-inflammatory cytokines may desensitize immune cells, not letting them respond properly during a real infection (Milner & Beck, 2012). Additionally, some studies showed that the presence of chronic-low grade inflammation may produce low-grade activation of signaling pathways like Janus kinase/signal transducers and activators of

transcription which can cause impaired immune responses, and affect the proliferation and development of immune cells, thus producing immune dysfunction (Shen-Orr et al., 2016).

The presence of obesity and type 2 diabetes has been related to impaired T cell function. For instance, a study showed a reduction in IL-2 production from splenocytes after mitogen stimulation in cafeteria diet-induced obesity (DIO) Wistar rats (Lamas et al., 2002). Another study from our lab showed similar results in humans, where people with obesity and type 2 diabetes had lower levels of IL-2, IL-6, and TNF-α from PBMCs after T cell mitogen stimulation compared to metabolically healthy individuals with obesity (Richard et al., 2017).

Immune dysfunction is highly associated with insulin resistance, as immune cells require energy for their metabolic functions, especially when they are activated and exert their effector function (van Niekerk et al., 2020). Cells from the innate and adaptive immune systems express the insulin receptor (InsR) which allows the uptake and use of glucose (Boucher et al., 2014; Helderman & Strom, 1978; Tsai et al., 2018). However, when T cells lack InsR, they exhibit decreased antigen-specific proliferation, reduced production of pro-inflammatory cytokines, diminished Th and T cytotoxic response following influenza virus exposure, and impaired Treg cells by reducing the production of IL-10 (Tsai et al., 2018; Viola et al., 2019). Consequently, in diabetes, insulin resistance may also weaken their overall effector function (Makhijani et al., 2023).

To summarize, chronic-low grade systemic inflammation, insulin resistance, and obesity are some factors that contribute to the development of immune dysfunction, causing low immune responses and a higher risk of infections. Therefore, modifications in these factors are important to improve the health of the individuals living with obesity and metabolic complications.

1.3 Diet and the immune system

As previously mentioned, diet plays an important role in the immune system as it provides nutrients that offer immune cells the necessary energy to carry out their functions; they give rise to the production of new cells, proteins, and metabolites that, in turn, protect the body against pathogens and oxidative stress. Thus, good nutrition is necessary to provide optimal functionality of the immune system (P. C. Calder, 2023).

1.3.1 Nutrients related to the immune system

Polyphenols

Polyphenols are aromatic substances with antioxidant properties. They are commonly found in fruits, vegetables, whole grains, tea, chocolate, and wine (Boccellino & D'Angelo, 2020). Studies have shown that polyphenols play a role in obesity by regulating hunger and satiety (H. Liu et al., 2024), and by reducing hyperinsulinemia (Leontieva et al., 2013), lipogenesis (Timmers et al., 2011), and adipocyte differentiation, while increasing lipolysis (Mamun et al., 2024), and promoting beneficial gut microbiota (Queipo-Ortuño et al., 2012).

In addition to their metabolic properties, polyphenols also have immunomodulatory effects such as promoting the anti-inflammatory polarization of macrophages into an M2 phenotype (Mohammadi et al., 2019) and inhibiting the NF-kB activation in adipocytes while promoting the production of anti-inflammatory cytokines (Gonzales & Orlando, 2008). Furthermore, some polyphenols may reduce macrophage infiltration into adipose tissue (Islam et al., 2021) and increase the ability of NK cells to destroy infected cells (Y. Lee et al., 2021). All together, polyphenols help improve the immune responses while controlling inflammation.

Fiber

Fibers, an essential component of the diet, are fermented in the gut by the microbiota (Jha & Berrocoso, 2016), resulting in the production of short-chain fatty acids that have properties that support the function of the intestinal barrier (Wrzosek et al., 2013). They have an action in intestinal homeostasis by fostering the differentiation of intestinal Treg cells that release IL-10 (P. M. Smith et al., 2013). Other studies have shown that fibers contribute to the inhibition of the NF-kB pathway (Kopczyńska & Kowalczyk, 2024), and are associated with

lower levels of CRP, white blood cell count, and systemic inflammation response (Qi et al., 2023).

Fatty acids

Fatty acids are other crucial nutrients that have immunomodulatory properties. They will be discussed in more detail in section 1.3.3.

1.3.2 Methods to measure food intake

Multiple dietary assessment tools have been designed to estimate the food and nutrient intake of individuals. These instruments are of great importance in research since they provide valuable information that leads to understanding the connection between diet and different health outcomes.

Diverse methods can be used depending on the context; however, for the purpose of this thesis, only those that evaluate consumption in individuals will be considered. The most common tools are self-reported, such as food frequency questionnaires (FFQ), 24-hour dietary recall, and food diaries/records. However, there are also other methods (e.g. the measurement of biomarkers) that provide information about food intake as well. Each method has its own strengths and limitations which should be considered to ensure the appropriate one is selected for the study. **Table 1-2**. Show a summary of these characteristics.

Food frequency questionnaires:

FFQs are tools that estimate the habitual dietary intake. They are designed with a list of foods and drinks and allow the participants to mention what of those foods they consume and how frequently. The most common FFQs are Harvard FFQ, Diet History questionnaire III, Block FFQ, and NHANES. FFQs are categorized as qualitative (the amount of food is not assessed), semi-quantitative (portion size is considered), and quantitative (when there is a correlation between frequency and food weight from a dietary record). FFQs are cost-effective and suitable tools for large sample sizes because participants can fill them out themselves. However, they are not precise in identifying absolute intakes, and it is challenging for participants to recall their estimated intake over time (Bailey, 2021). In addition to that, each FFQ uses specific databases for estimating food intake, bringing some limitations, since the databases may not be specific for the country in which the study was

conducted. This can lead to not reflecting accurately the nutrient composition of the foods due to different regulations in fortification, variations in agricultural practices, and may not reflect the cultural dietary differences across countries (NIH, 2024b).

24-Hour recall:

The 24-hour dietary recall provides information about the food consumed over a period of 24 hours. It encompasses portion sizes, preparations, and recipe ingredients including their brands. Some of the limitations of this tool is that it only reflects one day of consumption and they often require a trained interviewer to provide accurate results. However, to remedy these limitations it is possible to perform multiple recalls to have a better understanding of the usual intake (Bailey, 2021; Dao et al., 2019). Additionally, new technologies have emerged such as the Automated self-administrated 24-hour dietary assessment tool (ASA24), which is an automated web-based tool that participants can complete on their own, saving time and money with its usage (NIH, 2024a).

Dietary records/food diaries

Dietary records, also known as food diaries, are considered the gold standard method to measure food intake. It is used to register and describe all foods and drinks consumed over a period, typically 3-4 days; portion sizes should be specified by weight or estimation. An advantage of this tool is that participants record their intake during consumption, which helps eliminate memory bias since the information is immediately collected. Some often reported limitations are the change in participant's habitual food intake while completing their food record and the increased participant burden (Bailey, 2021). However, there are new technologies that facilitate the process of dietary records such as different apps and software (ex. Keenoa) that are able to give information about portion sizes through image-assisted technology and wearables, thereby giving more accurate information on food consumption (Eldridge et al., 2018).

Biomarkers:

Biomarkers are biological substances found in a sample that are used to reflect the consumption of a specific nutrient, food, or dietary pattern (Heinzmann et al., 2015; McGrath et al., 2016; Poppitt et al., 2005). Some of the most common are the measurement of fatty

acids in the blood, urinary sodium, potassium, vitamin D serum levels, and plasma carotenoids.

One benefit of using biomarkers is the elimination of recall bias that has the self-reported tools, giving a more objective measure of dietary intake (McNamara & Brennan, 2020). However, physiological and genetic factors can affect the outcomes (Bailey, 2021). Therefore, biomarkers must be validated in order to verify their accuracy in reflecting intake. This includes if there are time and dose-response relationships, their robustness against confounding factors, stability against degradation, accuracy of laboratory technique´s measurement, and reproducibility (Dragsted et al., 2018; McNamara & Brennan, 2020).

Table 1-2. Strengths and limitations of different methods to assess food intake.

Method	Strengths	Limitations
Food Frequency Questionnaire	● Assesses habitual intake over long periods. ● Useful for studies with a large sample size. ● Less expensive and time-consuming	● Potential recall bias ● Less precise ● Might be adjusted and validated to be used in different contexts.
24-Hour dietary recall	● Provides detailed intake data ● Easy to administer ● Accuracy can be improved by repetition	● Potential recall bias ● May not represent the usual intake ● Bias when formulating the questions
Food diaries/ records	● Provides detailed intake, including portion sizes. ● Can identify short-term intake variability	● Might be time-consuming ● There might be an alteration of eating behavior when recording.
Biomarkers	● Objective measurement ● Reduced reporting bias ● May reflect nutrient status and bioavailability	● Expensive ● Invasive method

1.3.3. Methods to assess fatty acid intake

The assessment of the intake of fatty acids can be done through different methods, including the self-assessment tools previously reviewed (FFQ, 24-hour recall, food diaries) or using biomarkers such as the measurement of plasma fatty acids, red blood cells (RBCs) membrane´s fatty acids, and adipose tissue biopsy that provide more objective outcomes.

Plasma fatty acids analysis

Measurement of total lipids and phospholipids in plasma or RBCs are techniques used to assess short-term and long-term fatty acid intake respectively. Analysis of plasma total lipids requires quantifying or determining the proportion of fatty acids found in the form of triglycerides, cholesterol esters, phospholipids, and free fatty acids. This method can be highly variable since it is affected by genetics, diet type, and fasting state. On the other hand, when analyzing RBCs phospholipids, which are major components of cell membranes, provide information on fatty acid status and membrane composition, being more stable since it eliminates the variability of the postprandial triglycerides (Brenna et al., 2018).

Red blood cell membrane fatty acid analysis

Assessing the fatty acid (FA) composition of RBC´s membrane offers an insight into the long-term dietary fat, reflecting the average dietary intake over the lifespan of these cells that approximately lasts 120 days, leading to a more stable assessment of habitual fat intake. (Brenna et al., 2018).

Adipose tissue biopsy

Another method to assess the FA intake is the adipose tissue biopsy. It consists of taking a small sample of adipose tissue, which provides both information about the FA composition and the long-term FA intake. However, this technique is more invasive than the previously discussed techniques. It is also important to consider that this technique only works to assess FAs that are not synthesized endogenously (e.g. polyunsaturated fatty acids (PUFA)). If other fatty acids are assessed then there could be errors of interpretation (Baylin et al., 2002).

1.3.4 Fatty acids and the immune system

Definition

FAs are hydrocarbon chains with carboxyl and methyl groups on each end. They differ from each other by having different numbers of carbons that vary from 2-30 and the presence or absence of double bonds. These molecules are components of lipids such as triglycerides, phospholipids, and cholesteryl esters, which are structural elements of cell membranes and serve to store energy (Burdge & Calder, 2015).

Classification

FAs can be classified based on their number of carbons: short-chain FAs have less than 6 carbons, medium-chain FAs between 6-12 carbons, and long-chain FAs have more than 12 carbons. They are also classified depending on their number of double bonds: Saturated fatty acids (SFA) do not have double bonds, monounsaturated fatty acids (MUFA) have one double bond, and PUFA have two or more double bonds in their composition (Burdge & Calder, 2015). **Table 1-3.** show the most important FAs and their classification.

Double bonds may also be located in different orientations. Cis isomers are those FAs that have their double bonds on the same side, while trans isomers have their double bonds on opposite sites (Burdge & Calder, 2015). This double bond orientation leads to different biological effects since cis isomers are characterized by having more fluid and flexible properties, contributing to optimal cell membrane fluidity. In contrast, trans isomers are more rigid, which leads them to interfere with normal cell function, thus being associated with adverse health effects like cardiovascular diseases, cancer, diabetes, and obesity (Dhaka et al., 2011; Valenzuela & Morgado, 1999).

Table 1-3. Names and classifications of fatty acids.

Notation	Trivial name	Classification	Main food sources
C12:0	Lauric acid	Saturated	Coconut oil
C13:0	Tridecanoic acid	Saturated	Dairy products
C14:0	Myristic acid	Saturated	Nutmeg, palm kernel, butter
C14:1	Myristoleic acid	Monounsaturated	Garfish, wheat, ginger
C15:0	Pentadecanoic acid	Saturated	Dairy, beef
C15:1	Pentadecenoic acid	Monounsaturated	Dairy
C16:0	Palmitic acid	Saturated	Palm oil
C16:1n9	Palmitoleic acid	Monounsaturated	Macadamia oil, fish
C16:1n7 cis-7	Hexadecenoic acid	Monounsaturated	Partially hydrogenated oils, dairy
C17:0	Heptadecanoic acid	Saturated	Dairy, fish
C17:1 cis-10	Heptadecenoic acid	Monounsaturated	Dairy
C18: 0	Stearic acid	Saturated	Beef, fish, milk, grains

C18:1n9	Oleic acid	Monounsaturated	Olive oil, avocado, nuts, seeds.
C18:1tn7 trans	Vaccenic acid	Monounsaturated	Dairy, beef
C18:2n6	Linoleic acid	Polyunsaturated	Safflower oil, nuts, seeds, meat, eggs
C18:3n3	Alpha-Linolenic acid	Polyunsaturated	Soybean oil, flaxseeds, canola oil
C20: 0	Arachidic acid	Saturated	Meat, eggs, organ meats
C20:1n9	Gadoleic acid	Monounsaturated	Onion, carrot
C20:2n6	Eicosadienoic acid	Polyunsaturated	Vegetable oils
C20:3n6	Eicosatrienoic acid	Polyunsaturated	Vegetable oils
C20:4n6	Arachidonic acid	Polyunsaturated	Meat, diary
C20:5n3	Eicosapentaenoic acid	Polyunsaturated	Fish oil
C24:0	Tetracosanoic acid	Saturated	Fats
C24:1n9	Nervonic acid	Monounsaturated	Seeds
C22:5n3	Docosapentaenoic acid	Polyunsaturated	Fish oil
C22:6n3	Docosahexaenoic acid	Polyunsaturated	Fish oil

Fatty acid metabolism

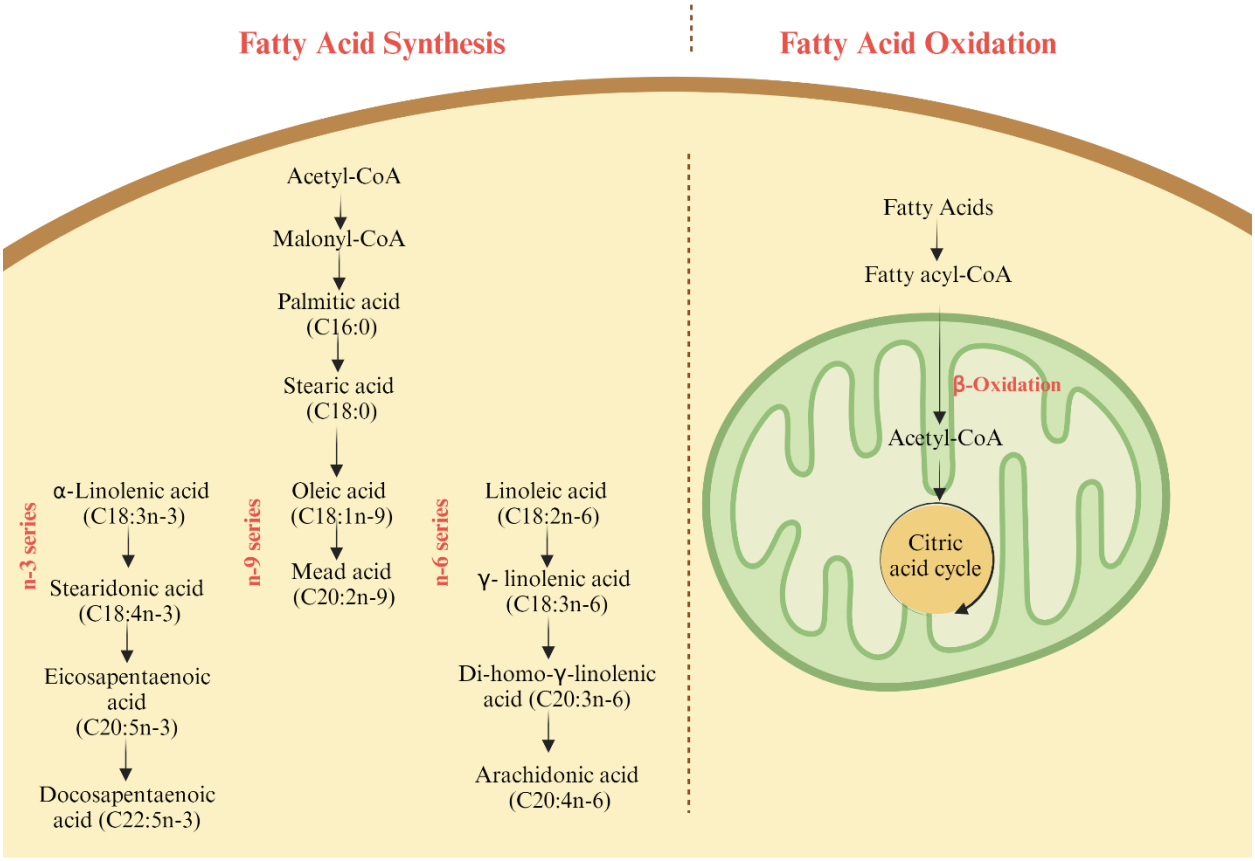
FA metabolism consists of both FA catabolism and anabolism, processes that are summarized in **Figure 1-7**.

The anabolic pathway leads to the synthesis of FA. This process occurs by carboxylation of acetyl-CoA to form malonyl-CoA, catalyzed by the enzyme acetyl-CoA carboxylase. Then, the enzyme fatty acid synthase complex adds two carbons in each cycle to elongate until palmitate acid with 16 carbons is obtained. After that, there can be more modifications, such as elongation or desaturation, to form other long-chain FAs from the family of n-9. However, the body cannot synthesize n-3 and n-6 fatty acids due to the lack of specific enzymes, therefore these fatty acids must be obtained from food dietary sources (Chandel, 2021).

The FA catabolism, also known as β-oxidation, starts with the formation of fatty acyl-CoA that is then transferred to the mitochondria, where it goes under oxidation, hydration, second

oxidation, and thiolysis, shortening two carbons each cycle, producing acetyl-CoA that enters in the citric acid cycle to finally produce energy (Chandel, 2021).

Figure 1-7. Fatty acid metabolism.[1]



[1] Abbreviations: Acetyl-CoA, Acetyl coenzyme A; n, omega. Adapted from Navdeep 2021. Created with Biorender.com

1.3.4.1 Effect of fatty acids on the immune system

Saturated fatty acids

SFAs might be harmful to human health since they are considered to have pro-inflammatory properties. Within this group, lauric acid is the one recognized as having the stronger inflammatory properties (Fritsche, 2015a). SFAs have a similar structure to LPS and bind to the TLR4, leading to the stimulation of an inflammatory response, secreting pro-inflammatory cytokines like TNF-α, IL-1B, IL-18, and IL-6. (Hwang et al., 2016a; J. Y. Lee et al., 2001a; J. Y. Lee, Ye, et al., 2003; Ravaut et al., 2020; Rogero & Calder, 2018a; Wong

et al., 2009). Other mechanisms that SFAs have to induce inflammation is through the increased expression of hepatic TLR4, which gives rise to high plasma levels of TNF-α, IL6, and MCP-1 and lower levels of IL-10 (Ravaut et al., 2020; Zaki et al., 2015). It has also been found that diets rich in SFAs produce larger lipid droplets in the adipocytes, producing higher storage of triglycerides that increase the secretion of leptin, leading to the secretion of pro-inflammatory cytokines from macrophages (Carbone et al., 2012; Jiménez-Gómez et al., 2009; Zhang & Chua, 2017).

Monounsaturated fatty acids

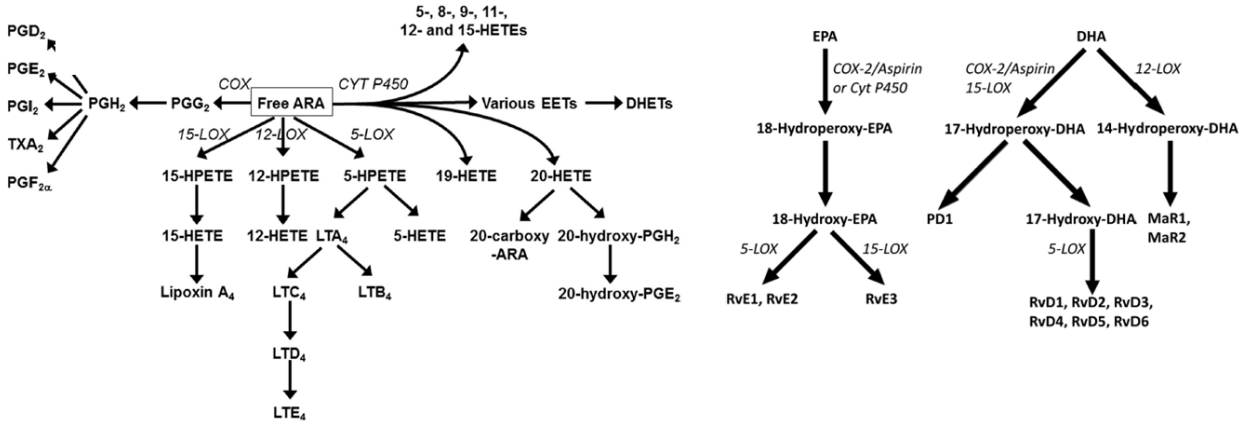
Less is known about the effects of MUFA on the immune system. They have been considered as being neutral compared to SFA and PUFA. However, recent studies have suggested a link to an anti-inflammatory response (Rocha et al., 2017). Some studies have shown that high consumption of olive oil can lead to suppressive effects of NK activity (Jeffery et al., 1997; Yaqoob et al., 1994a, 1998), a lower proliferation of lymphocytes after the stimulation with a T cell mitogen (Yaqoob et al., 1994b), a decrease in the production of IL-1B and TNF-α after LPS stimulation (Kien et al., 2015), and rise the proportion of M2 macrophages (Montserrat-de la Paz et al., 2019).

Other investigations have evaluated the actions of MUFA by using the Mediterranean diet (MedDiet), which contains high levels of oleic acid (Shively et al., 2019a). Casas et al. found a significant decrease in CRP, TNF-α, and IL-6 in two types of MedDiet, one supplemented with extra virgin olive oil and the other supplemented with nuts, compared to a low-fat diet (Casas et al., 2016). These changes might be attributable to weight loss; however, Richard et al. reported that the MedDiet was efficient at reducing the levels of CRP even without weight loss (Richard et al., 2013). Even though some studies have shown positive outcomes, it is still unknown if these effects are produced by MUFA or by the concurrent reduction in SFA intake when consuming more MUFA (Yaqoob, 2002a).

Polyunsaturated fatty acids

Omega 3 (n-3) and omega 6 (n-6) are the major families of PUFAs; they have anti and pro-inflammatory effects, respectively, primarily via the production of their lipid mediators (**Figure 1-8)**.

Figure 1-8. Overview of the production of lipid mediators.[1]



[1] Abbreviations: COX, cyclooxygenase; Cyt P450, cytochrome P450; LOX, lipoxygenase; MaR, maresin; PD, protectin D; Rv, resolvin. AEA, arachidonoyl ethanolamine (also called anandamide); 2-AG, 2-arachidonoyl glycerol; ARA, arachidonic acid; DHET, dihydroxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; LT, leukotriene; PG, prostaglandin; TX, thromboxane. Taken from (P. C. Calder, 2017; Innes & Calder, 2018) Permission from: Elsevier and Portlandpress.

Omega 3

A high consumption of n-3 has been associated with anti-inflammatory responses and with the resolution of inflammation (Dalli et al., 2013; Innes & Calder, 2018). Within this group, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been found to mitigate the activation of TLR4 and consequently NF-kB pathway (J. Y. Lee, Plakidas, et al., 2003; Y. Liu et al., 2012; Rogero & Calder, 2018a), reducing the production of pro-inflammatory cytokines (P. C. Calder, 2009) and increasing regulatory cytokines (P. C. Calder, 2015, 2017).

Another important characteristic of these FAs is their production of anti-inflammatory lipid mediators such as resolvins, protectins, and maresins. Some of these, like resolvin E1, resolvin D1 and protectin D1 inhibit migration of neutrophils into sites of inflammation, and resolvin D1 and protecting D1 inhibit IL-1B production (Bannenberg & Serhan, 2010; P. C. Calder, 2017; Serhan et al., 2008; Serhan & Chiang, 2013). Additionally, when there is an increased consumption of n-3, these FAs start accumulating in the cell membrane, replacing arachidonic acid (AA, an n-6), leading to a more inflammatory resolution environment (Djuricic & Calder, 2022; Innes & Calder, 2018; Rogero & Calder, 2018a).

EPA and DHA can also inhibit other aspects of inflammation, like leucocyte chemotaxis, expression of adhesion molecules VCAM-1, ICAM-1 (P. C. Calder, 2017; Miles et al., 2000; Sanderson & Calder, 1998), and modulation of microbiota acting as prebiotic agents (Djuricic & Calder, 2022).

Omega 6

N-6 fatty acids are considered pro-inflammatory due to the eicosanoids that are produced after stimulation, which are prostaglandins, thromboxanes, and leukotrienes (Epstein et al., 1990; Tilley et al., 2001). Some of the best studied are prostaglandin E2 and leukotriene B4 which increase vascular permeability, fever, vasodilatation, and chemotaxis (Rogero & Calder, 2018a). ARA is an important n-6 present in cell membrane phospholipids, which has an important role as a precursor of pro-inflammatory mediators (Innes & Calder, 2018). Interestingly, some of its mediators, like prostaglandin E2, can also reduce inflammation by lowering IL-1B and TNF-α levels (Rogero & Calder, 2018a). In addition, prostaglandin E3, thromboxane A3, and leukotriene B5 have lower inflammatory effects, leading n-6 to have both pro-inflammatory and anti-inflammatory responses (Miles et al., 2002; Yu et al., 1995).

Linoleic acid (LA) can also contribute to inflammation since it is metabolized by the LOX enzyme and limits the synthesis of EPA (Chan et al., 1993). However, it has been found that dietary intake of LA is not associated with increased inflammatory markers in plasma (Innes & Calder, 2018; Pischon et al., 2003); this could happen because the consumption of LA does not lead to an increase in ARA in PBMCs, even though it is its precursor (Yaqoob et al., 2000).

To sum up, different FAs have important functions that impact inflammation, and therefore immune function. SFAs are considered pro-inflammatory due to the fact that they bind to TLR4, MUFAs have a more neutral effect with some potential anti-inflammatory actions. Whereas within PUFAs, n-3 generally have anti-inflammatory and n-6 pro-inflammatory properties. However, they can both produce lipid mediators that may confer both effects.

1.4 Literature cited

Abdelaal, M., le Roux, C. W., & Docherty, N. G. (2017). Morbidity and mortality associated with obesity. Annals of Translational Medicine, 5(7), 161–161. https://doi.org/10.21037/atm.2017.03.107

Adam, L., López-González, M., Björk, A., Pålsson, S., Poux, C., Wahren-Herlenius, M., Fernández, C., & Spetz, A.-L. (2018). Early Resistance of Non-virulent Mycobacterial Infection in C57BL/6 Mice Is Associated With Rapid Up-Regulation of Antimicrobial Cathelicidin Camp. Frontiers in Immunology, 9. https://doi.org/10.3389/fimmu.2018.01939

Aguirre, V., Uchida, T., Yenush, L., Davis, R., & White, M. F. (2000). The c-Jun NH2-terminal Kinase Promotes Insulin Resistance during Association with Insulin Receptor Substrate-1 and Phosphorylation of Ser307. Journal of Biological Chemistry, 275(12), 9047–9054. https://doi.org/10.1074/jbc.275.12.9047

Aristizábal, B., & González, Á. (2013). Innate immune system. In J. Anaya, Y. Shoenfeld, & A. Rojas-Villarraga (Eds.), Autoimmunity: From Bench to Bedside. El Rosario University Press.

Ashen, M. D., & Blumenthal, R. S. (2005). Low HDL Cholesterol Levels. New England Journal of Medicine, 353(12), 1252–1260. https://doi.org/10.1056/NEJMcp044370

Aziz, M., Iheanacho, F., & Hashmi, M. (2023). Physiology, Antibody. StatPearls.

Bachmann, M. F., & Oxenius, A. (2007). Interleukin 2: from immunostimulation to immunoregulation and back again. EMBO Reports, 8(12), 1142–1148. https://doi.org/10.1038/sj.embor.7401099

Bailey, R. L. (2021). Overview of dietary assessment methods for measuring intakes of foods, beverages, and dietary supplements in research studies. Current Opinion in Biotechnology, 70, 91–96. https://doi.org/10.1016/j.copbio.2021.02.007

Bannenberg, G., & Serhan, C. N. (2010). Specialized pro-resolving lipid mediators in the inflammatory response: An update. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1801(12), 1260–1273. https://doi.org/10.1016/j.bbalip.2010.08.002

Baylin, A., Kabagambe, E. K., Siles, X., & Campos, H. (2002). Adipose tissue biomarkers of fatty acid intake. The American Journal of Clinical Nutrition, 76(4), 750–757. https://doi.org/10.1093/ajcn/76.4.750

BD biosciences. (2003). Techniques for Immune Function Analysis (1st ed.).

Bercovici, N., Duffour, M. T., Agrawal, S., Salcedo, M., & Abastado, J. P. (2000). New methods for assessing T-cell responses. Clinical and Diagnostic Laboratory Immunology, 7(6), 859–864. https://doi.org/10.1128/CDLI.7.6.859-864.2000

Blair, A. S., Hajduch, E., Litherland, G. J., & Hundal, H. S. (1999). Regulation of Glucose Transport and Glycogen Synthesis in L6 Muscle Cells during Oxidative Stress. Journal of Biological Chemistry, 274(51), 36293–36299. https://doi.org/10.1074/jbc.274.51.36293

Blumenreich, M. (1990). The White Blood Cell and Differential Count. In H. Walker, W. Hall, & J. Hurst (Eds.), The History, Physical, and Laboratory Examinations.

Boccellino, M., & D'Angelo, S. (2020). Anti-Obesity Effects of Polyphenol Intake: Current Status and Future Possibilities. International Journal of Molecular Sciences, 21(16), 5642. https://doi.org/10.3390/ijms21165642

Boi, S. K., Buchta, C. M., Pearson, N. A., Francis, M. B., Meyerholz, D. K., Grobe, J. L., & Norian, L. A. (2016). Obesity alters immune and metabolic profiles: New insight from obese-resistant mice on high-fat diet. Obesity, 24(10), 2140–2149. https://doi.org/10.1002/oby.21620

Boucher, J., Kleinridders, A., & Kahn, C. R. (2014). Insulin Receptor Signaling in Normal and Insulin-Resistant States. Cold Spring Harbor Perspectives in Biology, 6(1), a009191–a009191. https://doi.org/10.1101/cshperspect.a009191

Brenna, J. T., Plourde, M., Stark, K. D., Jones, P. J., & Lin, Y.-H. (2018). Best practices for the design, laboratory analysis, and reporting of trials involving fatty acids. The American Journal of Clinical Nutrition, 108(2), 211–227. https://doi.org/10.1093/ajcn/nqy089

Burdge, G. C., & Calder, P. C. (2015). Introduction to Fatty Acids and Lipids (pp. 1–16). https://doi.org/10.1159/000365423

Calder, P. C. (2009). Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. Biochimie, 91(6), 791–795. https://doi.org/10.1016/j.biochi.2009.01.008

Calder, P. C. (2015). Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1851(4), 469–484. https://doi.org/10.1016/j.bbalip.2014.08.010

Calder, P. C. (2017). Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochemical Society Transactions, 45(5), 1105–1115. https://doi.org/10.1042/BST20160474

Calder, P. C. (2022). Dietary factors and low-grade inflammation in relation to overweight and obesity revisted. British Journal of Nutrition, 127(10), 1455–1457. https://doi.org/10.1017/S0007114522000782

Calder, P. C. (2023). Nutrition and immunity: lessons from coronavirus disease-2019. Proceedings of the Nutrition Society, 1–16. https://doi.org/10.1017/S0029665123004792

Calder, P., & Kulkarni, A. (2018). Nutrition, immunity, and infection . Taylor & Francis Group.

Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A. M., Delzenne, N. M., & Burcelin, R. (2008). Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet–Induced Obesity and Diabetes in Mice. Diabetes, 57(6), 1470–1481. https://doi.org/10.2337/db07-1403

Carbone, F., La Rocca, C., & Matarese, G. (2012). Immunological functions of leptin and adiponectin. Biochimie, 94(10), 2082–2088. https://doi.org/10.1016/j.biochi.2012.05.018

Carpentier, A. C. (2021). 100 anniversary of the discovery of insulin perspective: insulin and adipose tissue fatty acid metabolism. American Journal of Physiology-Endocrinology and Metabolism, 320(4), E653–E670. https://doi.org/10.1152/ajpendo.00620.2020

Casas, R., Sacanella, E., Urpí-Sardà, M., Corella, D., Castañer, O., Lamuela-Raventos, R.-M., Salas-Salvadó, J., Martínez-González, M.-A., Ros, E., & Estruch, R. (2016). Long-Term Immunomodulatory Effects of a Mediterranean Diet in Adults at High Risk of Cardiovascular Disease in the PREvención con DIeta MEDiterránea (PREDIMED) Randomized Controlled Trial. The Journal of Nutrition, 146(9), 1684–1693. https://doi.org/10.3945/jn.115.229476

Chan, J. K., McDonald, B. E., Gerrard, J. M., Bruce, V. M., Weaver, B. J., & Holub, B. J. (1993). Effect of dietary α-linolenic acid and its ratio to linoleic acid on platelet and plasma fatty acids and thrombogenesis. Lipids, 28(9), 811–817. https://doi.org/10.1007/BF02536235

Chandel, N. S. (2021). Lipid Metabolism. Cold Spring Harbor Perspectives in Biology, 13(9), a040576. https://doi.org/10.1101/cshperspect.a040576

Chen, Y., & Junger, W. G. (2012). Measurement of Oxidative Burst in Neutrophils (pp. 115–124). https://doi.org/10.1007/978-1-61779-527-5_8

Chidambaram, A. G., & Josephson, M. (2019). Clinical research study designs: The essentials. Pediatric Investigation, 3(4), 245–252. https://doi.org/10.1002/ped4.12166

Corripio-Miyar, Y., Hayward, A., Lemon, H., Sweeny, A. R., Bal, X., Kenyon, F., Pilkington, J. G., Pemberton, J. M., Nussey, D. H., & McNeilly, T. N. (2022). Functionally distinct T-helper cell phenotypes predict resistance to different types of parasites in a wild mammal. Scientific Reports, 12(1), 3197. https://doi.org/10.1038/s41598-022-07149-9

Cunningham-Rundles, S. (1994). Malnutrition and gut immune function. Current Opinion in Gastroenterology, 10(6), 664–670.

Dalli, J., Colas, R. A., & Serhan, C. N. (2013). Novel n-3 Immunoresolvents: Structures and Actions. Scientific Reports, 3(1), 1940. https://doi.org/10.1038/srep01940

Dao, M. C., Subar, A. F., Warthon-Medina, M., Cade, J. E., Burrows, T., Golley, R. K., Forouhi, N. G., Pearce, M., & Holmes, B. A. (2019). Dietary assessment toolkits: an overview. Public Health Nutrition, 22(3), 404–418. https://doi.org/10.1017/S1368980018002951

de Heredia, F. P., Gómez-Martínez, S., & Marcos, A. (2012). Obesity, inflammation and the immune system. Proceedings of the Nutrition Society, 71(2), 332–338. https://doi.org/10.1017/S0029665112000092

Dhaka, V., Gulia, N., Ahlawat, K. S., & Khatkar, B. S. (2011). Trans fats—sources, health risks and alternative approach - A review. Journal of Food Science and Technology, 48(5), 534–541. https://doi.org/10.1007/s13197-010-0225-8

Djuricic, I., & Calder, P. C. (2022). Polyunsaturated fatty acids and metabolic health: novel insights. Current Opinion in Clinical Nutrition & Metabolic Care, 25(6), 436–442. https://doi.org/10.1097/MCO.0000000000000865

Dobrovolskaia, M. A., & Vogel, S. N. (2002). Toll receptors, CD14, and macrophage activation and deactivation by LPS. Microbes and Infection, 4(9), 903–914. https://doi.org/10.1016/S1286-4579(02)01613-1

Dragsted, L. O., Gao, Q., Scalbert, A., Vergères, G., Kolehmainen, M., Manach, C., Brennan, L., Afman, L. A., Wishart, D. S., Andres Lacueva, C., Garcia-Aloy, M., Verhagen, H., Feskens, E. J. M., & Praticò, G. (2018). Validation of biomarkers of food intake—critical assessment of candidate biomarkers. Genes & Nutrition, 13(1), 14. https://doi.org/10.1186/s12263-018-0603-9

Eldridge, A. L., Piernas, C., Illner, A.-K., Gibney, M. J., Gurinović, M. A., De Vries, J. H. M., & Cade, J. E. (2018). Evaluation of New Technology-Based Tools for Dietary Intake Assessment—An ILSI Europe Dietary Intake and Exposure Task Force Evaluation. Nutrients, 11(1), 55. https://doi.org/10.3390/nu11010055

Epstein, F. H., Lewis, R. A., Austen, K. F., & Soberman, R. J. (1990). Leukotrienes and Other Products of the 5-Lipoxygenase Pathway. New England Journal of Medicine, 323(10), 645–655. https://doi.org/10.1056/NEJM199009063231006

Everman, S., Meyer, C., Tran, L., Hoffman, N., Carroll, C. C., Dedmon, W. L., & Katsanos, C. S. (2016). Insulin does not stimulate muscle protein synthesis during increased plasma branched-chain amino acids alone but still decreases whole body proteolysis in humans. American Journal of Physiology-Endocrinology and Metabolism, 311(4), E671–E677. https://doi.org/10.1152/ajpendo.00120.2016

Fattahi, Y., Heidari, H. R., & Khosroushahi, A. Y. (2020). Review of short-chain fatty acids effects on the immune system and cancer. Food Bioscience, 38, 100793. https://doi.org/10.1016/j.fbio.2020.100793

Feingold, K., & Grunfeld, C. (2000). The Effect of Inflammation and Infection on Lipids and Lipoproteins (K. Feingold, B. Anawalt, & M. Blackman, Eds.). Endotext [Internet].

Folch, J., Busquets, O., Ettcheto, M., Sánchez-López, E., Pallàs, M., Beas-Zarate, C., Marin, M., Casadesus, G., Olloquequi, J., Auladell, C., & Camins, A. (2018). Experimental Models for Aging and their Potential for Novel Drug Discovery. Current Neuropharmacology, 16(10), 1466–1483. https://doi.org/10.2174/1570159X15666170707155345

Fritsche, K. L. (2015). The Science of Fatty Acids and Inflammation. Advances in Nutrition, 6(3), 293S-301S. https://doi.org/10.3945/an.114.006940

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. Nature Medicine, 25(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Gombart, A. F., Pierre, A., & Maggini, S. (2020). A Review of Micronutrients and the Immune System–Working in Harmony to Reduce the Risk of Infection. Nutrients, 12(1), 236. https://doi.org/10.3390/nu12010236

Gonzales, A. M., & Orlando, R. A. (2008). Curcumin and resveratrol inhibit nuclear factor-kappaB-mediated cytokine expression in adipocytes. Nutrition & Metabolism, 5(1), 17. https://doi.org/10.1186/1743-7075-5-17

Government of Canada. (2016, March 1). Leading causes of hospitalizations, Canada, 2009/10, males and females combined, counts.

Guo, S. (2014). Insulin signaling, resistance, and metabolic syndrome: insights from mouse models into disease mechanisms. Journal of Endocrinology, 220(2), T1–T23. https://doi.org/10.1530/JOE-13-0327

Han, M. S., Jung, D. Y., Morel, C., Lakhani, S. A., Kim, J. K., Flavell, R. A., & Davis, R. J. (2013). JNK Expression by Macrophages Promotes Obesity-Induced Insulin Resistance and Inflammation. Science, 339(6116), 218–222. https://doi.org/10.1126/science.1227568

Hartemink, N., Boshuizen, H. C., Nagelkerke, N. J. D., Jacobs, M. A. M., & van Houwelingen, H. C. (2006). Combining Risk Estimates from Observational Studies with Different Exposure Cutpoints: A Meta-analysis on Body Mass Index and Diabetes Type 2. American Journal of Epidemiology, 163(11), 1042–1052. https://doi.org/10.1093/aje/kwj141

Heinzmann, S. S., Holmes, E., Kochhar, S., Nicholson, J. K., & Schmitt-Kopplin, P. (2015). 2-Furoylglycine as a Candidate Biomarker of Coffee Consumption. Journal of Agricultural and Food Chemistry, 63(38), 8615–8621. https://doi.org/10.1021/acs.jafc.5b03040

Helderman, J. H., & Strom, T. B. (1978). Specific insulin binding site on T and B lymphocytes as a marker of cell activation. Nature, 274(5666), 62–63. https://doi.org/10.1038/274062a0

Hirosumi, J., Tuncman, G., Chang, L., Görgün, C. Z., Uysal, K. T., Maeda, K., Karin, M., & Hotamisligil, G. S. (2002). A central role for JNK in obesity and insulin resistance. Nature, 420(6913), 333–336. https://doi.org/10.1038/nature01137

Hossain, P., Kawar, B., & El Nahas, M. (2007). Obesity and Diabetes in the Developing World — A Growing Challenge. New England Journal of Medicine, 356(3), 213–215. https://doi.org/10.1056/NEJMp068177

Hsu, A., Aronoff, D. M., Phipps, J., Goel, D., & Mancuso, P. (2007). Leptin improves pulmonary bacterial clearance and survival in ob/ob mice during pneumococcal pneumonia. Clinical and Experimental Immunology, 150(2), 332–339. https://doi.org/10.1111/j.1365-2249.2007.03491.x

Hwang, D. H., Kim, J.-A., & Lee, J. Y. (2016). Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. European Journal of Pharmacology, 785, 24–35. https://doi.org/10.1016/j.ejphar.2016.04.024

Immunology of the rat. (1998). In Handbook of Vertebrate Immunology (pp. 137–222). Elsevier. https://doi.org/10.1016/B978-012546401-7/50007-X

Innes, J. K., & Calder, P. C. (2018). Omega-6 fatty acids and inflammation. Prostaglandins, Leukotrienes and Essential Fatty Acids, 132, 41–48. https://doi.org/10.1016/j.plefa.2018.03.004

Islam, T., Koboziev, I., Albracht-Schulte, K., Mistretta, B., Scoggin, S., Yosofvand, M., Moussa, H., Zabet-Moghaddam, M., Ramalingam, L., Gunaratne, P. H., & Moustaid-Moussa, N. (2021). Curcumin Reduces Adipose Tissue Inflammation and Alters Gut Microbiota in Diet-Induced Obese Male Mice. Molecular Nutrition & Food Research, 65(22). https://doi.org/10.1002/mnfr.202100274

Janeway, C., Travers, P., & Walport, M. (2001). The Immune System in Health and Disease (5th ed.). Garland Science.

Janssen, I., Katzmarzyk, P. T., & Ross, R. (2004). Waist circumference and not body mass index explains obesity-related health risk. The American Journal of Clinical Nutrition, 79(3), 379–384. https://doi.org/10.1093/ajcn/79.3.379

Jeffery, N. M., Cortina, M., Newsholme, E. A., & Calder, P. C. (1997). Effects of variations in the proportions of saturated, monounsaturated and polyunsaturated fatty acids in the rat diet on spleen lymphocyte functions. British Journal of Nutrition, 77(5), 805–823. https://doi.org/10.1079/BJN19970077

Jha, R., & Berrocoso, J. F. D. (2016). Dietary fiber and protein fermentation in the intestine of swine and their interactive effects on gut health and on the environment: A review. Animal Feed Science and Technology, 212, 18–26. https://doi.org/10.1016/j.anifeedsci.2015.12.002

Jiang, S.-Z., Lu, W., Zong, X.-F., Ruan, H.-Y., & Liu, Y. (2016). Obesity and hypertension. Experimental and Therapeutic Medicine, 12(4), 2395–2399. https://doi.org/10.3892/etm.2016.3667

Jiménez-Gómez, Y., López-Miranda, J., Blanco-Colio, L. M., Marín, C., Pérez-Martínez, P., Ruano, J., Paniagua, J. A., Rodríguez, F., Egido, J., & Pérez-Jiménez, F. (2009). Olive oil and walnut breakfasts reduce the postprandial inflammatory response in mononuclear cells compared with a butter breakfast in healthy men. Atherosclerosis, 204(2), e70–e76. https://doi.org/10.1016/j.atherosclerosis.2008.09.011

Jung, T., Schauer, U., Heusser, C., Neumann, C., & Rieger, C. (1993). Detection of intracellular cytokines by flow cytometry. Journal of Immunological Methods, 159(1–2), 197–207. https://doi.org/10.1016/0022-1759(93)90158-4

Karpe, F., Dickmann, J. R., & Frayn, K. N. (2011). Fatty Acids, Obesity, and Insulin Resistance: Time for a Reevaluation. Diabetes, 60(10), 2441–2449. https://doi.org/10.2337/db11-0425

Kersten, S. (2001). Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Reports, 2(4), 282–286. https://doi.org/10.1093/embo-reports/kve071

Kien, C. L., Bunn, J. Y., Fukagawa, N. K., Anathy, V., Matthews, D. E., Crain, K. I., Ebenstein, D. B., Tarleton, E. K., Pratley, R. E., & Poynter, M. E. (2015). Lipidomic evidence that lowering the typical dietary palmitate to oleate ratio in humans decreases the leukocyte production of proinflammatory cytokines and muscle expression of redox-sensitive genes. The Journal of Nutritional Biochemistry, 26(12), 1599–1606. https://doi.org/10.1016/j.jnutbio.2015.07.014

Kievit, P., Howard, J. K., Badman, M. K., Balthasar, N., Coppari, R., Mori, H., Lee, C. E., Elmquist, J. K., Yoshimura, A., & Flier, J. S. (2006). Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. Cell Metabolism, 4(2), 123–132. https://doi.org/10.1016/j.cmet.2006.06.010

Kim, C.-S., Park, H.-S., Kawada, T., Kim, J.-H., Lim, D., Hubbard, N. E., Kwon, B.-S., Erickson, K. L., & Yu, R. (2006). Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. International Journal of Obesity, 30(9), 1347–1355. https://doi.org/10.1038/sj.ijo.0803259

Kim, S. H., & Reaven, G. M. (2008). Insulin Resistance and Hyperinsulinemia. Diabetes Care, 31(7), 1433–1438. https://doi.org/10.2337/dc08-0045

Kintscher, U., Hartge, M., Hess, K., Foryst-Ludwig, A., Clemenz, M., Wabitsch, M., Fischer-Posovszky, P., Barth, T. F. E., Dragun, D., Skurk, T., Hauner, H., Blüher, M., Unger, T., Wolf, A.-M., Knippschild, U., Hombach, V., & Marx, N. (2008). T-lymphocyte Infiltration in Visceral Adipose Tissue. Arteriosclerosis, Thrombosis, and Vascular Biology, 28(7), 1304–1310. https://doi.org/10.1161/ATVBAHA.108.165100

Klop, B., Elte, J., & Cabezas, M. (2013). Dyslipidemia in Obesity: Mechanisms and Potential Targets. Nutrients, 5(4), 1218–1240. https://doi.org/10.3390/nu5041218

Kopczyńska, J., & Kowalczyk, M. (2024). The potential of short-chain fatty acid epigenetic regulation in chronic low-grade inflammation and obesity. Frontiers in Immunology, 15. https://doi.org/10.3389/fimmu.2024.1380476

Kornberg, M. D. (2020). The immunologic Warburg effect: Evidence and therapeutic opportunities in autoimmunity. WIREs Systems Biology and Medicine, 12(5). https://doi.org/10.1002/wsbm.1486

Kuby, J., Kindt, T., Goldsby, R., & Osborne, B. (2007). Kuby Immunology (6th ed.). W.H. Freeman.

Kyriakis, J. M., & Avruch, J. (1996). Sounding the Alarm: Protein Kinase Cascades Activated by Stress and Inflammation. Journal of Biological Chemistry, 271(40), 24313–24316. https://doi.org/10.1074/jbc.271.40.24313

Lamas, O., Martinez, J. A., & Marti, A. (2002). T-helper lymphopenia and decreased mitogenic response in cafeteria diet-induced obese rats. Nutrition Research, 22(4), 497–506. https://doi.org/10.1016/S0271-5317(02)00362-7

Lauterbach, M. A. R., & Wunderlich, F. T. (2017). Macrophage function in obesity-induced inflammation and insulin resistance. Pflügers Archiv - European Journal of Physiology, 469(3–4), 385–396. https://doi.org/10.1007/s00424-017-1955-5

Lee, J. Y., Plakidas, A., Lee, W. H., Heikkinen, A., Chanmugam, P., Bray, G., & Hwang, D. H. (2003). Differential modulation of Toll-like receptors by fatty acids: preferential inhibition by n-3 polyunsaturated fatty acids. Journal of Lipid Research, 44(3), 479–486. https://doi.org/10.1194/jlr.M200361-JLR200

Lee, J. Y., Sohn, K. H., Rhee, S. H., & Hwang, D. (2001). Saturated Fatty Acids, but Not Unsaturated Fatty Acids, Induce the Expression of Cyclooxygenase-2 Mediated through Toll-like Receptor 4. Journal of Biological Chemistry, 276(20), 16683–16689. https://doi.org/10.1074/jbc.M011695200

Lee, J. Y., Ye, J., Gao, Z., Youn, H. S., Lee, W. H., Zhao, L., Sizemore, N., & Hwang, D. H. (2003). Reciprocal Modulation of Toll-like Receptor-4 Signaling Pathways Involving MyD88 and Phosphatidylinositol 3-Kinase/AKT by Saturated and Polyunsaturated Fatty Acids. Journal of Biological Chemistry, 278(39), 37041–37051. https://doi.org/10.1074/jbc.M305213200

Lee, Y., Shin, H., & Kim, J. (2021). In vivo Anti-Cancer Effects of Resveratrol Mediated by NK Cell Activation. Journal of Innate Immunity, 13(2), 94–106. https://doi.org/10.1159/000510315

Leontieva, O. V, Paszkiewicz, G., Demidenko, Z. N., & Blagosklonny, M. V. (2013). Resveratrol potentiates rapamycin to prevent hyperinsulinemia and obesity in male mice on high fat diet. Cell Death & Disease, 4(1), e472–e472. https://doi.org/10.1038/cddis.2012.202

Levin, B. E., Dunn-Meynell, A. A., Balkan, B., & Keesey, R. E. (1997). Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 273(2), R725–R730. https://doi.org/10.1152/ajpregu.1997.273.2.R725

Li, C., Wang, G., Sivasami, P., Ramirez, R. N., Zhang, Y., Benoist, C., & Mathis, D. (2021). Interferon-α-producing plasmacytoid dendritic cells drive the loss of adipose tissue regulatory T cells during obesity. Cell Metabolism, 33(8), 1610-1623.e5. https://doi.org/10.1016/j.cmet.2021.06.007

Lindemann, M. (2014). Ex vivo assessment of cellular immune function – applications in patient care and clinical studies. Tissue Antigens, 84(5), 439–449. https://doi.org/10.1111/tan.12454

Lisby, A. N., Carlson, R. D., Baybutt, T. R., Weindorfer, M., & Snook, A. E. (2022). Evaluation of CAR-T cell cytotoxicity: Real-time impedance-based analysis (pp. 81–98). https://doi.org/10.1016/bs.mcb.2021.08.002

Liu, H., Guo, X., Jiang, K., Shi, B., Liu, L., Hou, R., Chen, G., Farag, M. A., Yan, N., & Liu, L. (2024). Dietary polyphenols regulate appetite mechanism via gut-brain axis and gut homeostasis. Food Chemistry, 446, 138739. https://doi.org/10.1016/j.foodchem.2024.138739

Liu, Y., Chen, F., Odle, J., Lin, X., Jacobi, S. K., Zhu, H., Wu, Z., & Hou, Y. (2012). Fish Oil Enhances Intestinal Integrity and Inhibits TLR4 and NOD2 Signaling Pathways in

Weaned Pigs after LPS Challenge3. The Journal of Nutrition, 142(11), 2017–2024. https://doi.org/10.3945/jn.112.164947

Long, A., Kleiner, A., & Looney, R. J. (2023). Immune dysregulation. Journal of Allergy and Clinical Immunology, 151(1), 70–80. https://doi.org/10.1016/j.jaci.2022.11.001

Lopez-Jimenez, F., Almahmeed, W., Bays, H., Cuevas, A., Di Angelantonio, E., le Roux, C. W., Sattar, N., Sun, M. C., Wittert, G., Pinto, F. J., & Wilding, J. P. H. (2022). Obesity and cardiovascular disease: mechanistic insights and management strategies. A joint position paper by the World Heart Federation and World Obesity Federation. European Journal of Preventive Cardiology, 29(17), 2218–2237. https://doi.org/10.1093/eurjpc/zwac187

Luck, H., Khan, S., Kim, J. H., Copeland, J. K., Revelo, X. S., Tsai, S., Chakraborty, M., Cheng, K., Tao Chan, Y., Nøhr, M. K., Clemente-Casares, X., Perry, M.-C., Ghazarian, M., Lei, H., Lin, Y.-H., Coburn, B., Okrainec, A., Jackson, T., Poutanen, S., … Winer, D. A. (2019). Gut-associated IgA+ immune cells regulate obesity-related insulin resistance. Nature Communications, 10(1), 3650. https://doi.org/10.1038/s41467-019-11370-y

Luck, H., Tsai, S., Chung, J., Clemente-Casares, X., Ghazarian, M., Revelo, X. S., Lei, H., Luk, C. T., Shi, S. Y., Surendra, A., Copeland, J. K., Ahn, J., Prescott, D., Rasmussen, B. A., Chng, M. H. Y., Engleman, E. G., Girardin, S. E., Lam, T. K. T., Croitoru, K., … Winer, D. A. (2015). Regulation of Obesity-Related Insulin Resistance with Gut Anti-inflammatory Agents. Cell Metabolism, 21(4), 527–542. https://doi.org/10.1016/j.cmet.2015.03.001

Lumeng, C. N., Bodzin, J. L., & Saltiel, A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of Clinical Investigation, 117(1), 175–184. https://doi.org/10.1172/JCI29881

Mak, T. W., & Saunders, M. E. (2006). T Cell Activation. In The Immune Response (pp. 373–401). Elsevier. https://doi.org/10.1016/B978-012088451-3.50016-8

Makhijani, P., Basso, P. J., Chan, Y. T., Chen, N., Baechle, J., Khan, S., Furman, D., Tsai, S., & Winer, D. A. (2023). Regulation of the immune system by the insulin receptor in health and disease. Frontiers in Endocrinology, 14. https://doi.org/10.3389/fendo.2023.1128622

Makki, K., Froguel, P., & Wolowczuk, I. (2013). Adipose Tissue in Obesity-Related Inflammation and Insulin Resistance: Cells, Cytokines, and Chemokines. ISRN Inflammation, 2013, 1–12. https://doi.org/10.1155/2013/139239

Mamun, M. A. Al, Rakib, A., Mandal, M., Kumar, S., Singla, B., & Singh, U. P. (2024). Polyphenols: Role in Modulating Immune Function and Obesity. Biomolecules, 14(2), 221. https://doi.org/10.3390/biom14020221

Maruyama, S. (2018). The Functional Assessment of T cells (pp. 177–199). https://doi.org/10.1007/978-1-4939-8802-0_19

Matia-Garcia, I., Vadillo, E., Pelayo, R., Muñoz-Valle, J. F., García-Chagollán, M., Loaeza-Loaeza, J., Vences-Velázquez, A., Salgado-Goytia, L., García-Arellano, S., & Parra-Rojas, I. (2021). Th1/Th2 Balance in Young Subjects: Relationship with Cytokine Levels and Metabolic Profile. Journal of Inflammation Research, Volume 14, 6587–6600. https://doi.org/10.2147/JIR.S342545

Mcdade, T. W., Adair, L., Feranil, A. B., & Kuzawa, C. (2011). Positive antibody response to vaccination in adolescence predicts lower C-reactive protein concentration in young adulthood in the philippines. American Journal of Human Biology, 23(3), 313–318. https://doi.org/10.1002/ajhb.21128

McGrath, A. J., Hamill, L. L., Cardwell, C. R., Draffin, C. R., Neville, C. E., Appleton, K. M., McEneny, J., McKinley, M. C., Young, I. S., & Woodside, J. V. (2016). Combining vitamin C and carotenoid biomarkers better predicts fruit and vegetable intake than individual biomarkers in dietary intervention studies. European Journal of Nutrition, 55(4), 1377–1388. https://doi.org/10.1007/s00394-015-0953-7

McNamara, A. E., & Brennan, L. (2020). Potential of food intake biomarkers in nutrition research. Proceedings of the Nutrition Society, 79(4), 487–497. https://doi.org/10.1017/S0029665120007053

McQuaid, S. E., Hodson, L., Neville, M. J., Dennis, A. L., Cheeseman, J., Humphreys, S. M., Ruge, T., Gilbert, M., Fielding, B. A., Frayn, K. N., & Karpe, F. (2011). Downregulation of Adipose Tissue Fatty Acid Trafficking in Obesity. Diabetes, 60(1), 47–55. https://doi.org/10.2337/db10-0867

Mestas, J., & Hughes, C. C. W. (2004). Of Mice and Not Men: Differences between Mouse and Human Immunology. The Journal of Immunology, 172(5), 2731–2738. https://doi.org/10.4049/jimmunol.172.5.2731

Miles, E. A., Allen, E., & Calder, P. C. (2002). In vitro effects of eicosanoids derived from different 20-carbon fatty acids on production of monocyte-derived cytokines in human whole blood cultures. Cytokine, 20(5), 215–223. https://doi.org/10.1006/cyto.2002.2007

Miles, E. A., Wallace, F. A., & Calder, P. C. (2000). Dietary fish oil reduces intercellular adhesion molecule 1 and scavenger receptor expression on murine macrophages. Atherosclerosis, 152(1), 43–50. https://doi.org/10.1016/S0021-9150(99)00446-3

Milner, J. J., & Beck, M. A. (2012). The impact of obesity on the immune response to infection. Proceedings of the Nutrition Society, 71(2), 298–306. https://doi.org/10.1017/S0029665112000158

Mohammadi, A., Blesso, C. N., Barreto, G. E., Banach, M., Majeed, M., & Sahebkar, A. (2019). Macrophage plasticity, polarization and function in response to curcumin, a diet-derived polyphenol, as an immunomodulatory agent. The Journal of Nutritional Biochemistry, 66, 1–16. https://doi.org/10.1016/j.jnutbio.2018.12.005

Montserrat-de la Paz, S., Naranjo, M. C., Millan-Linares, M. C., Lopez, S., Abia, R., Biessen, E. A. L., Muriana, F. J. G., & Bermudez, B. (2019). Monounsaturated Fatty Acids in a High-Fat Diet and Niacin Protect from White Fat Dysfunction in the Metabolic Syndrome. Molecular Nutrition & Food Research, 63(19). https://doi.org/10.1002/mnfr.201900425

Mörbe, U. M., Jørgensen, P. B., Fenton, T. M., von Burg, N., Riis, L. B., Spencer, J., & Agace, W. W. (2021). Human gut-associated lymphoid tissues (GALT); diversity, structure, and function. In Mucosal Immunology (Vol. 14, Issue 4, pp. 793–802). Springer Nature. https://doi.org/10.1038/s41385-021-00389-4

Mosca, S., Araújo, G., Costa, V., Correia, J., Bandeira, A., Martins, E., Mansilha, H., Tavares, M., & Coelho, M. P. (2022). Dyslipidemia Diagnosis and Treatment: Risk Stratification in Children and Adolescents. Journal of Nutrition and Metabolism, 2022, 1–10. https://doi.org/10.1155/2022/4782344

Mountain, I. M. (1955). Antibody Production by Spleen In Vitro. The Journal of Immunology, 74(4), 270–277. https://doi.org/10.4049/jimmunol.74.4.270

Mukherjee, P., Roy, S., Ghosh, D., & Nandi, S. K. (2022). Role of animal models in biomedical research: a review. Laboratory Animal Research, 38(1), 18. https://doi.org/10.1186/s42826-022-00128-1

NIH. (2024a). ASA24® Dietary Assessment Tool | EGRP/DCCPS/NCI/NIH. https://epi.grants.cancer.gov/asa24/

NIH. (2024b). Methods Used to Develop DHQ III Database | EGRP/DCCPS/NCI/NIH. https://epi.grants.cancer.gov/dhq3/database-methods.html

Pabst, R. (2020). The pig as a model for immunology research. Cell and Tissue Research, 380(2), 287–304. https://doi.org/10.1007/s00441-020-03206-9

Park, H. S., Park, J. Y., & Yu, R. (2005). Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-α and IL-6. Diabetes Research and Clinical Practice, 69(1), 29–35. https://doi.org/10.1016/j.diabres.2004.11.007

Paul, S., & Lal, G. (2017). The Molecular Mechanism of Natural Killer Cells Function and Its Importance in Cancer Immunotherapy. Frontiers in Immunology, 8. https://doi.org/10.3389/fimmu.2017.01124

Payne, K. J., & Crooks, G. M. (2007). Immune-Cell Lineage Commitment: Translation from Mice to Humans. Immunity, 26(6), 674–677. https://doi.org/10.1016/j.immuni.2007.05.011

Pearce, E. L. (2010). Metabolism in T cell activation and differentiation. Current Opinion in Immunology, 22(3), 314–320. https://doi.org/10.1016/j.coi.2010.01.018

Pearce, E. L., & Pearce, E. J. (2013). Metabolic Pathways in Immune Cell Activation and Quiescence. Immunity, 38(4), 633–643. https://doi.org/10.1016/j.immuni.2013.04.005

Pischon, T., Hankinson, S. E., Hotamisligil, G. S., Rifai, N., Willett, W. C., & Rimm, E. B. (2003). Habitual Dietary Intake of n-3 and n-6 Fatty Acids in Relation to Inflammatory Markers Among US Men and Women. Circulation, 108(2), 155–160. https://doi.org/10.1161/01.CIR.0000079224.46084.C2

Poppitt, S. D., Kilmartin, P., Butler, P., & Keogh, G. F. (2005). Assessment of erythrocyte phospholipid fatty acid composition as a biomarker for dietary MUFA, PUFA or saturated fatty acid intake in a controlled cross-over intervention trial. Lipids in Health and Disease, 4(1), 30. https://doi.org/10.1186/1476-511X-4-30

Prod´homme, M., Rieu, I., Balage, M., Dardevet, D., & Grizard, J. (2004). Insulin and amino acids both strongly participate to the regulation of protein metabolism. Current Opinion in Clinical Nutrition and Metabolic Care, 7(1), 71–77. https://doi.org/10.1097/00075197-200401000-00012

Purnell, J. (2000). Definitions, Classification, and Epidemiology of Obesity. (K. Feingold, B. Anawalt, & M. Blackman, Eds.). Endotext [Internet].

Qatanani, M., & Lazar, M. A. (2007). Mechanisms of obesity-associated insulin resistance: many choices on the menu. Genes & Development, 21(12), 1443–1455. https://doi.org/10.1101/gad.1550907

Qi, X., Li, Y., Fang, C., Jia, Y., Chen, M., Chen, X., & Jia, J. (2023). The associations between dietary fibers intake and systemic immune and inflammatory biomarkers, a multi-cycle study of NHANES 2015–2020. Frontiers in Nutrition, 10. https://doi.org/10.3389/fnut.2023.1242115

Queipo-Ortuño, M. I., Boto-Ordóñez, M., Murri, M., Gomez-Zumaquero, J. M., Clemente-Postigo, M., Estruch, R., Cardona Diaz, F., Andrés-Lacueva, C., & Tinahones, F. J. (2012). Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. The American Journal of Clinical Nutrition, 95(6), 1323–1334. https://doi.org/10.3945/ajcn.111.027847

Rahman, M. S., Hossain, K. S., Das, S., Kundu, S., Adegoke, E. O., Rahman, Md. A., Hannan, Md. A., Uddin, M. J., & Pang, M.-G. (2021). Role of Insulin in Health and Disease:

An Update. International Journal of Molecular Sciences, 22(12), 6403. https://doi.org/10.3390/ijms22126403

Ratajczak, W., Niedźwiedzka-Rystwej, P., Tokarz-Deptuła, B., & Deptuła, W. (2018). Immunological memory cells. Central European Journal of Immunology, 43(2), 194–203. https://doi.org/10.5114/ceji.2018.77390

Ravaut, G., Légiot, A., Bergeron, K.-F., & Mounier, C. (2020). Monounsaturated Fatty Acids in Obesity-Related Inflammation. International Journal of Molecular Sciences, 22(1), 330. https://doi.org/10.3390/ijms22010330

Reaven, G. M. (1988). Role of Insulin Resistance in Human Disease. Diabetes, 37(12), 1595–1607. https://doi.org/10.2337/diab.37.12.1595

Ren, G., Roberts, A. I., & Shi, Y. (2011). Adhesion molecules. Cell Adhesion & Migration, 5(1), 20–22. https://doi.org/10.4161/cam.5.1.13491

Resnick, E. S., Moshier, E. L., Godbold, J. H., & Cunningham-Rundles, C. (2012). Morbidity and mortality in common variable immune deficiency over 4 decades. Blood, 119(7), 1650–1657. https://doi.org/10.1182/blood-2011-09-377945

Richard, C., Couture, P., Desroches, S., & Lamarche, B. (2013). Effect of the mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. Obesity, 21(1), 51–57. https://doi.org/10.1002/oby.20239

Richard, C., Wadowski, M., Goruk, S., Cameron, L., Sharma, A. M., & Field, C. J. (2017). Individuals with obesity and type 2 diabetes have additional immune dysfunction compared with obese individuals who are metabolically healthy. BMJ Open Diabetes Research & Care, 5(1), e000379. https://doi.org/10.1136/bmjdrc-2016-000379

Rocha, D. M., Bressan, J., & Hermsdorff, H. H. (2017). The role of dietary fatty acid intake in inflammatory gene expression: a critical review. Sao Paulo Medical Journal, 135(2), 157–168. https://doi.org/10.1590/1516-3180.2016.008607072016

Rogero, M., & Calder, P. (2018). Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. Nutrients, 10(4), 432. https://doi.org/10.3390/nu10040432

SANDERSON, & CALDER. (1998). Dietary fish oil diminishes lymphocyte adhesion to macrophage and endothelial cell monolayers. Immunology, 94(1), 79–87. https://doi.org/10.1046/j.1365-2567.1998.00474.x

Serhan, C. N., & Chiang, N. (2013). Resolution phase lipid mediators of inflammation: agonists of resolution. Current Opinion in Pharmacology, 13(4), 632–640. https://doi.org/10.1016/j.coph.2013.05.012

Serhan, C. N., Chiang, N., & Van Dyke, T. E. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nature Reviews Immunology, 8(5), 349–361. https://doi.org/10.1038/nri2294

She, Y., Mangat, R., Tsai, S., Proctor, S. D., & Richard, C. (2022). The Interplay of Obesity, Dyslipidemia and Immune Dysfunction: A Brief Overview on Pathophysiology, Animal Models, and Nutritional Modulation. Frontiers in Nutrition, 9. https://doi.org/10.3389/fnut.2022.840209

Shen-Orr, S. S., Furman, D., Kidd, B. A., Hadad, F., Lovelace, P., Huang, Y.-W., Rosenberg-Hasson, Y., Mackey, S., Grisar, F. A. G., Pickman, Y., Maecker, H. T., Chien, Y., Dekker, C. L., Wu, J. C., Butte, A. J., & Davis, M. M. (2016). Defective Signaling in the JAK-STAT Pathway Tracks with Chronic Inflammation and Cardiovascular Risk in Aging Humans. Cell Systems, 3(4), 374-384.e4. https://doi.org/10.1016/j.cels.2016.09.009

Sheridan, P. A., Paich, H. A., Handy, J., Karlsson, E. A., Hudgens, M. G., Sammon, A. B., Holland, L. A., Weir, S., Noah, T. L., & Beck, M. A. (2012). Obesity is associated with impaired immune response to influenza vaccination in humans. International Journal of Obesity, 36(8), 1072–1077. https://doi.org/10.1038/ijo.2011.208

Shimobayashi, M., Albert, V., Woelnerhanssen, B., Frei, I. C., Weissenberger, D., Meyer-Gerspach, A. C., Clement, N., Moes, S., Colombi, M., Meier, J. A., Swierczynska, M. M., Jenö, P., Beglinger, C., Peterli, R., & Hall, M. N. (2018). Insulin resistance causes inflammation in adipose tissue. Journal of Clinical Investigation, 128(4), 1538–1550. https://doi.org/10.1172/JCI96139

Shively, C. A., Appt, S. E., Vitolins, M. Z., Uberseder, B., Michalson, K. T., Silverstein-Metzler, M. G., & Register, T. C. (2019). Mediterranean versus Western Diet Effects on Caloric Intake, Obesity, Metabolism, and Hepatosteatosis in Nonhuman Primates. Obesity, 27(5), 777–784. https://doi.org/10.1002/oby.22436

Shulman, G. I. (2000). Cellular mechanisms of insulin resistance. Journal of Clinical Investigation, 106(2), 171–176. https://doi.org/10.1172/JCI10583

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., Glickman, J. N., & Garrett, W. S. (2013). The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic T reg Cell Homeostasis. Science, 341(6145), 569–573. https://doi.org/10.1126/science.1241165

Smith, S. G., Smits, K., Joosten, S. A., van Meijgaarden, K. E., Satti, I., Fletcher, H. A., Caccamo, N., Dieli, F., Mascart, F., McShane, H., Dockrell, H. M., & Ottenhoff, T. H. M. (2015). Intracellular Cytokine Staining and Flow Cytometry: Considerations for Application in Clinical Trials of Novel Tuberculosis Vaccines. PLOS ONE, 10(9), e0138042. https://doi.org/10.1371/journal.pone.0138042

Song, H. K., & Hwang, D. Y. (2017). Use of C57BL/6N mice on the variety of immunological researches. Laboratory Animal Research, 33(2), 119. https://doi.org/10.5625/lar.2017.33.2.119

Srikakulapu, P., & McNamara, C. A. (2020). B Lymphocytes and Adipose Tissue Inflammation. Arteriosclerosis, Thrombosis, and Vascular Biology, 40(5), 1110–1122. https://doi.org/10.1161/ATVBAHA.119.312467

Stadler, J. T., & Marsche, G. (2020). Obesity-Related Changes in High-Density Lipoprotein Metabolism and Function. International Journal of Molecular Sciences, 21(23), 8985. https://doi.org/10.3390/ijms21238985

Statistics Canada. (2019, June 25). Overweight and obese adults, 2018.

Surmi, B., & Hasty, A. (2008). Macrophage infiltration into adipose tissue: initiation, propagation and remodeling. Future Lipidology, 3(5), 545–556. https://doi.org/10.2217/17460875.3.5.545

Talmadge, J. E., Meyers, K. M., Prieur, D. J., & Starkey, J. R. (1980). Role of natural killer cells in tumor growth and metastasis: C57BL/6 normal and beige mice. Journal of the National Cancer Institute, 65(5), 929–935.

Tanguay, S., & Killion, J. J. (1994). Direct comparison of ELISPOT and ELISA-based assays for detection of individual cytokine-secreting cells. Lymphokine and Cytokine Research, 13(4), 259–263.

Théry, C., & Amigorena, S. (2001). The cell biology of antigen presentation in dendritic cells. Current Opinion in Immunology, 13(1), 45–51. https://doi.org/10.1016/S0952-7915(00)00180-1

Tilley, S. L., Coffman, T. M., & Koller, B. H. (2001). Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. Journal of Clinical Investigation, 108(1), 15–23. https://doi.org/10.1172/JCI200113416

Timmers, S., Konings, E., Bilet, L., Houtkooper, R. H., van de Weijer, T., Goossens, G. H., Hoeks, J., van der Krieken, S., Ryu, D., Kersten, S., Moonen-Kornips, E., Hesselink, M. K. C., Kunz, I., Schrauwen-Hinderling, V. B., Blaak, E. E., Auwerx, J., & Schrauwen, P. (2011). Calorie Restriction-like Effects of 30 Days of Resveratrol Supplementation on Energy Metabolism and Metabolic Profile in Obese Humans. Cell Metabolism, 14(5), 612–622. https://doi.org/10.1016/j.cmet.2011.10.002

Tsai, S., Clemente-Casares, X., Zhou, A. C., Lei, H., Ahn, J. J., Chan, Y. T., Choi, O., Luck, H., Woo, M., Dunn, S. E., Engleman, E. G., Watts, T. H., Winer, S., & Winer, D. A. (2018).

Insulin Receptor-Mediated Stimulation Boosts T Cell Immunity during Inflammation and Infection. Cell Metabolism, 28(6), 922-934.e4. https://doi.org/10.1016/j.cmet.2018.08.003

Valenzuela, A., & Morgado, N. (1999). Trans fatty acid isomers in human health and in the food industry. Biological Research, 32(4). https://doi.org/10.4067/S0716-97601999000400007

van Niekerk, G., Christowitz, C., Conradie, D., & Engelbrecht, A.-M. (2020). Insulin as an immunomodulatory hormone. Cytokine & Growth Factor Reviews, 52, 34–44. https://doi.org/10.1016/j.cytogfr.2019.11.006

Vandevijvere, S., Chow, C. C., Hall, K. D., Umali, E., & Swinburn, B. A. (2015). Increased food energy supply as a major driver of the obesity epidemic: a global analysis. Bulletin of the World Health Organization, 93(7), 446–456. https://doi.org/10.2471/BLT.14.150565

Viola, A., Munari, F., Sánchez-Rodríguez, R., Scolaro, T., & Castegna, A. (2019). The Metabolic Signature of Macrophage Responses. Frontiers in Immunology, 10. https://doi.org/10.3389/fimmu.2019.01462

Vivier, E., Tomasello, E., Baratin, M., Walzer, T., & Ugolini, S. (2008). Functions of natural killer cells. Nature Immunology, 9(5), 503–510. https://doi.org/10.1038/ni1582

Wagar, L. E., DiFazio, R. M., & Davis, M. M. (2018). Advanced model systems and tools for basic and translational human immunology. Genome Medicine, 10(1), 73. https://doi.org/10.1186/s13073-018-0584-8

Wages, J. M. (2005). Polymerase chain reaction. In Encyclopedia of Analytical Science (pp. 243–250). Elsevier. https://doi.org/10.1016/B0-12-369397-7/00475-1

Wagner, N., Brandhorst, G., Czepluch, F., Lankeit, M., Eberle, C., Herzberg, S., Faustin, V., Riggert, J., Oellerich, M., Hasenfuss, G., Konstantinides, S., & Schäfer, K. (2013). Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk. Obesity, 21(3), 461–468. https://doi.org/10.1002/oby.20087

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation, 112(12), 1796–1808. https://doi.org/10.1172/JCI200319246

Weisheit, C. K., Engel, D. R., & Kurts, C. (2015). Dendritic Cells and Macrophages. Clinical Journal of the American Society of Nephrology, 10(10), 1841–1851. https://doi.org/10.2215/CJN.07100714

WHO. (2023, March 16). Hypertension.

WHO. (2024, March 1). Obesity and overweight.

Wilding, J. P. H., & Jacob, S. (2021). Cardiovascular outcome trials in obesity: A review. Obesity Reviews, 22(1). https://doi.org/10.1111/obr.13112

Wong, S. W., Kwon, M.-J., Choi, A. M. K., Kim, H.-P., Nakahira, K., & Hwang, D. H. (2009). Fatty Acids Modulate Toll-like Receptor 4 Activation through Regulation of Receptor Dimerization and Recruitment into Lipid Rafts in a Reactive Oxygen Species-dependent Manner. Journal of Biological Chemistry, 284(40), 27384–27392. https://doi.org/10.1074/jbc.M109.044065

Wrzosek, L., Miquel, S., Noordine, M.-L., Bouet, S., Chevalier-Curt, M. J., Robert, V., Philippe, C., Bridonneau, C., Cherbuy, C., Robbe-Masselot, C., Langella, P., & Thomas, M. (2013). Bacteroides thetaiotaomicron and Faecalibacterium prausnitziiinfluence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. BMC Biology, 11(1), 61. https://doi.org/10.1186/1741-7007-11-61

Wu, D., & Meydani, S. (2014). Age-Associated Changes in Immune Function: Impact of Vitamin E Intervention and the Underlying Mechanisms. Endocrine, Metabolic & Immune Disorders-Drug Targets, 14(4), 283–289. https://doi.org/10.2174/1871530314666140922143950

Yach, D., Stuckler, D., & Brownell, K. D. (2006). Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. Nature Medicine, 12(1), 62–66. https://doi.org/10.1038/nm0106-62

Yang, P.-C., & Mahmood, T. (2012). Western blot: Technique, theory, and trouble shooting. North American Journal of Medical Sciences, 4(9), 429. https://doi.org/10.4103/1947-2714.100998

Yang, W.-S., Chang, Y.-C., Chang, C.-H., Wu, L.-C., Wang, J.-L., & Lin, H.-H. (2021). The Association Between Body Mass Index and the Risk of Hospitalization and Mortality due to Infection: A Prospective Cohort Study. Open Forum Infectious Diseases, 8(1). https://doi.org/10.1093/ofid/ofaa545

Yaqoob, P. (2002). Monounsaturated fatty acids and immune function. European Journal of Clinical Nutrition, 56(S3), S9–S13. https://doi.org/10.1038/sj.ejcn.1601477

Yaqoob, P., Knapper, J., Webb, D., Williams, C., Newsholme, E., & Calder, P. (1998). Effect of olive oil on immune function in middle-aged men. The American Journal of Clinical Nutrition, 67(1), 129–135. https://doi.org/10.1093/ajcn/67.1.129

Yaqoob, P., Newsholme, E. A., & Calder, P. C. (1994a). Inhibition of natural killer cell activity by dietary lipids. Immunology Letters, 41(2–3), 241–247. https://doi.org/10.1016/0165-2478(94)90140-6

Yaqoob, P., Newsholme, E. A., & Calder, P. C. (1994b). The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. Immunology, 82(4), 603–610.

Yaqoob, Pala, Cortina-Borja, Newsholme, & Calder. (2000). Encapsulated fish oil enriched in α-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. European Journal of Clinical Investigation, 30(3), 260–274. https://doi.org/10.1046/j.1365-2362.2000.00623.x

Yiannikouris, F., Karounos, M., Charnigo, R., English, V. L., Rateri, D. L., Daugherty, A., & Cassis, L. A. (2012). Adipocyte-specific deficiency of angiotensinogen decreases plasma angiotensinogen concentration and systolic blood pressure in mice. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 302(2), R244–R251. https://doi.org/10.1152/ajpregu.00323.2011

Yu, K., Bayona, W., Kallen, C. B., Harding, H. P., Ravera, C. P., McMahon, G., Brown, M., & Lazar, M. A. (1995). Differential Activation of Peroxisome Proliferator-activated Receptors by Eicosanoids. Journal of Biological Chemistry, 270(41), 23975–23983. https://doi.org/10.1074/jbc.270.41.23975

Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z.-W., Karin, M., & Shoelson, S. E. (2001). Reversal of Obesity- and Diet-Induced Insulin Resistance with Salicylates or Targeted Disruption of Ikkβ. Science, 293(5535), 1673–1677. https://doi.org/10.1126/science.1061620

Zaki, S. M., Abdel Fattah, S., & Hassan, D. S. (2015). The differential effects of high-fat and high fructose diets on the liver of male albino rat and the proposed underlying mechanisms. Folia Morphologica. https://doi.org/10.5603/FM.a2018.0063

Zhang, Y., & Chua, S. (2017). Leptin Function and Regulation. In Comprehensive Physiology (pp. 351–369). Wiley. https://doi.org/10.1002/cphy.c160041

Zimmermann, P., & Curtis, N. (2019). Factors That Influence the Immune Response to Vaccination. Clinical Microbiology Reviews, 32(2). https://doi.org/10.1128/CMR.00084-18

Chapter 2: Research plan

2.1 Rationale

Obesity is a major public health problem as it is considered one of the most common non-communicable diseases worldwide (de Heredia et al., 2012). It is associated with different complications, including cardiovascular disease, T2D, cancer, and metabolic dysfunction-associated steatotic liver disease (Mokdad et al., 2003). Additionally, obesity has been related to a higher risk of post-operative infections (Lillenfeld et al., 1988a) and poorer outcomes of COVID-19 infection (Hoong et al., 2021).

Obesity has been associated with chronic low-grade systemic inflammation (de Heredia et al., 2012). Several factors contribute to this inflammatory state; one of them is adipose tissue hypertrophy, which leads to increased infiltration of macrophages in adipocytes acquiring an M1 pro-inflammatory phenotype (Weisberg et al., 2003a; Xu et al., 2003a). Consequently, pro-inflammatory cytokines such as interleukin (IL)-6, IL-1B, and TNF-α are secreted locally and systemically, resulting in systemic inflammation and insulin resistance (Hotamisligil, 2017).

Another factor that leads to systemic inflammation is the consumption of a HFD since it has been associated with increased gut permeability and LPS translocation, which causes endotoxemia (Luck et al., 2019a). LPS is a microbial component found in the outer membrane of gram-negative bacteria and recognized as being the primary agonist of TLR4 (Dobrovolskaia & Vogel, 2002a; Rogero & Calder, 2018a; Triantafilou & Triantafilou, 2002). The interaction between LPS and TLR4 induces the production of pro-inflammatory cytokines such as IL-6, TNF-α, IL-1B, IL-8, and IL-12. In addition to that, in response to LPS, macrophages secrete pro-inflammatory mediators to eliminate the pathogens (Basith et al., 2011; Könner & Brüning, 2011), thereby exacerbating systemic inflammation. In addition, insulin resistance intensifies inflammation by increasing the production of MCP-1 in adipocytes, which in turn recruits monocytes and triggers the activation of pro-inflammatory M1 macrophages (Shimobayashi et al., 2018).

On the other hand, when immune cells like T cells become insulin resistant, their proliferation diminishes, leading to decreased production of pro-inflammatory cytokines in response to

viral infections (Tsai et al., 2018). Consequently, all these factors contribute to an impaired immune response characterized by reduced proliferation, differentiation, and cytokine production by immune cells (Mito et al., 2000).

FAs exhibit different effects on the immune system. SFAs are recognized as being pro-inflammatory due to their structural resemblance to LPS, they act as non-microbial TLR4 agonists, producing an inflammatory response (Rocha et al., 2016). Conversely, PUFA possess both pro and anti-inflammatory actions depending on the lipid mediators that are synthesized from them. N-6 fatty acids such as arachidonic acid produce certain prostaglandins and leukotrienes, which promote inflammation, while n-3 fatty acids, particularly EPA and DHA, produce resolvins, protectins, and maresins, which facilitate inflammation resolution (P. C. Calder, 2017; Rogero & Calder, 2018a). MUFAs are often considered more neutral in their immunomodulatory effects.

For the context of this project, we were interested in investigating the effect of dietary FAs and their effect on immune function in the context of obesity and diabetes. While extensive research has explored the effects of SFA and PUFA on inflammation and the immune system, little is known about the specific effect of MUFA. Some studies have indicated the beneficial effects of diets rich in MUFA, such as the Mediterranean diet, in reducing systemic markers of inflammation like CRP and TNF-α (Casas et al., 2016; Hermsdorff et al., 2009). However, how the high consumption of MUFA modulates immune function in the context of obesity, is still unknown. Hence, our project address this gap by investigating the immunological effects of MUFA using both an animal model and a human cohort.

2.2 Objectives and hypotheses

The overall objective of this research is to investigate the role that dietary FAs have on immune function in the context of obesity. To address this overall aim, two specific objectives and their hypotheses were established:

1. The first objective of this research was to investigate the effect of a high MUFA diet on peripheral immune function (spleen) using a DIO rat model. We hypothesized that a high MUFA diet will reduce pro-inflammatory responses by immune cells that are generally associated with the consumption of a HFD, high in SFAs.
2. The second objective of this research was to explore the relationship between dietary fatty acids and immune function in people living with obesity and with and without metabolic complications. We hypothesized that a higher proportion of SFAs in plasma (reflective of food intake) will be associated with an increased secretion of pro-inflammatory cytokines by stimulated immune cells. A higher proportion of n-3 will lead to a reduction in pro-inflammatory cytokines while MUFAs will not lead to changes in the production of cytokines after immune cell stimulation exerting primarily a neutral effect.

2.3 Chapter format

These objectives and hypotheses were tested in different studies, which were organized into thesis chapters.

Chapter 3 evaluated the effect of a high MUFA diet on peripheral immune function (spleen) in the context of a DIO rat model. Objective 1 was addressed in this chapter. We found that MUFA reduced the production of pro-inflammatory cytokines after T cell stimulation, specifically reducing TNF-α and IL-6. No effect on APCs function was found. Additionally, high-fat diet (HF) with olive oil (HFOO) significantly diminished IL-2 secretion after ConA stimulation, suggesting a lower T cell proliferation when compared to HF.

Chapter 4 investigated the relationship between dietary FAs and immune function in people living with obesity and with and without metabolic complications. In summary, our results found that the proportions of SFAs and MUFAs in plasma increased across groups of obesity and metabolic complications, while PUFAs decreased. Additionally, plasma SFAs, mainly

palmitic acid, were positively associated with the production of IL-1B from APCs. Plasma MUFAs were not associated with changes in the production of pro-inflammatory cytokines. No significant association was found with n-3; however, PUFAs, especially linoleic acid, were negatively associated with the secretion of IL-1B by APC.

Chapter 5 provides an overall discussion that includes the results from each objective and hypotheses as well as directions for future research.

2.4 Literature cited

Basith, S., Manavalan, B., Lee, G., Kim, S. G., & Choi, S. (2011). Toll-like receptor modulators: a patent review (2006 – 2010). Expert Opinion on Therapeutic Patents, 21(6), 927–944. https://doi.org/10.1517/13543776.2011.569494

Calder, P. C. (2017). Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochemical Society Transactions, 45(5), 1105–1115. https://doi.org/10.1042/BST20160474

Casas, R., Sacanella, E., Urpí-Sardà, M., Corella, D., Castañer, O., Lamuela-Raventos, R.-M., Salas-Salvadó, J., Martínez-González, M.-A., Ros, E., & Estruch, R. (2016). Long-Term Immunomodulatory Effects of a Mediterranean Diet in Adults at High Risk of Cardiovascular Disease in the PREvención con DIeta MEDiterránea (PREDIMED) Randomized Controlled Trial. The Journal of Nutrition, 146(9), 1684–1693. https://doi.org/10.3945/jn.115.229476

de Heredia, F. P., Gómez-Martínez, S., & Marcos, A. (2012). Obesity, inflammation and the immune system. Proceedings of the Nutrition Society, 71(2), 332–338. https://doi.org/10.1017/S0029665112000092

Dobrovolskaia, M. A., & Vogel, S. N. (2002). Toll receptors, CD14, and macrophage activation and deactivation by LPS. Microbes and Infection, 4(9), 903–914. https://doi.org/10.1016/S1286-4579(02)01613-1

Hermsdorff, H. H. M., Zulet, M. Á., Abete, I., & Martínez, J. A. (2009). Discriminated benefits of a Mediterranean dietary pattern within a hypocaloric diet program on plasma RBP4 concentrations and other inflammatory markers in obese subjects. Endocrine, 36(3), 445–451. https://doi.org/10.1007/s12020-009-9248-1

Hoong, C. W. S., Hussain, I., Aravamudan, V. M., Phyu, E. E., Lin, J. H. X., & Koh, H. (2021). Obesity is Associated with Poor Covid-19 Outcomes: A Systematic Review and Meta-Analysis. Hormone and Metabolic Research, 53(02), 85–93. https://doi.org/10.1055/a-1326-2125

Hotamisligil, G. S. (2017). Foundations of Immunometabolism and Implications for Metabolic Health and Disease. Immunity, 47(3), 406–420. https://doi.org/10.1016/j.immuni.2017.08.009

Könner, A. C., & Brüning, J. C. (2011). Toll-like receptors: linking inflammation to metabolism. Trends in Endocrinology & Metabolism, 22(1), 16–23. https://doi.org/10.1016/j.tem.2010.08.007

Lillenfeld, D. E., Vlahov, D., Tenney, J. H., & McLaughlin, J. S. (1988). Obesity and diabetes as risk factors for postoperative wound infections after cardiac surgery. American Journal of Infection Control, 16(1), 3–6. https://doi.org/10.1016/0196-6553(88)90003-X

Luck, H., Khan, S., Kim, J. H., Copeland, J. K., Revelo, X. S., Tsai, S., Chakraborty, M., Cheng, K., Tao Chan, Y., Nøhr, M. K., Clemente-Casares, X., Perry, M.-C., Ghazarian, M., Lei, H., Lin, Y.-H., Coburn, B., Okrainec, A., Jackson, T., Poutanen, S., … Winer, D. A. (2019). Gut-associated IgA+ immune cells regulate obesity-related insulin resistance. Nature Communications, 10(1), 3650. https://doi.org/10.1038/s41467-019-11370-y

Mito, N., Hosoda, T., Kato, C., & Sato, K. (2000). Change of cytokine balance in diet-induced obese mice. Metabolism, 49(10), 1295–1300. https://doi.org/10.1053/meta.2000.9523

Rocha, D. M., Caldas, A. P., Oliveira, L. L., Bressan, J., & Hermsdorff, H. H. (2016). Saturated fatty acids trigger TLR4-mediated inflammatory response. Atherosclerosis, 244, 211–215. https://doi.org/10.1016/j.atherosclerosis.2015.11.015

Rogero, M., & Calder, P. (2018). Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. Nutrients, 10(4), 432. https://doi.org/10.3390/nu10040432

Shimobayashi, M., Albert, V., Woelnerhanssen, B., Frei, I. C., Weissenberger, D., Meyer-Gerspach, A. C., Clement, N., Moes, S., Colombi, M., Meier, J. A., Swierczynska, M. M., Jenö, P., Beglinger, C., Peterli, R., & Hall, M. N. (2018). Insulin resistance causes inflammation in adipose tissue. Journal of Clinical Investigation, 128(4), 1538–1550. https://doi.org/10.1172/JCI96139

Triantafilou, M., & Triantafilou, K. (2002). Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. Trends in Immunology, 23(6), 301–304. https://doi.org/10.1016/S1471-4906(02)02233-0

Tsai, S., Clemente-Casares, X., Zhou, A. C., Lei, H., Ahn, J. J., Chan, Y. T., Choi, O., Luck, H., Woo, M., Dunn, S. E., Engleman, E. G., Watts, T. H., Winer, S., & Winer, D. A. (2018). Insulin Receptor-Mediated Stimulation Boosts T Cell Immunity during Inflammation and Infection. Cell Metabolism, 28(6), 922-934.e4. https://doi.org/10.1016/j.cmet.2018.08.003

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation, 112(12), 1796–1808. https://doi.org/10.1172/JCI200319246

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., Sole, J., Nichols, A., Ross, J. S., Tartaglia, L. A., & Chen, H. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. Journal of Clinical Investigation, 112(12), 1821–1830. https://doi.org/10.1172/JCI200319451

Chapter 3: Consumption of a high-MUFA diet has an anti-inflammatory impact on T cell function in a DIO rat model

3.1 Introduction

Obesity is defined as the excess accumulation of body fat (Purnell, 2000). It has become a critical problem worldwide since it is related to the apparition of complications such as T2D, cardiovascular diseases, dyslipidemia, and hypertension. It has also been found that people living with obesity have a higher risk of post-operatory infections (Lillenfeld et al., 1988a) and more complications when contracting COVID-19 (Hoong et al., 2021) which suggests an impact on the immune system.

Obesity is characterized by having a state of chronic low-grade systemic inflammation, identified by having increased serum levels of CRP, TNF-α, and IL-6 (Bulló et al., 2003; Festa et al., 2001). This systemic inflammation appears due to various factors, that involve the consumption of a HFD, which increases gut permeability, leading to the translocation of LPS, a component of gram-negative bacteria, that binds the TLR4, thus starting an immune response (Dobrovolskaia & Vogel, 2002a; Luck et al., 2019b; Rogero & Calder, 2018a). In addition to that, adipocyte hypertrophy also plays an important role, since when adipocytes increase in size, they start attracting more immune cells, such as macrophages which shift their phenotype from an anti-inflammatory (M2) to a pro-inflammatory profile (M1), releasing cytokines such as IL-6, TNF-α that lead to insulin resistance and chemoattractants protein like MCP-1 that attracts more immune cells (C.-S. Kim et al., 2006; Lumeng et al., 2007; Makki et al., 2013; Weisberg et al., 2003a). Initially, this inflammation occurs locally in the adipose tissue, but eventually, these pro-inflammatory cytokines start moving to different tissues and travel through the bloodstream, generating systemic inflammation (Innes & Calder, 2018).

In the context of obesity, it is also known that even though there is a high level of inflammation, the immune response becomes less responsive to stimuli (Furman et al., 2019; Sheridan et al., 2012). This immune dysfunction may be due in part to a desensitization of immune cells to inflammatory markers (Milner & Beck, 2012). However, the presence of

insulin resistance often seen in obesity might also have a direct impact on immune function, given that when immune cells become insulin resistant, they are unable to get the necessary energy they need to carry out their functions leading to decreased proliferation and reduction in the production of pro-inflammatory cytokines upon virus exposure (Tsai et al., 2018).

FAs have immunomodulatory properties, playing crucial roles in regulating inflammation. SFAs are considered pro-inflammatory since it has been found that they can directly bind to the TLR4, inducing an immune response (Hwang et al., 2016a; Rogero & Calder, 2018a; Wong et al., 2009). PUFAs, specifically n-3 have anti-inflammatory properties due to the lipid mediators they generate such as resolvins, protectins, and maresins. (Bannenberg & Serhan, 2010; P. C. Calder, 2017; Serhan et al., 2008). On the other hand, n-6 fatty acids are considered more pro-inflammatory as they produce eicosanoids like prostaglandins, thromboxanes, and leukotrienes that increase inflammation (Epstein et al., 1990; Tilley et al., 2001). Nevertheless, little is known about MUFAs; traditionally they have been considered to possess neutral properties, however, some studies have shown that they could potentially have anti-inflammatory properties. For instance, it has been observed that the consumption of the MedDiet has beneficial health effects and helps decrease levels of CRP, TNF-α, and IL-6 improving the inflammatory state (Casas et al., 2016; Richard et al., 2013). Therefore, the aim of this study is to investigate the effect of a high MUFA diet on peripheral immune function (spleen) in the context of a DIO rat model. We hypothesized that a high MUFA diet will reduce pro-inflammatory responses by immune cells that are generally associated with the consumption of a HFD, high in SFAs.

3.2 Materials and Methods

3.2.1 Animals and Diets

All procedures were reviewed and approved by the Committee of Animal Policy and Welfare of the Faculty of Agriculture, Forestry and Home Economics at the University of Alberta and conducted following the Canadian Council on Animal Care guidelines (ACUCL092). The study was performed in 2011.

Male Sprague Dawley rats (n=24) of 6 weeks of age were obtained from Charles River Laboratories (Montreal, Quebec, Canada) and housed 2 per cage with 12/12-hour light/dark cycle and free access to water.

Three nutritionally adequate diets were designed for this study. A low-fat high carbohydrate diet (HC) providing 24% calories from fat (TD.06206, Harlan Teklad, Madison WI, with 11% w/w oil) (n=8). A standard high-fat diet (HF) providing 35% calories from fat (TD.84172, Harlan Teklad, Madison WI, with 17% w/w fat) (n=8) and a high-fat diet with olive oil (HFOO) high in MUFA (17% w/w fat) (n=8); where 33% of the fat mixture was replaced by olive oil. For the main outcomes, the glucose tolerance test, *ex vivo* cytokine production, and cell phenotyping were performed in 5-8 rats per group depending on the test and availability of immune cells.

The diet composition, fat mix, and macronutrient composition of the three experimental diets are presented in **Tables 3-1, 3-2,** and **3-3** respectively. The fatty acid composition of the diets was, analyzed by gas-liquid chromatography (GC) (Blewett et al., 2009a) which is presented in **Table 3-4**. All diets met the essential fatty acid requirements for rodents, had similar PUFA: SFA ratios and were vitamin E and phenol balanced.

During the first 3 weeks, HC and HF diets were used to induce obesity. After that time, animals in the HC group continued receiving the same diet, and those under the HF diet were randomized to either continue on the same HF control diet or to receive the HFOO for an additional 4 weeks.

Table 3-1. Composition of experimental diets (g/kg).[1]

Ingredient (g/kg diet)	HC	HF & HFOO
Casein	283,7	281,1
L-Methionine	2,6	2,6
Dextrose, monohydrate	245,6	217,4
Cornstarch	235,8	208,3
Cellulose	52,5	52,1
Fat mix[2]	107,2	166,6
Mineral Mix, Bemhart-Tomarelli (170750)	53,4	53,0
Sodium Selenite (0.0445% in sucrose)	0,3	0,3
Manganese Sulfate, monohydrate	0,3	0,2
Vitamin Mix, AOAC (40055)	10,5	10,4
Inositol	6,6	6,5
Choline Chloride	1,4	1,4

[1] Abbreviations: HC, High-carb diet; HF, High-fat diet; HFOO, High-fat diet with olive oil.
[2] See Table 3-4 for fatty acid composition.

Table 3-2. Fat mixture composition of experimental diets (%Fat provided by oil).[1]

Ingredient	HC	HF	HFOO
Sunflower oil, (%)	39	39	23
Flax oil, (%)	4	4	4
Tallow, (%)	57	57	40
Olive oil, (%)[2]	0	0	33

[1] Abbreviations: HC, High-carb diet; HF, High-fat diet; HFOO, High-fat diet with olive oil.
[2] Olive oil was a commercial, refined brand “Bertolli”.

Table 3-3. Macronutrient breakdown of experimental diets (%Energy).[1]

Macronutrient	HC	HF & HFOO
Carbohydrate, (%)	48	39
Fat, (%)	24	35
Protein, (%)	28	26

[1]Abbreviations: HC, High-carb diet; HF, High-fat diet; HFOO, High-fat diet with olive oil.

Table 3-4. Fatty acid composition of the experimental diets (g/100 g fatty acids).[1]

Fatty Acid	HC & HF	HFOO
16:0	15	16
16:1n9	0,35	0,28
18:0	45	36
18:1n9	14	27
18:1c11	0,23	1,1
18:2n6	23	17
20:0	0,39	0,39
18:3n3	2,3	1,9
20:3n6	0,38	0,25
22:4n6	0	0,1
Total SFA (%)	60	52
Total PUFA (%)	25	20
Total MUFA (%)	14	28
PUFA: SFA	0,4	0,4

[1] Abbreviations: SFA, Saturated fatty acids; PUFA, Polyunsaturated fatty acids; MUFA, Monounsaturated fatty acids; HC, High-carb diet; HF, High-fat diet; HFOO, High-fat diet with olive oil.

3.2.2 Glucose tolerance

An oral glucose tolerance test (OGTT) was performed one week prior to euthanasia. Animals were fasted overnight and blood was collected into a glucometer (Accu-chek Compact Plus, Roche, Laval, QC) strip to measure blood glucose concentration at baseline. The glucose dose per animal was 1g of glucose/kg of body weight which was administered orally and blood samples were taken at 10, 20, 30, 60, 90, and 120 minutes after the glucose was given and again measured using the glucometer.

3.2.3 Lymphocyte Isolation

Following four weeks on the experimental diets, animals were euthanized using a CO_2 chamber. At necroscopy, the spleen was collected aseptically, weighed and immune cells were isolated. The process of immune cell isolation has already been described (Field et al., 1990). Briefly, splenocyte cells were isolated by pushing cells through nylon mesh screens using the barrel of a sterile syringe in 5g/L Bovine serum albumin (BSA) (Sigma-Aldrich,

Oakville, ON, Canada) in Krebs-Ringer-Hepes (KRH) buffer. Each sample was centrifuged at 1000 rpm for 10 minutes to pellet cells (Jouan, Perkin Elmer, Woodbridge, ON, Canada). Splenocytes were treated with lysis buffer (ACK; 155 mM NH4Cl, 0.1mM EDTA, 10mM KHCO3, pH 7.4 (Simga Aldrich, Oakville, ON)) to lyse red blood cells and centrifuged. After discarding the supernatant the samples were washed with 10ml of KRH buffer, resuspended, and centrifuged again. 15ml of complete culture medium (CCM; RPMI 1640 with antibiotic and antimycotic, 5% v/v fetal calf serum (FCS; Invitrogen, Burlington, ON, Canada)) was added to the pellet of spleen samples, resuspended, and counted on a hemacytometer (Thermo Fisher Scientific, Ottawa, ON, Canada) using 20µl of the cell suspension and 20µl of trypan blue dye (Sigma).

3.2.4 Mitogen Stimulation

The ability to produce cytokines by splenocytes was determined following mitogen stimulation of isolated splenocytes, a process that has been previously described (Blewett et al., 2009b). Briefly, three ml of cells at a concentration of $1.25x10^6$cells/ml per mitogen for each sample was prepared with one of these mitogens: Con A (5µg/ml) (MP Biomedicals, Montreal PQ) and LPS (100 µg/ml) (Sigma, Oakville, ON) (eBioscience, San Diego, CA, US). Mitogen (300µl) was added to each sample at the specified concentration. Following the addition of mitogen, cell suspensions were incubated for 48 hours at 37°C and 5% CO_2. To stop the reaction, tubes were centrifuged at 1500 rpm for 10 minutes and the supernatant was collected and stored at -80°C until analysis for cytokines. Commercial enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, San Diego, US) were used to measure the concentration of IL-1B, IL-2, IL-6, IL-10, TNF-α, and IFN-γ concentration in supernatants following the manufacturer's instructions. All measurements were conducted in duplicates, with a coefficient of variation <15%.

3.2.5 Cell Phenotyping

To determine cell phenotypes an immunofluorescence assay was used as previously described (Field et al., 2000). Briefly, V-well plates were pre-conditioned with 4g/L FCS (Invitrogen) in phosphate-buffered saline (PBS) for 30 minutes. An aliquot of each sample (100,000 to 400,000 cells) was added to each well and washed by adding 200µl buffer

followed by centrifugation. A 20µl aliquot of fluorescent-labeled antibody was added to the wells according to the template (antibodies per panel: CD3/8/4, CD25/8/4, CD284/68, CD161/3, CD3/25/FoxP3/4, OX62/6) and incubated for 30 minutes in the refrigerator. The plate was washed twice with 200µl buffer and centrifuged. The supernatant was discarded and the remaining sample vortexed. Two hundred µl 1% w/v paraformaldehyde (Thermo Fisher Scienfic) was added to each well. The proportion of cells that were positive for each marker was determined by flow cytometry (FACSCalibur; Becton Dickinson, San Jose, CA, US) according to the relative fluorescence intensity using Cell-Quest software (Becton Dickinson, San Jose, CA, US). One, two, and three colors immunofluorescent analysis was performed.

3.2.6 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 29.0.2.0. Data are reported as means ± standard error of the mean (SEM). The study was powered to assess significant changes in immune function (*ex vivo* TNF-α production as the primary outcome). Incremental area under the curve (iAUC) was calculated by the trapezoidal method as the area under the curve above the baseline value. Differences between the diets were evaluated by using one-way ANOVA. In cases where there was a significant difference, a post hoc analysis was performed using Duncan to determine differences between groups. When data sets were not normally distributed the data was log transformed. If a normal distribution was determined following the log transformation statistical analysis was performed as described above. When a normal distribution was not obtained after using a log transformation, non-parametric statistical analysis was performed using a Kruskal-Wallace test to determine if differences existed between groups. Differences at $P \leq 0.05$ (two-sided) were considered significant.

3.3 Results

3.3.1 Animal Characteristics

After the 4 weeks of dietary intervention, there was no significant difference in body weight ($p > 0.05$) or spleen weight between the groups (**Table 3-5**). However, the HFOO group showed a significant reduction in liver weight when corrected for body weight compared to the control diets ($p < 0.05$).

Table 3-5. Anthropometric data of Sprague Dawley rats after intervention [1].

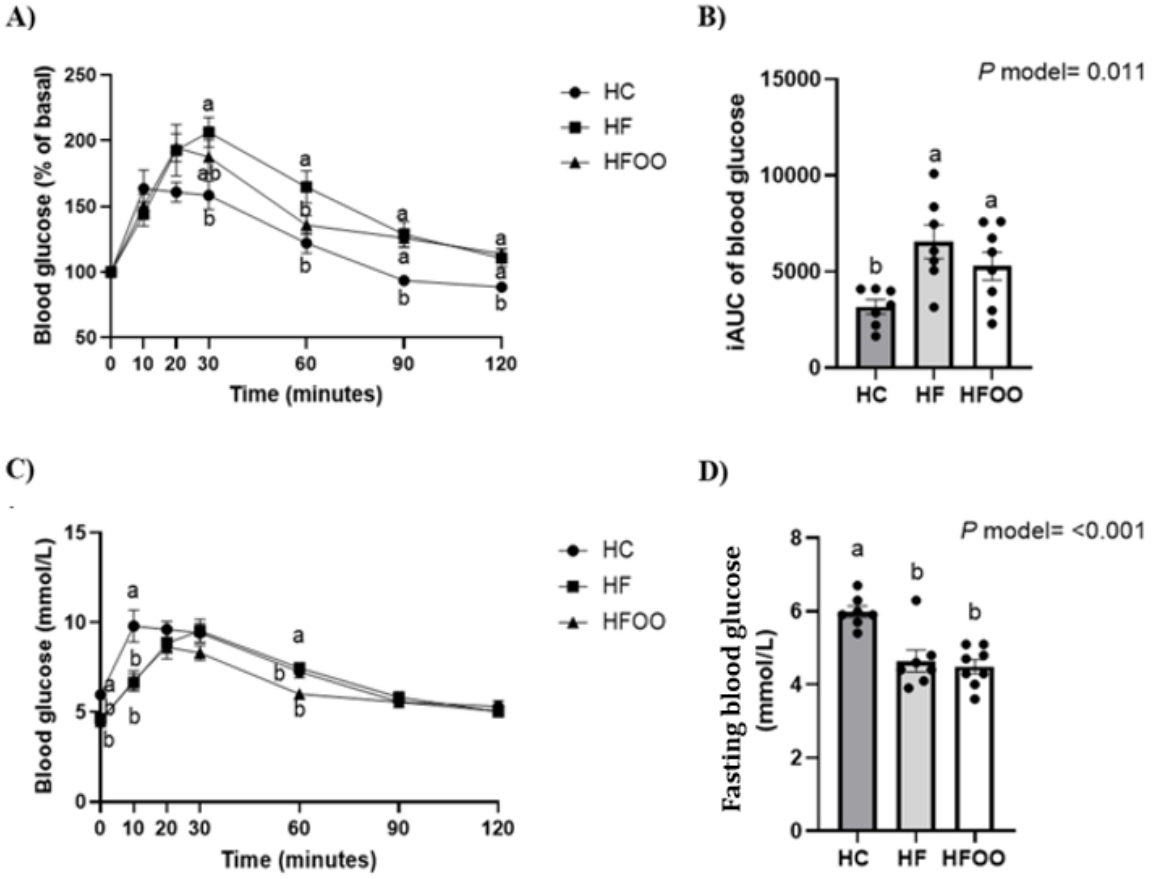
Variables	HC	HF	HFOO	*p* model
Body weight (g)	476,1 ± 5,6	464,1 ± 18,5	504,9 ± 11,9	0.101
Spleen weight (g)	0,9 ± 0,04	0,9 ± 0,08	0,9 ± 0,04	0.934
Liver weight (g)	18,8 ± 0,78	18,9 ± 1,08	15,8 ± 1,29	0.089
Spleen Weight: Body Weight (mg/g) [2]	1,9 ± 0,09	2,0 ± 0,11	1,9 ± 0,11	0.788
Liver Weight: Body Weight (mg/g) [2]	39,4 ± 1,33[a]	40,5 ± 1,08[a]	31,3 ± 2,65[b]	**0.009**
n /group	8	8	8	

[1]Values are means ± SEM. Groups that do not share the same letter are significantly different based on the one-way ANOVA test with the Duncan adjustment for multiple comparisons test ($p < 0.05$). HC, High-carb diet; HF, High-fat diet; HFOO, High-fat diet with olive oil.
[2]Analysis performed on log-transformed values.

3.3.2 Glucose levels

When rats were 12 weeks old, the OGTT test showed that the HC group had the highest fasting blood glucose levels (**Figure 3-1 C-D**) ($p < 0.05$). **Figure 3-1 A** shows the OGTT data normalized for baseline value. By 120 min the HC group had returned to baseline levels. However, neither HF nor HFOO returned to baseline levels by this time ($p < 0.05$). The iAUC showed that HC had the lowest overall glucose iAUC after 120 minutes compared to the other groups ($p < 0.05$) (**Figure 3-1 B**).

Figure 3-1. Effect of dietary interventions on blood glucose levels.[1]



(A) Blood glucose levels following an OGTT normalized for baseline value. (B) iAUC of blood glucose with baseline normalization, following an OGTT with the collection over 120 minutes. (C) Blood glucose levels following an OGTT with Log transformation (D) Fasting blood glucose after Log transformation. Values are means ± SEM p-model < 0.05 based on the one-way ANOVA test with the Duncan

[1]Abbreviations: HC, High-carb diet; HF, High-fat diet; HFOO, High-fat diet with olive oil; iAUC, incremental area under the curve.

3.3.3 *ex vivo* cytokine production by splenocytes

Cytokine production by splenocytes after mitogen stimulation is presented in **Table 3-6**. After the stimulation of T cells with ConA, a lower increase in IL-2, IL-6, and TNF-α was observed in the HFOO group compared to the control diets (p <0.05). There were no changes in IL-10 or the Th1:Th2 ratio represented by IFN-γ:IL-10 (p >0.05). Following the stimulation with a bacterial challenge LPS, there were no significant differences in IL-6, IL-10, or TNF-α across groups although the HC diet tended to lead to a higher production of IL-6 (p =0.065) and TNF-α (p =0.088) compared to both HF diets.

Table 3-6. *Ex vivo* cytokine production by mitogen-stimulated spleen cells.[1,2,3]

Cytokine	HC	HF	HFOO	*P-value*
		Con A (T cell mitogen)		
IL-2 [4]	3067± 72[a]	3054± 46[a]	2553± 105[b]	<0.001
IL-6	708± 65[a]	667± 106[a]	375± 41[b]	0.008
IL-10 [4]	336± 41	358± 69	245± 31	0.415
IFN-γ [4]	916± 79	706± 94	617± 41	0.061
TNF-α	163± 17[a]	177± 11[a]	97± 9[b]	<0.001
IFN-γ:IL-10	2,88± 0,36	2,8±0,85	2,8±0,34	0.995
		LPS (Bacterial challenge)		
IL-6 [4]	895± 88,40	621± 116	663± 35	0.065
IL-10 [4]	412± 9,89	442± 64	332± 55	0.249
TNF-α [4]	260± 27,79	194± 37	158± 11	0.088

[1]Values are means ± SEM p-model < 0.05 based on the one-way ANOVA test with the Duncan.
[2]Abbreviations: HC, High-carb diet; HF, High-fat diet; HFOO, High-fat diet with olive oil.
[3]The following supernatant cytokines were tested but resulted in nondetectable levels in the samples: ConA IL-1B, LPS IL-1B, and LPS IFN-γ.
[4]Analysis performed using non-parametric testing.

3.3.4 Splenocyte phenotype

Proportions of immune cell phenotypes from splenocytes are presented in **Table 3-7**. After diet intervention, results showed that rats fed the HFOO diet had a significantly lower amount of total T cells (CD3+) compared to the other group diets (p <0.05). Both high-fat diets HF and HFOO had a lower number of dendritic cells (OX6+ OX62+) and T cytotoxic cells (CD3+ CD8+) compared to HC. However, HFOO had the lowest number of T cytotoxic cells (p <0.05). HF diet was the group with less number of macrophages (CD68+) and natural killer (NK) cells (CD3- CD161+), whereas the HFOO group had the highest proportion of NK cells compared to the other groups (p <0.05).

There was no significant change in T helper cells (CD3+ CD4+), IL-2 receptor (CD25+), T regulatory cells (FoxP3+), or the expression of TLR4 on macrophages (CD68+ CD284+) (p >0.05) across groups.

Table 3-7. Splenocyte phenotype of male Sprague Dawley rats post-intervention.[1,2]

Phenotype	HC	HF	HFOO	*P-value*
CD3+ Total (T cell)	49,87±1,71[a]	46,07±1,25[a]	41,73±1,06[b]	0.002
CD3+ CD4+ (T Helper)	20,58±1,11	21,32±1,27	22,27±1,34	0.664
CD3+ CD8+ (T cytotoxic)	25,31±1,13[a]	22,24±0,78[b]	19,49±0,68[c]	0.001
CD25+ Total (IL-2 receptor)	9,09±0,49	8,92±0,45	8,15±0,57	0.389
CD4+ CD25+ [3]	6,23±0,48	6,41±0,35	6,2±0,52	0.888
CD8+ CD25+ [4]	3,03±0,08	3,07±0,17	2,74±0,12	0.197
FoxP3+ Total (T regulatory)	9,64±0,76	7,99±0,59	10,01±1	0.190
OX6+ (MHC class II+)[3]	48,44±2,39	51,94±2,27	53,62±1,08	0.172
OX6+ OX62+ (Dendritic cells)	1,44±0,12[a]	1,09±0,07[b]	1,14±0,08[b]	0.027
CD68+ (Macrophages)	9,3±0,28[a]	7,35±0,4[b]	10,74±0,99[a]	0.003
CD68+ CD284+ (Macrophages TLR4)	6,67±0,3	5,61±0,33	6,41±0,73	0.276
CD3- CD161+ (NK cells)	5,49±0,38[b]	3,72±0,26[c]	8,13±0,59[a]	<0.001

[1]Values are presented as mean ± SEM. Groups that do not share the same letter are significantly different based on the one-way ANOVA test with the Duncan adjustment for multiple comparisons test ($p < 0.05$).
[2]Abbreviations: HC, High-carb diet; HF, High-fat diet; HFOO, High-fat diet with olive oil.[1]
[3]Analysis performed on log-transformed values.
[4]Analysis performed using non-parametric testing.

3.4 Discussion

In this study, we evaluated the efficacy of a high-MUFA diet using olive oil in modulating immune function in a DIO rat model. Our results support our hypothesis, to the extent that MUFA seems to have immunomodulatory properties, particularly leading to a reduced pro-inflammatory response including lower production of IL-6 and TNF-α after immune cells stimulation.

First, our study showed that the rats in all of our 3 dietary interventions were obese since the normal weight of a male Sprague Dawley rat at 13 weeks of age is approximately 400g (*CD® (Sprague Dawley) Rats | Charles River*, n.d.) whereas our results showed body weights nearing 500g. We did not observe any change in body weight when comparing the groups, which is inconsistent with what the literature has reported. Romanguera et al. found that high adherence to the consumption of MedDiet, rich in olive oil, contributes to a reduction in body

weight and/or less likelihood of developing overweight and obesity (Romaguera et al., 2010). Zamora et al. (Zamora et al., 2018) reported in a meta-analysis a reduction in body weight with the consumption of olive oil. However, the MedDiet also has other components that could cause a decrease in body weight such as increased consumption of fiber and complex carbohydrates; nutrients that are limited in our diets. Additionally, we used refined olive oil, which did not contain phenolic components and antioxidants that have been proposed as having beneficial effects (Guo et al., 2017). It is important to stress, however, that our results need to be interpreted in the context of obesity as all three diets led to an obesity phenotype.

Nevertheless, the consumption of HFOO contributed to a significant reduction in liver weight corrected for body weight, which is an indicator of hepatic steatosis (Simon et al., 2020). Therefore, it suggests that olive oil may positively impact liver health. This is consistent with the results of previous studies. Ma et al. (Ma et al., 2023) found in a systematic review that 4 out of 6 studies reported a reduction in markers of hepatic steatosis with supplementation with olive oil; this effect might be due to the fact that MUFA decreases the accumulation of triglycerides in the liver (Hussein, 2007).

Regarding metabolic complications, we found that the consumption of the HC diet resulted in the highest blood glucose levels at baseline. One reason for that is the composition of our HC diet, which is high in simple sugars and low in fiber (Mao et al., 2021; Reiser et al., 1986). Despite that, HC had an overall better glucose tolerance compared to the other groups, since it had a smaller peak after glucose intake as well as a significantly lower iAUC. On the other hand, HF diet had the poorest overall glycemic control when looking at the iAUC by having a delayed peak and clearance of blood glucose levels. Additionally, glucose did not return to baseline levels after 2 hours, again pointing toward a lower glucose clearance (S. Y. Park et al., 2001). When looking at the high-MUFA group, we can see that the changes after glucose administration were not enough to improve the detrimental effects of HF. Some studies have found that MUFA improves insulin sensitivity (Moon et al., 2010). Moon JH et al. found significant improvements, although they used 79% of olive oil from total fat, compared to 33% given in our study (Moon et al., 2010). Therefore, perhaps the dose of MUFA in the context of a HFD should be considered for improving glucose metabolism, or

again the type of olive oil with extra virgin olive oil providing phenolic compounds. Indeed, phenolic and antioxidant are known for their anti-inflammatory effect and since inflammatory markers such as TNF-α and IL-6 can directly impair insulin signaling you could speculate that extra virgin olive oil would have a more beneficial effect on insulin sensitivity (Shahwan et al., 2022).

When evaluating T cell function after ConA stimulation, we found a decrease in the production of IL-2 suggesting a reduction in T cell proliferation in the HFOO group (Cheng et al., 2002). Other studies found similar results (Jeffery et al., 1996; Yaqoob, 2002a). Yaqoob et al. and Jeffery et al. found a lower proliferation of spleen lymphocytes after ConA stimulation, indicating that olive oil has immunosuppressive effects (Jeffery et al., 1996; Yaqoob et al., 1994b). Nevertheless, other studies found contradictory results, such as Von Hegedus et al. who used peripheral blood mononuclear cells (PBMCs) from human blood, showed that oleic acid can boost the proliferation of T cells by increasing the mobilization of calcium intracellularly (von Hegedus et al., 2024). However, this was an in vitro study that used a lower concentration of oleic acid which could be an explanation, since studies have found that using high amounts of exogenous FAs could lead to decreased cytokine production by apoptotic pathways (Zhu et al., 2005).

Our results also found that the consumption of HFOO lead to a significant reduction in TNF-α compared to the other control diets. TNF-α is a pro-inflammatory cytokine that protects the host against infections and injuries and it is also involved in the activation and proliferation of T cells by stimulating the secretion of IL-2 (Kasahara et al., 1983; Mehta et al., 2018; Steen et al., 2011). It is produced mainly by macrophages, but it is also produced by Th1 cells and T cytotoxic cells (Britt et al., 2019; Idriss & Naismith, 2000; Janeway et al., 2001a). The reduction in the production of TNF-α (along with a trend toward a reduction in IFN-γ) aligns with the reduced levels of IL-2 and highlights the anti-inflammatory properties of olive oil, especially the reduction of pro-inflammatory Th1 responses. Our results are consistent with previous studies. Kien et al. found that olive oil decreased the production of TNF-α after mitogen stimulation (Kien et al., 2015) and a systematic review made by Yarla et al. showed a reduction in TNF-α and IL-6 after the consumption of olive oil (Yarla et al., 2017).

Furthermore, we found a reduction in the numbers of total T cells (CD3+) and T cytotoxic cells (CD3+ CD8+), which are cells that secrete IL-2, TNF-α, and IFN-γ which could have contributed to the lower production of these cytokines.

In our study, we also demonstrated a decrease in the production of IL-6 after T cell stimulation in the group supplemented with olive oil. IL-6 modulates the secretion of cytokines and promotes the activation of T cells. It is produced mainly by macrophages, monocytes, and dendritic cells (Choy & Rose-John, 2017; Gabay, 2006), but also T helper cells (CD4+) (B. Li et al., 2018). IL-6 has both pro and anti-inflammatory properties. It contributes to the production of acute phase proteins that conduce to systemic inflammation (Gabay, 2006), but also promotes Th2 differentiation, inhibiting Th1 responses (Diehl & Rincón, 2002). In the context of chronic inflammation that is commonly seen in obesity (Khanna et al., 2022), IL-6 mainly plays a pro-inflammatory role. (Gabay, 2006). Therefore, a decrease in the production of this cytokine by using olive oil also suggests an anti-inflammatory property.

Both IL-6 and TNF-α, have an important role in the pathogenesis of insulin resistance, interfering with insulin signaling pathways (Xia et al., 2024). We found a decrease in both TNF-α and IL-6 but not a significant reduction in overall glucose iAUC. Yet, we did see a significant reduction in blood glucose concentration 60 min post-administration in the HFOO group relative to the HF group. It is possible that with a longer intervention or with the use of extra virgin olive oil, an overall effect of the AUC would have been observed.

We did not observe any changes in the production of IL-10 nor in the Th1:Th2 ratio. Therefore, even though olive oil is able to reduce the production of pro-inflammatory cytokines, it does not affect the Th2 response, maintaining a balance between pro and anti-inflammatory responses. This is important for allergy and atopic diseases since a higher Th2 response could lead to an increased risk of allergies and atopic diseases.

In the context of antigen-presenting cell (APC) function, there were no changes in the production of cytokines; however, it is interesting to see that there was an increased

proportion of macrophages (CD68+) in the HFOO group compared to HF. This finding could indicate that olive oil leads to a rise in M2 polarization preventing the production of pro-inflammatory cytokines by macrophages (Montserrat-de la Paz et al., 2019).

Limitations of this study include the usage of refined olive oil since some of the effects of the study could have been stronger with the use of extra virgin olive oil due to its various components such as polyphenols. Additionally, we lacked a control lean group, considering that all of our groups were obese with metabolic perturbations, which could have altered the immune response. Thus, it is not possible to compare the results with a normal lean phenotype. A longer feeding period could have also allowed us to see greater differences across groups. Although we were powered for TNF-α production after ConA stimulation, other cytokines with higher variability would have perhaps required a higher number of animals per group to see significant differences instead of trends.

3.5 Conclusion

In summary, the effect of the consumption of a diet high in MUFA in the context of obesity suggests an improvement in biomarkers of liver health, independent of a change in body weight or glucose tolerance. There was no effect on APC function, but there was a modulation in T cell response, showing a reduction of pro-inflammatory cytokines and a decrease in IL-2 after ConA stimulation, suggesting lower T cell proliferation and maintenance of the anti-inflammatory response. Therefore, the consumption of olive oil might be beneficial to attenuate the chronic inflammation found in obesity.

3.6 Literature cited

Bannenberg, G., & Serhan, C. N. (2010). Specialized pro-resolving lipid mediators in the inflammatory response: An update. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1801(12), 1260–1273. https://doi.org/10.1016/j.bbalip.2010.08.002

Blewett, H. J., Gerdung, C. A., Ruth, M. R., Proctor, S. D., & Field, C. J. (2009a). Vaccenic acid favourably alters immune function in obese JCR:LA- cp rats. British Journal of Nutrition, 102(04), 526. https://doi.org/10.1017/S0007114509231722

Blewett, H. J., Gerdung, C. A., Ruth, M. R., Proctor, S. D., & Field, C. J. (2009b). Vaccenic acid favourably alters immune function in obese JCR:LA- cp rats. British Journal of Nutrition, 102(04), 526. https://doi.org/10.1017/S0007114509231722

Britt, R. D., Thompson, M. A., Sasse, S., Pabelick, C. M., Gerber, A. N., & Prakash, Y. S. (2019). Th1 cytokines TNF-α and IFN-γ promote corticosteroid resistance in developing human airway smooth muscle. American Journal of Physiology-Lung Cellular and Molecular Physiology, 316(1), L71–L81. https://doi.org/10.1152/ajplung.00547.2017

Bulló, M., García-Lorda, P., Megias, I., & Salas-Salvadó, J. (2003). Systemic Inflammation, Adipose Tissue Tumor Necrosis Factor, and Leptin Expression. Obesity Research, 11(4), 525–531. https://doi.org/10.1038/oby.2003.74

Calder, P. C. (2017). Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochemical Society Transactions, 45(5), 1105–1115. https://doi.org/10.1042/BST20160474

Casas, R., Sacanella, E., Urpí-Sardà, M., Corella, D., Castañer, O., Lamuela-Raventos, R.-M., Salas-Salvadó, J., Martínez-González, M.-A., Ros, E., & Estruch, R. (2016). Long-Term Immunomodulatory Effects of a Mediterranean Diet in Adults at High Risk of Cardiovascular Disease in the PREvención con DIeta MEDiterránea (PREDIMED) Randomized Controlled Trial. The Journal of Nutrition, 146(9), 1684–1693. https://doi.org/10.3945/jn.115.229476

CD® (Sprague Dawley) Rats | Charles River. (n.d.). Retrieved July 12, 2024, from https://www.criver.com/products-services/find-model/cd-sd-igs-rat?region=24

Cheng, L. E., Öhlén, C., Nelson, B. H., & Greenberg, P. D. (2002). Enhanced signaling through the IL-2 receptor in CD8+ T cells regulated by antigen recognition results in preferential proliferation and expansion of responding CD8+ T cells rather than promotion of cell death. Proceedings of the National Academy of Sciences, 99(5), 3001–3006. https://doi.org/10.1073/pnas.052676899

Choy, E., & Rose-John, S. (2017). Interleukin-6 as a Multifunctional Regulator: Inflammation, Immune Response, and Fibrosis. Journal of Scleroderma and Related Disorders, 2(2_suppl), S1–S5. https://doi.org/10.5301/jsrd.5000265

Diehl, S., & Rincón, M. (2002). The two faces of IL-6 on Th1/Th2 differentiation. Molecular Immunology, 39(9), 531–536. https://doi.org/10.1016/S0161-5890(02)00210-9

Dobrovolskaia, M. A., & Vogel, S. N. (2002). Toll receptors, CD14, and macrophage activation and deactivation by LPS. Microbes and Infection, 4(9), 903–914. https://doi.org/10.1016/S1286-4579(02)01613-1

Epstein, F. H., Lewis, R. A., Austen, K. F., & Soberman, R. J. (1990). Leukotrienes and Other Products of the 5-Lipoxygenase Pathway. New England Journal of Medicine, 323(10), 645–655. https://doi.org/10.1056/NEJM199009063231006

Festa, A., D'Agostino Jr, R., Williams, K., Karter, A., Mayer-Davis, E., Tracy, R., & Haffner, S. (2001). The relation of body fat mass and distribution to markers of chronic inflammation. International Journal of Obesity, 25(10), 1407–1415. https://doi.org/10.1038/sj.ijo.0801792

Field, C. J., Thomson, C. A., Van Aerde, J. E., Parrott, A., Euler, &NA;, Lien, E., & Clandinin, M. T. (2000). Lower Proportion of CD45R0+ Cells and Deficient Interleukin-10 Production by Formula-Fed Infants, Compared With Human-Fed, Is Corrected With Supplementation of Long-Chain Polyunsaturated Fatty Acids. Journal of Pediatric Gastroenterology and Nutrition, 31(3), 291–299. https://doi.org/10.1097/00005176-200009000-00017

Field, C. J., Wu, G., Métroz-Dayer, M. D., Montambault, M., & Marliss, E. B. (1990). Lactate production is the major metabolic fate of glucose in splenocytes and is altered in spontaneously diabetic BB rats. The Biochemical Journal, 272(2), 445–452. https://doi.org/10.1042/bj2720445

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. Nature Medicine, 25(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Gabay, C. (2006). Interleukin-6 and chronic inflammation. Arthritis Research & Therapy, 8(Suppl 2), S3. https://doi.org/10.1186/ar1917

Guo, X., Tresserra-Rimbau, A., Estruch, R., Martínez-González, M., Medina-Remón, A., Fitó, M., Corella, D., Salas-Salvadó, J., Portillo, M., Moreno, J., Pi-Sunyer, X., & Lamuela-Raventós, R. (2017). Polyphenol Levels Are Inversely Correlated with Body Weight and

Obesity in an Elderly Population after 5 Years of Follow Up (The Randomised PREDIMED Study). Nutrients, 9(5), 452. https://doi.org/10.3390/nu9050452

Hoong, C. W. S., Hussain, I., Aravamudan, V. M., Phyu, E. E., Lin, J. H. X., & Koh, H. (2021). Obesity is Associated with Poor Covid-19 Outcomes: A Systematic Review and Meta-Analysis. Hormone and Metabolic Research, 53(02), 85–93. https://doi.org/10.1055/a-1326-2125

Hussein, O. (2007). Monounsaturated fat decreases hepatic lipid content in non-alcoholic fatty liver disease in rats. World Journal of Gastroenterology, 13(3), 361. https://doi.org/10.3748/wjg.v13.i3.361

Hwang, D. H., Kim, J.-A., & Lee, J. Y. (2016). Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. European Journal of Pharmacology, 785, 24–35. https://doi.org/10.1016/j.ejphar.2016.04.024

Idriss, H. T., & Naismith, J. H. (2000). TNFa and the TNF receptor superfamily: Structure-function relationship(s). Microscopy Research and Technique, 50(3), 184–195. https://doi.org/10.1002/1097-0029(20000801)50:3<184::AID-JEMT2>3.0.CO;2-H

Innes, J. K., & Calder, P. C. (2018). Omega-6 fatty acids and inflammation. Prostaglandins, Leukotrienes and Essential Fatty Acids, 132, 41–48. https://doi.org/10.1016/j.plefa.2018.03.004

Janeway, C., Travers, P., & Walport, M. (2001). Immunobiology: The Immune System in Health and Disease. (5th ed.). Garland Science.

Jeffery, N. M., Yaqoob, P., Kewsholme, E. A., & Calder, P. C. (1996). The Effects of Olive Oil upon Rat Serum Lipid Levels and Lymphocyte Functions Appear to Be Due to Oleic Acid. Annals of Nutrition and Metabolism, 40(2), 71–80. https://doi.org/10.1159/000177898

Kasahara, T., Hooks, J. J., Dougherty, S. F., & Oppenheim, J. J. (1983). Interleukin 2-mediated immune interferon (IFN-gamma) production by human T cells and T cell subsets. Journal of Immunology (Baltimore, Md. : 1950), 130(4), 1784–1789.

Khanna, D., Khanna, S., Khanna, P., Kahar, P., & Patel, B. M. (2022). Obesity: A Chronic Low-Grade Inflammation and Its Markers. Cureus. https://doi.org/10.7759/cureus.22711

Kien, C. L., Bunn, J. Y., Fukagawa, N. K., Anathy, V., Matthews, D. E., Crain, K. I., Ebenstein, D. B., Tarleton, E. K., Pratley, R. E., & Poynter, M. E. (2015). Lipidomic evidence that lowering the typical dietary palmitate to oleate ratio in humans decreases the leukocyte production of proinflammatory cytokines and muscle expression of redox-sensitive genes. The Journal of Nutritional Biochemistry, 26(12), 1599–1606. https://doi.org/10.1016/j.jnutbio.2015.07.014

Kim, C.-S., Park, H.-S., Kawada, T., Kim, J.-H., Lim, D., Hubbard, N. E., Kwon, B.-S., Erickson, K. L., & Yu, R. (2006). Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. International Journal of Obesity, 30(9), 1347–1355. https://doi.org/10.1038/sj.ijo.0803259

Li, B., Jones, L. L., & Geiger, T. L. (2018). IL-6 Promotes T Cell Proliferation and Expansion under Inflammatory Conditions in Association with Low-Level RORγt Expression. The Journal of Immunology, 201(10), 2934–2946. https://doi.org/10.4049/jimmunol.1800016

Lillenfeld, D. E., Vlahov, D., Tenney, J. H., & McLaughlin, J. S. (1988). Obesity and diabetes as risk factors for postoperative wound infections after cardiac surgery. American Journal of Infection Control, 16(1), 3–6. https://doi.org/10.1016/0196-6553(88)90003-X

Luck, H., Khan, S., Kim, J. H., Copeland, J. K., Revelo, X. S., Tsai, S., Chakraborty, M., Cheng, K., Tao Chan, Y., Nøhr, M. K., Clemente-Casares, X., Perry, M.-C., Ghazarian, M., Lei, H., Lin, Y.-H., Coburn, B., Okrainec, A., Jackson, T., Poutanen, S., … Winer, D. A. (2019). Gut-associated IgA+ immune cells regulate obesity-related insulin resistance. Nature Communications, 10(1), 3650. https://doi.org/10.1038/s41467-019-11370-y

Lumeng, C. N., Bodzin, J. L., & Saltiel, A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of Clinical Investigation, 117(1), 175–184. https://doi.org/10.1172/JCI29881

Ma, Y., Ding, X., Gu, J., Zhou, S., & Jiang, Y. (2023). Effects of olive oil on hepatic steatosis and liver enzymes: A systematic review. Journal of Functional Foods, 109, 105815. https://doi.org/10.1016/j.jff.2023.105815

Makki, K., Froguel, P., & Wolowczuk, I. (2013). Adipose Tissue in Obesity-Related Inflammation and Insulin Resistance: Cells, Cytokines, and Chemokines. ISRN Inflammation, 2013, 1–12. https://doi.org/10.1155/2013/139239

Mao, T., Huang, F., Zhu, X., Wei, D., & Chen, L. (2021). Effects of dietary fiber on glycemic control and insulin sensitivity in patients with type 2 diabetes: A systematic review and meta-analysis. Journal of Functional Foods, 82, 104500. https://doi.org/10.1016/j.jff.2021.104500

Mehta, A. K., Gracias, D. T., & Croft, M. (2018). TNF activity and T cells. Cytokine, 101, 14–18. https://doi.org/10.1016/j.cyto.2016.08.003

Milner, J. J., & Beck, M. A. (2012). The impact of obesity on the immune response to infection. Proceedings of the Nutrition Society, 71(2), 298–306. https://doi.org/10.1017/S0029665112000158

Montserrat-de la Paz, S., Naranjo, M. C., Millan-Linares, M. C., Lopez, S., Abia, R., Biessen, E. A. L., Muriana, F. J. G., & Bermudez, B. (2019). Monounsaturated Fatty Acids in a High-

Fat Diet and Niacin Protect from White Fat Dysfunction in the Metabolic Syndrome. Molecular Nutrition & Food Research, 63(19). https://doi.org/10.1002/mnfr.201900425

Moon, J. H., Lee, J. Y., Kang, S. B., Park, J. S., Lee, B. W., Kang, E. S., Ahn, C. W., Lee, H. C., & Cha, B. S. (2010). Dietary Monounsaturated Fatty Acids but not Saturated Fatty Acids Preserve the Insulin Signaling Pathway via IRS-1/PI3K in Rat Skeletal Muscle. Lipids, 45(12), 1109–1116. https://doi.org/10.1007/s11745-010-3475-3

Park, S. Y., Kim, Y. W., Kim, J. Y., Jang, E. C., Doh, K. O., & Lee, S. K. (2001). Effect of High Fat Diet on Insulin Resistance: Dietary Fat Versus Visceral Fat Mass. Journal of Korean Medical Science, 16(4), 386. https://doi.org/10.3346/jkms.2001.16.4.386

Purnell, J. (2000). Definitions, Classification, and Epidemiology of Obesity. (K. Feingold, B. Anawalt, & M. Blackman, Eds.). Endotext [Internet].

Reiser, S., Hallfrisch, J., Fields, M., Powell, A., Mertz, W., Prather, E., & Canary, J. (1986). Effects of sugars on Indices of glucose tolerance in humans. The American Journal of Clinical Nutrition, 43(1), 151–159. https://doi.org/10.1093/ajcn/43.1.151

Richard, C., Couture, P., Desroches, S., & Lamarche, B. (2013). Effect of the mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. Obesity, 21(1), 51–57. https://doi.org/10.1002/oby.20239

Rogero, M., & Calder, P. (2018). Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. Nutrients, 10(4), 432. https://doi.org/10.3390/nu10040432

Romaguera, D., Norat, T., Vergnaud, A.-C., Mouw, T., May, A. M., Agudo, A., Buckland, G., Slimani, N., Rinaldi, S., Couto, E., Clavel-Chapelon, F., Boutron-Ruault, M.-C., Cottet, V., Rohrmann, S., Teucher, B., Bergmann, M., Boeing, H., Tjønneland, A., Halkjaer, J., … Peeters, P. H. (2010). Mediterranean dietary patterns and prospective weight change in participants of the EPIC-PANACEA project. The American Journal of Clinical Nutrition, 92(4), 912–921. https://doi.org/10.3945/ajcn.2010.29482

Serhan, C. N., Chiang, N., & Van Dyke, T. E. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nature Reviews Immunology, 8(5), 349–361. https://doi.org/10.1038/nri2294

Shahwan, M., Alhumaydhi, F., Ashraf, G. Md., Hasan, P. M. Z., & Shamsi, A. (2022). Role of polyphenols in combating Type 2 Diabetes and insulin resistance. International Journal of Biological Macromolecules, 206, 567–579. https://doi.org/10.1016/j.ijbiomac.2022.03.004

Sheridan, P. A., Paich, H. A., Handy, J., Karlsson, E. A., Hudgens, M. G., Sammon, A. B., Holland, L. A., Weir, S., Noah, T. L., & Beck, M. A. (2012). Obesity is associated with

impaired immune response to influenza vaccination in humans. International Journal of Obesity, 36(8), 1072–1077. https://doi.org/10.1038/ijo.2011.208

Simon, G., Heckmann, V., Tóth, D., Pauka, D., Petrus, K., & Molnár, T. F. (2020). The effect of hepatic steatosis and fibrosis on liver weight and dimensions. Legal Medicine, 47, 101781. https://doi.org/10.1016/j.legalmed.2020.101781

Steen, B. M., Gerstenfeld, L. C., & Einhorn, T. A. (2011). The Role of the Immune System in Fracture Healing. In Osteoimmunology (pp. 343–367). Elsevier. https://doi.org/10.1016/B978-0-12-375670-1.10012-3

Tilley, S. L., Coffman, T. M., & Koller, B. H. (2001). Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. Journal of Clinical Investigation, 108(1), 15–23. https://doi.org/10.1172/JCI200113416

Tsai, S., Clemente-Casares, X., Zhou, A. C., Lei, H., Ahn, J. J., Chan, Y. T., Choi, O., Luck, H., Woo, M., Dunn, S. E., Engleman, E. G., Watts, T. H., Winer, S., & Winer, D. A. (2018). Insulin Receptor-Mediated Stimulation Boosts T Cell Immunity during Inflammation and Infection. Cell Metabolism, 28(6), 922-934.e4. https://doi.org/10.1016/j.cmet.2018.08.003

von Hegedus, J. H., de Jong, A. J., Hoekstra, A. T., Spronsen, E., Zhu, W., Cabukusta, B., Kwekkeboom, J. C., Heijink, M., Bos, E., Berkers, C. R., Giera, M. A., Toes, R. E. M., & Ioan-Facsinay, A. (2024). Oleic acid enhances proliferation and calcium mobilization of CD3/CD28 activated CD4+ T cells through incorporation into membrane lipids. European Journal of Immunology. https://doi.org/10.1002/eji.202350685

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation, 112(12), 1796–1808. https://doi.org/10.1172/JCI200319246

Wong, S. W., Kwon, M.-J., Choi, A. M. K., Kim, H.-P., Nakahira, K., & Hwang, D. H. (2009). Fatty Acids Modulate Toll-like Receptor 4 Activation through Regulation of Receptor Dimerization and Recruitment into Lipid Rafts in a Reactive Oxygen Species-dependent Manner. Journal of Biological Chemistry, 284(40), 27384–27392. https://doi.org/10.1074/jbc.M109.044065

Xia, J., Wang, Z., Yu, P., Yan, X., Zhao, J., Zhang, G., Gong, D., & Zeng, Z. (2024). Effect of Different Medium-Chain Triglycerides on Glucose Metabolism in High-Fat-Diet Induced Obese Rats. Foods, 13(2), 241. https://doi.org/10.3390/foods13020241

Yaqoob, P. (2002). Monounsaturated fatty acids and immune function. European Journal of Clinical Nutrition, 56(S3), S9–S13. https://doi.org/10.1038/sj.ejcn.1601477

Yaqoob, P., Newsholme, E. A., & Calder, P. C. (1994). The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. Immunology, 82(4), 603–610.

Yarla, N. S., Polito, A., & Peluso, I. (2017). Effects of Olive Oil on TNF-α and IL-6 in Humans: Implication in Obesity and Frailty. Endocrine, Metabolic & Immune Disorders - Drug Targets, 18(1), 63–74. https://doi.org/10.2174/1871530317666171120150329

Zamora, F., Martinez, J. M., Gaforio, J. J., & Delgado, M. (2018). Olive Oil and Body Weight. Systematic Review and Meta-Analysis of Randomized Controlled Trials. Rev Esp Salud Pública, 92. www.msc.es/resp

Zhu, Y., Schwarz, S., Ahlemeyer, B., Grzeschik, S., Klumpp, S., & Krieglstein, J. (2005). Oleic acid causes apoptosis and dephosphorylates Bad. Neurochemistry International, 46(2), 127–135. https://doi.org/10.1016/j.neuint.2004.08.003

Chapter 4: The effect of dietary lipids on immune function: data from the Nutrition and immunity (NutrIMM) study

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4.1 Introduction

Obesity is associated with the appearance of T2D, CVD, and cancer (Ogden, 2010; WHO, 2023a). Additionally, studies have shown a higher risk of post-surgical infections and increased COVID-19 mortality due to impaired immune responses (Dowsey & Choong, 2008; Lillenfeld et al., 1988b; Peters et al., 2021). This malfunction of the immune system is attributed to various factors associated with obesity. One of them is the chronic low-grade inflammation (She et al., 2022) present in obesity, which is recognized by elevated levels of circulating pro-inflammatory cytokines such as IL-6, TNF-α (Sproston & Ashworth, 2018), and CRP (Sproston & Ashworth, 2018). The production of these pro-inflammatory cytokines is caused in part by increased infiltration of macrophages in adipose tissue acquiring an M1 (pro-inflammatory) phenotype (Weisberg et al., 2003b; Xu et al., 2003b) that contributes to both local and systemic inflammation. The consumption of a HFD might also contribute to inflammation by increasing intestinal permeability (Luck et al., 2019a), leading to the translocation of LPS, which directly binds to the TLR4 (Dobrovolskaia & Vogel, 2002b), triggering the production of proinflammatory cytokines such as TNF-α, IL-1B, and IL-6 (Rogero & Calder, 2018a).

The mechanisms by which systemic inflammation affects immune function remain unclear; however, studies suggest immune cell exhaustion as one of the reasons, since cells are constantly activated, which impedes them in mounting an adequate immune response after a challenge (Geerlings & Hoepelman, 1999). Furthermore, hyperglycemia and insulin resistance also play a role in the impairment of immune function by reducing the number of T regulatory cells thereby preventing proper resolution of inflammation (C. Li et al., 2021; Wagner et al., 2013). Studies have also found that hyperglycemia reduces the production of IL-2 (a proliferation marker for T cells), and insulin resistance might diminish T cell

proliferation and the production of pro-inflammatory cytokines in the presence of influenza virus, demonstrating impaired immune function (Richard et al., 2017; Tsai et al., 2018). Altogether, this suggests that T cells are relying on glucose metabolism for energy when mounting an immune response.

Lipids are essential components of cell membranes, which provide immunomodulatory properties (Andersen, 2022). SFAs are recognized as pro-inflammatory, as some SFA structurally resemble LPS and can cause an inflammatory response by binding to the TLR4 (Fritsche, 2015b; Hwang et al., 2016b; J. Y. Lee et al., 2001b). In contrast, PUFA have both pro and anti-inflammatory properties, they can exhibit one or the other depending on the lipid mediators they generate. N-6 such as AA produces certain prostaglandins and leukotrienes that induce inflammation, whereas n-3, mainly EPA and DHA, produce resolvins, protectins, and maresins, promoting the resolution of inflammation (P. C. Calder, 2017; Rogero & Calder, 2018b). MUFA, on the other hand, are considered neutral, although they might exert an anti-inflammatory effect when replacing SFAs.

The effect of these FAs on inflammation and immune function depends on their relative amounts in cell membranes. Predicting the FA profile in body tissues can be achieved through different measurements, one of them being through plasma which contains lipoprotein-associated and non-esterified FAs, this measurement reflects the short-term consumption of these FAs (Brenna et al., 2018).

Overall, understanding the connection between obesity, inflammation, and immune function allows us to create strategies to improve individual's health. With this study, we aimed to explore the relationship between dietary FAs and immune function in people living with obesity and with and without metabolic complications. We hypothesized that a higher proportion of SFAs in plasma (reflective of food intake) will be associated with an increased secretion of pro-inflammatory cytokines by stimulated immune cells. A higher proportion of n-3 will lead to a reduction in pro-inflammatory cytokines while MUFAs will not lead to changes in the production of cytokines after immune cell stimulation exerting primarily a neutral effect. For this chapter, we have considered primarily baseline data from the Nutrition

and Immunity (NutrIMM) study since being a feeding study on the North American diet, post-intervention food intake is the same across groups.

4.2 Methods

4.2.1 Subjects and Study Design

The NutrIMM study is a prospective, non-randomized, four-arm, parallel-group, unicentre, controlled-feeding trial, designed to understand how nutrition, body weight, and glycemia influence the immune system. The detailed study protocol has been previously published (Braga Tibaes et al., 2023). The research protocol was approved by the University of Alberta Ethics Board (Pro00085839) and follows the standards proposed by the Canadian Tri-Council Policy statement on the use of human participants in research.

Briefly, participants were screened for eligibility where they were recruited based on their metabolic phenotype into 4 groups: Lean-normoglycemic (Lean), Obese-normoglycemic (Obese-NG), Obese-glucose intolerant (Obese-GI), Obese with type 2 diabetes (Obese-T2D). The criteria for allocation are presented in **Table 4-1**. At baseline, the Diet History Questionary III (DHQIII) was completed covering the last 4 weeks along with measurement of blood pressure, anthropometric (body weight, height, and waist circumference), and main CVD risk factors. Participants also completed several questionnaires including a physical activity questionnaire. During the feeding period, participants were instructed to maintain their physical activity level and consume an isocaloric standardized North American diet for 4 weeks (all foods provided to them). The nutrient composition of the NutrIMM menu is shown in **Table 4-2**. Body weight and food intake were measured regularly during the feeding period to ensure energy balance. All measurements were repeated at the end of the study.

Table 4-1. Eligibility criteria for group allocation.[1]

Criteria	Lean	Obese-NG	Obese-GI	Obese-T2D
BMI (kg/m²)	18.5–24.9 (±0.5)	≥ 30 (±0.5)		
Waist circumference (cm)	Males <102; Females <88	Males ≥102; Females ≥88		
Fasting blood glucose (mmol/L)[a]	<5.6		5.6–6.9	≥ 7.0
HbA1c (%)	<5.5		5.5–6.4	≥ 6.5[b]
BP (SBP/DBP, mmHg)	<130/85		NR	
Triglycerides (mmol/L)	<1.7		NR	
HDL-C (mmol/L)	Males ≥1.03; Females ≥1.29		NR	

[1] Abbreviations: BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein-cholesterol; NR, not required in it that could be normal or elevated; SBP, systolic blood pressure; T2D, type 2 diabetes. [a]If participants are at the upper or lower limits for fasting glucose, HbA1c will be used for group allocation. [b] Or a diagnosis of type 2 diabetes and use of medication.

Table 4-2. Nutrient composition of interventional diet [1]

Macronutrient	Interventional diet
% of Calories	
Carbohydrate (%)	47
Protein (%)	18
Fat (%)	36
% of Fat	
SFA (%)	35
MUFA (%)	38
PUFA (%)	18
n-6 (%)	14
n-3 (%)	2
Ratio SFA:PUFA	1,9
Ratio n-6:n-3	6,0

[1]Abbreviations: MUFA, Monounsaturated fatty acids; SFA, Saturated fatty acids; PUFA, Polyunsaturated fatty acids.

4.2.2 Blood analysis

Blood samples were collected after a 9-12 hours fast and analyzed by Alberta Precision Labs. Glycated hemoglobin (HbA1c), glucose, and insulin were measured and the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated to determine insulin resistance (Braga Tibaes et al., 2023). CRP was measured on two consecutive days to avoid including values suggestive of an infectious state.

4.2.3 Peripheral Blood Mononuclear Cells Isolation

Isolation of PBMCs has been previously described (Braga Tibaes et al., 2023). Briefly, blood samples were centrifuged, and the buffy coat and erythrocytes were resuspended in 1% BSA in PBS, layered onto Ficoll-Paque, and centrifuged for PBMC isolation. The lymphocyte band was transferred, washed, and centrifuged to pellet the PBMCs, which were then resuspended in a cryopreservation solution. PBMCs are then aliquoted to count using trypan blue and the remaining cell suspension is transferred to cryovials for freezing and transfer to liquid nitrogen.

4.2.4 Immune cell recovery

PBMC cryovials stored in liquid nitrogen were rapidly thawed in a 37°C water bath, wiped with ethanol, and transferred to 10% FCS RPMI to prewarmed, then were rinsed and resuspended to rest overnight. PBMCs were pelleted, resuspended, and counted using the trypan blue (Braga Tibaes et al., 2023).

4.2.5 Mitogen Stimulation of PBMC

To quantify the secretion of cytokines by PBMCs they were cultured in 10% FCS RPMI-1640 medium for 48–72 hours at 37°C without or with mitogen including PHA, a T cell stimulant (25 μg/mL; Sigma-Aldrich), and LPS an APC stimulant (5 μg/mL; Thermo Fisher Scientific, Carlsbad, CA, United States). After the incubation, PBMCs were centrifuged, and the supernatant was frozen until quantification by ELISA (Braga Tibaes et al., 2023).

4.3.6 Quantification of *ex-vivo* cytokine secretion

Concentrations of IL-2 (only PHA), IL-1B (only LPS), IL-6, IL-10, TNF-α, and IFN-γ in the supernatant were determined in duplicate by commercial ELISA kits (DuoSet®, R&D

Systems, Minneapolis, MN, United States). The detection limits for the cytokines were: IL-2 (1000–15.6 pg/mL), IL-1B (250 to 3.91 pg/mL), IL-6 (600–9.38 pg/mL), IL-10 (2000–31.3 pg/mL), TNF-α (1000–15.6 pg/mL), and IFN-γ (600–9.38 pg/mL). Cytokine concentrations are quantified using a microplate reader (Biotek® Synergy™ H1, Agilent Technologies, Inc., Santa Clara, CA, United States), with an intra-assay coefficient of variation (CV) <10% (Braga Tibaes et al., 2023).

4.3.7 Proliferation assay

The proliferation of T cells has already been described (Braga Tibaes et al., 2023). Briefly, Black flat-bottom 96-well plates were coated with 100µL of 5 µg/mL anti-CD3 stock solution and incubated overnight at 4°C, then rinsed with sterile PBS. Unstimulated and stimulated wells receive 200µL and 196µL of 10% RPMI respectively. The plate was set up to reach 1.00×10^6 cells/mL in 200µL, and 4µL of 50 µg/mL anti-CD28 was added. After incubating at 37°C with 5% CO2 for 72 hours, with alamarBlue® added in the final 4 hours. The plate was excited at 560 nm in a fluorescent microplate reader (Biotek® Synergy™ H1) and read at 590 nm fluorescence.

4.3.8 Fatty acid composition of plasma total lipids

Total plasma lipids (200µL) were extracted using the Folch method (Folch et al., 1957) with a 4:1 ratio (chloroform: methanol (8mL): 1.8mL of 0.1 M of potassium chloride was added. Samples were vortexed and incubated overnight at 4°C. They were centrifuged and the bottom solvent layer containing the total lipids was dried down under nitrogen gas. Samples were saponified by adding 1.5mL of Methanolic KOH, vortexing, and heating at 110°C for 1 hour. After that, samples were methylated by adding boron trifluoride: hexane (1:1, 1.5mL each) and heat a 110°C for 1 hour. After cooling, 1mL of double distilled water was added and samples were incubated at 4°C overnight. The next day, samples were centrifuged to take off the top layer containing the total lipids dried them down under nitrogen gas, and resuspended in 100ul of hexane for fatty acid methyl esters proportional analysis. A gas chromatograph Agilent 8,890 coupled with an autosampler (7693A) was used, with a 100m x 0.25mm x 0.2um CP-Sil 88 fused capillary column for long chain fatty acids. The standards used were GLC 502 and GLC37. The following fatty acids were determined: tetradecanoic/myristic (C14:0), tetradecenoic/myristoleic (C14:1),

pentadecanoic/pentadecylic (C15:0), hexadecanoic/palmitic (C16:0), hexadecenoic/palmitoleic (C16:1 n-9), (9Z)-hexadec-9-enoic/palmitoleic (C16:1 n-7), heptadecanoic/margaric (C17:0), 10-heptadecenoic (C17:1), octadecanoic/stearic (C18:0), octadecenoic/oleic (C18:1n-9), 11-octadecenoic acid/vaccenic (18:1 T n-7), octadecatrienoic /α-linolenic /ALA (C18:3 n-3), octadecadienoic/linoleic /LA (C18:2 n-6), eicosanoic/arachidic (C20:0), 11-eicosenoic/gondoic (C20:2 n-6), 11,14,17-eicosatrienoic/dihomo-γlinolenic acid (C20:3n-6), eicosatetraenoic/arachidonic/ARA (C20:4n-6), eicosapentaenoic/EPA (C20:5n-3), tetracosanoic/lignoceric (C24:0), tetracosenoic/nervonic (C24:1 n-9), docosapentaenoic/DPA (C22:5n-3), docosahexaenoic/DHA (C22:6 n-3) (Braga Tibaes et al., 2023). Data was expressed as fatty acid proportions from the combined area of all identified peaks.

4.3.9 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 29.0.2.0. Data are reported as means ± SEM. Data was analyzed using a one-way ANOVA, and a two-way ANOVA for repeated measures. In cases where there was a significant difference, a post hoc analysis was performed using Duncan to determine differences between groups. When data sets were not normally distributed, or homogeneity was violated the data was log-transformed. When a normal distribution was not obtained after using a log transformation, non-parametric statistical analysis was performed using a Kruskal-Wallace test to determine if differences existed between groups, and if homogeneity was still violated Games-Howell was used to see significant differences. In addition to that, partial correlations adjusted per group using baseline values for cytokines and fatty acids were performed. Correlations were performed only on cytokines and plasma FAs that changed significantly across the groups. Differences at $P \leq 0.05$ (two-sided) were considered significant.

4.4 Results

4.4.1 Subject characteristics

A total of 112 participants were included in the study, of which 51 were male and 61 were female. The characteristics of participants at baseline are shown in **Table 4-3**. As per the group allocation, significant changes in body mass index BMI, WC, glucose, HOMA-IR, and HbA1c were observed across groups (all p <0.001) Moreover, age was significantly higher in groups with metabolic complications and CRP increased in all groups with obesity (both p <0.001). Interestingly, even though the Obese-NG group had normal plasma glucose values, they had elevated insulin levels compared to the lean group indicative of IR (p <0.001).

Table 4-3. Baseline characteristics of participants.[1]

Variables	Lean (n=31)	Obese-NG (n=24)	Obese-GI (n=30)	Obese-T2D (n=27)	P-value
Sex (male/female)	15/16	9 / 15	14/16	13/14	
Age (years)	31,69±1,47^{c}	37,96±3,02^{bc}	44,96±2,40^{b}	55,89±1,89^{a}	<0.001
BMI (kg/m²)[2]	22,5±0,45^{b}	34,91±0,98^{a}	34,9±0,67^{a}	36,81±1,06^{a}	<0.001
WC (cm)[2]	75,89±1,46^{c}	106,57±2,94^{b}	110,91±2,36^{b}	121,4±1,93^{a}	<0.001
CRP (mg/L)[3]	0,95±0,28^{b}	4,88±0,92^{a}	3,93±0,67^{a}	4,94±1,18^{a}	<0.001
Glucose (mmol/L)[2]	4,88±0,06^{c}	5,09±0,07^{c}	5,64±0,11^{b}	8,37±0,44^{a}	<0.001
Insulin (pmol/L)[3]	54,1±6,45^{b}	117,38±14,78^{a}	169,48±23,21^{a}	170,74±21,51^{a}	<0.001
HbA1c (%)[3]	5,27±0,05^{c}	5,3±0,04^{c}	5,78±0,04^{b}	7,37±0,24^{a}	<0.001
HOMA-IR[3]	1,98±0,25^{c}	4,47±0,60^{b}	7,30±1,07^{ab}	10,63±1,34^{a}	<0.001

[1]Values are means ± SEM. Groups that do not share the same letter are significantly different based on the one-way ANOVA test, using Duncan as a post hoc analysis ($p < 0.05$). Abbreviations: BMI, Body Mass Index; CRP, C-Reactive Protein; HbA1c, Hemoglobin A1c; WC, Waist Circumference.
[2]Analysis performed on log-transformed values.
[3]Analysis performed using non-parametric testing.

4.4.2 Dietary intake

The habitual dietary intake of the participants before starting the study can be found in **Table 4-4**. Despite energy and macronutrient distribution not being statistically different among groups; the lean group reported consuming more calories per day (P >0.05). When looking at the proportion of fat coming from SFA, MUFA and PUFA, the lean group reported consuming more PUFA and less SFA compared to the other groups, due to a higher intake of n-6, which also led to a decreased ratio of SFA:PUFA in that groups (P <0.05).

Table 4-4. Habitual dietary intake at baseline from the DHQ III Questionnaire.[1]

Nutrient	Lean	Obese-NG	Obese-GI	Obese-T2D	P-value
Calories (Kcal/d)[2]	2740±400	2062±189	2017±155	1780±151	0.157
		% of Calories			
Carbohydrate (%)	45,91±1,14	44,47±1,17	46,07±1,55	47,44±1,69	0.548
Protein (%)[3]	16,87±0,37	16,44±0,65	16,67±0,58	16,5±0,62	0.954
Fat (%)	37,15±0,89	36,67±0,85	37,81±1,09	36,95±1,31	0.894
		% of Fat			
SFA (%)	31,83±0,65	34,24±0,78	34,55±1,05	34,23±0,75	0.059
MUFA (%)[3]	36,5±0,43	35,92±0,36	35,25±0,43	35,84±0,33	0.433
PUFA (%)	23,17±0,63[a]	21,11±0,61[b]	21,41±0,81[ab]	20,67±0,59[b]	0.039
n-6 (%)	20,58±0,6[a]	18,65±0,56[b]	18,88±0,72[ab]	18,3±0,54[b]	0.038
n-3 (%)[2]	2,18±0,14	2,05±0,13	2,22±0,18	1,95±0,13	0.515
Ratio SFA:PUFA[2]	1,42±0,06[b]	1,67±0,08[a]	1,73±0,13[a]	1,72±0,09[a]	0.046
Ratio n-6:n-3[3]	10,21±0,54	9,56±0,45	9,39±0,59	10,1±0,47	0.483

[1]Values are means ± SEM. Groups that do not share the same letter are significantly different based on the one-way ANOVA test, using Duncan as a post hoc analysis ($p < 0.05$). Abbreviations: Kcal, calories; d, day; DHQ III, diet history questionnaire III; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids. [2]Analysis performed on log-transformed values. [3]Analysis performed using non-parametric testing.

4.4.3 Cytokine production

Cytokine production after mitogen stimulation by PBMCs at baseline is presented in **Table 4-5**. T cell proliferation after CD3/CD28 stimulation was significantly lower in participants with GI and T2D (P <0.05). After T cell stimulation with PHA, there was a trend towards a reduced production of IL-2 and an increased production of IL-10 leading to a significant reduction in the TNF-α:IL-10 ratio in the groups with obesity (P <0.05). IL-6 which can be considered both a Th1 or Th2 cytokine was also increased in the Obese/T2D group compared to the lean group. After LPS stimulation, there was a significant increase in IL-1B across groups and a significant reduction in TNF-α in the participants with obesity and GI (P <0.05). When considering the diet effect on NutrIMM, baseline and post-intervention data have also been presented in the **Table 4-6**. Besides T cell proliferation going down after the intervention, there was no other diet effect nor interaction between group and diet.

Table 4-5. Ex vivo cytokine production by mitogen-stimulated PBMC cells at baseline.[1,2]

Cytokine	Lean	Obese NG	Obese GI	Obese T2D	P-value
			CD3/CD28		
Stimulation (S/U)	2,93±0,23[a]	2,69±0,24[ab]	2,43±0,15[b]	2,24±0,23[b]	**0.032**
			PHA (T cell mitogen)		
IL-2	7613±724[a]	8352±951[a]	6147±663[ab]	5052±812[b]	0.072
TNF-α[4]	8817±1909	6348±893	6477±593	5673±714	0.374
IFN-γ	3147±643	2469±427	2361±307	2982±371	0.213
IL-10[3]	1012±238[b]	1667±329[ab]	1959±248[a]	2177±428[ab]	**0.055**
IL-6	46069±8037[b]	63374±9537[ab]	57772±10965[ab]	74134±15605[a]	**0.051**
Ratio TNFα:IL-10 [3]	10,8±2,2[a]	5,3±1,1[b]	3,9±0,5[b]	3,8±1,0[b]	**<0.001**
			LPS (Bacterial challenge)		
IL-1B[4]	630±128[c]	1127±233[bc]	1874±361[ab]	2768±497[a]	**<0.001**
TNF-α[3]	2113±531[ab]	3677±855[a]	1577±490[b]	3555±758[a]	**0.019**
IL-10[3]	562±102	737±161	810±158	855±123	0.229
IL-6	49027±8088	57079±9627	58487±8029	53165±6737	0.739

[1]Values are means ± SEM. Groups that do not share the same letter are significantly different based on the one-way ANOVA test, using Duncan as a post hoc analysis ($p < 0.05$). Abbreviations: IFN-γ, interferon γ; IL, interleukin; LPS, lipopolysaccharide; NG, normoglycemic; GI, glucose intolerance; T2D, type 2 diabetes; PHA, phytohaemagglutinin; S:U, stimulated:unstimulated; TNF-α, tumour necrosis factor α

[2]The following plasma cytokines were tested but resulted in nondetectable levels in the samples: LPS IFN-γ.

[3]Analysis performed on log-transformed values [4]Analysis performed using non-parametric testing.

Table 4-6. Ex vivo cytokine production by mitogen-stimulated PBMC cells at baseline and post-intervention.[1,2]

Cytokines	Lean		Obese NG		Obese GI		Obese T2D		P-group	P-diet	P-group* diet
	Baseline	Post-intervention	Baseline	Post-intervention	Baseline	Post-intervention	Baseline	Post-intervention			
CD3 / CD28											
S:U	3,15±0,3	2,7±0,29	2,91±0,28	2,59±0,27	2,32±0,26	1,99±0,25	2,29±0,27	2,24±0,26	0,074	0,010	0,690
PHA (T cell mitogen)											
PHA IL-2[3]	8590±707 [a]	6728±658 [a]	7966±784 [a]	7177±730 [a]	6326±632 [ab]	6737±589 [ab]	5472±666 [b]	5433±621 [b]	0,022	0,152	0,152
PHA TNF-α[3]	10511±775	8311±845	6453±903	7323±984	6012±690	6965±751	6751±775	6206±845	0,078	0,336	0,067
PHA IFN-γ[3]	2854±379	2442±355	1744±485	1541±454	2157±335	2350±314	3314±379	3055±355	0,107	0,172	0,498
PHA IL-10[3]	1553±233	1382±261	1997±272	1951±304	1928±203	1895±227	2021±227	2189±255	0,231	0,378	0,804
PHA IL-6	47298±10999	53126±10177	68248±12121	71076±11215	70503±9070	60749±8393	76962±10141	68383±9383	0,372	0,534	0,361
Ratio PHA TNFα:IL-10[3]	8,97±0,94 [a]	7,22±0,78 [a]	4±1,09 [b]	4,38±0,91 [b]	4,17±0,84 [b]	4,77±0,7 [b]	4,1±0,97 [b]	3,83±0,8 [b]	0,001	0,814	0,190
LPS (Bacterial challenge)											
LPS IL-1B[3]	647±495 [b]	1084±762 [b]	1713±395 [ab]	2082±608 [ab]	2004±338 [ab]	2040±521 [ab]	2221±301 [a]	2925±463 [a]	0,040	0,636	0,537
LPS TNF-α[3]	2049±825	2595±691	3168±703	3195±589	1688±602	1372±505	3966±535	2939±449	0,056	0,278	0,123
LPS IL-10[3]	577±162	674±148	804±138	874±127	789±114	702±105	818±105	762±96	0,885	0,797	0,160
LPS IL-6[3]	47498±11440	50116±10722	61145±9341	62036±8755	58236±7848	47313±7355	59267±7627	60010±7148	0,685	0,994	0,515

[1]Values are means ± SEM. Groups that do not share the same letter are significantly different between groups based on the two-way repeated measures ANOVA test, using Duncan as a post hoc analysis ($p < 0.05$). Abbreviations: IFNγ, interferon γ; IL, interleukin; LPS, lipopolysaccharide; NG, normoglycemic; GI, glucose intolerance; T2D, type 2 diabetes; PHA, phytohaemagglutinin; S:U, stimulated:unstimulated; TNF-α, tumour necrosis factor α

[2]The following plasma cytokines were tested but resulted in nondetectable levels in the samples: LPS IFN-γ.

[3]Analysis performed on log-transformed values.

4.4.4 Fatty acid composition from plasma total lipids

Proportions of fatty acids from plasma total lipids at baseline are depicted in **Table 4-7**. The results showed a significant increase in total SFA in the obese-T2D group, coming mainly from a higher proportion of C16:0 in all groups with obesity (P <0.05). There were also significantly higher levels of total MUFA across groups resulting mainly from an increase in C18:1n9 (P <0.05). Conversely, in terms of PUFA, there were lower plasma levels across groups coming from a reduction in C18:2n6 and C22:6n3 (P <0.05). There was an overall reduction in the n-6:n-3 ratio in the group with T2D compared to the lean group while there was a higher SFA: PUFA ratio across groups (P <0.05).

When considering the diet effect on NutrIMM, baseline and post-intervention data have also been presented in **Table 4-8**. Although no group*diet interaction was observed, several diet effects were reported.

Table 4-7. Proportion of fatty acids from plasma total lipids at baseline (%).[1]

Fatty acid	Lean	Obese-NG	Obese-GI	Obese-T2D	P-value
C14:0[2]	0,88±0,06	0,90±0,07	1,03±0,06	0,98±0,06	0.068
C14:1[3]	0,05±0,01	0,05±0,01	0,06±0,01	0,05±0,01	0.546
C15:0	0,25±0,01	0,23±0,01	0,25±0,01	0,22±0,01	0.082
C16:0	23,29±0,44^{b}	24,33±0,37^{a}	23,67±0,35^{a}	25,79±0,39^{a}	<0.001
C16:1n9	0,32±0,01^{c}	0,34±0,01^{bc}	0,36±0,01^{ab}	0,39±0,02^{a}	0.001
C16:1n7[2]	1,50±0,11^{b}	2,08±0,15^{a}	1,93±0,14^{a}	1,95±0^{a}	<0.001
C17:0	0,25±0,01	0,24±0,01	0,25±0,01	0,26±0,01	0.100
C17:1	0,10±0,01^{b}	0,12±0,01^{ab}	0,13±0,01^{a}	0,13±0,01^{a}	0.021
C18:0[2]	7,02±0,13	6,95±0,18	6,87±0,15	6,82±0,16	0.634
C18:1n9	19,99±0,42^{c}	21,39±0,6^{b}	22,58±0,58^{b}	24,65±0,72^{a}	<0.001
C18:1tn7	2,01±0,09	2,12±0,10	2,13±0,09	2,05±0,12	0.886
C18:2n6 (LA)	32,14±0,67^{a}	29,07±0,71^{b}	28,98±0,57^{b}	24,69±0,69^{c}	<0.001
C18:3n3 (ALA)	0,52±0,03	0,56±0,03	0,53±0,04	0,53±0,03	0.912
C20:0[2]	0,78±0,04	0,83±0,05	0,95±0,06	0,83±0,05	0.156
C20:2n6[3]	0,15±0,01	0,15±0,01	0,15±0,01	0,15±0,01	0.910
C20:3n6	1,63±0,06	1,79±0,07	1,77±0,05	1,61±0,07	0.123
C20:4n6 (ARA)	5,87±0,23	5,78±0,30	5,39±0,25	5,87±0,36	0.445
C20:5n3 (EPA)	0,5±0,03	0,51±0,04	0,53±0,03	0,54±0,05	0.781
C24:0[3]	0,37±0,02	0,39±0,03	0,32±0,02	0,34±0,03	0.087
C24:1n9	0,77±0,05^{a}	0,79±0,04^{a}	0,68±0,04^{ab}	0,60±0,03^{b}	0.006

C22:5n3 (DPA)[2]	0,33±0,02	0,31±0,02	0,32±0,01	0,35±0,02	0.513
C22:6n3 (DHA)[3]	1,28±0,06[a]	1,1±0,07[b]	1,13±0,08[b]	1,21±0,06[ab]	0.047
Total SFA[3]	32,85±0,43[b]	33,86±0,40[b]	33,34±0,37[b]	35,24±0,34[a]	<0.001
Total MUFA	24,73±0,46[c]	26,88±0,57[b]	27,87±0,61[b]	29,82±0,74[a]	<0.001
Total PUFA	42,42±0,77[a]	39,26±0,68[b]	38,79±0,71[b]	34,93±0,83[c]	<0.001
Total n-6	39,79±0,76[a]	36,79±0,71[b]	36,28±0,68[b]	32,32±0,81[c]	<0.001
Total n-3[2]	2,63±0,07	2,48±0,11	2,51±0,12	2,62±0,12	0.576
Ratio n-6:n-3	15,41±0,52[a]	15,62±0,90[a]	15,22±0,68[a]	12,89±0,60[b]	0.011
Ratio SFA:PUFA[3]	0,79±0,03[c]	0,87±0,02[b]	0,87±0,02[b]	1,03±0,03[a]	<0.001

[1]Values are means ± SEM. Groups that do not share the same letter are significantly different based on the one-way ANOVA test, using Duncan as a post hoc analysis ($p < 0.05$). Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; LA, linoleic acid; MUFA, monounsaturated fatty acids; N, omega; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. [2]Analysis performed on log-transformed values. [3]Analysis performed using non-parametric testing.

Table 4-8. Proportion of fatty acids from plasma total lipids at baseline and post-intervention. [1,2,3]

Fatty acid	Lean		Obese NG		Obese GI		Obese T2D		P-group	P-diet	P group* diet
	Baseline	Post-intervention	Baseline	Post-intervention	Baseline	Post-intervention	Baseline	Post-intervention			
C14:0[2]	0,85±0,06[b]	0,73±0,04[b]	0,87±0,07[b]	0,78±0,05[b]	1,06±0,06 [a]	0,85±0,04 [a]	0,96±0,07 [ab]	0,8±0,05 [ab]	0,013	0,000	0,849
C14:1[2,3]	0,05±0,01 [b]	0,04±0,01 [b]	0,05±0,01[ab]	0,05±0,01[ab]	0,06±0,01[a]	0,05±0[a]	0,05±0,01 [ab]	0,04±0,01 [ab]	0,036	0,038	0,909
C15:0[2,3]	0,24±0,01	0,24±0,01	0,22±0,01	0,23±0,01	0,25±0,01	0,24±0,01	0,22±0,01	0,22±0,01	0,075	0,700	0,548
C16:0	22,99±0,37 [c]	22,56±0,34[c]	24,1±0,4[b]	23,54±0,38[b]	23,82±0,36[b]	23,42±0,34[b]	25,56±0,4[a]	24,47±0,38[a]	0,000	0,007	0,692
C16:1n9	0,32±0,01[b]	0,29±0,01[b]	0,33±0,01[b]	0,33±0,01[b]	0,37±0,01[a]	0,36±0,01[a]	0,38±0,01[a]	0,39±0,01[a]	0,000	0,265	0,609
C16:1n7[2,3]	1,47±0,12 [b]	1,43±0,11[b]	2,1±0,14[a]	2,06±0,12[a]	1,97±0,12[a]	1,98±0,1[a]	1,95±0,14[a]	1,78±0,12[a]	0,000	0,574	0,589
C17:0[2,3]	0,25±0,01	0,28±0,01	0,23±0,01	0,26±0,01	0,25±0,01	0,28±0,01	0,26±0,01	0,28±0,01	0,120	0,003	0,988
C17:1[2,3]	0,1±0,01[b]	0,11±0,01[b]	0,12±0,01[a]	0,14±0,01[a]	0,12±0,01[a]	0,14±0,01[a]	0,13±0,01[a]	0,13±0,01[a]	0,001	0,000	0,940
C18:0	7,11±0,15	7±0,11	6,95±0,17	6,65±0,12	6,85±0,15	6,74±0,11	6,86±0,17	6,84±0,12	0,363	0,049	0,546
C18:1n9	19,95±0,6 [c]	20,52±0,55[c]	21,37±0,65[b]	22,68±0,6[b]	22,59±0,59[b]	23,57±0,54[b]	24,92±0,65[a]	25,46±0,6[a]	0,000	0,001	0,649
C18:1tn7	2,13±0,11	2,18±0,12	2,1±0,12	2,1±0,13	2,14±0,11	2,05±0,12	1,93±0,12	2,16±0,13	0,873	0,481	0,484
C18:2n6 (LA)	32,19±0,64[a]	31,77±0,58[a]	29,23±0,7[b]	28,39±0,63[b]	28,65±0,63[b]	27,66±0,56[b]	24,84±0,7[c]	24,27±0,63[c]	0,000	0,011	0,865
C18:3n3 (ALA)	0,53±0,04	0,41±0,04	0,54±0,04	0,47±0,05	0,54±0,04	0,5±0,04	0,51±0,04	0,51±0,05	0,677	0,027	0,365
C20:0	0,79±0,05	0,9±0,04	0,83±0,06	0,89±0,05	0,93±0,05	0,96±0,04	0,87±0,06	0,89±0,05	0,382	0,020	0,524
C20:2n6[2]	0,15±0,01	0,15±0	0,15±0,01	0,15±0,01	0,15±0,01	0,15±0	0,15±0,01	0,14±0,01	0,581	0,373	0,516
C20:3n6	1,63±0,06[b]	1,57±0,05[b]	1,81±0,07[ab]	1,7±0,06[ab]	1,79±0,06[a]	1,8±0,05[a]	1,62±0,07[b]	1,6±0,06[b]	0,018	0,023	0,214
C20:4n6 (ARA)	6±0,28	6,39±0,3	5,9±0,3	6,37±0,32	5,44±0,27	5,98±0,29	5,8±0,3	6,61±0,32	0,471	0,000	0,725
C20:5n3 (EPA)	0,5±0,04	0,49±0,04	0,53±0,04	0,51±0,04	0,55±0,04	0,55±0,04	0,53±0,04	0,53±0,04	0,745	0,620	0,989
C24:0[2]	0,38±0,03	0,36±0,03	0,39±0,03	0,32±0,03	0,32±0,03	0,36±0,03	0,35±0,03	0,38±0,03	0,829	0,648	0,100
C24:1n9[2,3]	0,79±0,04	0,85±0,06	0,8±0,05	0,84±0,07	0,67±0,04	0,81±0,06	0,58±0,05	0,75±0,07	0,204	0,157	0,372
C22:5n3 (DPA)	0,33±0,02	0,37±0,02	0,32±0,02	0,33±0,02	0,33±0,02	0,36±0,02	0,36±0,02	0,38±0,02	0,342	0,003	0,617
C22:6n3 (DHA)[2]	1,28±0,07	1,35±0,07	1,1±0,08	1,19±0,07	1,15±0,07	1,18±0,06	1,19±0,08	1,25±0,07	0,148	0,023	0,980

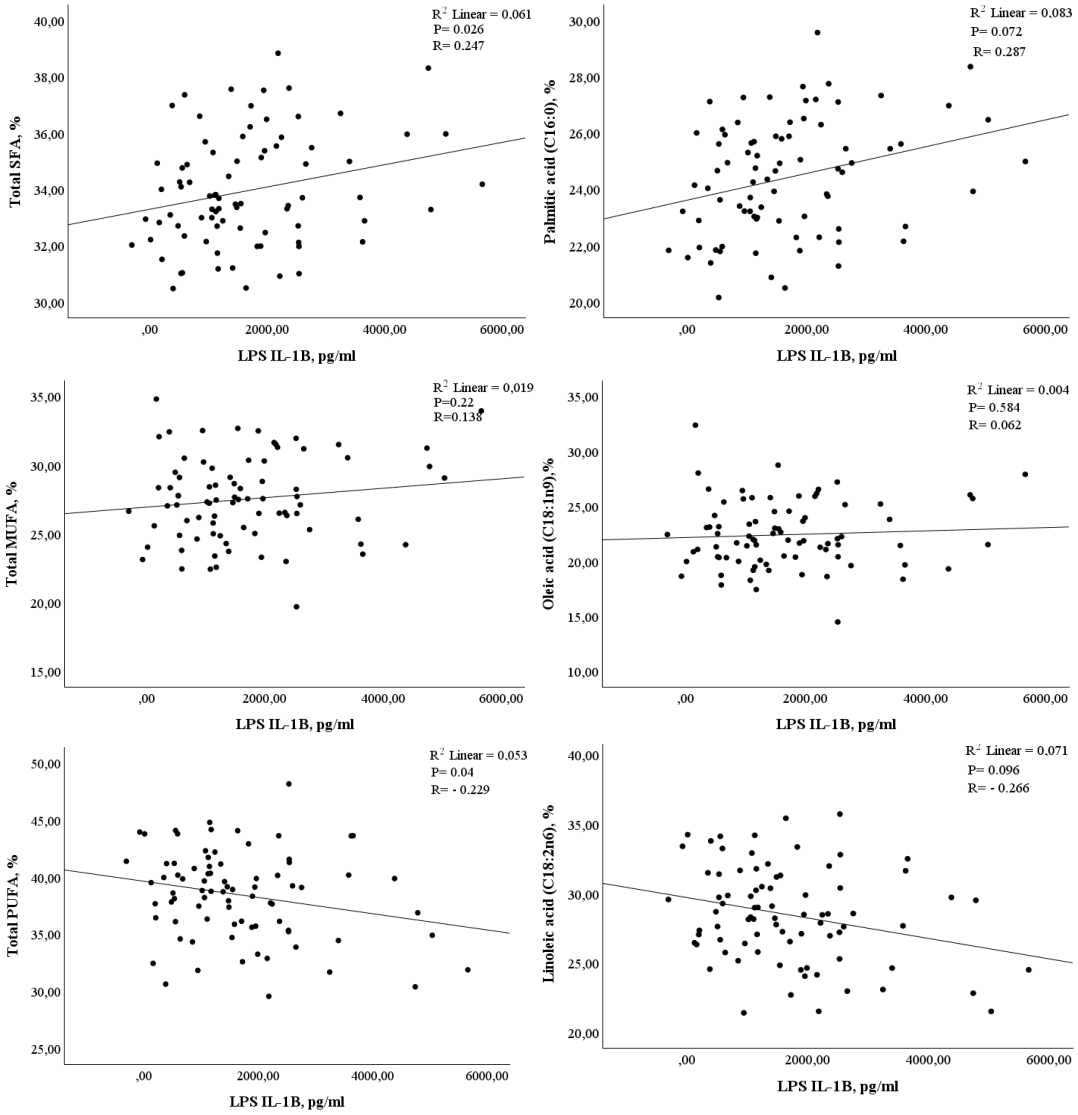
Total SFA	32,6±0,37[b]	32,07±0,35[b]	33,58±0,4[b]	32,67±0,38[b]	33,48±0,36[b]	32,85±0,34[b]	35,04±0,4[a]	33,85±0,38[a]	0,000	0,001	0,754
Total MUFA	24,8±0,61[c]	25,42±0,57[c]	26,83±0,66[b]	28,2±0,62[b]	27,92±0,59[b]	28,96±0,56[b]	29,95±0,66[a]	30,61±0,62[a]	0,000	0,000	0,674
Total PUFA	42,61±0,73[a]	42,51±0,68[a]	39,59±0,79[b]	39,12±0,73[b]	38,6±0,72[b]	38,18±0,66[b]	35,01±0,79[c]	35,29±0,73[c]	0,000	0,613	0,866
Total n6	39,98±0,72[a]	39,88±0,65[a]	37,1±0,78[b]	36,62±0,71[b]	36,03±0,7[b]	35,59±0,64[b]	32,41±0,78[c]	32,62±0,71[c]	0,000	0,552	0,882
Total n3	2,63±0,1	2,62±0,11	2,49±0,11	2,51±0,12	2,57±0,1	2,59±0,1	2,59±0,11	2,67±0,12	0,767	0,569	0,942
Ratio n6:n3[2]	15,54±0,65[a]	15,61±0,59[a]	15,66±0,71[a]	15,29±0,65[a]	14,73±0,64[a]	14,45±0,58[a]	13±0,71[b]	12,52±0,65[b]	0,002	0,451	0,927
Ratio SFA:PUFA[2]	0,78±0,02[c]	0,76±0,02[c]	0,85±0,03[b]	0,85±0,02[b]	0,88±0,02[b]	0,87±0,02[b]	1,01±0,03[a]	0,97±0,02[a]	0,000	0,169	0,874

[1]Values are means ± SEM. Groups that do not share the same letter are significantly different between groups based on the two-way ANOVA test, using Duncan as a post hoc analysis ($p < 0.05$). Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; LA, linoleic acid; MUFA, monounsaturated fatty acids; N, omega; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. [2]Analysis performed on log-transformed values. [3]Use of Games Howell as a Post Hoc test when homogeneity was violated.

4.4.5 Correlation between FA and cytokine production

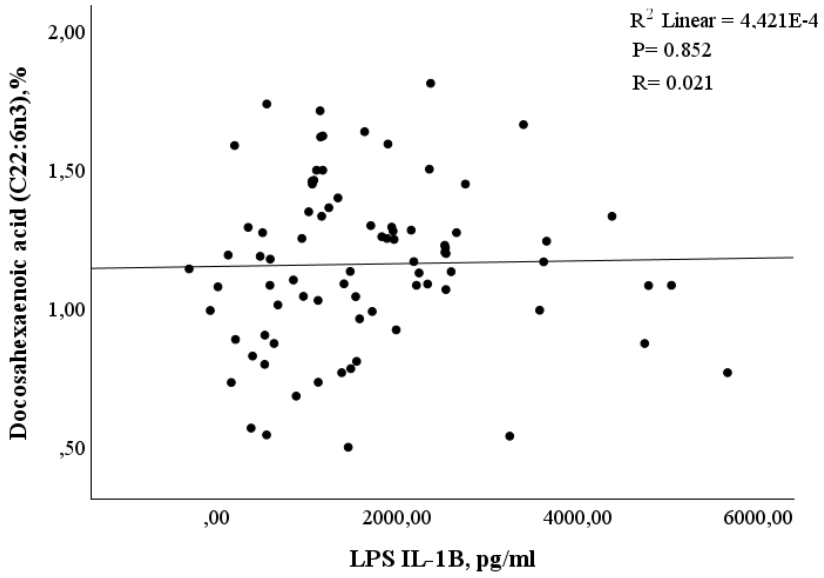
Correlations adjusted per group between the production of cytokines after mitogen stimulation and the proportion of FA in plasma total lipids at baseline are presented in **Figure 4-1**. Correlations were only performed for the main outcomes of NutrIMM (i.e. ex vivo cytokine production) and plasma FA that were significantly different across groups. Positive correlations were found between the production of IL-1B after LPS stimulation and total SFA (P <0.05, R= 0.247), mainly driven by a trend with palmitic acid (P =0.072, R= 0.287). In contrast, a negative correlation was found with total PUFA (P <0.05, R= -0,229), primarily due to a trend with LA (P =0.096, R= -0,266). No significant correlations were observed between IL-1B, and total MUFA or oleic acid, nor total n-3 or DHA (**Figure 4-2)** (P >0.05).

Figure 4-1. Scatter plots of the partial correlations adjusted per group between the production cytokines and plasma total lipids at baseline using Pearson's correlation.[1]



[1]Abbreviations : IL, Interleukin; LPS, lipopolysaccharide; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

Figure 4-2. Scatter plot of partial correlation adjusted per group between the production cytokines and plasma total lipids at baseline using Pearson's correlation coefficient.[1]



[1]Abbreviations : IL, Interleukin; LPS, lipopolysaccharide

4.5 Discussion

In this analysis of the NutrIMM study, we aimed to investigate the relationship between dietary FAs and immune function in people living with obesity and metabolic complications. Our results partially confirmed our hypothesis that SFAs are associated with increased production of pro-inflammatory cytokines from APCs, most likely due to palmitic acid while MUFA appears to have a neutral effect with no significant association with *ex vivo* cytokines production. However, total n-3, EPA, and DHA were not related to lower secretion of pro-inflammatory cytokines after stimulation. Instead, we reported that total PUFA and linoleic acid were inversely associated with IL-1B production. Altogether, our data suggest that a western-type diet, high in SFA and lower in PUFA (especially n-3) is associated with an increased production of pro-inflammation cytokine from APCs which could contribute to sustaining a state of chronic low-grade systemic inflammation in this population.

Indeed, considering systemic inflammation, we first demonstrated that plasma CRP level was elevated in all groups with obesity regardless of hyperglycemia (i.e. prediabetes or T2D).

This might be caused by the presence of adipocyte hypertrophy which eventually leads to a state of chronic low-grade systemic inflammation usually seen in obesity (de Heredia et al., 2012). Moreover, all groups with obesity had insulin resistance despite the obese-NG group having normal fasting blood glucose values and HbA1c, as assessed by both insulin levels and HOMA-IR. Inflammatory markers especially IL-6 and TNF-α have been shown to directly impair insulin signaling leading to insulin resistance and therefore contributing to the development of metabolic diseases (Carey & Febbraio, 2004; Hotamisligil et al., 1994; J. Kim et al., 2009). Additionally, our population with metabolic complications such as pre-diabetes and T2D was older than the obese-NG group with normal HbA1c. It is well known that age directly affects the immune system through a process called immunosenescence, while postmenopausal female experiences changes in the immune system through the reduction of estrogen levels. These changes are associated with increased levels of inflammatory markers (Abildgaard et al., 2020), therefore influencing our findings. Our data also suggest that the "obese metabolically healthy phenotype" found in the obese-NG group, could be transitional as around 74% of these individuals will end up developing the metabolic syndrome and/or T2D later in life (Ler et al., 2023; Tsatsoulis & Paschou, 2020).

As expected, we did not see major changes in immune function due to the NutrIMM menu (i.e. when comparing baseline to post-intervention values; **Table 4-6**). This is attributed to the fact that NutrIMM was providing a North American diet that was similar to most participants' habitual diet. Indeed, the NutrIMM menu had a macronutrient breakdown of 47% carbohydrates, 18% protein, and 36% fat, which mainly reflected participants' dietary habits as assessed by the DHQIII. Therefore, most of our analyses were focused on baseline data.

Regarding T cell stimulation, our results indicate an impaired T cell function in the groups with pre-diabetes and T2D, as evidenced by a lower T cell proliferation after CD3/CD28 stimulation and a trend towards a lower production of IL-2 after PHA stimulation (Cheng et al., 2002; Trickett & Kwan, 2003). This could be attributed to the fact that in the presence of insulin resistance, T cells are not able to obtain the necessary energy to perform their functions such as proliferation (Tsai et al., 2018). These results are consistent with our

previous studies where we showed impaired immune function in people living with obesity and T2D (Richard et al., 2017). Additionally, there was an increase in the Th2 response, while no change was observed in the Th1 response in all groups with obesity, leading to a lower Th1/Th2 ratio in the group with obesity and hyperglycemia. This imbalance could lead to a higher risk of infections and allergic/atopic diseases in this population (P. C. Calder, 2007; Gentile et al., 2008). Yet, when looking at the correlation between T cell function (i.e. proliferation, IL-2, IL-10, and the Th1/Th2 ratio) and plasma FA, no significant partial correlation was observed.

In terms of APC function, we found a higher production of pro-inflammatory cytokines with the secretion of IL-1B increasing gradually across the groups with obesity and T2D. TNF-α production after LPS stimulation was also higher in the group with obesity and normoglycemia and T2D (Ballak et al., 2015). As IL-1B and TNF-α are produced by macrophages/monocytes and dendritic cells, this increased production of pro-inflammatory cytokines after LPS stimulation could be attributed, at least partly, to the increased proportion of monocyte and APCs (i.e. cells expressing the MHC class II) in the group with obesity and hyperglycemia (data not shown in this chapter from the main NutrIMM paper). Regarding the relationship between FA and the production of pro-inflammatory cytokines by APCs, we demonstrated that the increased secretion of IL-1B after LPS stimulation was associated with the increased consumption of SFAs, especially palmitic acid in this cohort living with obesity. This result is consistent with the literature (Finucane et al., 2015; Zhou et al., 2013) since SFAs have been recognized to be natural ligands that bind to the TLR4 present on macrophages and dendritic cells, leading to the production of pro-inflammatory cytokines (Hwang et al., 2016a; J. Y. Lee et al., 2001a; J. Y. Lee, Ye, et al., 2003; Q. Li & Cherayil, 2003; Ravaut et al., 2020; Rogero & Calder, 2018a; Wong et al., 2009). Moreover, palmitic acid might induce the production of IL-1B by the activation of NLRP3 inflammasome (X. Li et al., 2024). In addition to that, the groups with obesity showed a trend towards an increase of SFA in plasma which is consistent with the increase reported from the DHQIII data collected, indicating that this population tends to consume more SFAs which has detrimental health effects.

Regarding MUFA, although there was an increase in plasma MUFA, particularly oleic acid, across groups, the DHQ III did not find significant changes. Additionally, no relationship was observed between plasma FAs and *ex vivo* cytokine production. This suggests an overall neutral effect of MUFA on cytokines production by immune cells. Yet, several studies have reported anti-inflammatory effects of a high MUFA diet such as the Mediterranean diet at least when assessing systemic inflammation including plasma CRP, IL-6, and TNF-α levels (Koelman et al., 2022; Quetglas-Llabrés et al., 2022; Richard et al., 2013; Urpi-Sarda et al., 2021). In the context of a MedDiet, it is important to consider the replacement effect where a high MUFA intake generally replaces high SFA intake in the diet, and therefore, the overall anti-inflammatory effect is more likely coming from the reduced intake of SFA rather than the increased intake of MUFA (Bédard et al., 2012; Shively et al., 2019b). Yet, other polyphenolic compounds present in extra virgin olive oil for instance could exert direct anti-inflammatory properties. (Bédard et al., 2012; Shively et al., 2019b)(Koelman et al., 2022; Quetglas-Llabrés et al., 2022; Richard et al., 2013; Urpi-Sarda et al., 2021)

Regarding PUFA, we were able to observe a decrease in the plasma proportion of PUFAs across the groups, which aligns with what these individuals reported in the DHQ III. Surprisingly, no relationship was found between n-3 and the production of pro-inflammatory cytokines despite a mountain of evidence that shows the anti-inflammatory of n-3, especially long-chain PUFA such as EPA and DHA (Dalli et al., 2013; Innes & Calder, 2018). Indeed, they have been reported to suppress IL-1B production by macrophages among other pro-inflammatory cytokines (Williams-Bey et al., 2014). One reason for this lack of relationship in our cohort might be the very low proportion of total n-3 relative to n-6 in plasma FA with an n-6/n-3 ratio of about 15 across groups (Risé et al., 2007). Conversely, a negative correlation between IL-1B secretion and total plasma PUFA and LA was found. LA, although generally recognized as a pro-inflammatory FA, can also exert some anti-inflammatory effects (Miles et al., 2002; Yu et al., 1995) by leading to the production of prostaglandin E2 which has been shown to reduce IL-1B levels (Rogero & Calder, 2018a). In fact, studies have found that dietary intake of LA is not associated with increased inflammatory markers in plasma (Innes & Calder, 2018; Pischon et al., 2003) especially when replacing high SFA intake.

The present study has some limitations, including the measurement of plasma total lipids which indicate the short-term consumption of FA, and does not fully represent the esterified FA composition in cell membranes, limiting the understanding of the relationship between membrane FA and immune function. FAs in red blood cells phospholipids should be assessed to have a better understanding of cell membrane FA and long-term consumption of dietary lipids. Another limitation is that we were only able to establish associations and no causation between the proportion of FA in plasma and cytokine production. Finally, despite the fact that our DHQIII data at baseline reflected the changes seen in plasma SFA and PUFA across groups, there are some limitations when using a food frequency questionnaire, since it might have recall bias or underreporting from the participants, affecting the reliability of the collected data.

In conclusion, our study suggests that plasma SFA and PUFA may have pro-inflammatory and anti-inflammatory properties when assessing the production of IL-1B by APCs, respectively while MUFAs have an overall neutral effect. This implies that the consumption of diets high in SFAs and low in PUFAs, such as the North American diet, may lead to increased production of pro-inflammatory cytokines that could contribute to chronic low-grade systemic inflammation and therefore contribute to immune dysfunction in a population living with obesity and metabolic complications.

4.6 Literature cited

Abildgaard, J., Tingstedt, J., Zhao, Y., Hartling, H. J., Pedersen, A. T., Lindegaard, B., & Dam Nielsen, S. (2020). Increased systemic inflammation and altered distribution of T-cell subsets in postmenopausal women. PLOS ONE, 15(6), e0235174. https://doi.org/10.1371/journal.pone.0235174

Andersen, C. J. (2022). Lipid Metabolism in Inflammation and Immune Function. Nutrients, 14(7), 1414. https://doi.org/10.3390/nu14071414

Ballak, D. B., Stienstra, R., Tack, C. J., Dinarello, C. A., & van Diepen, J. A. (2015). IL-1 family members in the pathogenesis and treatment of metabolic disease: Focus on adipose tissue inflammation and insulin resistance. Cytokine, 75(2), 280–290. https://doi.org/10.1016/j.cyto.2015.05.005

Bédard, A., Riverin, M., Dodin, S., Corneau, L., & Lemieux, S. (2012). Sex differences in the impact of the Mediterranean diet on cardiovascular risk profile. British Journal of Nutrition, 108(8), 1428–1434. https://doi.org/10.1017/S0007114511006969

Braga Tibaes, J. R., Barreto Silva, M. I., Makarowski, A., Cervantes, P. B., & Richard, C. (2023). The nutrition and immunity (nutrIMM) study: protocol for a non-randomized, four-arm parallel-group, controlled feeding trial investigating immune function in obesity and type 2 diabetes. Frontiers in Nutrition, 10. https://doi.org/10.3389/fnut.2023.1243359

Brenna, J. T., Plourde, M., Stark, K. D., Jones, P. J., & Lin, Y.-H. (2018). Best practices for the design, laboratory analysis, and reporting of trials involving fatty acids. The American Journal of Clinical Nutrition, 108(2), 211–227. https://doi.org/10.1093/ajcn/nqy089

Calder, P. C. (2007). Immunological Parameters: What Do They Mean?1,. The Journal of Nutrition, 137(3), 773S-780S. https://doi.org/10.1093/jn/137.3.773S

Calder, P. C. (2017). Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochemical Society Transactions, 45(5), 1105–1115. https://doi.org/10.1042/BST20160474

Carey, A. L., & Febbraio, M. A. (2004). Interleukin-6 and insulin sensitivity: friend or foe? Diabetologia, 47(7), 1135–1142. https://doi.org/10.1007/s00125-004-1447-y

Cheng, L. E., Öhlén, C., Nelson, B. H., & Greenberg, P. D. (2002). Enhanced signaling through the IL-2 receptor in CD8+ T cells regulated by antigen recognition results in preferential proliferation and expansion of responding CD8+ T cells rather than promotion of cell death. Proceedings of the National Academy of Sciences, 99(5), 3001–3006. https://doi.org/10.1073/pnas.052676899

Dalli, J., Colas, R. A., & Serhan, C. N. (2013). Novel n-3 Immunoresolvents: Structures and Actions. Scientific Reports, 3(1), 1940. https://doi.org/10.1038/srep01940

de Heredia, F. P., Gómez-Martínez, S., & Marcos, A. (2012). Obesity, inflammation and the immune system. Proceedings of the Nutrition Society, 71(2), 332–338. https://doi.org/10.1017/S0029665112000092

Dobrovolskaia, M. A., & Vogel, S. N. (2002). Toll receptors, CD14, and macrophage activation and deactivation by LPS. Microbes and Infection, 4(9), 903–914. https://doi.org/10.1016/S1286-4579(02)01613-1

Dowsey, M. M., & Choong, P. F. M. (2008). Obesity is a Major Risk Factor for Prosthetic Infection after Primary Hip Arthroplasty. Clinical Orthopaedics & Related Research, 466(1), 153–158. https://doi.org/10.1007/s11999-007-0016-3

Finucane, O. M., Lyons, C. L., Murphy, A. M., Reynolds, C. M., Klinger, R., Healy, N. P., Cooke, A. A., Coll, R. C., McAllan, L., Nilaweera, K. N., O'Reilly, M. E., Tierney, A. C., Morine, M. J., Alcala-Diaz, J. F., Lopez-Miranda, J., O'Connor, D. P., O'Neill, L. A., McGillicuddy, F. C., & Roche, H. M. (2015). Monounsaturated Fatty Acid–Enriched High-Fat Diets Impede Adipose NLRP3 Inflammasome–Mediated IL-1β Secretion and Insulin Resistance Despite Obesity. Diabetes, 64(6), 2116–2128. https://doi.org/10.2337/db14-1098

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. The Journal of Biological Chemistry, 226(1), 497–509.

Fritsche, K. L. (2015). The Science of Fatty Acids and Inflammation. Advances in Nutrition, 6(3), 293S-301S. https://doi.org/10.3945/an.114.006940

Geerlings, S. E., & Hoepelman, A. I. M. (1999). Immune dysfunction in patients with diabetes mellitus (DM). FEMS Immunology & Medical Microbiology, 26(3–4), 259–265. https://doi.org/10.1111/j.1574-695X.1999.tb01397.x

Gentile, D., Patel, A., Koehrsen, J., Schad, C., Schaffner, T., & Skoner, D. (2008). Elevations of Th2 Cytokines in CD8+ Lymphocytes from Overweight and Obese Allergic Subjects. Journal of Allergy and Clinical Immunology, 121(2), S120–S120. https://doi.org/10.1016/j.jaci.2007.12.478

Government of Canada. (2023). Type 2 diabetes. Type 2 Diabetes. https://www.canada.ca/en/public-health/services/diseases/type-2-diabetes.html

Hotamisligil, G. S., Murray, D. L., Choy, L. N., & Spiegelman, B. M. (1994). Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proceedings of the National Academy of Sciences, 91(11), 4854–4858. https://doi.org/10.1073/pnas.91.11.4854

Hwang, D. H., Kim, J.-A., & Lee, J. Y. (2016a). Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. European Journal of Pharmacology, 785, 24–35. https://doi.org/10.1016/j.ejphar.2016.04.024

Hwang, D. H., Kim, J.-A., & Lee, J. Y. (2016b). Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. European Journal of Pharmacology, 785, 24–35. https://doi.org/10.1016/j.ejphar.2016.04.024

Innes, J. K., & Calder, P. C. (2018). Omega-6 fatty acids and inflammation. Prostaglandins, Leukotrienes and Essential Fatty Acids, 132, 41–48. https://doi.org/10.1016/j.plefa.2018.03.004

Kim, J., Bachmann, R. A., & Chen, J. (2009). Chapter 21 Interleukin-6 and Insulin Resistance (pp. 613–633). https://doi.org/10.1016/S0083-6729(08)00621-3

Koelman, L., Egea Rodrigues, C., & Aleksandrova, K. (2022). Effects of Dietary Patterns on Biomarkers of Inflammation and Immune Responses: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Advances in Nutrition, 13(1), 101–115. https://doi.org/10.1093/advances/nmab086

Lee, J. Y., Sohn, K. H., Rhee, S. H., & Hwang, D. (2001a). Saturated Fatty Acids, but Not Unsaturated Fatty Acids, Induce the Expression of Cyclooxygenase-2 Mediated through Toll-like Receptor 4. Journal of Biological Chemistry, 276(20), 16683–16689. https://doi.org/10.1074/jbc.M011695200

Lee, J. Y., Sohn, K. H., Rhee, S. H., & Hwang, D. (2001b). Saturated Fatty Acids, but Not Unsaturated Fatty Acids, Induce the Expression of Cyclooxygenase-2 Mediated through Toll-like Receptor 4. Journal of Biological Chemistry, 276(20), 16683–16689. https://doi.org/10.1074/jbc.M011695200

Lee, J. Y., Ye, J., Gao, Z., Youn, H. S., Lee, W. H., Zhao, L., Sizemore, N., & Hwang, D. H. (2003). Reciprocal Modulation of Toll-like Receptor-4 Signaling Pathways Involving MyD88 and Phosphatidylinositol 3-Kinase/AKT by Saturated and Polyunsaturated Fatty Acids. Journal of Biological Chemistry, 278(39), 37041–37051. https://doi.org/10.1074/jbc.M305213200

Ler, P., Ojalehto, E., Zhan, Y., Finkel, D., Dahl Aslan, A. K., & Karlsson, I. K. (2023). Conversions between metabolically unhealthy and healthy obesity from midlife to late-life. International Journal of Obesity. https://doi.org/10.1038/s41366-023-01425-y

Li, C., Wang, G., Sivasami, P., Ramirez, R. N., Zhang, Y., Benoist, C., & Mathis, D. (2021). Interferon-α-producing plasmacytoid dendritic cells drive the loss of adipose tissue regulatory T cells during obesity. Cell Metabolism, 33(8), 1610-1623.e5. https://doi.org/10.1016/j.cmet.2021.06.007

Li, Q., & Cherayil, B. J. (2003). Role of Toll-Like Receptor 4 in Macrophage Activation and Tolerance during Salmonella enterica Serovar Typhimurium Infection. Infection and Immunity, 71(9), 4873–4882. https://doi.org/10.1128/IAI.71.9.4873-4882.2003

Li, X., Mai, K., & Ai, Q. (2024). Palmitic acid activates NLRP3 inflammasome through NF-κB and AMPK-mitophagy-ROS pathways to induce IL-1β production in large yellow croaker (Larimichthys crocea). Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1869(2), 159428. https://doi.org/10.1016/j.bbalip.2023.159428

Lillenfeld, D. E., Vlahov, D., Tenney, J. H., & McLaughlin, J. S. (1988). Obesity and diabetes as risk factors for postoperative wound infections after cardiac surgery. American Journal of Infection Control, 16(1), 3–6. https://doi.org/10.1016/0196-6553(88)90003-X

Luck, H., Khan, S., Kim, J. H., Copeland, J. K., Revelo, X. S., Tsai, S., Chakraborty, M., Cheng, K., Tao Chan, Y., Nøhr, M. K., Clemente-Casares, X., Perry, M.-C., Ghazarian, M., Lei, H., Lin, Y.-H., Coburn, B., Okrainec, A., Jackson, T., Poutanen, S., … Winer, D. A. (2019). Gut-associated IgA+ immune cells regulate obesity-related insulin resistance. Nature Communications, 10(1), 3650. https://doi.org/10.1038/s41467-019-11370-y

Miles, E. A., Allen, E., & Calder, P. C. (2002). In vitro effects of eicosanoids derived from different 20-carbon fatty acids on production of monocyte-derived cytokines in human whole blood cultures. Cytokine, 20(5), 215–223. https://doi.org/10.1006/cyto.2002.2007

Ogden, C. L. (2010). Prevalence of High Body Mass Index in US Children and Adolescents, 2007-2008. JAMA, 303(3), 242. https://doi.org/10.1001/jama.2009.2012

Peters, S. A. E., MacMahon, S., & Woodward, M. (2021). Obesity as a risk factor for COVID -19 mortality in women and men in the UK biobank: Comparisons with influenza/pneumonia and coronary heart disease. Diabetes, Obesity and Metabolism, 23(1), 258–262. https://doi.org/10.1111/dom.14199

Pischon, T., Hankinson, S. E., Hotamisligil, G. S., Rifai, N., Willett, W. C., & Rimm, E. B. (2003). Habitual Dietary Intake of n-3 and n-6 Fatty Acids in Relation to Inflammatory Markers Among US Men and Women. Circulation, 108(2), 155–160. https://doi.org/10.1161/01.CIR.0000079224.46084.C2

Quetglas-Llabrés, M. M., Monserrat-Mesquida, M., Bouzas, C., Gómez, C., Mateos, D., Ripoll-Vera, T., Tur, J. A., & Sureda, A. (2022). Inflammatory and Oxidative Stress Markers Related to Adherence to the Mediterranean Diet in Patients with Metabolic Syndrome. Antioxidants, 11(5), 901. https://doi.org/10.3390/antiox11050901

Ravaut, G., Légiot, A., Bergeron, K.-F., & Mounier, C. (2020). Monounsaturated Fatty Acids in Obesity-Related Inflammation. International Journal of Molecular Sciences, 22(1), 330. https://doi.org/10.3390/ijms22010330

Richard, C., Couture, P., Desroches, S., & Lamarche, B. (2013). Effect of the mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. Obesity, 21(1), 51–57. https://doi.org/10.1002/oby.20239

Richard, C., Wadowski, M., Goruk, S., Cameron, L., Sharma, A. M., & Field, C. J. (2017). Individuals with obesity and type 2 diabetes have additional immune dysfunction compared with obese individuals who are metabolically healthy. BMJ Open Diabetes Research & Care, 5(1), e000379. https://doi.org/10.1136/bmjdrc-2016-000379

Risé, P., Eligini, S., Ghezzi, S., Colli, S., & Galli, C. (2007). Fatty acid composition of plasma, blood cells and whole blood: Relevance for the assessment of the fatty acid status in humans. Prostaglandins, Leukotrienes and Essential Fatty Acids, 76(6), 363–369. https://doi.org/10.1016/j.plefa.2007.05.003

Rogero, M., & Calder, P. (2018a). Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. Nutrients, 10(4), 432. https://doi.org/10.3390/nu10040432

Rogero, M., & Calder, P. (2018b). Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. Nutrients, 10(4), 432. https://doi.org/10.3390/nu10040432

She, Y., Mangat, R., Tsai, S., Proctor, S. D., & Richard, C. (2022). The Interplay of Obesity, Dyslipidemia and Immune Dysfunction: A Brief Overview on Pathophysiology, Animal Models, and Nutritional Modulation. Frontiers in Nutrition, 9. https://doi.org/10.3389/fnut.2022.840209

Shively, C. A., Appt, S. E., Vitolins, M. Z., Uberseder, B., Michalson, K. T., Silverstein-Metzler, M. G., & Register, T. C. (2019). Mediterranean versus Western Diet Effects on Caloric Intake, Obesity, Metabolism, and Hepatosteatosis in Nonhuman Primates. Obesity, 27(5), 777–784. https://doi.org/10.1002/oby.22436

Sproston, N. R., & Ashworth, J. J. (2018). Role of C-Reactive Protein at Sites of Inflammation and Infection. Frontiers in Immunology, 9. https://doi.org/10.3389/fimmu.2018.00754

Trickett, A., & Kwan, Y. L. (2003). T cell stimulation and expansion using anti-CD3/CD28 beads. Journal of Immunological Methods, 275(1–2), 251–255. https://doi.org/10.1016/S0022-1759(03)00010-3

Tsai, S., Clemente-Casares, X., Zhou, A. C., Lei, H., Ahn, J. J., Chan, Y. T., Choi, O., Luck, H., Woo, M., Dunn, S. E., Engleman, E. G., Watts, T. H., Winer, S., & Winer, D. A. (2018). Insulin Receptor-Mediated Stimulation Boosts T Cell Immunity during Inflammation and Infection. Cell Metabolism, 28(6), 922-934.e4. https://doi.org/10.1016/j.cmet.2018.08.003

Tsatsoulis, A., & Paschou, S. A. (2020). Metabolically Healthy Obesity: Criteria, Epidemiology, Controversies, and Consequences. Current Obesity Reports, 9(2), 109–120. https://doi.org/10.1007/s13679-020-00375-0

Urpi-Sarda, M., Casas, R., Sacanella, E., Corella, D., Andrés-Lacueva, C., Llorach, R., Garrabou, G., Cardellach, F., Sala-Vila, A., Ros, E., Ruiz-Canela, M., Fitó, M., Salas-Salvadó, J., & Estruch, R. (2021). The 3-Year Effect of the Mediterranean Diet Intervention on Inflammatory Biomarkers Related to Cardiovascular Disease. Biomedicines, 9(8), 862. https://doi.org/10.3390/biomedicines9080862

Wagner, N., Brandhorst, G., Czepluch, F., Lankeit, M., Eberle, C., Herzberg, S., Faustin, V., Riggert, J., Oellerich, M., Hasenfuss, G., Konstantinides, S., & Schäfer, K. (2013). Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk. Obesity, 21(3), 461–468. https://doi.org/10.1002/oby.20087

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation, 112(12), 1796–1808. https://doi.org/10.1172/JCI200319246

WHO. (2023). Message from the Minister of Health – World Obesity Day 2023. https://www.canada.ca/en/public-health/news/2023/03/message-from-the-minister-of-health--world-obesity-day-2023.html

Williams-Bey, Y., Boularan, C., Vural, A., Huang, N.-N., Hwang, I.-Y., Shan-Shi, C., & Kehrl, J. H. (2014). Omega-3 Free Fatty Acids Suppress Macrophage Inflammasome Activation by Inhibiting NF-κB Activation and Enhancing Autophagy. PLoS ONE, 9(6), e97957. https://doi.org/10.1371/journal.pone.0097957

Wong, S. W., Kwon, M.-J., Choi, A. M. K., Kim, H.-P., Nakahira, K., & Hwang, D. H. (2009). Fatty Acids Modulate Toll-like Receptor 4 Activation through Regulation of Receptor Dimerization and Recruitment into Lipid Rafts in a Reactive Oxygen Species-dependent Manner. Journal of Biological Chemistry, 284(40), 27384–27392. https://doi.org/10.1074/jbc.M109.044065

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., Sole, J., Nichols, A., Ross, J. S., Tartaglia, L. A., & Chen, H. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. Journal of Clinical Investigation, 112(12), 1821–1830. https://doi.org/10.1172/JCI200319451

Yu, K., Bayona, W., Kallen, C. B., Harding, H. P., Ravera, C. P., McMahon, G., Brown, M., & Lazar, M. A. (1995). Differential Activation of Peroxisome Proliferator-activated Receptors by Eicosanoids. Journal of Biological Chemistry, 270(41), 23975–23983. https://doi.org/10.1074/jbc.270.41.23975

Zhou, B., Zhang, J., Zhang, Q., Permatasari, F., Xu, Y., Wu, D., Yin, Z., & Luo, D. (2013). Palmitic acid induces production of proinflammatory cytokines interleukin-6, interleukin-1β, and tumor necrosis factor-α via a NF-κB-dependent mechanism in HaCaT keratinocytes. Mediators of Inflammation, 2013, 1–11. https://doi.org/10.1155/2013/530429

Chapter 5: General discussion and future directions

5.1 Executive summary of findings

5.1.1. Consumption of a high-MUFA diet has an anti-inflammatory impact on T cell function in a diet-induced obesity (DIO) rat model

In Chapter 3 we examined the effect of a high MUFA diet on peripheral immune function (spleen) in the context of a DIO rat model. We hypothesized that a high MUFA diet will reduce pro-inflammatory responses by immune cells that are generally associated with the consumption of a HFD, high in SFAs. Our results support our hypothesis since we found that MUFA reduced the production of pro-inflammatory cytokines after T cell stimulation, specifically reducing TNF-α, and IL-6, with a trend towards a reduction in IFN-γ. However, there was no effect on APC function. In addition to that, HFOO in the context of a HFD, significantly reduced the secretion of IL-2 after ConA stimulation, suggesting diminished T cell proliferation compared to HFD high in SFAs. Taking it all together, our study suggests that in the context of a HFD leading to obesity, increased consumption of MUFA in the diet may have anti-inflammatory effects, which could contribute to attenuating the chronic-low grade inflammation often observed in obesity. However, systemic inflammation was not assessed in this animal study and no lean control group was included to understand how MUFA may normalize immune cell function relative to a healthy control.

5.1.2 The effect of dietary lipids on immune function: data from the nutrition and immunity (NutrIMM) study

In Chapter 4 we investigated the relationship between dietary FAs and immune function in people living with obesity and with and without metabolic complications. We hypothesized that 1) A higher proportion of SFAs in plasma (reflective of food intake) will be associated with an increased secretion of pro-inflammatory cytokines by stimulated immune cells. 2) A higher proportion of n-3 will be associated with a reduction in pro-inflammatory cytokines while 3) MUFAs will not lead to changes in the production of cytokines after immune cell stimulation exerting primarily a neutral effect. The results of this study partially supported our hypotheses. SFAs, mainly the proportion of palmitic acid in plasma, were positively

associated with the production of IL-1B from APCs while MUFAs were not associated with changes in the production of pro-inflammatory cytokines by immune cells. However, plasma levels of n-3 were not significantly associated with the reduction of pro-inflammatory cytokines. Indeed, our results rather showed that PUFAs, mainly driven by LA, were negatively associated with the secretion of IL-1B by APCs. This suggests that diets high in SFA and low in PUFAs can contribute to maintaining a state of chronic systemic inflammation by altering immune function.

5.2 General discussion and future directions

First, the work undertaken in this thesis was able to show that individuals with obesity and obesity-related metabolic complications, such as insulin resistance/glucose impairment (i.e. elevated insulin, HOMA-IR and HbA1c) and systemic inflammation (i.e. elevated circulating CRP levels) are associated with immune dysfunction. This immune dysfunction may explain in part the higher risk of infection including COVID-19 in this population (Ellulu et al., 2017; Wondmkun, 2020). These complications are thought to be attributable in part to the consumption of a HFD high in SFAs and lower in omega-3 fatty acids contributing to a state of chronic low-grade systemic inflammation impacting negatively the immune system (Luck et al., 2019a). In the human study (NutrIMM), we demonstrated that individuals with obesity and metabolic complications had impaired immune function both from T cells and APCs. This was evidenced by a reduction in T cell proliferation after CD3/CD28 stimulation, a lower secretion of IL-2 after T cell stimulation with PHA along with a decrease in the t helper Th1:Th2 ratio. Meanwhile, an increased production of pro-inflammatory cytokines including IL-1B and TNF-α after LPS stimulation was observed across the spectrum of obesity and its metabolic complications.

In the NutrIMM study, we were also able to relate these immune dysfunctions with changes in plasma FAs reflective, to some extent, to dietary lipids. This was the first human study to correlate plasma FAs to immune cell function beyond systemic inflammation. We also confirmed that the proportion of plasma SFAs, MUFAs, and PUFAs changed across the spectrum of obesity and metabolic complications when compared to lean individuals. Several research groups have contributed to documenting the role of different FAs in modulating

inflammatory pathways at the cellular level (Milanski et al., 2009; Sheppe & Edelmann, 2021). Here, we described the FA status in a cohort of individuals living with obesity with or without pre/T2D and correlated them with parameters of immune function.

In the case of SFAs, we were able to observe in our human study that people living with obesity and metabolic complications had a higher proportion of SFAs in plasma compared to lean individuals, particularly palmitic acid. These individuals also reported consuming more dietary SFAs from the DHQIII at baseline. SFAs have been highly recognized as being pro-inflammatory (Hwang et al., 2016a; J. Y. Lee et al., 2001a; J. Y. Lee, Ye, et al., 2003; Ravaut et al., 2020; Rogero & Calder, 2018a; Wong et al., 2009) which is consistent with our results since it was positively correlated with IL-1B production after LPS stimulation. This, in turn, could contribute to chronic systemic inflammation since macrophages and dendritic cells, which are major producers of IL-1B, have been shown to infiltrate the adipose tissue in the context of obesity (Lumeng et al., 2007; Weisberg et al., 2003a). Nevertheless, we were unable to confirm these findings in our animal studies since IL-1B wasn't measured following MUFA supplementation (with a lower SFAs intake) nor did we have a control lean group.

As it relates to MUFA, our animal study demonstrated that the HFOO had an anti-inflammatory effect by leading to a reduction in the secretion of IL-2, Th1 cytokines (TNF-α and, IFN-γ), and IL-6 after ConA stimulation compared to the HFD diet high in SFAs. These results are consistent with other studies that have suggested an anti-inflammatory effect of MUFA (Jeffery et al., 1996; Yaqoob et al., 1994). Conversely, in the NutrIMM study, despite a change in the proportion of MUFA in plasma across groups, which was primarily driven by oleic acid, we did not find any significant and meaningful association with cytokines production after immune cell stimulation. Considering both our animal and human studies, this suggests that MUFA itself may not have strong immunomodulatory properties but rather exert an overall anti-inflammatory effect when replacing SFAs in the diet. This was observed in the animal study, where a 5% replacement of SFA with MUFA led to positive outcomes, compared to the 1% replacement in the human study. Indeed, a high MUFA diet both from our animal experiment but also in humans following a Mediterranean

dietary pattern would lead to a reciprocal reduction in SFAs intake as MUFAs/PUFAs intake increases. This is particularly interesting because it suggests that MUFA have a neutral effect on inflammation and immune function while their reported protective effect likely comes from a reduction in the pro-inflammatory effect that SFAs confer (Yaqoob, 2002).

Concerning PUFAs, we did not evaluate their effects in our animal study since they were well-matched across all diet groups. However, in the NutrIMM study, we found a lower proportion of PUFAs in plasma in the groups with obesity and metabolic complications when compared to the lean group which was mainly explained by changes in LA proportions. LA in particular was negatively associated with IL-1B production after LPS stimulation. This is supported by previous reports showing that n-6 can also have anti-inflammatory properties (Miles et al., 2002; Yu et al., 1995) especially when replacing SFAs. Conversely, n-3 that have a well-documented anti-inflammatory effect (Dalli et al., 2013; Innes & Calder, 2018), showed no association with the production of pro-inflammatory cytokines by immune cells in our cohort of individuals living with obesity and metabolic complications. This may be due in part to the fact that n-3 intake relative to n-6 intake in our cohort was very low across all groups with a n6:n3 ratio in the range of 15.

Overall, our studies suggest that the consumption of a North American diet, a HFD characterized by a high intake of SFAs, and a low intake of PUFAs is associated with alteration in APCs function leading to a higher production of IL-1B, a pro-inflammatory cytokine. This in turn could contribute to the development of metabolic complications driven by inflammation as IL-1B, which has been shown to increase CRP production by the liver leading to systemic inflammation (Enquobahrie et al., 2009) but may also contribute to B cell dysfunction and therefore contributing to the development of T2D (Ibarra Urizar et al., 2019). Other dietary patterns such as the Mediterranean diet (MedDiet), distinguished by high intake of MUFAs, moderate intake of PUFAs, and low intake of SFAs, have been consistently associated with improvement in systemic inflammation (including a reduction in CRP, TNF-α, and IL-6) and overall cardiovascular health (Casas et al., 2016; Richard et al., 2013). Yet, no studies on the MedDiet have assessed its role in modulating immune function per se but

one could speculate that this overall anti-inflammatory effect of the MedDiet could be explained in part by reducing the production of pro-inflammatory cytokines by APCs.

Unfortunately, the rate of obesity in Canada and around the world has been increasing (WHO, 2024). The Canadian population tends to consume a North American diet, which is high in ultra-processed foods, processed meat, red meat, and sugar and low in fruits, legumes, vegetables, and whole grains (Clemente-Suárez et al., 2023). On average, Canadians consume about 12% of their daily energy intake from SFAs, whereas the recommendation is less than 10% (Harrison et al., 2019). In the case of MUFAs, the intake recommendation ranges between 10-25% of total energy. Canadians usually consume about 13%, which is consistent with our results (**Table 4-4**); however, as our studies suggest, rather than focusing only on the total amount of MUFAs consumed, it is necessary to have a significant replacement of SFA by MUFA to oberve positive health effects (at least 5% of total energy) (Y. Li et al., 2015). Meanwhile, the intake of EPA and DHA in the Canadian population is far below the recommended 250 mg per day (Meyer et al., 2003). Therefore, it is important to improve overall dietary habits through regular consumption of fatty fish or fish oil supplements along with increasing fiber intake while reducing SFA intake (Djuricic & Calder, 2022). In that regard, the new 2019 Canada´s food guide recommends a predominantly minimally processed plant-based diets (i.e. higher consumption of fruit, vegetables, whole grains, healthy fats, and vegetable oils) and reductions in the consumption of highly processed foods (Government of canada, 2024).

Although our research had several strengths such as examining the impact of FAs on immune function rather than just systemic inflammation like most of the current studies so far and employing both pre-clinical models of obesity and humans, a number of limitations can be highlighted. 1- in our human study, we only evaluated baseline parameters, without implementing interventions with different types of diets or FA compositions. As a result, we relied on correlation to identify associations, and cannot infer cause and effect relationship. Therefore, future studies should evaluate the effect of different FAs in humans on immune cell function using well-designed randomized controlled trials. 2- the use of plasma total lipids only reflects the short-term consumption of FAs and does not fully measure the

esterified phospholipid FAs in cell membranes (Brenna et al., 2018). This limitation affects our ability to determine the actual lipid pool available for immune cells to perform their activities such as the production of lipid mediators, thereby limitating our understanding of their effect on immune function. Future investigations should use different methods such as RBC which measure the proportion of FA in phospholipids present in the cell membrane, or even better the measurement of FAs in PBMCs that reflect the direct FA composition of immune cells (Kew et al., 2003). However, in our studies, we did not have enough immune cells left after all of our functional assays to perform lipid analysis on PBMCs. 3- another important aspect to consider would have been the use of intracellular staining after stimulation of our immune cells as part of our weekly protocol. Since cytokines are secreted by different types of immune cells, intracellular staining could be useful to identify exactly what type of cell secrete each cytokine, giving us a more detailed understanding of the changes in immune function (Jung et al., 1993; S. G. Smith et al., 2015).

Even though refined olive oil is high in MUFA and oleic acid (Khandouzi et al., 2021) which has some benefits for health (Khandouzi et al., 2020) other properties such as antioxidants and polyphenols present in extra virgin olive oil have been shown to exert stronger benefits. For example, Khandouzi et al. found that the consumption of extra virgin olive led to a reduction in plasma CRP levels and an increase in IL-10 production after LPS stimulation compared to refined olive oil (Khandouzi et al., 2021). Further investigations should compare the effect of olive oil to extra virgin olive oil to understand better the immunomodulatory properties of MUFA versus other anti-inflammatory components often present in high dietary sources of MUFA (i.e. extra virgin olive oil). Finally, in our research, we were not able to evaluate sex differences. Studies have shown that males in general have more pronounced impairment in immune response and higher levels of systemic inflammation after consuming a HFD compared to their female counterparts (Braga Tibaes et al., 2022). Some papers have determined that female hormones like estrogen may affect the metabolism of FAs. For instance, Paquette et al. found that estrogen appears to downregulate the expression of Stearoyl coenzyme A desaturase-1, an enzyme that catalyzes the biosynthesis of MUFA from SFA (Paquette et al., 2008; Paton & Ntambi, 2009). Therefore, future studies should assess

sex differences when trying to understand the effect of FAs intake on immune function and inflammation.

5.3 Conclusion

In conclusion, this research demonstrated the presence of immune dysfunction associated with obesity and metabolic complications and explored the immunomodulatory effects of the different types of fatty acids. SFAs exhibited pro-inflammatory effects by increasing the production of IL-1B after LPS stimulation. MUFAs did not show a direct relationship with the production of pro-inflammatory cytokines on their own; however, when replacing SFAs, MUFAs displayed anti-inflammatory properties by decreasing the Th1 response and diminishing IL-2 secretion after ConA stimulation. PUFAs, particularly linoleic acid, were found to also exert anti-inflammatory properties leading to the reduction in the secretion of IL-1B by APCs. Overall, it is recommended to avoid the consumption of high SFAs such as the North American diet, which may contribute to chronic low-grade systemic inflammation and immune dysfunction, and to prefer the consumption of diets high in MUFA and PUFA such as the Mediterranean diet.

5.4 Literature cited

Braga Tibaes, J. R., Azarcoya-Barrera, J., Wollin, B., Veida-Silva, H., Makarowski, A., Vine, D., Tsai, S., Jacobs, R., & Richard, C. (2022). Sex Differences Distinctly Impact High-Fat Diet-Induced Immune Dysfunction in Wistar Rats. The Journal of Nutrition, 152(5), 1347–1357. https://doi.org/10.1093/jn/nxac024

Brenna, J. T., Plourde, M., Stark, K. D., Jones, P. J., & Lin, Y.-H. (2018). Best practices for the design, laboratory analysis, and reporting of trials involving fatty acids. The American Journal of Clinical Nutrition, 108(2), 211–227. https://doi.org/10.1093/ajcn/nqy089

Casas, R., Sacanella, E., Urpí-Sardà, M., Corella, D., Castañer, O., Lamuela-Raventos, R.-M., Salas-Salvadó, J., Martínez-González, M.-A., Ros, E., & Estruch, R. (2016). Long-Term Immunomodulatory Effects of a Mediterranean Diet in Adults at High Risk of Cardiovascular Disease in the PREvención con DIeta MEDiterránea (PREDIMED) Randomized Controlled Trial. The Journal of Nutrition, 146(9), 1684–1693. https://doi.org/10.3945/jn.115.229476

Clemente-Suárez, V. J., Beltrán-Velasco, A. I., Redondo-Flórez, L., Martín-Rodríguez, A., & Tornero-Aguilera, J. F. (2023). Global Impacts of Western Diet and Its Effects on Metabolism and Health: A Narrative Review. Nutrients, 15(12), 2749. https://doi.org/10.3390/nu15122749

Dalli, J., Colas, R. A., & Serhan, C. N. (2013). Novel n-3 Immunoresolvents: Structures and Actions. Scientific Reports, 3(1), 1940. https://doi.org/10.1038/srep01940

Djuricic, I., & Calder, P. C. (2022). Polyunsaturated fatty acids and metabolic health: novel insights. Current Opinion in Clinical Nutrition & Metabolic Care, 25(6), 436–442. https://doi.org/10.1097/MCO.0000000000000865

Ellulu, M. S., Patimah, I., Khaza'ai, H., Rahmat, A., & Abed, Y. (2017). Obesity and inflammation: the linking mechanism and the complications. Archives of Medical Science, 4, 851–863. https://doi.org/10.5114/aoms.2016.58928

Enquobahrie, D. A., Rice, K., Williams, O. D., Williams, M. A., Gross, M. D., Lewis, C. E., Schwartz, S. M., & Siscovick, D. S. (2009). IL1B genetic variation and plasma C-reactive protein level among young adults: The CARDIA study. Atherosclerosis, 202(2), 513–520. https://doi.org/10.1016/j.atherosclerosis.2008.05.018

Government of canada. (2024). Canada's Food Guide. https://food-guide.canada.ca/en/

Harrison, S., Brassard, D., Lemieux, S., & Lamarche, B. (2019). Consumption and Sources of Saturated Fatty Acids According to the 2019 Canada Food Guide: Data from the 2015 Canadian Community Health Survey. Nutrients, 11(9), 1964. https://doi.org/10.3390/nu11091964

Hwang, D. H., Kim, J.-A., & Lee, J. Y. (2016). Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. European Journal of Pharmacology, 785, 24–35. https://doi.org/10.1016/j.ejphar.2016.04.024

Ibarra Urizar, A., Prause, M., Wortham, M., Sui, Y., Thams, P., Sander, M., Christensen, G. L., & Billestrup, N. (2019). Beta-cell dysfunction induced by non-cytotoxic concentrations of Interleukin-1β is associated with changes in expression of beta-cell maturity genes and associated histone modifications. Molecular and Cellular Endocrinology, 496, 110524. https://doi.org/10.1016/j.mce.2019.110524

Innes, J. K., & Calder, P. C. (2018). Omega-6 fatty acids and inflammation. Prostaglandins, Leukotrienes and Essential Fatty Acids, 132, 41–48. https://doi.org/10.1016/j.plefa.2018.03.004

Jung, T., Schauer, U., Heusser, C., Neumann, C., & Rieger, C. (1993). Detection of intracellular cytokines by flow cytometry. Journal of Immunological Methods, 159(1–2), 197–207. https://doi.org/10.1016/0022-1759(93)90158-4

Kew, S., Banerjee, T., Minihane, A. M., Finnegan, Y. E., Williams, C. M., & Calder, P. C. (2003). Relation between the fatty acid composition of peripheral blood mononuclear cells and measures of immune cell function in healthy, free-living subjects aged 25–72 y. The American Journal of Clinical Nutrition, 77(5), 1278–1286. https://doi.org/10.1093/ajcn/77.5.1278

Khandouzi, N., Zahedmehr, A., & Nasrollahzadeh, J. (2020). Effects of canola or olive oil on plasma lipids, lipoprotein-associated phospholipase A2 and inflammatory cytokines in patients referred for coronary angiography. Lipids in Health and Disease, 19(1), 183. https://doi.org/10.1186/s12944-020-01362-z

Khandouzi, N., Zahedmehr, A., & Nasrollahzadeh, J. (2021). Effect of polyphenol-rich extra-virgin olive oil on lipid profile and inflammatory biomarkers in patients undergoing coronary angiography: a randomised, controlled, clinical trial. International Journal of Food Sciences and Nutrition, 72(4), 548–558. https://doi.org/10.1080/09637486.2020.1841123

Lee, J. Y., Sohn, K. H., Rhee, S. H., & Hwang, D. (2001). Saturated Fatty Acids, but Not Unsaturated Fatty Acids, Induce the Expression of Cyclooxygenase-2 Mediated through Toll-like Receptor 4. Journal of Biological Chemistry, 276(20), 16683–16689. https://doi.org/10.1074/jbc.M011695200

Lee, J. Y., Ye, J., Gao, Z., Youn, H. S., Lee, W. H., Zhao, L., Sizemore, N., & Hwang, D. H. (2003). Reciprocal Modulation of Toll-like Receptor-4 Signaling Pathways Involving MyD88 and Phosphatidylinositol 3-Kinase/AKT by Saturated and Polyunsaturated Fatty Acids. Journal of Biological Chemistry, 278(39), 37041–37051. https://doi.org/10.1074/jbc.M305213200

Li, Y., Hruby, A., Bernstein, A. M., Ley, S. H., Wang, D. D., Chiuve, S. E., Sampson, L., Rexrode, K. M., Rimm, E. B., Willett, W. C., & Hu, F. B. (2015). Saturated Fats Compared With Unsaturated Fats and Sources of Carbohydrates in Relation to Risk of Coronary Heart Disease. Journal of the American College of Cardiology, 66(14), 1538–1548. https://doi.org/10.1016/j.jacc.2015.07.055

Luck, H., Khan, S., Kim, J. H., Copeland, J. K., Revelo, X. S., Tsai, S., Chakraborty, M., Cheng, K., Tao Chan, Y., Nøhr, M. K., Clemente-Casares, X., Perry, M.-C., Ghazarian, M., Lei, H., Lin, Y.-H., Coburn, B., Okrainec, A., Jackson, T., Poutanen, S., … Winer, D. A. (2019). Gut-associated IgA+ immune cells regulate obesity-related insulin resistance. Nature Communications, 10(1), 3650. https://doi.org/10.1038/s41467-019-11370-y

Lumeng, C. N., Bodzin, J. L., & Saltiel, A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of Clinical Investigation, 117(1), 175–184. https://doi.org/10.1172/JCI29881

Meyer, B. J., Mann, N. J., Lewis, J. L., Milligan, G. C., Sinclair, A. J., & Howe, P. R. C. (2003). Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. Lipids, 38(4), 391–398. https://doi.org/10.1007/s11745-003-1074-0

Milanski, M., Degasperi, G., Coope, A., Morari, J., Denis, R., Cintra, D. E., Tsukumo, D. M. L., Anhe, G., Amaral, M. E., Takahashi, H. K., Curi, R., Oliveira, H. C., Carvalheira, J. B. C., Bordin, S., Saad, M. J., & Velloso, L. A. (2009). Saturated Fatty Acids Produce an Inflammatory Response Predominantly through the Activation of TLR4 Signaling in Hypothalamus: Implications for the Pathogenesis of Obesity. The Journal of Neuroscience, 29(2), 359–370. https://doi.org/10.1523/JNEUROSCI.2760-08.2009

Miles, E. A., Allen, E., & Calder, P. C. (2002). In vitro effects of eicosanoids derived from different 20-carbon fatty acids on production of monocyte-derived cytokines in human whole blood cultures. Cytokine, 20(5), 215–223. https://doi.org/10.1006/cyto.2002.2007

Paquette, A., Wang, D., Jankowski, M., Gutkowska, J., & Lavoie, J.-M. (2008). Effects of ovariectomy on PPARα, SREBP-1c, and SCD-1 gene expression in the rat liver. Menopause, 15(6), 1169–1175. https://doi.org/10.1097/gme.0b013e31817b8159

Paton, C. M., & Ntambi, J. M. (2009). Biochemical and physiological function of stearoyl-CoA desaturase. American Journal of Physiology-Endocrinology and Metabolism, 297(1), E28–E37. https://doi.org/10.1152/ajpendo.90897.2008

Ravaut, G., Légiot, A., Bergeron, K.-F., & Mounier, C. (2020). Monounsaturated Fatty Acids in Obesity-Related Inflammation. International Journal of Molecular Sciences, 22(1), 330. https://doi.org/10.3390/ijms22010330

Richard, C., Couture, P., Desroches, S., & Lamarche, B. (2013). Effect of the mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. Obesity, 21(1), 51–57. https://doi.org/10.1002/oby.20239

Rogero, M., & Calder, P. (2018). Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. Nutrients, 10(4), 432. https://doi.org/10.3390/nu10040432

Sheppe, A. E. F., & Edelmann, M. J. (2021). Roles of Eicosanoids in Regulating Inflammation and Neutrophil Migration as an Innate Host Response to Bacterial Infections. Infection and Immunity, 89(8). https://doi.org/10.1128/IAI.00095-21

Smith, S. G., Smits, K., Joosten, S. A., van Meijgaarden, K. E., Satti, I., Fletcher, H. A., Caccamo, N., Dieli, F., Mascart, F., McShane, H., Dockrell, H. M., & Ottenhoff, T. H. M. (2015). Intracellular Cytokine Staining and Flow Cytometry: Considerations for Application in Clinical Trials of Novel Tuberculosis Vaccines. PLOS ONE, 10(9), e0138042. https://doi.org/10.1371/journal.pone.0138042

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. Journal of Clinical Investigation, 112(12), 1796–1808. https://doi.org/10.1172/JCI200319246

WHO. (2024, March 1). Obesity and overweight.

Wondmkun, Y. T. (2020). Obesity, Insulin Resistance, and Type 2 Diabetes: Associations and Therapeutic Implications. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, Volume 13, 3611–3616. https://doi.org/10.2147/DMSO.S275898

Wong, S. W., Kwon, M.-J., Choi, A. M. K., Kim, H.-P., Nakahira, K., & Hwang, D. H. (2009). Fatty Acids Modulate Toll-like Receptor 4 Activation through Regulation of Receptor Dimerization and Recruitment into Lipid Rafts in a Reactive Oxygen Species-dependent Manner. Journal of Biological Chemistry, 284(40), 27384–27392. https://doi.org/10.1074/jbc.M109.044065

Yaqoob, P. (2002). Monounsaturated fatty acids and immune function. European Journal of Clinical Nutrition, 56 Suppl 3, S9–S13. https://doi.org/10.1038/sj.ejcn.1601477

Yu, K., Bayona, W., Kallen, C. B., Harding, H. P., Ravera, C. P., McMahon, G., Brown, M., & Lazar, M. A. (1995). Differential Activation of Peroxisome Proliferator-activated Receptors by Eicosanoids. Journal of Biological Chemistry, 270(41), 23975–23983. https://doi.org/10.1074/jbc.270.41.23975

References

Abdelaal, M., le Roux, C. W., & Docherty, N. G. (2017). Morbidity and mortality associated with obesity. *Annals of Translational Medicine*, *5*(7), 161–161. https://doi.org/10.21037/atm.2017.03.107

Abildgaard, J., Tingstedt, J., Zhao, Y., Hartling, H. J., Pedersen, A. T., Lindegaard, B., & Dam Nielsen, S. (2020). Increased systemic inflammation and altered distribution of T-cell subsets in postmenopausal women. *PLOS ONE*, *15*(6), e0235174. https://doi.org/10.1371/journal.pone.0235174

Adam, L., López-González, M., Björk, A., Pålsson, S., Poux, C., Wahren-Herlenius, M., Fernández, C., & Spetz, A.-L. (2018). Early Resistance of Non-virulent Mycobacterial Infection in C57BL/6 Mice Is Associated With Rapid Up-Regulation of Antimicrobial Cathelicidin Camp. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.01939

Aguirre, V., Uchida, T., Yenush, L., Davis, R., & White, M. F. (2000). The c-Jun NH2-terminal Kinase Promotes Insulin Resistance during Association with Insulin Receptor Substrate-1 and Phosphorylation of Ser307. *Journal of Biological Chemistry*, *275*(12), 9047–9054. https://doi.org/10.1074/jbc.275.12.9047

Andersen, C. J. (2022). Lipid Metabolism in Inflammation and Immune Function. *Nutrients*, *14*(7), 1414. https://doi.org/10.3390/nu14071414

Aristizábal, B., & González, Á. (2013). Innate immune system. In J. Anaya, Y. Shoenfeld, & A. Rojas-Villarraga (Eds.), *Autoimmunity: From Bench to Bedside*. El Rosario University Press.

Ashen, M. D., & Blumenthal, R. S. (2005). Low HDL Cholesterol Levels. *New England Journal of Medicine*, *353*(12), 1252–1260. https://doi.org/10.1056/NEJMcp044370

Aziz, M., Iheanacho, F., & Hashmi, M. (2023). *Physiology, Antibody*. StatPearls.

Bachmann, M. F., & Oxenius, A. (2007). Interleukin 2: from immunostimulation to immunoregulation and back again. *EMBO Reports*, *8*(12), 1142–1148. https://doi.org/10.1038/sj.embor.7401099

Bailey, R. L. (2021). Overview of dietary assessment methods for measuring intakes of foods, beverages, and dietary supplements in research studies. *Current Opinion in Biotechnology*, *70*, 91–96. https://doi.org/10.1016/j.copbio.2021.02.007

Ballak, D. B., Stienstra, R., Tack, C. J., Dinarello, C. A., & van Diepen, J. A. (2015). IL-1 family members in the pathogenesis and treatment of metabolic disease: Focus on

adipose tissue inflammation and insulin resistance. *Cytokine*, *75*(2), 280–290. https://doi.org/10.1016/j.cyto.2015.05.005

Bannenberg, G., & Serhan, C. N. (2010). Specialized pro-resolving lipid mediators in the inflammatory response: An update. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1801*(12), 1260–1273. https://doi.org/10.1016/j.bbalip.2010.08.002

Basith, S., Manavalan, B., Lee, G., Kim, S. G., & Choi, S. (2011). Toll-like receptor modulators: a patent review (2006 – 2010). *Expert Opinion on Therapeutic Patents*, *21*(6), 927–944. https://doi.org/10.1517/13543776.2011.569494

Baylin, A., Kabagambe, E. K., Siles, X., & Campos, H. (2002). Adipose tissue biomarkers of fatty acid intake. *The American Journal of Clinical Nutrition*, *76*(4), 750–757. https://doi.org/10.1093/ajcn/76.4.750

BD biosciences. (2003). *Techniques for Immune Function Analysis* (1st ed.).

Bédard, A., Riverin, M., Dodin, S., Corneau, L., & Lemieux, S. (2012). Sex differences in the impact of the Mediterranean diet on cardiovascular risk profile. *British Journal of Nutrition*, *108*(8), 1428–1434. https://doi.org/10.1017/S0007114511006969

Bercovici, N., Duffour, M. T., Agrawal, S., Salcedo, M., & Abastado, J. P. (2000). New methods for assessing T-cell responses. *Clinical and Diagnostic Laboratory Immunology*, *7*(6), 859–864. https://doi.org/10.1128/CDLI.7.6.859-864.2000

Blair, A. S., Hajduch, E., Litherland, G. J., & Hundal, H. S. (1999). Regulation of Glucose Transport and Glycogen Synthesis in L6 Muscle Cells during Oxidative Stress. *Journal of Biological Chemistry*, *274*(51), 36293–36299. https://doi.org/10.1074/jbc.274.51.36293

Blewett, H. J., Gerdung, C. A., Ruth, M. R., Proctor, S. D., & Field, C. J. (2009a). Vaccenic acid favourably alters immune function in obese JCR:LA- cp rats. *British Journal of Nutrition*, *102*(04), 526. https://doi.org/10.1017/S0007114509231722

Blewett, H. J., Gerdung, C. A., Ruth, M. R., Proctor, S. D., & Field, C. J. (2009b). Vaccenic acid favourably alters immune function in obese JCR:LA- cp rats. *British Journal of Nutrition*, *102*(04), 526. https://doi.org/10.1017/S0007114509231722

Blumenreich, M. (1990). The White Blood Cell and Differential Count. In H. Walker, W. Hall, & J. Hurst (Eds.), *The History, Physical, and Laboratory Examinations*.

Boccellino, M., & D'Angelo, S. (2020). Anti-Obesity Effects of Polyphenol Intake: Current Status and Future Possibilities. *International Journal of Molecular Sciences*, *21*(16), 5642. https://doi.org/10.3390/ijms21165642

Boi, S. K., Buchta, C. M., Pearson, N. A., Francis, M. B., Meyerholz, D. K., Grobe, J. L., & Norian, L. A. (2016). Obesity alters immune and metabolic profiles: New insight from obese-resistant mice on high-fat diet. *Obesity*, *24*(10), 2140–2149. https://doi.org/10.1002/oby.21620

Boucher, J., Kleinridders, A., & Kahn, C. R. (2014). Insulin Receptor Signaling in Normal and Insulin-Resistant States. *Cold Spring Harbor Perspectives in Biology*, *6*(1), a009191–a009191. https://doi.org/10.1101/cshperspect.a009191

Braga Tibaes, J. R., Barreto Silva, M. I., Makarowski, A., Cervantes, P. B., & Richard, C. (2023). The nutrition and immunity (nutrIMM) study: protocol for a non-randomized, four-arm parallel-group, controlled feeding trial investigating immune function in obesity and type 2 diabetes. *Frontiers in Nutrition*, *10*. https://doi.org/10.3389/fnut.2023.1243359

Braga Tibaes, J. R., Azarcoya-Barrera, J., Wollin, B., Veida-Silva, H., Makarowski, A., Vine, D., Tsai, S., Jacobs, R., & Richard, C. (2022). Sex Differences Distinctly Impact High-Fat Diet-Induced Immune Dysfunction in Wistar Rats. *The Journal of Nutrition*, *152*(5), 1347–1357. https://doi.org/10.1093/jn/nxac024

Brenna, J. T., Plourde, M., Stark, K. D., Jones, P. J., & Lin, Y.-H. (2018). Best practices for the design, laboratory analysis, and reporting of trials involving fatty acids. *The American Journal of Clinical Nutrition*, *108*(2), 211–227. https://doi.org/10.1093/ajcn/nqy089

Britt, R. D., Thompson, M. A., Sasse, S., Pabelick, C. M., Gerber, A. N., & Prakash, Y. S. (2019). Th1 cytokines TNF-α and IFN-γ promote corticosteroid resistance in developing human airway smooth muscle. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *316*(1), L71–L81. https://doi.org/10.1152/ajplung.00547.2017

Bulló, M., García-Lorda, P., Megias, I., & Salas-Salvadó, J. (2003). Systemic Inflammation, Adipose Tissue Tumor Necrosis Factor, and Leptin Expression. *Obesity Research*, *11*(4), 525–531. https://doi.org/10.1038/oby.2003.74

Burdge, G. C., & Calder, P. C. (2015). *Introduction to Fatty Acids and Lipids* (pp. 1–16). https://doi.org/10.1159/000365423

Calder, P. C. (2007). Immunological Parameters: What Do They Mean?1,. *The Journal of Nutrition*, *137*(3), 773S-780S. https://doi.org/10.1093/jn/137.3.773S

Calder, P. C. (2009). Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. *Biochimie*, *91*(6), 791–795. https://doi.org/10.1016/j.biochi.2009.01.008

Calder, P. C. (2015). Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1851*(4), 469–484. https://doi.org/10.1016/j.bbalip.2014.08.010

Calder, P. C. (2017). Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochemical Society Transactions*, *45*(5), 1105–1115. https://doi.org/10.1042/BST20160474

Calder, P. C. (2022). Dietary factors and low-grade inflammation in relation to overweight and obesity revisted. *British Journal of Nutrition*, *127*(10), 1455–1457. https://doi.org/10.1017/S0007114522000782

Calder, P. C. (2023). Nutrition and immunity: lessons from coronavirus disease-2019. *Proceedings of the Nutrition Society*, 1–16. https://doi.org/10.1017/S0029665123004792

Calder, P., & Kulkarni, A. (2018). *Nutrition, immunity, and infection* . Taylor & Francis Group.

Cani, P. D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A. M., Delzenne, N. M., & Burcelin, R. (2008). Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet–Induced Obesity and Diabetes in Mice. *Diabetes*, *57*(6), 1470–1481. https://doi.org/10.2337/db07-1403

Carbone, F., La Rocca, C., & Matarese, G. (2012). Immunological functions of leptin and adiponectin. *Biochimie*, *94*(10), 2082–2088. https://doi.org/10.1016/j.biochi.2012.05.018

Carey, A. L., & Febbraio, M. A. (2004). Interleukin-6 and insulin sensitivity: friend or foe? *Diabetologia*, *47*(7), 1135–1142. https://doi.org/10.1007/s00125-004-1447-y

Carpentier, A. C. (2021). 100 anniversary of the discovery of insulin perspective: insulin and adipose tissue fatty acid metabolism. *American Journal of Physiology-Endocrinology and Metabolism*, *320*(4), E653–E670. https://doi.org/10.1152/ajpendo.00620.2020

Casas, R., Sacanella, E., Urpí-Sardà, M., Corella, D., Castañer, O., Lamuela-Raventos, R.-M., Salas-Salvadó, J., Martínez-González, M.-A., Ros, E., & Estruch, R. (2016). Long-Term Immunomodulatory Effects of a Mediterranean Diet in Adults at High Risk of Cardiovascular Disease in the PREvención con DIeta MEDiterránea (PREDIMED) Randomized Controlled Trial. *The Journal of Nutrition*, *146*(9), 1684–1693. https://doi.org/10.3945/jn.115.229476

CD® (Sprague Dawley) Rats | Charles River. (n.d.). Retrieved July 12, 2024, from https://www.criver.com/products-services/find-model/cd-sd-igs-rat?region=24

Chan, J. K., McDonald, B. E., Gerrard, J. M., Bruce, V. M., Weaver, B. J., & Holub, B. J. (1993). Effect of dietary α-linolenic acid and its ratio to linoleic acid on platelet and plasma fatty acids and thrombogenesis. *Lipids*, *28*(9), 811–817. https://doi.org/10.1007/BF02536235

Chandel, N. S. (2021). Lipid Metabolism. *Cold Spring Harbor Perspectives in Biology*, *13*(9), a040576. https://doi.org/10.1101/cshperspect.a040576

Chen, Y., & Junger, W. G. (2012). *Measurement of Oxidative Burst in Neutrophils* (pp. 115–124). https://doi.org/10.1007/978-1-61779-527-5_8

Cheng, L. E., Öhlén, C., Nelson, B. H., & Greenberg, P. D. (2002). Enhanced signaling through the IL-2 receptor in CD8+ T cells regulated by antigen recognition results in preferential proliferation and expansion of responding CD8+ T cells rather than promotion of cell death. *Proceedings of the National Academy of Sciences*, *99*(5), 3001–3006. https://doi.org/10.1073/pnas.052676899

Chidambaram, A. G., & Josephson, M. (2019). Clinical research study designs: The essentials. *Pediatric Investigation*, *3*(4), 245–252. https://doi.org/10.1002/ped4.12166

Choy, E., & Rose-John, S. (2017). Interleukin-6 as a Multifunctional Regulator: Inflammation, Immune Response, and Fibrosis. *Journal of Scleroderma and Related Disorders*, *2*(2_suppl), S1–S5. https://doi.org/10.5301/jsrd.5000265

Clemente-Suárez, V. J., Beltrán-Velasco, A. I., Redondo-Flórez, L., Martín-Rodríguez, A., & Tornero-Aguilera, J. F. (2023). Global Impacts of Western Diet and Its Effects on Metabolism and Health: A Narrative Review. *Nutrients*, *15*(12), 2749. https://doi.org/10.3390/nu15122749

Corripio-Miyar, Y., Hayward, A., Lemon, H., Sweeny, A. R., Bal, X., Kenyon, F., Pilkington, J. G., Pemberton, J. M., Nussey, D. H., & McNeilly, T. N. (2022). Functionally distinct T-helper cell phenotypes predict resistance to different types of parasites in a wild mammal. *Scientific Reports*, *12*(1), 3197. https://doi.org/10.1038/s41598-022-07149-9

Cunningham-Rundles, S. (1994). Malnutrition and gut immune function. *Current Opinion in Gastroenterology*, *10*(6), 664–670.

Dalli, J., Colas, R. A., & Serhan, C. N. (2013). Novel n-3 Immunoresolvents: Structures and Actions. *Scientific Reports*, *3*(1), 1940. https://doi.org/10.1038/srep01940

Dao, M. C., Subar, A. F., Warthon-Medina, M., Cade, J. E., Burrows, T., Golley, R. K., Forouhi, N. G., Pearce, M., & Holmes, B. A. (2019). Dietary assessment toolkits: an

overview. *Public Health Nutrition*, *22*(3), 404–418. https://doi.org/10.1017/S1368980018002951

de Heredia, F. P., Gómez-Martínez, S., & Marcos, A. (2012). Obesity, inflammation and the immune system. *Proceedings of the Nutrition Society*, *71*(2), 332–338. https://doi.org/10.1017/S0029665112000092

Dhaka, V., Gulia, N., Ahlawat, K. S., & Khatkar, B. S. (2011). Trans fats—sources, health risks and alternative approach - A review. *Journal of Food Science and Technology*, *48*(5), 534–541. https://doi.org/10.1007/s13197-010-0225-8

Diehl, S., & Rincón, M. (2002). The two faces of IL-6 on Th1/Th2 differentiation. *Molecular Immunology*, *39*(9), 531–536. https://doi.org/10.1016/S0161-5890(02)00210-9

Djuricic, I., & Calder, P. C. (2022). Polyunsaturated fatty acids and metabolic health: novel insights. *Current Opinion in Clinical Nutrition & Metabolic Care*, *25*(6), 436–442. https://doi.org/10.1097/MCO.0000000000000865

Dobrovolskaia, M. A., & Vogel, S. N. (2002a). Toll receptors, CD14, and macrophage activation and deactivation by LPS. *Microbes and Infection*, *4*(9), 903–914. https://doi.org/10.1016/S1286-4579(02)01613-1

Dobrovolskaia, M. A., & Vogel, S. N. (2002b). Toll receptors, CD14, and macrophage activation and deactivation by LPS. *Microbes and Infection*, *4*(9), 903–914. https://doi.org/10.1016/S1286-4579(02)01613-1

Dowsey, M. M., & Choong, P. F. M. (2008). Obesity is a Major Risk Factor for Prosthetic Infection after Primary Hip Arthroplasty. *Clinical Orthopaedics & Related Research*, *466*(1), 153–158. https://doi.org/10.1007/s11999-007-0016-3

Dragsted, L. O., Gao, Q., Scalbert, A., Vergères, G., Kolehmainen, M., Manach, C., Brennan, L., Afman, L. A., Wishart, D. S., Andres Lacueva, C., Garcia-Aloy, M., Verhagen, H., Feskens, E. J. M., & Praticò, G. (2018). Validation of biomarkers of food intake—critical assessment of candidate biomarkers. *Genes & Nutrition*, *13*(1), 14. https://doi.org/10.1186/s12263-018-0603-9

Eldridge, A. L., Piernas, C., Illner, A.-K., Gibney, M. J., Gurinović, M. A., De Vries, J. H. M., & Cade, J. E. (2018). Evaluation of New Technology-Based Tools for Dietary Intake Assessment—An ILSI Europe Dietary Intake and Exposure Task Force Evaluation. *Nutrients*, *11*(1), 55. https://doi.org/10.3390/nu11010055

Ellulu, M. S., Patimah, I., Khaza'ai, H., Rahmat, A., & Abed, Y. (2017). Obesity and inflammation: the linking mechanism and the complications. *Archives of Medical Science*, *4*, 851–863. https://doi.org/10.5114/aoms.2016.58928

Enquobahrie, D. A., Rice, K., Williams, O. D., Williams, M. A., Gross, M. D., Lewis, C. E., Schwartz, S. M., & Siscovick, D. S. (2009). IL1B genetic variation and plasma C-reactive protein level among young adults: The CARDIA study. *Atherosclerosis*, *202*(2), 513–520. https://doi.org/10.1016/j.atherosclerosis.2008.05.018

Epstein, F. H., Lewis, R. A., Austen, K. F., & Soberman, R. J. (1990). Leukotrienes and Other Products of the 5-Lipoxygenase Pathway. *New England Journal of Medicine*, *323*(10), 645–655. https://doi.org/10.1056/NEJM199009063231006

Everman, S., Meyer, C., Tran, L., Hoffman, N., Carroll, C. C., Dedmon, W. L., & Katsanos, C. S. (2016). Insulin does not stimulate muscle protein synthesis during increased plasma branched-chain amino acids alone but still decreases whole body proteolysis in humans. *American Journal of Physiology-Endocrinology and Metabolism*, *311*(4), E671–E677. https://doi.org/10.1152/ajpendo.00120.2016

Fattahi, Y., Heidari, H. R., & Khosroushahi, A. Y. (2020). Review of short-chain fatty acids effects on the immune system and cancer. *Food Bioscience*, *38*, 100793. https://doi.org/10.1016/j.fbio.2020.100793

Feingold, K., & Grunfeld, C. (2000). *The Effect of Inflammation and Infection on Lipids and Lipoproteins* (K. Feingold, B. Anawalt, & M. Blackman, Eds.). Endotext [Internet].

Festa, A., D'Agostino Jr, R., Williams, K., Karter, A., Mayer-Davis, E., Tracy, R., & Haffner, S. (2001). The relation of body fat mass and distribution to markers of chronic inflammation. *International Journal of Obesity*, *25*(10), 1407–1415. https://doi.org/10.1038/sj.ijo.0801792

Field, C. J., Thomson, C. A., Van Aerde, J. E., Parrott, A., Euler, &NA;, Lien, E., & Clandinin, M. T. (2000). Lower Proportion of CD45R0+ Cells and Deficient Interleukin-10 Production by Formula-Fed Infants, Compared With Human-Fed, Is Corrected With Supplementation of Long-Chain Polyunsaturated Fatty Acids. *Journal of Pediatric Gastroenterology and Nutrition*, *31*(3), 291–299. https://doi.org/10.1097/00005176-200009000-00017

Field, C. J., Wu, G., Métroz-Dayer, M. D., Montambault, M., & Marliss, E. B. (1990). Lactate production is the major metabolic fate of glucose in splenocytes and is altered in spontaneously diabetic BB rats. *The Biochemical Journal*, *272*(2), 445–452. https://doi.org/10.1042/bj2720445

Finucane, O. M., Lyons, C. L., Murphy, A. M., Reynolds, C. M., Klinger, R., Healy, N. P., Cooke, A. A., Coll, R. C., McAllan, L., Nilaweera, K. N., O'Reilly, M. E., Tierney, A. C., Morine, M. J., Alcala-Diaz, J. F., Lopez-Miranda, J., O'Connor, D. P., O'Neill, L. A., McGillicuddy, F. C., & Roche, H. M. (2015). Monounsaturated Fatty Acid–Enriched High-Fat Diets Impede Adipose NLRP3 Inflammasome–Mediated IL-1β

Secretion and Insulin Resistance Despite Obesity. *Diabetes*, *64*(6), 2116–2128. https://doi.org/10.2337/db14-1098

Folch, J., Busquets, O., Ettcheto, M., Sánchez-López, E., Pallàs, M., Beas-Zarate, C., Marin, M., Casadesus, G., Olloquequi, J., Auladell, C., & Camins, A. (2018). Experimental Models for Aging and their Potential for Novel Drug Discovery. *Current Neuropharmacology*, *16*(10), 1466–1483. https://doi.org/10.2174/1570159X15666170707155345

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, *226*(1), 497–509.

Fritsche, K. L. (2015a). The Science of Fatty Acids and Inflammation. *Advances in Nutrition*, *6*(3), 293S-301S. https://doi.org/10.3945/an.114.006940

Fritsche, K. L. (2015b). The Science of Fatty Acids and Inflammation. *Advances in Nutrition*, *6*(3), 293S-301S. https://doi.org/10.3945/an.114.006940

Furman, D., Campisi, J., Verdin, E., Carrera-Bastos, P., Targ, S., Franceschi, C., Ferrucci, L., Gilroy, D. W., Fasano, A., Miller, G. W., Miller, A. H., Mantovani, A., Weyand, C. M., Barzilai, N., Goronzy, J. J., Rando, T. A., Effros, R. B., Lucia, A., Kleinstreuer, N., & Slavich, G. M. (2019). Chronic inflammation in the etiology of disease across the life span. *Nature Medicine*, *25*(12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Gabay, C. (2006). Interleukin-6 and chronic inflammation. *Arthritis Research & Therapy*, *8*(Suppl 2), S3. https://doi.org/10.1186/ar1917

Geerlings, S. E., & Hoepelman, A. I. M. (1999). Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunology & Medical Microbiology*, *26*(3–4), 259–265. https://doi.org/10.1111/j.1574-695X.1999.tb01397.x

Gentile, D., Patel, A., Koehrsen, J., Schad, C., Schaffner, T., & Skoner, D. (2008). Elevations of Th2 Cytokines in CD8+ Lymphocytes from Overweight and Obese Allergic Subjects. *Journal of Allergy and Clinical Immunology*, *121*(2), S120–S120. https://doi.org/10.1016/j.jaci.2007.12.478

Gombart, A. F., Pierre, A., & Maggini, S. (2020). A Review of Micronutrients and the Immune System–Working in Harmony to Reduce the Risk of Infection. *Nutrients*, *12*(1), 236. https://doi.org/10.3390/nu12010236

Gonzales, A. M., & Orlando, R. A. (2008). Curcumin and resveratrol inhibit nuclear factor-kappaB-mediated cytokine expression in adipocytes. *Nutrition & Metabolism*, *5*(1), 17. https://doi.org/10.1186/1743-7075-5-17

Government of Canada. (2016, March 1). *Leading causes of hospitalizations, Canada, 2009/10, males and females combined, counts*.

Government of Canada. (2023). *Type 2 diabetes*. Type 2 Diabetes. https://www.canada.ca/en/public-health/services/diseases/type-2-diabetes.html

Government of canada. (2024). *Canada's Food Guide*. https://food-guide.canada.ca/en/

Guo, S. (2014). Insulin signaling, resistance, and metabolic syndrome: insights from mouse models into disease mechanisms. *Journal of Endocrinology*, *220*(2), T1–T23. https://doi.org/10.1530/JOE-13-0327

Guo, X., Tresserra-Rimbau, A., Estruch, R., Martínez-González, M., Medina-Remón, A., Fitó, M., Corella, D., Salas-Salvadó, J., Portillo, M., Moreno, J., Pi-Sunyer, X., & Lamuela-Raventós, R. (2017). Polyphenol Levels Are Inversely Correlated with Body Weight and Obesity in an Elderly Population after 5 Years of Follow Up (The Randomised PREDIMED Study). *Nutrients*, *9*(5), 452. https://doi.org/10.3390/nu9050452

Han, M. S., Jung, D. Y., Morel, C., Lakhani, S. A., Kim, J. K., Flavell, R. A., & Davis, R. J. (2013). JNK Expression by Macrophages Promotes Obesity-Induced Insulin Resistance and Inflammation. *Science*, *339*(6116), 218–222. https://doi.org/10.1126/science.1227568

Harrison, S., Brassard, D., Lemieux, S., & Lamarche, B. (2019). Consumption and Sources of Saturated Fatty Acids According to the 2019 Canada Food Guide: Data from the 2015 Canadian Community Health Survey. *Nutrients*, *11*(9), 1964. https://doi.org/10.3390/nu11091964

Hartemink, N., Boshuizen, H. C., Nagelkerke, N. J. D., Jacobs, M. A. M., & van Houwelingen, H. C. (2006). Combining Risk Estimates from Observational Studies with Different Exposure Cutpoints: A Meta-analysis on Body Mass Index and Diabetes Type 2. *American Journal of Epidemiology*, *163*(11), 1042–1052. https://doi.org/10.1093/aje/kwj141

Heinzmann, S. S., Holmes, E., Kochhar, S., Nicholson, J. K., & Schmitt-Kopplin, P. (2015). 2-Furoylglycine as a Candidate Biomarker of Coffee Consumption. *Journal of Agricultural and Food Chemistry*, *63*(38), 8615–8621. https://doi.org/10.1021/acs.jafc.5b03040

Helderman, J. H., & Strom, T. B. (1978). Specific insulin binding site on T and B lymphocytes as a marker of cell activation. *Nature*, *274*(5666), 62–63. https://doi.org/10.1038/274062a0

Hermsdorff, H. H. M., Zulet, M. Á., Abete, I., & Martínez, J. A. (2009). Discriminated benefits of a Mediterranean dietary pattern within a hypocaloric diet program on plasma RBP4 concentrations and other inflammatory markers in obese subjects. *Endocrine*, *36*(3), 445–451. https://doi.org/10.1007/s12020-009-9248-1

Hirosumi, J., Tuncman, G., Chang, L., Görgün, C. Z., Uysal, K. T., Maeda, K., Karin, M., & Hotamisligil, G. S. (2002). A central role for JNK in obesity and insulin resistance. *Nature*, *420*(6913), 333–336. https://doi.org/10.1038/nature01137

Hoong, C. W. S., Hussain, I., Aravamudan, V. M., Phyu, E. E., Lin, J. H. X., & Koh, H. (2021). Obesity is Associated with Poor Covid-19 Outcomes: A Systematic Review and Meta-Analysis. *Hormone and Metabolic Research*, *53*(02), 85–93. https://doi.org/10.1055/a-1326-2125

Hossain, P., Kawar, B., & El Nahas, M. (2007). Obesity and Diabetes in the Developing World — A Growing Challenge. *New England Journal of Medicine*, *356*(3), 213–215. https://doi.org/10.1056/NEJMp068177

Hotamisligil, G. S. (2017). Foundations of Immunometabolism and Implications for Metabolic Health and Disease. *Immunity*, *47*(3), 406–420. https://doi.org/10.1016/j.immuni.2017.08.009

Hotamisligil, G. S., Murray, D. L., Choy, L. N., & Spiegelman, B. M. (1994). Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proceedings of the National Academy of Sciences*, *91*(11), 4854–4858. https://doi.org/10.1073/pnas.91.11.4854

Hsu, A., Aronoff, D. M., Phipps, J., Goel, D., & Mancuso, P. (2007). Leptin improves pulmonary bacterial clearance and survival in ob/ob mice during pneumococcal pneumonia. *Clinical and Experimental Immunology*, *150*(2), 332–339. https://doi.org/10.1111/j.1365-2249.2007.03491.x

Hussein, O. (2007). Monounsaturated fat decreases hepatic lipid content in non-alcoholic fatty liver disease in rats. *World Journal of Gastroenterology*, *13*(3), 361. https://doi.org/10.3748/wjg.v13.i3.361

Hwang, D. H., Kim, J.-A., & Lee, J. Y. (2016a). Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. *European Journal of Pharmacology*, *785*, 24–35. https://doi.org/10.1016/j.ejphar.2016.04.024

Hwang, D. H., Kim, J.-A., & Lee, J. Y. (2016b). Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. *European Journal of Pharmacology*, *785*, 24–35. https://doi.org/10.1016/j.ejphar.2016.04.024

Ibarra Urizar, A., Prause, M., Wortham, M., Sui, Y., Thams, P., Sander, M., Christensen, G. L., & Billestrup, N. (2019). Beta-cell dysfunction induced by non-cytotoxic concentrations of Interleukin-1β is associated with changes in expression of beta-cell maturity genes and associated histone modifications. *Molecular and Cellular Endocrinology*, *496*, 110524. https://doi.org/10.1016/j.mce.2019.110524

Idriss, H. T., & Naismith, J. H. (2000). TNFa and the TNF receptor superfamily: Structure-function relationship(s). *Microscopy Research and Technique*, *50*(3), 184–195. https://doi.org/10.1002/1097-0029(20000801)50:3<184::AID-JEMT2>3.0.CO;2-H

Immunology of the rat. (1998). In *Handbook of Vertebrate Immunology* (pp. 137–222). Elsevier. https://doi.org/10.1016/B978-012546401-7/50007-X

Innes, J. K., & Calder, P. C. (2018). Omega-6 fatty acids and inflammation. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *132*, 41–48. https://doi.org/10.1016/j.plefa.2018.03.004

Islam, T., Koboziev, I., Albracht-Schulte, K., Mistretta, B., Scoggin, S., Yosofvand, M., Moussa, H., Zabet-Moghaddam, M., Ramalingam, L., Gunaratne, P. H., & Moustaid-Moussa, N. (2021). Curcumin Reduces Adipose Tissue Inflammation and Alters Gut Microbiota in Diet-Induced Obese Male Mice. *Molecular Nutrition & Food Research*, *65*(22). https://doi.org/10.1002/mnfr.202100274

Janeway, C., Travers, P., & Walport, M. (2001a). *Immunobiology: The Immune System in Health and Disease.* (5th ed.). Garland Science.

Janeway, C., Travers, P., & Walport, M. (2001b). *The Immune System in Health and Disease* (5th ed.). Garland Science.

Janssen, I., Katzmarzyk, P. T., & Ross, R. (2004). Waist circumference and not body mass index explains obesity-related health risk. *The American Journal of Clinical Nutrition*, *79*(3), 379–384. https://doi.org/10.1093/ajcn/79.3.379

Jeffery, N. M., Cortina, M., Newsholme, E. A., & Calder, P. C. (1997). Effects of variations in the proportions of saturated, monounsaturated and polyunsaturated fatty acids in the rat diet on spleen lymphocyte functions. *British Journal of Nutrition*, *77*(5), 805–823. https://doi.org/10.1079/BJN19970077

Jeffery, N. M., Yaqoob, P., Kewsholme, E. A., & Calder, P. C. (1996). The Effects of Olive Oil upon Rat Serum Lipid Levels and Lymphocyte Functions Appear to Be Due to Oleic

Acid. *Annals of Nutrition and Metabolism*, *40*(2), 71–80. https://doi.org/10.1159/000177898

Jha, R., & Berrocoso, J. F. D. (2016). Dietary fiber and protein fermentation in the intestine of swine and their interactive effects on gut health and on the environment: A review. *Animal Feed Science and Technology*, *212*, 18–26. https://doi.org/10.1016/j.anifeedsci.2015.12.002

Jiang, S.-Z., Lu, W., Zong, X.-F., Ruan, H.-Y., & Liu, Y. (2016). Obesity and hypertension. *Experimental and Therapeutic Medicine*, *12*(4), 2395–2399. https://doi.org/10.3892/etm.2016.3667

Jiménez-Gómez, Y., López-Miranda, J., Blanco-Colio, L. M., Marín, C., Pérez-Martínez, P., Ruano, J., Paniagua, J. A., Rodríguez, F., Egido, J., & Pérez-Jiménez, F. (2009). Olive oil and walnut breakfasts reduce the postprandial inflammatory response in mononuclear cells compared with a butter breakfast in healthy men. *Atherosclerosis*, *204*(2), e70–e76. https://doi.org/10.1016/j.atherosclerosis.2008.09.011

Jung, T., Schauer, U., Heusser, C., Neumann, C., & Rieger, C. (1993). Detection of intracellular cytokines by flow cytometry. *Journal of Immunological Methods*, *159*(1–2), 197–207. https://doi.org/10.1016/0022-1759(93)90158-4

Karpe, F., Dickmann, J. R., & Frayn, K. N. (2011). Fatty Acids, Obesity, and Insulin Resistance: Time for a Reevaluation. *Diabetes*, *60*(10), 2441–2449. https://doi.org/10.2337/db11-0425

Kasahara, T., Hooks, J. J., Dougherty, S. F., & Oppenheim, J. J. (1983). Interleukin 2-mediated immune interferon (IFN-gamma) production by human T cells and T cell subsets. *Journal of Immunology (Baltimore, Md. : 1950)*, *130*(4), 1784–1789.

Kersten, S. (2001). Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Reports*, *2*(4), 282–286. https://doi.org/10.1093/embo-reports/kve071

Kew, S., Banerjee, T., Minihane, A. M., Finnegan, Y. E., Williams, C. M., & Calder, P. C. (2003). Relation between the fatty acid composition of peripheral blood mononuclear cells and measures of immune cell function in healthy, free-living subjects aged 25–72 y. *The American Journal of Clinical Nutrition*, *77*(5), 1278–1286. https://doi.org/10.1093/ajcn/77.5.1278

Khandouzi, N., Zahedmehr, A., & Nasrollahzadeh, J. (2020). Effects of canola or olive oil on plasma lipids, lipoprotein-associated phospholipase A2 and inflammatory cytokines in patients referred for coronary angiography. *Lipids in Health and Disease*, *19*(1), 183. https://doi.org/10.1186/s12944-020-01362-z

Khandouzi, N., Zahedmehr, A., & Nasrollahzadeh, J. (2021). Effect of polyphenol-rich extra-virgin olive oil on lipid profile and inflammatory biomarkers in patients undergoing coronary angiography: a randomised, controlled, clinical trial. *International Journal of Food Sciences and Nutrition*, *72*(4), 548–558. https://doi.org/10.1080/09637486.2020.1841123

Khanna, D., Khanna, S., Khanna, P., Kahar, P., & Patel, B. M. (2022). Obesity: A Chronic Low-Grade Inflammation and Its Markers. *Cureus*. https://doi.org/10.7759/cureus.22711

Kien, C. L., Bunn, J. Y., Fukagawa, N. K., Anathy, V., Matthews, D. E., Crain, K. I., Ebenstein, D. B., Tarleton, E. K., Pratley, R. E., & Poynter, M. E. (2015). Lipidomic evidence that lowering the typical dietary palmitate to oleate ratio in humans decreases the leukocyte production of proinflammatory cytokines and muscle expression of redox-sensitive genes. *The Journal of Nutritional Biochemistry*, *26*(12), 1599–1606. https://doi.org/10.1016/j.jnutbio.2015.07.014

Kievit, P., Howard, J. K., Badman, M. K., Balthasar, N., Coppari, R., Mori, H., Lee, C. E., Elmquist, J. K., Yoshimura, A., & Flier, J. S. (2006). Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. *Cell Metabolism*, *4*(2), 123–132. https://doi.org/10.1016/j.cmet.2006.06.010

Kim, C.-S., Park, H.-S., Kawada, T., Kim, J.-H., Lim, D., Hubbard, N. E., Kwon, B.-S., Erickson, K. L., & Yu, R. (2006). Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. *International Journal of Obesity*, *30*(9), 1347–1355. https://doi.org/10.1038/sj.ijo.0803259

Kim, J., Bachmann, R. A., & Chen, J. (2009). *Chapter 21 Interleukin-6 and Insulin Resistance* (pp. 613–633). https://doi.org/10.1016/S0083-6729(08)00621-3

Kim, S. H., & Reaven, G. M. (2008). Insulin Resistance and Hyperinsulinemia. *Diabetes Care*, *31*(7), 1433–1438. https://doi.org/10.2337/dc08-0045

Kintscher, U., Hartge, M., Hess, K., Foryst-Ludwig, A., Clemenz, M., Wabitsch, M., Fischer-Posovszky, P., Barth, T. F. E., Dragun, D., Skurk, T., Hauner, H., Blüher, M., Unger, T., Wolf, A.-M., Knippschild, U., Hombach, V., & Marx, N. (2008). T-lymphocyte Infiltration in Visceral Adipose Tissue. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *28*(7), 1304–1310. https://doi.org/10.1161/ATVBAHA.108.165100

Klop, B., Elte, J., & Cabezas, M. (2013). Dyslipidemia in Obesity: Mechanisms and Potential Targets. *Nutrients*, *5*(4), 1218–1240. https://doi.org/10.3390/nu5041218

Koelman, L., Egea Rodrigues, C., & Aleksandrova, K. (2022). Effects of Dietary Patterns on Biomarkers of Inflammation and Immune Responses: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Advances in Nutrition*, *13*(1), 101–115. https://doi.org/10.1093/advances/nmab086

Könner, A. C., & Brüning, J. C. (2011). Toll-like receptors: linking inflammation to metabolism. *Trends in Endocrinology & Metabolism*, *22*(1), 16–23. https://doi.org/10.1016/j.tem.2010.08.007

Kopczyńska, J., & Kowalczyk, M. (2024). The potential of short-chain fatty acid epigenetic regulation in chronic low-grade inflammation and obesity. *Frontiers in Immunology*, *15*. https://doi.org/10.3389/fimmu.2024.1380476

Kornberg, M. D. (2020). The immunologic Warburg effect: Evidence and therapeutic opportunities in autoimmunity. *WIREs Systems Biology and Medicine*, *12*(5). https://doi.org/10.1002/wsbm.1486

Kuby, J., Kindt, T., Goldsby, R., & Osborne, B. (2007). *Kuby Immunology* (6th ed.). W.H. Freeman.

Kyriakis, J. M., & Avruch, J. (1996). Sounding the Alarm: Protein Kinase Cascades Activated by Stress and Inflammation. *Journal of Biological Chemistry*, *271*(40), 24313–24316. https://doi.org/10.1074/jbc.271.40.24313

Lamas, O., Martinez, J. A., & Marti, A. (2002). T-helper lymphopenia and decreased mitogenic response in cafeteria diet-induced obese rats. *Nutrition Research*, *22*(4), 497–506. https://doi.org/10.1016/S0271-5317(02)00362-7

Lauterbach, M. A. R., & Wunderlich, F. T. (2017). Macrophage function in obesity-induced inflammation and insulin resistance. *Pflügers Archiv - European Journal of Physiology*, *469*(3–4), 385–396. https://doi.org/10.1007/s00424-017-1955-5

Lee, J. Y., Plakidas, A., Lee, W. H., Heikkinen, A., Chanmugam, P., Bray, G., & Hwang, D. H. (2003). Differential modulation of Toll-like receptors by fatty acids: preferential inhibition by n-3 polyunsaturated fatty acids. *Journal of Lipid Research*, *44*(3), 479–486. https://doi.org/10.1194/jlr.M200361-JLR200

Lee, J. Y., Sohn, K. H., Rhee, S. H., & Hwang, D. (2001a). Saturated Fatty Acids, but Not Unsaturated Fatty Acids, Induce the Expression of Cyclooxygenase-2 Mediated through Toll-like Receptor 4. *Journal of Biological Chemistry*, *276*(20), 16683–16689. https://doi.org/10.1074/jbc.M011695200

Lee, J. Y., Sohn, K. H., Rhee, S. H., & Hwang, D. (2001b). Saturated Fatty Acids, but Not Unsaturated Fatty Acids, Induce the Expression of Cyclooxygenase-2 Mediated through

Toll-like Receptor 4. *Journal of Biological Chemistry*, *276*(20), 16683–16689. https://doi.org/10.1074/jbc.M011695200

Lee, J. Y., Ye, J., Gao, Z., Youn, H. S., Lee, W. H., Zhao, L., Sizemore, N., & Hwang, D. H. (2003). Reciprocal Modulation of Toll-like Receptor-4 Signaling Pathways Involving MyD88 and Phosphatidylinositol 3-Kinase/AKT by Saturated and Polyunsaturated Fatty Acids. *Journal of Biological Chemistry*, *278*(39), 37041–37051. https://doi.org/10.1074/jbc.M305213200

Lee, Y., Shin, H., & Kim, J. (2021). In vivo Anti-Cancer Effects of Resveratrol Mediated by NK Cell Activation. *Journal of Innate Immunity*, *13*(2), 94–106. https://doi.org/10.1159/000510315

Leontieva, O. V, Paszkiewicz, G., Demidenko, Z. N., & Blagosklonny, M. V. (2013). Resveratrol potentiates rapamycin to prevent hyperinsulinemia and obesity in male mice on high fat diet. *Cell Death & Disease*, *4*(1), e472–e472. https://doi.org/10.1038/cddis.2012.202

Ler, P., Ojalehto, E., Zhan, Y., Finkel, D., Dahl Aslan, A. K., & Karlsson, I. K. (2023). Conversions between metabolically unhealthy and healthy obesity from midlife to late-life. *International Journal of Obesity*. https://doi.org/10.1038/s41366-023-01425-y

Levin, B. E., Dunn-Meynell, A. A., Balkan, B., & Keesey, R. E. (1997). Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *273*(2), R725–R730. https://doi.org/10.1152/ajpregu.1997.273.2.R725

Li, B., Jones, L. L., & Geiger, T. L. (2018). IL-6 Promotes T Cell Proliferation and Expansion under Inflammatory Conditions in Association with Low-Level RORγt Expression. *The Journal of Immunology*, *201*(10), 2934–2946. https://doi.org/10.4049/jimmunol.1800016

Li, C., Wang, G., Sivasami, P., Ramirez, R. N., Zhang, Y., Benoist, C., & Mathis, D. (2021). Interferon-α-producing plasmacytoid dendritic cells drive the loss of adipose tissue regulatory T cells during obesity. *Cell Metabolism*, *33*(8), 1610-1623.e5. https://doi.org/10.1016/j.cmet.2021.06.007

Li, Q., & Cherayil, B. J. (2003). Role of Toll-Like Receptor 4 in Macrophage Activation and Tolerance during Salmonella enterica Serovar Typhimurium Infection. *Infection and Immunity*, *71*(9), 4873–4882. https://doi.org/10.1128/IAI.71.9.4873-4882.2003

Li, X., Mai, K., & Ai, Q. (2024). Palmitic acid activates NLRP3 inflammasome through NF-κB and AMPK-mitophagy-ROS pathways to induce IL-1β production in large yellow

croaker (Larimichthys crocea). *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1869*(2), 159428. https://doi.org/10.1016/j.bbalip.2023.159428

Li, Y., Hruby, A., Bernstein, A. M., Ley, S. H., Wang, D. D., Chiuve, S. E., Sampson, L., Rexrode, K. M., Rimm, E. B., Willett, W. C., & Hu, F. B. (2015). Saturated Fats Compared With Unsaturated Fats and Sources of Carbohydrates in Relation to Risk of Coronary Heart Disease. *Journal of the American College of Cardiology*, *66*(14), 1538–1548. https://doi.org/10.1016/j.jacc.2015.07.055

Lillenfeld, D. E., Vlahov, D., Tenney, J. H., & McLaughlin, J. S. (1988a). Obesity and diabetes as risk factors for postoperative wound infections after cardiac surgery. *American Journal of Infection Control*, *16*(1), 3–6. https://doi.org/10.1016/0196-6553(88)90003-X

Lillenfeld, D. E., Vlahov, D., Tenney, J. H., & McLaughlin, J. S. (1988b). Obesity and diabetes as risk factors for postoperative wound infections after cardiac surgery. *American Journal of Infection Control*, *16*(1), 3–6. https://doi.org/10.1016/0196-6553(88)90003-X

Lindemann, M. (2014). Ex vivo assessment of cellular immune function – applications in patient care and clinical studies. *Tissue Antigens*, *84*(5), 439–449. https://doi.org/10.1111/tan.12454

Lisby, A. N., Carlson, R. D., Baybutt, T. R., Weindorfer, M., & Snook, A. E. (2022). *Evaluation of CAR-T cell cytotoxicity: Real-time impedance-based analysis* (pp. 81–98). https://doi.org/10.1016/bs.mcb.2021.08.002

Liu, H., Guo, X., Jiang, K., Shi, B., Liu, L., Hou, R., Chen, G., Farag, M. A., Yan, N., & Liu, L. (2024). Dietary polyphenols regulate appetite mechanism via gut-brain axis and gut homeostasis. *Food Chemistry*, *446*, 138739. https://doi.org/10.1016/j.foodchem.2024.138739

Liu, Y., Chen, F., Odle, J., Lin, X., Jacobi, S. K., Zhu, H., Wu, Z., & Hou, Y. (2012). Fish Oil Enhances Intestinal Integrity and Inhibits TLR4 and NOD2 Signaling Pathways in Weaned Pigs after LPS Challenge3. *The Journal of Nutrition*, *142*(11), 2017–2024. https://doi.org/10.3945/jn.112.164947

Long, A., Kleiner, A., & Looney, R. J. (2023). Immune dysregulation. *Journal of Allergy and Clinical Immunology*, *151*(1), 70–80. https://doi.org/10.1016/j.jaci.2022.11.001

Lopez-Jimenez, F., Almahmeed, W., Bays, H., Cuevas, A., Di Angelantonio, E., le Roux, C. W., Sattar, N., Sun, M. C., Wittert, G., Pinto, F. J., & Wilding, J. P. H. (2022). Obesity and cardiovascular disease: mechanistic insights and management strategies. A joint position paper by the World Heart Federation and World Obesity Federation. *European*

Journal of Preventive Cardiology, *29*(17), 2218–2237. https://doi.org/10.1093/eurjpc/zwac187

Luck, H., Khan, S., Kim, J. H., Copeland, J. K., Revelo, X. S., Tsai, S., Chakraborty, M., Cheng, K., Tao Chan, Y., Nøhr, M. K., Clemente-Casares, X., Perry, M.-C., Ghazarian, M., Lei, H., Lin, Y.-H., Coburn, B., Okrainec, A., Jackson, T., Poutanen, S., … Winer, D. A. (2019a). Gut-associated IgA+ immune cells regulate obesity-related insulin resistance. *Nature Communications*, *10*(1), 3650. https://doi.org/10.1038/s41467-019-11370-y

Luck, H., Khan, S., Kim, J. H., Copeland, J. K., Revelo, X. S., Tsai, S., Chakraborty, M., Cheng, K., Tao Chan, Y., Nøhr, M. K., Clemente-Casares, X., Perry, M.-C., Ghazarian, M., Lei, H., Lin, Y.-H., Coburn, B., Okrainec, A., Jackson, T., Poutanen, S., … Winer, D. A. (2019b). Gut-associated IgA+ immune cells regulate obesity-related insulin resistance. *Nature Communications*, *10*(1), 3650. https://doi.org/10.1038/s41467-019-11370-y

Luck, H., Tsai, S., Chung, J., Clemente-Casares, X., Ghazarian, M., Revelo, X. S., Lei, H., Luk, C. T., Shi, S. Y., Surendra, A., Copeland, J. K., Ahn, J., Prescott, D., Rasmussen, B. A., Chng, M. H. Y., Engleman, E. G., Girardin, S. E., Lam, T. K. T., Croitoru, K., … Winer, D. A. (2015). Regulation of Obesity-Related Insulin Resistance with Gut Anti-inflammatory Agents. *Cell Metabolism*, *21*(4), 527–542. https://doi.org/10.1016/j.cmet.2015.03.001

Lumeng, C. N., Bodzin, J. L., & Saltiel, A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *The Journal of Clinical Investigation*, *117*(1), 175–184. https://doi.org/10.1172/JCI29881

Ma, Y., Ding, X., Gu, J., Zhou, S., & Jiang, Y. (2023). Effects of olive oil on hepatic steatosis and liver enzymes: A systematic review. *Journal of Functional Foods*, *109*, 105815. https://doi.org/10.1016/j.jff.2023.105815

Mak, T. W., & Saunders, M. E. (2006). T Cell Activation. In *The Immune Response* (pp. 373–401). Elsevier. https://doi.org/10.1016/B978-012088451-3.50016-8

Makhijani, P., Basso, P. J., Chan, Y. T., Chen, N., Baechle, J., Khan, S., Furman, D., Tsai, S., & Winer, D. A. (2023). Regulation of the immune system by the insulin receptor in health and disease. *Frontiers in Endocrinology*, *14*. https://doi.org/10.3389/fendo.2023.1128622

Makki, K., Froguel, P., & Wolowczuk, I. (2013). Adipose Tissue in Obesity-Related Inflammation and Insulin Resistance: Cells, Cytokines, and Chemokines. *ISRN Inflammation*, *2013*, 1–12. https://doi.org/10.1155/2013/139239

Mamun, M. A. Al, Rakib, A., Mandal, M., Kumar, S., Singla, B., & Singh, U. P. (2024). Polyphenols: Role in Modulating Immune Function and Obesity. *Biomolecules*, *14*(2), 221. https://doi.org/10.3390/biom14020221

Mao, T., Huang, F., Zhu, X., Wei, D., & Chen, L. (2021). Effects of dietary fiber on glycemic control and insulin sensitivity in patients with type 2 diabetes: A systematic review and meta-analysis. *Journal of Functional Foods*, *82*, 104500. https://doi.org/10.1016/j.jff.2021.104500

Maruyama, S. (2018). *The Functional Assessment of T cells* (pp. 177–199). https://doi.org/10.1007/978-1-4939-8802-0_19

Matia-Garcia, I., Vadillo, E., Pelayo, R., Muñoz-Valle, J. F., García-Chagollán, M., Loaeza-Loaeza, J., Vences-Velázquez, A., Salgado-Goytia, L., García-Arellano, S., & Parra-Rojas, I. (2021). Th1/Th2 Balance in Young Subjects: Relationship with Cytokine Levels and Metabolic Profile. *Journal of Inflammation Research*, *Volume 14*, 6587–6600. https://doi.org/10.2147/JIR.S342545

Mcdade, T. W., Adair, L., Feranil, A. B., & Kuzawa, C. (2011). Positive antibody response to vaccination in adolescence predicts lower C-reactive protein concentration in young adulthood in the philippines. *American Journal of Human Biology*, *23*(3), 313–318. https://doi.org/10.1002/ajhb.21128

McGrath, A. J., Hamill, L. L., Cardwell, C. R., Draffin, C. R., Neville, C. E., Appleton, K. M., McEneny, J., McKinley, M. C., Young, I. S., & Woodside, J. V. (2016). Combining vitamin C and carotenoid biomarkers better predicts fruit and vegetable intake than individual biomarkers in dietary intervention studies. *European Journal of Nutrition*, *55*(4), 1377–1388. https://doi.org/10.1007/s00394-015-0953-7

McNamara, A. E., & Brennan, L. (2020). Potential of food intake biomarkers in nutrition research. *Proceedings of the Nutrition Society*, *79*(4), 487–497. https://doi.org/10.1017/S0029665120007053

McQuaid, S. E., Hodson, L., Neville, M. J., Dennis, A. L., Cheeseman, J., Humphreys, S. M., Ruge, T., Gilbert, M., Fielding, B. A., Frayn, K. N., & Karpe, F. (2011). Downregulation of Adipose Tissue Fatty Acid Trafficking in Obesity. *Diabetes*, *60*(1), 47–55. https://doi.org/10.2337/db10-0867

Mehta, A. K., Gracias, D. T., & Croft, M. (2018). TNF activity and T cells. *Cytokine*, *101*, 14–18. https://doi.org/10.1016/j.cyto.2016.08.003

Mestas, J., & Hughes, C. C. W. (2004). Of Mice and Not Men: Differences between Mouse and Human Immunology. *The Journal of Immunology*, *172*(5), 2731–2738. https://doi.org/10.4049/jimmunol.172.5.2731

Meyer, B. J., Mann, N. J., Lewis, J. L., Milligan, G. C., Sinclair, A. J., & Howe, P. R. C. (2003). Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. *Lipids*, *38*(4), 391–398. https://doi.org/10.1007/s11745-003-1074-0

Milanski, M., Degasperi, G., Coope, A., Morari, J., Denis, R., Cintra, D. E., Tsukumo, D. M. L., Anhe, G., Amaral, M. E., Takahashi, H. K., Curi, R., Oliveira, H. C., Carvalheira, J. B. C., Bordin, S., Saad, M. J., & Velloso, L. A. (2009). Saturated Fatty Acids Produce an Inflammatory Response Predominantly through the Activation of TLR4 Signaling in Hypothalamus: Implications for the Pathogenesis of Obesity. *The Journal of Neuroscience*, *29*(2), 359–370. https://doi.org/10.1523/JNEUROSCI.2760-08.2009

Miles, E. A., Allen, E., & Calder, P. C. (2002). In vitro effects of eicosanoids derived from different 20-carbon fatty acids on production of monocyte-derived cytokines in human whole blood cultures. *Cytokine*, *20*(5), 215–223. https://doi.org/10.1006/cyto.2002.2007

Miles, E. A., Wallace, F. A., & Calder, P. C. (2000). Dietary fish oil reduces intercellular adhesion molecule 1 and scavenger receptor expression on murine macrophages. *Atherosclerosis*, *152*(1), 43–50. https://doi.org/10.1016/S0021-9150(99)00446-3

Milner, J. J., & Beck, M. A. (2012). The impact of obesity on the immune response to infection. *Proceedings of the Nutrition Society*, *71*(2), 298–306. https://doi.org/10.1017/S0029665112000158

Mito, N., Hosoda, T., Kato, C., & Sato, K. (2000). Change of cytokine balance in diet-induced obese mice. *Metabolism*, *49*(10), 1295–1300. https://doi.org/10.1053/meta.2000.9523

Mohammadi, A., Blesso, C. N., Barreto, G. E., Banach, M., Majeed, M., & Sahebkar, A. (2019). Macrophage plasticity, polarization and function in response to curcumin, a diet-derived polyphenol, as an immunomodulatory agent. *The Journal of Nutritional Biochemistry*, *66*, 1–16. https://doi.org/10.1016/j.jnutbio.2018.12.005

Montserrat-de la Paz, S., Naranjo, M. C., Millan-Linares, M. C., Lopez, S., Abia, R., Biessen, E. A. L., Muriana, F. J. G., & Bermudez, B. (2019). Monounsaturated Fatty Acids in a High-Fat Diet and Niacin Protect from White Fat Dysfunction in the Metabolic Syndrome. *Molecular Nutrition & Food Research*, *63*(19). https://doi.org/10.1002/mnfr.201900425

Moon, J. H., Lee, J. Y., Kang, S. B., Park, J. S., Lee, B. W., Kang, E. S., Ahn, C. W., Lee, H. C., & Cha, B. S. (2010). Dietary Monounsaturated Fatty Acids but not Saturated Fatty Acids Preserve the Insulin Signaling Pathway via IRS-1/PI3K in Rat Skeletal Muscle. *Lipids*, *45*(12), 1109–1116. https://doi.org/10.1007/s11745-010-3475-3

Mörbe, U. M., Jørgensen, P. B., Fenton, T. M., von Burg, N., Riis, L. B., Spencer, J., & Agace, W. W. (2021). Human gut-associated lymphoid tissues (GALT); diversity, structure, and function. In *Mucosal Immunology* (Vol. 14, Issue 4, pp. 793–802). Springer Nature. https://doi.org/10.1038/s41385-021-00389-4

Mosca, S., Araújo, G., Costa, V., Correia, J., Bandeira, A., Martins, E., Mansilha, H., Tavares, M., & Coelho, M. P. (2022). Dyslipidemia Diagnosis and Treatment: Risk Stratification in Children and Adolescents. *Journal of Nutrition and Metabolism*, *2022*, 1–10. https://doi.org/10.1155/2022/4782344

Mountain, I. M. (1955). Antibody Production by Spleen In Vitro. *The Journal of Immunology*, *74*(4), 270–277. https://doi.org/10.4049/jimmunol.74.4.270

Mukherjee, P., Roy, S., Ghosh, D., & Nandi, S. K. (2022). Role of animal models in biomedical research: a review. *Laboratory Animal Research*, *38*(1), 18. https://doi.org/10.1186/s42826-022-00128-1

NIH. (2024a). *ASA24® Dietary Assessment Tool | EGRP/DCCPS/NCI/NIH*. https://epi.grants.cancer.gov/asa24/

NIH. (2024b). *Methods Used to Develop DHQ III Database | EGRP/DCCPS/NCI/NIH*. https://epi.grants.cancer.gov/dhq3/database-methods.html

Ogden, C. L. (2010). Prevalence of High Body Mass Index in US Children and Adolescents, 2007-2008. *JAMA*, *303*(3), 242. https://doi.org/10.1001/jama.2009.2012

Pabst, R. (2020). The pig as a model for immunology research. *Cell and Tissue Research*, *380*(2), 287–304. https://doi.org/10.1007/s00441-020-03206-9

Paquette, A., Wang, D., Jankowski, M., Gutkowska, J., & Lavoie, J.-M. (2008). Effects of ovariectomy on PPARα, SREBP-1c, and SCD-1 gene expression in the rat liver. *Menopause*, *15*(6), 1169–1175. https://doi.org/10.1097/gme.0b013e31817b8159

Park, H. S., Park, J. Y., & Yu, R. (2005). Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-α and IL-6. *Diabetes Research and Clinical Practice*, *69*(1), 29–35. https://doi.org/10.1016/j.diabres.2004.11.007

Park, S. Y., Kim, Y. W., Kim, J. Y., Jang, E. C., Doh, K. O., & Lee, S. K. (2001). Effect of High Fat Diet on Insulin Resistance: Dietary Fat Versus Visceral Fat Mass. *Journal of Korean Medical Science*, *16*(4), 386. https://doi.org/10.3346/jkms.2001.16.4.386

Paton, C. M., & Ntambi, J. M. (2009). Biochemical and physiological function of stearoyl-CoA desaturase. *American Journal of Physiology-Endocrinology and Metabolism*, *297*(1), E28–E37. https://doi.org/10.1152/ajpendo.90897.2008

Paul, S., & Lal, G. (2017). The Molecular Mechanism of Natural Killer Cells Function and Its Importance in Cancer Immunotherapy. *Frontiers in Immunology*, *8*. https://doi.org/10.3389/fimmu.2017.01124

Payne, K. J., & Crooks, G. M. (2007). Immune-Cell Lineage Commitment: Translation from Mice to Humans. *Immunity*, *26*(6), 674–677. https://doi.org/10.1016/j.immuni.2007.05.011

Pearce, E. L. (2010). Metabolism in T cell activation and differentiation. *Current Opinion in Immunology*, *22*(3), 314–320. https://doi.org/10.1016/j.coi.2010.01.018

Pearce, E. L., & Pearce, E. J. (2013). Metabolic Pathways in Immune Cell Activation and Quiescence. *Immunity*, *38*(4), 633–643. https://doi.org/10.1016/j.immuni.2013.04.005

Peters, S. A. E., MacMahon, S., & Woodward, M. (2021). Obesity as a risk factor for COVID -19 mortality in women and men in the UK biobank: Comparisons with influenza/pneumonia and coronary heart disease. *Diabetes, Obesity and Metabolism*, *23*(1), 258–262. https://doi.org/10.1111/dom.14199

Pischon, T., Hankinson, S. E., Hotamisligil, G. S., Rifai, N., Willett, W. C., & Rimm, E. B. (2003). Habitual Dietary Intake of n-3 and n-6 Fatty Acids in Relation to Inflammatory Markers Among US Men and Women. *Circulation*, *108*(2), 155–160. https://doi.org/10.1161/01.CIR.0000079224.46084.C2

Poppitt, S. D., Kilmartin, P., Butler, P., & Keogh, G. F. (2005). Assessment of erythrocyte phospholipid fatty acid composition as a biomarker for dietary MUFA, PUFA or saturated fatty acid intake in a controlled cross-over intervention trial. *Lipids in Health and Disease*, *4*(1), 30. https://doi.org/10.1186/1476-511X-4-30

Prod´homme, M., Rieu, I., Balage, M., Dardevet, D., & Grizard, J. (2004). Insulin and amino acids both strongly participate to the regulation of protein metabolism. *Current Opinion in Clinical Nutrition and Metabolic Care*, *7*(1), 71–77. https://doi.org/10.1097/00075197-200401000-00012

Purnell, J. (2000). *Definitions, Classification, and Epidemiology of Obesity.* (K. Feingold, B. Anawalt, & M. Blackman, Eds.). Endotext [Internet].

Qatanani, M., & Lazar, M. A. (2007). Mechanisms of obesity-associated insulin resistance: many choices on the menu. *Genes & Development*, *21*(12), 1443–1455. https://doi.org/10.1101/gad.1550907

Qi, X., Li, Y., Fang, C., Jia, Y., Chen, M., Chen, X., & Jia, J. (2023). The associations between dietary fibers intake and systemic immune and inflammatory biomarkers, a

multi-cycle study of NHANES 2015–2020. *Frontiers in Nutrition*, *10*. https://doi.org/10.3389/fnut.2023.1242115

Queipo-Ortuño, M. I., Boto-Ordóñez, M., Murri, M., Gomez-Zumaquero, J. M., Clemente-Postigo, M., Estruch, R., Cardona Diaz, F., Andrés-Lacueva, C., & Tinahones, F. J. (2012). Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. *The American Journal of Clinical Nutrition*, *95*(6), 1323–1334. https://doi.org/10.3945/ajcn.111.027847

Quetglas-Llabrés, M. M., Monserrat-Mesquida, M., Bouzas, C., Gómez, C., Mateos, D., Ripoll-Vera, T., Tur, J. A., & Sureda, A. (2022). Inflammatory and Oxidative Stress Markers Related to Adherence to the Mediterranean Diet in Patients with Metabolic Syndrome. *Antioxidants*, *11*(5), 901. https://doi.org/10.3390/antiox11050901

Rahman, M. S., Hossain, K. S., Das, S., Kundu, S., Adegoke, E. O., Rahman, Md. A., Hannan, Md. A., Uddin, M. J., & Pang, M.-G. (2021). Role of Insulin in Health and Disease: An Update. *International Journal of Molecular Sciences*, *22*(12), 6403. https://doi.org/10.3390/ijms22126403

Ratajczak, W., Niedźwiedzka-Rystwej, P., Tokarz-Deptuła, B., & Deptuła, W. (2018). Immunological memory cells. *Central European Journal of Immunology*, *43*(2), 194–203. https://doi.org/10.5114/ceji.2018.77390

Ravaut, G., Légiot, A., Bergeron, K.-F., & Mounier, C. (2020). Monounsaturated Fatty Acids in Obesity-Related Inflammation. *International Journal of Molecular Sciences*, *22*(1), 330. https://doi.org/10.3390/ijms22010330

Reaven, G. M. (1988). Role of Insulin Resistance in Human Disease. *Diabetes*, *37*(12), 1595–1607. https://doi.org/10.2337/diab.37.12.1595

Reiser, S., Hallfrisch, J., Fields, M., Powell, A., Mertz, W., Prather, E., & Canary, J. (1986). Effects of sugars on Indices of glucose tolerance in humans. *The American Journal of Clinical Nutrition*, *43*(1), 151–159. https://doi.org/10.1093/ajcn/43.1.151

Ren, G., Roberts, A. I., & Shi, Y. (2011). Adhesion molecules. *Cell Adhesion & Migration*, *5*(1), 20–22. https://doi.org/10.4161/cam.5.1.13491

Resnick, E. S., Moshier, E. L., Godbold, J. H., & Cunningham-Rundles, C. (2012). Morbidity and mortality in common variable immune deficiency over 4 decades. *Blood*, *119*(7), 1650–1657. https://doi.org/10.1182/blood-2011-09-377945

Richard, C., Couture, P., Desroches, S., & Lamarche, B. (2013). Effect of the mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. *Obesity*, *21*(1), 51–57. https://doi.org/10.1002/oby.20239

Richard, C., Wadowski, M., Goruk, S., Cameron, L., Sharma, A. M., & Field, C. J. (2017). Individuals with obesity and type 2 diabetes have additional immune dysfunction compared with obese individuals who are metabolically healthy. *BMJ Open Diabetes Research & Care*, *5*(1), e000379. https://doi.org/10.1136/bmjdrc-2016-000379

Risé, P., Eligini, S., Ghezzi, S., Colli, S., & Galli, C. (2007). Fatty acid composition of plasma, blood cells and whole blood: Relevance for the assessment of the fatty acid status in humans. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *76*(6), 363–369. https://doi.org/10.1016/j.plefa.2007.05.003

Rocha, D. M., Bressan, J., & Hermsdorff, H. H. (2017). The role of dietary fatty acid intake in inflammatory gene expression: a critical review. *Sao Paulo Medical Journal*, *135*(2), 157–168. https://doi.org/10.1590/1516-3180.2016.008607072016

Rocha, D. M., Caldas, A. P., Oliveira, L. L., Bressan, J., & Hermsdorff, H. H. (2016). Saturated fatty acids trigger TLR4-mediated inflammatory response. *Atherosclerosis*, *244*, 211–215. https://doi.org/10.1016/j.atherosclerosis.2015.11.015

Rogero, M., & Calder, P. (2018a). Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. *Nutrients*, *10*(4), 432. https://doi.org/10.3390/nu10040432

Rogero, M., & Calder, P. (2018b). Obesity, Inflammation, Toll-Like Receptor 4 and Fatty Acids. *Nutrients*, *10*(4), 432. https://doi.org/10.3390/nu10040432

Romaguera, D., Norat, T., Vergnaud, A.-C., Mouw, T., May, A. M., Agudo, A., Buckland, G., Slimani, N., Rinaldi, S., Couto, E., Clavel-Chapelon, F., Boutron-Ruault, M.-C., Cottet, V., Rohrmann, S., Teucher, B., Bergmann, M., Boeing, H., Tjønneland, A., Halkjaer, J., … Peeters, P. H. (2010). Mediterranean dietary patterns and prospective weight change in participants of the EPIC-PANACEA project. *The American Journal of Clinical Nutrition*, *92*(4), 912–921. https://doi.org/10.3945/ajcn.2010.29482

SANDERSON, & CALDER. (1998). Dietary fish oil diminishes lymphocyte adhesion to macrophage and endothelial cell monolayers. *Immunology*, *94*(1), 79–87. https://doi.org/10.1046/j.1365-2567.1998.00474.x

Serhan, C. N., & Chiang, N. (2013). Resolution phase lipid mediators of inflammation: agonists of resolution. *Current Opinion in Pharmacology*, *13*(4), 632–640. https://doi.org/10.1016/j.coph.2013.05.012

Serhan, C. N., Chiang, N., & Van Dyke, T. E. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nature Reviews Immunology*, *8*(5), 349–361. https://doi.org/10.1038/nri2294

Shahwan, M., Alhumaydhi, F., Ashraf, G. Md., Hasan, P. M. Z., & Shamsi, A. (2022). Role of polyphenols in combating Type 2 Diabetes and insulin resistance. *International Journal of Biological Macromolecules*, *206*, 567–579. https://doi.org/10.1016/j.ijbiomac.2022.03.004

She, Y., Mangat, R., Tsai, S., Proctor, S. D., & Richard, C. (2022). The Interplay of Obesity, Dyslipidemia and Immune Dysfunction: A Brief Overview on Pathophysiology, Animal Models, and Nutritional Modulation. *Frontiers in Nutrition*, *9*. https://doi.org/10.3389/fnut.2022.840209

Shen-Orr, S. S., Furman, D., Kidd, B. A., Hadad, F., Lovelace, P., Huang, Y.-W., Rosenberg-Hasson, Y., Mackey, S., Grisar, F. A. G., Pickman, Y., Maecker, H. T., Chien, Y., Dekker, C. L., Wu, J. C., Butte, A. J., & Davis, M. M. (2016). Defective Signaling in the JAK-STAT Pathway Tracks with Chronic Inflammation and Cardiovascular Risk in Aging Humans. *Cell Systems*, *3*(4), 374-384.e4. https://doi.org/10.1016/j.cels.2016.09.009

Sheppe, A. E. F., & Edelmann, M. J. (2021). Roles of Eicosanoids in Regulating Inflammation and Neutrophil Migration as an Innate Host Response to Bacterial Infections. *Infection and Immunity*, *89*(8). https://doi.org/10.1128/IAI.00095-21

Sheridan, P. A., Paich, H. A., Handy, J., Karlsson, E. A., Hudgens, M. G., Sammon, A. B., Holland, L. A., Weir, S., Noah, T. L., & Beck, M. A. (2012). Obesity is associated with impaired immune response to influenza vaccination in humans. *International Journal of Obesity*, *36*(8), 1072–1077. https://doi.org/10.1038/ijo.2011.208

Shimobayashi, M., Albert, V., Woelnerhanssen, B., Frei, I. C., Weissenberger, D., Meyer-Gerspach, A. C., Clement, N., Moes, S., Colombi, M., Meier, J. A., Swierczynska, M. M., Jenö, P., Beglinger, C., Peterli, R., & Hall, M. N. (2018). Insulin resistance causes inflammation in adipose tissue. *Journal of Clinical Investigation*, *128*(4), 1538–1550. https://doi.org/10.1172/JCI96139

Shively, C. A., Appt, S. E., Vitolins, M. Z., Uberseder, B., Michalson, K. T., Silverstein-Metzler, M. G., & Register, T. C. (2019a). Mediterranean versus Western Diet Effects on Caloric Intake, Obesity, Metabolism, and Hepatosteatosis in Nonhuman Primates. *Obesity*, *27*(5), 777–784. https://doi.org/10.1002/oby.22436

Shively, C. A., Appt, S. E., Vitolins, M. Z., Uberseder, B., Michalson, K. T., Silverstein-Metzler, M. G., & Register, T. C. (2019b). Mediterranean versus Western Diet Effects on Caloric Intake, Obesity, Metabolism, and Hepatosteatosis in Nonhuman Primates. *Obesity*, *27*(5), 777–784. https://doi.org/10.1002/oby.22436

Shulman, G. I. (2000). Cellular mechanisms of insulin resistance. *Journal of Clinical Investigation*, *106*(2), 171–176. https://doi.org/10.1172/JCI10583

Simon, G., Heckmann, V., Tóth, D., Pauka, D., Petrus, K., & Molnár, T. F. (2020). The effect of hepatic steatosis and fibrosis on liver weight and dimensions. *Legal Medicine*, *47*, 101781. https://doi.org/10.1016/j.legalmed.2020.101781

Smith, P. M., Howitt, M. R., Panikov, N., Michaud, M., Gallini, C. A., Bohlooly-Y, M., Glickman, J. N., & Garrett, W. S. (2013). The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic T reg Cell Homeostasis. *Science*, *341*(6145), 569–573. https://doi.org/10.1126/science.1241165

Smith, S. G., Smits, K., Joosten, S. A., van Meijgaarden, K. E., Satti, I., Fletcher, H. A., Caccamo, N., Dieli, F., Mascart, F., McShane, H., Dockrell, H. M., & Ottenhoff, T. H. M. (2015). Intracellular Cytokine Staining and Flow Cytometry: Considerations for Application in Clinical Trials of Novel Tuberculosis Vaccines. *PLOS ONE*, *10*(9), e0138042. https://doi.org/10.1371/journal.pone.0138042

Song, H. K., & Hwang, D. Y. (2017). Use of C57BL/6N mice on the variety of immunological researches. *Laboratory Animal Research*, *33*(2), 119. https://doi.org/10.5625/lar.2017.33.2.119

Sproston, N. R., & Ashworth, J. J. (2018). Role of C-Reactive Protein at Sites of Inflammation and Infection. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.00754

Srikakulapu, P., & McNamara, C. A. (2020). B Lymphocytes and Adipose Tissue Inflammation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *40*(5), 1110–1122. https://doi.org/10.1161/ATVBAHA.119.312467

Stadler, J. T., & Marsche, G. (2020). Obesity-Related Changes in High-Density Lipoprotein Metabolism and Function. *International Journal of Molecular Sciences*, *21*(23), 8985. https://doi.org/10.3390/ijms21238985

Statistics Canada. (2019, June 25). *Overweight and obese adults, 2018*.

Steen, B. M., Gerstenfeld, L. C., & Einhorn, T. A. (2011). The Role of the Immune System in Fracture Healing. In *Osteoimmunology* (pp. 343–367). Elsevier. https://doi.org/10.1016/B978-0-12-375670-1.10012-3

Surmi, B., & Hasty, A. (2008). Macrophage infiltration into adipose tissue: initiation, propagation and remodeling. *Future Lipidology*, *3*(5), 545–556. https://doi.org/10.2217/17460875.3.5.545

Talmadge, J. E., Meyers, K. M., Prieur, D. J., & Starkey, J. R. (1980). Role of natural killer cells in tumor growth and metastasis: C57BL/6 normal and beige mice. *Journal of the National Cancer Institute*, *65*(5), 929–935.

Tanguay, S., & Killion, J. J. (1994). Direct comparison of ELISPOT and ELISA-based assays for detection of individual cytokine-secreting cells. *Lymphokine and Cytokine Research*, *13*(4), 259–263.

Théry, C., & Amigorena, S. (2001). The cell biology of antigen presentation in dendritic cells. *Current Opinion in Immunology*, *13*(1), 45–51. https://doi.org/10.1016/S0952-7915(00)00180-1

Tilley, S. L., Coffman, T. M., & Koller, B. H. (2001). Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *Journal of Clinical Investigation*, *108*(1), 15–23. https://doi.org/10.1172/JCI200113416

Timmers, S., Konings, E., Bilet, L., Houtkooper, R. H., van de Weijer, T., Goossens, G. H., Hoeks, J., van der Krieken, S., Ryu, D., Kersten, S., Moonen-Kornips, E., Hesselink, M. K. C., Kunz, I., Schrauwen-Hinderling, V. B., Blaak, E. E., Auwerx, J., & Schrauwen, P. (2011). Calorie Restriction-like Effects of 30 Days of Resveratrol Supplementation on Energy Metabolism and Metabolic Profile in Obese Humans. *Cell Metabolism*, *14*(5), 612–622. https://doi.org/10.1016/j.cmet.2011.10.002

Triantafilou, M., & Triantafilou, K. (2002). Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. *Trends in Immunology*, *23*(6), 301–304. https://doi.org/10.1016/S1471-4906(02)02233-0

Trickett, A., & Kwan, Y. L. (2003). T cell stimulation and expansion using anti-CD3/CD28 beads. *Journal of Immunological Methods*, *275*(1–2), 251–255. https://doi.org/10.1016/S0022-1759(03)00010-3

Tsai, S., Clemente-Casares, X., Zhou, A. C., Lei, H., Ahn, J. J., Chan, Y. T., Choi, O., Luck, H., Woo, M., Dunn, S. E., Engleman, E. G., Watts, T. H., Winer, S., & Winer, D. A. (2018). Insulin Receptor-Mediated Stimulation Boosts T Cell Immunity during Inflammation and Infection. *Cell Metabolism*, *28*(6), 922-934.e4. https://doi.org/10.1016/j.cmet.2018.08.003

Tsatsoulis, A., & Paschou, S. A. (2020). Metabolically Healthy Obesity: Criteria, Epidemiology, Controversies, and Consequences. *Current Obesity Reports*, *9*(2), 109–120. https://doi.org/10.1007/s13679-020-00375-0

Urpi-Sarda, M., Casas, R., Sacanella, E., Corella, D., Andrés-Lacueva, C., Llorach, R., Garrabou, G., Cardellach, F., Sala-Vila, A., Ros, E., Ruiz-Canela, M., Fitó, M., Salas-Salvadó, J., & Estruch, R. (2021). The 3-Year Effect of the Mediterranean Diet Intervention on Inflammatory Biomarkers Related to Cardiovascular Disease. *Biomedicines*, *9*(8), 862. https://doi.org/10.3390/biomedicines9080862

Valenzuela, A., & Morgado, N. (1999). Trans fatty acid isomers in human health and in the food industry. *Biological Research*, *32*(4). https://doi.org/10.4067/S0716-97601999000400007

van Niekerk, G., Christowitz, C., Conradie, D., & Engelbrecht, A.-M. (2020). Insulin as an immunomodulatory hormone. *Cytokine & Growth Factor Reviews*, *52*, 34–44. https://doi.org/10.1016/j.cytogfr.2019.11.006

Vandevijvere, S., Chow, C. C., Hall, K. D., Umali, E., & Swinburn, B. A. (2015). Increased food energy supply as a major driver of the obesity epidemic: a global analysis. *Bulletin of the World Health Organization*, *93*(7), 446–456. https://doi.org/10.2471/BLT.14.150565

Viola, A., Munari, F., Sánchez-Rodríguez, R., Scolaro, T., & Castegna, A. (2019). The Metabolic Signature of Macrophage Responses. *Frontiers in Immunology*, *10*. https://doi.org/10.3389/fimmu.2019.01462

Vivier, E., Tomasello, E., Baratin, M., Walzer, T., & Ugolini, S. (2008). Functions of natural killer cells. *Nature Immunology*, *9*(5), 503–510. https://doi.org/10.1038/ni1582

von Hegedus, J. H., de Jong, A. J., Hoekstra, A. T., Spronsen, E., Zhu, W., Cabukusta, B., Kwekkeboom, J. C., Heijink, M., Bos, E., Berkers, C. R., Giera, M. A., Toes, R. E. M., & Ioan-Facsinay, A. (2024). Oleic acid enhances proliferation and calcium mobilization of CD3/CD28 activated CD4+ T cells through incorporation into membrane lipids. *European Journal of Immunology*. https://doi.org/10.1002/eji.202350685

Wagar, L. E., DiFazio, R. M., & Davis, M. M. (2018). Advanced model systems and tools for basic and translational human immunology. *Genome Medicine*, *10*(1), 73. https://doi.org/10.1186/s13073-018-0584-8

Wages, J. M. (2005). Polymerase chain reaction. In *Encyclopedia of Analytical Science* (pp. 243–250). Elsevier. https://doi.org/10.1016/B0-12-369397-7/00475-1

Wagner, N., Brandhorst, G., Czepluch, F., Lankeit, M., Eberle, C., Herzberg, S., Faustin, V., Riggert, J., Oellerich, M., Hasenfuss, G., Konstantinides, S., & Schäfer, K. (2013). Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk. *Obesity*, *21*(3), 461–468. https://doi.org/10.1002/oby.20087

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. (2003a). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation*, *112*(12), 1796–1808. https://doi.org/10.1172/JCI200319246

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W. (2003b). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation*, *112*(12), 1796–1808. https://doi.org/10.1172/JCI200319246

Weisheit, C. K., Engel, D. R., & Kurts, C. (2015). Dendritic Cells and Macrophages. *Clinical Journal of the American Society of Nephrology*, *10*(10), 1841–1851. https://doi.org/10.2215/CJN.07100714

WHO. (2023a). *Message from the Minister of Health – World Obesity Day 2023*. https://www.canada.ca/en/public-health/news/2023/03/message-from-the-minister-of-health--world-obesity-day-2023.html

WHO. (2023b, March 16). *Hypertension*.

WHO. (2024, March 1). *Obesity and overweight*.

Wilding, J. P. H., & Jacob, S. (2021). Cardiovascular outcome trials in obesity: A review. *Obesity Reviews*, *22*(1). https://doi.org/10.1111/obr.13112

Williams-Bey, Y., Boularan, C., Vural, A., Huang, N.-N., Hwang, I.-Y., Shan-Shi, C., & Kehrl, J. H. (2014). Omega-3 Free Fatty Acids Suppress Macrophage Inflammasome Activation by Inhibiting NF-κB Activation and Enhancing Autophagy. *PLoS ONE*, *9*(6), e97957. https://doi.org/10.1371/journal.pone.0097957

Wondmkun, Y. T. (2020). Obesity, Insulin Resistance, and Type 2 Diabetes: Associations and Therapeutic Implications. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *Volume 13*, 3611–3616. https://doi.org/10.2147/DMSO.S275898

Wong, S. W., Kwon, M.-J., Choi, A. M. K., Kim, H.-P., Nakahira, K., & Hwang, D. H. (2009). Fatty Acids Modulate Toll-like Receptor 4 Activation through Regulation of Receptor Dimerization and Recruitment into Lipid Rafts in a Reactive Oxygen Species-dependent Manner. *Journal of Biological Chemistry*, *284*(40), 27384–27392. https://doi.org/10.1074/jbc.M109.044065

Wrzosek, L., Miquel, S., Noordine, M.-L., Bouet, S., Chevalier-Curt, M. J., Robert, V., Philippe, C., Bridonneau, C., Cherbuy, C., Robbe-Masselot, C., Langella, P., & Thomas, M. (2013). Bacteroides thetaiotaomicron and Faecalibacterium prausnitziiinfluence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biology*, *11*(1), 61. https://doi.org/10.1186/1741-7007-11-61

Wu, D., & Meydani, S. (2014). Age-Associated Changes in Immune Function: Impact of Vitamin E Intervention and the Underlying Mechanisms. *Endocrine, Metabolic &*

Immune Disorders-Drug Targets, *14*(4), 283–289. https://doi.org/10.2174/1871530314666140922143950

Xia, J., Wang, Z., Yu, P., Yan, X., Zhao, J., Zhang, G., Gong, D., & Zeng, Z. (2024). Effect of Different Medium-Chain Triglycerides on Glucose Metabolism in High-Fat-Diet Induced Obese Rats. *Foods*, *13*(2), 241. https://doi.org/10.3390/foods13020241

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., Sole, J., Nichols, A., Ross, J. S., Tartaglia, L. A., & Chen, H. (2003a). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation*, *112*(12), 1821–1830. https://doi.org/10.1172/JCI200319451

Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., Sole, J., Nichols, A., Ross, J. S., Tartaglia, L. A., & Chen, H. (2003b). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation*, *112*(12), 1821–1830. https://doi.org/10.1172/JCI200319451

Yach, D., Stuckler, D., & Brownell, K. D. (2006). Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nature Medicine*, *12*(1), 62–66. https://doi.org/10.1038/nm0106-62

Yang, P.-C., & Mahmood, T. (2012). Western blot: Technique, theory, and trouble shooting. *North American Journal of Medical Sciences*, *4*(9), 429. https://doi.org/10.4103/1947-2714.100998

Yang, W.-S., Chang, Y.-C., Chang, C.-H., Wu, L.-C., Wang, J.-L., & Lin, H.-H. (2021). The Association Between Body Mass Index and the Risk of Hospitalization and Mortality due to Infection: A Prospective Cohort Study. *Open Forum Infectious Diseases*, *8*(1). https://doi.org/10.1093/ofid/ofaa545

Yaqoob, P. (2002a). Monounsaturated fatty acids and immune function. *European Journal of Clinical Nutrition*, *56*(S3), S9–S13. https://doi.org/10.1038/sj.ejcn.1601477

Yaqoob, P. (2002b). Monounsaturated fatty acids and immune function. *European Journal of Clinical Nutrition*, *56 Suppl 3*, S9–S13. https://doi.org/10.1038/sj.ejcn.1601477

Yaqoob, P., Knapper, J., Webb, D., Williams, C., Newsholme, E., & Calder, P. (1998). Effect of olive oil on immune function in middle-aged men. *The American Journal of Clinical Nutrition*, *67*(1), 129–135. https://doi.org/10.1093/ajcn/67.1.129

Yaqoob, P., Newsholme, E. A., & Calder, P. C. (1994a). Inhibition of natural killer cell activity by dietary lipids. *Immunology Letters*, *41*(2–3), 241–247. https://doi.org/10.1016/0165-2478(94)90140-6

Yaqoob, P., Newsholme, E. A., & Calder, P. C. (1994b). The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. *Immunology*, *82*(4), 603–610.

Yaqoob, Pala, Cortina-Borja, Newsholme, & Calder. (2000). Encapsulated fish oil enriched in α-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. *European Journal of Clinical Investigation*, *30*(3), 260–274. https://doi.org/10.1046/j.1365-2362.2000.00623.x

Yarla, N. S., Polito, A., & Peluso, I. (2017). Effects of Olive Oil on TNF-α and IL-6 in Humans: Implication in Obesity and Frailty. *Endocrine, Metabolic & Immune Disorders - Drug Targets*, *18*(1), 63–74. https://doi.org/10.2174/1871530317666171120150329

Yiannikouris, F., Karounos, M., Charnigo, R., English, V. L., Rateri, D. L., Daugherty, A., & Cassis, L. A. (2012). Adipocyte-specific deficiency of angiotensinogen decreases plasma angiotensinogen concentration and systolic blood pressure in mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *302*(2), R244–R251. https://doi.org/10.1152/ajpregu.00323.2011

Yu, K., Bayona, W., Kallen, C. B., Harding, H. P., Ravera, C. P., McMahon, G., Brown, M., & Lazar, M. A. (1995). Differential Activation of Peroxisome Proliferator-activated Receptors by Eicosanoids. *Journal of Biological Chemistry*, *270*(41), 23975–23983. https://doi.org/10.1074/jbc.270.41.23975

Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z.-W., Karin, M., & Shoelson, S. E. (2001). Reversal of Obesity- and Diet-Induced Insulin Resistance with Salicylates or Targeted Disruption of *Ikkβ*. *Science*, *293*(5535), 1673–1677. https://doi.org/10.1126/science.1061620

Zaki, S. M., Abdel Fattah, S., & Hassan, D. S. (2015). The differential effects of high-fat and high fructose diets on the liver of male albino rat and the proposed underlying mechanisms. *Folia Morphologica*. https://doi.org/10.5603/FM.a2018.0063

Zamora, F., Martinez, J. M., Gaforio, J. J., & Delgado, M. (2018). Olive Oil and Body Weight. Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Rev Esp Salud Pública*, *92*. www.msc.es/resp

Zhang, Y., & Chua, S. (2017). Leptin Function and Regulation. In *Comprehensive Physiology* (pp. 351–369). Wiley. https://doi.org/10.1002/cphy.c160041

Zhou, B., Zhang, J., Zhang, Q., Permatasari, F., Xu, Y., Wu, D., Yin, Z., & Luo, D. (2013). Palmitic acid induces production of proinflammatory cytokines interleukin-6, interleukin-1β, and tumor necrosis factor-α via a NF-κB-dependent mechanism in HaCaT keratinocytes. *Mediators of Inflammation*, *2013*, 1–11. https://doi.org/10.1155/2013/530429

Zhu, Y., Schwarz, S., Ahlemeyer, B., Grzeschik, S., Klumpp, S., & Krieglstein, J. (2005). Oleic acid causes apoptosis and dephosphorylates Bad. *Neurochemistry International*, *46*(2), 127–135. https://doi.org/10.1016/j.neuint.2004.08.003

Zimmermann, P., & Curtis, N. (2019). Factors That Influence the Immune Response to Vaccination. *Clinical Microbiology Reviews*, *32*(2). https://doi.org/10.1128/CMR.00084-18