Aus dem Lehrstuhl für Epidemiologie der Universität Augsburg
am Universitätsklinikum Augsburg
ehem. Lehrstuhl für Epidemiologie am Universitären Zentrum für
Gesundheitswissenschaften am Universitätsklinikum Augsburg (UNIKA-T)

Obesity and circulating markers of low-grade systemic inflammation: An exploratory study using population-based data

Dissertation
zum Erwerb des Doktorgrades der Humanbiologie
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität München

vorgelegt von
Mariana Ponce de León Rodríguez
aus
Morelia, Mexiko
2023

Mit Genehmigung der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

Erster Gutachter:	Prof. Dr. med. Christine Meisinger
Zweiter Gutachter:	Prof. Dr. Michael Vogeser
Dritter Gutachter:	Prof. Dr. Sebastian E. Baumeister
Dekan:	Prof. Dr. med. Thomas Gudermann
Tag der mündlichen Prüfung:	15.02.2023

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Promotionsbüro
Medizinische Fakultät

MMRS

Affidavit

Ponce de León Rodríguez, Mariana
Surname, first name

I hereby declare, that the submitted thesis entitled:

"Obesity and circulating markers of low-grade systemic inflammation: An exploratory study using population-based data"

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

Munich, 2023-02-27 Mariana Ponce de Leon Rodriguez

Place, date Signature doctoral candidate

To my family

Contents

List of abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATLAS	Automatic Tissue and Labeling Analysis Software
BIA	Body impedance analysis
BMI	Body mass index
BVSII	Bayerische Verzehrsstudie II
CCL19	C-C motif chemokine 19
CCL28	C-C motif chemokine 28
CHI3L1	Chitinase-3-like protein 1
CNS	Central nervous system
CRP	C-reactive protein
CSF-1	Macrophage colony-stimulating factor 1
CVD	Cardiovascular disease
DNER	Delta and Notch-like epidermal growth factor related receptor
DXA	Dual-energy X-ray absorptiomertry
ECM	Extracellular matrix
eGFR	Estimated glomerular filtration rate
ESTHER	Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und Optimierten Therapie Chronischer Erkrankungen in der Älteren Bevölkerung
FCR	False coverage rate
FDR	False discovery rate

FGF-21	Fibroblast growth factor 21
HbA1c	Hemoglobin A1c
HGF	Hepatocyte growth factor
IL-1	Interleukin-1
IL-1 beta	Interleukin-1 beta
IL-10RB	Interleukin-10 receptor subunit beta
IL-15	Interleukin-15
IL-18	Interleukin-18
IL-18R1	Interleukin-18 receptor 1
IL-33	Interleukin-33
IL-6	Interleukin-6
IPAQ	International Physical Activity Questionnaire
KORA	Kooperative Gesundheitsforschung in der Region Augsburg
LDL-C	Low-density lipoprotein cholesterol
LOD	Limit of detection
MCP-1	Monocyte chemoattractant protein 1
MMP-2	Matrix metalloproteinase-2
MMPs	Matrix metalloproteinases
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NAFPD	Non-alcoholic fatty pancreas disease
NASH	Non-alcoholic steatohepatits
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NPX	Normalized protein expression
OGTT	Oral glucose tolerance test
OLS	Ordinary least square
OSTCN	Osteocalcin
PAI-1	Plasminogen activator inhibitor 1
PDFF	Proton density fat fraction

PEA	Proximity extension assay
PTX3	Pentraxin-related protein 3
qPCR	Quantitiative polymerase chain reaction
RANKL	Receptor activator of nuclear factor kappa-beta ligand
ROI	Region of interest
SAT	Subcutaneous adipose tissue
sCD163	Soluble CD163 antigen
SCF	Stem cell factor
SD	Standard deviation
SHIP	Study of Health in Pomerania
SLAMF1	Signaling lymphocytic activation molecule 1
sTNFR1	Soluble tumor necrosis factor receptor 1
sTNFR2	Soluble tumor necrosis factor receptor 2
TIMPs	Tissue inhibitors of metalloproteinases
TNF-alpha	Tumor necrosis factor alpha
TNFSF12	Tumor necrosis factor ligand superfamily member 12
TNFSF13B	Tumor necrosis factor ligand superfamily member 13B
TNFSF14	Tumor necrosis factor ligand superfamily member 14
Tregs	Regulatory T cells
VAT	Visceral adipose tissue
VEGF-A	Vascular endothelial growth factor A
WC	Waist circumference
WHO	World Health Organization

List of publications

1. **Ponce-de-Leon M**, Linseisen J, Peters A, Linkohr B, Heier M, Grallert H, Schöttker B, Trares K, Bhardwa M, Gào X, Brenner H, Kamiński K, Paniczko M, Kowalska I, Baumeister SE, Meisinger C. Novel associations between inflammation-related proteins and adiposity: A targeted proteomics approach across four population-based studies. Translational Research, 2022; 242:93–104. DOI: https://doi.org/10.1016/j.trsl.2021.11.004

2. **Ponce-de-Leon M**, Hannemann A, Linseisen J, Nauck M, Lerch M, Bülow R, Völzke H, Friedrich N, Kassubek J, Müller HP, Baumeister SE, Meisinger C. Links between ectopic and abdominal fat and systemic inflammation: New insights from the SHIP-Trend study. Digestive and Liver Disease, 2022; 54; 1030-1037. DOI: https://doi.org/10.1016/j.dld.2022.02.003

Contribution to publications

Publication 1

Novel associations between inflammation-related proteins and adiposity: A targeted proteomics approach across four population-based studies.

Prof. Meisinger had the original research idea. Together with Prof. Baumeister, I contributed to the final study design. The original study design included the KORA-Fit study for discovery and the BVSII study for replication. My concern for the age difference between the study samples prompted the inclusion of the ESTHER and Bialystok-PLUS studies for further replication and validation. I performed quality control on normalized and standardized protein measurements. I calculated the measures of body fat distribution based on preprocessed body fat measurements. For the Bialystok-PLUS study sample, I calculated the physical activity scores based on the International Physical Activity Questionnaire (IPAQ). For this, I wrote an algorithm based on the Guidelines for Data Processing and Analysis of the IPAQ [1] using the software environment R. I subsequently developed the R package *IPAQlong* [2] based on it and made the package publicly available (see Appendix). I planned the main and secondary analyses with input from Profs. Meisinger and Baumeister. I performed all the statistical analyses and developed the visualization ideas for the results. I created and interpreted all the figures and tables. I wrote the manuscript which was finalized based on comments from coauthors. I submitted and revised the manuscript and communicated with *Translational Research* during the entire publication process.

Publication 2

Links between ectopic and abdominal fat and systemic inflammation: New insights from the SHIP-Trend study

The research idea originated during discussions between Profs. Meisinger and Baumeister and me. I performed transformation and scaling of preprocessed protein and fat measurements, respectively. I planned and executed all the statistical analyses with input from Profs. Meisinger and Baumeister. I proposed the ideas to adjust the fat measurements for whole-body fat, to test the potential effect-measure modification by sex and to calculate the amount of biomarker variance explained by fat content in each depot. I discussed with Prof. Baumeister the most appropriate methods to use in secondary analyses and carried them out. I developed, created and interpreted all the figures and tables. I wrote the manuscript which was reviewed by the coauthors. I submitted and revised the manuscript and communicated with *Digestive and Liver Disease* during the entire publication process.

Introduction

Obesity is a complex disease defined as an excessive accumulation of body fat mass. The current definition by the World Health Organization (WHO) uses the body mass index (BMI) as an estimate for body fat and establishes a BMI cutoff of 25 for overweight and of 30 for obesity [3]. Obesity rates, according to this definition, have increased steadily in almost every country in the world since the 1980s making it a public health issue of major concern [4]. In 2015, over half a billion adults were obese worldwide [5] and, if trends remain unchanged, this number is estimated to double by 2030 [6].

The number of factors influencing and regulating metabolism contribute to the complexity of the disease and its treatment. For many years it was believed that obesity was a simple imbalance between food intake and physical activity. However, research during the last decades has highlighted the importance of other factors such as appetite regulation, adipose tissue biology, host-microbiome interactions as well other genetic, epigenetic and environmental factors [7].

Obesity is in itself a chronic, progressive and relapsing disease that is associated with functional impairment, reduced quality of life and an increased all-cause mortality [8–10]. In addition, it increases the risk of developing other conditions such as type 2 diabetes, fatty liver disease, hypertension, myocardial infarction, stroke, dementia and several cancers [7]. Obesity is also accompanied by chronic systemic inflammation, which is linked to the development of some of these comorbidities [11].

Adipose tissue and its distribution

Body fat is stored in localized depots of adipose tissue, which is a specialized tissue distributed throughout the body. By its function and morphology, it can be classified into brown and white adipose tissue. Brown adipose tissue is characterized by its heat production capacity, whereas the

main function of white adipose tissue is to store lipids in the form of triglycerides and release them as free fatty acids. In addition, white adipose tissue produces several signaling proteins such as leptin, FGF21 and adiponectin that contribute to the regulation of energy homeostasis [12]. Throughout the rest of the present text, adipose tissue will refer exclusively to white adipose tissue.

An important feature of adipose tissue is its remarkable capacity to adapt to the nutritional status of the individual and change its size accordingly. Expansion of adipose tissue for excess nutrient storage is achieved through the enlargement of adipocytes (hypertrophy) and, to a lesser extent, through adipogenesis (hyperplasia) [13]. Over a decade ago, Spalding et al. showed that adipocyte number is mostly determined during childhood and adolescence and that it remains tightly regulated during adulthood [14]. Soon after, however, it was observed that this is not the case for all adipose tissue depots and that they respond differently to excess nutrition [15]. Indeed, adipose tissue depots differ in several characteristics including mechanism of expansion, secretion patterns, blood flow, fat storage efficiency and lipolysis rates [16]. Some of these differences might partly explain why body fat distribution is a major determinant of metabolic health.

By its location, adipose tissue can be classified into subcutaneous and visceral (SAT and VAT, respectively). SAT is the most abundant type of adipose tissue in normal conditions and is distributed across the body. By location, it can be divided into upper and lower body SAT. According to its structure, it can be classified into deposit, structural and fibrous SAT and serves different functions, including heat loss prevention and protection against physical stress [18]. VAT comprises several depots located in the abdominal cavity; these include omental, mesenteric, retroperitoneal, gonadal and pericardial. VAT is metabolically very active and releases free fatty acids into the circulation. For this reason, and due to its proximity to inner organs, its expansion is particularly detrimental [12,16].

For many decades now, it has been known that the accumulation of fat in the abdomen is linked to negative effects on metabolic health as well as an increased risk of type 2 diabetes and cardiovascular disease [19]. This was initially described in the 1950s using the concepts of gynoid and android obesity, which reference the fat distribution differences observed between men and women [20]. Gynoid obesity is characterized by fat accumulation in the lower body in contrast to android obesity, in which accumulation occurs in the upper body.

Abdominal or central obesity occurs due to the expansion of VAT depots and/or abdominal SAT. The expansion of VAT has received special attention due to its association with insulin resistance, low-grade inflammation, dyslipidemia and hypertension [21]. Adipose tissue accumulation in the

extremities, as opposed to the trunk, has been shown to have a protective effect on metabolic risk. Recent studies have suggested impaired fat accumulation in the extremities as a factor involved in the development of insulin resistance [22].

Although BMI is widely used to diagnose obesity due to its simplicity, it is a poor predictor of the development of comorbidities and of metabolic health [23]. By its nature, BMI does not distinguish between fat mass and fat-free mass, nor does it provide any information about body fat distribution. Other indices and ratios, such as waist circumference (WC) and waist-to-hip ratio, have been developed and recommended [24]. Further characterization of obesity can be achieved through imaging methods such as dual-energy X-ray absorptiometry and magnetic resonance imaging [25].

Ectopic fat accumulation

Despite the fact that adipose tissue is the only specialized tissue with the primary function of storing lipids, fat can accumulate in other organs under certain conditions such as obesity. Surprisingly, ectopic lipid accumulation is not always correlated with the amount of overall adiposity. To explain this phenomenon, Vidal-Puig and others proposed the "adipose tissue expandability hypothesis" [26,27]; according to which, adipose tissue has a limited capacity for lipid storage that depends on genetic and environmental factors. When this capacity is reached, fat accumulates in other organs causing inflammation and metabolic disturbances. Well-studied ectopic sites are the liver, the pancreas and the muscle; other sites include pericardial fat, kidney fat and perivascular adipose tissue [28].

In the liver, triglycerides can accumulate inside hepatocytes in lipid droplets; hepatic steatosis occurs when fat in the liver exceeds five percent. Steatosis can be the result of drug treatments, viral infections and alcohol intake. In the absence of any of these or other causes, steatosis is considered part of a spectrum of progressive liver disorders known as non-alcoholic fatty liver disease (NAFLD) or metabolic dysfunction-associated fatty liver disease (MAFLD), as it has been recently called [29]. Although NAFLD is closely related to obesity, it can occur without obesity and is referred to as lean NAFLD [30]. NAFLD includes simple steatosis, steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma [31]. The exact causes for the progression of NAFLD are still not well understood. One of the leading hypotheses is the "multi-hit hypothesis", which considers that several events contribute to the development of an inflammatory and toxic environment that eventually leads to NASH and, in some individuals, to the more severe forms of the disease. Some of these events

include local and systemic insulin resistance, adipose tissue dysfunction and inflammation, genetic and epigenetic predisposition, changes in the microbiome as well as lipotoxicity and mitochondrial dysfunction in the liver [32].

Similar to NAFLD, obesity-associated pancreatic fat accumulation in the absence of other causes has been defined as non-alcoholic fatty pancreas disease (NAFPD). Compared to NAFLD, NAFPD has been less studied and, in consequence, a consensus about its natural history is lacking. In the pancreas, in contrast to the liver, fat can accumulate through intracellular fat deposition as well as through adipocyte infiltration. Some studies have shown that fat infiltration is not homogeneous and that there are differences in the distribution of fat within the pancreas related to ethnicity [34]. For many years pancreatic steatosis was thought to be merely an imaging finding, which has led to a poor understanding of its clinical relevance [35]. Whether pancreatic steatosis has a direct impact on glucose homeostasis is still debated [34], although it is closely related to NAFLD, VAT and the metabolic syndrome [38]. It has also been associated with other negative outcomes such as more severe episodes of acute pancreatitis as well as the development of pancreatic cancer and pancreatic fistula [40].

In skeletal muscle, fat can accumulate within muscle cells (intramyocellular) or in extramyocellular adipose tissue compartments which include adipose tissue between muscle fibers (intermuscular) as well as adipose tissue around large muscles (perimuscular). Both types of accumulation have been associated with insulin resistance [41,42]. Notably, intermuscular adipose tissue is a strong predictor of fasting glucose and insulin levels independently of BMI [43].

Adipose tissue inflammation

Inflammation is the coordinated activation of several cellular and non-cellular mechanisms in response to an insult or an injury to the organism. Different types of cells and molecules participate in the response, depending on the nature of the initial trigger. The classic inflammatory pathway is characterized by the recruitment of immune cells to the site of the insult as well by as the production of pro-inflammatory cytokines, such as TNF-alpha and IL-6, that induce further responses locally and, in some cases, systemically.

The first link between inflammation and obesity was established over 50 years ago with the observation that adipose tissue of obese mice was infiltrated by macrophages [44]; and although we now know that the presence of macrophages and other immune cells in adipose tissue is essential for

homeostasis [45], the relationship between excess adipose tissue and inflammation has been widely recognized.

How the inflammatory state in adipose tissue is initially triggered is still subject of debate but several hypotheses have been put forward. Rapid expansion of adipose tissue, particularly through hypertrophy, is thought to cause hypoxia and to induce mechanical stress on adipocytes, promoting the upregulation of inflammation-related genes [46]. In addition, stressed and dead adipocytes promote macrophage infiltration and the formation of multinucleate giant cells, which cause persistent macrophage activation [47]. Furthermore, inflammation could be triggered by increased circulating levels of gut-derived antigens as well as the presence of free fatty acids [46].

Regardless of the event (or events) triggering inflammation, adipose tissue inflammation is characterized by several changes in the number and the type of resident immune cells as well as by abnormal cytokine production by immune cells and adipocytes. Macrophages are the most abundant immune cell type in adipose tissue. During obesity, the total number of macrophages increases through the recruitment of circulating monocytes [48]. In addition, there is an increase in the number of M1 or pro-inflammatory macrophages in relation to the number of M2 or anti-inflammatory macrophages [49]. These macrophage populations are distinguished by the expression of cytokines and cell surface markers; however, recent studies have shown that macrophage populations exist in a spectrum rather than in two distinct populations [50]. Other immune cell populations affected include B and T cells, regulatory T cells (Tregs) and mast cells. However, their main contribution to the inflammatory state seems to be through the regulation of macrophage activity, function and recruitment [49]. Importantly, in mice, macrophage depletion corrects some of the adverse metabolic effects linked to inflammation [51], which underlies their importance.

Elevated TNF-alpha expression in adipose tissue was the first cytokine alteration observed in adipose tissue [52,53]. This was fundamental evidence of the role that inflammation plays in the development of obesity-associated insulin resistance, since TNF-alpha negatively regulates insulin signaling [54,55]. Other cytokines produced by adipose tissue that show increased levels in obesity are IL-6 and MCP-1. Altered levels of other proteins secreted by adipose tissue such as omentin, leptin, FGF-21 and adiponectin have also been observed [18].

Obesity-induced inflammation in other organs

In addition to adipose tissue inflammation, obesity-induced inflammation has been observed in other organs including, the pancreas, the liver and the muscle [56].

In the liver, there is increased macrophage and neutrophil recruitment as well as changes in resident macrophages, also known as Kupfer cells. Macrophage recruitment occurs mainly through MCP-1, which can be produced by hepatocytes and kupfer cells. Once in the liver, recruited macrophages show a pro-inflammatory profile as well as increased cytokine production that includes TNF-alpha, IL-1 beta and IL-6 [57]. The overall number of kupfer cells, on the other hand, does not seem to be affected. Kupfer cells exist in a spectrum, with some cells displaying pro-inflammatory and others anti-inflammatory profiles. This balance seems to be affected, resulting in increased inflammation [58]. Liver inflammation is accompanied by decreased insulin sensitivity [49,50] and is also a key component of the progression of simple steatosis to steatohepatitis [32].

Obesity-induced macrophage recruitment also occurs in the pancreas. It is thought that increased glucose and free fatty acids trigger cytokine and chemokine production in beta cells, which promote macrophage infiltration [59]. However, the proliferation of resident intra-islet macrophages seems to be more important than macrophage recruitment, in contrast to what occurs in the liver. Accumulated macrophages show an altered transcriptomic profile and can affect beta cell function through several mechanisms [60]. Recruited pro-inflammatory macrophages also seem to contribute to beta-cell dysfunction associated with type 2 diabetes [59].

In skeletal muscle, increased macrophage accumulation is similar to what has been observed within VAT. Macrophages exhibit a pro-inflammatory profile and contribute to increased local levels of inflammatory cytokines [11]. Like adipocytes, myocytes are also capable of secreting cytokines and other factors called myokines. There is some evidence of changes in myokine expression during obesity, although it does not seem to be the main driver of muscle inflammation. Obesity-induced muscle inflammation is linked to muscle insulin resistance and, given the importance of muscle in insulin-mediated glucose disposal, it is likely that it is involved in the development of type 2 diabetes [61].

Finally, recent research shows evidence of inflammation in the central nervous system as well as pro-inflammatory changes in the gastrointestinal tract associated with obesity [62]. Neuroinflammation affects several structures such as the hypothalamus, the amygdala and the cerebellum, and could be implicated in obesity-related cognitive impairment and mood disorders [63]. Changes in the

gastrointestinal tract include altered microbiome composition as well as increased gut permeability [49].

Low-grade chronic systemic inflammation

Inflammatory responses can lead to tissue damage, therefore their extent and duration are tightly regulated by several mechanisms that, in normal conditions, lead to a resolution phase. When the resolution fails, however, the inflammatory response can become chronic. Low-grade chronic systemic inflammation is characterized by a persistent and mild increased level of circulatory inflammatory proteins. Systemic responses to an inflammatory process are triggered by the production and secretion of cytokines such as IL-1, TNF-alpha and IL-6. These cytokines signal the liver to produce so-called acute phase proteins.

One of the most important acute phase proteins and biomarkers of inflammation is C-reactive protein (CRP). In acute conditions, it can increase up to a thousand-fold whereas moderate increases are observed in low-grade chronic inflammation [64]. CRP production is mainly regulated by IL-6. Thus, the discovery that IL-6 is produced by adipose tissue of healthy individuals and that it is released into circulation led researchers to the hypothesis that obesity might induce a state of systemic inflammation [65]. Indeed, several studies showed that CRP is elevated in obesity [66,67] and that it is associated with insulin resistance [67]. Later research showed that the circulating level of other proteins is also increased in obesity including MCP-1, FGF-21, leptin, IL-18 and PAI-1 [68–72].

Importantly, there are also alterations in circulating proteins that are not pro-inflammatory, which makes it relevant to include proteins with a wide range of functions in exploratory studies. An important example is adiponectin, which was unexpectedly found to be negatively correlated with BMI despite being secreted by adipose tissue [73]. Adiponectin has many target cells and is an important regulator of energy homeostasis; in addition, it displays anti-inflammatory, insulin-sensitizing and anti-atherosclerotic properties [74]. Similarly, omentin, also produced by adipose tissue, shows beneficial effects on inflammation and cardiovascular disease and is decreased in obesity [75,76]. Another example is IL-15 which is negatively associated with total and trunk fat mass and is thought to have protective effects in obesity by promoting fat mass loss [77]. Furthermore, IL-10 is a known anti-inflammatory and regulatory cytokine, with a key role in the termination of the inflammatory response. Several studies have reported altered levels in obesity, although some of

them are conflicting [78–80].

Recent studies have identified altered levels of other proteins implicated in inflammation, such as IL-33 which was only characterized in 2005 [81] and linked to obesity during the last decade [82–84]; or granzyme B, a serine protease with multiple functions in the immune system, which shows elevated levels in obesity [86].

Low-grade chronic systemic inflammation has repercussions on multiple tissues and organs and impacts overall health by directly contributing to disease development or by negatively affecting disease outcomes. In addition to the well-known role of inflammation in insulin resistance and type 2 diabetes, chronic inflammation links obesity with an increased risk of multiple diseases including, but not limited to, cancer, bone-related and autoimmune disorders and cardiovascular disease (CVD).

Cardiovascular disease encompasses several conditions including atherosclerotic CVD, heart failure, stroke and arrhythmias; how obesity-induced inflammation affects their development and progression is disease-dependent and in some instances not yet clear. Early research showed that low-grade systemic inflammation is an independent predictor of the risk of myocardial infarction [87]; subsequent research underlined the importance of inflammation in CVD by showing that antiinflammatory agents could help prevent cardiovascular events and that this effect was independent of lipid levels [88,89]. This, and other research, helped to change the notion that CVD, particularly atherosclerotic CVD, was mainly a lipid storage disease. Inflammation participates in the development of the atherosclerotic plaque as well as the progression to advanced lesions through several mechanisms including T-cell and macrophage activity [90–92]. In other types of cardiovascular disease, such as heart failure, the involvement of obesity-induced chronic inflammation is not as clear. Some studies suggest that adipokines, which regulate myocardial function, are implicated; other studies point to the involvement of inflammation in pericardial and epicardial adipose tissue [91,93].

Obesity is a risk factor contributing to the development of bone-related disorders through weight-dependent mechanisms as well as by altering molecular pathways [94]. Low-grade systemic inflammation has emerged as an important mediator in this relationship. TNF-alpha and IL-6, for instance, are thought to induce bone loss by modulating osteoclastogenesis [95]. Furthermore, systemic inflammation contributes to local joint inflammation, which has been recently recognized as an important factor in the pathogenesis of osteoarthritis [96]. Other factors with immune-regulating properties such as leptin and adiponectin also contribute to bone and joint homeostasis

[94,97]. Lastly, recent research suggests an interplay between obesity-induced inflammation and bone marrow homeostasis [98].

In addition to its involvement in the pathogenesis of some diseases, chronic inflammation can also exacerbate and affect the outcome of diseases that are accompanied by an inflammatory process. Two examples are infections and autoimmune disorders. Obesity is known to affect both the susceptibility and the severity of certain bacterial and viral infections [99]. A pertinent example is SARS-CoV-2 infection, in which obesity is a major determinant of disease severity [100]. Chronic inflammation has been proposed as a key mediator of negative outcomes in infections by interfering with the adaptive and innate immune responses [101]. Moreover, obesity increases the risk of several autoimmune disorders such as rheumatoid arthritis, multiple sclerosis and psoriasis and, in some cases, it affects disease progression and treatment response [102]. Although several mechanisms are likely to be involved, adipokines seem to play an important role through their pro-inflammatory properties. Other mechanisms involve changes in different immune cell populations as well as the activation of the inflammasome, a protein complex that can be activated by several factors altered in obesity [102].

Some types of cancer are another example in which inflammation has been identified as a central component in disease development and progression. Obesity increases the risk of several cancers including breast cancer, hepatocellular carcinoma, colorectal and pancreatic cancer [103]. An important predictor of increased cancer risk is metabolic health, which is tightly connected to chronic inflammation [104]. In addition, the chronic inflammatory environment promotes tumor growth and progression through multiple mechanisms. The inflammation-mediated activation of some transcription factors, in particular NF-kB, plays an important role in tumor initiation, promotion and dissemination [105]. Inflammation also promotes angiogenesis through the activity of pro-angiogenic factors like IL-6, leptin, VEGF-A and TNF-alpha [103].

Finally, low-grade chronic inflammation has been implicated in the intergenerational transmission of obesity and metabolic disturbances as well as in the development of perinatal complications [106,107]. Normal pregnancy is accompanied by multiple changes in the immune system that help regulate implantation, placental development and delivery [107]. An altered obesity-induced inflammatory state is, therefore, hypothesized to be an important factor driving perinatal complications such as gestational diabetes, preeclampsia and miscarriage [107]. Furthermore, recent studies have shown that maternal inflammation affects fetal immune and metabolic programming which predisposes the offspring to metabolic disorders later in life [106,108].

Project outline and objectives

The relationship between excess adipose tissue and inflammation has been widely studied in the last decades. Nevertheless, our understanding of the molecular players involved remains incomplete. Recent technology improvements have allowed the development of multiplex immunoassays that allow the simultaneous quantification of a broad number of proteins in low sample volumes. Therefore, it is now feasible to use these methods in large-scale human studies, which facilitates the identification of biomarkers as well as the elucidation of the molecular basis of diseases. The primary goal of this doctoral project was to further explore the relationship between obesity and systemic inflammation using data from five European population-based studies: KORA-Fit, BVSII, ESTHER, SHIP and Bialystok PLUS. These data included measurements of circulating proteins carried out using inflammation-specific multiplex panels as well as data obtained by medical imaging techniques. In particular, we had the following objectives:

1) To identify novel associations between circulating inflammation-related proteins and general adiposity.

2) To explore the relationship between circulating inflammation-related proteins and body fat distribution.

3) To investigate the relationship between circulating inflammatory biomarkers and abdominal, hepatic and pancreatic fat.

The first two objectives were addressed in publication 1 *"Novel associations between inflammation-related proteins and adiposity: A targeted proteomics approach across four population-based studies"* and the third objective in publication 2 *"Links between ectopic and abdominal fat and systemic inflammation: New insights from the SHIP-Trend study"*.

Summary

Obesity affects more than half a billion people worldwide and its prevalence is estimated to double by 2030 if trends remain unchanged. Obesity is often associated with a state of low-grade chronic inflammation, which in turn has been proposed as a linking mechanism to the risk of developing comorbidities, such as insulin resistance, cardiovascular disease and some types of cancer. It is, therefore, crucial to better understand the relationship between excess adiposity and inflammation. The objective of this doctoral project was to explore the relationship between several features of obesity and low-grade systemic inflammation using data from five different European population-based studies (KORA-Fit, BVSII, ESTHER, Bialystok PLUS and SHIP).

In the first part of the project, we investigated the association between anthropometric measures of adiposity and an extensive panel of 72 circulating inflammation-related proteins using KORA-Fit data for discovery. We replicated our results in BVSII, ESTHER and Bialystok PLUS and further validated them using Dual-energy X-ray absorptiometry fat mass measurements from the Bialystok PLUS study. This also allowed us to investigate how the proteins were affected by body fat distribution. We found four novel associations between adiposity and inflammation-related proteins (DNER, SLAMF1, RANKL and CSF-1) and confirmed ten that had been previously reported (CCL19, CCL28, FGF-21, HGF, IL-10RB, IL-18, IL18R1, IL-6, SCF and VEGF-A). Most of these proteins were associated with visceral fat as well as fat accumulation in the trunk.

In the second part, we explored the association between fat accumulation in the abdomen and 31 circulating biomarkers of inflammation using data from the SHIP study. We focused on abdominal subcutaneous and visceral adipose tissue as well as hepatic and pancreatic fat. We used fat mass measurements obtained by magnetic resonance imaging, which is the most accurate method available. We found associations between sTNFR1, sTNFR2, and sCD163 and pancreatic fat that have not been reported in the literature. However, further studies are necessary to replicate them in independent populations. We also reported several associations with subcutaneous, visceral and

hepatic fat.

In conclusion, the work presented in this cumulative dissertation provides new insights into the complex relationship between fat accumulation and systemic inflammation. In addition to identifying novel associations, we explored the role of body fat distribution as well as the involvement of hepatic and pancreatic fat. The latter is particularly relevant since it has been poorly studied in the past. Further studies are needed to investigate causality as well as to establish the clinical significance of our findings.

Zusammenfassung

Weltweit sind mehr als eine halbe Milliarde Menschen von Fettleibigkeit betroffen und die Prävalenz wird sich Schätzungen zufolge bis 2030 verdoppeln. Adipositas geht oft mit geringgradigen chronischen Entzündungen einher, was wiederum als Verbindungsmechanismus mit dem Risiko für Komorbiditäten wie Insulinresistenz, Herz-Kreislauf-Erkrankungen und einigen Krebsarten vorgeschlagen wurde. Es ist daher wichtig, die Beziehung zwischen Adipositas und Entzündung besser zu verstehen. In dieser Dissertation wurden Daten aus fünf verschiedenen europäischen bevölkerungsbasierten Studien (Kora-Fit, BVSII, ESHTER, Bialystok PLUS und SHIP) verwendet, um die Beziehung zwischen mehreren Merkmalen von Adipositas und systemischer Entzündung zu untersuchen.

Im ersten Teil des Projekts wurde der Zusammenhang zwischen anthropometrischen Messungen der Adipositas und einem Panel zirkulierender entzündungsbezogener Proteine in KORA-Fit untersucht. Die Ergebnisse wurden in BVSII, ESTHER und Bialystok PLUS repliziert und weiter validiert mittels Dual-Röntgen-Absorptiometrie Fettmassenmessungen aus der Bialystok PLUS Studie. Dadurch wurde auch der Einfluss der Körperfettverteilung auf den Proteingehalt untersucht. Wir fanden vier neue Assoziationen zwischen Adipositas und entzündungsbezogener Proteine (DNER, SLAMF1, RANKL und CSF-1) und bestätigten zehn zuvor berichtete (CCL19, CCL28, FGF-21, HGF, IL-10RB, IL-18, IL18R1, IL-6, SCF und VEGF-A). Die meisten dieser Proteine waren sowohl mit viszeralem Fett als auch mit Fettansammlungen im Rumpf verbunden.

Im zweiten Teil wurde der Zusammenhang zwischen der Fettansammlung im Abdomen und zirkulierenden Entzündungsmarkern in der SHIP Studie untersucht. Wir konzentrierten uns auf abdominales subkutanes und viszerales Fett sowie Leber- und Pankreasfett. Die Fettkompartimente wurden mittels MRT quantifiziert, was derzeit als die genaueste Methode gilt. Wir fanden Assoziationen zwischen sTNFR1, sTNFR2 und sCD163 und Pankreasfett, die in der Literatur

bisher nicht beschrieben wurden. Es sind jedoch weitere Studien notwendig, um sie zu bestätigen. Wir berichteten auch über mehrere Assoziationen mit subkutanem, viszeralem und hepatischem Fett.

Abschließend geben unsere Ergebnisse neue Einblicke in die komplexe Beziehung zwischen Adipositas und systemischer Entzündung. Neben der Identifizierung neuer Assoziationen untersuchten wir die Rolle der Körperfettverteilung sowie die Beteiligung von Leber- und Pankreasfett. Letzteres ist besonders relevant, da es in der Vergangenheit kaum untersucht wurde. Weitere Studien sind erforderlich, um die Kausalität zu untersuchen und die klinische Relevanz unserer Ergebnisse zu etablieren.

Publication 1

Novel associations between inflammation-related proteins and adiposity: A targeted proteomics approach across four population-based studies

Ponce-de-Leon M, Linseisen J, Peters A, Linkohr B, Heier M, Grallert H, Schöttker B, Trares K, Bhardwa M, Gào X, Brenner H, Kamiński K, Paniczko M, Kowalska I, Baumeister SE, Meisinger C. Novel associations between inflammation-related proteins and adiposity: A targeted proteomics approach across four population-based studies. Translational Research, 2022; 242:93–104. DOI: https://doi.org/10.1016/j.trsl.2021.11.004

Publication 2

Links between ectopic and abdominal fat and systemic inflammation: New insights from the SHIP-Trend study

Ponce-de-Leon M, Hannemann A, Linseisen J, Nauck M, Lerch M, Bülow R, Völzke H, Friedrich N, Kassubek J, Müller HP, Baumeister SE, Meisinger C. Links between ectopic and abdominal fat and systemic inflammation: New insights from the SHIP-Trend study. Digestive and Liver Disease, 2022; 54; 1030-1037. DOI: https://doi.org/10.1016/j.dld.2022.02.003

References

1. The IPAQ group. IPAQ scoring protocol [Internet]. 2005. Available from: https://sites.google.com/site/theipaq/scoring-protocol

2. Ponce-de-Leon M. IPAQlong: Calculates the Scores for the 'International Physical Activity Questionnaire (IPAQ)' Long Form (Version 0.1.0) [Internet]. 2022. Available from: https://github.com/Mariana-plr/IPAQlong

3. World Health Organization. Obestiy [Internet]. Available from: https://www.who.int/health-topics/obesity%7B/#%7Dtab=tab%7B/_%7D1

4. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet (London, England). 2014 Aug;384(9945):766–81.

5. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. The New England journal of medicine. 2017 Jul;377(1):13–27.

6. World Obesity Federation. World Obesity Atlas 2022 [Internet]. 2022 p. 19. Available from: https://www.worldobesityday.org/policy-makers

7. Blüher M. Obesity: global epidemiology and pathogenesis. Nature Reviews Endocrinology [Internet]. 2019;15(5):288–98. Available from: https://doi.org/10.1038/s41574-019-0176-8

8. Collaboration GBMIM, Di Angelantonio E, Bhupathiraju S, Wormser D, Gao P, Kaptoge S, et al. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. Lancet (London, England) [Internet]. 2016/07/13 ed. 2016 Aug;388(10046):776–86. Available from: https://pubmed.ncbi.nlm.nih.gov/27423262 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4995441/

9. Bray GA, Kim KK, Wilding JPH. Obesity: a chronic relapsing progressive disease process. A position statement of the World Obesity Federation. Vol. 18. World Obesity Federation; 2017. p. 715–23.

10. Allison DB, Downey M, Atkinson RL, Billington CJ, Bray GA, Eckel RH, et al. Obesity as a disease: a white paper on evidence and arguments commissioned by the Council of the Obesity Society. Obesity (Silver Spring, Md). 2008 Jun;16(6):1161–77.

11. Lee YS, Wollam J, Olefsky JM. An Integrated View of Immunometabolism. Cell. 2018 Jan;172(1-2):22–40.

12. Rosen ED, Spiegelman BM. What we talk about when we talk about fat. Cell [Internet]. 2014 Jan;156(1-2):20–44. Available from: https://pubmed.ncbi.nlm.nih.gov/24439368 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3934003/

13. Ghaben AL, Scherer PE. Adipogenesis and metabolic health. Nature Reviews Molecular Cell Biology [Internet]. 2019;20(4):242–58. Available from: https://doi.org/10.1038/s41580-018-0093-z

14. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. Nature [Internet]. 2008;453(7196):783–7. Available from: https://doi.org/10.1038/nature06902

15. Tchoukalova YD, Votruba SB, Tchkonia T, Giorgadze N, Kirkland JL, Jensen MD. Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. Proceedings of the National Academy of Sciences of the United States of America. 2010 Oct;107(42):18226–31.

16. Tchkonia T, Thomou T, Zhu Y, Karagiannides I, Pothoulakis C, Jensen MD, et al. Mechanisms and metabolic implications of regional differences among fat depots. Cell metabolism [Internet]. 2013/04/11 ed. 2013 May;17(5):644–56. Available from: https://pubmed.ncbi.nlm.nih.gov/23583168 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3942783/

17. Sbarbati A, Accorsi D, Benati D, Marchetti L, Orsini G, Rigotti G, et al. Subcutaneous adipose tissue classification. European journal of histochemistry : EJH. 2010 Nov;54(4):e48.

18. Chait A, Hartigh LJ den. Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease. Frontiers in cardiovascular medicine [Internet]. 2020 Feb;7:22. Available from: https://pubmed.ncbi.nlm.nih.gov/32158768 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7052117/

19. Smith U. Abdominal obesity: a marker of ectopic fat accumulation. The Journal of clinical investigation. 2015 May;125(5):1790–2.

20. VAGUE J. The Degree of Masculine Differentiation of Obesities: A FACTOR DETERMINING PREDISPOSITION TO DIABETES, ATHEROSCLEROSIS, GOUT, AND URIC CALCULOUS DISEASE. The American Journal of Clinical Nutrition [Internet]. 1956 Jan;4(1):20–34. Available from: https://doi.org/10.1093/ajcn/4.1.20

21. Karastergiou K, Fried SK. Multiple adipose depots increase cardiovascular risk via local and systemic effects. Current atherosclerosis reports [Internet]. 2013 Oct;15(10):361. Available from: https://pubmed.ncbi.nlm.nih.gov/23982264 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3997174/

22. Lotta LA, Gulati P, Day FR, Payne F, Ongen H, Bunt M van de, et al. Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. Nature genetics. 2017 Jan;49(1):17–26.

23. Rothman KJ. BMI-related errors in the measurement of obesity. International Journal of Obesity [Internet]. 2008;32(3):S56–9. Available from: https://doi.org/10.1038/ijo.2008.87

24. Ross R, Neeland IJ, Yamashita S, Shai I, Seidell J, Magni P, et al. Waist circumference as a vital sign in clinical practice: a Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. Nature Reviews Endocrinology [Internet]. 2020;16(3):177–89. Available from: https://doi.org/10.1038/s41574-019-0310-7

25. Borga M, West J, Bell JD, Harvey NC, Romu T, Heymsfield SB, et al. Advanced body composition assessment: from body mass index to body composition profiling. Journal of Investigative Medicine [Internet]. 2018;66(5):1–9. Available from: https://jim.bmj.com/content/66/5/1.10

26. Virtue S, Vidal-Puig A. It's not how fat you are, it's what you do with it that counts. PLoS biology. 2008 Sep;6(9):e237.

27. Tan CY, Vidal-Puig A. Adipose tissue expandability: the metabolic problems of obesity may arise from the inability to become more obese. Biochemical Society Transactions [Internet]. 2008 Sep;36(5):935–40. Available from: https://doi.org/10.1042/BST0360935

28. Ferrara D, Montecucco F, Dallegri F, Carbone F. Impact of different ectopic fat depots on cardiovascular and metabolic diseases. Journal of Cellular Physiology [Internet]. 2019 Dec;234(12):21630–41. Available from: https://doi.org/10.1002/jcp.28821

29. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. Journal of Hepatology [Internet]. 2020;73(1):202–9. Available from: https://www.sciencedirect.com/science/article/pii/S0168827820302014

30. Kumar R, Mohan S. Non-alcoholic Fatty Liver Disease in Lean Subjects: Characteristics and Implications. Journal of clinical and translational hepatology. 2017 Sep;5(3):216–23.

31. Nassir F, Rector RS, Hammoud GM, Ibdah JA. Pathogenesis and Prevention of Hepatic Steatosis. Gastroenterology & hepatology. 2015 Mar;11(3):167–75.

32. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism [Internet]. 2016;65(8):1038–48. Available from: https://www.sciencedirect.com/science/article/pii/S0026049515003832

33. Hakim O, Bonadonna RC, Mohandas C, Billoo Z, Sunderland A, Boselli L, et al. Associations Between Pancreatic Lipids and β-Cell Function in Black African and White European Men With Type 2 Diabetes. The Journal of Clinical Endocrinology & Metabolism [Internet]. 2019 Apr;104(4):1201–10. Available from: https://doi.org/10.1210/jc.2018-01809

34. Begovatz P, Koliaki C, Weber K, Strassburger K, Nowotny B, Nowotny P, et al. Pancreatic adipose tissue infiltration, parenchymal steatosis and beta cell function in humans. Diabetologia [Internet]. 2015;58(7):1646–55. Available from: https://doi.org/10.1007/s00125-015-3544-5

35. Majumder S, Philip NA, Takahashi N, Levy MJ, Singh VP, Chari ST. Fatty Pancreas: Should We Be Concerned? Pancreas [Internet]. 2017;46(10):1251–8. Available from: https://pubmed.ncbi.nlm.nih.gov/29040194 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6689238/

36. Gerst F, Wagner R, Oquendo MB, Siegel-Axel D, Fritsche A, Heni M, et al. What role do fat cells play in pancreatic tissue? Molecular Metabolism [Internet]. 2019;25:1–10. Available from: https://www.sciencedirect.com/science/article/pii/S2212877819301760

37. Geenen E-JM van, Smits MM, Schreuder TCMA, Peet DL van der, Bloemena E, Mulder CJJ. Nonalcoholic Fatty Liver Disease Is Related to Nonalcoholic Fatty Pancreas Disease. Pancreas [Internet]. 2010;39(8). Available from: https://journals.lww.com/pancreasjournal/Fulltext/2010/11000/Nonalcoholic%7B/_%7DFatty%7B/_%7DLiver%7B/_%7DDisease%7B/_%7DIs%7B/_%7DRelated%7B/_%7Dto.11.aspx

38. Wagner R, Eckstein SS, Yamazaki H, Gerst F, Machann J, Jaghutriz BA, et al. Metabolic implications of pancreatic fat accumulation. Nature Reviews Endocrinology [Internet]. 2022;18(1):43–54. Available from: https://doi.org/10.1038/s41574-021-00573-3

39. Smits MM, Geenen EJM van. The clinical significance of pancreatic steatosis. Nature reviews Gastroenterology & hepatology. 2011 Mar;8(3):169–77.

40. Truong E, Pandol S, Jeon C. Uniting epidemiology and experimental models: pancreatic steatosis and pancreatic cancer. eBioMedicine [Internet]. 2022;79:103996. Available from: https://www.sciencedirect.com/science/article/pii/S2352396422001803

41. Park SS, Seo Y-K. Excess Accumulation of Lipid Impairs Insulin Sensitivity in Skeletal Muscle. Vol. 21. 2020.

42. Khan IM, Perrard XY, Brunner G, Lui H, Sparks LM, Smith SR, et al. Intermuscular and perimuscular fat expansion in obesity correlates with skeletal muscle T cell and macrophage infiltration and insulin resistance. International journal of obesity (2005). 2015 Nov;39(11):1607–18.

43. Addison O, Marcus RL, Lastayo PC, Ryan AS. Intermuscular fat: a review of the consequences and causes. International journal of endocrinology. 2014;2014:309570.

44. Hausberger FX. Pathological changes in adipose tissue of obese mice. The Anatomical record. 1966 Mar;154(3):651–60.

45. Hotamisligil GS. Foundations of Immunometabolism and Implications for Metabolic Health and Disease. Immunity. 2017 Sep;47(3):406–20.

46. Reilly SM, Saltiel AR. Adapting to obesity with adipose tissue inflammation. Nature Reviews Endocrinology [Internet]. 2017;13(11):633–43. Available from: https://doi.org/10.1038/nrendo.2017.90

47. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. Journal of Lipid Research [Internet]. 2005;46(11):2347–55. Available from: https://www.sciencedirect.com/science/article/pii/S0022227520328728

48. Lumeng CN, Deyoung SM, Bodzin JL, Saltiel AR. Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes. 2007 Jan;56(1):16–23.

49. Lee YS, Olefsky J. Chronic tissue inflammation and metabolic disease. Genes & development. 2021 Mar;35(5-6):307–28.

50. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. Immunity [Internet]. 2022;55(1):31–55. Available from: https://www.sciencedirect.com/science/article/pii/S1074761321005495

51. Bu L, Gao M, Qu S, Liu D. Intraperitoneal injection of clodronate liposomes eliminates visceral adipose macrophages and blocks high-fat diet-induced weight gain and development of insulin resistance. The AAPS journal. 2013 Oct;15(4):1001–11.

52. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science (New York, NY). 1993 Jan;259(5091):87–91.

53. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. The Journal of clinical investigation [Internet]. 1995 May;95(5):2409–15. Available from: https://pubmed.ncbi.nlm.nih.gov/7738205 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC295872/

54. Plomgaard P, Fischer CP, Ibfelt T, Pedersen BK, Hall G van. Tumor necrosis factor-alpha modulates human in vivo lipolysis. The Journal of clinical endocrinology and metabolism. 2008 Feb;93(2):543–9.

55. Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. Diabetes. 2005 Oct;54(10):2939–45.

56. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. The Journal of Clinical Investigation [Internet]. 2011 Jun;121(6):2111–7. Available from: https://doi.org/10.1172/JCI57132

57. Morinaga H, Mayoral R, Heinrichsdorff J, Osborn O, Franck N, Hah N, et al. Characterization of distinct subpopulations of hepatic macrophages in HFD/obese mice. Diabetes. 2015 Apr;64(4):1120–30.

58. Wan J, Benkdane M, Teixeira-Clerc F, Bonnafous S, Louvet A, Lafdil F, et al. M2 Kupffer cells promote M1 Kupffer cell apoptosis: a protective mechanism against alcoholic and nonalcoholic fatty liver disease. Hepatology (Baltimore, Md). 2014 Jan;59(1):130–42.

59. Eguchi K, Manabe I, Oishi-Tanaka Y, Ohsugi M, Kono N, Ogata F, et al. Saturated Fatty Acid and TLR Signaling Link β Cell Dysfunction and Islet Inflammation. Cell Metabolism [Internet]. 2012 Apr;15(4):518–33. Available from: https://doi.org/10.1016/j.cmet.2012.01.023

60. Ying W, Lee YS, Dong Y, Seidman JS, Yang M, Isaac R, et al. Expansion of Islet-Resident Macrophages Leads to Inflammation Affecting β Cell Proliferation and Function in Obesity. Cell metabolism. 2019 Feb;29(2):457–474.e5.

61. Wu H, Ballantyne CM. Skeletal muscle inflammation and insulin resistance in obesity. The Journal of clinical investigation. 2017 Jan;127(1):43–54.

62. Winer DA, Winer S, Dranse HJ, Lam TKT. Immunologic impact of the intestine in metabolic disease. The Journal of clinical investigation [Internet]. 2017/01/03 ed. 2017 Jan;127(1):33–42. Available from: https://pubmed.ncbi.nlm.nih.gov/28045403 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5199708/

63. Guillemot-Legris O, Muccioli GG. Obesity-Induced Neuroinflammation: Beyond the Hypothalamus. Trends in neurosciences. 2017 Apr;40(4):237–53.

64. Du Clos TW, Mold C. C-reactive protein. Immunologic Research [Internet]. 2004;30(3):261–77. Available from: https://doi.org/10.1385/IR:30:3:261

65. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, et al. Subcutaneous Adipose Tissue Releases Interleukin-6, But Not Tumor Necrosis Factor-α, in Vivo1. The Journal of Clinical Endocrinology & Metabolism [Internet]. 1997 Dec;82(12):4196–200. Available from: https://doi.org/10.1210/jcem.82.12.4450

66. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-Reactive Protein Levels in Overweight and Obese Adults. JAMA [Internet]. 1999 Dec;282(22):2131–5. Available from: https://doi.org/10.1001/jama.282.22.2131

67. Yudkin JS, Stehouwer CDA, Emeis JJ, Coppack SW. C-Reactive Protein in Healthy Subjects: Associations With Obesity, Insulin Resistance, and Endothelial Dysfunction . Arteriosclerosis, Thrombosis, and Vascular Biology [Internet]. 1999 Apr;19(4):972–8. Available from: https://doi.org/10.1161/01.ATV.19.4.972

68. Barnard SA, Pieters M, De Lange Z. The contribution of different adipose tissue depots to plasma plasminogen activator inhibitor-1 (PAI-1) levels. Blood reviews. 2016 Nov;30(6):421–9.

69. Zhang X, Yeung DCY, Karpisek M, Stejskal D, Zhou Z-G, Liu F, et al. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes. 2008 May;57(5):1246–53.

70. Kim C-S, Park H-S, Kawada T, Kim J-H, Lim D, Hubbard NE, et al. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. International Journal of Obesity [Internet]. 2006;30(9):1347–55. Available from: https://doi.org/10.1038/sj.ijo.0803259

71. Esposito K, Pontillo A, Ciotola M, Di Palo C, Grella E, Nicoletti G, et al. Weight Loss Reduces Interleukin-18 Levels in Obese Women. The Journal of Clinical Endocrinology & Metabolism [Internet]. 2002 Aug;87(8):3864–6. Available from: https://doi.org/10.1210/jcem.87.8.8781

72. Iikuni N, Lam QLK, Lu L, Matarese G, La Cava A. Leptin and Inflammation. Current immunology reviews. 2008 May;4(2):70–9.

73. MATSUZAWA Y, FUNAHASHI T, NAKAMURA T. Molecular Mechanism of Metabolic Syndrome X: Contribution of Adipocytokines · Adipocyte-derived Bioactive Substances. Annals of the New York Academy of Sciences [Internet]. 1999 Nov;892(1):146–54. Available from: https://doi.org/10.1111/j.1749-6632.1999.tb07793.x

74. Nguyen TMD. Adiponectin: Role in Physiology and Pathophysiology. International journal of preventive medicine. 2020;11:136.

75. Tan Y-L, Zheng X-L, Tang C-K. The protective functions of omentin in cardiovascular diseases. Clinica Chimica Acta [Internet]. 2015;448:98–106. Available from: https://www.sciencedirect.com/science/article/pii/S000989811500282X

76. Tan BK, Adya R, Randeva HS. Omentin: A Novel Link Between Inflammation, Diabesity, and Cardiovascular Disease. Trends in Cardiovascular Medicine [Internet]. 2010;20(5):143–8. Available from: https://www.sciencedirect.com/science/article/pii/S1050173810001593

77. Nielsen AR, Hojman P, Erikstrup C, Fischer CP, Plomgaard P, Mounier R, et al. Association between Interleukin-15 and Obesity: Interleukin-15 as a Potential Regulator of Fat Mass. The Journal of Clinical Endocrinology & Metabolism [Internet]. 2008 Nov;93(11):4486–93. Available from: https://doi.org/10.1210/jc.2007-2561

78. Esposito K, Pontillo A, Giugliano F, Giugliano G, Marfella R, Nicoletti G, et al. Association of Low Interleukin-10 Levels with the Metabolic Syndrome in Obese Women. The Journal of Clinical Endocrinology & Metabolism [Internet]. 2003 Mar;88(3):1055–8. Available from: https://doi.org/10.1210/jc.2002-021437

79. Manigrasso MR, Ferroni P, Santilli F, Taraborelli T, Guagnano MT, Michetti N, et al. Association between Circulating Adiponectin and Interleukin-10 Levels in Android Obesity: Effects of Weight Loss. The Journal of Clinical Endocrinology & Metabolism [Internet]. 2005 Oct;90(10):5876–9. Available from: https://doi.org/10.1210/jc.2005-0281

80. Fain JN. Release of Inflammatory Mediators by Human Adipose Tissue Is Enhanced in Obesity and Primarily by the Nonfat Cells: A Review. Fantuzzi G, editor. Mediators of Inflammation [Internet]. 2010;2010:513948. Available from: https://doi.org/10.1155/2010/513948

81. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 2005 Nov;23(5):479–90.

82. Wood IS, Wang B, Trayhurn P. IL-33, a recently identified interleukin-1 gene family member, is expressed in human adipocytes. Biochemical and biophysical research communications. 2009 Jun;384(1):105–9.

83. Darkhal P, Gao M, Ma Y, Liu D. Blocking high-fat diet-induced obesity, insulin resistance and fatty liver by overexpression of Il-13 gene in mice. International Journal of Obesity [Internet]. 2015;39(8):1292–9. Available from: https://doi.org/10.1038/ijo.2015.52

84. Schmidt FM, Weschenfelder J, Sander C, Minkwitz J, Thormann J, Chittka T, et al. Inflammatory cytokines in general and central obesity and modulating effects of physical activity. PloS one. 2015;10(3):e0121971.

85. Cimini FA, Barchetta I, Ceccarelli V, Chiappetta C, Di Biasio A, Bertoccini L, et al. Granzyme B Expression in Visceral Adipose Tissue Associates With Local Inflammation and Glyco-Metabolic Alterations in Obesity. Frontiers in Immunology [Internet]. 2020;11. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2020.589188

86. El Mesallamy HO, Hamdy NM, Mostafa DM, Amin AI. The serine protease granzyme B as an inflammatory marker, in relation to the insulin receptor cleavage in human obesity and type 2 diabetes mellitus. Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research. 2014 Mar;34(3):179–86.

87. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. The New England journal of medicine. 1997 Apr;336(14):973–9.

88. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. The New England journal of medicine. 2000 Mar;342(12):836–43.

89. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. The New England journal of medicine. 2017 Sep;377(12):1119–31.

90. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002 Mar;105(9):1135–43.

91. Powell-Wiley TM, Poirier P, Burke LE, Després J-P, Gordon-Larsen P, Lavie CJ, et al. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. Circulation. 2021 May;143(21):e984–1010.

92. Rocha VZ, Libby P. Obesity, inflammation, and atherosclerosis. Nature Reviews Cardiology [Internet]. 2009;6(6):399–409. Available from: https://doi.org/10.1038/nrcardio.2009.55

93. Ebong IA, Goff DCJ, Rodriguez CJ, Chen H, Bertoni AG. Mechanisms of heart failure in obesity. Obesity research & clinical practice. 2014;8(6):e540–8.

94. Hou J, He C, He W, Yang M, Luo X, Li C. Obesity and Bone Health: A Complex Link [Internet]. Vol. 8. 2020. Available from: https://www.frontiersin.org/articles/10.3389/fcell.2020.600181

95. Gkastaris K, Goulis DG, Potoupnis M, Anastasilakis AD, Kapetanos G. Obesity, osteoporosis and bone metabolism. Journal of musculoskeletal & neuronal interactions. 2020 Sep;20(3):372–81.

96. Robinson WH, Lepus CM, Wang Q, Raghu H, Mao R, Lindstrom TM, et al. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. Nature reviews Rheumatology. 2016 Oct;12(10):580–92.

97. Roy B, Curtis ME, Fears LS, Nahashon SN, Fentress HM. Molecular Mechanisms of Obesity-Induced Osteoporosis and Muscle Atrophy [Internet]. Vol. 7. 2016. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2016.00439

98. Benova A, Tencerova M. Obesity-Induced Changes in Bone Marrow Homeostasis [Internet]. Vol. 11. 2020. Available from: https://www.frontiersin.org/articles/10.3389/fendo.2020.00294

99. Falagas ME, Kompoti M. Obesity and infection. The Lancet Infectious Diseases [Internet]. 2006;6(7):438–46. Available from: https://www.sciencedirect.com/science/article/pii/S1473309906705230

100. Popkin BM, Du S, Green WD, Beck MA, Algaith T, Herbst CH, et al. Individuals with obesity and COVID-19: A global perspective on the epidemiology and biological relationships. Obesity Reviews [Internet]. 2020 Nov;21(11):e13128. Available from: https://doi.org/10.1111/obr.13128

101. Guglielmi V, Colangeli L, D'Adamo M, Sbraccia P. Susceptibility and Severity of Viral Infections in Obesity: Lessons from Influenza to COVID-19. Does Leptin Play a Role? International Journal of Molecular Sciences [Internet]. 2021;22(6). Available from: https://www.mdpi.com/1422-0067/22/6/3183

102. Versini M, Jeandel P-Y, Rosenthal E, Shoenfeld Y. Obesity in autoimmune diseases: Not a passive bystander. Autoimmunity Reviews [Internet]. 2014;13(9):981–1000. Available from: https://www.sciencedirect.com/science/article/pii/S1568997214001414

103. Deng T, Lyon CJ, Bergin S, Caligiuri MA, Hsueh WA. Obesity, Inflammation, and Cancer. Annual review of pathology. 2016 May;11:421–49.

104. Moore LL, Chadid S, Singer MR, Kreger BE, Denis GV. Metabolic Health Reduces Risk of Obesity-Related Cancer in Framingham Study Adults. Cancer Epidemiology, Biomarkers & Prevention [Internet]. 2014 Sep;23(10):2057–65. Available from: https://doi.org/10.1158/1055-9965.EPI-14-0240

105. Taniguchi K, Karin M. NF-κB, inflammation, immunity and cancer: coming of age. Nature Reviews Immunology [Internet]. 2018;18(5):309–24. Available from: https://doi.org/10.1038/nri.2017.142

106. Parisi F, Milazzo R, Savasi VM, Cetin I. Maternal Low-Grade Chronic Inflammation and Intrauterine Programming of Health and Disease. Vol. 22. 2021.

107. St-Germain LE, Castellana B, Baltayeva J, Beristain AG. Maternal Obesity and the Uterine Immune Cell Landscape: The Shaping Role of Inflammation. International journal of molecular sciences. 2020 May;21(11).

108. Ingvorsen C, Brix S, Ozanne SE, Hellgren LI. The effect of maternal Inflammation on foetal programming of metabolic disease. Acta Physiologica [Internet]. 2015 Aug;214(4):440–9. Available from: https://doi.org/10.1111/apha.12533

Appendix

Reference manual for the R package 'IPAQlong'

Package 'IPAQlong'

March 23, 2022

Package: IPAQlong
Type: Package
Title: Calculates the Scores for the 'International Physical Activity Questionnaire (IPAQ)' Long Form
Version: 0.1.0
Author: Mariana Ponce-de-Leon
Maintainer: Mariana Ponce-de-Leon mariana.ponce-de-leon@outlook.com
Description: Calculates the scores for the 'International Physical Activity Questionnaire (IPAQ)' long form, based on the "Guidelines for the data processing and analysis of the IPAQ" https://sites.google.com/site/theipaq/home.
License: MIT + file LICENSE
Encoding: UTF-8
LazyData: true
RoxygenNote: 7.1.2
Suggests:
knitr,
rmarkdown
Imports: *dplyr*

R topics documented

ipaq_scores

Description

ipaq_scores() calculates the continuous and categorical scores for the 'International Physical Activity Questionnaire (IPAQ)' long form.

Usage

```
ipaq_scores(data, truncate = F)
```

Arguments

Argument	Description
`data`	A data frame object containing 25 columns with the replies to the IPAQ long format (parts 1-4). Yes/no replies should be coded as yes-1, no-0. Time should be in minutes.
`truncate`	Logical vector. If TRUE all walking, moderate and vigorous time variables are truncated following the IPAQ short rule. Variables exceeding 180 minutes are truncated to be equal to 180 minutes. Default FALSE.

Value

A data frame object with the continuous (metabolic equivalent of task minutes (MET-min)/week) and categorical scores (low, moderate, high). Returns NA for cases with missing values.

ipaq_subScores

Description

ipaq_subscores() calculates the domain and intensity sub scores for the 'International Physical Activity Questionnaire (IPAQ)' long form.

Usage

```
ipaq_subScores(data, truncate = F)
```

Arguments

Argument	Description
`data`	A data frame object containing 25 columns with the replies to the IPAQ long format (parts 1-4). Yes/no replies should be coded as yes-1, no-0. Time should be in minutes.
`truncate`	Logical vector. If TRUE all walking, moderate and vigorous time variables are truncated following the IPAQ short rule. Variables exceeding 180 minutes are truncated to be equal to 180 minutes.Default FALSE.

Value

A data frame object with the domain (work, transportation, domestic, leisure) and intensity (walking, moderate, vigorous) sub scores in metabolic equivalent of task minutes (MET-min)/week. Sub scores are calculated for all cases, even in the presence of missing values.

References

The IPAQ Group (2005). Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire. Retrieved from https://sites.google.com/site/theipaq/home

Acknowledgements

First of all, I would like to thank my supervisor, Prof. Meisinger, as well as Prof. Linseisen, for giving me the opportunity to carry out my doctoral project in the group and for their steadfast support and guidance. Equally, I would like to thank Prof. Baumeister for his advice and support during the last 3 years and Prof. Seißler for being part of my thesis advisory committee.

I want to use this opportunity to thank the coauthors of the publications included in this dissertation as well as all of those involved in the KORA, SHIP, BVSII, ESTHER and Bialystok PLUS studies.

I would like to acknowledge the LMU Graduate Center for awarding me the LMU Completion Grant, which provided support during the last phase of my doctoral project.

I am grateful to the UNIKA-T team in Augsburg for welcoming me into the group. Special thanks to Marion Kötzner for her constant help and support and to Dennis Freuer for his assistance in everything computer-related. Many thanks to Taylor Breuninger, Chetana Dahal and Nina Grundmann for the nice moments we shared in the office. I would also like to extend my thanks to Dr. Rui Wang-Sattler and her team for welcoming me as a guest at Helmholtz Munich.

I would like to thank my friends, old and new, for their support and for all the adventures we shared throughout these years. Thank you, Aurora, Nancy, David, Andrea, Esmeralda, Alex, Oleg and Monica. I also want to thank the members of the doctoral students' initiative at Helmholtz Munich for being a source of joy and fun, especially during the SARS-CoV-2 pandemic.

Finally, I would like to thank my parents, Mela and Javier, for their dedication to my education and for their unconditional love and support; my sisters, Maricarmen and Sara, for always being there for me, no matter the circumstances; and Florian for his love and support.