Thesis

Longterm Dietary Pattern, Quality and Inflammation Index in Immunocompromised and Healthy Individuals

submitted by

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Graz, 08.01.2025 *Lukas Haintz m.p.*

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Zusammenfassung

Eine nährstoffsbedarfsdeckende Ernährungsweise ist die Grundlage für eine optimal funktionierende Physiologie sowie Immunfunktion. Mehrere nicht übertragbare Krankheiten werden mit chronischen, niedrig-gradigen Entzündungen in Verbindung gebracht. Es gibt zunehmend Hinweise darauf, dass eine gesunde Ernährung den Ausbruch und das Fortschreiten von Krankheiten beeinflussen kann. Bestimmte Nahrungsbestandteile sind in der Lage, die Stoffwechselreaktion zu verändern und können entzündungsfördernde oder -hemmende Bedingungen auslösen. Einige Nährstoffe, wie mehrfach ungesättigte Fettsäuren oder Phytonährstoffe, haben entzündungshemmende Eigenschaften, während andere eine entzündungsfördernde Reaktion begünstigen können. Ziel dieser Arbeit ist es, das Nährstoffprofil und die entzündlichen Eigenschaften der Ernährung bei immungeschwächten Personen und gesunden Kontrollpersonen der CoVVac-Studie nach einer einjährigen Nachbeobachtung zu analysieren.

Methoden

Zum ersten Untersuchungstermin absolvierten insgesamt 213 Teilnehmer ein 4-tägiges Ernährungsprotokoll. Außerdem wurde die Anthropometrie bestimmt und Blutproben entnommen, um Entzündungsmarker im Serum zu bestimmen. Nach einem Jahr wurden die Teilnehmer erneut gebeten, das Wiener Ernährungsprotokoll auszufüllen, dem 122 Personen nachkamen. Darüber hinaus wurden die für die Berechnung des Dietary Inflammatory Index relevanten Ernährungskomponenten ermittelt. Die Ernährungsdaten wurden mit der Ernährungssoftware nut.s ausgewertet und der Dietary Inflammatory Index berechnet. Die ermittelten Daten wurden im Längsschnitt ausgewertet. Außerdem verglichen wir den Dietary Inflammatory Index mit dem PAIFIS-Index und versuchten, den Dietary Inflammatory Index mit 7 Entzündungsmarkern im Serum und im Fettgewebe zum ersten Untersuchungszeitpunkt in Verbindung zu bringen.

Ergebnisse

Die Ergebnisse der Studie zeigten, dass es in der gesunden Kontrollgruppe nach einem Jahr Nachbeobachtung keine Veränderungen in der Nährstoffaufnahme gab. In der immungeschwächten Gruppe gab es eine signifikante Veränderung in der Aufnahme von

Flavonolen ($p = 0,019$), aber ansonsten auch keine Veränderung in der Energie- und Nährstoffaufnahme. In unserer Sub-Analyse konnten wir einen signifikant höheren DII ($p = 0,031$) und signifikant höhere Konzentrationen von IL-6 ($p = 0,04$) und IL-18 ($p = 0,014$) in der adipösen Gruppe im Vergleich zu einer alters- und geschlechts-gematchten nicht-adipösen Gruppe feststellen. Wir haben auch gezeigt, dass der DII und der PAIFIS sich in ihrer Aussagekraft unterscheiden, aber miteinander korrelieren ($rs(147) = 0,358$, $p < 0,001$).

Schlussfolgerung

Unsere Studie ergab, dass sich die Nährstoffzufuhr in der immungeschwächten Gruppe ebenso wie in der gesunden Kontrollgruppe nach einem Jahr Nachbeobachtung nicht oder nur geringgradig verändert hat. Weiter konnten wir die entzündungsfördernde Wirkung des überschüssigen Fettgewebes untermauern. Wir plädieren daher für eine bessere Ernährungsschulung der Patient*innen und betonen die Rolle eines gesunden Ernährungsmusters und der Vermeidung von überschüssigem Fettgewebe als zwei wichtige Ansätze zur Verringerung chronischer Entzündungen.

Abstract

A dietary pattern that meets nutrient requirements is the basis for optimal physiology, appropriate immune function, and health. Several non-communicable diseases are associated with chronic low-grade inflammation. There is emerging evidence that a healthy diet has the ability to influence disease onset and progression. Certain dietary components have the ability to shift the metabolic response and may trigger pro- and anti-inflammatory conditions. Several nutrients, such as polyunsaturated fatty acids or phytonutrients exhibit anti-inflammatory properties, whereas others might favor a pro-inflammatory response. The aim of this thesis is to analyze the diet's nutritive profile and inflammatory properties in immunocompromised individuals and healthy controls of the CoVVac Study after a one-year follow-up.

Methods

At the first investigation date a total of 213 participants completed a 4-day dietary protocol. Moreover, anthropometry was assessed, and blood samples were taken, to determine inflammatory markers in serum. After one year, participants were asked again to complete the Vienna Food Record, which 122 people complied with. In addition, dietary components relevant for the calculation of the Dietary Inflammatory Index were assessed. The nutritional data were analyzed using the nutrition software nut.s and the Dietary Inflammatory Index was calculated. The assessed data were analyzed longitudinally. Furthermore, we compared the Dietary Inflammatory Index to the PAIFIS Index and aimed to associate the Dietary Inflammatory Index with 7 serum inflammatory markers and adipose tissue at the first investigation date.

Results

The results of the study showed that there were no changes in the nutrient intake in the healthy control group after a one-year follow-up. In the immunocompromised group, there was a significant change in the intake of flavonols ($p = 0{,}019$), but also no significant change in energy and nutrient intake. In our sub-analysis, we could show a significant higher DII ($p = 0.031$) and significant higher concentrations of IL-6 ($p = 0.04$) and IL-18 ($p = 0.014$) in the obese group compared to an age- and sex matched non-obese group. We also showed that the DII and the PAIFIS differed in their informative value, but correlated with each other ($r_s(147) = 0.358$, $p < 0.001$).

Conclusion

Our study revealed that there is no or only a minor change in the nutrient intake in the immunocompromised group as well as in the healthy control group after a one-year follow-up. We also underlined the pro-inflammatory effect of excess adipose tissue. We therefore call for better nutritional training for patients and highlight the role of a healthy dietary pattern and avoiding excess adipose tissue as two main approaches to reduce chronic low-grade inflammation.

Details of publications already made

Parts of this thesis have been presented at scientific conferences. A short oral abstract presentation was given at the Nutrition 2023 congress in Bregenz, Austria. This congress is the largest nutritional medicine conference in the German-speaking region and is organized in cooperation with the AKE (Arbeitsgemeinschaft für Klinische Ernährung), DGEM (Deutsche Gesellschaft für Ernährungsmedizin), and GESKES (Gesellschaft für Ernährungsmedizin und Metabolismus Schweiz). The corresponding abstract, titled "Association between the dietary inflammatory index (DII) and serum inflammatory markers in healthy obese and non-obese individuals," was published in the journal *Aktuelle Ernährungsmedizin*.

In addition, a poster presentation was given at the 10th Conference of the ÖGE (Austrian Society for Nutrition) Southern Section in Graz, Austria. The corresponding abstract, titled "Zusammenhänge des Dietary Inflammatory Index (DII) und des Pro-Inflammatory/Anti-Inflammatory Food Intake Score (PAIFIS) mit Entzündungsmarkern im Serum und der Ausprägung des Fettgewebes bei gesunden Personen," was published in the journal *Ernährung Aktuell*.

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Abbreviations

ALA	alpha-linolenic acid
ACR	American College of Rheumatology
AA	arachidonic acid
ACPAs	autoantibodies against citrullinated peptide
AKE	Arbeitsgemeinschaft für klinische Ernährung
bDMARD	biologic synthetic disease-modifying antirheumatic drug
BMI	body mass index
BR	brachioradialis
BAT	brown adipose tissue
CRP	C-reactive protein
CDAI	clinical disease activity index
C’	complement
ctDMARD	conventional synthetic disease-modifying antirheumatic drugs
COXs	cyclooxygenases
DAMPs	damage-associated molecular patterns
DGEM	Deutsche Gesellschaft für Ernährungsmedizin
DII	Dietary Inflammatory Index
DRI	dietary reference intakes
DMARDs	disease-modifying antirheumatic drugs
DT	distal triceps
DHA	docosahaexonic acid
EPA	eicosapentaenoic acid
ES	erector spinae
EFSA	European Food Safety Authority
EULAR	European League Against Rheumatism
EVOO	extra-virgin olive oil
FFQ	Food Frequency Questionary
FT	front thigh
GP	General Practitioner
GESKES	Gesellschaft für Ernährungsmedizin und Metabolismus Schweiz

GSH	glutathione
IOM	Institute of Medicine
INF-γ	Interferon-gamma
IL-1	interleukin-1
IL-17	interleukin-17
IL-2	interleukin-2
IL-4	interleukin-4
IL-5	interleukin-5
IL-13	interleukin-13
IL-22	interleukin-22
IL-6	interleukin-6
JAK	Janus-kinase
LT	lateral thigh
LA	linoleic acid
LOXs	lipoxygenases
LA	lower abdomen
MHC	Major-Histocompatibility Complexes
MC	medial calf
MedDiet	Mediterranean Diet
MAC	Membrane Attack Complex
MTX	methotrexate
MAPk	modulate mitogen-activated protein kinase
PAMPs	molecular patterns
NOX	NADPH oxidase
NHANES	National Health and Nutrition Examination Survey
NK cells	Natural Killer Cells
NCDs	non-communicable diseases
NF-κB	nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
RANKL	receptor activator of nuclear factor κ B ligand
ROS	Reactive oxygen species
O3-I	Omega-3-index

PRRs	pattern recognition receptors
PPO	polyphenol oxidase
PUFAs	polyunsaturated fatty acids
PAIFIS	Pro-Inflammatory / Anti-Inflammatory Food Intake Score
RCT	randomized controlled trial
ROS	reactive oxygen species
RDA	recommended dietary allowances
RA	Rheumatoid arthritis
RF	rheumatoid factor
RAPID3	Routine Assessment of Patient Index Data 3
SCFAs	short chain fatty acids
SDAI	simplified disease activity index
SAT	subcutaneous adipose tissue
SLE	Systemic Lupus erythematosus
sWAT	subcutaneous white adipose tissue
SOD	superoxide dismutase
TLR	toll-like receptor
TCM	Traditional Chinese Medicine
TNF-α	tumor necrosis factor alpha
USDA	U.S. Department of Agriculture
UA	upper abdomen
WAT	white adipose tissue
WHO	World Health Organization

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1 Introduction

Several non-communicable diseases (NCDs), such as ischemic heart disease, cancer, chronic kidney disease, type 2 diabetes, neurodegenerative diseases, and autoimmune diseases are associated with systemic chronic inflammation (1). Acknowledging that these chronic inflammatory diseases have been the major causes of death worldwide in the last two decades (2), there is urgent need to understand systemic chronic inflammation in more depth as well as to find therapeutic strategies against it.

Acute inflammation is a critical and beneficial transient response to harmful stimuli, such as infection and injury. Reasonable progress has been made in understanding underlying mechanisms of acute inflammation, whereas systemic chronic inflammation is still less well understood (3).

The purpose of the introduction of this work is to provide an overview of the immune system and to provide a basic understanding of the main topic of inflammation. The subtypes of inflammation will be discussed, specifically systemic chronic inflammation, as well as its potential triggers with a focus on nutritional determinants. Furthermore, Rheumatoid arthritis will be explained and the potential association with the Dietary Inflammatory Index (DII) will be discussed (4).

1.1 Overview of the immune system

Since the human organism is permanently exposed to foreign substances, that could potentially lead to detrimental damage or disease, it is critical to have protective mechanisms. The immune system is composed of mechanical barriers, lymphoid organs, cells, humoral factors and cytokines. All components are interactive and together fulfill the task of recognizing and fighting pathogens and therefore maintaining homeostasis (5).

The immune system is generally divided into two parts, the innate and the adaptive immune system. While the innate part is mostly considered to be responsible for the acute general first-line response, the adaptive part is much more precise in combating pathogens, which comes at the expense of its speed. In reality both parts are highly interactive and work together in unity (6).

1.1.1 The innate immune system

1.1.1.1 Barriers

The very ancient innate immune system comprises a wide variety of defense mechanisms. First, it consists of specialized surface cells, called epithelial cells, which form a mechanical barrier against intruders with their tight junctions. The mucus on mucous membranes represents another mechanical barrier. In addition, it contains antibacterial peptides and enzymes such as lysozyme, which damage the bacterial cell wall. Antimicrobial peptides, such as defensins directed against bacteria, are found on the skin and mucous membranes and in the secretions of exocrine glands. Antimicrobial peptides have a direct bactericidal or fungicidal effect on a broad spectrum of pathogens. Moreover, several mechanism supporting the mechanical barrier are known. For example, pathogens are also removed by the natural flow of secretions, which is, for instance, maintained by the ciliated epithelium of the respiratory tract. Larger parasites can also be removed via the sneezing reflex and diarrhea, which are started by IgE-triggered histamine release from mast cells. The flow of acidic urine prevents bacteria from ascending through the urethra into the bladder. More examples include the acidic milieu on the skin or in the stomach, which impedes or prevents the multiplication of pathogens (6).

1.1.1.2 Cellular components of innate immunity

Once the pathogen invades through the mechanical barriers, the innate response is primarily characterized by the recruitment and activation of neutrophils. This results from the recognition of an infection or tissue damage by macrophages, which consequently become activated, release cytokines, and lead to the characteristic neutrophil leukocytosis. To get to the site of infection, neutrophils use a versatile process including pro-inflammatory mediators, adhesion molecules, chemoattractants, and chemokines. Neutrophils can phagocytose organisms, form phagolysosomes as well as destructing them via respiratory burst or specific enzymes. This process is much more effective if the object is opsonized with an antibody or complement (C') (5). Additionally, neutrophils can secrete various messenger substances such as eicosanoids, cytokines and chemokines that can augment innate as well as adaptive immunity. They also can present Major-Histocompatibility Complexes (MHC) that enable them to directly interact with specific cells of the adaptive immune system (6).

While neutrophils are the first line response to acute damage (7), there exists a broad range of cell types involved in innate immunity.

Monocytes are the precursor cells of macrophages that major function is also phagocytosis. They are longer lasting than neutrophils and therefore constitute the later major effector cell population of the innate defense after the initial neutrophil infiltration. These phagocytes are characterized by specific pattern recognition receptors (PRRs) that are capable of detecting pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) and consequently get activated (6, 8). After activation and phagocytosis of the pathogen, they form same as neutrophils phagolysosomes, and proteolytic enzymes degrade the microbe (6).

Eosinophils have the ability to phagocytose, but it is much less efficient compared to granulocytes (6). Their main role is fighting parasites by releasing their cytotoxic proteins which they store in large granules (5).

Basophils and mast cells are morphologically similar and share also functional similarities. IgE and allergens activate mast cells and basophils via the high-affinity IgE receptor, causing them to degranulate and release histamine and a variety of other mediators. Mast cells are mostly located in the body's surface organs, whereas basophils are found in the blood and move into the inflammatory tissues as a result of inflammatory events. While being relatively few compared to other white blood cells, basophils and mast cells are responsible for some of the most serious immunological responses, including anaphylaxis (5).

Natural Killer Cells (NK cells) morphologically resemble lymphoid cells but do not express T- or B-cell receptors (5). They contain azurophil granulas containing a variety of cytotoxic molecules. If the number of MHC-I molecules on a given cell declines or disappears, which weakens the inhibitory signals sent to NK cells, the NK cell gets activated and induces apoptosis in the target cell. NK cells also produce various cytokines, primarily Interferon- gamma (INF-γ), chemokines and growth factors. These mediators allow NK cells to interact with macrophages, T-cells and dendritic cells (9). Dendritic cells are important links between innate and adaptive immunity (8).

Innate immunity is strongly influenced by nutrition and therefore healthy dietary patterns, such as the Mediterranean diet, have the ability to modulate and improve immune function (10, 11).

1.1.1.3 Complement system

Parallel to directly fighting pathogens through phagocytosis, neutrophils and macrophages further activate the complement system through the secretion of cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) (6, 12). It serves the innate immunity through several mechanisms and consists of proteins produced by the liver. Its function augments that of phagocytes and antibodies. The complement system produces the Membrane Attack Complex (MAC), which perforates the cell wall of bacteria and therefore leads to their destruction. Moreover, it marks bacteria through opsonization, which provides easier access for phagocytosis. In addition, it enhances the recruitment and activation of leukocytes, especially neutrophiles and macrophages but also serves as a co-stimulator of B-cells and therefore augments innate immunity as well as adaptive immunity (12).

The initiation of the proteolytic complement cascade leads to a step-by-step activation of previously inactive proteases, whose activity activates the next inactive enzyme precursor of the complement cascade and finally leads to the so-called lytic terminal sequence and the formation of the previously mentioned MAC. Insertion of the MAC into the cell membrane leads to lysis of the target cell. Depending on the initiation of complement activation, three complement activation pathways can be distinguished from each other. First, the classical pathway, which is initiated by C1q recognizing specific target molecules and forming an antigen-antibody-complex; second, the lectin binding pathway, which can be triggered by circulating PRRs; and third, the alternative pathway, that can be activated by spontaneous hydrolysis of C3 or by C3 via the classical- and lectin-pathway. All three pathways lead to the formation of the C3 convertase and then share a common terminal pathway, where the complement cascade proceeds and induces a series of inflammatory and pathogen-fighting factors (6, 12).

1.1.2 The adaptive immune system

The adaptive immune system takes longer to fully develop and increases enormously in effectiveness as humans mature. Our lymphocytes' capacity to recognize and get rid of any imaginable infection is essential to our existence. The immune system must also avoid harming its own organism. Hence, the adaptive immune system must fulfill both maximal diversity as well as avoidance of autoreactivity (13). There are two primary divisions working closely together. On the one hand, the humoral defense, whose main actors are the B lymphocytes, and

the cell-mediated immune defense, which is represented by the T lymphocytes (8). The adaptive immune system, also called specific immune system, is characterized by high specificity through antigen specific receptors (5).

The development of B- and T-cells, in contrast to the cells of the innate immune system, begins from the lymphoid progenitor cell. At the beginning their descendants reside in the primary lymphoid organs; T-cells primarily in the thymus and B-cells primarily in the bone marrow. They multiply and go through a maturing process. Numerous DNA segments are randomly rearranged and spliced together in a unique mechanism to produce antigen-specific receptors in both cell types. Later they get released as immunocompetent but still naive lymphocytes to the periphery. Only 5% of the cells starting the maturing process develop the needed characteristics and are therefore being classified as suitable and get released from the organs. Being naive means that the cells did not come across their specific antigen yet. The secondary lymphoid tissues provide a suitable environment for the naive cells to encounter an antigen and therefore express adhesion molecules to allow the cells to move through the tissue (5, 8).

1.1.2.1 B-Lymphocytes

The main function of B-lymphocytes, also known as B-cells, is the production of antibodies. Since they get expressed on the surface of B-cells, they are also known as B-cell-receptors. Antibodies interact with antigens in a highly specific manner, and this bonding results in the activation of various functions of the B-cells, including the secretion of cytokines. Cytokines are messengers that can activate other immune cells. Furthermore, the bonding between antigen and B-cell receptor can lead to somatic hypermutation and class switching. Somatic hypermutation is a process that enables B-cells during an immune response to produce antibodies with higher affinity for the antigen. This can be explained by mutations in the genes coding for the variable region of the antibody, that is responsible for binding the antigen and known as the Fab-region. There is also a constant region in antibodies, the Fc-part, which changes in the course of an immune response from IgM to other isotypes such as IgG, IgA or IgE, a process known as class switching. Antibodies, specifically the constant region, provide a link to the innate immune system by activating effector cells, such as macrophages, granulocytes, or mast cells. These cells can therefore lead to phagocytosis of the antigen,

cytolysis of the antibody loaden cell or to the production of histamine. Moreover, antibodies can activate the complement system.

The first adaptive immune response to an antigen is slow and inefficient, taking about a week for detectable IgM antibodies and longer for IgG antibodies. However, subsequent exposures to the same pathogen result in a faster and higher increase in IgG antibody levels, due to immune memory. This is a unique property of the adaptive immune system. Innate immune cells do not form memory. Plasma cells are the primary mediators of the antibody memory by continuously producing antibodies in the bone marrow, even after the antigen is gone. Such plasma cells can survive for decades under favorable conditions (13).

1.1.2.2 T-Lymphocytes

T-Lymphocytes or T-cells are the second main subgroup of the adaptive immune system. Most responses of the adaptive immune system involve T-cells and a malfunction can therefore lead to severe loss of function. Whereas a diminished function can lead to a suppressed immune state, a hyperfunction can lead to damage of body tissue and therefore autoimmunity. Hence, the right balance is critical (6).

The detection of antigens differs fundamentally from that in B-cells. T-cells express a T-cell receptor on their membrane that is not able to directly detect soluble antigens, but only specific peptides generated by antigens and bound to a MHC-molecule. These antigen-presenting MHC molecules are glycoproteins that are either found on nearly every nucleus-containing cell in the organism (MHC I) or are limited to antigen presenting cells (MHC II). T-cells can be divided into CD4+ T-helper-cells and CD8+ cytotoxic T-cells. CD4+ T-helper-cells recognize antigens bound to MHC-II-class-molecules, that are presented by antigen-presenting-cells such as dendritic cells, macrophages, and B-cells. They present antigens originating from extracellular bacteria or serum proteins such as viral proteins and toxins. MHC-I-class-molecules present only antigens produced by the cell itself and interact with CD8+ T-cells. Besides recognition of the antigen by the T-cell-receptors some other co-stimulatory signals, also produced by the antigen presenting cells, are necessary to activate T-cells. Thus, it is ensured that the presentation of microbial antigens, but not the presentation of self-antigens, leads to T-cell activation (6, 13).

T-helper cells (Th-cells) can be divided into several subtypes, characterized by a different pattern of produced cytokines. Th-0 cells can be seen as precursors of Th-1 and Th-2 cells. They become activated at the beginning of an immune response and secrete a broad variety of cytokines in low concentrations. Chronic stimulation then leads either to the differentiation of Th1-cells, mainly secreting IL-1 and IFN-y, which leads to activation of T-cell proliferation and phagocytosis (5). Th-2 cells mainly produce interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13) which provide signals for antibody class switching, such as IgE, making them a critical pathogenetic factor for allergies. Another subclass are Th-17 cells, mainly producing interleukin-17 (IL-17) and interleukin-22 (IL-22), stimulate neutrophil granulocytes. A dysregulated production of IL-17 is associated with several chronic inflammatory and autoimmune diseases (13).

Regulatory T-cells, also named Tregs, are also CD4+ T-cells that can suppress the immune response. They produce anti-inflammatory cytokines such as IL-10 or Transforming growth factor (TGF) beta and consume interleukin-2 (IL-2), which is necessary for the survival of T-cells. If the Th-cell is going to differentiate into Th-1, Th-2 or Th-17 cells is mainly decided by the antigen and by the antigen presenting cell produced cytokines. It is important to note that the classification is not strict and that Th-cells are highly plastic and can transform into another subclass. Nevertheless, some diseases are characterized by a disturbed Th-cell-balance, what makes the classification justified (6, 13).

CD8+-cytotoxic-T-cells interact with MHC-1 and therefore only recognizes antigens produced by the cell itself. Therefore, they primarily lead to lysis of tumor cells, virus-infected cells and allogeneic cells (13).

In order to avoid autoreactivity, immature T-cells coming from the bone marrow get selected in the thymus. They start proliferating and go through positive selection. If the thymocyte binds to MHC with low to medium affinity a growth signal gets released. Depending on whether MHC class I or MHC class II complexes are recognized, CD8+ or CD4+ T cells are produced. Thymocytes that do not bind or have low affinity for these complexes do not receive any signals for growth, and they undergo cell death known as "death by neglect". After positive selection, negative selection occurs which is characterized by the apoptosis of thymocytes that can bind with high affinity to self-peptide/ MHC-molecules. This process reduces the risk of autoreactivity due to the low affinity to autoantigens of the released T-cells (6, 13).

1.2 Inflammation

Usually, acute inflammation is triggered by infections or tissue damage and involves the delivery of plasma and leukocytes to the affected area. This is mainly observed in microbial infections where the innate immune system's receptors PRRs play a crucial role in detecting PAMPs and in tissue lesions by detecting DAMPs. Tissue-resident macrophages and mast cells recognize the infection and produce various inflammatory mediators, such as chemokines, cytokines and eicosanoids, leading to an inflammatory exudate. This exudate allows plasma proteins and leukocytes, mainly neutrophils, to enter the site of infection or damage. When leukocytes are recruited from the bloodstream into the tissue, they first approach the vascular endothelium, a process known as margination. To facilitate this process, vasodilation slows down the blood flow velocity, allowing leukocytes to shear off from the central axial flow and move towards the endothelium. Once they reach the endothelium, leukocytes firmly adhere to it through an integrin-mediated process, known as adhesion (3, 6). The neutrophils then strive to kill the invader by releasing their granules which include reactive oxygen species (ROS), reactive nitrogen species and proteinase 3. These toxic executors cannot distinguish between microbial and host targets, which makes collateral damage inevitable (3). This process is accompanied by the acute-phase reaction, where cytokines, such as IL-6, stimulate the liver to produce acute-phase proteins, that help the inflammatory reaction to occur efficiently (6). Some of these acute-phase proteins also play an important clinical role in diagnosing an inflammatory status. The phenomenological signs of inflammation were described already 2000 years ago: *rubor* (redness), *tumor* (swelling), *calor* (heat), *dolor* (pain) and *functio laesa* (loss of function) (14).

Inflammation is usually thought to be linked with severe disruptions to the body's balance, such as infection or tissue injury. However, these extreme cases are just one part of a spectrum of conditions that can activate inflammation. They cause the strongest responses, which is why they are the most well-known and characterized inflammatory reactions. However, there are several inflammation-triggers that can be divided into exogenous (microbes, virulence factors, allergens, irritants, foreign bodies, toxic compounds) and endogenous inducers (stressed, damaged or malfunctioning tissue). Generally, every detection of tissue abnormalities is likely to trigger an inflammatory response. These types of responses are expected to occur more often

but with less severity than the typical inflammatory reactions induced by infection or injury. Therefore, the severity, characteristics and duration of an inflammatory response are also likely to be variable depending on the severity of malfunction, with the general purpose being to regain homeostasis (3).

After a successful acute inflammatory response that eliminates the infectious agents, the repair phase begins and is mainly mediated by tissue-resident and recruited macrophages. To move from inflammation to resolution, a shift in lipid mediators from pro-inflammatory prostaglandins to anti-inflammatory lipoxins, as well as the induction of the anti-inflammatory M2-macrophages, is vital. This shift hinders the recruitment of neutrophils and facilitates the recruitment of monocytes, which remove dead cells and start the tissue remodeling process. However, if the inducer of the inflammation is not resolved through the acute-phase reaction and the abnormal conditions persist, chronic inflammation occurs. Chronic inflammation results in the infiltration of neutrophil granulocytes and monocytes, the formation of granulomas by the clustering of macrophages, constant activation of the adaptive immune system, tissue reconstruction, growth factor-triggered formation of new blood vessels, and fibrosis. The adaptive changes that occur in the short term during acute inflammation often serve a transient benefit whereas the same mechanism can become counterproductive. Inflammation can temporarily reduce insulin sensitivity, which allows glucose to be redirected from skeletal muscle to leukocytes and other cells with higher energy demands during infection and tissue repair. But if skeletal muscle remains insulin resistant for too long, it can cause type 2 diabetes (3, 6). Indeed, several metabolic diseases, including type 2 diabetes, are characterized by chronic inflammation (15). Chronic inflammation and factors influencing it will be discussed in depth later, with a primary focus on nutrition.

1.3 Autoimmunity and autoinflammatory diseases

While several NCDs are associated with chronic inflammation (1), autoimmune and autoinflammatory diseases tend to be primarily characterized by chronic inflammation in their clinical presentation (16).

Autoimmune disorders are identified by the failure of the immune system to distinguish self-antigens, leading to the activation of T cells and B cells. This process results in the production of autoantibodies that target specific organs, leading to their damage due to the dysfunctional adaptive immune response (17). In contrast, autoinflammatory diseases do not involve specific antigens and have a systemic chronic inflammation that persists without any disruption in immune tolerance or the formation of specific autoantibodies. They tend to develop based on an overly active immune response based to environmental stimuli such as stress, infections or smoking while certain genetic mutations can be a predisposing factor. In contrast to autoimmunity, it is primarily the innate immunity that is dysfunctional, and the formation of specific autoantibodies is usually not seen (6, 16).

Systemic Lupus erythematosus (SLE) represents a classical example for an autoimmune disease while gout is characteristic for autoinflammation. However, many rheumatic and musculoskeletal diseases may have overlapping features of both autoimmunity and autoinflammation, which can make their classification difficult. Moreover, it is possible for a single disorder to display features of both at different points in its progression (16).

Autoimmune diseases can be divided into systemic, such as SLE, where multiple organ systems are involved, and organ-specific, such as Type 1 diabetes. Although useful in clinical practice, the cause and pathophysiology do not seem to differ (18). The inability to distinguish between self and non-self leads to the concept of dysregulated immune tolerance. The human body has developed several mechanisms to prevent autoimmunity and maintain immune tolerance. The antigen-specific deletion of T-cells in the maturing process during negative selection, the need for co-stimulation of lymphocytes, the existence of regulatory cells and the elimination of chronic active T- and B-cell represent a few (6). A certain level of autoreactivity always seems to occur and is physiologic. Hence, the detection of low levels of autoantibodies in the blood should not automatically be considered as being pathological, making the diagnosis of diseases challenging (18).

The mechanisms behind the development of autoimmune diseases remain to be fully understood. However, certain associations possibly explaining causality have been described in the literature. For example, certain diseases are linked to specific MHC and their gene products

known as human leucocyte antigens (HLA). The percentage of monozygotic twins who have the same autoimmune disease ranges from 12% to 67%, indicating that there might be other factors besides genetic susceptibility that are involved. Indeed, various environmental factors are associated with autoimmunity. These factors include dietary habits, the microbiota, infections and exposure to toxins (17).

Mechanisms that aim to explain the development of autoimmune diseases are the inability to control autoreactive T- and B-cells, development of neoantigens, molecular mimicry and persistence of neoantigens (6).

1.4 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is one of the most widespread chronic inflammatory autoimmune diseases and is characterized by painful joint swelling, caused by inflammatory processes in the synovia leading to cartilage and bone damage. While the manifestation of RA is primarily in the joints, systemic manifestations including cardiovascular, pulmonary, psychological and skeletal disorders are common (19) which further emphasizes the assumption of detrimental systemic effects of chronic inflammation (20).

The incidence of rheumatoid arthritis is estimated to be between 0.5% and 1% and exhibits a notable decrease from north to south as well as from urban to rural areas. An increased risk due to positive family history as well as a concordance rate between twins, suggests the suspicion of genetic influence, with HLA-DRB1 being the most researched and established inherited association (19). This association gets further strengthened by the fact that having the shared epitope is linked with seropositivity for autoantibodies against citrullinated peptide (ACPAs) and autoantibodies against IgG (rheumatoid factor [RF]) (21). In 50-70% of RA-patients, these autoantibodies can be detected in the blood and compared to seronegative patients, seropositive patients are also exposed to a higher risk for an unfavorable course of disease (19, 21).

Geographic risk disparities suggest environmental risk factors, with tobacco use, socioeconomical status and educational attainment being among the most established ones. RA is also associated with periodontal disease. A possible explanation is that a certain bacterium often detected in periodontitis favors loss of tolerance to citrullinated peptides. Possibly due to

molecular mimicry, being infected with certain other microbes, such as Epstein-Barr virus, is also associated with RA (21) as well as other autoimmune diseases (22, 23). In recent years growing evidence suggests an association between the development of RA and the gastrointestinal microbiome (24) as well as dietary habits (25), opening new doors for possible dietary and orthomolecular therapeutic approaches (26, 27). Figure 1 gives an overview of pathways contributing to the development of RA.

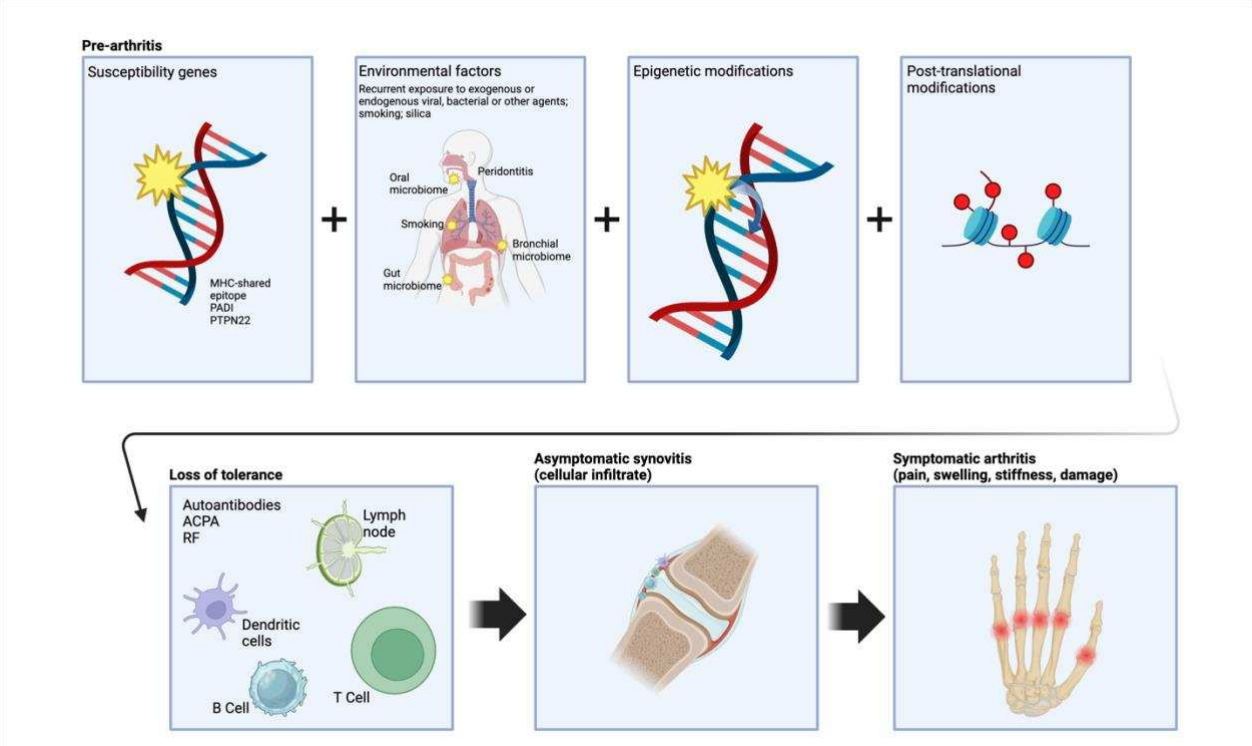

Figure 1: Pathways to Rheumatoid Arthritis; adjusted from Smollen et al. 2016 with © 2024 BioRender

The typical joint swelling in RA, accompanied by stiffness and pain, arises from leukocyte infiltration into the synovial compartment, which lacks immunocompetent cells in healthy individuals. The resulting synovitis is triggered by adaptive immune cells as well as innate immune cells. The inflammatory environment, where TNF-α and IL-6 probably play an essential role, leads to the activation of endothelial cells and attracts immune cells to accumulate within the synovial compartment, resulting in the initiation or exacerbation of the inflammatory response. The consequent activation of fibroblasts, T- and B-cells, monocytes and macrophages leads to the initiation of osteoclasts via receptor activator of nuclear factor κ B ligand (RANKL).

Bone erosions follow, as well as cartilage damage via chondrocytes getting stimulated by cytokines (21).

In order to diagnose RA, the American College of Rheumatology (ACR), together with the European League Against Rheumatism (EULAR), developed the so-called ACR/EULAR classification criteria. Based on a scoring system, the diagnosis of RA can be made (Table 1).

Table 1: ACR/ EULAR criteria for the diagnosis of Rheumatoid Arthritis; adjusted from Aletaha et al. 2010

Domain	**Categorie**	**Points**
A	Joint involvement (0-5 points)	
	1 large joint	0
	2-10 large joints	1
	1-3 small joints	2
	4-10 small joints	3
	>10 joints including at least one small joint	5
B	Serology (at least one test needed for classification; 0-3 points)	
	Negative RF and negative ACPA	0
	Low positive RF or low positive ACPA	2
	High positive RF or hight positive ACPA	3
C	Acute-phase reactants (at least one test needed for classification; 0-1 points)	
	Normal CRP and normal ESR	0
	Abnormal CRP and abnormal ESR	1
D	Duration of symptoms	
	< 6 weeks	0
	≥ 6 weeks	1

According to this joint ACR and EULAR RA-classification criteria the scoring system can be defined as follows: "'Large joints' refers to shoulders, elbows, hips, knees, and ankles. 'Small joints' refers to the metacarpophalangeal joints, proximal interphalangeal joints, second to fifth metatarsophalangeal joints, thumb interphalangeal joints and wrists. A score of ≥6/10 is needed for classification of a patient as having definite RA." (Aletaha et al., 2010, p. 2573) (28)

Being the primary driver of pathogenesis, the resolution and reduction in inflammation is the main goal in pharmacologic therapy. Regular assessment and classification of disease activity are necessary to adapt medications as needed. If low disease activity or nearly 80% improvements in the simplified disease activity index (SDAI) or clinical disease activity index (CDAI) occurs after 3 months the treatment should be continued whereas if the improvement is low, the therapeutic approach should be reconsidered (21).

Due to the heterogeneity of the pathophysiology of RA, patients often require different successive therapies to achieve remission or a decline in symptoms. A combination of pharmacologic substances should be used, with disease-modifying antirheumatic drugs (DMARDs) and glucocorticoids (GCs) being the main representatives. As soon as the diagnosis is made, the therapy should be initiated with a conventional synthetic disease-modifying antirheumatic drug (ctDMARD), where the first-line recommendation is methotrexate (MTX). If patients cannot tolerate MTX or have a contraindication, leflunomide or sulfasalazine are the recommended alternatives. The initiation or the change to another DMARD should be accompanied by short-term GCs. However, due to side effects GCs should be gradually reduced and stopped as soon as clinically possible. If the symptoms have improved after 3 months and the target goal is achieved after 6 months, the therapy should be continued, and dose reduction should be considered in sustained remission. If this is not the case, however, depending on prognostic factors, another ctDMARD should be added or either a biologic DMARD (bDMARD) or a Janus-kinase- (JAK) inhibitor should be added. If this approach is still not successful, the bDMARD or the JAK-inhibitor should be changed (29).

1.5 Factors influencing chronic inflammation

1.5.1 Adipose tissue and obesity

Obesity represents a multifactorial and intricate pathological state characterized by excess adipose tissue, giving rise to adverse health consequences. It is defined by a body mass index (BMI) equal to or greater than 30 kg/m^2, whereas being overweight is defined by having a BMI of 25 – 29.9 kg/m^2. The BMI is calculated by dividing the bodyweight in kg by the square of the height in m (kg/m^2) (30). Being overweight or obese is linked to a higher risk of developing several diseases, including diabetes, coronary heart disease, several types of cancer, hypertension and osteoarthritis. The worldwide rising prevalence of obesity is a major public-health concern with threatening socio-economic consequences (31). Currently, the World Health Organization (WHO) reports that excess weight and obesity impact nearly 60% of adults and approximately one in three children in the European Region (32).

The pathogenesis of obesity is complex and has many causes and risk factors. An imbalance in consumed and expended energy, poor lifestyle and exercise habits, genetic and epigenetic features, as well as factors related to the microenvironment and the gut microbiome are among them (30).

Against previous beliefs, adipocytes are not only a reservoir for lipids, but also form a highly active metabolic, immune, and endocrine tissue. Adipose tissue secretes proteins such as leptin, which is a major appetite regulator, it produces enzymes contributing to the metabolism of steroid hormones and it secretes many bioactive peptides called adipokines (33). Adipokines are cellular signaling proteins that play a regulatory role in numerous biological processes within target organs such as the brain, liver, heart, and immune system, among others. Adipokines contribute to diverse functions and have the potential to impact various physiological processes, including the regulation of energy and appetite, modulation of lipid and glucose metabolism, insulin sensitivity, regulation of endothelial cell function, inflammatory responses, angiogenesis, blood pressure, hemostasis, development of atherosclerosis, and metabolic syndrome (34).

Adipose tissue can be divided into white adipose tissue (WAT) and brown adipose tissue (BAT). In contrast to BAT, which is associated with positive health benefits (35), the increase in WAT in obesity influences the secretion of adipokines, which then compromise the beforementioned metabolic and cardiovascular health complications. Interestingly, this is especially true for

adipose tissue around the trunk, namely visceral white adipose tissue (vWAT), and not for subcutaneous white adipose tissue (sWAT) (34). This meaningful differentiation is one of the reasons why the BMI alone is considered to be insufficient in assessing comorbidity risk and the measurement of the waist circumference is highly recommended in clinical practice to evaluate and manage overweight and obese patients (36).

In a state of progressive obesity and therefore increasing WAT, the quantity of adipogenic stem cells diminishes, adipogenesis gets impaired, and triglycerides accumulate leading to hypertrophying adipocytes. The adipocytes expand, capillary density gets reduced, leading to less availability of nutrients and oxygen. These hypoxic, hypertrophic cells are more prone to necrosis, additionally resulting in a hypoxic and pro-inflammatory environment. Cells then start behaving aberrantly, macrophages, physiologically resident in the adipocyte, undergo a phenotype switch from M2 to M1, a more pro-inflammatory phenotype, and more monocytes infiltrate the adipocyte also evolving towards the M1 phenotype. These macrophages primarily produce IL-6 and TNF-α which consequently influence the production of other adipokines (37). It is estimated that the percentage of macrophages in adipose tissue in lean people is below 10%, whereas in obese individuals they can be as high as 40% (38). This explains why pro- and anti-inflammatory adipokines in lean people usually balance each other, whilst in obese people, the balance is shifted towards pro-inflammatory adipokines, inducing chronic low-grade inflammation (34).

1.5.2 Nutrition

1.5.2.1 Healthy dietary patterns

Although NCDs, which are among the most common causes of death worldwide, have genetic and environmental components, adjustable lifestyle-factors, including nutrition, can contribute to their development. Indeed, a causal link between the increased prevalence of chronic NCDs and westernized dietary patterns has been described. Dietary patterns can lead to the occurrence of hypertension, dyslipidemia, obesity, inflammation, and more, which contribute to an increased risk of death (39).

The science around nutrition and the knowledge of the meaningful impact of food ingredients has emerged immensely in the last few decades, so that today, nutrition is scientifically

recognized as being of far greater importance than it was in the 1980s. Based on the current state of knowledge, different bodies around the world make recommendations for nutrient and energy intake. In the D-A-CH region, this is done by the German Society for Nutrition in cooperation with the Austrian Society for Nutrition and the Swiss Association for Nutrition. The "recommended dietary allowances" (RDA) and the "dietary reference intakes" (DRI) of the US Institute of Medicine (IOM) and the WHO are used as overarching guidelines (40).

A health-promoting diet generally ensures macronutrients are consumed in suitable proportions to meet energy and physiological requirements without excessive intake. Additionally, it ensures adequate intake of micronutrients and hydration to fulfill the body's physiological needs (39). In order to assess the association of dietary patterns and all-cause mortality, a systematic review was conducted to inform the Dietary Guidelines for Americans. Based on the reviewed evidence, the researchers suggested that nutrient-dense diets, independently of pattern label or name, were associated with a decreases risk for all-cause mortality. Commonalities of these health promoting dietary patterns included a comparatively higher intake of vegetables, legumes, fruit, nuts, cereals, either unprocessed or whole grains, fish, seafood, lean meat and poultry (if included), and unsaturated fat compared to saturated fat. In addition, they contained relatively lower intake of red and processed meat, high-fat dairy, refined grains, added sugars and/ or sugar-sweetened beverages, solid fats, saturated fats and/ or trans-fat, and excessive sodium (41). One specific health promoting dietary pattern that is supported by a large body of evidence is the Mediterranean Diet (MedDiet). It is composed of ample seasonal vegetables, olive oil as the main fat source, fresh seasonal fruits, legumes several times per week, daily whole grains, fish two - three times a week, dairy several times weekly, spices and herbs, infrequent consumption of sweets, minimal inclusion of red and processed meat, three to four eggs per week, plenty of water as beverage and moderate consumption of wine always with meals. The main characteristic therefore is the focus on unprocessed foods, mostly plant-foods, which come with a large amount of healthy nutrients (42). Indeed, systematic reviews and meta-analyses support the health-promoting and disease-preventing effect of the Mediterranean Diet, including a decreased risk of all-cause mortality (43-46). As mentioned before, many nutrient-dense and plant-based diets can be health-promoting. However, the evidence around the MedDiet is unique due to the fact that its benefits are supported by a large, randomized controlled trial with a low risk of bias looking at hard clinical endpoints. This level of evidence

is not available for any other dietary pattern (47). The study in question is called the PREDIMED (Prevención con Dieta Mediterránea) trial, a randomized, controlled, multicenter trial, designed to determine the long-term effects of a MedDiet on the primary prevention of cardiovascular disease in subjects with high cardiovascular risk. The intervention groups were either randomized to a MedDiet supplemented with extra-virgin olive oil (EVOO) or with mixed nuts. Health outcomes were compared to a control group, which received the advice to reduce dietary fat intake. The primary end point was a major cardiovascular event. Although the planned duration of the trial was 6 years, the trial was stopped after 4.8 years due to sufficient evidence for the benefit of the MedDiet. The incidence of the primary endpoint was reduced by 31% when supplemented with EVOO and by 28% when supplemented with nuts. Benefits were also seen for the metabolic syndrome, adiposity, cognition, breast cancer, incidence of diabetes complications, peripheral arterial disease, lipid profile and inflammatory status (48).

1.5.2.2 Anti-inflammatory dietary components

Multiple mechanisms might explain the observed benefit of a MedDiet, however, they have not been explained adequately. Though, one mechanism that has been discussed extensively is the anti-inflammatory effect of the MedDiet. (42) Several dietary compounds that are part of the MedDiet, including omega-3 fatty acids and certain phytochemicals, have anti-inflammatory potential (49), (50).

1.5.2.3 Omega-3 fatty acids

Fatty acids are a crucial part of phospholipids located in the cell membrane, playing a critical role in functional, metabolic, and signaling processes. Depending on the cell type, the fatty acid composition is different, influencing its flexibility and fluidity, as well as the function of membrane proteins. Depending on the dietary intake of polyunsaturated fatty acids (PUFAs), the level of PUFAs in the cell membrane phospholipids can vary and consequently change the influence on antioxidative pathways and the modulation of inflammatory processes.

Two families of PUFAs are of special interest related to human health: omega-3 PUFAs and omega-6 PUFAs. Two of them are considered to be essential, namely alpha-linolenic acid (ALA), representive for the omega-3 PUFAs, and the omega-6 PUFA linoleic acid (LA). A deficiency is rare but can contribute to the development of various diseases. However, also a

deficiency of their metabolic products, the omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahaexonic acid (DHA) and the omega-6 PUFA arachidonic acid (AA), can lead to adverse health outcomes. Western dietary patterns usually contain LA 5 to 15 times higher than ALA. Due to sharing the same enzymes to form their metabolic products, the metabolism of ALA to EPA and DHA seems to be limited in humans, resulting in much higher AA concentrations in many cell types than those of EPA and DHA (51).

The omega-PUFAs fulfill various kinds of functions in human metabolism. One function that holds special significance is their role in inflammatory processes. In reaction to stimuli that trigger inflammation, AA gets released from the cell membrane and via enzymes such as cyclooxygenases (COXs), lipoxygenases (LOXs) and cytochrome P450, it is converted into eicosanoids, such as prostaglandins, leukotrienes and lipoxins, which are important regulators and mediators of inflammation. On the other hand, the omega-3 fatty acid EPA also serves as a substrate for the same enzymes to form bioactive eicosanoids, however with a different structure and therefore less biologically potent. Other anti-inflammatory mechanisms are that both EPA and DHA get integrated into the cell membrane at the expense of AA, leading to less substrate to produce eicosanoids. Moreover, EPA has been shown to decrease the expression of the COX-2 gene. Omega-3 fatty acids also affect the production of inflammatory proteins such as cytokines and adhesion molecules, generally favoring a more anti-inflammatory milieu, probably due to downregulation of genes encoding those proteins, such as nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB). In addition, EPA and DHA can produce pro-resolving lipid mediators such as resolvins, protectins, and maresins that could further explain their anti-inflammatory potential (52). Indeed, several meta-analyses of randomized controlled trials suggest that supplementation with omega-3 PUFAs is associated with a decrease in inflammatory biomarkers in various diseases, such as cancer, COVID-19, heart failure, HIV-infected patients, or patients with polycystic ovary syndrome (PCOS) (53), (54), (55), (56), (57). Both EPA, but more so DHA supplementation can increase the Omega-3-index (O3-I) (58). The O3-I is a marker defined as the percentage of EPA and DHA of total fatty acids found in the membrane of red blood cells (59). Higher blood concentrations of EPA and DHA have been associated with a decreased risk of sudden cardiac death in primary prevention in men (60). Consequently, several large interventional trials assessing the potential of supplementation with long chained omega-3 fatty acids on hard cardiovascular endpoints have been performed. Due

to different dosages, different trial durations and different baseline dietary habits, they revealed mixed results, though suggesting that EPA and DHA supplementation is an effective strategy to prevent CVD (61, 62).

1.5.2.4 Phytochemicals

As elucidated, the MedDiet is, inter alia, rich in plant-based foods such as fruits, vegetables, legumes, nuts and olive oil (42). Besides vitamins, minerals, and other essential nutrients, they contain a variety of phytochemicals. Phytochemicals are bioactive compounds found in numerous plant-based foods, that have been associated with decreasing the risk of chronic diseases (63). They arise in the secondary metabolism of plants and function as coloring agents, growth regulators and protect against predators. Phytochemicals are non-essential nutrients and have mostly health-promoting effects such as being antioxidative and antimicrobial, but depending on the dose, can also have negative functions such as inhibiting digestive enzymes or binding minerals. They have a heterogeneous chemical structure, which allows them to be categorized into different subclasses (40). Especially their anti-inflammatory potential makes them interesting in the prevention and treatment of NCDs (64).

Polyphenols are one subgroup of phytochemicals, and a high intake is especially characteristic for the MedDiet. A re-analysis of the PREDIMED trial showed that participants with a high intake had a lower risk for overall mortality compared to those who had lower intakes of polyphenols (65). Polyphenols can again be categorized into a number of sub-classes such as phenolic-acids, flavonoids, stilbenes, or lignans. Numerous biochemical and molecular mechanisms have been described, that can explain the beneficial effects of polyphenols (66). In relation to inflammation, it has been shown that they can downregulate NF-κB, modulate mitogen-activated protein kinase (MAPk) and arachidonic acid pathways, inhibit enzymes involved in the production of prostaglandins and leukotriens such as COX and LOX and suppress toll-like receptor (TLR) signaling. Their antioxidative potential comes through their ability to inhibit enzymes involved in ROS-production such as xanthin oxidase and NADPH oxidase (NOX) as well as the ability to upregulate endogenous antioxidant systems such as the superoxide dismutase (SOD), catalase, and glutathione (GSH) peroxidase (49).

1.5.2.5 Fiber

Another non-essential nutrient that is considered to be a main player in a healthy dietary pattern is fiber. Dietary fiber is a form of carbohydrate that is not able to be hydrolyzed by intestinal digestive enzymes and is therefore non-digestible. To further distinguish between different sources of fiber, their solubility in water is used to classify them into soluble and insoluble. Soluble fiber is mainly found in fruits and vegetables, while insoluble fiber is foremost part of whole grains (67). However, most foods with high amounts of fiber contain a mixture of both forms. Another classification is based on the gel-forming ability and categorizes fiber into viscous and non-viscous, which may be more appropriate for assessing the relevance for certain health-promoting effects of fiber (68).

The benefits of dietary fiber are broad and include the improvement of gut motility and therefore the prevention and resolution of constipation, a positive effect on insulin sensitivity, promotion of a healthy gut microbiota, favorable effects on weight loss and a decreased risk for cardiovascular diseases and colorectal cancer (67). Due to its physiological functions, prospective cohort studies consistently associate dietary fiber with a decreased risk of all-cause mortality, with a mean decrease of 22% for an increased fiber intake (69).

Another health-promoting influence of fiber is its anti-inflammatory effect. When reaching the colon, soluble fiber gets fermented by gut microbiota into metabolites such as short chain fatty acids (SCFAs) such as butyrate. Butyrate is known to inhibit the transcription factor NF-κB and therefore leads to a downregulation of the transcription of pro-inflammatory genes. Additionally, butyrate may alleviate oxidative stress in the colon by modulating the expression of genes associated with the metabolism of glutathione and uric acid (67). SCFAs are also essential for the proper functioning of the gut barrier. If the integrity of this barrier is compromised, it could result in the translocation of bacteria and their components, such as lipopolysaccharides, into the bloodstream, leading to a state of chronic, low-grade inflammation (70).

1.5.2.6 Diet and Rheumatoid Arthritis

As discussed above, the development of RA is of multifactorial genesis, including environmental factors such as nutrition. Hence, in recent years, there is an emerging interest in the role of diet in preventing as well as treating rheumatoid arthritis. Although there is no

evidence that diet and nutritional interventions can replace pharmacological therapy, there is a decent amount of literature suggesting that diet can influence the risk of developing RA and has a role in treating RA (71).

For example, a study that looked at the association between fiber intake as well as the Dietary Inflammatory Index (DII) of a cohort of the National Health and Nutrition Examination Survey (NHANES) including a total of 15144 participants, could show an inverse relationship between fiber consumption and the incidence of RA, with the consumption of cereal fiber appearing as the main mitigating factor. Moreover, a higher DII was associated with a decreased risk of developing RA (72).

The intake of an anti-inflammatory dietary component of high interest, long chain omega-3 fatty acids, was put into association with the risk of devolping RA in a Swedish cohort including 32232 women. An intake of dietary long-chain n-3 PUFAs of more than 0.21 g/day was associated with a significant decreased risk for RA. Further, consumption of fish ≥1 serving per week compared with <1 was associated with a diminished likelihood of RA (73). These findings are supported by a systematic review that included seventy-one studies including RCTs, observational studies and cohort studies. The authors concluded that the presence of omega-3 fatty acids in one's diet, along with the intake of fish, their primary source, might play a role in lowering the occurrence of rheumatoid arthritis (74). However, these findings should be considered with caution (75).

Moreover, the hypothesis that certain dietary patterns and components can decrease the occurrence of RA, gets supported by a study that prospectively followed 76 597 women in the Nurses' Health Study and 93 392 women in the Nurses' Health Study II. Better long-term dietary quality, assessed by the 2010 Alternative Healthy Eating Index, was associated with a decreased risk for RA. However, these findings were only present in women aged below 55 and not for women older than 55 years (76). In addition, a systematic review, including nineteen cohort studies, and eight case-control studies, that looked at different dietary patterns and food groups and how they relate to the development of RA, showed, although limited, evidence of a protective role of fish, vegetables, and Mediterranean-style diets (77).

Due to a noticeable change towards more motivated patients taking on a more active role in their health, striving to engage in as much self-management as possible to regulate their illness, the interest in managing RA through nutrition has gained much attention, not only in patients but

also in the literature (71). For example, one randomized controlled trial (RCT) could show that weight loss in obese RA patients resulted in significant beneficial differences in Routine Assessment of Patient Index Data 3 (RAPID3), patient global, patient pain, leptin, and adiponectin. Although there was no significant difference in the DAS28 reduction in the intervention group compared to the control group, it trended toward better improvement in in the weight-loss group (78). In order to assess if weight loss or the dietary pattern is more important in reducing the DAS28 in RA patients, one RCT compared the MedDiet to a low-fat diet. Interestingly, although weight loss did not differ significantly between the groups, the MedDiet group had a significantly better improvement in the DAS28 compared to the low-fat group as well as the control group, once more suggesting the anti-inflammatory potential of the MedDiet (79). The benefits of the MedDiet in improving RA symptoms are supported by other interventional trials (80), (81). In order to assess the role of diet and dietary supplements in attenuating RA symptoms, a systematic review including 27 RCT's showed moderate evidence that a MedDiet, ginger powder, cinnamon, saffron, quercetin, ubiquinone and probiotics containing L. casei reduce DAS28 in patients with RA. However, overall the evidence was considered to be limited (82) and is therefore in line with a previously published Cochrane review (83). Another systematic review that included both RCT's and observational studies reported positive effects of omega-3 supplementation, Vitamin-D supplementation, dietary sodium restriction and the MedDiet in some RA disease activity measures (84). A systematic review that looked at whole dietary patterns, such as Mediterranean, vegetarian, vegan, or ketogenic diets, which the authors defined as anti-inflammatory diets, showed significant improvements in pain in RA patients compared to ordinary diets (85).

Combining all the available evidence, the 2022 ACR guideline for exercise, rehabilitation, diet, and additional integrative interventions for RA recommends the adherence to a Mediterranean-style diet without the addition of dietary supplements (86).

1.6 Tools to assess the inflammatory potential of a dietary pattern

1.6.1 The Dietary Inflammatory Index

Due to the increasing understanding of inflammation in the pathophysiology of diseases as well as the impact of diet on inflammation, the Dietary Inflammatory Index (DII) was created with

the goal of providing a tool, that can classify a dietary pattern based on its ability to shift the metabolic response towards a pro- or anti-inflammatory direction. The first version was based on 927 peer reviewed scientific articles linking diet to at least one of six inflammatory markers: IL-1β, IL-4, IL-6, IL-10, TNF-α and CRP. After recognizing some limitations and distortions of the original DII, a new, revised DII was published based on 1943 peer-reviewed articles. Forty-five food parameters, including whole foods, nutrients and other bioactive compounds were applied to the scoring system. In order to compare consumption data across these forty-five food parameters, eleven sets of food consumption data were selected from various regions worldwide, representing a diverse range of dietary habits among humans to serve as the baseline reference. In addition, a percentile scoring system was created to act as the real values against which the intakes of individuals are multiplied, resulting in the calculation of everyone's DII score. Each food parameter was provided with a specific inflammatory score, that is either positive, meaning pro-inflammatory, negative, meaning anti-inflammatory or neutral. This allows researchers to assess the DII of any dietary data collected through calculating and adding up each value. It can also be used for assessing and assisting individuals in establishing dietary choices that aim to lower inflammation and potentially mitigate the risk of specific chronic ailments (4, 87).

1.6.2 PAIFIS

Another index, that is less established, is the Pro-Inflammatory / Anti-Inflammatory Food Intake Score (PAIFIS). Compared to the DII, the PAIFIS aims to characterize a dietary pattern not based on single nutrients and foods, but on certain food groups. Based on a Food Frequency Questionary (FFQ) or on other dietary assessments, the PAIFIS is calculated by summing all pro-inflammatory and anti-inflammatory foods in grams and after that, the sum of all pro-inflammatory foods gets subtracted by the sum of all anti-inflammatory foods.

Pro-inflammatory food groups are sugar-sweetened beverages, red meat, processed meat, candies, and snacks whereas anti-inflammatory food groups that are considered are fruits and vegetables, in which pulses are included as well (88).

1.7 Aims and Hypothesis

The aim of this thesis is to analyze and re-evaluate the diet's nutritive profile and inflammatory properties in immunocompromised individuals and healthy controls of the CoVVac Study after a one-year follow-up.

Moreover, we aim to evaluate the cross-sectional association between the DII, anthropometric measurements and seven serum markers of inflammation in a healthy sub-cohort at the first investigation date.

Further, we aim to compare the DII and the PAIFIS in their informative value about the inflammatory potential and evaluate their association with seven serum markers of inflammation as well as with anthropometric measurements in a healthy sub-cohort at the first investigation date.

It is hypothesized that

- the diet's nutritive profile and its inflammatory properties will have remained constant between the first and the second investigation.
- The DII differs between individuals with normal BMI compared to individuals with BMI > 30 kg/m².
- The DII correlates with serum inflammatory markers.
- Serum inflammatory markers correlate with BMI.
- DII and PAIFIS correlate with each other and do not differ in their informative value.

2 Materials and Methods

2.1 Study design and study population

The CoVVac Study is an open label, prospective, monocentric cohort study, conducted at the Medical University of Graz, that aims to examine, among other things, the extent to which the quality and diversity of the diet and the calculated Dietary Inflammatory Index from the nutritional protocols differs between a group of autoimmune patients and a healthy control group.

The nutritional protocols were collected as part of the CoVVac study, which was approved by the Graz Ethics Committee (EK 1128/2021). A sub-collective of the CoVVac study was analyzed for this diploma thesis. The patients were recruited at the Clinical Department of Rheumatology and Immunology and at the University Clinic for Blood Group Serology and Transfusion Medicine at Graz University Hospital as part of their treatment: A total of 213 subjects completed dietary protocols, including 65 patients with an autoimmune-compromised immune system and 148 healthy controls. The immunocompromised study cohort comprised 21 males, 41 females, and one individual identifying as diverse gender. This diverse participant was excluded from gender-based comparisons due to its singular representation (n=1), rendering any comparison with other genders statistically inconsequential. The subjects in this group are undergoing treatment for their autoimmune disease at the Department of Rheumatology and Immunology at Graz University Hospital and were recruited for the CoVVac study as part of this programme. Participation in the study was voluntary and no compensation was paid to the subjects.

After a one-year follow-up, all the participants were asked again to complete a food protocol.

A total of 122 participants completed the protocols.

2.2 The Vienna Food Record

This prospective food record was crafted following the approach of the Freiburg Food Record (Freiburger Ernährungsprotokoll), aiming to devise a straightforward, dependable, and accurate food diary tailored to Austrian eating habits. The objective was to eliminate the need for expert interviews or guidance, simplifying both the completion and assessment of the record across

private, scientific, clinical, and commercial domains (89, 90). Utilizing regional culinary specialties and food names ensures a swift and easy nutritional assessment. Following the data collected, a nutrient profile is generated and subsequently compared with the D-A-C-H reference values for a well-rounded diet. This analysis enables the formulation of recommendations concerning ideal energy and nutrient consumption levels (89).

In essence, the food record can be maintained over a flexible duration, but the minimum recommended documentation period to draw substantive conclusions about dietary behavior is 4 days, encompassing 3 weekdays and 1 weekend day. The more detailed the information provided by the respondents, the more meaningful the analysis will be. To optimize user-friendliness, such as facilitating searches for specific foods, dishes, and beverages, items are categorized by food group. Examples of these groups include fruits, vegetables & salads, dairy products, fish, meat, meat products, side dishes, and sweet & savory foods (89, 90).

2.3 The nutritional software *nut.s*

The nut.s software emerged from a collaboration between nutrition scientists and serves to accurately calculate nutritional values (both micronutrients and macronutrients) and handle allergen information. It finds application across various sectors including science, food industry, nutritional guidance, catering, and culinary endeavors. It offers five distinct versions, with *nut.s science* primarily employed for statistical analysis of nutritional protocols, primarily in research contexts (91).

The basis for calculating nutritional values using this software are nutritional value tables, which contain the average nutritional values of foods based on laboratory chemical analyses. Specifically, the Austrian nutritional value table (Österreichische Nährwerttabelle ÖNWT) is used as a supplement to the Federal Food Code (Bundeslebensmittelschlüssel BLS), as it contains additional Austria-specific foods and products as well as additional data per food. It contains 120 nutrients, 14 allergens of the Food Information Regulation, a categorization of foods according to the principle of Traditional Chinese Medicine (TCM) and is also supplemented by packaging and portion sizes, synonyms, product names and company names (92).

2.4 Calculation of the Dietary Inflammatory Index (DII) and PAIFIS

In order to assess the pro- or anti-inflammatory potential of the participants' dietary patterns, the DII was calculated. Since the DII is based on single nutrients, we aimed to compare it to an index that is based on whole food groups, rather than on single nutrients (4). Therefore, we also calculated the Pro-Inflammatory / Anti-Inflammatory Food Intake Score (PAIFIS) (88).We aimed to compare the DII and the PAIFIS in their informative value about the inflammatory potential and evaluate their association with seven serum markers of inflammation as well as with adipose tissue in a healthy cohort.

2.5 Measurement of inflammatory biomarkers

To objectify the inflammatory status of each individual, the inflammation markers IL- 6, IL-8, IL-18, IL1-RA, TNF-α, IFN-α, and CRP were determined in serum by clinically established protocols. CRP and IL-6 were measured via an analyzer from Roche Diagnostics GmbH (*cobas 8000 e801*). For CRP, an immunological turbidity test was used, and for determining IL-6, an "ECLIA" (Electrochemiluminescence Immunoassay) was performed. For the measurement of IL-8, IL-18, IL1-RA, TNF-α, and IFN-α a Multiplex Immunoassay was performed. The ProcartaPlex™ from Invitrogen™ has been adjusted (Customized ProcartaPlex™) and was measured via a MAGPIX™ device. The measurement of inflammatory biomarkers was conducted in collaboration with the Clinical Institute for Medical and Chemical Laboratory Diagnostics Graz and the Division of Rheumatology and Immunology from the Medical University of Graz.

2.6 Ultrasound measurement of subcutaneous adipose tissue

To measure subcutaneous adipose tissue, an ultrasound measurement was conducted. The standardized ultrasound method developed by Müller et al. 2016, is considered the most precise approach for determining both the precise quantity and distribution of body fat. In this technique, ultrasound images are captured at eight predefined locations, which are upper abdomen (UA), lower abdomen (LA), erector spinae (ES), distal triceps (DT), brachioradialis (BR), lateral thigh (LT), front thigh (FT) and medial calf (MC) (93). Before conducting ultrasounds, each site is precisely defined and marked, considering factors like body size, percentage, body position, and a standardized reference muscle. Additionally, parameters including body weight, seat height, leg length, and various circumferences are measured to accurately calculate body fat mass and

distribution, along with recording participants' ethnicity and sports activity levels for statistical analysis.

The ultrasound images must be taken in specific predefined positioning for comparable results. Due to the fact, that the procedure was primarily developed for athletes, occasionally, our patients encountered difficulty maintaining specific positions. Despite this, the images were taken as closely to the standard positions as possible and documented accordingly. In order to edit the images properly with a specialized software, all images must include skin, subcutaneous adipose tissue, muscle fascia, and muscle. An example is shown in the figure below. Ultrasound transducers are placed atop a thick gel layer to accommodate the adipose tissue's compressibility.

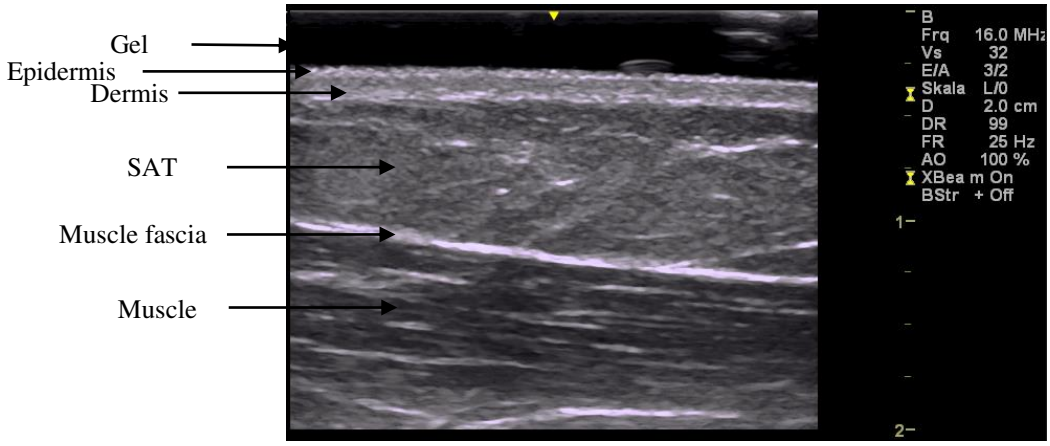

Figure 2: Example of an ultrasound image showing the different layers; adjusted from Müller et al. 2016

One key benefit of the ultrasound method is its ability to exclude embedded fibrous structures from measurements, as depicted in the figure below. Athletes and regular sports participants often have more of these structures in their subcutaneous adipose tissue, but it would be inaccurate to include them in body fat mass calculations (93).

The ultrasound investigations utilized a conventional ultrasound system (*GE Logiq-e, General Electric*) with linear probes (*L8-18i RS and 12L RS*) operating at 8-16 MHz. To analyze the ultrasound images, the NISOS body composition analysis software version 4.1 (Rotosport, Stattegg Austria) was utilized. This software computes two values: D_{INCL}, which is the total of

the eight measurement sites, and D_{EXCL}, which is the total of the eight measurement sites excluding embedded fibrous structures. The evaluation results, depicted in a document, encompass BMI, total subcutaneous fat (DINCL) with and without fibres, and the fibres alone. This breakdown is provided for each measurement point, offering individuals insight into their personal body fat distribution. Furthermore, a specific algorithm yields data on subcutaneous adipose tissue (SAT) in kilograms and as a percentage of total body weight.

2.7 Statistical analysis

Statistical analysis was conducted utilizing SPSS Statistics v27 software (IBM, Armonk, NY, USA). The dataset underwent assessment for normal distribution via the Shapiro-Wilk test. Given that the majority of the data did not exhibit normal distribution, non-parametric tests were employed for subsequent analysis. The average nutrient intake findings were juxtaposed against the reference range for recommended nutrient intake. The longitudinal change of dietary intake values from the first to the second investigation was tested by Wilcoxon signed-rank test, and the delta between first and second dietary protocols were calculated and used for group comparison. Group comparisons were conducted using the Mann-Whitney U-Test. Correlations between body fat values and inflammation were assessed by Spearmen-rank correlation coefficient. The Chi-Square Test was used for comparison of categorial data non-metric data such das DII and PAIFIS categories.

3 Results

3.1 Study population characteristics

In total, 122 participants provided a dietary protocol at the one-year follow-up (second investigation date). Of these, 29 had an immunodeficiency and took B-cell depleting medication (rituximab or ocrelizumab) and 93 were healthy controls. 65 were male und 56 were female. We observed no significant difference in age (Md = 53 years, IQR =19 vs. Md = 51 years, IQR = 18; p = 0,058) and neither in BMI (Md = 23,63 [kg/m²], IQR = 6,87 vs. Md = 24,6 [kg/m²], IQR = 4,3; p = 0,936) in the immunosuppressed group compared to the healthy controls. For 3 people in the control group, BMI was not available. The study characteristics are shown in Table 2.

Table 2: Characteristics of the study population

Characteristics	Immunosuppressed	Healthy Controls	p-value
n=122	29	93	-
female = 65	18	47	-
male = 56	10	46	-
divers = 1	1	0	-
age [years]	53 (19)	51 (18)	0,058
BMI [kg/m²]	23,63 (6,87)	24,6 (4,3) *	0,936

*BMI was not available for 3 participants

Of the whole CoVVac cohort, a total of 209 participants provided a dietary protocol at the first investigation date. Of these, 11 obese participants and 22 sex- and age-matched healthy participants were assessed in a sub analysis looking at the association between the DII, body fat and inflammatory serum markers. Another sub analysis looked at 147 healthy participants and aimed to examine the association between the DII and the PAIFIS. A flowchart of the whole cohort is shown in Figure 3.

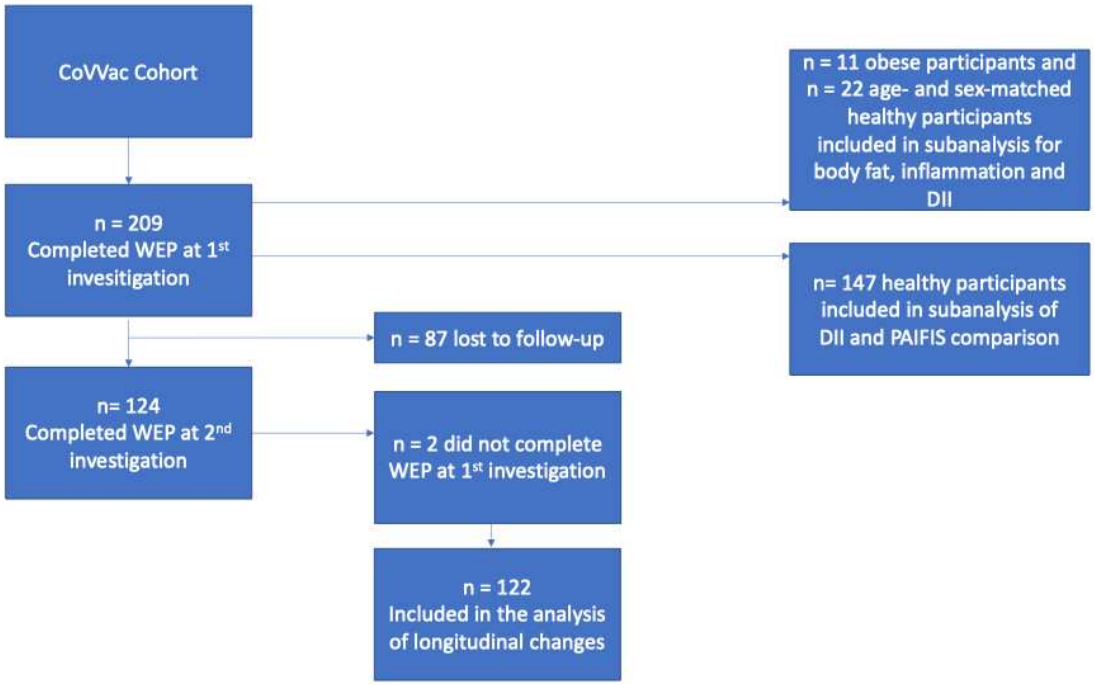

Figure 3: Flowchart showing the emergence of the study population

3.2 Comparison of the nutrient intake after a one-year follow-up

3.2.1 The healthy control group

Compared to the initial assessment, after a one-year follow-up, we did not observe any differences in energy and macronutrient intake, as well as in the intake of fiber, omega-3 fatty acids, omega-6 fatty acids, EPA, DHA, and phytonutrients (Flavan-3-ol, Flavones, Flavonols, Flavanones, Anthocyanidins, Isoflavones and Carotenoids) in the healthy control group (Table 3).

Table 3: Longitudinal changes in nutrient intake in the healthy control group

Nutrient	2021 Healthy Controls	2022 Healthy Controls	p-value
DII	-1.202 (2.671)	-0.962 (2.636)	0.746
kcal (IQR)	2122 (932)	1905 (1013)	0.132
KH [g]/Tag (IQR)	216.5 (92.7)	189.28 (105.58)	0.108
Protein [g]/Tag (IQR)	88.5 (42.8)	76.1 (39.5)	0.318
Fett [g]/Tag (IQR)	90.7 (40)	81.3 (48.2)	0.302
Ballaststoffe [g]/Tag (IQR)	21.6 (9.3)	19.1 (12.1)	0.152
Omega 3 [mg]/Tag (IQR)	1925.9 (1069.7)	1603.1 (1064.6)	0.298
Omega 6 [mg]/Tag (IQR)	12502.3 (8879.2)	12441.7 (9460.2)	0.826
EPA [mg]/Tag (IQR)	78.9 (140)	64.1 (117.4)	0.195
DHA [mg]/Tag (IQR)	122.18 (187.6)	121.95 (185.6)	0.549
Anthocyanidine [µg]/Tag (IQR)	301563 (904477)	301908 (870392)	0.772
beta-Carotin [µg]/Tag (IQR)	5184 (4744)	4960 (5073)	0.519
Flavone [µg]/Tag (IQR)	1355 (2987)	1411 (3265)	0.68
Flavan-3-ol [µg]/Tag (IQR)	41281 (74868)	41713 (66213)	0.695
Flavonone [µg]/Tag (IQR)	2672 (37358)	1052 (37306)	0.916
Flavonol [µg]/Tag (IQR)	201984 (401047)	205701 (374175)	0.562
Isoflavone [µg]/Tag (IQR)	222 (186)	263 (280)	0.152

3.2.2 The immunocompromised group

In the immunocompromised group, we could show a significant change in the intake of Flavonols (Md = 216417µg, IQR = 302299 vs. Md = 198327 µg IQR = 297127; p = 0.019) after a one-year follow-up. No differences were seen in the other nutrients (Table 4).

Table 4: Longitudinal changes in the nutrient intake in the immunosuppressed group

Nutrient	2021 Immunosuppressed	2022 Immunosuppressed	p-value
DII (IQR)	-0.967 (2.103)	-0.820 (2.292)	0.82
kcal (IQR)	1981.7 (542)	1901.82 (896.4)	0.705
KH [g]/Tag (IQR)	201.9 (46.8)	196.5 (85.3)	0.991
Protein [g]/Tag (IQR)	73.9 (37.8)	76.5 (42.4)	0.482
Fett [g]/Tag (IQR)	88.5 (29.4)	81.3 (58.7)	0.854
Ballaststoffe [g]/Tag (IQR)	19.8 (13.4)	17.9 (12.6)	0.804
Omega 3 [mg]/Tag (IQR)	1450.7 (661.5)	1693.2 (826.1)	0.098
Omega 6 [mg]/Tag (IQR)	13922.6 (5593.6)	14309.1 (12109.5)	0.37
EPA [mg]/Tag (IQR)	41.1 (105.2)	58.6 (101.9)	0.336
DHA [mg]/Tag (IQR)	79.4 (136)	89.2 (175)	0.77
Anthocyanidine [µg]/Tag (IQR)	23013 (306014)	43902 (404651)	0.524
beta-Carotin [µg]/Tag (IQR)	4131(5841)	4968 (5849)	0.991
Flavone [µg]/Tag (IQR)	1123 (3918)	2040(4739)	0.787
Flavan-3-ol [µg]/Tag (IQR)	42354 (70472)	32611 (51840)	0.08
Flavonone [µg]/Tag (IQR)	7563 (49834)	24769 (60012)	0.469
Flavonol [µg]/Tag (IQR)	216417 (302299)	198327 (297127)	0.019*
Isoflavone [µg]/Tag (IQR)	213 (131)	188 (138)	0.905

* $p<0.05$

3.3 DII and serum inflammatory markers in healthy obese and non-obese individuals

At the first investigation date, we identified a total of 11 obese participants (BMI: Md = 32.5 kg/m², IQR = 6.0) in the healthy control group. 22 sex- and age-matched non-obese controls (BMI: Md = 24.5 kg/m², IQR = 5.0) were included for this analysis.

We compared the DII of the obese group (Md = -0.739, IQR = 1.137) to the non-obese group (Md = -2.136, IQR = 2.711) and could show that it was significantly higher in the obese group ($p = 0.031$) (Figure 4).

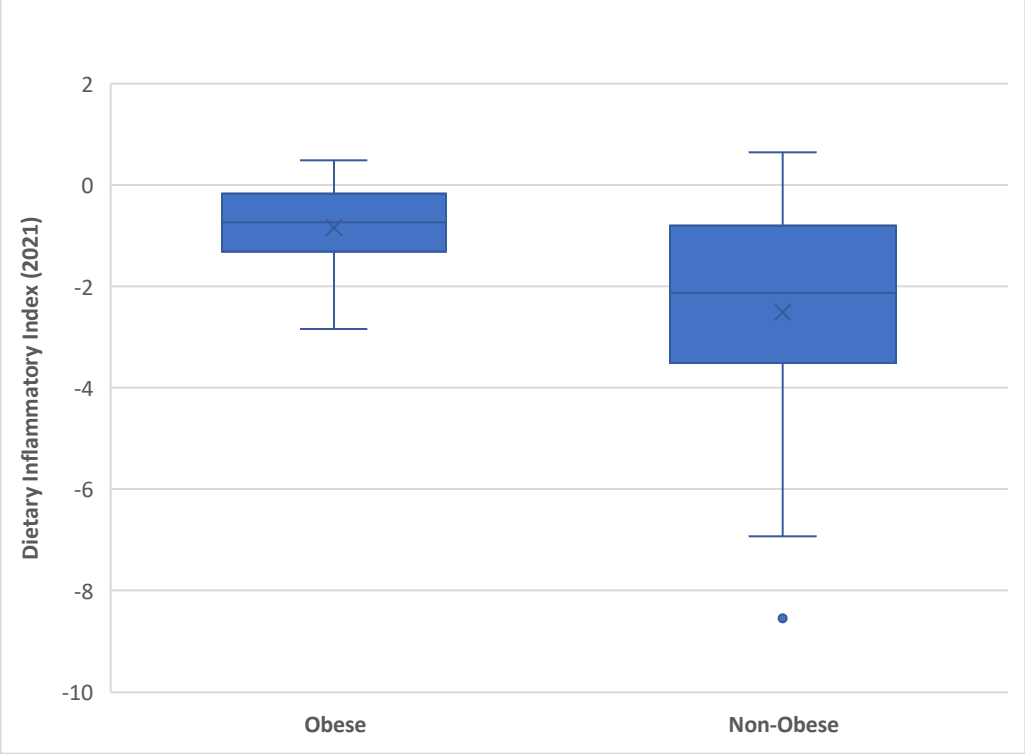

Figure 4: Difference of the DII in the obese group compared to non-obese controls

Moreover, the inflammation markers IL-6 (Md = 3.4 pg/ml, IQR = 2.4 vs Md = 2.4 pg/ml, IQR = 1.4; p = 0.04) (Figure 5) and IL-18 (Md = 10.33 pg/ml, IQR = 7.71 vs Md = 4.89 pg/ml, IQR = 5.29; p = 0.014) (Figure 6) were significantly higher in the obese group. No differences were seen for the other inflammation markers.

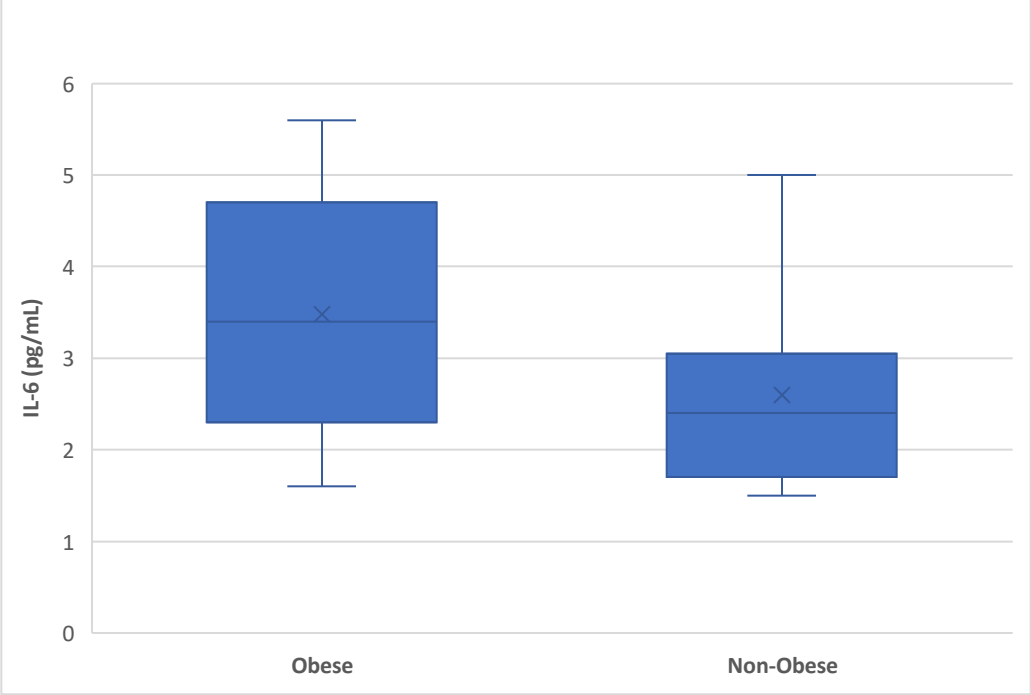

Figure 5: Difference in IL-6 in the obese group compared to non-obese controls

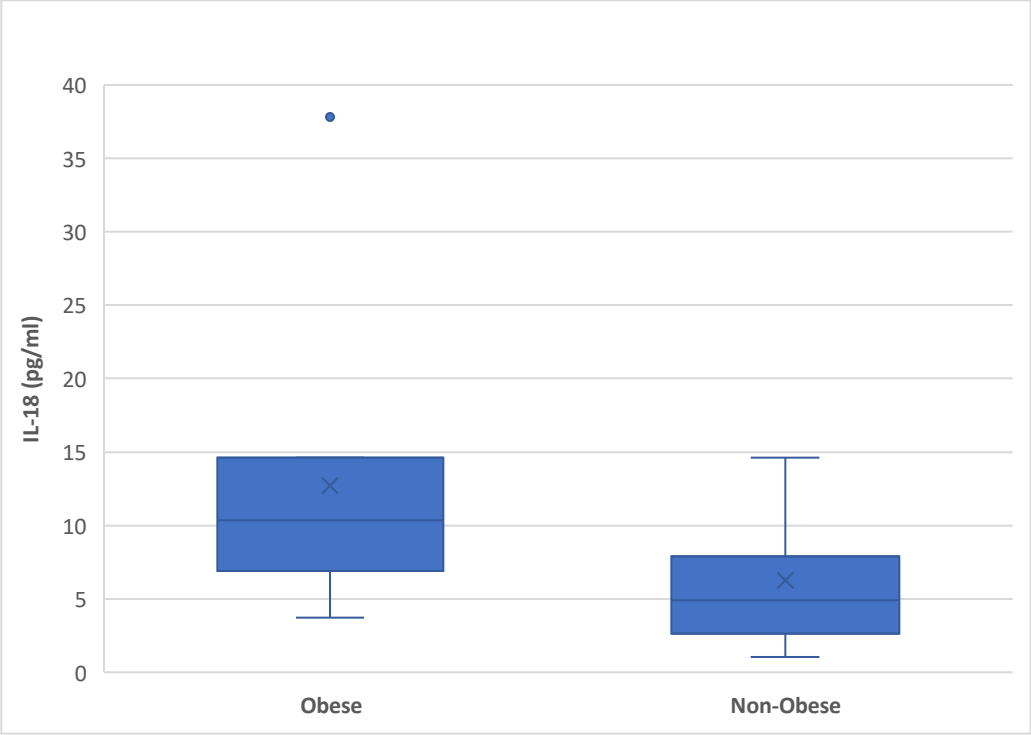

Figure 6: Difference in IL-18 in the obese group compared to non-obese controls

The DII did not correlate with any of the inflammatory markers, however, DII was significantly correlated with BMI ($r_s(32) = 0.421$, $p = 0.016$), SAT ($r_s(32) = 0.386$, $p = 0.029$) and abdominal fat ($r_s(32) = 0.404$, $p = 0.022$) (Figure 7).

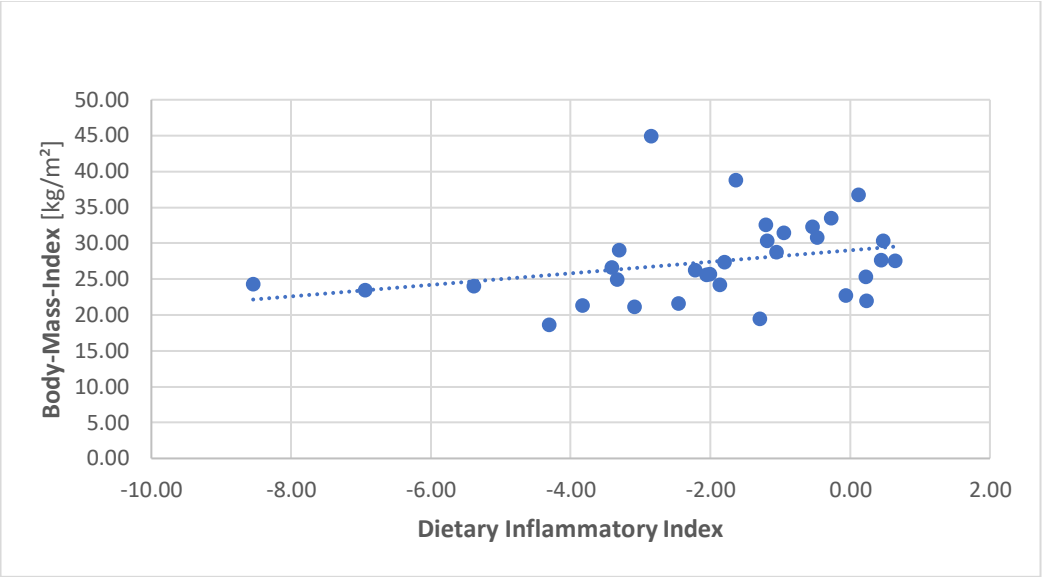

Figure 7: Correlation of the BMI with the DII

3.4 Comparison of the DII and the PAIFIS

At the first investigation date we identified 147 healthy individual and based on their four-day dietary protocol their DII and PAIFIS were calculated. In order to predict their informative value, we categorized both DII and PAIFIS into three categories, namely “pro-inflammatory“, “anti-inflammatory“ and “highly-anti-inflammatory“. For the PAIFIS we defined “pro-inflammatory“ as a value < 0, “anti-inflammatory“ between -500 and -0 and “highly-anti-inflammatory“ as a value < 500. The range in our cohort was between 503.79 and -1000. For the DII we defined “pro-inflammatory“ as a value < 0, “anti-inflammatory“ between -5 and -0, and “highly-anti-inflammatory“ as a value < -5. The range in our cohort was between 0.74 and -10.12.

The DII and the PAIFIS differed significantly in their informative value about the inflammatory potential ($\chi^2 < 0.001$), nevertheless, they correlated with each other ($r_s(147) = 0.358$, $p < 0.001$) (Figure 8).

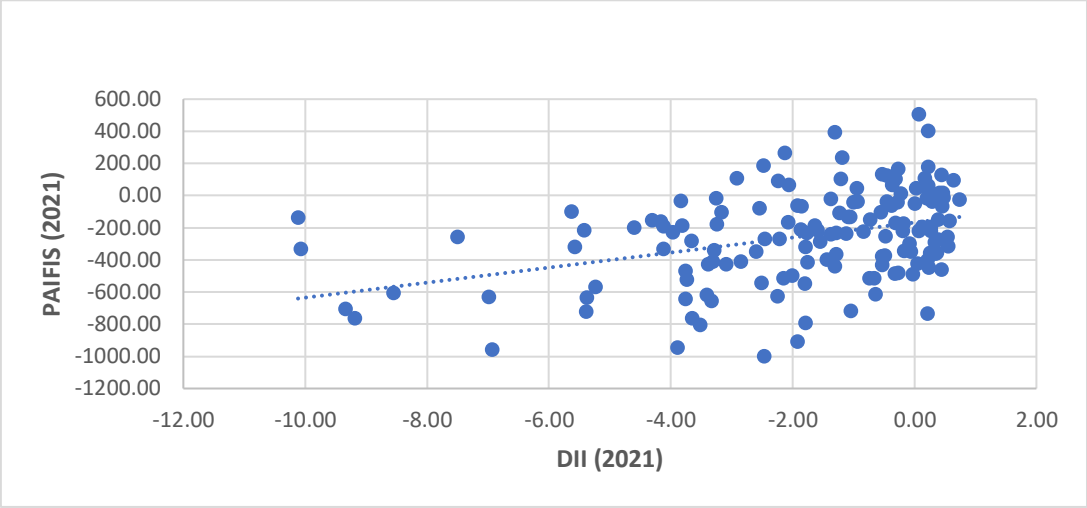

Figure 8: Correlation of the PAIFIS with the DII

There was a modest correlation with the PAIFIS and IL-6 ($r_s(147) = 0.180$, $p = 0.032$) (Figure 9).

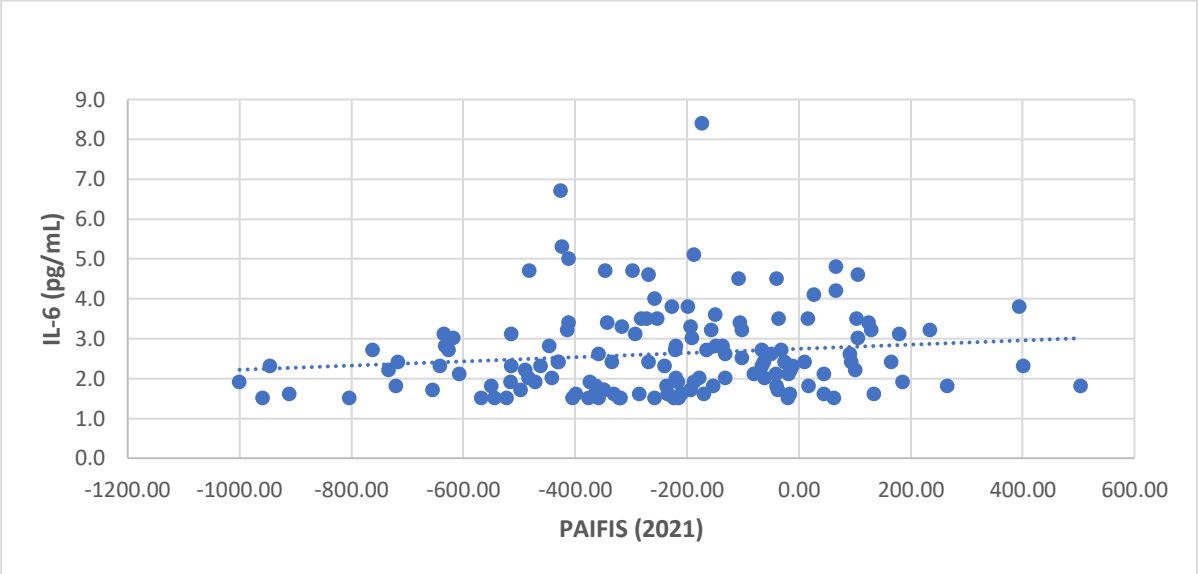

Figure 9: Correlation of the PAIFIS with IL-6

Moreover, the PAIFIS correlated with trunk body-fat ($r_s(147) = 0.246$, $p = 0.005$) and was inversely correlated with body fat on the extremities ($r_s(147) = 0.246$, $p = 0.032$) whereas these correlations were not seen with the DII.

4 Discussion

As part of this diploma thesis, the dietary protocols of the CoVVac study, a prospective cohort study at the Medical University of Graz, were analyzed longitudinally. The dietary protocols from the first investigation date were compared with those from the second measurement point, which was one year later, and were assessed for changes. The aim of this diploma thesis was to record differences in nutrient intake and the inflammatory potential of the diet, objectified using the DII, between first and second investigation dates. Both the group of immunocompromised participants and the healthy participants were considered. For this purpose, the aforementioned parameters were quantified using a validated 4-day dietary protocol.

In addition to the longitudinal analysis, sub-analyses were also conducted at the first measurement date.

Firstly, an attempt was made to establish an association between the DII, seven different inflammatory parameters and the amount of adipose tissue in obese and healthy normal-weight study participants.

In addition, a less-established, food-based index for assessing the inflammatory potential of a dietary pattern, the PAIFIS, was compared with the well-established, nutrient-based DII and their differences in significance were assessed.

On the first investigation date, the participants' anthropometric data were collected, and blood samples were taken. These were used to determine seven different inflammatory parameters in the serum. The 4-day food consumption of the participants was documented using the Vienna dietary protocol. The nutrient content of the participants' individual diets was then calculated using the nut.s nutrition software, a software that uses an extensive nutrient database, which was supplemented by an additional data set for the Dietary Inflammatory Index. Based on these data, the DII as well as the PAIFIS were calculated for each participant.

On the second investigation date, a 4-day dietary record was again obtained, and the nutrient intake and DII were analyzed. The collected data was then analyzed using the SPSS statistics program.

The results of the statistical analysis are summarized below:

In the healthy control group, we could not observe any changes in the intake of calories and macronutrient intake, as well as of the intake of fiber, omega-3 fatty acids, omega-6 fatty acids, EPA, DHA, and phytonutrients (Flavan-3-ol, Flavones, Flavonols, Flavanones, Anthocyanidins,

Isoflavones and Carotenoids) after a one-year follow up. However, in the immunocompromised group, a significant change in the intake of Flavonols was observed after a one-year follow-up. No differences were seen in the intake of energy and the other nutrients. In one sub-analysis of the participants assessed at the first investigation date, where we compared 11 healthy obese individuals to 22 healthy sex- and age-matched controls we found that the DII was significantly higher in the obese group. Additionally, the inflammation markers IL-6 and IL-18 were significantly higher in the obese group. No differences were seen for the other inflammation markers. Moreover, the DII did not correlate with any of the inflammatory markers, however, DII was significantly correlated with BMI and abdominal fat In another sub-analysis, we looked at the difference between the DII and the PAIFIS in 147 healthy participants at the first investigation date. The DII and the PAIFIS differed significantly in their informative value about the inflammatory potential however, they did correlate with each other There was a modest correlation with the PAIFIS and IL-6 and the PAIFIS also correlated with trunk body-fat and was inversely correlated with body fat on the extremities whereas these correlations were not observed with the DII.

4.1 Nutrient intake in the immunocompromised group

Autoimmune diseases impact roughly 10% of the population. Interestingly, over the past thirty years, there has been a reported rise in certain autoimmune conditions. This trend prompts an inquiry into the main contributors of this trend (94). While genetic factors cannot be understated, it is important to note that it is often only unfavorable environmental factors that make certain genes problematic. The current consensus is that autoimmune diseases arise from a complex interplay of numerous genetic and environmental risk factors over extended periods. This interplay progresses from genetic susceptibility through early immune system activation and autoimmunity to the initial clinical manifestations, ultimately resulting in a phenotype that meets classification or diagnostic standards. The considered unfavorable environmental factors include stress, sleep deprivation, xenobiotic contact, and lifestyle changes, however, also nutritional factors are thought to be one of the main drivers that ultimately lead to the increased prevalence of autoimmune diseases (95). As mentioned before, an anti-inflammatory diet has promising evidence in the treatment of RA, an autoimmune disease which affects some of our

participants in the immunocompromised group (85). In a case-control study, also for other autoimmune diseases, such as MS and other demyelinating autoimmune diseases, the adherence to a pro-inflammatory diet, assessed by the DII, is associated with a higher risk for disease development (96). Therefore, a healthy dietary pattern, not only to prevent disease outbreak, but also in the treatment of autoimmune disease, seems to be sensible and is indeed recommended (86).

As already mentioned and discussed in the diploma thesis of Wimmer 2023, the CoVVac cohort revealed, that the healthy control group has a significantly higher intake of zinc, selenium, β-carotene, vitamin A and vitamin D compared to the group with suppressed immune function. This emphasizes the importance of a nutritional consultation for the immunocompromised group, since the study results mentioned above particularly suggest the possible favorable effects of an anti-inflammatory diet in this group (97).

Another important and anti-inflammatory nutrient also discussed before is fiber. As mentioned above, there is an inverse relationship between the intake of fiber and the development of RA (72). There is also some evidence that increasing fiber intake results in a decreased disease activity if RA is already present. One randomized controlled crossover trial looked at the impact of an anti-inflammatory diet, which due to increased intake of vegetables, fruits, legumes, and whole grains resulted in triple the amount of fiber in the intervention diet versus the control diet (24 g/d vs. 8.3 g/d). In addition, they increased other anti-inflammatory foods such as fish and herbs. After the intervention period, the DAS28-ESR decreased significantly, suggesting a positive effect on disease activity due to an anti-inflammatory diet, partly due to the increase in fiber (98). A small feasibility study also demonstrated an improvement in laboratory markers as well as in patient-related outcomes in RA patients following a diet supplemented by high-fiber bars or cereals. However, it is important to note that this study was unblinded (99). Taking these findings together, one can conclude that it is particularly important for patients to reach at least the suggested daily intake of fiber of 30g/day or 14.3g /1000 kcal (100). However, in our cohort the median daily dietary fiber intake was 19.8g /day at the first investigation date and even decreased to 17.9g/ day. This highlights the urgent need to improve and establish better nutritional interventions for patients with rheumatoid arthritis.

As already elucidated in the introduction, omega-3 fatty acids, especially EPA and DHA, have interesting and promising anti-inflammatory properties. Of all the omega-3 fatty acids only ALA

is considered to be essential, because it can be converted into the longer chained EPA and DHA (52). The German Nutrition society recommends the intake of 0.5% ALA of the total energy intake (101). However, it is important to note that the conversions rates of ALA into EPA and DHA are fairly low. While the quantities of ALA needed to effectively convert to EPA can be readily obtained by the general population through foods high in ALA, the amount needed for sufficient conversion into DHA is most likely not achievable (102). The European Food Safety Authority (EFSA) does not only provide recommendations of ALA intake but also for EPA and DHA. The intake of 250 mg of combined EPA and DHA is recommended (103). With a median daily intake of 58.6 mg EPA and a median intake of 89.2 mg of DHA, the immunosuppressed group in our cohort clearly and does not reach this recommendation of 250 mg EPA + DHA, once again highlighting the missed potential of adequate intake of anti-inflammatory nutrients in this group. However, one limitation of our analysis is that we did not assess the intake of any dietary supplements. This may falsify the intake of EPA and DHA or any other nutrients.

It is important to note that individuals with RA have approximately a 1.5 times higher risk of developing cardiovascular diseases (104). The International Society for the Study of Fatty Acids and Lipids recommends healthy people a minimum of 500 mg/d of combined EPA and DHA to sustain cardiovascular health. Therefore this recommendation is double the amount of the EFSA recommendation (105). A recent meta-analysis looked at the plasma levels of DHA in over 160 000 individuals. After a follow-up of 14 years, the highest quintile of DHA levels, compared to the lowest quintile, had a 17% risk reduction in all-cause mortality as well as a 21% reduction in risk for mortality due to cardiovascular disease. This evidence provides strong support for the hypothesis that DHA could contribute significantly to cardiovascular health and longevity (106). To make sense of all these findings, a dietary pattern rich in vegetables, fruits, pulses, edible seeds, plant protein and fatty fish, and hence high in fiber and omega-3 is an optimal approach to prevent cardiovascular disease (107).

4.2 Strengths and Limitations of the DII

As discussed before, the DII has been explored in many observational studies and was linked to the risk of chronic diseases. Considering the totality of evidence, a more pro-inflammatory Index is associated with a higher risk of chronic disease outcomes (108).

Before the Dietary Inflammatory Index was established, apart from the glycemic index, all dietary indices were either based on the adherence to dietary recommendations, on the adherence to a particular way of eating or a cuisine or the indices were derived from a specific study employing a type of regression analysis. Each approach, however, is hindered by unique idiosyncrasies, with a shared limitation being a restricted range of exposure variability. In contrast, the DII was developed to incorporate all available evidence from diverse human populations, utilizing various study designs and dietary assessment methods. Besides human studies, the DII also integrates evidence from eligible laboratory animal and cell culture experiments, although these are given less weight. After the first publication of the DII in 2009 several adjustments were made. Additional three years of publications about the DII were added into the calculation and although the number of studies included had nearly doubled, the overall estimation of the pro- or anti-inflammatory effect of all food groups did not change. That means, no parameters that were assessed to be pro-inflammatory before, were found to be null or anti-inflammatory in the revised second version. Further, no values that were found to be anti-inflammatory in the first version, were then shown to be null or pro-inflammatory. This strengthened the methodology used by the authors (87).

It is important to note, that the DII uses the amount of single nutrients in order to assess the pro- or anti-inflammatory potential of a dietary pattern (4). Although this makes the index applicable to calculating a variety of dietary assessments, the limitations of this approach should be considered as well.

In recent years, the conventional way of looking at food as a delivery of single nutrients has been questioned. Now there is emerging evidence, that the food matrix, defined by the U.S. Department of Agriculture (USDA) as the “nutrient and non-nutrient components of foods and their molecular relations”, is important as well and that the health effects of certain foods are not only influenced by their individual single nutrients but also partly on the interaction between those and therefore the resulting bio accessibility. A representive example are dairy foods, which often get thrown into one bucket in clinical guidelines, but there is evidence to suggest that they have different effects on certain health outcomes depending on a person's risk of disease. This difference is for example due to fermentation, favoring for example the consumption of yoghurt for health reasons (109, 110).

This concept is applicable for other food groups and nutrients as well. One example are anthocyanins, a subgroup of polyphenols also considered in the DII: as the research of the health benefits of anthocyanins expanded, understanding how anthocyanins were absorbed in the body and their mechanisms of action became more and more important. It is clear nowadays that anthocyanin availability varies depending on a number of parameters, including their source, chemical structure, dietary composition, processing techniques, and individual physiological characteristics (111). A recent randomized controlled trial investigated the bioavailability of another subclass of polyphenols, namely flavan-3-ols, in fruit smoothies. The authors could show that a polyphenol-rich berry smoothie combined with fruits high in polyphenol oxidase (PPO) such as bananas, results in a significant lower peak plasma concentration of flavan-3-ol metabolites compared to a berry-only smoothie without fruits high in PPO. This means that combining polyphenol rich fruits with fruits high in PPO could decrease the health benefit of the ingested polyphenols (112). Although this was a small study that looked at only one subclass of polyphenols and preliminary public health messages should be considered with caution, it highlights the complexity of assessing the metabolic effect of nutrition and emphasizes that looking at single nutrients separately, might be too short-sighted.

Despite being based on a large amount of evidence, the authors of the DII underscore the importance of understanding that nutrition is by far not the only contributor to inflammation and call for other indices for different modulator such as stress and physical activity. Although the DII correlates with other indicators of diet quality, such as the Healthy Eating Index and Mediterranean Dietary Index, it is not exclusively explained by just choosing healthy food groups (87). One analysis of the previously discussed PREDIMED trial, that had one arm with a Mediterranean diet supplemented with extra-virgin olive oil, one arm with a Mediterranean diet supplemented with mixed nuts and one arm with a low-fat diet, so in total three intervention arms where all participants received dietary training leading to a “healthy“ diet, examined the association between the DII and the incidence of cardiovascular disease. The authors could show that the higher the DII, the higher the risk of cardiovascular diseases. This association was quite homogenous across all three intervention arms, highlighting that a healthy Mediterranean diet with a lower DII is very likely to be superior to a Mediterranean diet with a higher DII (113).

4.3 Comparison of DII and PAIFIS

The comparison of the DII with another index, the PAIFIS, was made in order to compare their informative value, but also due to the fact that the PAIFIS does not focus on single nutrients, which might has some downsides, but instead takes whole food groups into the calculation to assess a pro- or anti-inflammatory dietary pattern. This approach is also in line with recent dietary guidelines, such as the newest recommendations from the German Nutrition Society, that focus on food-based recommendations rather than a nutrient-based guidance (114).

Although our findings revealed that the DII and the PAIFIS differed significantly in their informative value about the inflammatory potential, they did correlate with each other. In addition to that, in our cohort, only the PAIFIS, but not the DII correlated with IL-6, which even seems to favor the PAIFIS in its expressiveness to predict the metabolic shift towards a pro- or anti-inflammatory direction.

Even though the evidence underlying the PAIFIS is by far not as robust as the one underlying the DII and the calculation of the PAIFIS does not consider important anti-inflammatory food groups such as phytochemicals or omega-3-fatty acids, we could show that the PAIFIS might have some value in nutritional sciences. Especially in view of the easy calculation and applicability, which becomes even more obvious if we compare it to the rather complex calculation of the DII, it should be considered in future research to assess the inflammatory potential of a dietary pattern.

4.4 Relevance of body-fat reduction as an anti-inflammatory approach

Another important finding we could show in our analysis, was the higher concentration of the pro-inflammatory cytokines IL-6 and IL-18 in an obese sub-group, compared to an age- and sex-matched non-obese group. These findings can be explained by examining the pathophysiological processes involved in the hypertrophy of adipose tissue. As previously detailed, excess accumulation of subcutaneous adipose tissue results in physiological changes in adipocytes, shifting them towards a more pro-inflammatory milieu. This leads to increased production of pro-inflammatory adipokines, contributing to chronic low-grade inflammation (34).

Therefore, in order to reduce systemic chronic inflammation, the logical consequence is not only to consume an anti-inflammatory dietary pattern, but also to avoid caloric overfeeding (87). If overweight or obesity is already present, caloric restriction should be recommended due to its

weight-lowering and therefore health-promoting potential (115). Indeed, it has been shown in clinical trials, that weight loss in obese subjects leads to a reduction in C-reactive protein (CRP) (116).

When weight loss and fat reduction are the goal, a multidisciplinary team including an obesity-specialized physician, a nutritionist or dietician, a physical activity specialist, a psychological health professional, a nurse and the patient's General Practitioner (GP) should be involved in the treatment of the individual. Before working on a therapeutic approach for the patient, it should be emphasized that stigmatization of obese patients should be avoided at all costs, nevertheless it is remarkably common not only in day-to-day life, but also among specialists in health care settings. This not only leads to poor treatment responses, but also to an increased risk of depression, diminished self-esteem, elevated risk of eating disorders and abstention of medical consultation. Therefore, considering that obesity is a multifactorial disease, both influenced by individual and external factors, to refrain from the obsolete and wrong opinion that people with obesity are generally lazy and lack self-discipline, is the basis of all therapeutic action (117).

When treating obesity, it is important to consider potential underlying endocrine dysfunctions that favor the development and progression of the disease. For example, due to the high prevalence of malfunction, it is recommended that all patients with obesity get tested for thyroid function. If clinical features are apparent, other hormonal dysfunctions should be considered and then tested (118).

Current guidelines suggest that treatment of obesity is indicated if the BMI is ≥ 30 kg/m² or if BMI is between 25 and < 30 kg/m2 with the simultaneous presence of obesity-related health disorders (e.g., hypertension, Type-2 Diabetes), abdominal obesity, diseases that are aggravated by obesity or high psychosocial distress. The first goal of therapy should be to reach 5-10% weight-loss of the initial weight and to avoid consequent weight regain. To achieve that, an individualized approach is necessary. Therefore, extremely unbalanced diets should not be advocated. It is recommended to suggest a diet, that leads to a long-term energy deficit and that does not lead to negative health consequences. Aiming for a daily energy reduction of 500 kcal or more is advised. Different approaches to reduce energy intake, such as the reduction of carbohydrates, the reduction of fat or the reduction of carbohydrate and fat are possible. There is consistence evidence that shows that the ratio of the macronutrients used for weight loss is

not as important as long as it results in a caloric deficit. Hence, personal preferences should be endorsed in order to improve long term adherence. Depending on the individual situation of the patient, the temporary limited use of formulated products with an energy intake of 800 - 1200 kcal/day can be considered. The involvement of a physician should be ensured. Last but definitely not least, current guidelines suggest, among other recommendations, the encouragement of physical activity to improve weight loss and health (119).

5 Conclusion

This diploma thesis investigated dietary protocols of participants of the CoVVac study and analyzed them longitudinally. Changes in nutrient intake and the diet's inflammatory potential after a one-year follow-up, in both the immunocompromised group as well as the healthy control group were studied.

Additionally, sub-analysis were made at the first investigation date. Besides comparing the well-established Dietary Inflammatory Index to the less established PAIFIS, both markers that aim to categorize the inflammatory potential of a dietary pattern, a special focus was made to bring the DII into association with inflammatory markers in serum and with adipose tissue.

Our results showed that there were no differences in nutrient intake after a one-year follow-up in the healthy group, and apart from a significant change in flavanols, a non-essential phytonutrient, there were also no changes in the immunocompromised group.

A sub-analysis that compared a small group of healthy obese to healthy non-obese participants, showed that the DII was significantly higher in the obese group. Moreover, the inflammation markers IL-6 and IL-18 were significantly higher in the obese group. We could also show that the DII was significantly correlated with BMI and adipose tissue.

Taken these findings together, we could show, that although scientific progress has been made in understanding the potential of an anti-inflammatory diet as a therapeutic approach in chronic inflammatory diseases, the DII did not change to the better in the immunocompromised group. As elucidated in the diploma thesis of Wimmer 2023, there is no difference in the inflammatory potential of the diet in the immunocompromised group compared to the healthy group, suggesting that there is a lack of information of the potential of an anti-inflammatory dietary approach in people suffering from chronic-inflammatory diseases (97).

Further, we substantiated the pro-inflammatory effect of excess adipose tissue. Overweight and obesity do not only contribute to the development of several NCD's such as hypertension, dyslipidemia and diabetes (120) but also to chronic low-grade inflammation (34). Hence, the importance of reducing excess adipose tissue in reducing chronic inflammation is inevitable.

In conclusion, this thesis outlined the importance of reducing chronic low-grade inflammation and suggested a healthy dietary pattern, rich in anti-inflammatory components, as well as maintaining or achieving a normal body composition as two main approaches. This refers primarily to people suffering from chronic inflammatory diseases, but also to public health.

6 List of literature

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