REPRODUCIBILITY AND VALIDITY OF ULTRASOUND-ASSESSED VISCERAL AND SUBCUTANEOUS BODY FAT AND ASSOCIATIONS WITH INFLAMMATORY PARAMETERS AND METABOLITES

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Inga Schlecht
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Dekan:	Prof. Dr. Dr. Torsten E. Reichert
Betreuer:	Prof. Dr. Dr. Michael F. Leitzmann
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AP	affinity propagation
ATC	Anatomical Therapeutic Chemical
BCAA	branched chain amino acid
BMI	body mass index
CRP	C-reactive protein
CT	computer tomography
DXA	dual x-ray absorptiometry
eGFR	estimated glomerular filtration rate
EPIC	European Prospective Investigation into Cancer and Nutrition
FFA	free fatty acid
FID	free induction decay
GNC	German National Cohort
HMDB	Human Metabolome Database
HPFS	Health Professionals' Follow-up Study
HSQC	heteronuclear single quantum coherence
IGF-1	insulin-like growth factor-1
IL-6	interleukin-6
LLOQ	lower limits of quantification
MDRD4	four-variable Modification of Diet in Renal Disease
MHO	metabolically healthy obese
MRI	magnetic resonance imaging
MS	mass spectrometry
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser enhancement spectroscopy
NSAIDs	non-steroidal anti-inflammatory drugs
SAT	subcutaneous adipose tissue
SD	standard deviation
SOP	standard operation procedure
TG	triglyceride

TNF-α	tumor necrosis factor alpha
TOCSY	total correlated spectroscopy
TCA	tricarboxylic acid
TSP	3-trimethylsilyl-2,2,3,3-tetradeuteropropionate
VAT	visceral adipose tissue
VSR	visceral-to-subcutaneous-fat ratio
WC	waist circumference
WHO	World Health Organization
WHR	waist-to-hip ratio

1 Introduction

1.1 The public health impact of obesity

The prevalence of overweight and obesity is increasing substantially worldwide at an alarming rate, in both developed and developing countries among adults and children (1, 2). According to the World Health Organization (WHO), a body mass index (BMI) of <18.5 kg/m² is classified as underweight, a BMI of 18.5-24.9 kg/m² as normal weight is, and a BMI of 25.0-29.9 kg/m² as overweight. A BMI of ≥30.0 kg/m² is classified as obesity. Globally, the age-standardized prevalence of overweight increased from 24.6% in 1980 to 34.4% in 2008 (3). Moreover, during the same time period, the age-standardized prevalence of obesity nearly doubled from 6.4% to 12.0%. Currently, excess weight is causing about 3.4 million annual deaths and 3.8% of the global burden of disease (4).

In Germany, more than 50% of individuals aged 18 to 79 years are overweight (5). Further, 67.1% of men and 53.0% of women are overweight, and 23.3% of men and 23.9% of women are obese (5). From a societal perspective, the total costs of obesity are estimated to total up to euro 5.7 million annually, imposing a major burden on the healthcare system (6). Several meta-analyses have provided a comprehensive estimate of the co-morbidities attributable to overweight and obesity and confirm that overweight and obesity carry a profound health burden and have a significant impact on health expenditures (7). Specifically, overweight and obesity were found to be associated with the incidence of type 2 diabetes mellitus, numerous cancers, cardiovascular disease, asthma, gallbladder disease, osteoarthritis, and chronic back pain (7-13). Moreover, the risks (as defined by hazard ratios) of diabetic, renal, and hepatic mortality associated with overweight and obesity are as high as 60–120% (14). In addition, about 40% of cardiovascular mortality is caused by obesity, and obesity is responsible for almost 20% of respiratory and all other mortality (14).

To some extent, the burden of obesity-associated diseases may be attributable to prolonged life expectancy and may additionally be determined by non-modifiable factors, such as sex, ethnicity and genetic predisposition. Nevertheless, obesity-associated diseases are also a result of behavior and lifestyle choices, including a hyper-caloric

diet, physical inactivity, and smoking (15). These modifiable factors also offer a great potential to develop and implement prevention strategies to counteract the global burden of obesity (16, 17). The early application of intervention strategies may enhance quality of life and reduce healthcare costs. Consequently, it is important to understand the underlying biological mechanisms and to identify individuals at risk for obesity-associated diseases.

1.2 How obesity causes chronic diseases

Adipose tissue consists of a large number of adipocytes, other non-fat cells, connective tissue matrix, vascular and neural tissues (18). The non-adipocytes cellular component includes inflammatory cells (macrophages), immune cells, pre-adipocytes, and fibroblasts. The main cellular components of adipose tissue are adipocytes that represent the primary storage depots of energy in form of triglyceride (TG) droplets. New smaller adipocytes can act as buffers to absorb free fatty acids (FFAs) and TGs in the postprandial period. As adipocytes grow larger, they become dysfunctional. Specifically, large adipocytes are insulin-resistant, hyperlipolytic, and resistant to the anti-lipolytic effect of insulin (18). Adipose tissue is recognized as an endocrine organ secreting cytokines, chemokines, growth factors, and hormones. Moreover, adipose tissue dysfunction is a central component of obesity-related inflammation and the main instigator of the pathological consequences of obesity, mostly through its association with insulin resistance (19-21). In addition, there has been an increasing appreciation of the role of inflammation both in the pathogenesis of atherosclerosis (22, 23) and as a key factor in insulin resistance (24).

Low-grade chronic inflammation can be characterized by increased systemic levels of some inflammatory parameters, including interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), C-reactive protein (CRP), and resistin, and decreased levels of adiponectin. These inflammatory parameters have been proposed as intermediates that might explain the association between obesity and obesity-associated diseases. In particular, a number of studies have confirmed an association of inflammatory parameters with atherosclerosis, type 2 diabetes, and numerous cancers (25, 26). For instance, increased levels of TNF-α, IL-6, and CRP have been associated with type 2 diabetes (27, 28). Moreover, increased circulating levels of TNF-α, IL-6, and CRP play a major

role in all stages of atherosclerosis, from plaque formation over progression to rupture of atherosclerotic plaques (29) and are associated with worse cardiovascular outcomes (30, 31). In addition, a recent meta-analysis of prospective cohort studies reported that elevated levels of CRP are associated with an increased risk of all-cancer, lung cancer, and possibly breast, prostate, and colorectal cancer (25). Yet another study found positive associations of resistin and TNF-α with colorectal cancer (32). Decreased adiponectin levels have been related to type 2 diabetes, cardiovascular diseases, and numerous cancers, including breast, endometrial, prostate, colon, gastric, pancreatic, and hematological malignancies (33, 34).

Obesity is also associated with increased macrophage infiltration of adipose tissue, and these macrophages may be an important component of the inflammatory response, playing a crucial role in the development of insulin resistance (35). Adipose tissue inflammatory factors are produced by both adipocytes and associated inflammatory cells, such as adipose tissue-related macrophages. Evidence suggests that adipose tissue macrophages may be responsible for a large proportion of adipose tissue TNF-α expression and significant amounts of other inflammatory factors such as IL-6, resistin, and adiponectin (36-38). Moreover, increased inflammatory parameters derived from adipose tissue, are also promoting inflammatory responses from other body organs. For instance, CRP is secreted by the liver in response to adipocyte or adipose tissue IL-6 release (12).

However, it has been recognized that the level of risk to develop obesity-associated diseases differs among obese individuals. Specifically, research suggests that about 20% of people, who are characterized as obese, are apparently insulin sensitive and metabolically normal (39). These individuals have been categorized as metabolically healthy but obese (MHO) (40). In contrast, approximately 18% of the general population that have been defined as being normal weight, are metabolically obese displaying several of the metabolic abnormalities associated with the metabolic syndrome (40). It has been shown that MHO often have less visceral adipose tissue (VAT) than obese patients with cardiometabolic diseases (40, 41). Conversely, patients who are normal weight, but metabolically obese, tend to have more VAT than individuals of similar weight and no metabolic disease (40). Mechanisms explaining such clinical findings are not fully understood.

Part of the problem is due to the fact that BMI has been used to characterize the obese phenotype. However, BMI is not a biological trait but a calculated value (i.e., body weight in kilograms divided by height in meters squared), which is widely used in clinical practice to categorize individuals (42). Body weight is the sum of the different body components; however each body component (e.g., adipose tissue, skeletal muscle mass, and fat-free mass) has its own regulatory basis (43). Thus, emerging research has begun to address body fat distribution as an important factor that might help explain the robust association of BMI with various diseases and mortality that has been found in observational research. Abdominal obesity, as characterized by waist circumference (WC) and waist-to-hip ratio (WHR), were shown to more accurately measure body fat distribution than BMI, which is more closely related to general adiposity (44). Moreover, abdominal obesity is a strong correlate of adverse metabolic profiles and their resulting diseases (45). WC and WHR have been suggested to be better predictors of the risk of disease than BMI (43, 44, 46). For instance, according to the Health Professionals' Follow-up Study (HPFS) among men, WC is a more powerful predictor of type 2 diabetes than WHR or BMI (47). Moreover, WC or WHR better discriminated and calibrated 5-year mortality risk than BMI in men and for women from the European Prospective Investigation into Cancer and Nutrition (EPIC) study (48). Further, evidence suggests that WC and WHR but not general adiposity were associated with increased risk of death in diabetic men and women (49).

Nevertheless, there are regional differences between abdominal adipose tissues in relation to metabolic outcomes (50). VAT has been considered to have multiple endocrine, metabolic, and immunological functions and may be more strongly associated with metabolic risk factors than abdominal subcutaneous adipose tissue (SAT) (51-55). Moreover, an independent curvilinear association between VAT and mortality has been reported for men (56), suggesting that a large amount of VAT is required for an increased risk of mortality (57). Although it remains unclear what drives the association between VAT and obesity-associated diseases, the most accepted theory is that VAT releases excess FFAs, which results in insulin resistance in the liver and acts as a trigger for obesity-associated diseases such as type 2 diabetes (58, 59), cardiovascular diseases (57-61), and some cancers, including colorectal (62), hepatic (63), pancreatic (64), breast (65), endometrial (66), and urinary tract malignancies (67).

Specifically, elevated FFAs in the plasma cause decreased glucose transport into the muscle cells and increased fat breakdown, subsequently leading to elevated hepatic glucose production (59). A combination of insulin resistance and pancreatic β-cell dysfunction is responsible for the development of type 2 diabetes (68). Insulin resistance increases cardiovascular risk through dyslipidaemia, hypertension, and glucose dysmetabolism. Moreover, the aforementioned inflammatory parameters secreted by adipocytes and macrophages infiltrating adipose tissue may lead to increased oxidative stress and endothelial dysfunction, which promotes atherosclerosis (57). Several studies proposed possible mechanisms explaining the underlying mechanisms of increased VAT and the increased risk of cancer (61, 69-71). These studies have demonstrated sustained hyperglycaemia, hyperinsulinemia, insulin resistance, and hyperinsulinemia-related increase of insulin-like growth factor-1 (IGF-1) in cancer promotion and progression.

Anatomic location is one of the more important reasons why different fat depots have different pathogenic potential. VAT accounts for up to 10–20% of total fat in men and 5–8% in women (72). The amount of VAT increases with age in both sexes (72). Because VAT is predominantly located in the mesentery and omentum, it drains directly through the portal circulation to the liver (18). By comparison, SAT presents the largest fat storage site (approximately 80% of total body fat) and occurs over the whole body below the skin and between the skeletal muscles (intermuscular fat) (73). VAT contains a greater number of large adipocytes in contrast to SAT that contains small adipocytes (74). The deposition in non-adipose tissue of small adipocytes is prevented by the enhanced insulin-sensitivity and a high affinity for FFA and TG uptakes (74, 75). In contrast, during positive caloric balance, the recruitment and proliferation of smaller and more functional subcutaneous adipocytes occurs and the risk of developing metabolic diseases may be decreased (75) and thus may explain the proposed protective effects of SAT.

However, the role of abdominal SAT regarding the development of obesity-associated diseases is not well understood (28). Some studies have suggested that SAT in the abdominal region has metabolic activity, such as lipolysis and the release of inflammatory factors if subcutaneous adipocytes become sufficiently enlarged (28, 38, 51, 76). Yet, data examining potential differences between VAT and SAT are not entirely

consistent (28). Moreover, because the absolute quantification of VAT or SAT does not reflect its relative distribution in the human body, the assessment of the visceral-to-subcutaneous-fat ratio (VSR) may provide additional information with respect to metabolic regulation perturbed by obesity (77). However, the propensity whether fat is stored viscerally rather than subcutaneously has rarely been targeted by previous research (77-79). Most previous studies investigating the associations between obesity and inflammatory parameters used BMI, WC, or WHR as an underlying metric of adiposity (80-92). Although a number of studies did consider body fat distribution and examined relations of VAT (79, 83, 85, 93-98) and/or SAT (83, 93-104) to parameters of systemic chronic inflammation, results are inconsistent and only few studies (94, 98, 102) reported results from multivariable analyses that adjusted for common confounding factors (Supplementary Table 1). Only one previous study (79) among obese adolescents examined the VSR in relation to hs-CRP and IL-6. Moreover, to date no previous study has compared different measures of obesity with regards to their relations to parameters of chronic inflammation and no previous study examined numerous inflammatory parameters at the same time. However, to better understand biological mechanisms that link obesity to obesity-associated diseases and to further improve the prediction of those diseases, it is indispensable to comprehensively characterize the obese phenotype by including the examination of adipose tissue compartments.

1.3 Transition from single biomarker investigation to systems epidemiology

Although a number of important risk factors of obesity-associated diseases, including inflammatory parameters, have been identified, the underlying metabolic pathways of obesity-associated diseases are not yet fully understood (105). Obesity itself is a complex disease and very challenging with respect to its pathophysiologic consequences, heterogeneity in phenotypes, and multifactorial origin (106). To enhance the understanding of the pathophysiologic consequences of obesity and to better address potential biological mechanisms underlying obesity-associated diseases, a more comprehensive approach seems to be necessary that does not only focus on individual biomarkers. A more systematic approach to investigate biological mechanisms and pathways has been introduced by the “–omics”-sciences, such as genomics

(genetic predisposition), transcriptomics (expression of genes), proteomics (enzymes), and metabolomics (substrates and products of enzymes) (Figure 1). In general, the focus of the –"omic"-sciences is directed on the entire systems (e.g., the complete human metabolism) rather than on single biomarkers.

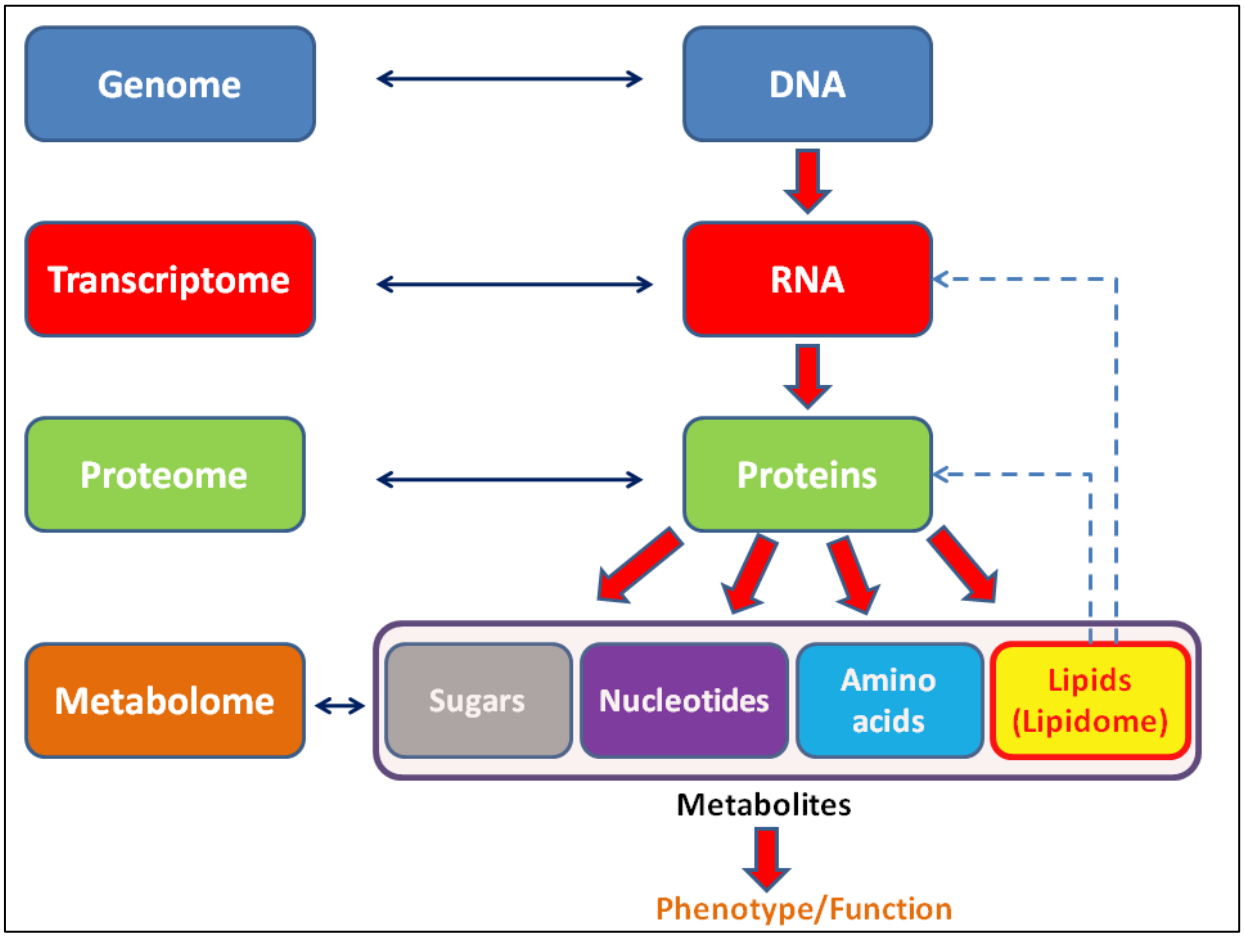

Figure 1: Hierarchy of the "-omic"-sciences
Source: (107).

Metabolomics is described as the concurrent study of all measurable low-weight molecular compounds present in a biological system such as biological fluids (e.g., urine or blood), tissues, or cells (108). Metabolites are defined as compounds with less than 1 kDa of molecular weight (e.g., amino acids, mono- and di-saccharides, lipids, organic acids, steroids, nucleotides); the entity of metabolites represents the metabolome (108). Depending on their origin, metabolites can further be categorized as endogenous (e.g., derived from de-novo synthesis) or exogenous (e.g., ingested with diet or medication) (109). Metabolomics focuses on substrates, intermediates, and end products of

metabolic pathways and it reflects cellular processes, such as substrate fluxes and enzyme activities. Furthermore, by representing a unique snapshot of metabolic phenotypes, metabolomics has the potential to depict genetic predisposition and modulation as well as environmental and lifestyle exposures, including obesity. Thus, metabolic perturbations linked to chronic disease risk can be identified (108, 110) and biological mechanisms may be unraveled to gain information about how habitual factors and phenotypes are linked to metabolism (105). Knowledge about these mechanisms may offer great potential in terms of understanding the development and consequently the prevention of chronic diseases, which are strongly linked to human behavior and phenotypes (16, 111).

The exploratory nature, covering a whole biological system with multiple factors, is an important attribute of the systemic metabolomics approach. To date, “-omic”-sciences have mostly been applied in the context of experimental studies, e.g., animal studies or small scale clinical trials. However, the combination of an observational study design with the innovative measurements from molecular biology, might offer the potential to unravel the “black box” of biological mechanisms and pathways that underlie the observed associations between environmental and lifestyle exposures and chronic disease risk (105, 112, 113). To describe this concept, the term “systems epidemiology” has been proposed (114). Systems epidemiology concatenates the molecular underpinnings with multiple environmental interaction, including behavioral and sociodemographic levels that may influence health and disease, with the aim of a comprehensive and more complete description of the physical state of an individual (115, 116).

However, the description of the obese phenotype often fails to capture the diverse subclasses of those phenotypes that predict the outcome or response to treatment (117). Specifically, although BMI and WC are frequently used to categorize individuals as overweight or obese, these parameters cannot reflect biological mechanisms because they are no biological traits (43). Overcoming these difficulties requires an exhaustive examination of the discrete components of the obese phenotypes that goes beyond BMI and WC measurements. Integrating the examination of different body fat tissues, including VAT and SAT, such deep phenotyping, gathers details about related disease mechanisms more precisely (118). In line with a more comprehensive

characterization of the obese phenotype by considering general adiposity, abdominal adiposity, different abdominal adipose tissue compartments, and the relative distribution of different abdominal adipose tissue compartments, the thorough investigation of the metabolic phenotype by considering the explorative examination of the metabolome, may further enhance the understanding of metabolic consequences of obesity and mechanisms linking obesity to obesity-related diseases.

Intermediate metabolic markers may be identified that mirror different stages of progression from a healthy state to disease and the development of obesity-associated diseases may be more comprehensible. Similar to the findings that WC, WHR, and VAT are better predictors of morbidity and mortality from obesity-associated diseases than BMI (43, 48, 52, 56, 119-123), the investigation of the complex human metabolism in addition to already known obesity-related biomarkers such as inflammatory parameters, may also improve the prediction of morbidity and mortality attributable to obesity (105, 115, 116). Taken together, the described deep phenotyping of the exposure and outcome adds to our ability to unravel the “black box” between obesity and obesity-related diseases. Ultimately, metabolic markers may contribute to the identification of individuals at high-risk for the development of obesity-associated diseases and facilitate administering adequate prevention and treatment strategies.

Including metabolomics in epidemiologic studies requires a shift in paradigm of research design from hypothesis-driven approaches and the investigation of single biomarkers, towards exploratory approaches to examine the complex human metabolism and provide important information on underlying biochemical pathways and interrelations, thus enriching the biological context and clinical relevance. The exploratory approach does not require an a priori hypothesis and can be used to discover novel metabolic associations and disease pathways to gain scientific insight in an incremental manner. However, metabolomic data pose a challenge in epidemiologic research in that they possess high dimensionality, some degree of collinearity and missing data, non-linearity, and non-normality (124). Moreover, effect sizes and most metabolites are unknown in the exploratory mode, which also challenge power calculations because of the unknown number of features and tests that will be performed. Finally, findings need to be validated in external, independent data sets following exact analytical procedures and identical preprocessing methods (124).

Metabolomics has raised particular interest of clinicians and epidemiologists (112, 125, 126). A number of obesity-related metabolites have been identified by metabolomics that affect metabolic pathways in humans (127). However, to date no consistent results have been identified and little is known about the relations of VAT and SAT or VSR to metabolic profiles and whether the contributions of those parameters differ regarding their relation to metabolic regulation compared to simpler obesity measurements, such as BMI and WC. Previous studies examining the association of obesity with metabolite levels in human blood or urine specimens reported positive relations of BMI to branched chain amino acids (BCAA) (128-133), and carnitine concentrations (131, 132) (Supplementary Table 2). Moreover, inverse relations were found between BMI and microbial metabolites, such as hippuric acid (131, 133, 134). Both, positive and inverse relations were found between obesity and amino acid-related metabolites (129-133, 135). Two studies measured abdominal obesity by dual x-ray absorptiometry (DXA) and reported positive relations to intermediates of BCAA among older adults (136) and BCAAs among overweight men and women (130). Only one study measured VAT and SAT by magnetic resonance imaging (MRI) and reported a positive relation of VAT to serum isoleucine in overweight men and women (130).

Thus, there is a need to describe biologically sound, multifaceted, and robust phenotypes to provide useful information about physiological and pathological states. In particular, to overcome the limitations of BMI, the investigation of relations of abdominal obesity, abdominal adipose tissues, and the relative distribution of abdominal adipose tissues with metabolite regulation aims at a better understanding of metabolic pathways that are perturbed by obesity and may consequently improve the understanding of the development of obesity-related diseases.

1.4 Measurement of visceral and subcutaneous adipose tissue

The recognition of the importance of body fat distribution has led to a variety of methods to assess abdominal fat tissue, spanning from WC and WHR, to more sophisticated methods, such as computer tomography (CT) (137) and MRI (138). Population-based epidemiologic studies have often used BMI, WC, and WHR to characterize body weight and the distribution of body fat. Despite being cost and time efficient, these parameters are indirect measurements that have serval methodological

shortcomings because these simple measures are not capable of adequately discriminating between VAT and SAT. However, to achieve a thorough understanding of obesity-related chronic diseases, new measurements that apply deep phenotyping of obesity measurements are needed.

For the investigation of the pathophysiology of obesity accurate measures to differentiate and quantify VAT and SAT are of major importance as they improve the understanding of mechanisms underlying obesity-related morbidity (139). CT and MRI are considered the reference methods for the measurement of VAT and SAT (140). However, MRI and CT approaches are limited in field conditions due to their associated costs and issues regarding accessibility, contraindications, and in terms of CT examinations, potential adverse effects of radiation. One possible non-invasive and cost-effective alternative to differentiate and quantify VAT and SAT is ultrasound.

1.4.1 Ultrasound to measure visceral and subcutaneous adipose tissue

Although ultrasound has been commonly used to assess soft tissue structures in clinical diagnoses, ultrasound as an imaging modality can also measure tissue thicknesses such as SAT and VAT. Sound waves of high frequency (1-10MHz) are produced by vibrations of an electrically stimulated piezoelectric crystal within a transducer and are used by brightness-modulation (B-mode) instruments (141). The interfaces of dissimilar underlying tissues partially reflect the ultrasound beam that is disseminated through the skin. One part of the beam is reflected back to the transducer as an echo, while the remainder of the beam continues to travel through the interface to deeper tissues. The strength of the reflection, which in turn depends on the acoustic impedance properties of different tissues and the number of tissue interfaces that the ultrasonic beam transverses, determine the quality of an image (142). The density of the medium times the velocity of ultrasound wave propagation in the medium defines the intrinsic physical property of a medium (143). The lowest acoustic impedance is produced by air-containing organs such as the lung, while dense organs such as bone have very high-acoustic impedance (144). Finally, the transducer converts and amplifies the echoes into electric signals to form an enlarged image on a display device (145).

1.4.2 Reliability and validity of ultrasound-based measurements of visceral and subcutaneous adipose tissue

The sonographic-based quantification of VAT and SAT offers great potential for epidemiological research. As a non-invasive method, abdominal fat can time- and cost-efficiently be examined and body fat compartments can be differentiated and quantified (Table 1). However, limitations of the ultrasound method include the requirement of experienced technicians with considerable skills. In addition, the ultrasound examination is of higher cost compared to field methods, although it is of lower cost compared to laboratory methods such as CT and MRI.

Table 1: Advantages and limitations using ultrasound for assessing body fat.

Advantages	Limitations
- Lower costs than laboratory methods - High accuracy and precision in the hands of an experienced technician - Capable of regional and segmental measurements - Minimal tissue compression - Noninvasive and no ionizing radiation - Applicable for testing in the field - Short testing time, rapid procedure	- Higher costs than field methods - Requires experienced technician, considerable skill is necessary - Measurement procedures and techniques are not yet standardized - Inherent artifacts (e.g., fascia)

Reproduced from Wagner, 2013 (146).

In 1966, ultrasound was introduced for the measurement of SAT (147). The first application of ultrasound for the measurement of VAT in a population-based epidemiological study dates back to 1990 (148). Ever since, the ultrasound method to quantify VAT and SAT has been subject to research to explore the reliability and validity of using different measurement protocols to differentiate and quantify VAT and SAT. Some studies that have examined the validity of ultrasound for the assessment of VAT

and SAT compared these measures with reference methods (CT or MRI) and concluded that ultrasound is a suitable technique to accurately measure VAT and SAT (51, 52, 149, 150), while other studies showed inconsistent results for the precision of the ultrasound measurement of VAT and SAT (52, 140, 148-158) (Supplementary Table 3). Moreover, only few studies examined the reproducibility of the sonographic-based measurement of VAT (153, 157, 159-161), and none of those studies examined the reproducibility of measuring SAT. In addition, results were inconsistent showing favorable results, with intra-class correlation coefficients of 0.90-0.99 (157, 159) and mean differences ranging from 0.25-0.69 mm (153, 160) and less favorable results with a correlation coefficient of 0.64 (161).

1.5 Objectives

Against this background, the present thesis aimed to examine metabolic differences between visceral and abdominal subcutaneous adipose tissue. A systematic, multiple step approach was taken and the following objectives were of interest:

1) Reproducibility of the sonographic quantification of VAT and SAT
2) Validity of the sonographic quantification of VAT and SAT
3) Relations of VAT, SAT, VSR, BMI, and WC to parameters of chronic inflammation
4) Associations between VAT, SAT, VSR, BMI, and WC with concentrations of quantified urinary and serum metabolites applying a targeted metabolomic approach
5) Using an untargeted metabolomic approach to detect differences between VAT, SAT, VSR, BMI, and WC regarding a) their relations to urinary and serum metabolic fingerprints adopting cluster analyses and related methods to identify groups of metabolites and b) their relations to individual urinary and serum bins applying multiple regression analyses and subsequent metabolite identification

To examine the inter- and intra-observer reproducibility of the ultrasound-based quantification of VAT and SAT, a reproducibility study was conducted among 127 participants from two study centers in Germany. In total, 30 participants were recruited for the validity study to evaluate the validity of the ultrasound method to measure VAT and SAT compared with the gold standard method MRI. The inflammation study was

conducted in a sample of 97 participants. In this context, VAT, SAT, VSR, BMI, and WC were surveyed and related to serum concentrations of parameters of chronic inflammation, including hs-CRP, TNF-α, IL-6, resistin, and adiponectin. Nuclear magnetic resonance spectroscopy (NMR) was used to measure metabolites in 228 urinary and 200 serum specimens in the metabolomics study. A targeted metabolomics approach was used to quantify concentrations of urinary and serum metabolites and the relations of VAT, SAT, VSR, BMI, and WC to metabolite concentrations were examined. An untargeted metabolomics approach was used to detect differences between obesity measures with respect to their relations to urinary and serum features (bins).

2 Methods

2.1 Study population

The present thesis emerged from the pre-test studies of the German National Cohort study (GNC). The study aim of the GNC, a joint interdisciplinary endeavor of scientists from the Helmholtz and the Leibniz Associations, universities and other German research institutes, is to investigate the development of major chronic diseases, the subclinical stages and functional changes (162). Therefore, a population-based sample of 200,000 men and women was drawn in in 18 study centers across Germany (162). The logistics, feasibility and other elements of the study, including measurement instruments, were tested during pre-test and pilot studies. These pre-test studies were conducted from June 2011 to April 2013 in all 18 GNC study centers. The pre-test studies were based on a cross-sectional study design.

An age- and sex stratified sample of the population was drawn by the local population registries in Regensburg and adjacent areas. Participants that were included in the pre-test studies were aged between 20 and 70 years, were living in Regensburg or adjacent areas, and were German speaking. Participants were recruited in two phases (pre-test I and pre-test II). Pre-test I was conducted between June and August 2011 and pre-test II was conducted between October 2012 and April 2013. Participants were invited to take part of the study by invitation letters and no incentives were given to the participants. Overall, 233 of the 949 sampled individuals agreed to participate, which corresponds to a response proportion of 24.6%. Of those, five participants were

excluded from analyses due to pregnancy (n=1) or diagnosed chronic diseases including diabetes (n=2), pancreatic cancer (n=1), or hepatitis (n=1). Urine samples were collected from all participants (n=228) and blood samples were collected from 88% of participants (n=200). In addition, data from 30 subjects participating in the GNC pre-test studies in Kiel were included for specific data analyses. In Kiel, the same recruiting strategies were used as in Regensburg. Another sample of 30 subjects also aged between 20 and 70 years were patients of the local hospital in Regensburg undergoing MRI examination of the abdomen or they were volunteers. All pre-test studies were conducted according to the Declaration of Helsinki guidelines and were approved by the ethics committee of the local hospitals. Written informed consent was obtained from all participants.

Each previously described objective (chapter 1.5) is examined based on a study specific population comprised from the GNC pre-test studies. Information about each study population considered for each objective is described in the flow chart (Figure 2). In detail, data from pre-test I and the additional data from 30 subjects participating in the GNC pre-test studies in Kiel were included in the data analyses for the reproducibility study (n=127). For the validation study, data from volunteers and patients of the local hospital in Regensburg was included (n=30). The inflammation study was conducted with data from pre-test one (n=97) and for the metabolomic studies data from both pre-test studies were considered, resulting in a total study sample of 228 participants.

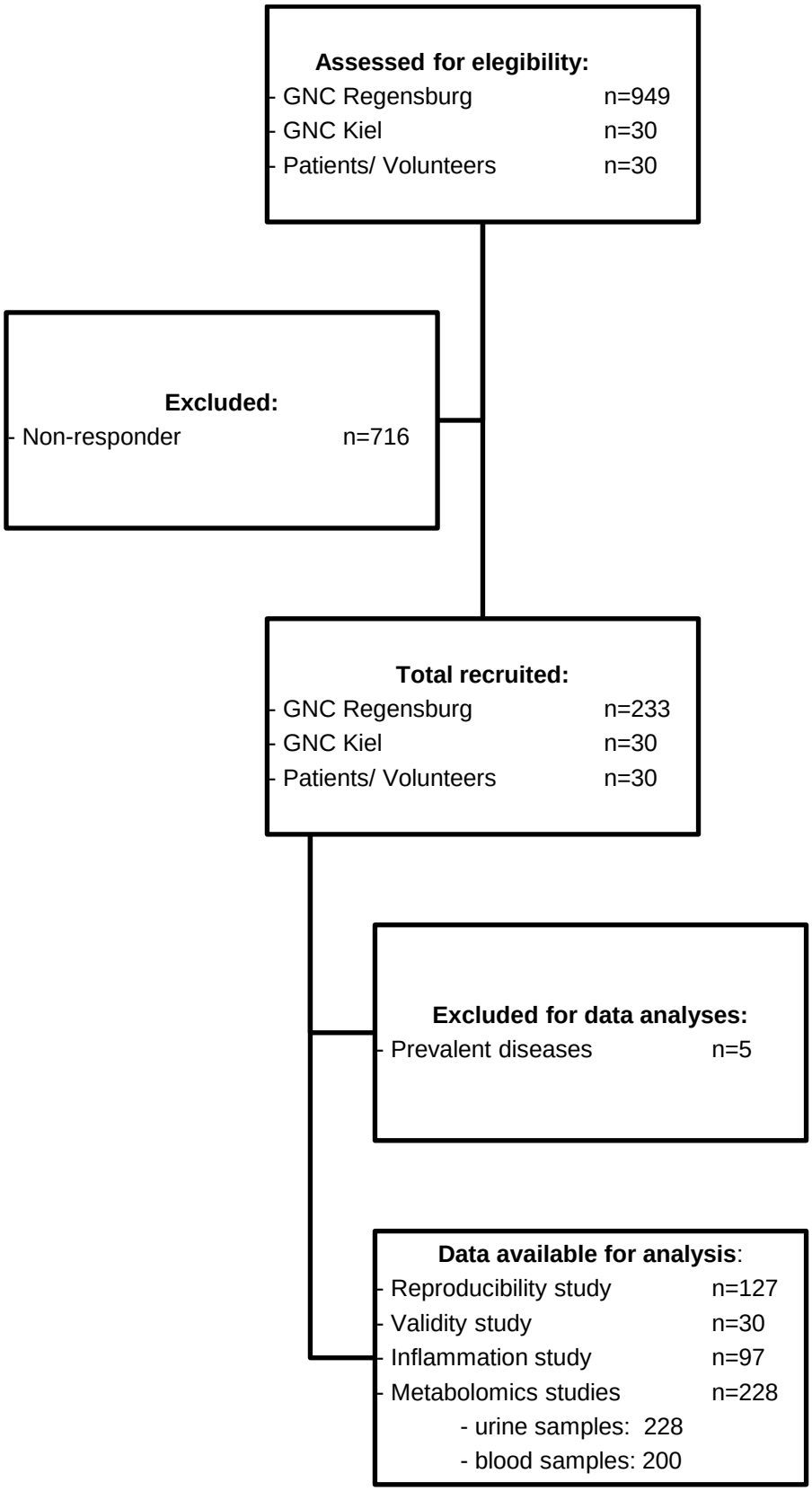

Figure 2: Study population for each objective.

2.2 Measurements

2.2.1 Sonographic-based measurement of visceral and subcutaneous adipose tissue

A standard operation procedure (SOP) (Appendix 1) for the ultrasound measurements was developed based on the protocol introduced by Stolk et al., who reported reproducibility correlation coefficients of 0.97 ($p<0.001$), mean differences of 0.3 cm (±0.6), and a coefficient of variation of 4.3% for the measurement of VAT. They also reported Pearson correlation coefficients of 0.81 ($p<0.001$) for validity of the ultrasound measurement of VAT compared to CT and MRI measurements of VAT (157).

VAT and SAT were quantified using a Mindray DP-50 (Regensburg) or GE Healthcare Logic 700 (Kiel) B-mode ultrasound machine with a 3.5 – 5.0 MHz convex array transducer. Examiners were trained to perform the measurements according to the developed ultrasound SOP. Measurements involved multiple image planes that provided information on adipose tissue thickness. For all images, the transducer was placed on a marked position drawn at the cut-point between the left and right midpoint of the lower rib and the iliac crest on the median line of the abdomen (Figure 3).

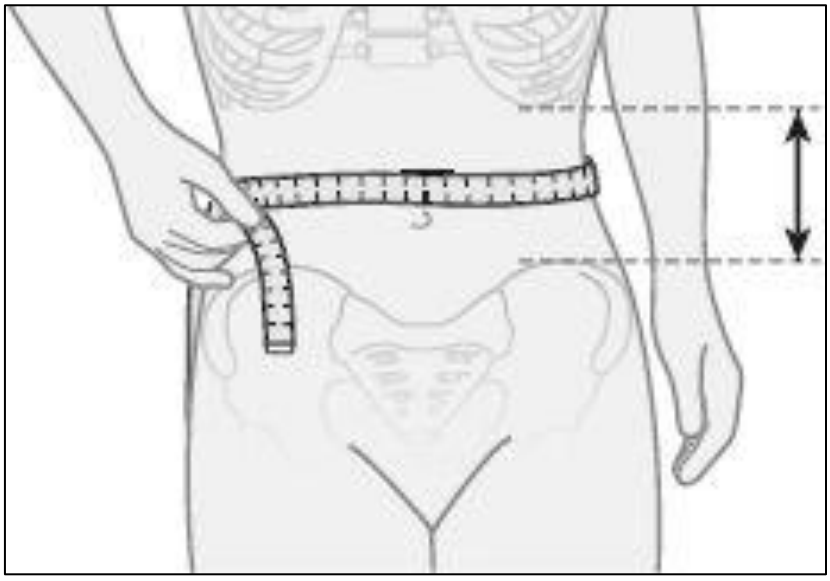

Figure 3: Position for ultrasound measurements and waist circumference measurement.

The measurement of SAT involved one individual image plane at the median line extending from the skin to the linea alba (Figure 4).

Figure 4: Sonographic-based measurement of subcutaneous adipose tissue.

VAT was measured as a second image plane from the linea alba to the lumbar vertebra corpus at the median line (Figure 5).

Figure 5: Sonographic-based measurement of visceral adipose tissue.

All measurements were performed manually by the same examiner at the end of normal expiration applying minimal pressure without displacement of the abdominal contents as observed by the ultrasound image. The parameters assessed were extracted manually from the images with the electronic onboard caliper and stored in a database. Reproducibility of the ultrasonographic quantification of VAT and SAT was assessed by examining each participant twice by two observers, each using the same examination protocol. The examinations were performed consecutively without any other examinations in-between. The second observer was blinded to the results of the first observer. The VSR was calculated by dividing visceral thickness in centimeters by subcutaneous thickness in centimeters.

2.2.2 MRI-based measurement of visceral and subcutaneous adipose tissue

MRI was performed with a clinical whole body 3T system (Magneton Sykra, Siemens Healthcare, Erlangen, Germany). Subjects were placed in supine position and three T1-weighted turbo spin echo, water suppressed, transaxial slices with a thickness of 10 mm were acquired and centered on the L2-L3 vertebral body as well as 10 mm above (L2-L3+) and 10 mm below (L2-L3-) by trained radiographers. The in-plane resolution was 1.3 × 1.3 mm, field of view 500 × 500 mm, repetition time = 400 ms, echo time = 21 ms, 2 averages, 3 concatenations. The software analyze 11.0 (BIR; Mayo Clinic, Rochester, MN) was used to quantify VAT and SAT areas. Areas of VAT and SAT were calculated using a semi-automated method and a threshold map in combination with manual input to distinguish between the VAT and SAT compartments (Figure 6). To avoid inter-reader variation, all images were reviewed and calculations performed by the same person. Because the ultrasound-based parameters are one-dimensional and the MRI measures are two-dimensional, the validity of ultrasound measurement to predict VAT and SAT cannot be directly assessed. Thus, in addition to the calculated area of VAT and SAT, MRI images of VAT and SAT thicknesses were determined using the *MicroDicom Viewer*.

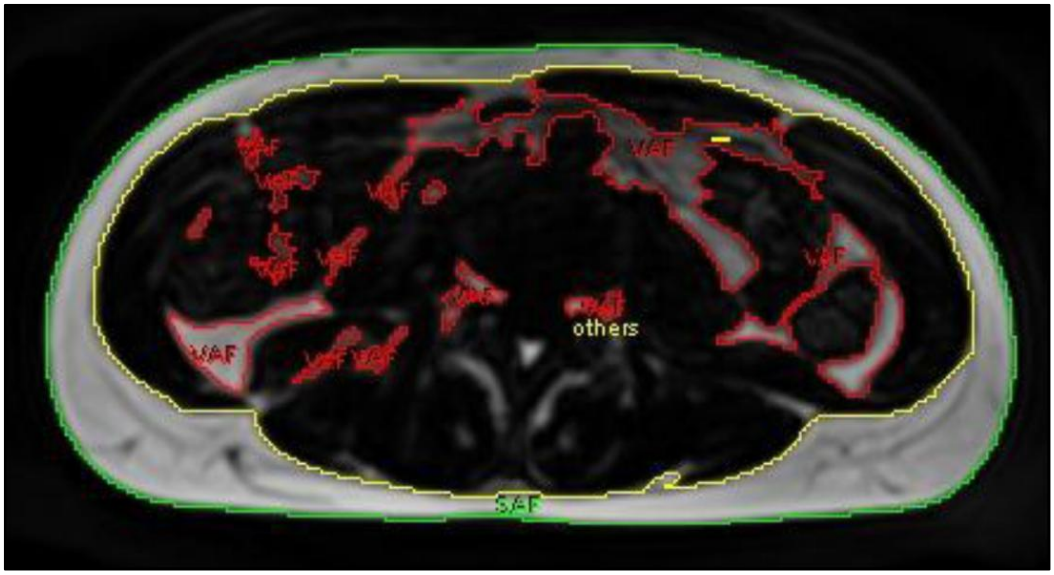

Figure 6: MRI-based measurement of visceral and subcutaneous adipose tissue.
VAF=visceral adipose tissue (red circled); SAF=subcutaneous adipose tissue (green and yellow circled).

2.2.3 Anthropometric measurements

Height in centimeters and weight in kilograms were measured with two decimal places using a digital measuring station (seca285 measuring station, SECA, Hamburg, German). Participants were wearing underwear without shoes and were positioned on the measurement station according to the GNC anthropometric SOP. BMI was calculated by dividing body weight in kilograms by height in meters squared. WC in centimeters was measured using an inelastic tape (SECA measuring tape 201, SECA, Hamburg, Germany) with two decimal places and was measured at the mid-point between the lower rib and the iliac crest. Measurements were taken with the participant standing in an upright position (Figure 3).

2.2.4 Blood and urine measurements

Venous blood was drawn by qualified medical staff. Blood was immediately fractionated into serum, plasma, buffy coat, and erythrocytes, and aliquoted into specimens of 0.5 mL each according to a standardized protocol. During blood withdrawal and processing, time and room temperature were steadily documented. The serum specimens were tilted twice and kept at room temperature for 30 minutes before they were centrifuged by 2500g for exactly 10 minutes at 15°C. Serum aliquots of 0.5 mL each were stored at -80°C. Serum concentrations of TNF-α, IL-6, resistin, and

adiponectin were measured by an external laboratory (Synlab, Labordienstleistungen, Labor Zentrum München) using an enzyme linked immunosorbent assay (ELISA) (Immundiagnostik, Bensheim, Germany), hs-CRP was determined by immunonephelometry (Behring Nephelometer II, Dade Behring, Marburg, Germany), and creatine was measured using a calorimetric method according to the Jaffé reaction. Midstream urine specimens were collected from participants and centrifuged by 2000xg for exactly 10 minutes at 15°C. Supernatant urine was immediately stored at -80°C.

2.2.5 NMR Analyses

Four hundred microliters of urine were mixed with 200 µL of phosphate buffer, pH 7.4, and 50 µL of D_2O containing 0.75 (w%) 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate (TSP) as internal standard. Serum specimens (0.5 mL) were ultrafiltered at 4000xg for 60 minutes at 8°C using Millipore Amicon 10kDa-Filters, which had been prewashed once with 3 mL of distilled water by centrifugation at 4000g for 30 minutes at 22°C to remove filter preservatives. Subsequently, 400µL of the serum ultrafiltrate were mixed with 200 µL of phosphate buffer, pH 7.4, and 50 µL of D_2O containing 0.75 (w%) TSP. NMR experiments were carried out on a Bruker Avance III 600 MHz spectrometer employing a triple-resonance (^{1}H, ^{13}C ^{31}P, ^{2}H lock) cryogenic probe equipped with *z*-gradients and an automatic sample changer. All spectra were acquired following established protocols (163). For each sample, the probe was automatically locked, tuned, matched, and shimmed, using shim files optimized for urine and serum as starting point for the automated shimming procedure.

All spectra were measured at 298 K, and every sample was allowed to equilibrate for 5 minutes in the magnet before measurement. 1D ^{1}H and 2D $^{1}H-^{13}C$ heteronuclear single quantum coherence (HSQC) spectra of each sample were automatically collected using the Bruker automated acquisition suite ICON-NMR. 1D ^{1}H NMR spectra were obtained using a 1D nuclear Overhauser enhancement spectroscopy (NOESY) pulse sequence with presaturation during relaxation and mixing time and additional spoil gradients for water suppression. 1D spectra were automatically Fourier transformed and phase corrected, applying a line broadening of 0.3 Hz and zero filling to 128k points. A flat baseline was obtained by using the “baseopt” option of the program TopSpin, which performs a correction of the first points of the free induction decay (FID). For the 2D

HSQC spectra, water suppression was achieved using presaturation during the relaxation delay. 2D spectra were semi-automatically processed employing a 90^{o} shifted squared sine-bell window function in both dimensions. For increased resolution in the indirect dimension, forward linear prediction was used to double the number of data points prior to Fourier transformation. Spectra were manually phase corrected and a polynomial baseline correction was applied. All spectra were chemical shift referenced relative to the TSP signal.

Signal assignment was performed by overlaying 1D and 2D spectra with reference spectra of pure compounds taken in most cases from the commercially available Bruker Biofluid Reference Compound Database BBIOREFCODE 2-0-3. Assignments were validated by high-resolution 2D ^{1}H-^{13}C HSQC, 2D ^{1}H-^{13}C HMBC and 2D ^{1}H-^{1}H TOCSY spectra.

2.2.6 Targeted metabolomics approach

Employing the Analytical Profiler module of AMIX 3.9.13 (BrukerBioSpin), 30 urine and 20 serum metabolites were quantified by integrating well-resolved signals of these metabolites in the acquired 2D ^{1}H-^{13}C HSQC and 1D ^{1}H spectra relative to the TSP reference signal. From the obtained relative integrals, absolute concentrations were calculated employing individual peak calibration factors using the NMR quantification tool MetaboQuant (164). All metabolites quantitated yielded concentration values at or above the individual lower limits of quantification (LLOQ) for at least 10% of the spectra. For a detailed list of the 30 urinary metabolites quantitated and the corresponding information for the 20 serum metabolites can be found in Table 2 and Table 3**.**

Table 2: Sex-dependent urinary metabolite concentrations.

Metabolite (µmol/mmol creatinine)	LLOQ	Total conc. range min	Total conc. range max	N/228	Men conc. range min	Men conc. range max	N/107	Women conc. range min	Women conc. range max	N/121
[2]Alanine	0.078	6.52	76.70	227	6.52	76.70	106	6.91	70.73	121
[2]Glycine	0.156	13.06	923.12	204	28.99	341.51	92	13.06	923.12	112
[2]Taurine	0.312	17.44	324.20	88	17.44	186.10	60	18.71	324.20	28
[2]Glutamine	0.312	19.10	98.87	80	19.83	82.58	49	19.10	98.87	31
[2]3-Methylhistidine	0.078	7.25	650.17	125	7.25	406.68	68	9.84	650.17	57
[2]Betaine	0.078	2.76	109.74	112	6.14	54.68	63	2.76	109.74	49
[2]Phenylcompound*	0.141	17.12	416.80	154	17.12	416.80	71	21.16	274.71	83
[2]Serine	0.281	13.18	323.41	65	27.77	323.41	42	13.18	139.48	23
[2]D-Glucose	0.563	36.27	593.79	45	40.14	593.79	26	36.27	467.01	19
[2]Choline^{+}	0.141	6.71	97.19	90	10.93	97.19	59	6.71	55.48	31
[2]Lactic acid	0.078	5.94	954.05	97	9.04	512.95	44	5.94	954.05	53
[2]Methanol	0.141	4.17	217.43	78	8.42	155.69	31	4.17	217.43	47
[2]Ascorbic acid	0.312	13.15	1125.48	55	17.92	1125.48	27	13.15	569.02	28
[2]Creatine	0.156	7.83	1131.94	68	7.83	391.44	22	11.07	1131.94	46
[2]L-Pyroglutamic acid	0.312	12.98	106.57	32	16.48	59.15	16	12.98	106.57	16
[2]Hippuric acid	0.312	20.02	1947.11	208	20.02	1467.01	93	25.43	1947.11	115
[2]Ethanolamine	0.312	14.69	190.90	101	18.45	95.71	57	14.69	114.18	44
[2]Trimethylamine-N-oxide	0.039	7.40	494.26	210	7.40	494.26	100	10.84	166.39	110
[2]Citricacid	0.312	50.52	838.99	212	54.30	654.03	94	50.52	838.99	118
[2]Dimethylamine	0.156	19.26	98.70	130	20.90	98.70	76	19.26	77.10	54
[2]D-Mannitol	0.268	21.46	1138.04	130	21.46	1138.04	66	21.99	717.36	64
[2]Guanidinoacetic acid	0.312	16.43	171.40	78	16.43	104.02	38	16.75	162.41	40
[2]Glycolic acid	0.312	23.78	137.74	97	36.03	114.23	59	23.78	137.74	38
[1]Formic acid	0.003	3.97	188.19	227	4.02	59.89	106	3.97	188.19	121
[1]Tyrosine	0.002	0.82	31.53	196	2.09	31.53	95	0.82	24.85	101

Table 2 continued: Sex-dependent urinary metabolite concentrations.

Metabolite (µmol/mmol creatinine)	LLOQ	Total conc. range min	Total conc. range max	N/228	Men conc. range min	Men conc. range max	N/107	Women conc. range min	Women conc. range max	N/121
[1]Fumaric acid	0.002	0.08	4.36	107	0.08	1.94	50	0.13	4.36	57
[1]Orotic acid	0.002	0.11	12.32	68	0.11	12.32	38	0.15	1.45	30
[1]Leucine	0.002	0.54	11.56	217	0.54	7.35	106	1.13	11.56	114
[1]N,N-Dimethylglycine	0.020	2.38	17.99	157	2.50	13.40	80	2.38	17.99	77
[1]Trigonelline	0.020	0.87	187.33	196	0.87	133.54	90	1.11	187.33	106

All metabolites were quantified by 1D or 2D nuclear magnetic resonance spectroscopy: [1]Metabolites quantified from 1D spectra, [2]Metabolites quantified from 2D spectra, LLOQ=lower limit of quantification, conc.=concentration, min=minimum, max=maximum, N=number of values above LLOQ.
*Concentrations are influenced by Phenylalanine, Phenylacetyl-glycine und Phenylacetyl-glutamine.
[+]Choline concentrations are likely to be influenced by other compounds including creatinine.

Table 3: Sex-dependent serum metabolite concentration.

Metabolite (µmol/L)	LLOQ	Total conc. range min	Total conc. range max	N/200	Men conc. range min	Men conc. range max	N/90	Women conc. range min	Women conc. range max	N/110
[2]Alanine	0.078	144.94	596.96	200	144.94	596.96	90	174.20	576.65	110
[2]Glycine	0.156	157.63	535.34	157	158.13	535.34	71	157.63	445.24	86
[2]Glutamine	0.312	316.92	800.05	186	322.97	718.42	81	316.92	800.05	105
[2]D-glucose	0.563	2614.40	10283.96	200	2628.09	10006.38	90	2614.40	10283.96	110
[2]Lactic acid	0.078	875.12	3865.71	200	937.85	3205.56	90	875.12	3865.71	110
[2]Methanol	0.141	142.84	401.21	105	144.98	401.21	55	142.84	364.13	50
[1]L-isoleucine	0.002	24.06	120.62	200	24.06	120.62	90	29.57	119.39	110
[1]Threonine	0.002	26.28	205.32	200	47.30	200.00	90	26.28	205.32	110
[1]Valine	0.002	118.01	348.86	200	118.01	346.28	90	146.22	348.86	110
[1]Acetone	0.002	5.60	197.62	200	5.60	197.62	90	8.11	106.92	110
[1]Formic acid	0.003	5.23	63.52	200	5.23	41.78	90	5.72	63.52	110
[1]Tyrosine	0.002	23.81	123.39	200	26.03	123.39	90	23.81	109.13	110
[1]Pyruvic acid	0.001	8.33	132.23	200	8.33	64.89	90	9.53	132.23	110
[1]Creatinine	0.003	35.33	148.86	200	35.55	120.32	90	35.33	148.86	110
[1]Acetic acid	0.003	7.79	87.69	200	8.96	87.69	90	7.79	83.55	110
[1]Creatine	0.005	6.36	148.87	200	6.36	123.24	90	7.06	148.87	110
[1]Ketoleucin	0.001	2.58	11.00	150	2.63	10.69	70	2.58	11.00	80
[1]Phenylalanine	0.003	13.63	72.95	184	14.21	71.39	83	13.63	72.95	101
[1]Leucine	0.003	45.78	202.29	200	45.78	202.29	90	55.84	189.02	110
[1]3-Hydroxybutyric acid	0.002	9.48	978.88	189	9.48	978.88	87	10.42	329.56	102

All metabolites were quantified by 1D or 2D nuclear magnetic resonance spectroscopy: [1]Metabolites quantified from 1D spectra, [2]Metabolites quantified from 2D spectra, LLOQ=lower limit of quantification, conc.=concentration, min=minimum, max=maximum, N=number of values above LLOQ.

2.2.7 Untargeted metabolomics approach

The untargeted approach involves a large number of metabolites and does not depend upon a priori assumptions. Urinary and serum data sets were subjected to a comparable pre-processing routine, starting with equidistant binning of the 1D ^{1}H spectra to compensate for slight shifts in signal positions across spectra due to small variations in sample pH, salt concentration, or temperature. The spectral regions 9.5−0.5 ppm were evenly split into bins of 0.01 ppm, employing Amix 3.9.13 (BrukerBioSpin). The region between 6.2−4.6 ppm, which contains the broad urea and water signals, was excluded, resulting in a total of 740 spectral bins. After exclusion of additional bins that had yielded in less than 90% spectra signals, a total of 701 urinary bins remained. In case of serum, the region corresponding to residual glycerol, which served as a filter preservative (3.81–3.76 ppm, 3.68–3.52 ppm), was excluded in addition, resulting in 678 bins. All urine metabolites were scaled and normalized to creatinine, and serum samples were scaled and normalized to the reference signal (TSP) to remove disturbing technical or biological variances.

Chemical compounds in biological specimens were identified by their characteristic peak patterns and signal positions in the NMR spectra using reference spectra from the commercially available Bruker Biofluid Reference Compound Database BBIOREFCODE 2-0-3 and the Human Metabolome Database (HMDB). The HMDB is an extensive, freely available electronic database that stores >40,000 different metabolite entries, with exhaustive biological metadata and NMR spectral references (165). Bins were identified when relations of adiposity measures to urinary or serum bins were statistically significant and reproduced in sensitivity analyses. Confirmation of metabolite identification was achieved by spike-in experiments. Specifically, 2.5 millimolar standard solutions were added to serum or urine and spectra checked for increase of the corresponding signals. In addition, concentrations of well-resolved compounds were calculated using calibration curves that had been generated from serially diluted external standards over a concentration range of 0.0012 – 10.0 mmol/L. For compounds like urinary creatinine that exceed this concentration range in some instances spike-in experiments were used to verify that measurements were still in the linear range.

2.3 Covariate assessment

Data were collected by certified personnel with respect to participants' socio-demographic characteristics, medical history, health related behavior, and lifestyle factors. Potential confounding variables including age, sex, current smoking status, physical activity, fasting status (overnight fasting), use of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) (WHO Anatomical Therapeutic Chemical (ATC) Classification System codes: A01AD05, B01AC06, N02BA01), and menopausal status (women only) were assessed by standardized computer-assisted personal interviews. Physical activity levels were calculated from Metabolic Equivalents of Task (METs) by a 24-hour physical activity recall (166). The estimated glomerular filtration rate (eGFR) was calculated from serum creatinine using the four-variable Modification of Diet in Renal Disease (MDRD4) (167). Overnight fasting status (yes or no) was determined from self-reports of the participants before collecting urine and blood specimens.

2.4 Statistical analyses

For each study aim, descriptive statistics were used to characterize the study population. Arithmetic means (and standard deviations) were provided for continuous variables and percent values for categorical variables. In addition, the target population of the inflammation study was directly standardized to the age distribution of the study population and stratified by VAT and SAT tertiles. Differences between men and women, lean (BMI≤25.0 kg/m²) and overweight/obese (BMI>25.0 kg/m²) individuals, smokers and non-smokers, users and non-users of aspirin or NSAIDs, fasting and non-fasting participants, subjects with elevated urinary glucose and subjects with no urinary glucose were tested using Kruskal-Wallis and χ^2 tests. In sensitivity analyses, age and sex interaction was examined by fitting multiplicative interaction terms in the regression models and testing their significance using likelihood ratio tests. All reported p values are two-tailed and values <0.05 were deemed statistically significant. IBM SPSS 19.0 and the statistical software R version 3.1.2 were used for analyses.

2.4.1 Reproducibility study

The test-retest reliability of ultrasound-based measurements of VAT and SAT was quantified using intra-class correlation coefficients (ICC) with 95% confidence intervals

(CI), estimated using a linear random effects model (168). The intra-rater reproducibility for VAT and SAT was assessed by comparing the mean of the two measurements from image 1 and the mean of the two measurements from image 2. Inter-rater reproducibility was calculated by comparing the mean of 4 measurements from two images by observer 1 and the mean of 4 measurements from two images by observer 2. Results were classified as high, modest, and low when ICC≥0.8, ICC≥0.7-0.79 and ICC≤0.69, respectively (169). The Bland-Altman method was used to illustrate the difference between the two measurements for each sample (vertical axis) and the average of the two measurements (horizontal axis) (170). In addition, three horizontal reference lines represent the mean difference between the measurements and the upper and lower control limits of the standard deviation. Results were regarded as strong agreement of intra- and inter-rater reproducibility when 5% or fewer values were seen outside the upper and lower levels of agreement. Findings were rated as good agreement when 10% or fewer values were seen outside the upper and lower levels of agreement (171).

2.4.2 Validity study

The validity of the sonographic-based quantification of VAT and SAT was assessed by comparing VAT and SAT thicknesses with VAT and SAT areas at vertebrae L2/L3 as obtained by three single MRI slices. In addition to the calculated area of VAT and SAT, thicknesses of MRI measured VAT and SAT were also compared to ultrasound measured VAT and SAT thicknesses. The VAT and SAT thicknesses on the MRI slice and those for the ultrasound measures were based on the same anatomical landmarks. Validity was examined calculating ICCs with 95% CI and Bland-Altman plots were produced when possible to show the difference between the two methods of measuring VAT and SAT thicknesses with ultrasound and MRI. For the evaluation of the ICCs with 95% CI and Bland-Altman plots, the same classification as in the reproducibility study was applied (high: ICC≥0.8; modest: ICC≥0.7-0.79; low: ICC≤0.69; strong agreement of Bland-Altman plots: disagreement ≤5%; good agreement of Bland-Altman plots: disagreement ≤10%) (169-171). In addition, Spearman's rank ordered correlation coefficient (ρ) was calculated to evaluate the correlation between thicknesses and area parameters of VAT and SAT. Correlation coefficients were classified as high when ρ≥0.8, as modest when ρ≥0.7-0.79, and as low when ρ≤0.69 (169, 172).

2.4.3 Inflammation study

The relation of body composition to parameters of chronic inflammation was examined by associating VAT, SAT, VSR, BMI, and WC with the inflammatory parameters hs-CRP, TNF-α, IL-6, resistin, and adiponectin. To assure that distributions follow an approximate normal distribution, hs-CRP, TNF-α, IL-6, resistin, and adiponectin were $\log_n$ transformed. Partial correlation coefficients were calculated between inflammatory parameters adjusted for age (continuous), sex (men, women), smoking status (current, former, never smokers), physical activity (continuous), menopausal status (pre-, peri-, or postmenopausal), and aspirin or NSAID use (drug use during the past 7 days: yes; no).

Multiple linear regression analysis was performed to estimate relations of VAT, SAT, VSR, BMI, and WC (independent variables) to log hs-CRP, log IL-6, log TNF-α, log resistin, and log adiponectin (dependent variables). Model 1 was adjusted for age (continuous), sex (men, women), smoking status (currently smoking; non-smoking), physical activity (continuous), menopausal status (pre-, peri-, or postmenopausal), and aspirin or NSAID use (drug use during the past 7 days: yes; no). In a second model, all parameters of chronic inflammation were mutually adjusted, in addition to the adjustments described in the first model. In a third model, VAT and SAT were mutually adjusted and BMI and WC were mutually adjusted. Also, exploratory analyses were run stratified by sex, BMI, smoking status, and aspirin or NSAID use. Before fitting the linear regression models, all variables (independent and dependent) were standardized by subtracting the mean and dividing by the standard deviation to assure that effect sizes are comparable across regression models. When reporting the results from the linear regression models, ß-coefficients were considered weak (ß≤0.3), moderate (ß>0.3-≤0.6), or strong (ß>0.6) (173).

2.4.4 Metabolomics studies

An untargeted metabolomics approach was used to detect relations of VAT, SAT, VSR, BMI, and WC, respectively, to individual bins of urinary and serum metabolic fingerprints. For the detection of unbalanced regulation of compounds, the total spectral area for each NMR spectrum was calculated and tested for normal distribution using the Shapiro-Wilk test (174). To reduce heteroscedasticity, urinary and serum metabolic data

were normalized using variance stabilization (VSN) with 60% of the urinary and 65% of the serum features, respectively. To detect general trends in the data and to reduce the large amount of metabolic data, cluster analysis was performed. Thereby, subjects with similar metabolic profiles are grouped into clusters. Subsequently, clusters were investigated to detect whether these clusters, which differ in their metabolic profile, also differ regarding the obese phenotype. Affinity propagation (AP) clustering was used to determine clusters by taking negative squared Euclidean distances as input measures of similarity between pairs of data (175). AP clustering algorithm automatically finds a subset of exemplar points, which can best describe the data groups by exchanging messages. Each data point is assigned to its nearest exemplar with the aim of splitting a whole data set into clusters. Thereby, the maximization of the overall sum of the similarities between data points and their exemplars is obtained. Input preferences were set to q=0.1 to obtain a minimum number of clusters. To detect differences regarding distributions of VAT, SAT, VSR, BMI, and WC between clusters compared to the grand mean of these adiposity measures, marginal effects at the mean for each cluster were estimated using a linear regression model adjusted for age and sex. Predicted mean values of VAT, SAT, VSR, BMI, and WC were plotted for each cluster.

In addition, multiple linear regression analyses were applied to estimate relations of VAT, SAT, VSR, BMI, and WC (independent variables) to urinary and serum bins (dependent variables). In sensitivity analyses, it was examined whether the model fit was improved by inclusion of squared and cubic terms of the independent variables as a test of linearity. Multiple linear regression models were adjusted for study (pre-test I or II), age (10 year age categories) and sex (men, women) (Model 1). In a second model (fully adjusted model), additional adjustments for multiplicative age-sex interaction, smoking status (former, current, never smoking), physical activity level (continuous), menopausal status (pre-, peri-, postmenopausal), fasting status (overnight fasting: yes, no), urinary glucose (elevated glucose: yes, no), and eGFR (continuous) were applied (Model 2). Stratified analyses were performed regarding sex, fasting status, and elevated urinary glucose.

Multiple linear regression models were also used to examine associations of VAT, SAT, VSR, BMI, and WC to the quantified urinary and serum metabolite concentrations using a targeted metabolomics approach. In addition, a third regression model (mutually

adjusted model) was applied to mutually adjust for VAT and SAT, VSR and BMI, and BMI and WC respectively, in addition to the adjustments in Model 2 (Model 3). Prior to further analyses, quantified metabolites were log_2 transformed to achieve normally distributed data and to reduce heteroscedasticity. To account for the considerable amount of values below the LLOQ of some metabolites among the set of quantified urinary and serum metabolites (Tables 2 and 3), linear regression models were only calculated for those metabolites, for which at least 75% of data were available. Otherwise, metabolites were dichotomized as detectable and undetectable and logistic regression models were calculated considering the same adjustment variables as described for linear models. Pearson correlations between all quantified, log_2 transformed metabolites were calculated. Pearson correlation coefficients were considered weak ($r<0.4$), moderate ($r\geq0.4-<0.6$), or strong ($r\geq0.6$) (173). Among serum metabolic data, logistic regression models were additionally calculated to detect differences between the age and sex-specific lower and upper quartiles of VAT, SAT, VSR, BMI, and WC (dependent variables) regarding quantified serum metabolite concentrations (independent variables).

In order to increase the comparability of results from both, targeted and untargeted regression models, all variables (independent and dependent) were standardized by subtracting the mean and dividing by the standard deviation. Regression models were calculated for each bin or metabolite separately. Analyses were corrected for multiple testing. Because the analytical metabolomics dataset includes partly highly correlated metabolites and to reduce the type II error, multiplicity was corrected by controlling the false discovery rate (FDR) according to Benjamini and Hochberg (176) at a level of 5%.

3 Results

3.1 Reproducibility study

The mean age of the population of the reproducibility study from both study centers was 51.15 years and there were 72 women (57%) and 55 men (43%). The mean BMI was 26.54 kg/m² and the mean WC was 91.21 cm. Mean VAT thickness measured by ultrasound was 6.82 cm (±3.47 cm) and the mean thickness of SAT was 1.89 cm (Table 4).

Table 4: Characteristics of the study population.

	Min	Max	Mean (SD)
Age (years)	20	70	51.15 (13.50)
VAT thickness US (cm)	2.93	19.08	6.82 (3.47)
SAT thickness US (cm)	0.52	4.70	1.89 (0.79)
WC (cm)	64.31	124.84	91.21 (14.01)
Hip circumference (cm)	83.03	134.07	101.70 (8.95)
BMI (kg/m²)	17.49	43.75	26.54 (4.70)

US=ultrasound, MRI=magnetic resonance imaging, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, WC=waist circumference, BMI=body mass index, Min=minimal, Max=maximal, SD=standard deviation, n=127.

Results from the reproducibility study are presented in Table 5. For VAT, the ICCs for the intra-rater reproducibility of observers 1 and 2 were 0.996 and 0.999, respectively. The Bland-Altman method for comparing the intra-rater reproducibility of VAT measurements showed a mean difference of -0.219 mm for observer 1 (8.7% disagreement) and a mean difference of -0.087 mm for observer 2 (5.5% disagreement) (Supplementary Figure 1).

Table 5: Intra- and inter-rater reproducibility of ultrasound-based measurements of visceral and subcutaneous fat thickness.

	Intra-rater reproducibility				Inter-rater reproducibility	
	Observer 1		Observer 2		Observer 1 – 2	
	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)
VAT	0.996 (0.994-0.997)	-0.219 (-3.43-2.99)	0.999 (0.999-0.999)	-0.087 (-3.07-2.89)	0.998 (0.997-0.999)	-0.095 (-3.57-3.34)
SAT	0.992 (0.988-0.994)	-0.076 (-2.08-1.99)	0.992 (0.989-0.995)	0.008 (-1.95-1.97)	0.989 (0.985-0.992)	0.168 (-2.13-2.46)

VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, ICC=Intra-class correlation coefficient, CI=confidence interval, LOA=limit of agreement, n=127.

For SAT, the ICCs for the intra-rater reproducibility of observers 1 and 2 were 0.992 and 0.992, respectively. The mean difference of SAT measurements was -0.076 mm for observer 1 (4.7% disagreement) and 0.008 mm for observer 2 (6.3% disagreement) (Table 5). The ICCs for the inter-rater reproducibility for SAT and VAT were 0.989 and 0.998, respectively. The Bland-Altman method for comparing the inter-rater reproducibility showed a mean difference of -0.095 mm for VAT measurements (3.9% disagreement) and a mean difference of 0.168 mm for SAT measurements (5.5% disagreement). Results from the Bland-Altman plots showed no systematic differences for the measurements of VAT and SAT (Figure 7). Results for reproducibility were similar when both study centers were analyzed separately (Supplementary Table 4). Subgroup analyses stratified by BMI, WC, and VAT tertiles showed similar results for intra-and inter-rater reproducibility of VAT and SAT measurements (Supplementary Table 5).

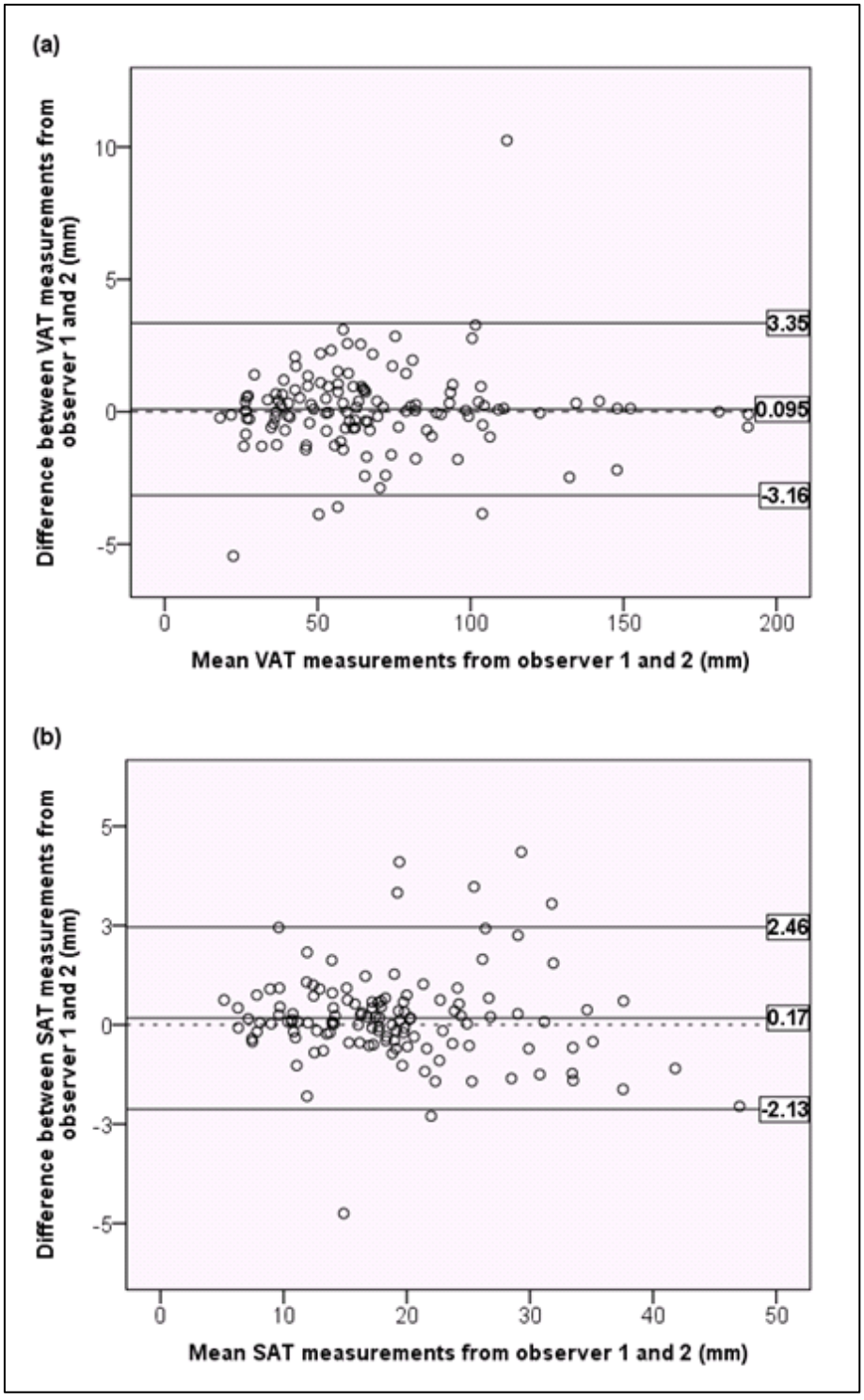

Figure 7: Bland-Altman Plots for the inter-observer agreement between visceral adipose tissue respectively subcutaneous adipose tissue measurements.

(a) Agreement between VAT measurements from observer 1 and 2; (b) Agreement between SAT measurements from observer 1 and 2; VAT=visceral adipose tissue; SAT=subcutaneous adipose tissue, n=127

3.2 Validity study

The mean age of subjects included in the validation study was 43.50 years. There were 16 women (53%) and 14 men (47%), with a mean BMI of 24.87 kg/m² and a mean WC of 86.05 cm. The mean VAT area measured by MRI was 114.05 cm². The mean VAT thickness was 5.89 cm when measured by MRI and it showed the same value of 5.89 cm when measured by ultrasound. The mean SAT area measured by MRI was 130.06 cm². The mean SAT thickness measured by MRI was 1.78cm and it was 1.74 cm when measured by ultrasound (Table 6).

Table 6: Study population characteristics.

	Min	Max	Mean (SD)
Age (years)	20	70	43.50 (16.21)
VAT area MRI (cm²)	22.89	408.94	114.05 (90.82)
VAT thickness MRI (cm)	3.52	12.71	5.89 (2.16)
VAT thickness US (cm)	3.38	13.15	5.89 (2.36)
SAT area MRI (cm²)	35.64	336.74	130.06 (69.98)
SAT thickness MRI (cm)	0.47	3.82	1.78 (0.89)
SAT thickness US (cm)	0.47	3.75	1.74 (0.88)
WC (cm)	67.20	116.50	86.05 (15.01)
Hip circumference (cm)	85.50	116.50	98.87 (6.61)
BMI (kg/m²)	19.48	36.02	24.87 (4.45)

US=ultrasound, MRI=magnetic resonance imaging, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, WC=waist circumference, BMI=body mass index, Min=minimal, Max=maximal, SD=standard deviation, n=30.

The correlation of VAT thickness as measured by ultrasound with VAT area as measured by MRI was 0.898. A comparison of VAT thickness measurements and VAT area, both measured by MRI, resulted in a correlation of ρ=0.863 (Table 7). By comparison, BMI and WC showed similar correlations with VAT area (BMI: ρ=0.841; WC: ρ=0.834). The correlation of SAT thickness measured by ultrasound with SAT area measured by MRI was 0.705. A slightly higher correlation of ρ=0.781 was noted between SAT thickness and SAT area, both measured by MRI. The correlation coefficients of BMI and WC with SAT area were 0.697 and 0.663, respectively.

Table 7: Correlation of MRI-based visceral and subcutaneous adipose tissue areas with ultrasound-based and MRI-based visceral and subcutaneous adipose tissue thicknesses and anthropometric measures of adiposity.

	VAT area MRI (cm²)			SAT area MRI (cm²)	
	ρ	p-value		ρ	p-value
VAT thickness US (cm)	0.898	<0.001	SAT thickness US (cm)	0.705	<0.001
VAT thickness MRI (cm)	0.863	<0.001	SAT thickness MRI (cm)	0.781	<0.001
WC (cm)	0.834	<0.001	WC (cm)	0.663	<0.001
Hip circumference (cm)	0.634	<0.001	Hip circumference (cm)	0.717	<0.001
BMI (kg/m²)	0.841	<0.001	BMI (kg/m²)	0.697	<0.001

US=ultrasound, MRI=magnetic resonance imaging, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, WC=waist circumference, BMI=body mass index, r=Spearman correlation coefficient (ρ), n=30.

A comparison of measurements of VAT thickness by ultrasound and MRI yielded an ICC of 0.990 (Table 8). The Bland-Altman plot showed a mean difference of -0.124 mm (±3.21 mm) for the measurement of VAT. In addition, Bland-Altman plots showed no systematic differences between methods when measuring VAT thickness (Figure 8). By, comparison, when comparing measurements of SAT thickness by ultrasound and MRI, the correlation between the two was 0.976. The Bland-Altman method showed a mean difference of 0.392 mm (±1.96 mm) for the measurement of SAT and no systematic differences between both methods measuring SAT thickness.

Table 8: Intra-class correlation between measurements of visceral respectively subcutaneous adipose tissue thickness by ultrasound.

	ICC (95% CI)	Mean difference (mm) (95% LOA)
VAT thickness (US/MRI)	0.990 (0.979-0.995)	-0.124 (-6.42-6.17)
SAT thickness (US/MRI)	0.976 (0.950-0.988)	0.392 (-3.45-4.24)

US=ultrasound, MRI=magnetic resonance imaging, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, ICC=Intra-class correlation coefficient, CI=confidence interval, LOA=limit of agreement, n=30.

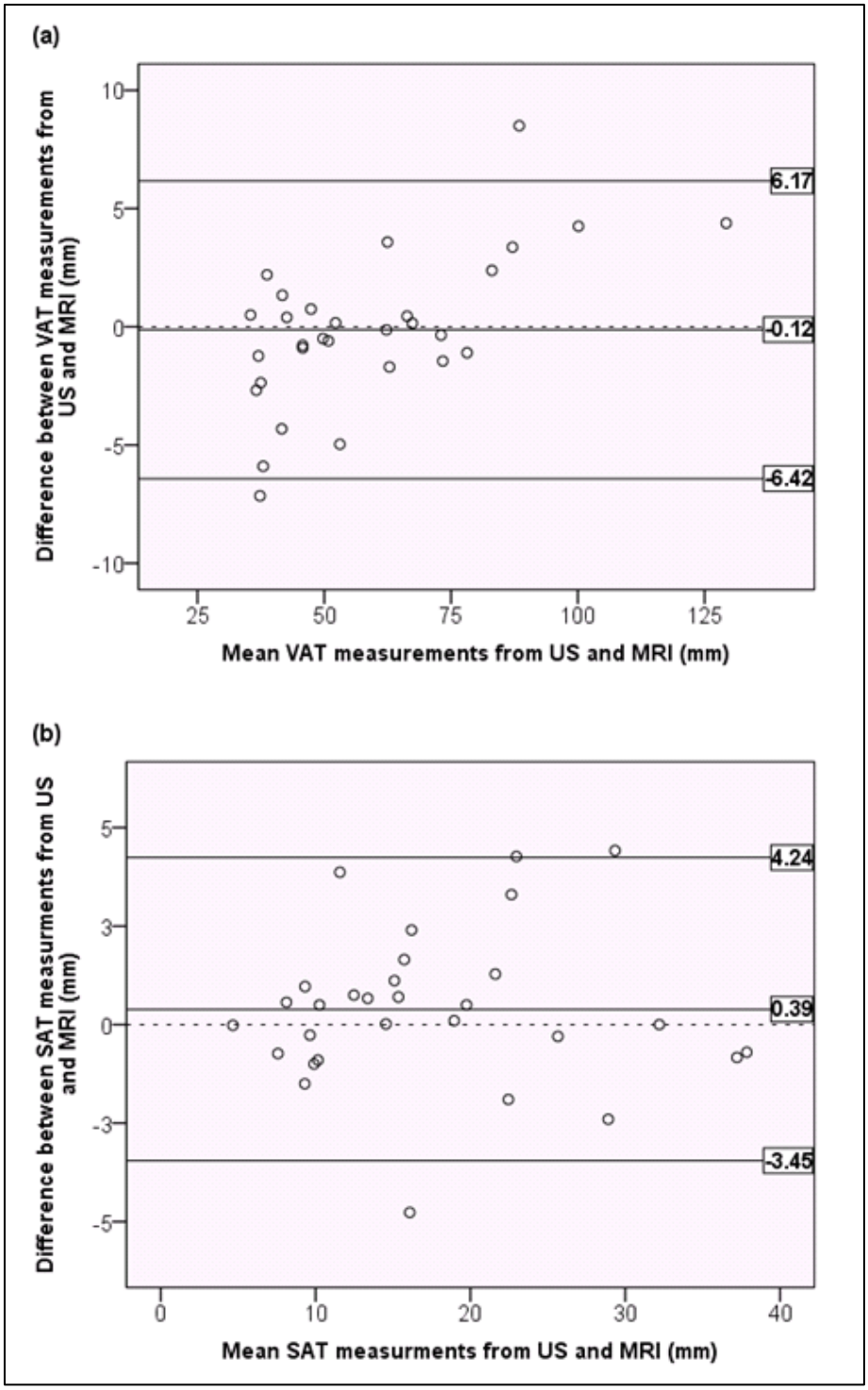

Figure 8: Bland-Altman Plots on the agreement between MRI and US measurements of visceral and subcutaneous adipose tissue.

(a) Agreement between measurements of VAT thickness by US and MRI; (b) Agreement between measurements of SAT thickness by US and MRI; VAT=visceral adipose tissue; SAT=subcutaneous adipose tissue; US=ultrasound; MRI=magnetic resonance spectroscopy; n=30.

Subgroup analyses stratified by BMI showed slightly weaker correlations in overweight/obese than lean subjects for the measurements of VAT (overweight/obese: ρ=0.643, lean: ρ=0.777) and SAT (overweight/obese: ρ=0.594; lean: ρ=0.802) (Supplementary Table 6-11). By comparison, the correlation between WC and VAT area was stronger among overweight/obese (ρ=0.783) than among lean subjects (ρ=0.383). Similar relations were found between BMI and VAT area among overweight/obese (ρ=0.587) and lean (ρ=0.480) subjects. Relations between WC and SAT area were weaker among overweight/obese (ρ=0.070) and lean subjects (ρ=0.398). Also, relations between BMI and SAT area were weaker in the group of overweight/obese (ρ=0.189) than in lean subjects (ρ=0.527).

3.3 Inflammation study

Characteristics of the study population are presented in Table 9 and Table 10. The mean age of study participants was 53.49 years. Compared to those in the lower VAT tertile, participants in the upper VAT tertile were older (p=0.008), had a higher VSR (p<0.001), BMI (p<0.001), and a higher WC (p<0.001) Table 9. There were no differences in physical activity levels, smoking, or use of aspirin or NSAIDs according to VAT. Participants in the upper SAT tertile were more likely to have higher levels of BMI (p<0.001) and WC (p<0.001) but a lower VSR (p<0.001). There were no differences in smoking status, physical activity levels, or use of aspirin or NSAIDs according to SAT.

Table 9: Characteristics of participants according to tertiles of visceral and subcutaneous adipose tissue.

Characteristic	VAT				SAT			
	T1 (≤5.66 cm)	T2 (5.67-≤8.06 cm)	T3 (≥8.07 cm)	p value	T1 (≤1.62 cm)	T2 (1.63-≤2.14 cm)	T3 (≥2.15 cm)	p value
N	32	33	32		32	33	32	
Age (years)	48	55	58	0.008	49	59	52	0.003
Sex (% men)	29	46	59	0.074	47	44	35	0.719
VSR	3.1	3.7	5.7	<0.001	5.9	4.3	3.0	<0.001
BMI (kg/m²)	23.5	26.1	31.6	<0.001	24.0	27.6	29.4	<0.001
WC (cm)	80.9	90.1	105.0	<0.001	84.1	92.6	97.6	<0.001
Smoking (%)	24	19	19	0.923	24	23	14	0.788
Physical activity level	1.7	1.7	1.7	0.903	1.7	1.8	1.7	0.716
Use of aspirin or NSAIDs (%)	5	21	21	0.165	11	19	16	0.634

Entries are mean (standard deviation) for continuous variables and percentages for categorical variables. VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, VSR=visceral-to-subcutaneous-fat ratio, BMI=body mass index, WC=waist circumference, T1-3=tertiles of VAT, respectively SAT, n=97, p-value from Kruskal-Wallis test (continuous variables) or X^2 test (categorical variables).

The mean concentrations of selected parameters of chronic inflammation were similar in men and women, with the exception of adiponectin, which was higher in women than men (p=0.003) (Table 10). In addition, differences between lean and obese subjects were found for hs-CRP (p<0.001) and IL-6 (p=0.020) concentrations. Smokers and non-smokers showed differences in mean hs-CRP (p=0.031) and adiponectin (p=0.047) concentrations, and aspirin or NSAID users differed in their mean hs-CRP concentration (p=0.009) (Supplementary Table 12).

Table 10: Sex-dependent distribution of age, anthropometric variables, and concentrations of inflammatory markers.

	Total Mean (SD)	Women Mean (SD)	Men Mean (SD)	p-value
Age (years)	53.49 (12.41)	55.55 (10.18)	50.81 (14.52)	0.188
VAT (cm)	7.48 (3.48)	6.79 (3.22)	8.39 (3.64)	0.009
SAT (cm)	1.98 (0.82)	2.05 (0.77)	1.89 (0.89)	0.207
VSR	4.27 (2.47)	3.61 (2.12)	5.12 (2.64)	0.001
BMI (m/kg²)	26.94 (4.68)	26.46 (4.91)	27.57 (4.39)	0.201
WC (cm)	91.51 (13.86)	86.60 (12.30)	97.83 (13.30)	<0.001
hs-CRP (mg/dl)	0.17 (0.19)	0.15 (0.12)	0.20 (0.26)	0.878
TNF-α (pg/ml)	6.49 (2.31)	6.18 (2.10)	6.89 (2.53)	0.101
IL-6 (pg/ml)	2.67 (3.76)	2.42 (2.48)	3.00 (4.97)	0.051
Resistin (ng/ml)	3.56 (1.12)	3.49 (1.16)	3.65 (1.07)	0.457
Adiponectin (µg/ml)	12.00 (5.28)	14.04 (5.25)	9.34 (4.04)	0.003

Entries are mean value (standard deviation). VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, VSR=visceral-to-subcutaneous-fat ratio, BMI=body mass index, WC=waist circumference, n=97 (women: n=55; men: n=42), p-value from Kruskal-Wallis test.

Hs-CRP was positively correlated with TNF-α (r=0.21; p=0.04) but was not correlated with IL-6, resistin, or adiponectin (Table 11). After adjustment for age, sex,

smoking, physical activity, menopausal status and aspirin or NSAID use, the only significant partial correlation that was found was between adiponectin and TNF-α (r=0.31; p=0.02).

Table 11: Correlation matrix with selected parameters of systemic chronic inflammation.

	log hs-CRP	log TNF-α	log IL-6	log Resistin
log hs-CRP				
Model 1	1			
Model 2	1			
log TNF-α				
Model 1	**r=0.21, p=0.04**	1		
Model 2	r=0.18, p=0.15	1		
log IL-6				
Model 2	r=0.16, p=0.11	r=0.10, p=0.34	1	
Model 2	r=0.11, p=0.42	r=-0.07, p=0.58	1	
log Resistin				
Model 2	r=0.10, p=0.30	r=0.09, p=0.37	r=-0.01, p=0.90	1
Model 2	r=0.09, p=0.52	r=-0.01, p=0.97	r=0.03, p=0.84	1
log Adiponectin				
Model 1	r=-0.14, p=0.16	r=0.06, p=0.53	r=-0.00, p=0.97	r=0.06, p=0.56
Model 2	r=-0.11, p=0.41	**r=0.31, p=0.02**	r=0.02, p=0.89	r=0.08, p=0.53

Model 1: Pearson correlation between parameters of systemic chronic inflammation.
Model 2: Pearson correlation between parameters of systemic chronic inflammation adjusted for age, physical activity level, sex, menopausal status (women only), smoking, and aspirin or NSAID use.

Multiple linear regression analyses adjusted for age, physical activity level, sex, smoking, menopausal status, and aspirin or NSAIDs but unadjusted for parameters of systemic chronic inflammation (Model 1) showed that VAT was positively associated with hs-CRP (ß=0.12; p=0.004), IL-6 (ß=0.33; p=0.002) and inversely associated with adiponectin (ß=-0.20; p=0.048) (Table 12). SAT was positively associated with hs-CRP (ß=0.60; p=0.0005) but it was unrelated to any of the other parameters. VSR was inversely related to resistin (ß=-0.31; p=0.023). No associations were found between VSR and any of the other parameters. BMI showed positive associations with hs-CRP (ß=0.39; p=0.004) and IL-6 (ß=0.22; p=0.034). An inverse association was found

between BMI and adiponectin (ß=-0.32; p=0.002). WC was positively associated with hs-CRP (ß=0.46; p=0.001) and inversely associated with adiponectin (ß=-0.28; p=0.012).

Table 12: Relations of adiposity measures to inflammatory parameters.

	VAT		SAT		VSR		BMI		WC	
	ß	p	ß	p	ß	p	ß	p	ß	p
log hs-CRP										
Model 1	**0.12**	**0.004**	**0.60**	**0.0005**	-0.06	0.657	**0.39**	**0.004**	**0.46**	**0.001**
Model 2	**0.33**	**0.02**	**0.42**	**0.001**	-0.08	0.608	**0.36**	**0.013**	**0.41**	**0.006**
Model 3	0.17	0.25	**0.35**	**0.01**	-0.19	0.183	0.05	0.88	0.36	0.26
log TNF-α										
Model 1	0.14	0.31	-0.07	0.61	0.13	0.263	0.01	0.94	0.08	0.61
Model 2	0.16	0.27	-0.11	0.44	0.13	0.244	0.05	0.73	0.07	0.69
Model 3	0.22	0.15	-0.19	0.22	0.13	0.269	-0.05	0.88	0.11	0.76
log IL-6										
Model 1	**0.33**	**0.002**	0.13	0.22	0.02	0.910	**0.22**	**0.034**	0.14	0.32
Model 2	0.22	0.12	0.17	0.23	0.05	0.745	0.11	0.44	0.13	0.42
Model 3	0.18	0.24	0.10	0.50	0.01	0.942	0.03	0.92	0.10	0.77
log Resistin										
Model 1	-0.09	0.49	0.15	0.26	**-0.31**	**0.023**	-0.05	0.73	-0.05	0.74
Model 2	-0.14	0.38	0.15	0.33	**-0.31**	**0.030**	-0.07	0.66	-0.09	0.62
Model 3	-0.22	0.20	0.22	0.18	**-0.32**	**0.034**	0.04	0.91	-0.13	0.74

Table 12 continued: Relations of adiposity measures to inflammatory parameters.

	VAT		SAT		VSR		BMI		WC	
	ß	p	ß	p	ß	p	ß	p	ß	p
log Adiponectin										
Model 1	**-0.20**	**0.048**	-0.15	0.12	0.03	0.806	**-0.32**	**0.002**	**-0.28**	**0.012**
Model 2	-0.19	0.17	-0.12	0.37	-0.01	0.978	**-0.28**	**0.035**	-0.18	0.24
Model 3	-0.16	0.26	-0.06	0.68	0.09	0.493	**-0.58**	**0.035**	0.39	0.19

Model 1 was adjusted for age, physical activity level, sex, smoking, menopausal status (women only), and aspirin or NSAID use.
Model 2 was adjusted for age, physical activity level, sex, smoking, menopausal status (women only), aspirin or NSAID use, and mutually adjusted for all parameters of systemic chronic inflammation.
Model 3 was adjusted for age, physical activity level, sex, smoking, menopausal status (women only), aspirin or NSAID use, and mutually adjusted for all parameters of systemic chronic inflammation and mutually for VAT and SAT, VSR and BMI, and BMI and WC respectively. VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, VSR=visceral-to-subcutaneous-fat ratio, BMI=body mass index, WC=waist circumference. ß=standardized linear regression coefficient, p=p-value.

After mutual adjustment of parameters of systemic chronic inflammation (Model 2), VAT remained significantly associated with hs-CRP but not with IL-6 or adiponectin (Table 12). Likewise, SAT was associated with hs-CRP after adjustment for the other inflammatory parameters. VSR remained significantly inversely related to resistin and BMI remained significantly associated with hs-CRP and adiponectin. WC remained significantly associated with hs-CRP. When VAT and SAT were simultaneously included in the model (Model 3), only SAT remained significantly associated with hs-CRP. After mutually adjusting for VSR and BMI, VSR remained significantly inversely related to resistin when BMI was additionally included in the model. When BMI and WC were simultaneously included in the model, BMI remained significantly inversely related to adiponectin.

Results from sex-stratified analyses are presented in Table 13. In women, an inverse association was noted between BMI and adiponectin (ß=-0.42; p=0.045). In men, VAT and BMI were positively related to IL-6 (ß=0.47; p=0.01 and ß=0.36; p=0.049, respectively). No relations were observed between VAT, SAT, VSR, BMI, or WC and other inflammatory parameters in men or women, although some sex differences for all inflammatory parameters were noted. With the exception of SAT and VSR, relations of VAT, BMI, and WC to hs-CRP appeared to be stronger in women than men. VAT, SAT, VSR, BMI, and WC were inversely related to TNF-α and to IL-6 in women, whereas in men only SAT was inversely related to TNF-α. Relations of VAT, SAT, VSR, BMI, and WC to IL-6 were stronger in men than women.

Table 13: Associations between adiposity measures with parameters of systemic chronic inflammation in subgroups defined by sex, BMI, smoking status, and use of aspirin or NSAIDs.

	Women (n=55)										**Men (n=42)**									
	VAT		SAT		VSR		BMI		WC		VAT		SAT		VSR		BMI		WC	
	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p
log hs-CRP	0.18	0.40	0.22	0.24	0.04	0.86	0.19	0.42	0.21	0.31	0.03	0.90	0.31	0.14	-0.22	0.23	0.12	0.58	0.19	0.39
log TNF-α	-0.03	0.89	-0.14	0.49	-0.06	0.76	-0.22	0.38	-0.24	0.32	0.32	0.18	-0.05	0.85	0.12	0.58	0.23	0.32	0.30	0.17
log IL-6	-0.17	0.93	-0.03	0.84	-0.01	0.98	-0.15	0.43	-0.14	0.46	**0.47**	**0.01**	0.25	0.19	0.16	0.37	**0.36**	**0.049**	0.33	0.09
log Re-sistin	0.11	0.67	0.28	0.23	-0.20	0.40	0.14	0.63	-0.19	0.85	-0.44	0.07	0.02	0.95	-0.36	0.08	-0.25	0.32	-0.22	0.39
log Adipo-nectin	-0.30	0.14	0.05	0.79	-0.28	0.13	**-0.42**	**0.045**	-0.16	0.47	-0.27	0.26	-0.22	0.31	0.11	0.60	-0.35	0.11	-0.30	0.19
	BMI<25.0 kg/m² (n=36)										**BMI≥25.0 kg/m² (n=61)**									
	VAT		SAT		VSR		BMI		WC		VAT		SAT		VSR		BMI		WC	
	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p
log hs-CRP	0.05	0.83	0.38	0.11	-0.48	0.10	-0.22	0.40	0.22	0.39	0.16	0.41	**0.33**	**0.04**	-0.10	0.56	0.32	0.08	0.18	0.37
log TNF-α	0.05	0.84	0.30	0.29	-0.55	0.10	0.17	0.59	0.25	0.40	0.30	0.17	-0.09	0.62	0.24	0.18	0.19	0.39	0.29	0.16
log IL-6	0.07	0.65	0.18	0.31	-0.15	0.49	-0.16	0.39	0.08	0.64	**0.39**	**0.03**	0.04	0.82	0.19	0.28	0.33	0.13	0.34	0.09
log Re-sistin	-0.11	0.67	0.32	0.26	-0.53	0.11	0.27	0.37	0.06	0.84	-0.22	0.30	0.15	0.46	-0.25	0.15	-0.34	0.14	0.00	0.99
log Adipo-nectin	-0.07	0.78	0.28	0.29	0.07	0.82	-0.42	0.10	-0.15	0.60	-0.34	0.08	-0.25	0.18	-0.04	0.84	**-0.47**	**0.02**	**-0.46**	**0.01**

Table 13 continued: Associations between adiposity measures with parameters of systemic chronic inflammation in subgroups defined by sex, BMI, smoking status, and use of aspirin or NSAIDs.

	Current non-smoking (n=78)										Current smoking (n=19)									
	VAT		SAT		VSR		BMI		WC		VAT		SAT		VSR		BMI		WC	
	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p
log hs-CRP	0.18	0.22	**0.35**	**0.01**	-0.13	0.37	0.23	0.12	0.22	0.12	**0.74**	**0.001**	**0.49**	**0.049**	0.56	0.08	**0.80**	**0.00003**	**0.88**	**0.0003**
log TNF-α	0.19	0.23	-0.12	0.44	0.19	0.10	0.07	0.65	0.11	0.48	0.34	0.18	-0.25	0.34	0.49	0.09	0.08	0.74	0.29	0.30
log IL-6	**0.36**	**0.01**	0.13	0.39	0.11	0.39	**0.29**	**0.048**	0.24	0.10	0.43	0.13	-0.01	0.99	0.28	0.60	0.20	0.46	0.17	0.61
log Re-sistin	-0.18	0.28	0.13	0.41	-0.16	0.23	-0.08	0.66	-0.06	0.74	0.43	0.12	0.42	0.14	-0.40	0.38	0.46	0.08	0.59	0.05
log Adipo-nectin	-0.25	0.09	-0.11	0.44	0.02	0.85	**-0.34**	**0.01**	**-0.27**	**0.049**	-0.27	0.28	-0.09	0.72	0.07	0.86	-0.19	0.43	-0.37	0.18
	Non-use of aspirin or NSAIDs (n=81)										**Use of aspirin or NSAIDs (n=16)**									
	VAT		SAT		VSR		BMI		WC		VAT		SAT		VSR		BMI		WC	
	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p	ß	p
log hs-CRP	0.30	0.06	**0.41**	**0.01**	0.01	0.92	**0.31**	**0.04**	**0.31**	**0.04**	**0.57**	**0.04**	0.53	0.13	0.29	0.27	0.63	0.12	**0.72**	**0.03**
log TNF-α	**0.30**	**0.049**	-0.05	0.74	0.25	0.30	0.17	0.30	0.17	0.28	-0.42	0.27	-0.46	0.30	0.07	0.86	-0.85	0.30	-0.31	0.41
log IL-6	**0.29**	**0.046**	0.15	0.32	0.15	0.23	0.17	0.25	0.12	0.41	-0.27	0.50	-0.78	0.46	0.31	0.44	-0.73	0.66	-0.07	0.87
log Re-sistin	-0.14	0.39	0.16	0.34	-0.10	0.42	-0.03	0.85	0.01	0.93	-0.20	0.90	0.13	0.86	0.05	0.87	0.42	0.67	-0.30	0.89
log Adipo-nectin	-0.26	0.08	-0.06	0.73	-0.25	0.83	**-0.30**	**0.04**	-0.24	0.10	-0.22	0.84	-0.05	0.93	0.09	0.78	-0.55	0.28	**-0.89**	**0.001**

Models were adjusted for age, physical activity level, sex, menopausal status (women only), smoking, aspirin or NSAID use, and mutually adjusted for all parameters of systemic chronic inflammation. In these models, VAT and SAT, respectively VSR and BMI, and BMI and WC were not mutually adjusted. In each case, the stratification variable was excluded from the model. VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, VSR=visceral-to-subcutaneous-fat ratio, BMI=body mass index, WC=waist circumference, ß=standardized linear regression coefficient, p=p-value.

In non-obese participants, no significant relations were found for VAT, SAT, VSR, BMI, or WC to any of the inflammatory parameters (Table 13). In overweight/obese participants, VAT showed a positive association with IL-6 (ß=0.39; p=0.03), and SAT was positively associated with hs-CRP (ß=0.33; p=0.04). BMI and WC were inversely associated with adiponectin (ß=-0.47; p=0.02 and ß=-0.46; p=0.01, respectively). In general, VAT and BMI showed stronger relations to inflammatory parameters in overweight/obese participants than in normal weight participants, whereas associations between SAT and inflammatory parameters were stronger in normal weight than in overweight/obese participants.

In an analysis restricted to current non-smokers, VAT was positively associated with IL-6 (ß=0.36; p=0.01) and SAT showed a significant association with hs-CRP (ß=0.35; p=0.01) (Table 13). BMI was positively associated with IL-6 (ß=0.29; p=0.048) and it showed an inverse association with adiponectin (ß=-0.34; p=0.01). WC also showed an inverse association with adiponectin (ß=-0.27; p=0.049). By comparison, in an analysis restricted to current smokers, VAT, BMI, and WC showed strong positive associations with hs-CRP (ß=0.74; p=0.001, ß=0.80; p=0.00003, and ß=0.88; p=0.0003, respectively) and a positive association was found between SAT and hs-CRP (ß=0.49; p=0.049). Associations between VAT and inflammatory markers were stronger in current smokers than non-smokers. No associations were found between VSR and any of the inflammatory parameters in smokers or non-smokers.

In an analysis limited to non-users of aspirin or NSAIDs, VAT showed positive associations with IL-6 (ß=0.29; p=0.046) and TNF-α (ß=0.30; p=0.049) (Table 13). SAT, BMI, and WC showed positive associations with hs-CRP (ß=0.41; p=0.01, ß=0.31; p=0.04, and ß=0.31; p=0.04, respectively). BMI was inversely related to adiponectin (ß=-0.30; p=0.04). In an analysis restricted to participants using aspirin and NSAIDs, VAT showed a positive association with hs-CRP (ß=0.57; p=0.04). WC showed a positive association with hs-CRP (ß=0.72; p=0.03) and an inverse association with adiponectin (ß=-0.89; p=0.001). In general, VAT, SAT, BMI, and WC were inversely related to TNF-α and IL-6 in aspirin or NSAID users, whereas VAT, BMI, and WC were positively related to those parameters in non-users of aspirin and NSAIDs. No significant relations were found for VSR to any of the inflammatory in users or non-users of aspirin and NSAIDs.

3.4 Metabolomics study

Characteristics of the study population are presented in Table 14. The mean age of study participants was 51.96 years and 13.2% of the participants were fasting. Women had lower VAT (p<0.001), VSR (p<0.001), BMI (p=0.008) and WC (p<0.001) than men. In contrast, women had a higher SAT (p=0.011) than men. Differences between men and women, fasting and non-fasting participants as well as between subjects with elevated urinary glucose and subjects with no urinary glucose were found for urinary and serum metabolites (Supplementary Table 13 and 14). There were no significant differences in the distribution of the characteristics between urinary (n=228) and serum (n=200) study population.

Table 14: Sex-dependent characteristics of the study population.

	Total mean (SD)	Women mean (SD)	Men mean (SD)	p-value
N	228	121	107	
Age (years)	51.96 (12.55)	52.80 (12.00)	50.97 (13.15)	0.388
Non-fasting	198	105	93	0.511
Current smoking	31	12	19	0.075
Physical activity level*	1.67 (0.27)	1.67 (0.27)	1.68 (0.28)	0.888
VAT thickness US (cm)	6.79 (2.85)	6.29 (2.80)	7.38 (2.81)	<0.001
SAT thickness US (cm)	2.06 (0.85)	2.17 (0.89)	1.93 (0.78)	0.011
VSR	3.77 (2.14)	3.23 (1.70)	4.41 (2.41)	<0.001
BMI (kg/m²)	26.61 (4.66)	25.97 (4.99)	27.36 (4.13)	0.008
WC (cm)	91.16 (13.56)	85.53 (11.99)	97.75 (12.30)	<0.001

Entries are mean (standard deviation) for continuous variables and absolute numbers for categorical variables. VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, BMI=body mass index, WC=waist circumference, US=ultrasonography, n=228. *Calculated from Metabolic Equivalents of Task. p-value from Kruskal-Wallis test (continuous variable) or X^2 test (categorical variables).

3.4.1 Results from targeted metabolomics approach

Pearson correlation analyses to assess correlations between metabolites showed moderate (r≥0.4) or strong (r≥0.6) correlations between 36% of the urinary metabolites

(Figure 9). By comparison, Figure 10 displays Pearson correlation coefficients between serum metabolites, showing that 66% of all serum metabolites were weakly correlated with one another and only few metabolites (12%) being strongly correlated with one another.

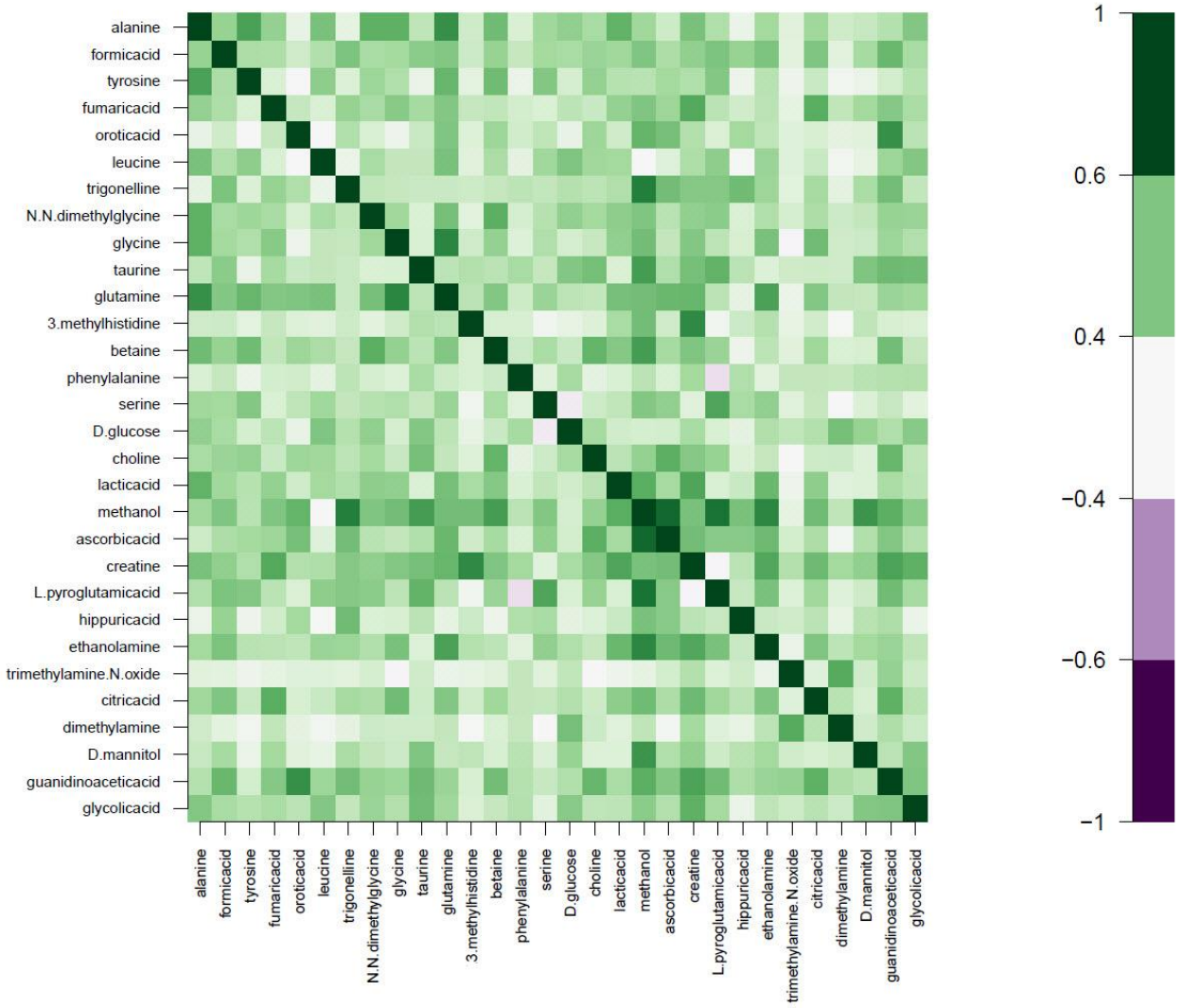

Figure 9: Pearson correlation matrix between creatinine-normalized urinary metabolite concentrations.

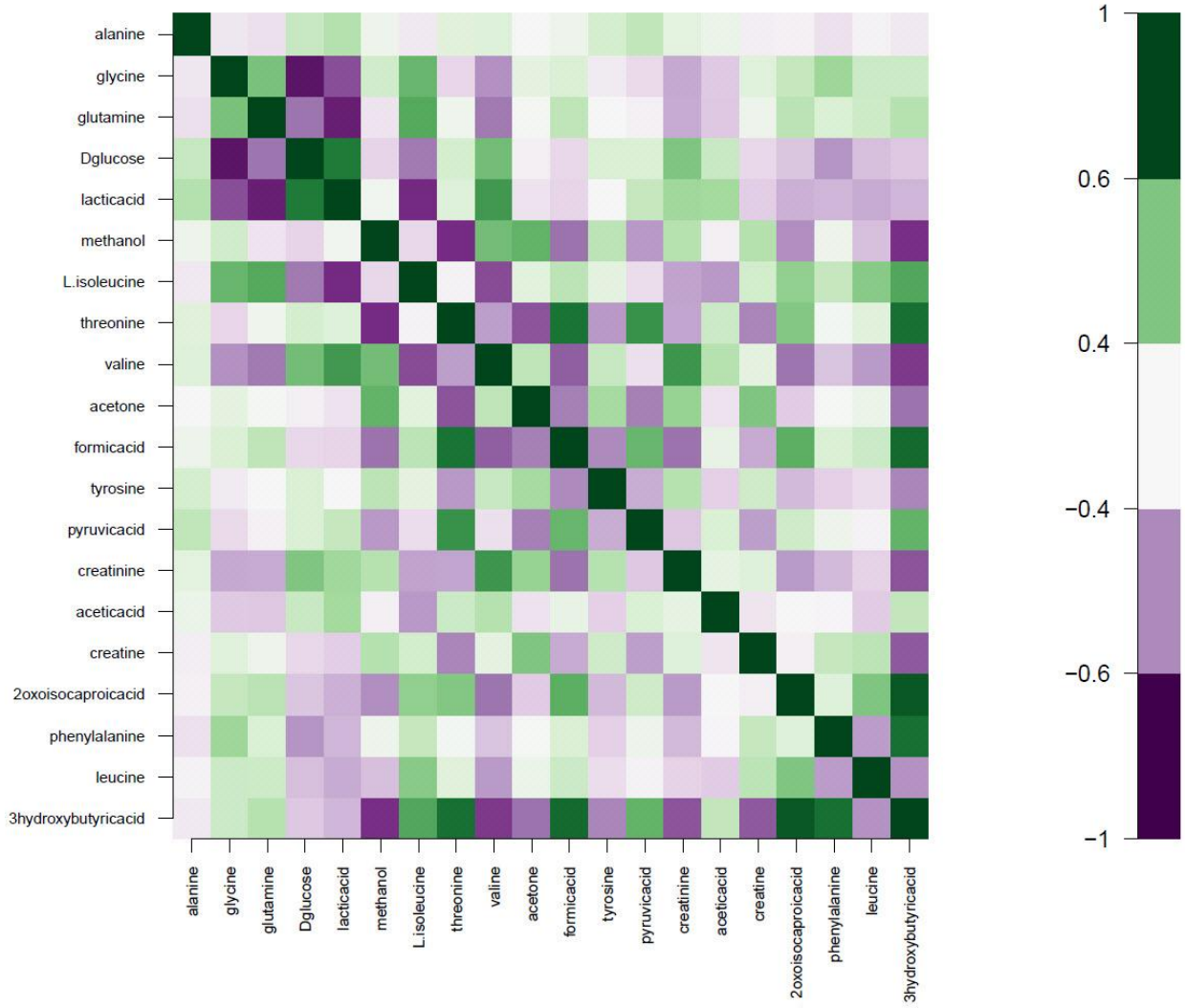

Figure 10: Pearson correlation matrix between serum metabolite concentrations.

Linear regression analyses of urinary metabolites revealed significant inverse associations of VAT, BMI, and WC with urinary formic acid after adjustment for study, age and sex and correcting p-values for multiple testing (Table 15). These associations did not remain significant in fully adjusted models considering further adjustments for smoking, menopause status, physical activity, urinary glucose, and eGFR (Model 2). Logistic regression models of urinary metabolites dichotomized as detectable and undetectable showed significant inverse and positive relations of VAT, BMI, and WC to urinary metabolites in age and sex adjusted models (Model 1) and in fully adjusted models (Model 2). Specifically, VAT was inversely related to the detection of choline (ß=-0.18, $p=2.73*10^{-3}$), guanidinoacetic acid (ß=-0.12, p=0.04), and glycolic acid

(ß=-0.20, p=0.02), and positively related to the detection of ethanolamine (ß=0.18, p=0.02) and dimethylamine (ß=0.32, p=0.02) in fully adjusted models (Model 2). Note that the obtained choline concentrations were most likely influenced by related molecules due to the presence of partial overlap in the analyzed NMR spectra. Further, BMI and WC were inversely related to the detection of glutamine and lactic acid in fully adjusted models (Model 2). Moreover, WC was inversely associated with the detection of serine in fully adjusted models (Model 2). However, when obesity measures were mutually adjusted (Model 3), only VAT remained significantly inversely related to the detection of choline (ß=-0.18; p=0.01) and glycolic acid (ß=-0.20, p=0.046) and positively related to the detection of dimethylamine (ß=0.33, p=0.046). No relations were found between SAT or VSR and urinary metabolites in any of the analyses.

Table 15: Significant[#] associations between obesity measures and urinary metabolite levels.

	Metabolite	Model1		Model 2		Model 3	
		ß	p	ß	p	ß	p
All subjects (n=228)							
VAT	Formic acid*	-0.09	$4.81*10^{-4}$	-0.07	0.19	-0.05	0.76
	Choline	-0.12	$5.26*10^{-4}$	-0.18	$2.73*10^{-3}$	-0.18	0.01
	Methanol	0.23	0.01	0.10	0.29	0.14	0.30
	Ethanolamine	0.15	0.03	0.18	0.02	0.23	0.05
	Dimethylamine	0.23	0.01	0.32	0.02	0.33	$4.69*10^{-2}$
	Guanidinoacetic acid	-0.14	0.03	-0.12	0.04	-0.27	0.05
	Glycolic acid	-0.16	0.03	-0.20	0.02	-0.20	$4.64*10^{-2}$
BMI	Formic acid*	-0.04	0.03	-0.04	0.20	-0.01	0.92
	Alanine	0.10	0.01	0.17	0.01	0.01	0.99
	Betaine	-0.10	0.01	-0.13	0.03	-0.00	0.99
	Choline	-0.11	0.01	-0.17	0.01	0.04	0.99
	Creatine	-0.10	0.01	-0.10	0.10	-0.08	0.94
	Dimethylamine	0.18	$5.51*10^{-4}$	0.26	0.01	0.11	0.94
	Ethanolamine	0.13	$2.10*10^{-3}$	0.15	0.02	0.01	0.99
	Glutamine	-0.12	$3.32*10^{-3}$	-0.16	0.01	-0.05	0.04
	Glycolic acid	-0.15	$6.74*10^{-4}$	-0.19	0.01	-0.16	0.60
	Guanidinoacetic acid	-0.14	$1.30*10^{-3}$	-0.17	0.01	-0.07	0.94
	Lactic acid	-0.08	0.02	-0.16	0.01	-0.09	0.94
	Methanol	0.12	0.01	0.05	0.36	0.00	0.99
	Taurine	-0.08	0.03	-0.10	0.10	-0.06	0.94
WC	Formic acid*	-0.02	0.01	-0.02	0.21	-0.01	0.94
	Alanine	0.05	$1.93*10^{-3}$	0.07	0.05	0.07	0.08
	Betaine	-0.05	$1.67*10^{-3}$	-0.05	0.12	-0.05	0.12
	Choline	-0.05	$1.67*10^{-3}$	-0.08	$3.99*10^{-3}$	-0.10	0.21
	Creatine	-0.04	0.01	-0.03	0.13	-0.02	0.51
	Dimethylamine	0.07	$7.71*10^{-5}$	0.09	$3.99*10^{-3}$	0.11	0.06
	Ethanolamine	0.06	$6.87*10^{-4}$	0.06	0.01	0.06	0.05
	Glutamine	-0.06	$8.49*10^{-4}$	-0.06	0.01	-0.06	0.87
	Glycolic acid	-0.05	$1.67*10^{-3}$	-0.06	0.01	-0.06	0.05
	Guanidinoacetic acid	-0.06	$6.87*10^{-4}$	-0.07	0.01	-0.06	0.05

Table 15 continued: Significant[#] associations between obesity measures and urinary metabolite levels.

	Metabolite	Model1		Model 2		Model 3	
		ß	p	ß	p	ß	p
WC	Lactic acid	-0.03	0.02	-0.06	0.01	-0.05	0.16
	Methanol	0.03	0.04	0.02	0.30	0.02	0.48
	Phenylalanine	-0.04	0.03	-0.02	0.28	-0.02	0.45
	Serine	-0.04	0.02	-0.05	0.02	-0.04	0.20
	Taurine	-0.03	0.02	-0.04	0.10	-0.02	0.34

Model 1: linear or logistic regression model adjusted for study, age (non-linear) and sex.
Model 2: linear or logistic regression model adjusted for study, age and sex interaction (non-linear), smoking status, menopausal status (women only), physical activity, urinary glucose, and eGFR. In logistic regression models: 0=metabolite undetected, 1=metabolite detected.
Model 3: linear regression or logistic model adjusted for all variables from model 2 plus VAT and SAT mutually adjusted and BMI and WC mutually adjusted.
VAT=visceral adipose tissue, BMI=body mass index, WC=waist circumference, ß=standardized regression coefficient, p-value=corrected for multiple testing by controlling the false discovery rate.
*linear regression model was applied.
[#]p-values<0.05 after correction for multiple testing were considered significant

After stratifying by sex, results from logistic regression models showed inverse relations of VAT to the detection of choline (ß=-0.18; $p=2.73*10^{-3}$), glycolic acid (ß=-0.58, p=0.04), guanidinoacetic acid (ß=-0.36, p=0.04), lactic acid (ß=-0.32, p=0.04), and serine (ß=-0.41, p=0.04) and positive relations to the detection of ethanolamine (ß=0.71, p=0.04) and dimethylamine (ß=0.65, p=0.04) in fully adjusted models among men (Table 16). These relations remained significant after mutual adjustment for SAT (Model 3). By comparison, BMI and WC were additionally inversely associated with the detection of betaine and glutamine in fully adjusted models, but no relation was found between BMI and serine in fully adjusted models among men. In addition, none of the relations remained statistically significant when BMI and WC were mutually adjusted (Model 3). No significant associations were found between SAT or VSR and urinary metabolites among men. Among women, VAT was inversely associated with formic acid (ß=-0.11, $p=3.22*10^{-3}$) in model 1. However, this relation did not remain significant in fully adjusted models (Model 2) or mutually adjusted models (Model 3). In addition, VAT was positively related with the detection of dimethylamine (ß=0.12; p=0.04) in the fully adjusted model. BMI was significantly inversely related to the detection of creatine and glycolic acid and positively related to the detection of dimethylamine and methanol in the age and sex adjusted models. However, after full adjustment, estimated relations were attenuated and associations of BMI with the detection of urinary metabolites were no longer statistically significant. WC was positively associated with the detection of dimethylamine in fully adjusted models (Model 2) among women. No significant relations were found between SAT or VSR and urinary metabolites among men or women.

After stratifying by fasting status and full adjustment for covariates (Model 2), VAT, BMI, and WC were inversely associated with the detection of choline and positively associated with the detection of dimethylamine and ethanolamine in non-fasting subjects (Table 16). After mutual adjustment for other anthropometric variables (Model 3), only VAT remained significantly related to the detection of choline (ß=-0.17; $p=1.62*10^{-4}$), dimethylamine (ß=0.34; $p=4.90*10^{-2}$), and ethanolamine (ß=0.34; $p=4.69*10^{-2}$). By comparison, no significant relations were found for VAT, SAT, VSR, BMI, or WC with urinary metabolites among fasting participants.

Analyses stratified by urinary glucose revealed an inverse relation between VAT and the detection of choline (ß=-0.24; $p=9.34*10^{-5}$) in fully adjusted models (Model 2)

among subjects with no urinary glucose (Table 16). The inverse association between VAT and choline (ß=-0.22; $p=1.94*10^{-4}$) remained statistically significant after further adjustment for SAT (Model 3). By comparison, BMI and WC showed positive relations to the detection of dimethylamine and ethanolamine and inverse relations to glutamine and glycolic acid in fully adjusted models. No significant results were found between SAT or VSR and urinary metabolites. In addition, no significant results were found in glucose subjects.

Table 16: Significant[#] associations between measures of obesity and urinary metabolite levels among subgroups.

	Metabolite	Model 1		Model 2		Model 3	
		ß	p	ß	p	ß	p
Men (n=107)							
VAT	Formic acid*	-0.11	$3.21*10^{-3}$	-0.04	0.87	-0.03	0.91
	Choline	-0.21	$4.23*10^{-4}$	-0.18	$2.73*10^{-3}$	-0.18	$3.21*10^{-5}$
	Dimethylamine	0.41	0.01	0.65	0.04	0.63	0.03
	Ethanolamine	0.21	0.03	0.71	0.04	0.71	0.01
	Glycolic acid	-0.17	0.06	-0.58	0.04	-0.57	0.03
	Guanidinoacetic acid	-0.19	0.03	-0.36	0.04	-0.41	0.02
	Lactic acid	-0.13	0.12	-0.32	0.04	-0.38	0.04
	Serine	-0.20	0.03	-0.41	0.04	-0.39	0.04
BMI	Alanine	0.17	0.04	0.16	0.10	0.09	0.93
	Betaine	-0.30	0.01	-0.35	0.01	-0.22	0.49
	Choline	-0.22	0.01	-0.48	0.01	-0.28	0.49
	Dimethylamine	0.27	0.01	0.28	0.04	0.02	0.94
	Ethanolamine	0.24	0.01	0.44	0.01	0.25	0.49
	Glutamine	-0.37	0.01	-0.46	0.01	-0.55	0.03
	Glycolic acid	-0.24	0.01	-0.34	0.01	-0.23	0.49
	Guanidinoacetic acid	-0.24	0.01	-0.36	0.01	-0.23	0.49
	Lactic acid	-0.16	0.04	-0.31	0.01	-0.30	0.49
WC	Alanine	0.05	0.04	0.05	0.08	0.03	0.80
	Betaine	-0.11	0.00	-0.10	0.01	-0.05	0.73
	Choline	-0.08	0.01	-0.14	0.01	-0.08	0.56
	D-Glucose	-0.06	0.03	-0.14	0.01	-0.15	0.53
	Dimethylamine	0.11	0.01	0.11	0.02	0.12	0.51
	Ethanolamine	0.09	0.01	0.14	0.01	0.08	0.56
	Glutamine	-0.10	0.01	-0.11	0.01	0.03	0.80
	Glycolic acid	-0.07	0.01	-0.10	0.01	-0.04	0.73
	Guanidinoacetic acid	-0.08	0.01	-0.11	0.01	-0.05	0.73
	Lactic acid	-0.05	0.04	-0.08	0.02	0.00	0.96
	Phenylalanine	-0.05	0.05	-0.01	0.66	0.01	0.96
	Serine	-0.06	0.03	-0.09	0.02	-0.07	0.56

Table 16 continued: Significant[#] associations between measures of obesity and urinary metabolite levels among subgroups.

	Metabolite	Model 1		Model 2		Model 3	
		ß	p	ß	p	ß	p
Women (n=121)							
VAT	Formic acid*	-0.11	$3.22*10^{-3}$	-0.04	0.87	-0.02	0.89
	Dimethylamine	0.16	0.02	0.12	0.04	0.12	0.25
BMI	Creatine	-0.12	$4.51*10^{-2}$	-0.10	0.42	-0.09	0.95
	Dimethylamine	0.15	0.03	0.30	0.08	0.17	0.89
	Glycolic acid	-0.12	$4.51*10^{-2}$	-0.12	0.42	-0.13	0.89
	Methanol	0.15	$4.51*10^{-2}$	0.06	0.63	-0.01	0.96
WC	Dimethylamine	0.06	0.03	0.11	$4.46*10^{-3}$	0.06	0.70
Non-fasting subjects (n=198)							
VAT	Formic acid*	-0.11	$6.63*10^{-5}$	-0.09	0.04	-0.07	0.23
	Choline	-0.20	$6.68*10^{-6}$	-0.17	$1.84*10^{-4}$	-0.17	$1.62*10^{-4}$
	Dimethylamine	0.24	0.03	0.43	0.02	0.34	$4.90*10^{-2}$
	Ethanolamine	0.20	0.03	0.36	0.02	0.34	$4.69*10^{-2}$
BMI	Formic acid*	-0.05	0.01	-0.06	0.09	-0.04	0.86
	Choline	-0.10	0.04	-0.19	0.02	-0.02	0.96
	Creatine	-0.11	0.02	-0.12	0.08	-0.21	0.83
	Dimethylamine	0.17	0.01	0.25	0.01	0.11	0.83
	Ethanolamine	0.13	0.01	0.19	0.02	0.06	0.94
	Glutamine	-0.14	0.01	-0.19	0.01	-0.12	0.04
	Glycolic acid	-0.14	0.01	-0.18	0.02	-0.13	0.83
	Guanidinoacetic acid	-0.13	0.01	-0.15	0.03	-0.01	0.96
	Lactic acid	-0.09	0.05	-0.21	0.01	-0.15	0.83
	Methanol	0.13	0.04	0.04	0.46	-0.12	0.83
WC	Formic acid*	-0.02	0.01	-0.01	0.12	-0.01	0.93
	Alanine	0.03	0.03	0.06	0.06	0.06	0.38
	Betaine	-0.04	0.02	-0.05	0.02	-0.06	0.38
	Choline	-0.04	0.02	-0.08	0.01	-0.07	0.38
	Creatine	-0.04	0.03	-0.03	0.21	0.04	0.67
	Dimethylamine	0.07	$1.84*10^{-3}$	0.09	0.01	0.06	0.39
	Ethanolamine	0.05	$4.72*10^{-3}$	0.07	0.01	0.05	0.39
	Glutamine	-0.05	$4.72*10^{-3}$	-0.07	0.01	-0.03	0.67

Table 16 continued: Significant[#] associations between measures of obesity and urinary metabolite levels among subgroups.

	Metabolite	Model 1		Model 2		Model 2	
		ß	p	ß	p	ß	p
WC	Glycolic acid	-0.04	0.02	-0.06	0.01	-0.02	0.68
	Guanidinoacetic acid	-0.05	$4.72*10^{-3}$	-0.06	0.01	-0.06	0.38
	Lactic acid	-0.03	0.04	-0.07	0.01	-0.03	0.67
	Methanol	0.04	0.04	0.03	0.22	0.07	0.38
	Serine	-0.04	0.03	-0.07	0.01	-0.07	0.38
Subjects with no urinary glucose (n=183)							
VAT	Choline	-0.26	$8.21*10^{-7}$	-0.24	$9.34*10^{-5}$	-0.22	$1.94*10^{-4}$
	Dimethylamine	0.61	0.05	0.64	0.03	0.45	0.62
	Ethanolamine	0.60	0.05	0.29	0.01	0.10	0.96
BMI	Alanine	-0.10	0.03	-0.15	0.06	0.04	0.83
	Creatine	-0.12	0.03	-0.11	0.19	-0.14	0.83
	Dimethylamine	0.17	$1.86*10^{-3}$	0.27	0.01	0.09	0.83
	Ethanolamine	0.15	$4.02*10^{-3}$	0.20	0.02	0.05	0.83
	Glutamine	-0.14	0.02	-0.22	0.01	-0.18	0.03
	Glycolic acid	-0.19	$1.85*10^{-3}$	-0.25	0.01	-0.23	0.52
WC	Alanine	0.04	0.02	0.07	0.08	0.08	0.32
	Betaine	-0.04	0.04	-0.04	0.12	-0.05	0.51
	Choline	-0.05	0.04	-0.09	0.05	-0.10	0.32
	Creatine	-0.04	0.05	-0.03	0.28	0.01	0.90
	Dimethylamine	0.07	$7.85*10^{-4}$	0.11	0.01	0.08	0.32
	Ethanolamine	0.06	$2.48*10^{-3}$	0.08	0.02	0.07	0.39
	Glutamine	-0.05	0.02	-0.08	0.02	-0.02	0.85
	Glycolic acid	-0.06	$3.45*10^{-3}$	-0.07	0.02	-0.01	0.90
	Guanidinoacetic acid	-0.04	0.04	-0.05	0.08	-0.04	0.72
	Taurine	-0.04	0.05	-0.04	0.20	-0.04	0.72

Model 1: linear or logistic regression model adjusted for study, age (non-linear) and sex.
Model 2: linear or logistic regression model adjusted for study, age and sex interaction (non-linear), smoking status, menopausal status (women only), physical activity, urinary glucose, and eGFR. In logistic regression models: 0=metabolite undetected, 1=metabolite detected.
Model 3: linear regression or logistic model adjusted for all variables from model 2 plus VAT and SAT mutually adjusted and BMI and WC mutually adjusted.
In each case, the stratification variable was excluded from the model. VAT=visceral adipose tissue, BMI=body mass index, WC=waist circumference, ß=standardized regression coefficient, p-value=corrected for multiple testing by controlling the false discovery rate.
*linear regression model was applied.
[#]p-values<0.05 after correction for multiple testing were considered significant.

When linear and logistic regression models were applied to serum metabolites, results showed no significant results between VAT, SAT, VSR, BMI, and WC and concentrations of serum metabolites for the overall population and among subgroups. Also no significant results were found between the highest and lowest quartiles of VAT, SAT, VSR, BMI, or WC and serum metabolites in the overall population or among subgroups.

3.4.2 Results from untargeted metabolomics approach

Urine and serum spectral areas showed only a small degree of unbalanced data (Supplementary Figures 2 and 3). To normalize unbalanced data 60% of the urinary and 65% of the serum features were transformed using variance stabilization (VSN) (177). To obtain a general overview about differences between fingerprints among subjects, AP clustering analyses with no a priori assumption was applied. For the whole study population, AP clustering based on urinary metabolic fingerprints formed nine clusters. Results from age and sex adjusted regression analyses showed that there were no significant differences between clusters in the distribution of VAT, SAT, VSR, or WC (Table 17). By comparison, the regression-adjusted BMI of cluster 4 ($p=0.01$) and cluster 6 ($p=0.02$) differed statistically significantly compared to the predicted grand mean of BMI. The distributions of the predicted mean values of phenotypes among clusters are presented in Figure 11.

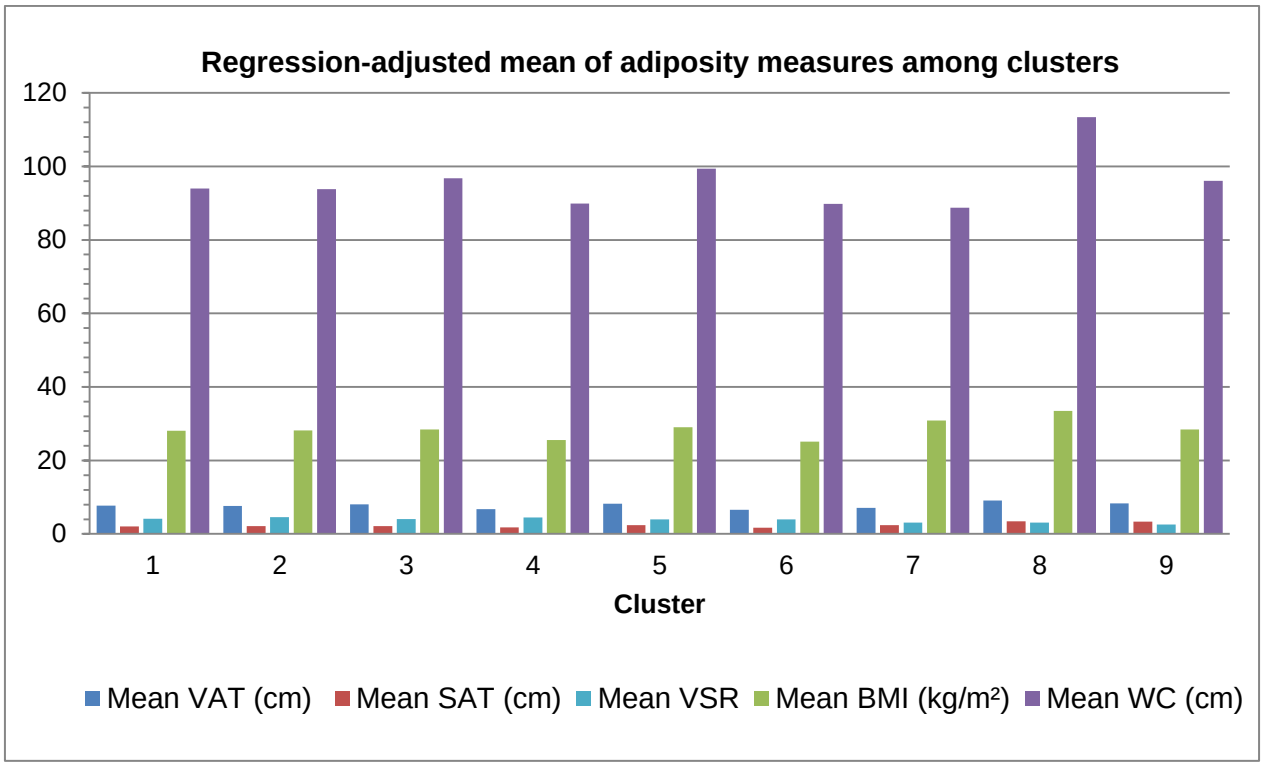

Figure 11: Age and sex adjusted distribution of phenotypes among clusters formed from urinary fingerprints (overall study population).

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Table 17: Clusters formed from urinary fingerprints (overall study population).

Cluster	Mean VAT (95% CI)	p	Mean SAT (95% CI)	p	Mean VSR (95% CI)	p	Mean BMI (95% CI)	p	Mean WC (95 % CI)	p
1	7.69 (6.80-8.57)	0.50	2.04 (1.78-2.30)	0.31	4.16 (3.50-4.83)	0.43	28.06 (26.65-29.46)	0.48	94.00 (90.44-97.57)	0.54
2	7.59 (6.61-8.57)	0.86	2.11 (1.82-2.40)	0.65	4.57 (3.83-5.30)	0.34	28.14 (26.60-29.67)	0.93	93.77 (89.89-97.66)	0.92
3	8.01 (7.02-9.00)	0.59	2.17 (1.88-2.46)	0.45	4.04 (3.29-4.78	0.78	28.39 (26.83-29.95)	0.72	96.78 (92.82-100.73)	0.24
4	6.77 (5.70-7.85)	0.14	1.78 (1.47-2.10)	0.16	4.49 (3.68-5.31)	0.47	25.59 (23.88-27.30)	**0.01**	89.91 (85.57-94.25)	0.10
5	8.25 (6.98-9.53)	0.41	2.36 (1.99-2.73)	0.10	3.94 (2.98-4.90)	0.66	28.99 (26.98-31.00)	0.39	99.36 (94.26-104.45)	0.05
6	6.55 (5.10-8.00)	0.14	1.73 (1.31-2.16)	0.18	3.99 (2.90-5.09)	0.77	25.12 (22.82-27.43)	**0.02**	89.82 (83.96-95.68)	0.18
7	7.05 (1.64-12.47)	0.82	2.42 (0.83-4.00)	0.64	3.11 (0.97-7.19)	0.62	30.86 (22.24-39.48)	0.53	88.75 (66.87-110.62)	0.64
8	9.06 (3.40-14.72)	0.63	3.41 (1.75-5.07)	0.10	3.09 (1.18-7.35)	0.62	33.49 (24.49-42.50)	0.24	113.36 (90.51-136.21)	0.10
9	8.33 (2.82-13.84)	0.82	3.34 (1.72-4.95)	0.11	2.53 (1.62-6.68)	0.44	28.44 (19.67-37.21)	0.93	96.11 (73.86-118.36)	0.85

Mean VAT, SAT, VSR, BMI and WC=predictive margins from linear regression adjusted for age and sex, grand mean=reference category, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, VSR=visceral-to-subcutaneous-fat ratio, BMI=body mass index, WC=waist circumference, p=p-value for the comparison to the grand mean.

AP-clustering was repeated in men and women separately and detected six clusters for men and women, respectively (Supplementary Table 15). Among men and women, clusters did not differ with regard to VAT, SAT, VSR, BMI, and WC. When analyses were restricted to non-fasting participants, eight clusters were formed and showed that the predicted mean VAT of cluster 5 ($p=0.03$) differed statistically significantly compared to the overall mean VAT. No significant differences between clusters were found for SAT, VSR, BMI, and WC. By comparison, three clusters were formed in the subsample of fasting participants. In fasting participants, clusters did not differ statistically significantly with regard to VAT, SAT, VSR, BMI, or WC. Further stratification of non-fasting participants by sex resulted in six clusters for men and women respectively. None of the formed clusters differed statistically significantly in any of the considered phenotypes. When the study population was stratified by urinary glucose, seven clusters were generated among subjects with no urinary glucose and three clusters were formed among subjects with elevated glucose. No significant differences between clusters among these groups were found for VAT, SAT, VSR, BMI, or WC.

Multiple linear regression models to detect relations of VAT and SAT to the urinary metabolic fingerprints in the overall population showed statistically significant associations between VAT and 39 urinary bins in fully adjusted models (Model 2) (Table 18). Metabolite identification was performed by comparison with reference spectra of pure compounds. In case that more than one compound contributed to a given bin all corresponding molecules are shown in Table 18. Positive relations were found to lysine, dimethylamine, and tyrosine/creatinine, while negative relations were found to 4-hydroxyhippuric acid, acetone, citric acid, glycine, L-pyroglutamic acid, methylmalonic acid, scyllo-inositol, and formic acid. In addition, significant associations were found between BMI and 35 bins and between WC and 62 bins (Figure 12 and Figure 13). Most bins that were significantly associated with VAT were also found to be significant in models with BMI and WC. However, VAT but none of the other anthropometric parameters was inversely related to 4-hydroxyhippuric acid in the overall study population in fully adjusted models (Model 2). By comparison, no significant relations were found between SAT or VSR and urinary bins.

Table 18: Significant[#] results from multiple regression analyses on the relation of VAT to urinary bins (all subjects).

		Model 1		Model 2		
Anthropometric variable	bin (ppm)	ß	p	ß	p	metabolite identification
VAT	3.795	0.0341	0.0106	0.0388	0.0155	Lysine
	1.485	0.0981	0.0500	0.0918	0.0285	Lysine
	1.355	0.0234	0.0271	0.0226	0.0392	unknown
	2.435	-0.0354	0.0316	-0.0329	0.0318	3-Hydroxy-3-methylglutaric acid, others
	2.475	-0.0334	0.0695	-0.0355	0.0102	3-Hydroxy-3-methylglutaric acid/others
	3.945	-0.0452	0.0695	-0.0475	0.0320	4-Hydroxyhippuric acid/others
	6.955	-0.0717	0.0277	-0.0860	0.0100	4-Hydroxyhippuric acid
	6.965	-0.0803	0.0277	-0.0843	0.0227	4-Hydroxyhippuric acid
	2.235	-0.0480	0.0562	-0.0526	0.0318	Acetone
	2.725	0.0518	0.0546	0.0601	0.0304	Dimethylamine/citrate
	3.205	-0.0324	0.0546	-0.0458	0.0097	Choline, others
	3.525	-0.0455	0.0562	-0.0463	0.0344	Choline, others
	4.065	-0.1101	0.0546	-0.1337	0.0175	Choline, creatinine, others
	2.605	-0.0119	0.0346	-0.0508	0.0217	unknown
	2.615	-0.0157	0.0446	-0.0613	0.0162	unknown
	2.625	-0.0198	0.0018	-0.0511	0.0045	unknown
	2.645	-0.0468	0.0546	-0.0454	0.0449	unknown
	2.675	-0.1161	0.0546	-0.1358	0.0304	Citric acid
	2.735	0.0633	0.0772	0.0813	0.0304	Dimethylamine
	3.565	-0.0661	0.0546	-0.0669	0.0349	Glycine, others
	2.405	-0.0540	0.0277	-0.0583	0.0100	L-Pyroglutamic acid
	2.515	-0.0430	0.0313	-0.0430	0.0297	L-Pyroglutamic acid
	1.235	-0.0428	0.0546	-0.0457	0.0318	Methylmalonic acid
	2.395	-0.0283	0.1068	-0.0308	0.0465	L-Pyroglutamic acid

Table 18 continued: Significant[#] results from multiple regression analyses on the relation of VAT to urinary bins (all subjects).

		Model 1		Model 2		
Anthropometric variable	bin (ppm)	ß	p	ß	p	metabolite identification
VAT	3.355	-0.0503	0.0277	-0.0561	0.0223	scyllo-Inositol
	3.055	0.0952	0.0587	0.1149	0.0304	Tyrosine, creatinine
	3.535	-0.0324	0.0695	-0.0320	0.0465	Sugar compounds, unknown
	2.325	-0.0303	0.0661	-0.0307	0.0320	unknown
	2.335	-0.0259	0.1025	-0.0276	0.0320	unknown
	2.365	-0.0362	0.0562	-0.0377	0.0320	unknown
	2.425	-0.0318	0.0695	-0.0348	0.0320	L-Pyroglutamic acid
	2.645	-0.0368	0.0562	-0.0386	0.0320	unknown
	2.965	-0.0347	0.0772	-0.0394	0.0435	Asparagine, unknown
	2.975	-0.0331	0.0772	-0.0674	0.0381	Asparagine, unknown
	6.585	-0.0657	0.0277	-0.0692	0.0223	unknown
	6.785	-0.0369	0.0772	-0.0439	0.0320	unknown
	6.805	-0.0632	0.0433	-0.0807	0.0223	unknown
	6.945	-0.0384	0.1147	-0.0489	0.0320	unknown
	8.455	-0.0911	0.0316	-0.1013	0.0223	Formic acid

Model 1: linear regression model adjusted for study, age (non-linear) and sex.
Model 2: linear regression model adjusted for study, age and sex interaction (non-linear), eGFR, smoking status, menopausal status (women only), fasting status, urinary glucose, and physical activity. In each case, the stratification variable was excluded from the model. VAT=visceral adipose tissue, bin=spectra position (ppm), ß=standardized linear regression coefficient, p=corrected p-value by controlling the false discovery rate, n=228.
[#]p-values<0.05 after correction for multiple testing were considered significant.

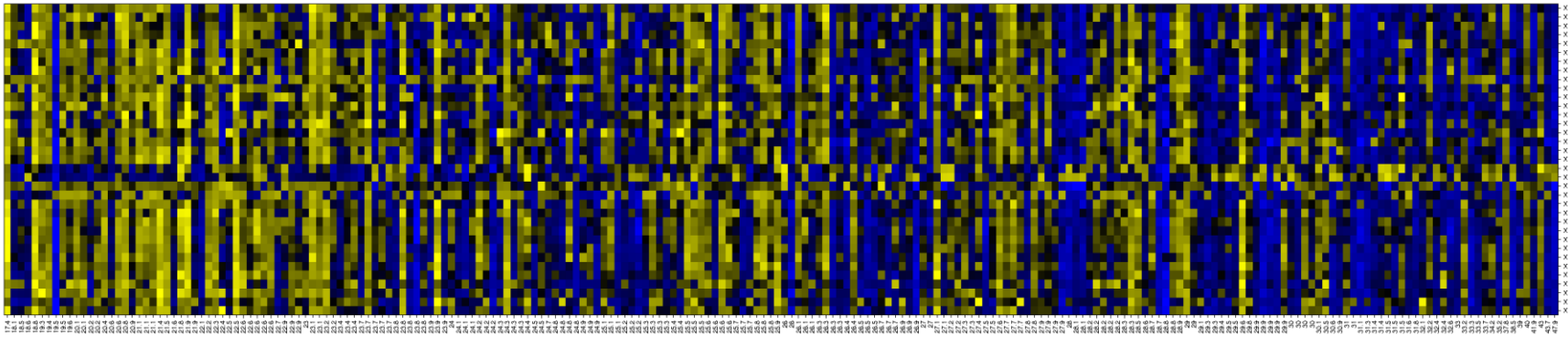


Figure 12: Heat map showing significant[#] relations of BMI and urinary bins in the overall study population.
X axis shows individual BMI value from lowest (left corner) to highest (right corner). [#] p-values<0.05 after correction for multiple testing are considered significant.

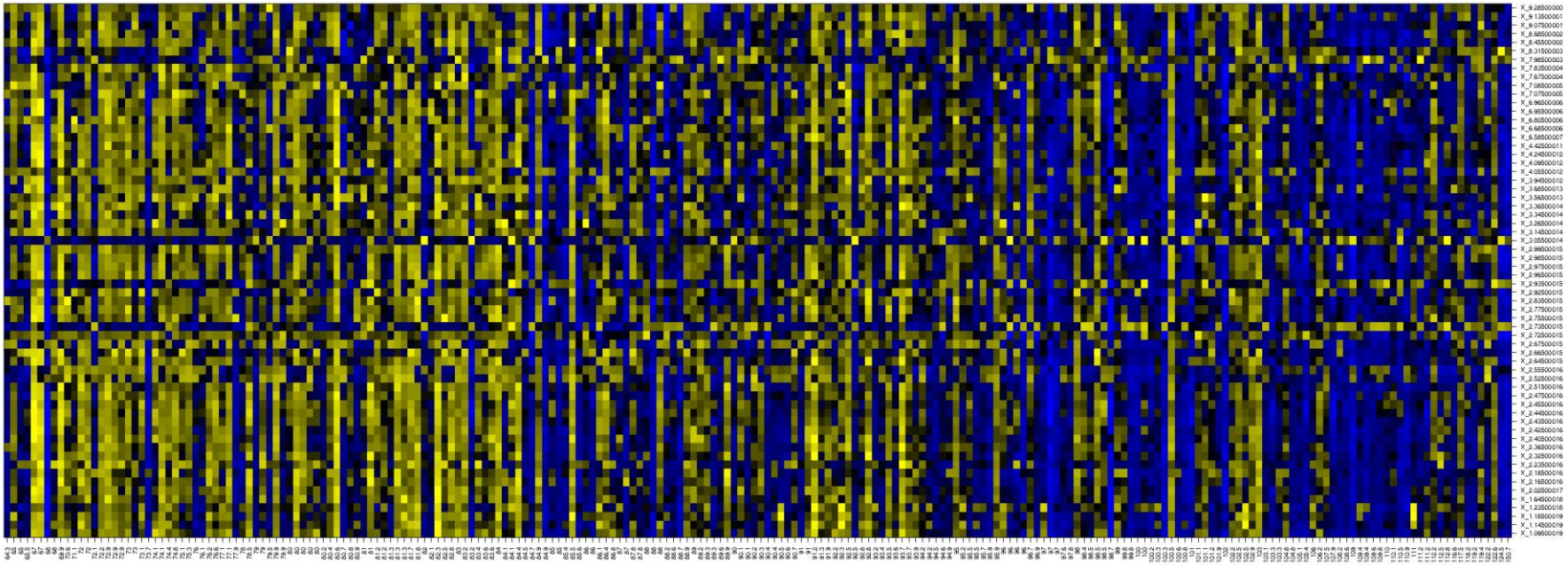


Figure 13: Heat map showing significant[#] relations of waist circumference and urinary bins in the overall study population.
X axis shows individual value of waist circumference from lowest (left corner) to highest (right corner). [#] p-values<0.05 after correction for multiple testing are considered significant.

In subgroup analyses stratified by sex, no significant relations were found between VAT, SAT, VSR, BMI, or WC and urinary bins. However, when analyses were restricted to non-fasting participants, significant associations were found between VAT and 80 bins in fully adjusted models (Model 2) (Supplementary Table 17). No relations were found between SAT or VSR and urinary bins. By comparison, BMI, and WC were significantly related to 99 and 70 bins, respectively (Supplementary Table 18 and Supplementary Table 19). Significant results from analyses among all subjects were reproduced in sensitivity analyses among non-fasting subjects. For instance, the positive relations of VAT, BMI and WC to dimethylamine/citrate were also found among non-fasting participants. Moreover, VAT, BMI and WC were inversely associated with acetone, choline, citric acid, asparagine/unknown, and L-pyroglutamic acid in this group. No significant results were found between VAT, SAT, VSR, BMI, or WC and urinary bins after further stratification of the non-fasting group by sex. Significant associations were found between VAT, BMI, and WC to urinary bins in analyses restricted to subjects with no urinary glucose in fully adjusted models (Model 2). Specifically, VAT was related to 11 bins among subjects with no urinary glucose (Supplementary Table 17). These results were also found to be significant in the overall study population (e.g., 4-hydroxyhippuric acid or L-pyroglutamic acid). By comparison, among the subjects with no urinary glucose, BMI and WC were related to 29 and 12 bins, respectively. No relations were found between SAT or VSR and urinary bins in subjects with no urinary glucose and no significant relations were found between any of the obesity measures and urinary bins among subjects with elevated glucose.

Based on serum metabolic fingerprints, the AP algorithm formed six clusters for the overall study population. Results from age and sex adjusted regression analyses showed that there were no significant differences between clusters in the distribution of VAT, SAT, BMI, or WC (Table 19). The distributions of the age and sex adjusted mean values of phenotypes among clusters are presented in Figure 14.

Table 19: Distribution of phenotypes among clusters formed from serum fingerprints (overall study population).

Cluster	Mean VAT (95% CI)	p	Mean SAT (95% CI)	p	Mean VSR (95% CI)	p	Mean BMI (95% CI)	p	Mean WC (95% CI)	p
1	7.41 (6.57-8.26)	0.67	2.04 (1.78-2.30)	0.48	4.26 (3.66-4.86)	0.51	27.25 (25.83-28.67)	0.59	93.81 (90.25-97.37)	0.61
2	7.09 (6.14-8.05)	0.53	2.10 (1.81-2.39)	0.69	3.68 (3.01-4.34)	0.11	27.93 (26.33-29.54)	0.42	94.72 (90.70-98.74)	0.67
3	7.07 (5.93-8.21)	0.56	1.94 (1.59-2.29)	0.59	4.15 (3.34-4.95)	0.79	27.44 (25.56-29.32)	0.85	93.00 (88.29-97.72)	0.74
4	7.87 (6.60-9.15)	0.50	2.06 (1.68-2.45)	0.91	4.10 (3.19-4.99)	0.72	28.13 (25.99-30.28)	0.44	93.31 (87.93-98.69)	0.86
5	7.39 (6.05-8.73)	0.97	1.67 (1.26-2.08)	0.08	4.92 (3.97-5.87)	0.17	27.31 (25.05-29.57)	0.96	92.14 (86.47-97.81)	0.56
6	6.64 (1.29-12.00)	0.78	0.70 (0.93-2.33)	0.11	6.16 (4.37-9.95)	0.46	22.02 (12.99-31.04)	0.26	79.34 (56.72-101.97)	0.21

Predictive margins from linear regression adjusted for age and sex, grand mean=reference category, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, VSR=visceral-to-subcutaneous-fat ratio, BMI=body mass index, WC=waist circumference, p=p-value for the comparison to the grand mean.

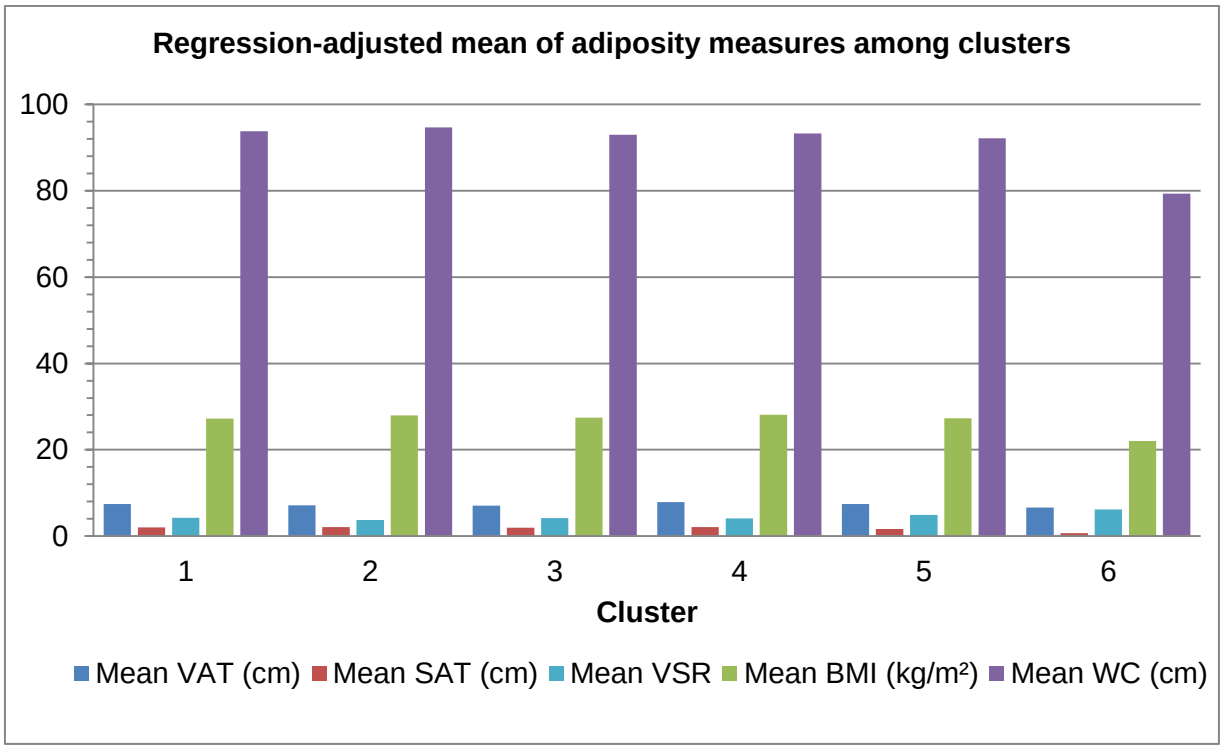


Figure 14: Age and sex adjusted distribution of phenotypes among clusters formed from serum fingerprints (overall study population).

In sensitivity analyses (Supplementary Table 16), four clusters were formed for men and three clusters were formed for women. Among women, the regression-adjusted mean SAT of cluster 2 (p=0.02) and 3 (p=0.002) differed statistically significantly compared to the overall predicted mean SAT. No significant differences between clusters were found for VAT, VSR, BMI, or WC. In addition, no significant differences between clusters regarding the considered phenotypes were found among men. Analyses stratified by fasting status formed four clusters and showed that in cluster 3, the regression-adjusted mean SAT differed statistically significantly compared to the grand mean of SAT among non-fasting participants. No significant differences between clusters were found for VAT, VSR, BMI, or WC. In addition, no significant results were found among fasting participants. A further stratification of the non-fasting participants by sex showed that cluster 3 differed statistically significantly regarding the regression-adjusted mean SAT (p=0.02) compared to the overall predicted mean SAT among non-fasting women. No statistically significant differences between clusters were found regarding VAT, VSR, BMI, or WC. Also no significant differences between clusters with regard to the adiposity measures were found among non-fasting men. Finally, when the

study population was stratified by urinary glucose, three clusters were formed among subjects with no urinary glucose. No significant differences between clusters among these groups were detected for VAT, SAT, VSR, BMI, or WC.

Linear regression models to detect relations of VAT and SAT to serum bins showed no significant results in the overall population. VSR, BMI, and WC also showed no significant relations to individual serum bins. When analyses were stratified by sex, significant results were found between VAT and 20 serum bins among men in fully adjusted models (Model 2) (Table 20). Of these significantly related 20 bins, twelve could be assigned to specific metabolites. In case that more than one compound contributed to a significant bin all corresponding molecules were indicated (e.g., isobutyric acid and unknown compound at 1.065 ppm). VAT was positively related to tryptophan, and showed an inverse relation to ketoleucine. In addition, positive relations were found between VAT and essential amino acids, including valine, phenylalanine, and isoleucine. Note that the bin corresponding to isoleucine shows partial overlap with ketoleucine. By comparison, among women VAT was inversely related to three serum bins in fully adjusted models (Model 2). Those bins were not related to VAT among men. However, only one of the significantly inversely related bins was identified as lipid-cholesterol. No relations were found between SAT, VSR, BMI, or WC and serum bins among men or women.

Among non-fasting participants, there were also no significant relations of VAT, SAT, VSR, BMI, or WC to serum bins. When the non-fasting group was further stratified by sex, VAT showed significant relations to 17 bins among non-fasting men in fully adjusted models (Model 2) (Table 20). By comparison, among non-fasting women, VAT was inversely related to three bins (Model 2). Significantly related bins in both non-fasting men and women were also significantly related in all men and women irrespectively of their fasting status. No relations were found between SAT, VSR, BMI, or WC and serum bins among non-fasting men or women. When analyses were restricted to subjects with no urinary glucose, no significant results were found for any of the obesity measures.

Table 20: Significant results[#] from multiple regression analyses on the relation between VAT and serum bins.

		Model 1		Model 2		
Anthropometric variable	bin (ppm)	ß	p	ß	p	metabolite identification
Men (n=98)						
VAT	0.995	0.0413	0.0146	0.0588	0.0015	Valine
	0.985	0.0381	0.0476	0.0138	0.0285	Valine
	0.935	0.0008	0.9952	0.0466	0.0178	Isoleucine, ketoleucine
	1.065	0.0444	0.8602	0.0993	0.0006	Isobutyric acid, unknown
	1.075	0.0314	0.8602	0.0798	0.0017	Isobutyric acid, unknown
	1.085	0.0271	0.8602	0.0638	0.0202	unknown
	1.105	0.0102	0.9818	0.0826	0.0161	unknown
	1.035	0.0813	0.0246	0.0562	0.0128	Valine
	2.595	0.0203	0.9746	0.0538	0.0285	unknown
	2.605	-0.0160	0.9746	-0.0508	0.0117	Ketoleucine
	2.615	-0.0175	0.9746	-0.0613	0.0060	Ketoleucine
	2.625	-0.0084	0.9818	-0.0502	0.0047	Ketoleucine
	2.635	0.0167	0.9746	0.0672	0.0003	unknown
	2.655	0.0186	0.8602	0.0442	0.0093	Methionine, unknown
	4.345	-0.2167	0.7141	-0.4572	0.0013	unknown
	6.745	0.4734	0.8602	0.7706	0.0351	unknown
	7.375	0.0170	0.9746	0.0669	0.0202	Phenylalanine, unknown
	7.485	-0.2613	0.8602	-0.4372	0.0388	unknown
	7.555	0.0485	0.9802	0.1351	0.0274	Tryptophan
	7.675	-0.2390	0.7141	-0.3350	0.0082	unknown
Women (n=102)						
	0.605	-0.4264	0.1599	-0.3640	0.0136	unknown
	0.735	-0.0242	0.5579	-0.0482	0.0136	Lipid-cholesterol
	7.845	-0.2337	0.1888	-0.4020	0.0368	unknown

Table 20 continued: Significant results from multiple regression analyses on the relation between VAT and serum bins.

		Model 1		Model 2		
Anthropometric variable	bin (ppm)	ß	p	ß	p	metabolite identification
Non-fasting men (n=78)						
VAT	0.935	0.0413	0.0146	0.0588	0.0015	Isoleucine, ketoleucine
	0.985	0.0381	0.0476	0.0138	0.0285	Valine
	0.995	0.0210	0.5806	0.0454	0.0309	Valine
	1.035	0.0694	0.0993	0.0962	0.0020	unknown
	1.075	0.0494	0.2005	0.0764	0.0034	Isobutyric acid, unknown
	1.085	0.0399	0.3770	0.0594	0.0467	unknown
	1.105	0.0311	0.3770	0.0507	0.0219	unknown
	2.595	0.0409	0.3770	0.0548	0.0467	unknown
	2.605	0.0405	0.1133	0.0505	0.0136	Ketoleucine
	2.615	0.0410	0.1447	0.0606	0.0085	Ketoleucine
	2.625	0.0321	0.3385	0.0486	0.0034	Ketoleucine
	2.635	0.0358	0.1447	0.0571	0.0006	unknown
	2.655	0.0282	0.3384	0.0584	0.0023	Methionine, unknown
	4.345	-0.2383	0.2005	-0.3690	0.0125	unknown
	6.535	0.4483	0.3770	0.7294	0.0467	unknown
	7.375	0.0356	0.4929	0.0761	0.0134	Phenylalanine, unknown
	7.675	-0.2247	0.3932	-0.3475	0.0117	unknown
Non-fasting women (n=94)						
VAT	0.605	-0.4988	0.1530	-0.4837	0.0033	unknown
	0.735	-0.0341	0.5236	-0.0631	0.0049	Lipid-cholesterol
	7.845	-0.2551	0.3720	-0.5066	0.0373	unknown

Model 1: linear regression model adjusted for study, age (non-linear) and sex.
Model 2: linear regression model adjusted for study, age and sex interaction (non-linear), smoking status, menopausal status (women only), fasting status, urinary glucose, and physical activity. In each case, the stratification variable was excluded from the model.
VAT=visceral adipose tissue, bin= spectra positions (ppm), ß=standardized linear regression coefficient, p=corrected p-value by controlling the false discovery rate.
[#]p-values<0.05 after correction for multiple testing were considered significant.

4 Discussion

The research underlying present thesis was conducted using a systematic and multistep approach to address several challenges of obesity research in an epidemiologic setting. First, a method capable of differentiating and quantifying abdominal adipose tissue compartments was investigated with respect to its reproducibility, validity, and feasibility in the context of epidemiological studies. Further, different measures of obesity and their associated metabolic consequences were examined extensively by numerous investigations, proceeding from hypothesis-driven analyses of inflammatory parameters in serum samples to more complex and unsupervised approaches studying urinary and serum metabolomic profiles.

4.1 Summary of the findings

Results from the current study revealed that ultrasound represents a reproducible and valid method for assessing VAT and SAT. The intra- and inter-observer reproducibility was high for measurements of VAT and SAT. Further, the correlations of ultrasonographic measurements of VAT and SAT with measurements of VAT and SAT derived from MRI were strong. The present findings add to the growing evidence that ultrasound is a useful method for estimating abdominal fat compartments and represents a suitable method for large-scale epidemiologic studies.

When examining relationships of different obesity phenotypes, distinct associations with selected parameters of chronic inflammation and urinary and serum metabolites were found for VAT, SAT, VSR, BMI, and WC, indicating that each of the anthropometric variables provides distinct information regarding metabolic processes related to inflammatory parameters and metabolite regulation. VAT most consistently demonstrated relations to inflammatory parameters, individual urinary and serum metabolites. SAT was the strongest correlate for increased hs-CRP concentrations but was not related to urinary or serum metabolites. VSR represented a stable association with decreased resistin levels but was not related to urinary or serum metabolites. By comparison, BMI was the strongest correlate for decreased adiponectin levels and was consistently related to urinary metabolites. WC represented less consistent relations

when examining associations with inflammatory parameters and serum metabolites. However, WC was consistently related to urinary metabolites.

4.1.1 Reproducibility and validity of the sonographic-based quantification of visceral and subcutaneous adipose tissue [1]

In the present study, the reproducibility of the ultrasound measurements of VAT and SAT was high, as indicated by ICC values exceeding 0.9. Both study centers performed the ultrasound measurements according to a protocol that provided detailed procedures regarding the precise anatomic placement of the transducer, the specific amount of pressure to be applied to the abdomen by the transducer, and the exact timing of the ultrasound measurement in relation to the timing of respiration. Other studies using a similar protocol also reported favorable results, with ICCs of 0.90-0.99 (157, 159) and mean differences ranging from 0.25-0.69 mm (153, 160). An earlier study not employing a standardized protocol reported less favorable results for reproducibility (r=0.64) (161).

According to the results presented in this thesis, ultrasound can be considered a valid method for assessing VAT and SAT when compared to MRI measurements of VAT and SAT area. The correlation between the two methods was high, particularly for VAT. Our results are consistent with previous studies that reported modest to high correlations between VAT thickness as assessed by ultrasound and VAT measured by MRI or CT, with correlation coefficients ranging from 0.67 to 0.91 (52, 140, 148-158). Two previous studies reported a modest correlation coefficient of 0.67. Both studies were conducted among overweight women with sample sizes of 50 (150) and 101 subjects (148). Authors from these investigations reported slightly higher correlations of 0.71 and 0.74 in a second study they conducted among 100 and 119 overweight women (151, 156). Other studies included men and women and showed correlations of ≥0.79 (52, 140, 149, 152-155, 157, 158).

In subgroup analyses stratified by BMI, the correlations between the two methods were lower for obese than lean subjects. Similarly, a previous study showed a slightly

[1] In the framework of this thesis, parts of the discussion on reproducibility and validity of the quantification of VAT and SAT have already been published in: Schlecht, I., et al., Reproducibility and validity of ultrasound for the measurement of visceral and subcutaneous adipose tissues. Metabolism, 2014. 63(12): p. 1512-1519.

weaker correlation for VAT of 0.75 among obese subjects (BMI ≥30) compared to an overall correlation of 0.82 (140). One possible reason for a weaker correlation among obese than lean subjects is that measuring a greater distance (fat thickness) may be more prone to measurement error. In addition, in this study might be underpowered to detect subtle differences across BMI groups.

In general, higher correlations were found in studies that used a protocol that included instructions to perform the examination at the end of a normal exhalation and to place a minimal amount of pressure on the probe during the ultrasound examination (152, 154, 157). Although the use of different anatomic landmarks in measuring visceral fat may influence the results, no clear recommendations can be made as to whether visceral fat thickness should be defined as the distance between the internal face of the rectus abdominis muscle and the anterior wall of the aorta (r=0.67-0.86) (52, 140, 148-150, 154, 156, 178) or the corpus of the lumbar vertebra (r=0.73-0.91) (152, 153, 155, 157).

The correlation coefficients between SAT thickness as assessed by ultrasound and SAT as assessed by MRI or CT ranged from 0.33 to 0.83 in previous studies (149, 152, 153, 155, 156). One study reported a correlation coefficient as low as 0.33 in their study among overweight women (156). In that study (156), ultrasound measurements were performed with the transducer placed 1 cm above the umbilicus, which is differed from another study that showed a correlation of 0.52 when they took measurements 5 cm cranial to the umbilicus (153). Other studies showed higher correlations of ≥0.63 when they placed the transducer at the cut-point between the left and right midpoint of the lower rib and the iliac crest on the median line of the abdomen (152) or placed it at the umbilicus level (149, 155). In addition, the highest correlation of 0.82 was yielded when ultrasound measurements of SAT were performed according to a protocol that emphasized the need for applying minimal pressure to the skin (155). Another reason for the higher correlations found in the validation study may be the location of the MRI examination at L2-L3 (L2-L3±). Previous validation studies used L4-L5 or L4 for VAT and SAT measurements by MRI (52, 140, 148-158). In the present study, L2-L3 was chosen because a recent study found the highest correlation (r=0.96) at this location (179). In addition, the locations of L2-L3, L2-L3- and L2-L3+ are anatomically closer to

our ultrasound measurement at the cut-point between the left and right midpoint of the lower rib and the iliac crest on the median line of the abdomen.

In summary, CT and MRI have been considered the most accurate and reproducible instruments for the measurement of VAT and SAT. However, CT and MRI scans are costly and time-consuming. In addition, CT exposes subjects to ionizing radiation. Thus, those methods are not feasible for use in large-scale epidemiologic studies of non-diseased individuals. BMI, WC, and WHR are the most common methods for estimating body fat, and several studies have reinforced the role of those parameters in the prediction of morbidity and mortality of obesity-associated diseases (45, 120, 180). Although BMI, WC and WHR are convenient and cost-effective methods for estimating body fat, they are inadequate for individuals with high levels of BMI (156) and the fact that WHR might not change with weight loss, reduces their utility in follow-up studies (181). In addition, BMI, WC, and WHR cannot distinguish between VAT and SAT, which is essential for distinguishing between abdominal fat components as they relate to the development of obesity and associated chronic diseases. It has been recognized that obesity-related chronic diseases, including cardiovascular disease, type 2 diabetes, and several types of cancer, are more strongly related to body fat distribution than to total body fat (43, 139, 182). Several reports have revealed the significance of VAT in obesity-related diseases, whereas SAT may play a protective role for the development of type 2 diabetes (18, 51, 183). The addition of reproducible, accurate, and feasible methods to quantify abdominal fat components in large-scale epidemiologic studies contributes to increased statistical power and reduced measurement error that may help advance the field of obesity epidemiology that seeks to identify the etiology and sequelae of obesity.

4.1.2 Relations of adiposity measures to parameters of chronic inflammation

The present population-based study of healthy adults showed distinct associations between VAT, SAT, VSR, BMI, and WC with selected parameters of chronic inflammation. Specifically, VAT, SAT, BMI, and WC demonstrated a positive relation to hs-CRP. The strongest relation was found between SAT and hs-CRP. Compared to the other anthropometric variables, BMI showed a stronger inverse association with adiponectin. Albeit not statistically significant, VAT was the strongest correlate for

increased levels of IL-6 and TNF-α. VSR presented the strongest associations with increased resistin levels. WC was only weakly related to most of the inflammatory parameters. These findings were fairly consistent throughout subgroups defined by sex, BMI, current smoking, and use of aspirin and NSAIDs.

Similar to the presented results, previous studies among healthy adults reported that VAT, SAT, BMI, or WC were positively associated with CRP (83, 93-95, 98, 184). Several investigations reported comparable relations of VAT and SAT to CRP (94, 98) or a stronger association with VAT (93, 95, 97), whereas other studies found a stronger relation with SAT (96, 98, 184). However, none of the aforementioned studies mutually adjusted for inflammatory parameters or VAT and SAT (93-98, 184). When VAT and SAT were mutually adjusted, only SAT remained significantly positively associated with hs-CRP, indicating that abdominal SAT may have pathogenic function, as additionally evidenced by endocrine and inflammatory responses (28, 38, 51, 76). No relation was found between VSR and hs-CRP which is in line with the only previous study that examined the association between those two parameters (79).

Associations of VAT, BMI, and WC with hs-CRP were stronger in women than men, which is in agreement with previous studies (80, 81, 94, 98). This finding is likely to be the result of enhanced estrogen production in the adipose tissue with upregulation of pro-inflammatory gene expression in women (185, 186). The findings in women of similar relations of VAT, SAT, BMI, and WC, but weaker relations of VSR to hs-CRP suggest that in women, associations with CRP are more strongly determined by overall fat mass than by fat distribution. In contrast, in men, SAT, but not VAT, BMI, or WC was moderately associated with CRP, which is consistent with previous studies (96, 98, 184). Together with the observed inverse association between VSR and hs-CRP, these findings indicate that adiposity relations with CRP in men may be less strongly influenced by overall fat mass.

The observation of a more pronounced relation of SAT to hs-CRP than VAT in overweight/obese subjects has not yet been reported. Only one previous study stratified by BMI and found no significant association between SAT and CRP in obese subjects (97). However, different from that study, adjustments for potential confounding variables were considered in the present analyses. In obese individuals, the limited ability of

abdominal SAT to store excess energy may cause an increase in FFA flux to the portal vein and the systemic circulation (55). Increased FFA levels may then raise CRP (187).

All anthropometric variables showed stronger associations with hs-CRP in current smokers than non-smokers. Cigarette smoking is associated with increased CRP levels (188), which may, at least in part, reflect the mechanisms believed to underlie the adverse effects of smoking on cardiovascular disease and several types of cancer (189). None of the previous studies that examined the relation between adiposity and CRP reported results stratified by smoking status (83, 93-95, 98, 184). Also, previous studies examining the relations of obesity to CRP and other inflammatory parameters did not report findings stratified by aspirin or NSAIDs use (83, 93-95, 98, 184). Associations of anthropometric factors to hs-CRP were found to be more pronounced among users of aspirin or NSAIDs. Because NSAIDs down-regulate inflammatory cytokine production including CRP (190, 191), it was expected that associations with inflammatory parameters among users of aspirin or NSAIDs were less pronounced than among non-users. This unexpected finding may be explained by the unequal distribution of aspirin or NSAIDs users and non-users and the small number of participants particularly in the group the aspirin or NSAIDs users.

The findings from multivariable analyses are consistent with those from previous studies reporting a positive association between VAT and IL-6 (94, 95, 97, 98, 104) and no relation between SAT and IL-6 (97, 98, 104). However, the positive relation of VAT to IL-6 was rendered non-significant after mutual adjustment for other parameters of systemic chronic inflammation and when SAT was included in the model. Only one previous study examined the relation between VSR and IL-6 and also found no significant association between the two parameters (79). Positive associations between VAT and IL-6 emerged in additional analyses among men, overweight/obese individuals, current non-smokers, and participants not using aspirin and NSAIDs. In these analyses, we additionally found that associations between VAT and IL-6 were stronger than those between BMI and IL-6, indicating that collecting data on VAT may represent metabolic information captured by IL-6 that is not accounted for by BMI. Only one previous study stratified their population by sex and reported a stronger relation between VAT and IL-6 in women than men (98). However, that study was limited to elderly individuals, which may explain the difference in comparison to the results of the present study. Men have

larger visceral fat depots than women (192, 193) and IL-6 is predominantly expressed and secreted by VAT (194).

No overall relations of VAT, SAT, VSR, BMI, or WC to TNF-α were found, which is similar to other studies that addressed these associations (94, 95, 97). Albeit not statistically significant, the relation of VAT to TNF-α was stronger than the relations of other obesity measures to TNF-α. In further exploratory analyses, positive relations of VAT to TNF-α and IL-6 among non-users of aspirin or NSAIDs was noted, which may be due to NSAID-mediated down-regulation of inflammatory cytokine production (190, 191). The available literature includes one study that reported a positive relation between VAT and TNF-α in adults aged 70 to 79 years, but no association between SAT and TNF-α (98), and another study that found positive relations of both VAT and SAT to TNF-α among obese adolescents (104). However, none of those studies mutually adjusted for inflammatory parameters or VAT and SAT.

No associations were detected between VAT, SAT, BMI, or WC and resistin levels. This is consistent with most previous studies that found no correlations between markers of adiposity and resistin (88-90, 195-200), whereas other studies reported a positive relation of obesity to resistin levels (100, 101, 201-204). Only one population-based study that examined the relation of VAT and SAT to resistin reported results from multivariable analyses and found similar relations of VAT and SAT to resistin in women and no association between VAT and resistin in men (101). No previous study has targeted the relation between VSR and resistin before. In the presents study, VSR showed a moderate inverse relation to resistin. Albeit not statistically significant, the relations of VAT (inversely) and SAT (positively) appeared in opposite directions. Taken together, these findings indicate, that the distribution of VAT relative to SAT may be more relevant regarding the secretion of resistin than the individual amount of VAT or SAT alone. Moreover, resistin is not expressed by adipocytes, but is secreted by macrophages located within adipose tissue depots (205). Hence, circulating resistin is not directly related to adiposity levels, but to the degree of inflammation within the adipose tissue depots (55).

Largely similar to results in the present thesis, previous studies reported that VAT, SAT, BMI, or WC were inversely associated with adiponectin (96, 102-104, 206). In the present study, the inverse relation of VAT to adiponectin was attenuated and rendered

statistically non-significant after mutual adjustment for other parameters of systemic chronic inflammation and when SAT was included in the model. BMI was found to be a stable indicator of decreased adiponectin levels, showing an inverse association in the overall population before and after adjustment for other variables and across a number of stratified analyses. In addition, the relation of BMI to adiponectin was found to be stronger than those with other markers of adiposity in all analyses. This suggests that adiponectin may better represent metabolic processes that are associated with BMI than those related to VAT or SAT.

To summarize, in the present thesis VAT, SAT, VSR, BMI, and WC showed distinct associations with selected parameters of chronic inflammation. These results suggest that each of the anthropometric variables provides distinct information regarding metabolic processes related to inflammatory parameters. Compared to VAT, BMI, and WC, SAT presented the strongest association for increased hs-CRP concentrations. BMI was the strongest correlate for decreased adiponectin levels and VSR showed the strongest associations with decreased resistin levels. Albeit not statistically significant, VAT was the strongest correlate for increased levels of IL-6 and TNF-α. WC represented a less consistent parameter when examining relations to inflammatory parameters. Subgroup analyses showed that sex, BMI, current smoking, and use of aspirin or NSAIDs modify the relations of adiposity measures to levels of inflammation parameters. The distinct relations of VAT, SAT, VSR, BMI, and WC to selected parameters of systemic chronic inflammation emphasize the importance of accurately differentiating between body fat compartments when evaluating the role of adiposity-associated systemic chronic inflammation in the development of metabolic diseases.

4.1.3 Relations of adiposity measures to quantified urinary and serum metabolites

Results from the targeted metabolomics approach showed that only urinary but no serum metabolites were significantly related to adiposity measures. VAT, in particular, was associated with urinary metabolites in fully and mutually adjusted models among the overall population and stratified subgroups. SAT showed no significant relations to urinary or serum metabolites in any of the analyses. By comparison, BMI and WC were consistently associated with urinary metabolites in the overall study population and

among subgroups. In this study, the absolute number, the effect sizes, and significance levels of relations between measures of obesity and urinary metabolites were generally greater among men than women. This is concordant with previous studies that reported sexual dimorphism of obesity and the metabolic profile (132, 207). Prior studies reported significant positive or inverse associations between obesity and lipids, amino acids including alanine, glutamine, and valine, and gut flora-derived metabolites such as hippuric acid (128, 129, 131, 133-135) or dimethylamine (133) (Supplementary Table 2).

In the present study, significant positive and inverse relations of VAT, BMI, and WC to urinary metabolites were detected in the overall study population and in stratified analyses among men and women, non-fasting subjects, and subjects with no urinary glucose. Specifically, VAT, BMI, and WC were consistently inversely related to the detection of urinary choline in the overall study population and stratified subgroups including men, non-fasting participants, and subjects with no urinary glucose. The strongest and most significant associations were found between VAT and choline. In addition, when VAT and SAT on the one hand and BMI and WC on the other hand were mutually adjusted, only VAT but none of the other parameters remained statistically significantly and inversely related to choline in the overall study population and in stratified subgroups. This indicates that predominantly VAT is related to choline metabolism. Choline is an essential nutrient, playing a complex role in human metabolism (208). Specifically, choline is needed for neurotransmitter synthesis (acetylcholine), cell-membrane signaling (phospholipids), lipid transport (lipoproteins), and methyl-group metabolism (homocysteine reduction) (209).

Previous metabolomics studies reported inverse relations of serum choline to type 2 diabetes (210) and cardiovascular disease (211). A lack of choline can lead to liver fat accumulation and consequently to non-alcoholic hepatic steatosis (NAFLD) including non-alcoholic steatohepatitis (NASH) due to lack of very low density lipoprotein (VLDL), which shuttles lipids away from the liver (208). NAFLD and NASH are considered components of hepatic manifestation of the metabolic syndrome and a potential causal factor in the development of type-2 diabetes and cardiovascular disease (212, 213). In addition, choline deficiency is associated with increased levels of several inflammatory markers that link obesity to obesity-associated diseases, including CRP, homocysteine, IL-6, and TNF (208, 214). A possible mechanism is that with inadequate choline stores,

the capacity to methylate homocysteine to methionine is diminished and consequently, plasma levels of homocysteine increase (208). Homocysteine was demonstrated to contribute to the initiation and progression of vascular disease by activating monocytes, resulting in the secretion of cytokines that amplify the inflammatory response (215).

In addition, VAT, BMI, and WC were positively related to the detection of urinary dimethylamine in the overall population, among men and women, non-fasting participants, and subjects with no urinary glucose. Compared to other measures of obesity, the strongest relations were found between VAT and dimethylamine and only weak relations were found between WC and dimethylamine. This indicates that VAT may be the main indicator for increased dimethylamine levels. A positive association of BMI with urinary dimethylamine was reported recently by the international study of macro- and micro-nutrients and blood pressure (INTERMAP), which was replicated in INTERMAP UK (133). Dimethylamine is a gut microbial metabolite that is related to the gut microbial metabolism of choline. The impact of the gut microbiota on dietary choline, for the most part originating from carnitine and lecithin, to produce trimethylamine by choline trimethylamine-lyases and subsequently the conversion of trimethylamine to trimethylamine-N-oxide to dimethylamine describes a pathway that has been linked to the development of atherosclerosis and cardiovascular disease (216, 217).

Increased urinary excretion of microbial metabolites from co-metabolic processing of choline has also been related to steatosis and insulin resistance in animals fed high-fat diets (218). In humans, an increase in energy intake from high-fat diets rapidly influences the composition of the gut microbiota (219). These compositional changes in microbiota may lead to increases in systemic endotoxin levels (220) that consequently may contribute to the low-grade inflammation, insulin resistance, adipocyte hyperplasia, and decreased β-cell function that characterizes the metabolic syndrome (219). Another source of dimethylamine is the catabolic pathway of asymmetric dimethylarginine that is hydrolyzed by dimethylarginine dimethylaminohydrolases (221). Elevated levels of asymmetric dimethylarginine have been reported to cause endothelial dysfunction in various etiologies of cardiovascular disease such as essential hypertension and metabolic syndrome (222, 223) thus leading to atherosclerosis (224) and development of coronary artery disease (225).

BMI and WC, but none of the other adiposity measures showed inverse relations to the detection urinary glutamine among the overall study population and in stratified analyses among men, non-fasting participants, and subjects with no urinary glucose. BMI showed stronger relations with glutamine than WC in the analyses, and when WC and BMI were mutually adjusted, only BMI remained significantly inversely related to glutamine. This suggests that glutamine may better represent metabolic processes that are associated with BMI than other obesity measures. This finding is in agreement with previous studies that reported inverse associations between obesity and serum (135, 136) or urinary (133) glutamine. Glutamine is a non-essential amino acid and presents the most abundant free amino acid in skeletal muscle tissue and plasma (226), playing an important role in maintaining skeletal muscle, immune system function as well as glucose and glycogen metabolism (227).

No significant relations were found between VAT, SAT, VSR, BMI, or WC and quantified serum metabolites. Although the human urine metabolome is generally believed to constitute a subset of the human serum metabolome (228), a few hundred compounds identified in urine still await their detection in blood (229). The fact that so many compounds seem to be unique to urine most likely reflects the ability of the kidney to concentrate certain metabolites from blood with urinary levels of some metabolites exceeding those in serum more than a 1000-fold (e.g., histamine) (229). In addition, the use of ultrafiltration in sample pretreatment allows only measurement of the protein-unbound fraction of metabolites (230). As a result, many serum metabolites may go undetected. This holds particularly true for NMR spectroscopy, a fairly insensitive method with lower limits of quantification in the low to medium micromolar range (231).

In conclusion, when different measures of adiposity are considered, VAT showed the strongest and most significant relations to urinary metabolites, even when VAT and SAT were mutually adjusted. By comparison, SAT showed no associations with urinary metabolites. Additional important relations were found between BMI or WC and urinary metabolites. However, when BMI and WC were mutually adjusted, most of the relations were altered and did not remain significant. Only BMI remained significantly related to urinary glutamine in mutually adjusted models. In addition, stronger and more significant associations were noted between adiposity measures and urinary metabolites compared to the relations to serum metabolites. In general, the present findings support the notion

that VAT is more strongly involved in metabolite regulation than abdominal SAT. The distinct relations of VAT, BMI, and WC to metabolites and the null-findings for associations of SAT and VSR with metabolites emphasize the importance of accurately differentiating between body fat compartments when evaluating the potential role of adiposity-associated metabolic regulation in the development of obesity-related diseases.

4.1.4 Relations of adiposity measures to the urinary and serum metabolic fingerprints

Results from the untargeted metabolomics approach showed that the unsupervised classification of urinary and serum metabolic fingerprints is not capable of grouping subjects by their VAT or SAT thickness in the overall study population. However, in stratified analyses among women, clusters from serum fingerprints were formed that differed statistically significantly by SAT. Specifically, among women, SAT, but none of the other measures of obesity influenced the metabolic fingerprint that significantly differed between groups of high, medium, and low SAT. This is to be expected, as the metabolic fingerprints of generally healthy individuals are influenced by a multitude of factors including among others diet, exercise, time of the day, and gut flora composition. In addition, although obesity is frequently associated with metabolic disorders (11-13), not all obese individuals display a clustering of metabolic risk factors. Recent interest has focused on an obese subgroup with a healthy metabolic profile despite increased adiposity and has been investigated by several researchers (232-234).

When multiple linear regression models were applied, VAT, BMI, and WC were consistently related to numerous urinary bins in the overall study population and in stratified subgroups. No relations were found between SAT or VSR and urinary bins. By comparison, VAT but none of the other measures of obesity was related to serum bins. Moreover, relations to serum bins were found in stratified analyses only, indicating that serum metabolites might be more strongly influenced by sex differences than urinary metabolites. With the application of the untargeted metabolomics approach in combination with multiple linear regression models, further candidate metabolites were identified that were not included in the set of quantitated metabolites. For instance, in

addition to choline, VAT was inversely related to numerous urinary bins identified as 4-hydroxyhippuric acid, acetone, L-pyroglutamic acid, citric acid, and scyllo-inositol. Moreover, in addition to urinary dimethylamine, VAT was positively related to lysine and creatinine. Note that for the latter bin, below the dominating creatinine signal a minor contribution of tyrosine was observed. These significant relations were also reproduced in stratified analyses among non-fasting subjects. Notably, VAT but none of the other adiposity measures was significantly inversely related to 4-hydroxyhippuric acid. As a glycine conjugate of 4-hydroxybenzoic acid, 4-hydroxyhippuric acid is an end-product derived of polyphenol metabolism by the intestinal microflora (235). A previous study suggested that the intake of fruits containing polyphenols rich in anthocyanins (i.e., berries and grapes) may increase 4-hydroxyhippuric acid in the urine (236). Anthocyanins are naturally occurring polyphenols with anti-inflammatory activity (237, 238). Hence, the observed inverse relations of VAT to 4-hydroxyhippuric acid found in the present study may reflect a diet lacking in health benefitting nutrients among participants with increased VAT. However, concentrations of 4-hydroxyhippuric acid were not quantitated in the present study. Nevertheless, 4-hydroxyhippuric acid may be a promising candidate metabolite for future obesity research.

Associations between VAT and serum bins were found in sex-stratified analyses only. This indicates a possible interaction of sex on the association between VAT and serum metabolites that needs further investigation. Among men and non-fasting men, VAT was significantly related to serum bins that were not significantly related in women. Two previous studies demonstrated major differences in metabolic fingerprints between men and women (130, 132). Specifically, the authors reported that the serum metabolic profile showed no significant difference between lean and obese participants in the overall study population. Moreover, similar to the present study, one study reported differences in abundance of serum metabolites between lean and obese men, but not between lean and obese women (132). Interestingly, no relations were found between SAT, VSR, BMI, or WC and serum metabolites, indicating that VAT may be predominantly responsible for metabolite alterations in serum among men. Note that the significant relations found between VAT and serum bins were detected in untargeted analyses could not be corroborated by targeted analyses. However, results from targeted analyses showed that the association between VAT and valine (ß=0.07;

p=0.06) was “borderline significant” after the correction for multiple testing in fully adjusted models. These differences may be explained by the fact that in targeted analyses only those integrals are considered that were above the LLOQ, whereas in untargeted analyses all integrals for one bin were considered. Moreover, the additional normalization of spectral areas using VSN on untargeted data may have attenuated some of the relations.

The positive relations found between VAT and serum valine is consistent with findings from previous studies that reported significantly higher levels of BCAA, including valine in obese participants compared to lean participants (128, 131, 132, 239). Moreover, plasma BCAA levels were positively correlated with insulin resistance in a previous study (128). Another study concluded, that BCAAs and aromatic amino acids could serve as predictors for the development of diabetes (240). No relations of SAT, VSR, BMI, or WC to serum BCAAs were found in the present study, indicating that VAT is predominantly involved in the BCAA metabolism. This supports findings from previous studies that identified adipose tissue and adipocytes to be a main contributor to whole-body, branched-chain amino acid catabolism (241). However, the mechanisms explaining the elevated levels of BCAAs in obesity are not fully understood. Because BCAAs are essential amino acids, a diet high in protein may be one possible explanation (242). Yet, two previous studies examining the associations of BCAAs with the risk for diabetes and insulin resistance concluded that elevated BCAA levels were not influenced by protein consumption (240, 243). Another possible underlying mechanism may be a genetic variation in the expression of genes encoding key BCAA catabolic enzymes or proteins that control protein synthesis and turnover (242). Interestingly, a recent study postulated a potential role of inflammation in controlling the BCAA metabolism pathway, indicating that the accumulation of serum leucine, isoleucine, and valine may partly be explained by decreased BCAA metabolism in inflamed visceral adipose tissue (244).

In conclusion, in the present study, neither the urinary nor the serum metabolic fingerprint was capable to steadily cluster groups that differed by their age and sex adjusted mean VAT, SAT, VSR, BMI, or WC. However, VAT was consistently related to urinary and serum metabolites in multiple regression analyses. By comparison, SAT and VSR showed no relation to urinary or serum metabolites, further indicating that VAT is a

major contributor to metabolite regulation. In addition, relations between VAT and serum metabolites occurred in sex stratified analyses only. The present findings suggest potential pathways that may explain possible mechanisms underlying obesity-associated diseases. Specifically, metabolites and their related pathways could be identified that may explain mechanisms of obesity-induced low-grade inflammation. In addition, the accumulation of VAT may be, at least in part, responsible for dysregulation of BCAAs in serum. BMI and WC were consistently related to urinary metabolites but no relations were found between BMI or WC and serum metabolites. Compared to the relations of WC and BMI to urinary metabolites, relations between VAT and metabolites were generally stronger. In addition, some relations of metabolites such as 4-hydrxyhippuric acid were unique to VAT. Nevertheless, the number of metabolites that were significantly associated with VAT was smaller than the number of metabolites related to BMI and WC. This might indicate that the impact of VAT, BMI, and WC differs regarding metabolic regulation and metabolic pathways associated with VAT cannot precisely be reflected by BMI or WC alone.

4.2 Strengths and limitations

The present study is based on a cross-sectional study design. All information refers to the same point in time and represents a snapshot of the study population regarding the exposure and outcome variables. Thus, the cross-sectional nature of our study design precludes an assessment of temporality and reverse causality, i.e., that the metabolite changes were due to overt and undiagnosed disease conditions and are not explained by body fat composition. Nevertheless, using a data-driven approach, as chosen in this study, cross-sectional studies can be instrumental in generating hypotheses for future studies. The study population consisted of a rather small sample size, potentially resulting in insufficient statistical power to detect associations, particularly in subgroup analyses. In addition, the present study population was limited to Caucasians, which bears the potential shortcoming that findings may not strictly apply to non-Caucasian ethnicities.

Although gold standard methods to measure VAT and SAT are MRI or CT, these approaches are limited in field conditions due to the associated costs and issues regarding accessibility, contraindications, and in terms of CT examinations, potential

adverse effects of radiation. The major strength regarding the exposure assessment in the present thesis is that by the use of ultrasound, abdominal fat compartments could be differentiated and quantified. However, the ultrasound method to quantify VAT and SAT is not trivial and it requires experienced examiners with considerable skills. A further asset of the present study is that all analyses also included classical anthropometric variables, such as BMI and WC as exposures, which enabled comparison of results with those from VAT and SAT.

To evaluate the reproducibility and validity of the sonographic-based quantification of VAT and SAT, a single MRI slice was used to quantify the area of VAT and SAT. The volume of the body fat compartments in the entire abdomen would have been a more suitable gold standard criterion because individual variation in the distribution of VAT and SAT across the abdominal area may exist (245, 246). However, validation studies with large sample sizes (n>668) showed high correlations between single slice MRI or CT measurements and volume of abdominal fat compartments (r>0.96) (179, 247). Additionally, it was not possible to examine the true agreement between the two methods because the ultrasound and MRI measurements were based on different dimensions or units (thickness vs. area). However, there was strong agreement between ultrasound and MRI measures when using thickness measurements as a substitute for abdominal fat area. The ICC was calculated as a measure of reproducibility in the present thesis. An advantage of the ICC is that it accounts for both variance components: between- and within-person variation and their relation (173, 248).

The present data is based on data retrieved from the pre-test studies of the GNC. Therefore, compromises had to be taken in biological specimen collection because of logistic reasons and cost constraints. Participants of the study were examined only once and consequently, urinary and serum analyses were based on a single laboratory measurement and they may not represent the true long-term average urinary and serum concentrations of inflammatory parameters and metabolites. In addition, all participants of pre-test I were non-fasted and only a small proportion of participants from pre-test II provided fasting blood and urine samples. Hence, the inflammation study included non-fasting participants only, whereas the metabolomics study population was heterogeneous and it included fasting and non-fasting participants. However, the focus was directed on inflammatory parameters that are unaffected by fasting status (249,

250) and a previous study on the reliability of metabolite concentrations over time stated that for most metabolites, a single measurement may be sufficient (251).

The inflammation study accounted for correlations between individual parameters of systemic inflammation by mutual adjustment in multivariable models, which has not been done in any previous study. In addition, numerous informative exploratory subgroup analyses were conducted. Due to the numerous additional analyses performed, some of the findings may have been the result of multiple testing.

In the main metabolomics analysis, data of fasted and non-fasted participants were combined. Differences in diet (e.g., meat or dairy consumption) may induce variation in both serum and urinary metabolites (252). However, one study reported that habitual diet had only a small impact on serum metabolites (253). Unfortunately, the majority of the urine and blood specimens were non-fasting, and stratifying by fasting status may have resulted in reduced statistical power in fasting participants. Stratified analyses among the non-fasting participants showed that associations between anthropometric measurements and urinary and serum metabolites had the same direction and were of similar strength compared to the overall study population, which indicates that these associations might not be affected by fasting status. Nevertheless, among non-fasting participants, more significant results were found between VAT and urinary bins in untargeted analyses than in the overall study population, indicating that fasting status may affect some urinary metabolites.

The metabolomics study included both, a targeted and an untargeted approach on urine and serum specimens and it represents an innovative and comprehensive approach to study biological mechanisms related to VAT and SAT. However, the statistical approach of NMR-obtained metabolomics data is challenging due to large and complex datasets. Data normalization is an essential step in NMR-based metabolomics to improve data quality and to remove unwanted bias such as differences in fluid intake, experimental error, or technical variation (177). In the present thesis, urinary data were creatinine-normalized to account for dilution effects due to differences in fluid intake. The assumption is that creatinine is an indicator of the concentration of urine due to a constant excretion of creatinine into urine (254). However, the application of the creatinine normalization is faced by technical and biological difficulties. For instance, from a technical perspective, the determination of the creatinine concentration can be

interfered by metabolites with peaks overlapping with creatinine signals (e.g., creatine at 3.04 ppm) (255). Moreover, the pH of samples determines the chemical shift of creatinine at 4.05 ppm necessitate a sophisticated peak-picking algorithm. Biological challenges in the context of creatinine normalization are changes of the concentrations of creatinine caused by metabolomic responses, which has been shown in previous studies (256, 257).

In the present thesis, two main statistical strategies were followed. On the one hand, data reduction strategies by AP clustering were applied. AP uses pairwise similarities as inputs and iteratively determines clusters along with samples that are representative for the clusters, so-called exemplars (175). The aim of AP clustering was to determine if this unsupervised classification was capable of separating groups based on their VAT or SAT thicknesses. Advantages of this method are that the number of clusters does not need to be pre-specified, that exemplars are real samples instead of hypothetical averages, and that the algorithm is rather deterministic and not sensitive to initialization (258). However, the interpretation of the results from cluster analyses is difficult and most often, clusters cannot be reproduced by other data sets. In general, a cluster is simply a collection of cases that are more “similar” to each other than they are to cases in other clusters.

By comparison, regression models are easier to interpret with the drawback of multiple testing. Specifically, by conducting multiple analyses, the results are expected to be false positive by chance due to type I error. Based on a common significance level of $\alpha=0.05$, there is a 5% chance of having a falsely significant result. One possibility to address these concerns is to adjust the significance level α by setting a lower threshold. In general, multiple testing corrections successfully decrease type I error and the number of false positives but on the cost of increasing type II error and the number of false negative results. A very common but conservative approach to correct for multiple testing among independent tests is the use of the Bonferroni method (259). This procedure controls the overall experiment-wise type I error rate through adjusting the threshold of the significance level by dividing this level by the number of all performed tests. However, the analytical metabolomics dataset includes in part highly correlated metabolites, so that the assumption of independent tests is not appropriate. In case of highly correlated metabolites, the type II error and therefore the number of false negative

results would increase even more when applying this method. In the present thesis, an alternative approach was applied to control the problem of multiple testing by controlling the FDR according to the Benjamini-Hochberg method (260). This approach is less conservative compared to Bonferroni and type II error appears less often (261).

Another challenge of high throughput metabolomic analysis is the subsequent identification of compounds. This is crucial in order to confer a biological meaning to the associated features from data analyses. However, biological specimens contain hundreds of metabolites that vary considerably in quantity and can produce highly overlapping peaks (262). In addition, it is not unusual that for one bin a number of database records exist, leading to an exploration of a combinatorially large space of candidate metabolites. Further, numerous factors, e.g., changes in pH and ionic strength, or a potential pH and ionic interaction, may shift generated peaks from the expected position of the metabolite, thereby introducing “positional uncertainty” (262, 263).

In general, the major strength of the present thesis is the interdisciplinary nature of the study, drawing on methods from radiology, clinical chemistry, functional genomics, and epidemiology. Certainly, every discipline represented in this study offers virtually unlimited potential itself for explicit research with respect to obesity and obesity-related diseases. However, the applied interdisciplinary approach provides a unique comprehensive insight into the complex domain of obesity research, which none of the individual disciplines could have done on their own.

4.3 Conclusion and outlook

The present thesis represents one of the first studies that successfully adopted the ultrasonographic measurement of VAT and SAT in a population-based context to investigate correlations with systemic low-grade inflammation as well as urinary and serum metabolite regulation. In particular, VAT and SAT measured in the pre-test studies of the GNC were systematically studied with regard to their reproducibility and validity. Results showed that the intra- and inter-observer reproducibility of the ultrasound measurements of VAT and SAT was high. Compared to MRI measurements of VAT and SAT area, ultrasound can be evaluated as a valid method for assessing VAT and SAT. The correlation between the two methods was high, particularly for VAT.

However, among obese subjects, the correlation between the two methods was somewhat lower compared to lean subjects. Nevertheless, this work has shown that sonographic measurements of VAT and SAT are a reliable measurement technique that can be implemented in population-based studies that may help to better understand the mechanisms underlying the correlation with crude obesity phenotypes, such as BMI. Future research might also investigate whether VAT and SAT might help identify individuals at elevated risk of obesity-related diseases and whether these measures have clinical utility and are cost-effective. As a result, intervention strategies can be developed and applied early to enhance quality of life and reduce health care costs.

The distinct relationships of VAT, SAT, VSR, BMI, and WC to selected parameters of systemic chronic inflammation and urinary and serum metabolites further emphasize the importance of accurately differentiating between body fat compartments in obesity research. VAT, but not SAT was most consistently related to systemic low-grade inflammation as well as to urinary and serum metabolite regulation. Interestingly, in the present study, WC represented a less consistent measure of adiposity. Confounders regarding WC may exist by the assumption that VAT represents a pathogenic fat depot and SAT represents a more protective or at least less metabolic active fat depot, showing only few relations with urinary or serum compounds in the present study. Hence, it could be argued that the measure of WC is inherently flawed, given that WC would represent a mixture of both pathogenic and protective fat depots (28). This would support why relations of WC to inflammatory parameters and metabolite levels were found to be less strong and less consistent in the present study. This may also explain the rare, respectively null findings for the associations between VSR and inflammatory parameters and urinary and serum metabolites.

Deep phenotyping enables the precise examination of biological consequences of obesity (118). Specifically, biological mechanisms that are perturbed by obesity can precisely be linked to the obese phenotype and detect different dimensions of obesity. In the present study, the obese phenotype has been extensively characterized through examining VAT and SAT, calculating the ratio between these tissues, and measuring BMI and WC. Moreover, in addition to studying individual risk factors, more systematic approaches may help to better address and counteract the multifactorial genesis of obesity (105, 114, 115). Accordingly, in addition to the study of individual inflammatory

parameters, a targeted and untargeted metabolomics approach on urine and serum specimens was chosen to identify potential metabolite candidates that may explain mechanisms related to the development of obesity-associated diseases. In particular, compositional changes of gut microbial metabolites and dysregulation of BCAAs describe potential pathways that have been linked to the occurrence of low-grade systemic inflammation. Notably, urinary choline, dimethylamine, and 4-hydroxyhippuric acid may represent promising candidates that seem worthy of being addressed in future research. In addition, a replication study needs to be conducted, preferably in multiple independent population-based studies covering a broader geographic area and various ethnicities, to validate the findings of the present thesis. If the identified metabolites are replicated in independent data, future research should examine whether these novel metabolome biomarkers are related to disease onset.

Summary

Obesity is a complex multifactorial disease influencing several metabolic pathways. To better understand biological mechanisms that link obesity to obesity-associated diseases, it is indispensable to comprehensively characterize the obese phenotype by including the examination of adipose tissue compartments. In line with the exhaustive characterization of the obese phenotype, the thorough investigation of the metabolic phenotype may enhance our understanding of the metabolic consequences of unhealthy body fat distribution. Taken together, these approaches may further improve the prediction of obesity-related diseases in the future. Consequently, persons at high risk for the development of those diseases may be identified early before the actual disease onset. Thus, intervention strategies can be implemented to prevent obesity-related morbidity and mortality.

Ultrasound represents a low-cost and widely available field method for assessing visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). Findings from the present study demonstrate that VAT and SAT thicknesses quantified by ultrasound provide highly reproducible estimates independent of observer, subject, or ultrasound device. For instance, the inter-rater reproducibility values for VAT and SAT were 0.998 and 0.990, respectively. Moreover, compared to gold standard methods (magnetic

resonance imaging), the ultrasound measured VAT and SAT provides valid estimates with correlation coefficients of 0.898 ($p<0.001$) and 0.705 ($p<0.001$), respectively. Hence, the sonographic-based measurement of VAT and SAT can be considered a suitable method to assess abdominal fat in large-scale epidemiologic studies.

Adipose tissue is characterized as an endocrine organ and its dysfunction has been considered a central component of obesity-related inflammation and the main instigator of the pathological consequences of obesity. There is a need of describing biologically sound, multifaceted, and robust phenotypes to provide useful information about physiological and pathological states. In particular, to negotiate the limitations of the body mass index (BMI), relations of abdominal obesity and different adipose tissues to metabolic regulation are examined to unravel metabolic pathways perturbed by obesity and may consequently improve our understanding of the development of obesity-related diseases.

Results from the present thesis showed that different measures of body fat distribution differed in their relations to parameters of chronic inflammation. Specifically, VAT, SAT, BMI, and waist circumference (WC) were all associated with high sensitive C-reactive protein (hs-CRP). Additionally, BMI was inversely related to adiponectin and the ratio of visceral to subcutaneous body fat (VSR) was inversely related to resistin. In exploratory subgroup analyses, VAT was the strongest indicator for increased levels of interleukin-6 (IL-6). SAT was the most consistent indicator for increased levels of hs-CRP and BMI was the most consistent indicator for decreased levels of adiponectin. WC represented a weak indicator for increased levels of hs-CRP and decreased levels of adiponectin. In addition, by investigation of the urine and serum metabolom, a more comprehensive approach that does not only focus on single biomarkers was applied in the present thesis. Nuclear magnetic resonance spectroscopy (NMR) based metabolomics simultaneously examines all measurable low-weight molecular compounds present in biological samples and aims to unravel biological mechanisms to identify pathways through which behavioral factors and phenotypes are linked to metabolism.

VAT, BMI, and WC were inversely related to urinary metabolites including choline and also positively related to metabolites including dimethylamine, in multivariable regression models. In addition, inverse relations to the newly discovered urinary 4-

hydrxyhippuric acid were solely found for VAT. By comparison, only BMI and WC showed inverse relations to urinary glutamine. VAT, but none of the other anthropometric parameters was related to serum metabolites including essential amino acids, such as valine and phenylalanine among men. Compared to SAT, VSR, BMI, and WC, VAT demonstrated the strongest and most often the most significant relations to urinary and serum metabolites. In addition, relations between VAT and serum metabolites occurred sex-specific. The distinct relations of VAT, SAT, VSR, BMI, and WC to inflammatory parameters and metabolites emphasize the importance of accurately differentiating between body fat compartments when evaluating the potential role of adiposity-associated metabolic regulation in the development of obesity-related diseases. The precise attribution of metabolic consequences to obesity measures is crucial when investigating the etiology of obesity-associated diseases.

Zusammenfassung

Adipositas stellt eine komplexe, multifaktorielle Erkrankung dar, die verschiedene Stoffwechselwege beeinflusst. Um die biologischen Mechanismen, der Zusammenhänge von Adipositas mit Adipositas-assoziierten Erkrankungen besser zu verstehen, ist es unumgänglich, den adipösen Phänotypen durch die Bestimmung der viszeralen und subkutanen Körperfettschichten umfangreich zu charakterisieren. Zusammen mit der umfassenden Charakterisierung des adipösen Phänotyps, sollen durch die systemische Untersuchung des metabolischen Phänotyps die Auswirkungen der Körperfettverteilung auf das metabolische Profil besser verstanden werden. Beide Ansätze in Kombination, können Adipositas-assoziierte Erkrankungen in der Zukunft möglicherweise besser vorhersagen als bisher. Daraus würde resultieren, dass Personen mit einem hohen Risiko für die Entwicklung Adipositas-assoziierter Erkrankungen früher erkannt werden könnten. Entsprechend könnte diesen Erkrankungen durch entsprechende Interventionen vorgebeugt werden.

Ultraschall ist eine kostengünstige und weitgehend verfügbare Feldmethode zur Quantifizierung der viszeralen (VAT) und subkutanen (SAT) Körperfettkompartimente. Ergebnisse aus der vorliegenden Arbeit haben gezeigt, dass unter Anwendung eines standardisierten Untersuchungsprotokolls die mit Ultraschall gemessenen VAT und SAT

Dicken unabhängig von Untersucher, Studienteilnehmer und Ultraschallgerät reproduzierbar sind. Zum Beispiel waren die Intraklassenkorrelationskoeffizienten von VAT und SAT für die Inter-Untersucher Reproduzierbarkeit mit 0.998 und 0.990 besonders hoch. Des Weiteren liefert die sonographische Quantifizierung valide Messwerte für VAT (ρ=0.898; $p<0.001$) und SAT (ρ=0.705; $p<0.001$) im Vergleich zur Goldstandardmethode (Magnetresonanztomographie). Die ultraschallbasierte Erhebung von VAT und SAT kann somit als geeignete Methode zur Anwendung in epidemiologischen Studien bewertet werden.

Das Fettgewebe wird als endokrines Organ bezeichnet, dessen Anomalien zentraler Bestandteil Adipositas-assoziierter Inflammation darstellen und als wesentliche Auslöser der pathologischen Konsequenz von Adipositas gelten. Eine wichtige Voraussetzung bei der Ermittlung nützlicher Informationen zum physiologischen und pathologischen Status ist die biologisch gründliche, vielfältige und robuste Charakterisierung der Phänotypen. Bei der Untersuchung der durch Adipositas gestörten Stoffwechselvorgänge, müssen die Limitationen des Body Mass Index (BMI) beachtet werden und sollten mit der Erhebung der abdominellen Adipositas und den verschiedenen Körperfettkompartimenten erweitert werden. Somit werden genauere Informationen gesammelt und die Mechanismen bei der Entstehung von Adipositas-assoziierten Erkrankungen besser erklärt.

Die Ergebnisse aus der vorliegenden Arbeit haben gezeigt, dass verschiedene Messungen der Körperfettverteilung unterschiedlich mit chronischen Inflammationsmarkern assoziiert sind. So waren VAT, SAT, BMI und der Taillenumfang (WC) positiv mit dem hochsensitiven C-reaktiven Protein (hs-CRP) assoziiert. BMI zeigte zusätzlich einen inversen Zusammenhang mit Adiponektin. Das Verhältnis viszerales Fett zu subkutanem Fett (VSR) war invers mit Resistin assoziiert. In explorativen Subgruppenanalysen zeigte VAT die stärkste Assoziation mit erhöhten Interleukin-6 (IL-6) Konzentrationen. SAT war eine konsistente Messgröße für erhöhte hs-CRP Level und BMI war die konsistenteste Messgröße für niedrige Adiponektinspiegel. WC zeigte schwache Zusammenhänge mit erhöhten hs-CRP Spiegeln und niedrigen Adiponektinkonzentrationen. Zusätzlich zur Untersuchung von einzelnen Biomarkern wurde in der vorliegenden Arbeit die eingehende Analyse des Urin- und Serummetaboloms angewandt. Die Kernresonanzspektroskopische (NMR)

Messung des Metaboloms ermöglicht die gleichzeitige Untersuchung aller messbaren niedermolekularen Stoffe in Bioproben mit dem Ziel, biologische Mechanismen und Stoffwechselwege zu identifizieren, welche durch Lebensstilfaktoren und Phänotypen beeinflusst werden.

VAT, BMI und WC waren in multivariablen Regressionsmodellen unter anderem invers mit Cholin und positiv mit Dimethylamin im Urin assoziiert. Darüber hinaus konnte die inverse Beziehung zu dem neu entdeckten Metaboliten 4-hydroxy-hippursäure im Urin einzig für VAT gezeigt werden. Im Vergleich dazu waren nur BMI und WC invers mit Glutamin im Urin assoziiert. VAT, jedoch keine der anderen anthropometrischen Parameter, war mit Serummetaboliten, wie beispielsweise den essentiellen Aminosäuren Valin und Phenylalanin, bei Männern assoziiert. Im Vergleich zu SAT, VSR, BMI und WC waren die Zusammenhänge zwischen VAT und Urinmetaboliten am stärksten und oftmals am signifikantesten. Des Weiteren konnten geschlechtsspezifische Zusammenhänge zwischen VAT und Serummetaboliten gezeigt werden. Die unterschiedlichen Assoziationen von VAT, SAT, VSR, BMI und WC mit Inflammationsmarkern sowie Urin- und Serummetaboliten unterstreichen die Notwendigkeit, bei der Untersuchung der von Adipositas beeinflussten Stoffwechselvorgänge im Zusammenhang mit der Entstehung Adipositas-assoziierter Erkrankungen die Körperfettzusammensetzung umfassend zu betrachten.

Supplements

Appendix 1: Standard operation procedures (SOP) ultrasound

Standardarbeitsanweisung (SOP)

Kürzel: UN25-SD_V03

Titel: Ultraschalluntersuchung Abdomen (Fettgewebe)

Anlagen:
UN25-SD-A1 Dokumentationsbogen Ultraschalluntersuchung Abdomen
UN25-SD-A2 Leitfaden für Untersuchung Ultraschalluntersuchung Abdomen

Gültigkeitszeitraum: ab: 11.05.2012

Ersetzte Version: Version aus Pretest/Interimsphase SOP_L2_12_SOP-Abdomen_V1-3

Änderungshinweis: N.A.

Verteiler: Original: Qualitätsmanagement / Quality Officer stellvertretend für Geschäftsstelle
Autorisierte Kopien: Studienzentren (SZ-Leiter u. Qualitätsbeauftragte), Zentrales Datenmanagement und externe QS via Sharepoint

Autoren: Inga Schlecht

SOP-Verantwortliche:	**ReviewerInnen:**	**Freigegeben durch:**
Inga Schlecht	Beate Fischer	QS-Officer im Auftrag des Vorstands
Datum / Unterschrift	Datum / Unterschriften	Datum / Unterschrift

Inhaltsverzeichnis

Ziel

Das Ziel ist bei TN der Nationalen Kohorte die abdominalen Körperfettdepots mittels Ultraschall zu differenzieren und zu quantifizieren.

Geltungsbereich der SOP

Die SOP gilt für alle Mitarbeiter, die mit diesem Modul befasst sind. Dies sind insbesondere Trainer/Monitore und das Untersuchungspersonal.

Abkürzungen

BMI	Body Mass Index
Mango	Eingabetool zur Datenerfassung
QS	Qualitätssicherung
SAT	Subkutanes adipöses Fettgewebe
SOP	Standard Operating Procedure
SZ	Studienzentrum
TN	Teilnehmer
VAT	Viszerales adipöses Fettgewebe
ZDM	Zentrales Datenmanagement

Hintergrund

Die Adipositas stellt ein multifaktorielles Geschehen dar und ist aufgrund ihrer zentralen Rolle bei der Entwicklung von bedeutenden chronischen Erkrankungen, wie z. B. kardiovaskuläre Erkrankungen, Typ 2 Diabetes und Krebs, für epidemiologische Forschungsprojekte von wesentlichem Interesse. Vor allem das viszerale adipöse Fettgewebe (VAT) gilt als ein unabhängiger Risikofaktor für die Entwicklung Adipositas-assoziierter Erkrankungen. Hingegen weist das subkutane adipöse Fettgewebe (SAT) einen antiatherogenen Effekt auf und ist mit einer erhöhten Insulinsensitivität sowie mit einem geringeren Risiko für die Entwicklung von Typ 2 Diabetes und Dyslipidämien assoziiert.

Die gängigen anthropometrischen Methoden zur Messung des Körperfetts wie z.B. Körpermassenindex (BMI), Hüftumfang oder Taillen-Hüft-Verhältnis unterscheiden nicht zwischen der viszeralen und subkutanen abdominellen Körperfettverteilung. Inzwischen ist jedoch bekannt, dass bei den Fettmessungen nicht nur die Größe bzw. Menge, sondern v.a. auch die anatomische/regionale Verteilung der Fettdepots entscheidend ist. Die präziseren Methoden wie Computertomographie und Magnetresonanztherapie eignen sich jedoch nicht für den Einsatz in großen Kohorten, da diese Methoden invasiv und kostenintensiv sind. Eine mögliche Alternative bietet die Ultrasonographie. Die Ultrasonographie ermöglicht die Quantifizierung der abdominalen Körperfettdepots. Sie verwendet Ultraschallwellen, die über einen Schallkopf in den Körper gestrahlt werden und im Körper reflektiert oder absorbiert werden. Die reflektierten Wellen werden anschließend vom Schallkopf aufgenommen. Die Schallwellen, die von festem Gewebe wie z. B. Knochen reflektiert werden, sind am größten und erscheinen weiß auf dem Bildschirm. Weiches Gewebe wird grau oder gefleckt dargestellt, während Flüssigkeiten schwarz erscheinen, da sie kein Echo abgeben. Aus diesen Wellen wird ein Bild berechnet und auf dem Monitor sichtbar gemacht. Diese bildgestützte Methode liefert

somit Informationen über die Dicke der verschiedenen abdominellen Körperfett-kompartimente. Ultrasonographie gilt als valide Methode zur Quantifizierung von SAT und VAT, die nicht invasiv, schmerzfrei und kosteneffizient ist.

Verantwortlichkeiten

Tabelle 1 Übersicht Verantwortlichkeiten

Funktion	**Verantwortlichkeiten**
Modulverantwortliche: Inga Schlecht Universität Regensburg Tel. 0941 944 52 16 inga.schlecht@ukr.de	• Autor des SOPs • Schulung und Zertifizierung von Trainern • Ansprechpartner
Wissenschaftlicher Stab	• Beteiligung am Review Prozess
Trainer / Monitore	• Lokale Schulung der Untersucher im eigenen SZ • Site Visits und Zertifizierung der Untersucher eines benachbarten SZ
Untersuchungspersonal	• Durchführung der Untersuchung
Zentrales Qualitätsmanagement (zQM)	• Begleitung und Überwachung des Reviewing-Prozesses • Dokumentenverwaltung
Externe QS	• Site Visits und Meldung von Problemen bzgl. QS an jeweiliges SZ und an zQM

Verweise

Literaturhinweise zur Ultraschallmethode:

- Manual für das Mindray DP 50
- Stolk, R.P., et al., *Validity and reproducibility of ultrasonography for the measurement of intra-abdominal adipose tissue.* Int J Obes Relat Metab Disord, 2001. **25**(9): p. 1346-51.

Prozesse

Ausstattung des Untersuchungsplatzes / Messumgebung

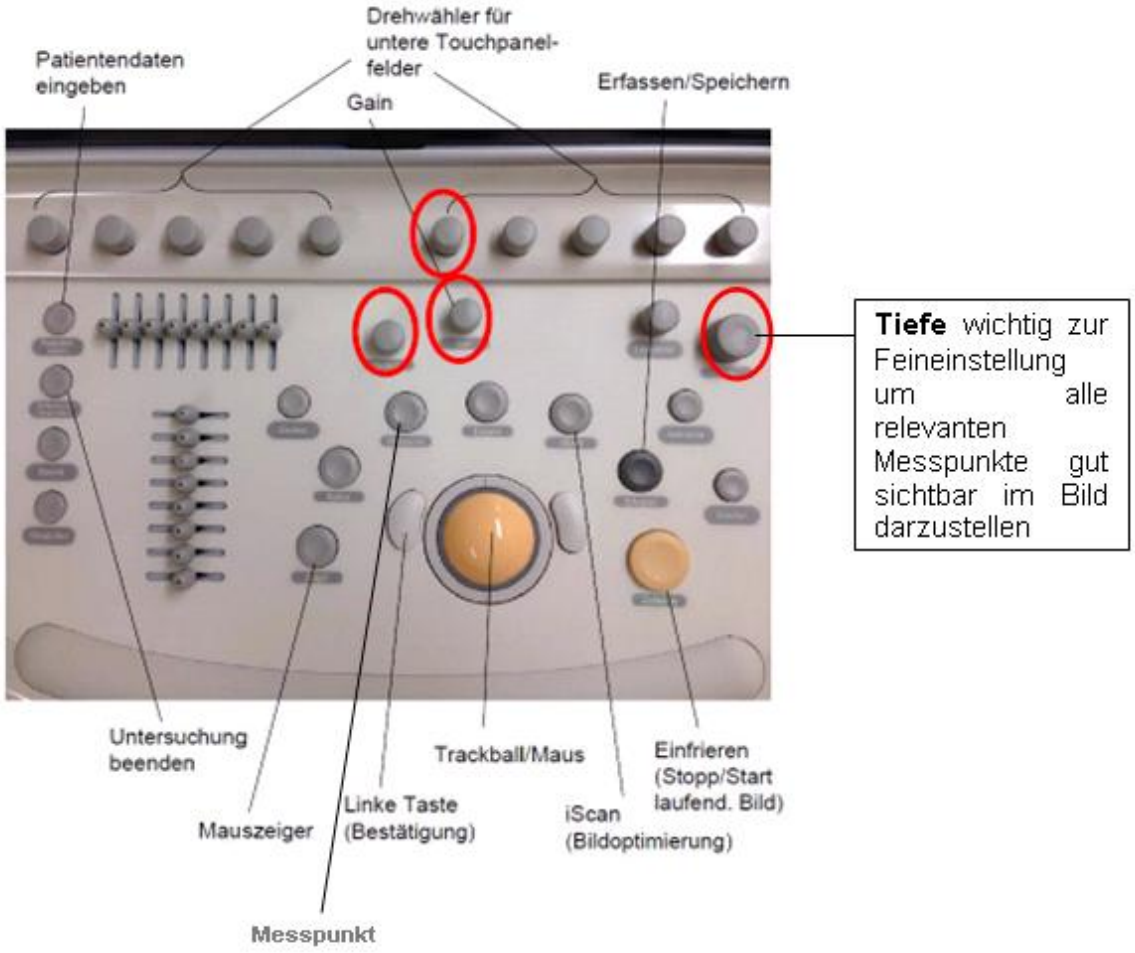
- **Raumsituation**

Der Raum sollte für die Ultraschalluntersuchung abgedunkelt werden und es sollte eine angenehme Raumtemperatur erreicht sein.

- **Messgeräte**

Mindray DP 50

Relevante Bedienfelder:



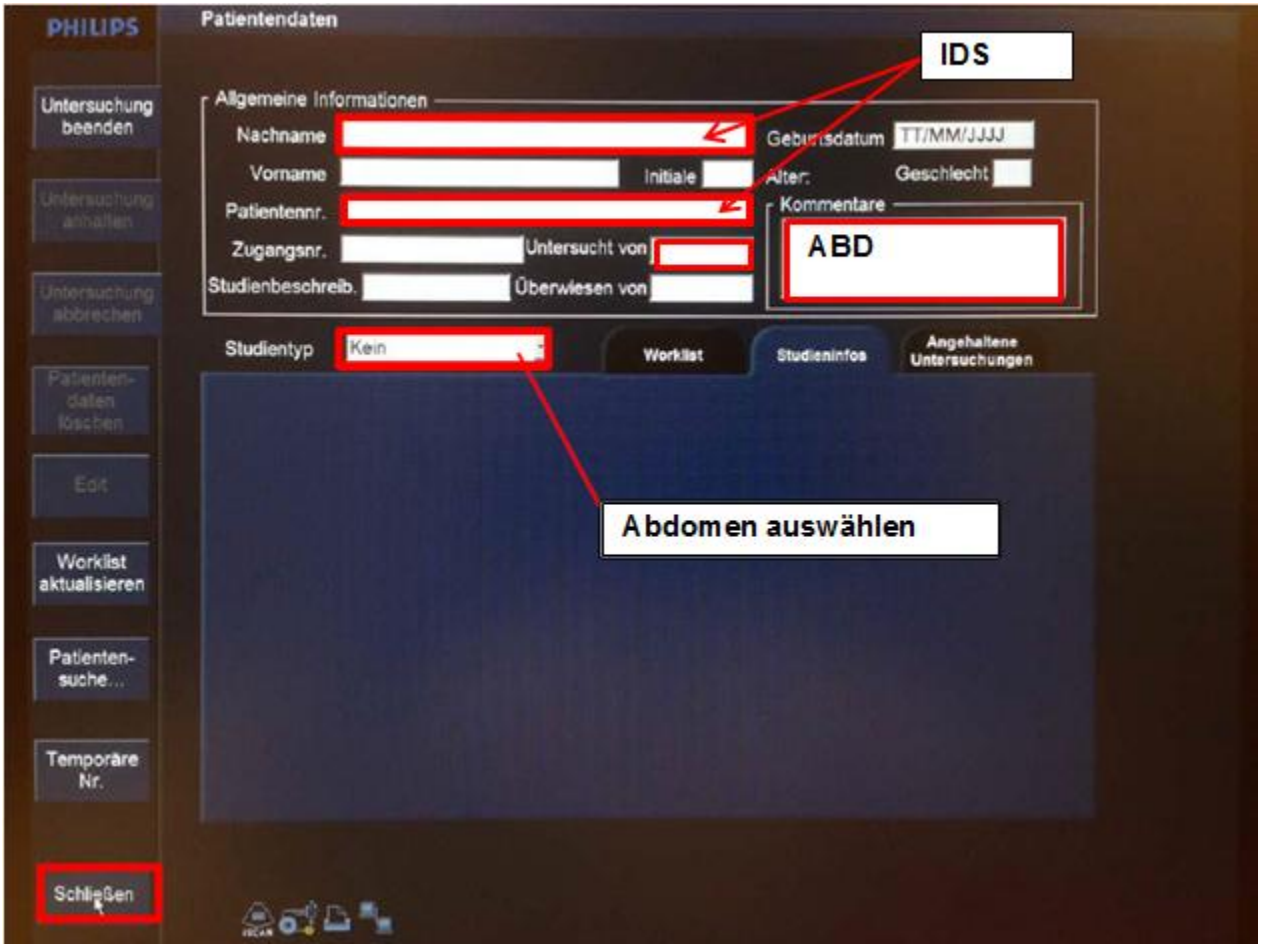
1. **An- und Ausschalter**
   ACHTUNG: Caps Lock Taste beim erstem Einschalten deaktivieren!

2. **Mauszeiger**
   - Aktiviert Bewegung mit Trackball/Maus

3. **Linke Taste**
   - ermöglicht Bestätigung

4. **Konvexschallkopf auswählen**
   - Preset/Schallkopf (Touchpanel) anwählen
   - C5-1 3,5-5 MHz (meist voreingestellt)
   - Abdomen Gefäße anwählen

5. **Patientendaten eingeben**
   - Eingabe Nachname: IDS
   - Eingabe Patientennr.: IDS
   - Eingabe Untersucht von: Untersuchernummer
   - Auswahl Studientyp: Abdomen → Sortieren von Echo und Abdomen zu ermöglichen
   - Eingabe Kommentare.: ABD → Sortieren von Echo und Abdomen zu ermöglichen
   - Mit Schließen zurück zu Monitor



6. **iScan:**
   - Automatische Bildoptimierung

7. **Gain:**
   - Heller oder dunkler

8. **Komprim.:**
   - Kontraste härter oder weicher einstellen

9. **Frequenz (Touchpanel):**
   Voreinstellung HAllg.
   - Tiefer: Pen, HPen
   - Näher: HAufl.

10. **Tiefe:**
    - Anpassung der Eindringtiefe um relevante Messpunkte gut sichtbar darzustellen

11. **Einfrieren (Freezing Mode):**
    - Erzeugt stehendes Bild
    - Distanzmessung erfolgt am stehenden Bild

12. **Messpunkt:**
    - Ermöglicht Distanzmessung durchzuführen

13. **Eingeben:**
    - Speichern des Bildes

14. **Untersuchung beenden**

15. **Überprüfen:**
    - Um abgespeicherte/abgeschlossene Untersuchungen aufzurufen

Weitere Einrichtungs- und Ausstattungsgegenstände

1. Untersuchungsliege
2. Ganzkörperspiegel
3. PC für die Dateneingabe ins Mango
4. Xcelera Servive als Speicherplatz für Bilder

Verbrauchsmaterialien

1. Papierauflage für Untersuchungsliege
2. Gel für die Messungen (z.B. Sonosid von ASID Bonz GmbH)
3. Maßband
4. Kajalstift zur Markierung der Messpunkte auf der Haut
5. Geeignetes Desinfektionsmittel zur Reinigung des Schallkopfs (z.B. Cleanisept Wipes Desinfektionstücher)
6. Tücher zum Abwischen des Gels

Software

1. Gerätesoftware: Mindray DP 50
2. Mango Datenbank in der aktuellsten Version

Die Eingabe der Daten erfolgt zunächst manuell auf dem vorgesehenen Papierbogen. Die Daten müssen anschließend in das vorgesehene Formular im Mango übertragen werden.

Vorbereitung vor Studienbeginn

- **Aufstellung der Geräte**

  Das Ultraschallgerät sollte unmittelbar nahe, rechts oder links der Untersuchungsliege aufgestellt werden. Für die Ultraschalluntersuchung des Abdomen muss die C5-1 3,5-5 MHz Sonde angeschlossen sein. Es kann ein Barcode Scanner am Ultraschallgerät angeschlossen werden.

- **Grundeinstellungen der Gerätesoftware**

  Das Ultraschallgerät wird mit einer Netzwerkverbindung zum Xcelera Server verbunden, sodass eine automatische Übertragung der Aufnahmen zum Server erfolgen kann. Die Ergebnisse der Distanzmessungen von VAT und SAT werden händisch in das vorgesehene Formular im Mango eingetragen.

Durchführung

Zusammenhänge mit anderen Modulen und Messbedingungen

Die Untersuchung kann gut mit der anthropometrischen Untersuchung verknüpft werden, da die Messung des Taillenumfangs mit der Markierung des Messpunkts für die Ultraschalluntersuchung einhergeht.

Vorbereitung vor jeder Messung

Der Raum sollte für die Ultraschalluntersuchung abgedunkelt werden und es sollte eine angenehme Raumtemperatur erreicht sein. Das Ultraschallgerät Mindray DP 50 (2D) mit 3,5 – 5 MHz Konvexschallkopf muss vorbereitet und angeschaltet sein. Untersuchungsliege (mit Papier ausgelegt), Gel für die Messungen, Maßband, Stift zur Markierung der Messpunkte auf der Haut, Desinfektionsmittel zur Reinigung des Schallkopfs und ausreichend Tücher zum Abwischen des Gels sollten zur Verfügung stehen. Auch sollte der vorhandene Speicherplatz für Bilder überprüft werden.

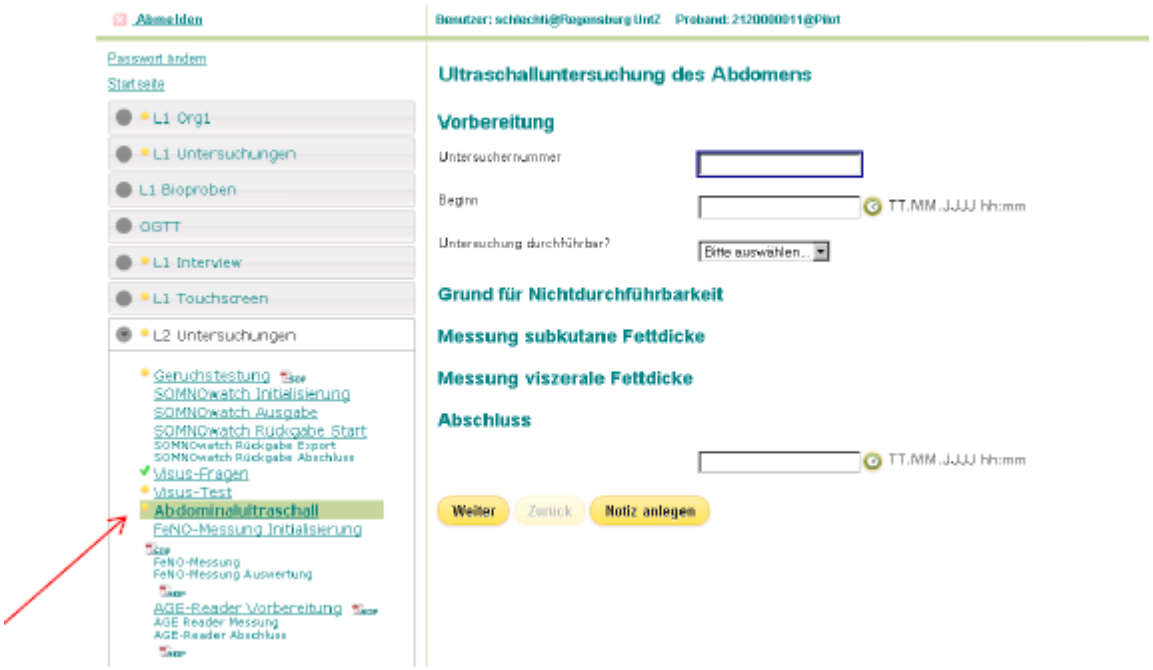
Ausschlusskriterien

Es gibt keine Ausschlusskriterien für die Ultraschalluntersuchung mit der Ausnahme einer fehlenden Einwilligung des TN für dieses Modul. Eine Messung bei fortgeschrittener Schwangerschaft (ab SSW 15) macht jedoch wenig Sinn.

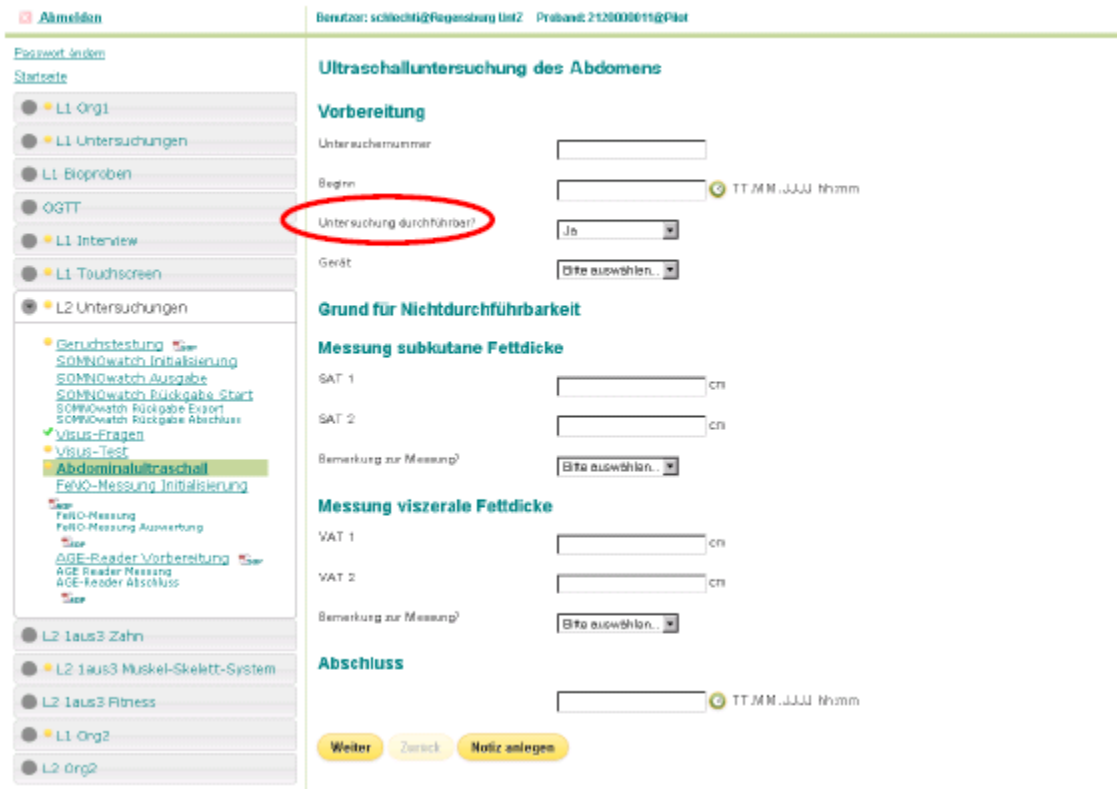
Aufklärung des Studienteilnehmers

Zunächst wird dem TN das Verfahren und Vorgehen erklärt (siehe Punkt 4 Hintergrund und 7.3.7 Messprinzip). Die Sonographie sollte direkt im Rahmen der anthropometrischen Untersuchung durchgeführt werden. Eine Prozedur wird ca. 5-10 Minuten dauern. Es sind keine möglichen Risiken bekannt.

Start der Messungen über Mango



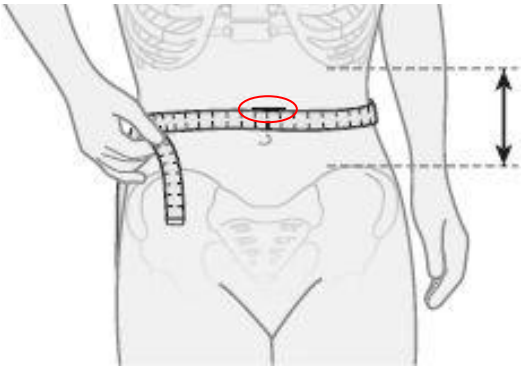
Bei der Menüauswahl „Untersuchung durchführbar“ muss Ja ausgewählt werden damit sich die Eingabemaske für die SAT und VAT Werte öffnet.



Etwaige Auffälligkeiten können unter „Bemerkung zur Messung“ angelegt werden oder es kann eine Notiz zur Untersuchung angelegt werden.

Durchführung der Messung

Vor der Ultraschalluntersuchung wird am stehenden TN ein Messpunkt mit einem Kajalstift auf die Haut aufgetragen. Der Messpunkt ergibt sich aus der Messung des Taillenumfangs: Am Schnittpunkt aus den beiden lateralen Messpunkten beidseits exakt mittig zwischen unterem Rippenbogen und höchstgelegenem Punkt des Beckenkammes mit der ventralen Mittellinie (die Medianlinie und der Schwertfortsatz dienen hier zur Orientierung). Der Messpunkt wird direkt am oberen Rand des Maßbands gesetzt.



Während der Untersuchung liegt der TN in Rückenlage auf der Untersuchungsliege. Die Schulter, Arme, Fersen und das Gesäß müssen auf der Liege aufliegen. Die Oberbekleidung kann ausgezogen oder nach oben geschoben werden, so dass der Bauch für die Messung freiliegt.

Messprinzip

Die subkutane und viszerale Messung bilden eine schräg verlaufende Sichtweise zur Darstellung der Bauchmuskulatur (rectus abdominis) und Linea alba ab. Die Messung ist **ohne Verformung** (durch Kompression) auf der Haut mit der Nutzung von entsprechendem Gel durchzuführen. Die Linea alba ist bei der Messung horizontal im Zentrum des Bildes. Die Messung mit dem Caliper erfolgt ebenfalls senkrecht im Zentrum des Bildes.

Das Ultrasonographieprotokoll beinhaltet 4 zweidimensionale Bilder mit insgesamt 4 Distanzmessungen. Durch Regulierung der Eindringtiefe („Tiefe“ Drehtaste) werden die Feineinstellung zur Darstellung der Messpunkte von SAT und VAT vorgenommen. Für die Bestimmung von SAT und VAT werden jeweils 2 fixierte Bilder (Freezing Mode) gespeichert. Bei allen Bildern erfolgt eine Distanzmessung mit Hilfe des elektronischen Calipers/Trackball und der „Messpunkt“ Taste am Ultraschallgerät. Die Bilder sollen mit der Distanzmessung abgespeichert werden („Eingeben“ Taste).

Alle Messungen erfolgen als stehendes Bild am Ende des (gewöhnlichen) Ausatmens (Hinweis für den TN: „Am Ende der Ausatmung die Luft anhalten“); das elektronische Caliper ist senkrecht im Zentrum des Bildes positioniert.

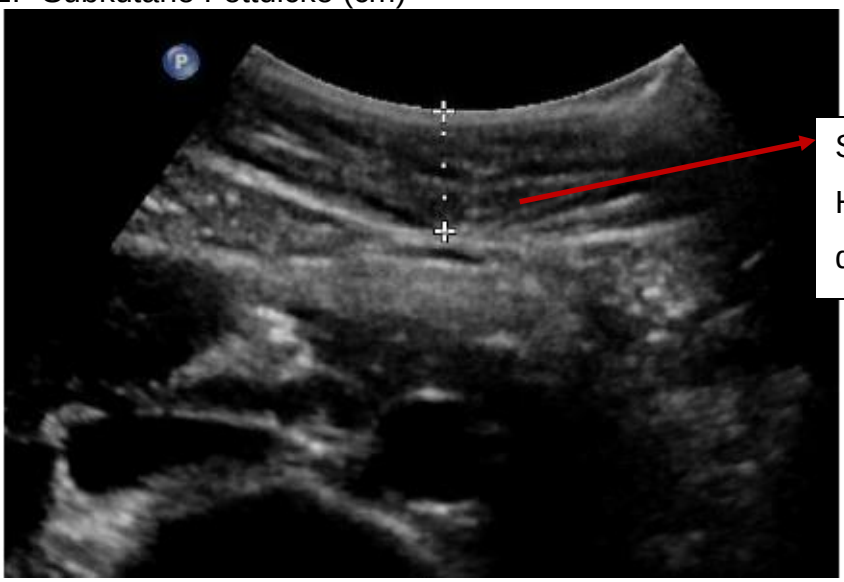
Nach der ersten Distanzmessung wird die Sonde für das zweite Bild jeweils neu angesetzt.

1. **SAT:**
   Zwei fixierte Bilder die jeweils auf Distanz vermessen werden. Für die Analyse des SAT erfolgt die Distanzmessung an der **Medianlinie von** der **Hautoberfläche bis** zur **Linea alba** (siehe Abbildung, S.12).

2. **VAT:**
   Zwei fixierte Bilder die jeweils auf Distanz vermessen werden. Die Distanzmessungen von VAF erfolgen von der **Linea alba** zur **Vorderkante des Lendenwirbelkörpers mittig**, am Schnittpunkt aus den beiden lateralen Messpunkten und der ventralen Mittellinie (siehe Abbildung, S. 12).

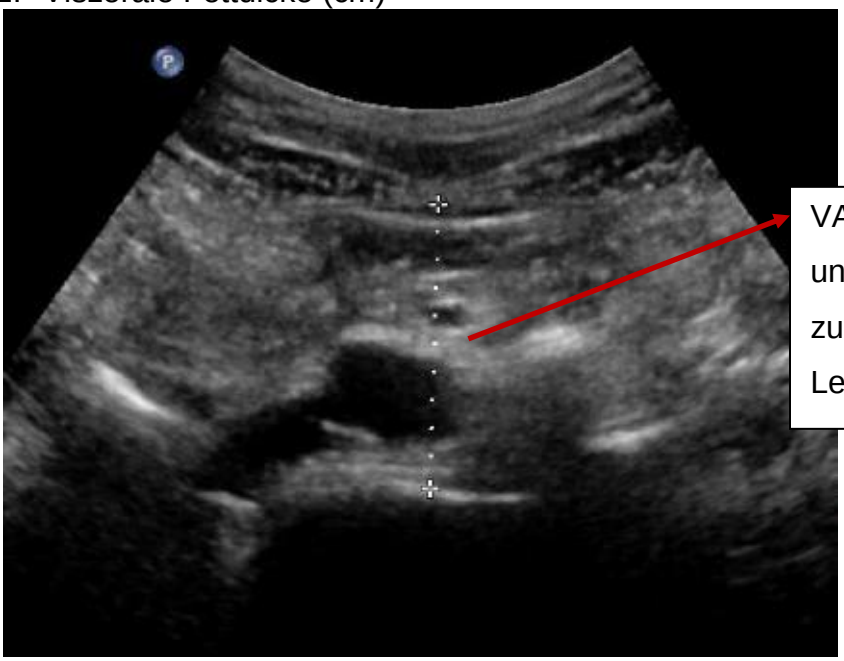
Durch die Verwendung der Ultrasonographie können folgende Parameter bestimmt werden:

1. Subkutane Fettdicke (cm)



SAT: Distanzmessung von der Hautoberfläche bis oberhalb der Linea alba.

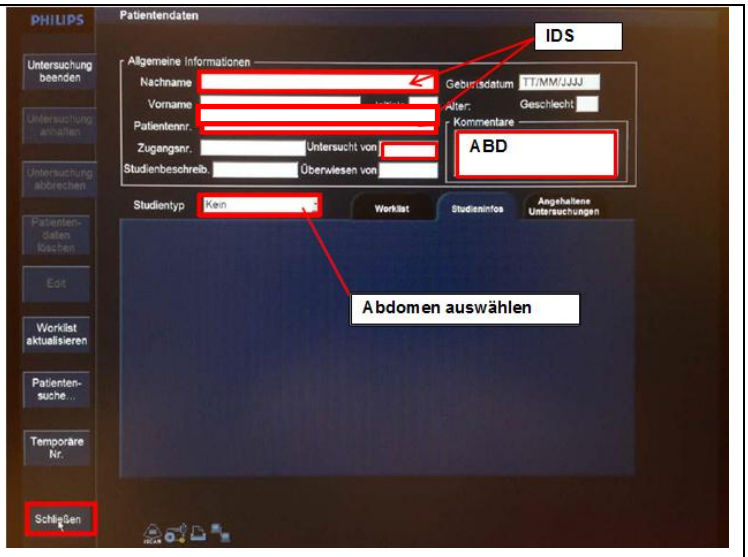
2. Viszerale Fettdicke (cm)



VAT: Distanzmessung von unterhalb der Linea alba bis zur Vorderkante des Lendenwirbelkörpers.

Tabelle 2 Schritte bei der Durchführung der Untersuchung

1.	**Gerät einschalten**	Siehe Ein-Ausschalter (Kapitel 7.1.2: Messgeräte)
2.	**TN lagern**	Markierung des Messpunkts auf der Haut ist erfolgt. TN in Rückenlage auf der Untersuchungsliege platzieren. Schultern, Arme, Fersen und das Gesäß müssen auf der Liege aufliegen. Oberbekleidung kann ausgezogen oder nach oben geschoben werden, so dass der Bauch für die Messung freiliegt.
3.	**Position des Untersuchers**	Die Sitzposition kann der Untersucher selbst wählen. Es kann von rechts als auch von links geschallt werden.
4.	**Schallkopf auswählen**	C 5-1 
5.	**TNdaten eingeben**	Eingabe der IDS, Untersuchernummer, des Studientyps und Kommentar (Kapitel 7.1.2: Messgeräte)

		
6.	**Zur Untersuchung/Monitor**	Mit „Schließen“ gelangt man aus der Maske Patientendaten zur Untersuchung
7.	**Schallkopf nehmen**	Schallkopf aus Halterung nehmen und Gel auf Schallfläche geben
8.	**Positionierung des Schallkopfes**	Auf vormarkierte Stelle Dunkelgraue Markierung am Schallkopf zeigt vom Untersucher weg wenn dieser auf der linken Seit vom TN positioniert ist
9.	**1. Subkutanes Fett**	Gestartet wird mit der Messung von SAT. **Druck vermeiden und Atemkommando geben!** **<u>Vorgehen:</u>** **erstes Ultraschallbild SAT** 1.Messung: eine Messung am stehenden Bild (Tiefe und iScan Optimierung, siehe Kapitel 7.1.2: Messgeräte) ➔ Messpunkte mit Hilfe des Trackballs und der Messpunkt Taste setzten (siehe Kapitel 7.3.7: Messprinzip) ➔ Bild mit Distanzmessung speichern (Eingeben) ➔ Messwert mit zwei bzw. drei

		Nachkommastellen auf dem Dokubogen eintragen (**bei SAT Werten kleiner 1.00cm bitte drei Nachkommastellen eintragen**) **zweites Ultraschallbild SAT** selbiges Vorgehen 
10.	**2. Viszerales Fett**	Anschließend erfolgt die Messung von VAT. **Druck vermeiden und Atemkommando geben!** **<u>Vorgehen:</u>** **erstes Ultraschallbild VAT** 1.Messung: eine Messung am stehenden Bild (Tiefe iScan Optimierung, siehe Kapitel 7.1.2: Messgeräte) ➔ Messpunkte mit Hilfe des Trackballs und der Messpunkt Taste setzten (siehe Kapitel 7.3.7: Messprinzip) ➔ Bild mit Distanzmessung speichern (Eingeben) ➔ Messwert auf dem Dokubogen eintragen **zweites Ultraschallbild VAT** selbiges Vorgehen

		
11.	**Messprotokoll**	Alle 4 Werte aus 4 erfassten Bildern werden in das Messprotokoll bzw. Mango übertragen.
12.	**Untersuchung beenden**	Taste „Untersuchung beenden“ drücken (Kapitel 7.1.2: Messgeräte)

Rückmeldung an Teilnehmer

Für den Ergebnisbericht sollen jeweils die Mittelwerte aus den 2 Distanzmessungen von SAT und VAT mitgeteilt werden. Zur Erklärung sollten folgende Informationen mitgeteilt werden:

Ergebnis Ultraschalluntersuchung

Bisher gibt es keine Referenzwerte für die gemessenen Fettdicken in der Bauchregion. Mit Ihrer Studienteilnahme tragen sie wesentlich zur Erforschung möglicher Grenzwerte im Zusammenhang mit Gesundheit und Krankheit bei.

Subkutane Fettdicke _____cm

Das subkutane Fett wird auch als Unterhautfett bezeichnet und befindet sich direkt unter der Haut und über den Bauchmuskeln.

Viszerale Fettdicke _____cm

Das viszerale Fett wird als Organfett bezeichnet. Es liegt unterhalb der Bauchmuskeln und umhüllt die Organe.

Nachbereitung

- Das Gel nach der Untersuchung vom Teilnehmer und vom Schallkopf entfernen
- Den Schallkopf desinfizieren
- Das Papier von der Untersuchungsliege wechseln
- Die Messwerte in das vorgesehene Formular im Mango übertragen
- Die Konfiguration für den Datenexport der Untersuchung an den Zentralen Datenspeicher steht noch nicht fest und wird später mitgeteilt

Problembehandlung

In einigen Fällen verdeckt der Darminhalt die Wirbelsäule oder andere Strukturen. Unter gesteuertem Druck mit dem Schallkopf kann der Darminhalt verlagert werden, um die verdeckten Strukturen visualisieren zu können. Die gezielte Kompression kann behilflich sein, die exakte Position der Wirbelsäule zu bestimmen, indem langsam weniger Druck ausgeübt wird, wobei simultan versucht wird die Wirbelsäule sichtbar zu halten. Der Darminhalt bewegt sich konstant, so dass eine sich eine schlechte Bildqualität durch störenden Darminhalt innerhalb von wenigen Minuten ändern kann und eine bessere Messung möglich macht.

Glossar

VAT Das viszerale Fett auch intraabdominales Fett genannt, bezeichnet das in der freien Bauchhöhle eingelagerte Fett, das die inneren Organe, vor allem des Verdauungssystems, umhüllt. Vor allem das VAT steht mit der Entwicklung von Adipositas-assoziierten Erkrankungen in direktem Zusammenhang.

SAT Die Subkutis ist die untere Schicht der Haut. SAT wird mit potenziell protektiven Funktionen assoziiert. Es weist einen antiatherogenen Effekt auf und ist mit einer erhöhten Insulinsensitivität sowie mit einem geringeren Risiko für die Entwicklung von Typ 2 Diabetes und Dyslipidämien assoziiert.

Linea alba Die Linea alba ist eine senkrechte Bindegewebsnaht in der Mitte des Bauches, die durch die Vereinigung der seitlichen Bauchmuskeln entsteht.

Supplementary Table 1: Association of adiposity measures with chronic inflammation.

Reference	Study population	VAT and SAT measurement	Association VAT	Association SAT	Association BMI	Association WC	Adjustment variables
				hs-CRP			
Festa et al. 2001 (84)	1559 tri-ethnic population				r=0.41; p=0.0001	r=0.42; p=0.0001	age, gender, ethnicity, clinic, smoking, diabetic status
					r=0.28; p=0.0001	r=0.29; p=0.0001	+insulin sensitivity
Forouhi et al. 2001 (83)	113 healthy adults 57 healthy Europeans	CT	ß=0.95; p=0.013		ß=0.14; 0.005	ß=0.04; p=0.027	age, sex, smoking status
	56 healthy South Asians		ß=0.78; p=0.0004		ß=0.07; 0.058	ß=0.04; p=0.002	
Lemieux et al. 2001 (184)	159 healthy men	CT	r=0.28; p<0.0003	r=0.33; p<0.0001	r=0.36; p<0.0001	r=0.37; p<0.0001	
Saijo et al. 2004 (93)	119 healthy Japanese	CT	ß=0.39; p<0.001	ß=0.28; p<0.001	ß=0.35; <0.0001	ß=0.46; <0.0001	sex, age, smoking status
Panagiotakos et al. 2005 (80)	3043 healthy adults men				r=0.21; p<0.05	r=0.30; p<0.01	age, diet score, total cholesterol and glucose levels
	women				r=0.22; p<0.05	r=0.34; p<0.01	
Park et al. 2005 (97)	46 obese and 54 non-obese Koreans	CT			r=0.69; p<0.0001	r=0.75; p<0.0001	
	obese		r=0.40; p<0.05	r=0.30; n.s.			
Piché et al. 2005 (95)	112 postmeno-pausal women	CT	r=0.55; p<0.0001	r=0.50; p<0.0001	r=0.60; p<0.0001	r=0.61; p<0.0001	

Supplementary Table 1 continued: Association of adiposity measures with chronic inflammation.

Reference	Study population	VAT and SAT measurement	Association VAT	Association SAT	Association BMI	Association WC	Adjustment variables
Thorand et al. 2006 (81)	1238 healthy adults (55-74y)						age, smoking habits, alcohol consumption and physical activity
	men				ß=0.015; p<0.001	ß=0.002; p<0.001	
	women				ß=0.018; p<0.001	ß=0.003; p<0.001	
Pou et al. 2007 (94)	1250 healthy adults	CT					age
	men		r=0.33; p<0.001 r^2=0.19; p<0.0001	r=0.30; p<0.001 r^2=0.19; p<0.0001	r=0.38; p<0.001	r=0.37; p<0.001	age, smoking, aspirin, alcohol intake, physical activity
	women		r=0.47; p<0.001 r^2=0.29; p<0.0001	r=0.45; p<0.001 r^2=0.28; p<0.0001	r=0.52; p<0.001	r=0.51; p<0.001	+ menopausal status, hormone replacement therapy
Taksali et al. 2008 (79)	obese adolescents	MRI	VAT/SAT ratio p=0.40				age, sex, race/ethnicity
Beasley et al. 2009 (98)	2651 black and white elderly	CT					clinic site, age, BMI, marital status, education level, pack years, medical condiction, anti-inflammatory drugs, oral steroids, physical acticity
	white men		ß=0.12; p=0.01	ß=0.17; p=0.00			
	black men		ß=-0.01;p=0.84	ß=0.09; p=0.24			
	white women		ß=0.18; p=0.00	ß=0.18; p=0.00			
	black women		ß=0.03; p=0.56	ß=-0.09;p=0.23			

Supplementary Table 1 continued: Association of adiposity measures with chronic inflammation.

Reference	Study population	VAT and SAT measurement	Association VAT	Association SAT	Association BMI	Association WC	Adjustment variables
Saito et al. 2012 (96)	130 type 2 diabetic adults men women	CT	r=0.346; p<0.001 r=0.363; p<0.001 r=0.341; p<0.001	r=0.319; p<0.001 r=0.412; p<0.001 r=0.209; n.s.			
				IL-6			
Panagiotakos et al. 2005 (80)	3043 healthy adults men women				r=0.29; p<0.01 r=0.29; p<0.01	r=0.12; p<0.05 r=0.42; p<0.01	age, diet score, total cholesterol and glucose levels
Park et al. 2005 (97)	46 obese and 54 non-obese Koreans obese	CT	r=0.46; p<0.05	r=0.13; n.s	r=0.37; p<0.005	r=0.42; p<0.005	
Piché et al. 2005 (95)	112 postmeno-pausal women	CT	r=0.49; p<0.0001	r=0.34; p<0.01	r=0.49; p<0.0001	r=0.56; p<0.0001	
Thorand et al. 2006 (81)	1238 healthy adults (55-74y) men women				ß=0.015; p<0.01 ß=0.012; p<0.001	ß=0.003; p<0.001 ß=0.003; p<0.001	age, smoking habits, alcohol consumption and physical activity
Pou et al. 2007 (94)	1250 healthy adults	CT	r=0.23; p<0.001 r^2=0.12; p<0.0001	r=0.23; p<0.001 r^2=0.12; p<0.0001	r=0.27; p<0.001	r=0.25; p<0.001	age, sex age, smoking, aspirin, alcohol intake, menopausal status, hormone replacement therapy, physical activity
Taksali et al. 2008 (79)	obese adolescents	MRI	VAT/SAT ratio p=0.16				age, sex, race/ethnicity

Supplementary Table 1 continued: Association of adiposity measures with chronic inflammation.

Reference	Study population	VAT and SAT measurement	Association VAT	Association SAT	Association BMI	Association WC	Adjustment variables
Beasley et al. 2009 (98)	2651 black and white aged 70-79 white men black men white women black women	CT	ß=0.10; p=0.02 ß=-0.11; p=0.05 ß=0.22; p=0.00 ß=0.11; p=0.05	ß=-0.01; p=0.77 ß=0.07; p=0.34 ß=0.00; p=0.963 ß=-0.07; p=0.34			clinic site, age, BMI, marital status, education level, pack years, medical condiction, anti-inflammatory drugs, physical acticity
Lira et al. 2011 (104)	obese adolescents	US	r=0.42; p<0.02	n.s.			
				TNF-α			
Panagiotakos et al. 2005 (80)	3043 healthy adults men women				r=0.19; p<0.05 r=0.29; p<0.05	r=0.21; p<0.05 r=0.42; p<0.01	age, diet score, total cholesterol and glucose levels
Park et al. 2005 (97)	46 obese and 54 non-obese Koreans obese	CT	 r=0.27; n.s.	 r=-0.02; n.s.	r=0.24; p<0.05	r=0.32; p<0.05	
Piché et al. 2005 (95)	112 postmenopausal women	CT	r=-0.03; n.s.	r=-0.02; n.s.	r=-0.009;n.s.	r=-0.03; n.s.	
Pou et al. 2007 (94)	1250 healthy adults	CT	r=0.05; n.s. r^2=0.04; p=0.06	r=0.06; n.s. r^2=0.03; p=0.08	r=0.09; p<0.001	r=0.09; p<0.001	age, sex age, smoking, aspirin, alcohol intake, menopausal status, hormone replacement therapy, physical activity

Supplementary Table 1 continued: Association of adiposity measures with chronic inflammation.

Reference	Study population	VAT and SAT measurement	Association VAT	Association SAT	Association BMI	Association WC	Adjustment variables
Beasley et al. 2009 (98)	2651 black and white aged 70-79 white men black men white women black women	CT	ß=0.13; p=0.00 ß=-0.09; p=0.12 ß=0.19; p=0.00 ß=0.20; p=0.00	ß=-0.05; p=0.29 ß=0.05; p=0.51 ß=0.07; p=0.26 ß=-0.04; p=0.61			clinic site, age, BMI, marital status, education level, pack years, medical condiction, anti-inflammatory drugs, oral steroids, physical acticity
Lira et al. 2011 (104)	obese adolescents	US	r=0.40; p<0.05	r=-0.46; p<0.01			
				Resistin			
Zhang et al. 2002 (202)	71 adults				r=n.s.	r=n.s.	
Fehmann et al. 2002 (198)	76 healthy controls 76 type 2 diabetics				r=n.s. r=n.s.		
Azuma et al. 2003 (100)	79 adults	CT	r=0.5; p<0.01	r=0.32; p=0.06	r= 0.35, p < 0.01		
Degawa_Yamauchi et al. 2003 (203)	27 lean, 50 obese				r = 0.37; p = 0.002		
Heilbronn et al. 2003 (89)	38 non-obese 12 obese 22 type 2 dibetics 34 obese				r=n.s. r=n.s. r=n.s. ß=n.s.		

Supplementary Table 1 continued: Association of adiposity measures with chronic inflammation.

Reference	Study population	VAT and SAT measurement	Association VAT	Association SAT	Association BMI	Association WC	Adjustment variables
Lee et al. 2003 (88)	123 middle aged women				ß=0.11; n.s.	ß=0.11; n.s.	age, menopausal status, estradiol level
	120 healthy students				ß=0.07; n.s.	ß=0.17; n.s.	age, gender
Pfutzner et al. 2003 (199)	78 type 2 diabetics				r=n.s.		
Yang et al. 2003 (200)	51 untreated type 2 diabetic patients 52 sex and age-matched normal control subjects				r=n.s.		
Fujinami et al. 2004 (204)	90 type 2 diabetics				r=0.412, p<0.0003		
	74 healthy controls				r=0.395, p<0.0001		
Seow et a. 2004 (196)	17 PCOS women 10 non-PCOS women				r= 0.184, p= 0.479		
Chen et al. 2005 (195)	71 healthy adults						
	36 males				r=0.084; p=n.s.	r=0.046, p=n.s.	age
	35 females				r=-0.005; p=n.s.	r=-0.097; p=n.s.	
Jain et al. 2009 (101)	916 adults men women	CT	ES=1.04; p=0.07 ES=1.06; p<0.001	ES=1.05; p=0.008 ES=1.06; p<0.001			age, smoking, alcohol use, menopausal status (women only), and hormone replacement therapy

Supplementary Table 1 continued: Association of adiposity measures with chronic inflammation.

Reference	Study population	VAT and SAT measurement	Association VAT	Association SAT	Association BMI	Association WC	Adjustment variables
				Adiponectin			
Cnop et al. 2003 (102)	182 adults (USA)	CT	ß=-0.011; p=0.009	ß=0.001; p=0.77	ß=-0.115; p=0.084		Age, Sex, BMI, VAT, SAT,
Taksali et al. 2008 (79)	obese adolescents	MRI	VAT/SAT ratio p=0.08				age, sex, race/ethnicity
Mazzali et al. 2006 (206)	35 women				r=-0.306; n.s.	r=0.3783; p<0.05	
Lira et al. 2011 (104)	obese adolescents	US	r=-0.4; p<0.03				
Saito et al. 2012 (96)	130 type 2 diabetic men and women	CT	r=-0.332; p<0.01	r=-0.262; p<0.01			
	men		r=-0.360; p<0.01	r=-0.293; p<0.05			
	women		r=-0.327; p<0.01	r=-0.390; p<0.01			
Guenther et. Al. 2014 (103)	424 obese	CT	r=-0.22; p<0.001	r=-0.2; p<0.001	r=-0.23; p<0.001	r=-0.29; p<0.001	Age, Sex
	men		r=-0.25; p<0.001	r=-0.12; n.s.	r=-0.12; n.s.	r=-0.21; p<0.01	Age
	women		r=-0.22; p<0.001	r=-0.23; p<0.001	r=-0.27; p<0.001	r=-0.33; p<0.001	Age

VAT= visceral adipose tissue; SAT= subcutaneous adipose tissue; BMI= body mass index; WC= waist circumference, CT=computer tomography, US=Ultrasound, PCOS=Polycystic ovary syndrome, ß=regression coefficient, r=Pearson correlation coefficient.

Supplementary Table 2: Metabolomics studies on obesity.

Reference	Anthropometric variable	Metabolomics technique	Metabolites positive associated with obesity	Metabolites inverse associated with obesity
Newgard et al. 2009 (128)	BMI (74 lean vs. 67 obese)	serum, targeted metabolomics, MS/MS	leucine/isoleucine, valine, glutamate/glutamine, phenylalanine, tyrosine, C3 and C5 acylcarnitines	
Calvani et al. 2010 (134)	BMI (10 lean vs. 15 obese)	urine, untargeted metabolomics, NMR	2-hydroxyisobutyrate	hippuric acid, trigonelline, xanthine
Oberbach et al. 2011 (135)	BMI (15 lean vs. 15 obese)	serum, targeted metabolomics, MS/MS	glycerophosphocholine,	diacylglycerophosphocholine, glycine, glutamine
Cheng et al. 2012 (129)	BMI, WC (1015 men and women)	serum, targeted metabolomics, LC/MS	BMI: lysine, N-carbomoyl-beta-alanine, ornithine, phenylalanine, proline, tyrosine, valine, xanthosine WC: methionine, N-carbomoyl-beta-alanine, ornithine, phenylalanine, proline, tyrosine, valine, xanthosine	
Szymansky et al. 2012 (130)	abdominal obesity by DXA, VAT, SAT by MRI (215 overweight men and women)	serum, targeted 1D NMR	<u>Men</u> A/G ratio: alanine, isoleucine, tyrosine VAT: isoleucine <u>Women</u> A/G ratio: isoleucine, valine VAT: isoleucine	

Supplementary Table 2 continued: Metabolomics studies on obesity.

Reference	Anthropometric variable	Metabolomics technique	Metabolites positive associated with obesity	Metabolites inverse associated with obesity
Wahl et al. 2012 (264)	BMI (40 lean vs. 80 obese children)	serum, targeted metabolomics LC/MS/MS	acylcarnitine, acyl-alkyl-PC	
Lustgarten et al. 2013 (136)	Abdominal obesity by DXA image (73 elderly)	serum, ultrahigh performance LC/MS/MS, GC/MS	2-hydroxy-3-methylvalerate, 3-hydroxyisobutyrate, Isovalerylcarnitine, 3-dehydrocarnitine	glycine, glutamine, 3-methoxytyrosine
Martin et.al. 2013 (239)	VAT/SAT, VAT/total fat by iDXA and CT (40 obese women)	urine and serum, untargeted NMR and targeted MS	tyrosine, glutamine, PC-O 44:6, PC-O 44:4, PC-O 42:4, PC-O 40:4, and PC-O 40:3 lipid species	
Moore et al. 2014 (131)	BMI (974 men and women)	serum and plasma, targeted metabolomics, LC/MS/MS, GC-MS	3-(4-hydroxyphenyl)lactate, gamma-glutamyltyrosine, butyrylcarnitine, 2-hydroxybutyrate (AHB), 3-methyl-2-oxobutyrate, gamma-glutamyl-isoleucine, 7-alpha-hydroxy-3-oxo-4-cholestenoate, Alpha-hydroxyi-sovalerate, 3-methyl-2-oxovalerate, 4-methyl-2-oxopentanoate, 3-hydroxyisobutyrate, glutamate, valine, mannose, Isoleucine, Isovalerylcarnitine, tyrosine, leucine, andro steroid monosulfate 2, lathosterol, kynurenine, glycerol, phenylalanine, hexanoylcarnitine, propionylcarnitine, carnitine, lactate	1-oleoylglycerophosphocholine, 1-eicosadienoylglycerophosphocholine, 2-linoleoylglycerophosphocholine, benzoic acid, N-acetylglycine, palmitoyl sphingomyelin, histidine, 1-5-anhydroglucitol, asparagine, glycine

Supplementary Table 2 continued: Metabolomics studies on obesity.

Reference	Anthropometric variable	Metabolomics technique	Metabolites positive associated with obesity	Metabolites inverse associated with obesity
Xie et al. 2014 (265)	BMI (105 lean vs. 106 healthy obese)	serum, targeted metabolomics, LC/MS, GC/MS	valine, creatine, beta-tyrosine, isoleucine, 4-hydroxycinnamic acid, adenine, leucine, delta-hydroxylysine, kynurenine, glutamic acid, 12a-hydroxy-3-oxocholadienic acid, tryptophan, tyrosine, 8-hydroxy-7-methylguanine, glycerylphosphate, carnitine, lysoPC, alanine, propionylcarnitine, glycolithocholic acid, allantoin, N-actylneuraminic acid, phenylalanine	n-octadecanoic acid, histidinol, palmitoleic acid, bilirubin, cis-11,14-eicosadienoic acid, 2-hydroxyglutaric acid, carnosine, myristic acid
Elliot et al. 2015 (133)	BMI (1880 U.S. men and women plus 444 UK men and women)	urine, targeted and untargeted metabolomics, NMR	urinary glycoproteins, tyrosine+4hydroxymandelate, N-acetyl neuraminate, 3-methylhistidine, citrate, creatinine, leucine, pseudourine, lysine, 2-hydroxyisobutyrate, TMA, ethanolamine, glucose, dimethylamine, dimethylglycine, valine, alanine, O-acetyl carnitine	ketoleucine, succinate, glutamine, hippuric acid, NMNA, formate, 4-Cresyl sulfate, glycine, proline betaine, phenylacetylglutamine, 3-hydroxymandalate, ketoleucine/leucine ratio

BMI=body mass index, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, DXA=Dual X-ray absorptiometry LC/MS=liquid chromatography combined with mass spectroscopy, GC/MS=gas chromatography combined with mass spectroscopy, NMR=nuclear magnetic resonance spectroscopy.

Supplementary Table 3: Validation studies on sonographic-based measurement of visceral and subcutaneous adipose tissue.

Reference	Study population	Measurement technique and location of measurement of reference method	Measurement technique and location of ultrasound measurement	Ultrasound vs. CT / MRI VAT	SAT
Armellini et al. 1990 (148)	50 overweight women	CT single slice at L4 (cm^2)	VAT: linea alba – aorta SAT: skin – linea alba	0.67	
Armellini et al. 1993 (151)	119 overweight women	CT single slice at L4 (cm^2)	VAT: linea alba – aorta SAT: skin – linea alba	0.74	
Tornaghi et al. 1994 (158)	24 volunteers	CT single slice at L4-5 (cm^2)	VAT: internal face of rectus abdominis – lumbar vertebrar	0.89-0.91	
Ribeiro-Filho et al. 2001 (150)	101 overweight women	CT single slice at L4-5 (cm^2)	VAT: internal face of rectus abdominis - anterior wall of the aorta SAT: skin – external face of rectus abdominis 1 cm above umbilicus	0.67	
Stolk et al. 2001 (157)	19 overweight patients	CT single slice at L4-5 (cm^2), MRI multiple slices at L4-L5 (cm^3)	VAT: linea alba – lumbar spine 5 different angles Quiet expiration, minimal pressure	0.81-0.83	
Leite et al. 2002 (149)	29 volunteers	CT single slice at L4-5 (cm^2)	VAT: linea alba – aorta SAT: skin – linea alba 1 cm above umbilicus	0.84	0.79
Ribeiro-Filho et al. 2003 (156)	100 overweight women	CT single slice at L4-5 (cm^2)	VAT: internal face of rectus abdominis – anterior wall of the aorta SAT: skin – external face of rectus abdominis 1 cm above umbilicus	0.71	0.33
Kim et al. 2004 (52)	75 adults	CT single slice at L4-5 (cm^2)	VAT: linea alba – aorta 1 cm above umbilicus	0.80	

Supplementary Table 3 continued: Validation studies on sonographic-based measurement of visceral and subcutaneous adipose tissue.

Reference	Study population	Measurement technique and location of measurement of reference method	Measurement technique and location of ultrasound measurement	Ultrasound vs. CT / MRI VAT	SAT
Hirooka et al. 2005 (154)	87 patents	CT single slice at L4 (cm^2)	VAT: linea alba – aorta End of normal exhalation	0.86	
Koda et al. 2007 (155)	17 adults	MRI volume of abdomen (cm^3)	VAT: linea alba – lumbar vertebra SAT: skin – external face of linea alba Umbilicus level	0.73	0.82
Berker et al. 2010 (140)	104 adults, stratified by BMI (>/< 30 kg/m^2)	CT single slice at L4-5 (cm^2)	VAT: internal face of linea alba – aorta at umbilicus	0.82	
De Lucia Rolfe et al. 2010 (152)	41 men 33 women	MRI single slice at L4 (cm^2)	VAT: peritoneal boundry – corpus of lumbar vertebra SAT: cutaneous boundry – linea alba Xiphoid line intercept WC Respiration protocol	0.82 0.80	0.63 0.68
Gradmark et al. 2010 (153)	29 men and women	CT single slice 5 cm cranial to umbilicus (cm^2)	VAT: inside bowell wall-spine SAT: posterior line of dermis to outer bowell wall 5 cm cranial to umbilicus	0.79	0.52

CT=computer tomography, MRI=magnetic resonance imaging, BMI=body mass index, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue. L4=lumbar vertebra 4, L4-5= lumbar vertebra 4-5.

Supplementary Table 4: Intra- and inter-rater reproducibility of ultrasound-based measurements of VAT and SAT thickness by study centers.

	Intra-rater reproducibility				Inter-rater reproducibility	
	Observer 1		Observer 2		Observer 1 – 2	
(a) Reproducibility in southern study center, n=97						
	ICC (95% CI)	Mean difference (mm) (95% LOA)	ICC (95% CI)	Mean difference (mm) (95% LOA)	ICC (95% CI)	Mean difference (mm) (95% LOA)
VAT	0.995 (0.993-0.997)	-0.303 (-3.62-3.01)	0.999 (0.998-0.999)	-0.029 (-3.20-3.26)	0.998 (0.997-0.999)	-0.073 (-2.60-2.45)
SAT	0.993 (0.989-0.995)	-0.091 (-2.11-1.93)	0.991 (0.987-0.994)	0.039 (-2.15-2.23)	0.992 (0.989-0.994)	0.155 (-1.88-2.19)
(b) Reproducibility in northern study center, n=30						
	Intra-rater reproducibility				Inter-rater reproducibility	
	Observer 1		Observer 2		Observer 1 – 2	
	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)
VAT	0.998 (0.996-0.998)	0.052 (-2.82-2.97)	0.999 (0.998-0.999)	-0.462 (-2.31-1.43)	0.995 (0.989-0.998)	0.637 (-3.25-6.38)
SAT	0.988 (0.974-0.994)	-0.027 (-1.94-1.99)	0.998 (0.995-0.999)	0.122 (-0.89-0.79)	0.971 (0.941-0.986)	0.209 (-2.67-3.31)

VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, ICC=Intra-class correlation coefficient, CI=confidence interval, LOA=limit of agreement.

Supplementary Table 5: Intra- and inter-rater reproducibility of ultrasound-based measurements of VAT and SAT thickness by subgroups.

(a) Reproducibility among lean subjects: BMI ≤ 25.0, n=55

	Intra-rater reproducibility				Inter-rater reproducibility	
	Observer 1		Observer 2		Observer 1 – 2	
	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)
VAT	0.997 (0.994-0.998)	-0.106 (-3.13-2.91)	0.997 (0.994-0.998)	-0.168 (-3.09-2.75)	0.994 (0.990-0.997)	-0.095 (-3.57-3.34)
SAT	0.988 (0.979-0.993)	-0.134 (-2.04-1.78)	0.991 (0.985-0.995)	0.052 (-1.60-1.50)	0.977 (0.962-0.987)	0.312 (-2.20-2.82)

(b) Reproducibility among obese subjects: BMI > 25.0, n=72

	Intra-rater reproducibility				Inter-rater reproducibility	
	Observer 1		Observer 2		Observer 1 – 2	
	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)
VAT	0.994 (0.990-0.996)	-0.060 (-7.78-7.66)	0.999 (0.999-0.999)	0.017 (-3.00--3.04)	0.998 (0.997-0.999)	-0.170 (-4.77-4.43)
SAT	0.992 (0.987-0.995)	-0.031 (-2.07-2.01)	0.991 (0.986-0.994)	0.041 (-2..14-2.22)	0.992 (0.987-0.995)	0.063 (-2.04-2.01)

(c) Reproducibility among lean subjects: Waist circumference < 102 cm in men and < 88 cm in women, n=61

	Intra-rater reproducibility				Inter-rater reproducibility	
	Observer 1		Observer 2		Observer 1 – 2	
	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)
VAT	0.977 (0.962-0.986)	-0.038 (-8.27-8.35)	0.997 (0.995-0.998)	-0.002 (-3.13-3.13)	0.993 (0.988-0.996)	-0.465 (-5.15-4.22)
SAT	0.989 (0.983-0.994)	-0.050 (-1.87-1.77)	0.990 (0.983-0.994)	0.0221 (-1.72-1.76)	0.988 (0.981-0.993)	0.321 (-1.56-2.21)

(d) Reproducibility among obese subjects: Waist circumference ≥ 102 cm in men and ≥ 88 cm in women, n=66

	Intra-rater reproducibility				Inter-rater reproducibility	
	Observer 1		Observer 2		Observer 1 – 2	
	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)
VAT	0.999 (0.998-0.999)	-0.364 (-4.03-3.30)	0.999 (0.998-0.999)	0.153 (-3.20--3.51)	0.999 (0.999-0.999)	-0.014 (-2.74-2.71)
SAT	0.991 (0.983-0.995)	-0.149 (-2.40-2.11)	0.988 (0.977-0.994)	0.065 (-2..65-2.78)	0.992 (0.985-0.996)	-0.102 (-2.24-2.04)

(e) Reproducibility among VAT tertile 1: VAT ≤ 5.09 cm, n=43

	Intra-rater reproducibility				Inter-rater reproducibility	
	Observer 1		Observer 2		Observer 1 – 2	
	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)
VAT	0.989 (0.980-0.994)	-0.193 (-2.94-2.55)	0.988 (0.979-0.994)	-0.238 (-3.00-2.53)	0.990 (0.981-0.994)	-0.057 (-2.70-2.59)
SAT	0.987 (0.977-0.993)	-0.001 (-2.20-2.19)	0.994 (0.989-0.997)	-0.181 (-1.61-1.24)	0.974 (0.953-0.986)	0.469 (-2.57-3.51)

(f) Reproducibility among VAT tertile 2: VAT > 5.09 cm - ≤ 7.25 cm, n=42

	Intra-rater reproducibility				Inter-rater reproducibility	
	Observer 1		Observer 2		Observer 1 – 2	
	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)
VAT	0.689 (0.493-0.819)	0.304 (-8.77-9.38)	0.972 (0.948-0.985)	0.050 (-2.67--2.77)	0.881 (0.791-0.934)	-0.283 (-5.63-5.07)
SAT	0.990 (0.983-0.995)	-0.0814 (-1.92-1.75)	0.985 (0.973-0.992)	0.034 (-2..29-2.36)	0.993 (0.988-0.996)	0.112 (-1.44-1.67)

(g) Reproducibility among VAT tertile 3: VAT > 7.25 cm, n=42

	Intra-rater reproducibility				Inter-rater reproducibility	
	Observer 1		Observer 2		Observer 1 – 2	
	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)	ICC (95 % CI)	Mean difference (mm) (95% LOA)
VAT	0.996 (0.998-0.997)	-0.347 (-5.44-4.75)	0.998 (0.997-0.999)	0.007 (-3.43-3.44)	0.997 (0.995-0.999)	-0.272 (-4.03-3.48)
SAT	0.994 (0.989-0.997)	-0.142 (-2.07-1.79)	0.994 (0.989-0.997)	0.157 (-1.80-2.11)	0.995 (0.990-0.997)	-0.083 (-1.89-1.73)

VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, ICC=Intra-class correlation coefficient, CI=confidence interval, LOA=limit of agreement

Supplementary Table 6: Spearman correlation coefficients between VAT measurements and anthropometric measurements in lean subjects (BMI ≤25.0 kg/m²).

	VAT area (mm²) MRT	VAT thickness (cm) US	VAT thickness (cm) MRT	WC (cm)
VAT thickness (cm) US	ρ=0.777, p=<0.001			
VAT thickness (cm) MRT	ρ=0.626, p=0.005	ρ=0.876, p=<0.001		
WC (cm)	ρ=0.383, p=0.116	ρ=0.388, p=0.112	ρ=0.440, p=0.068	
BMI (kg/m²)	ρ=0.480, p=0.044	ρ=0.482, p=0.043	ρ=0.417, p=0.085	ρ=0.780, p=<0.001

US=ultrasound, MRI=magnetic resonance imaging, VAT=visceral adipose tissue, BMI=body mass index, WC=waist circumference, ρ=Spearman correlation coefficient (ρ), p=p-value, n=18.

Supplementary Table 7: Spearman correlation coefficients between VAT measurements and anthropometric measurements in overweight/obese subjects (BMI >25.0 kg/m²).

	VAT area (mm²) MRT	VAT thickness (cm) US	VAT thickness (cm) MRT	WC (cm)
VAT thickness (cm) US	ρ=0.643, p=0.024			
VAT thickness (cm) MRT	ρ=0.524, p=0.080	ρ=0.979, p=<0.001		
WC (cm)	ρ=0.783, p=0.003	ρ=0.455, p=0.138	ρ=0.378, p=0.226	
BMI (kg/m²)	ρ=0.587, p=0.045	ρ=0.322, p=0.308	ρ=0.280, p=0.379	ρ=0.916, p=<0.001

US=ultrasound, MRI=magnetic resonance imaging, VAT=visceral adipose tissue, BMI=body mass index, WC=waist circumference, ρ =Spearman correlation coefficient (ρ), p=p-value, n=12.

Supplementary Table 8: Spearman correlation coefficients between VAT measurements and anthropometric measurements in all subjects.

	VAT area (mm²) MRT	VAT thickness (cm) US	VAT thickness (cm) MRT	WC (cm)
VAT thickness (cm) US	ρ=0.898, p=<0.001			
VAT thickness (cm) MRT	ρ=0.863, p=<0.001	ρ=0.971, p=<0.001		
WC (cm)	ρ=0.834, p=<0.001	ρ=0.772, p=<0.001	ρ=0.781, p=<0.001	
BMI (kg/m²)	ρ=0.841, p=<0.001	ρ=0.800, p=<0.001	ρ=0.793, p=<0.001	ρ=0.931, p=<0.001

US=ultrasound, MRI=magnetic resonance imaging, VAT=visceral adipose tissue, BMI=body mass index, WC=waist circumference, ρ=Spearman correlation coefficient (ρ), p=p-value, n=30.

Supplementary Table 9: Spearman correlation coefficients between SAT measurements and anthropometric measurements in lean subjects (BMI ≤25.0 kg/m²).

	SAT area (mm²) MRT	SAT thickness (cm) US	SAT thickness (cm) MRT	WC (cm)
SAT thickness (cm) US	ρ=0.802, p=<0.001			
SAT thickness (cm) MRT	ρ=0.793, p=<0.001	ρ=0.969, p=<0.001		
WC (cm)	ρ=0.398, p=0.102	ρ=0.301, p=0.225	ρ=0.345, p=0.161	
BMI (kg/m²)	ρ=0.527, p=0.025	ρ=0.478, p=0.045	ρ=0.511, p=0.030	ρ=0.780, p=<0.001

US=ultrasound, MRI=magnetic resonance imaging, SAT=subcutaneous adipose tissue, BMI=body mass index, WC=waist circumference, ρ=Spearman correlation coefficient (ρ), p=p-value, n=18.

Supplementary Table 10: Spearman correlation coefficients between SAT measurements and anthropometric measurements in overweight/obese subjects (BMI > 25.0 kg/m²).

	SAT area (mm²) MRT	SAT thickness (cm) US	SAT thickness (cm) MRT	WC (cm)
SAT thickness (cm) US	ρ=0.594, p=0.042			
SAT thickness (cm) MRT	ρ=0.685, p=0.014	ρ=0.972, p=<0.001		
WC (cm)	ρ=0.070, p=0.829	ρ=-0.091, p=0.779	ρ=-0.035, p=0.914	
BMI (kg/m²)	ρ=0.189, p=0.557	ρ=0.063, p=0.846	ρ=0.126, p=0.697	ρ=0.916, p=<0.001

US=ultrasound, MRI=magnetic resonance imaging, SAT=subcutaneous adipose tissue, BMI=body mass index, WC=waist circumference, ρ=Spearman correlation coefficient (ρ), p=p-value, n=12.

Supplementary Table 11: Spearman correlation coefficients between SAT measurements and anthropometric measurements in all subjects.

	SAT area (mm²) MRT	SAT thickness (cm) US	SAT thickness (cm) MRT	WC (cm)
SAT thickness (cm) US	ρ=0.705, p=<0.001			
SAT thickness (cm) MRT	ρ=0.781, p=<0.001	ρ=0.965, p=<0.001		
WC (cm)	ρ=0.663, p=<0.001	ρ=0.357, p=0.053	ρ=0.395, p=0.031	
BMI (kg/m²)	ρ=0.697, p=<0.001	ρ=0.443, p=0.014	ρ=0.489, p=0.007	ρ=0.931, p=<0.001

US=ultrasound, MRI=magnetic resonance imaging, SAT=subcutaneous adipose tissue, BMI=body mass index, WC=waist circumference, ρ=Spearman correlation coefficient (ρ), p=p-value, n=30.

Supplementary Table 12: Inflammatory parameter interaction with sex, BMI, smoking, and medication use.

	Sex p-value	BMI p-value	Smoking p-value	Aspirin or NSAIDs use p-value
hs-CRP (mg/dl)	0.878	<0.001	0.031	0.009
TNF-α (pg/ml)	0.101	0.578	0.261	0.834
IL-6 (pg/ml)	0.051	0.020	0.423	0.575
Resistin (ng/ml)	0.457	0.374	0.841	0.630
Adiponectin (µg/ml)	0.003	0.580	0.047	0.553

BMI=body mass index (lean ≤ 25 vs. obese > 25), NSAIDs=non-steroidal anti-inflammatory drugs, p-value from Kruskal-Wallis test.

Supplementary Table 13: Urinary metabolite interaction with sex, fasting status, and urinary glucose.

	Sex p value	Fasting status p value	Urinary glucose p value
Alanine	0.142	0.002	0.548
Glycine	<0.001	0.125	0.056
Taurine	0.795	0.780	0.006
Glutamine	0.204	0.013	0.246
3-Methylhistidine	0.024	0.218	0.003
Betaine	0.413	0.078	0.028
Phenylalanine	<0.001	0.668	0.041
Serine	0.459	0.146	0.007
D-glucose	0.301	0.252	-
Choline	0.302	0.768	0.017
Lactic acid	<0.001	0.180	0.005
Methanol	0.007	0.003	<0.001
Ascorbic acid	0.096	0.009	<0.001
Creatine	0.001	0.165	<0.001
L-Pyroglutamic acid	0.546	0.024	0.001
Hippuricacid	<0.001	0.043	<0.001
Ethanolamine	0.019	0.463	0.001
Trimethylamine-N-oxide	0.026	0.401	0.075
Citric acid	<0.001	0.032	0.013
Dimethylamine	<0.001	0.412	0.133
D-Mannitol	0.002	0.037	0.001
Guanidinoacetic acid	<0.001	0.048	<0.001
Glycolic acid	0.209	0.001	0.659
Formic acid	<0.001	0.004	<0.001
Tyrosine	0.582	0.026	0.295
Fumaric acid	<0.001	0.061	0.853
Orotic acid	0.217	0.442	0.002
Leucine	0.112	0.325	0.668
N,N-Dimethylglycine	0.013	0.083	0.070
Trigonelline	<0.001	<0.001	<0.001

p-value from Kruskal-Wallis (continuous variables) or X^2 test (categorical variables).

Supplementary Table 14: Serum metabolite interaction with sex, fasting status, and urinary glucose.

	Sex p value	Fasting status p value	Urinary glucose p value
Alanine	0.412	0.357	0.115
Glycine	0.659	0.145	0.337
Glutamine	0.934	0.514	0.705
D-Glucose	0.854	0.776	0.015
Lacticacid	0.352	0.466	0.029
Methanol	0.568	0.374	0.291
L-isoleucine	0.593	0.051	0.785
Threonine	0.824	0.016	0.248
Valine	0.894	0.038	0.741
Acetone	0.396	0.430	0.317
Formic acid	0.737	0.188	0.950
Tyrosine	0.850	0.018	0.480
Pyruvic acid	0.784	0.247	0.007
Creatinine	0.042	0.024	0.118
Acetic acid	0.688	0.939	0.227
Creatine	0.234	0.800	0.948
Ketoleucine	0.999	0.098	0.058
Phenylalanine	0.909	0.010	0.145
Leucine	0.410	0.055	0.712
3-Hydroxybutyricacid	0.856	0.924	0.256

p-value from Kruskal-Wallis test (continuous variables) or X^2 test (categorical variables).

Supplementary Table 15: Differences between phenotypes among clusters formed from urinary fingerprints in subgroup analyses.

	VAT		SAT		VSR		BMI		WC	
Cluster	ß	p	ß	p	ß	p	ß	p	ß	p
Men										
1	-0.33	0.60	0.43	0.33	0.91	0.34	0.87	0.51	2.27	0.48
2	0.33	0.62	-0.15	0.37	1.08	0.06	1.00	0.27	3.23	0.21
3	0.31	0.73	0.38	0.09	-0.57	0.46	1.37	0.26	5.80	0.10
4	-0.89	0.31	-0.06	0.78	-0.41	0.59	-0.70	0.57	-2.62	0.45
5	-1.89	0.50	0.66	0.36	-1.84	0.46	1.49	0.70	0.88	0.94
6	0.50	0.86	1.34	0.07	-1.48	0.55	1.19	0.76	4.08	0.71
Women										
1	-0.12	0.66	0.29	0.50	0.45	0.93	1.61	0.38	2.59	0.43
2	0.14	0.83	0.01	0.96	0.32	0.42	0.91	0.43	0.69	0.80
3	0.32	0.68	0.25	0.31	0.07	0.89	1.19	0.39	3.92	0.23
4	-0.79	0.30	-0.27	0.28	-0.13	0.78	-1.95	0.16	-3.13	0.33
5	-1.03	0.71	0.36	0.69	-1.11	0.53	3.12	0.53	-5.84	0.62
6	0.77	0.80	1.12	0.26	-0.62	0.74	4.78	0.38	17.31	0.18
Non-fasting participants										
1	0.74	0.43	0.45	0.26	0.14	0.82	1.79	0.37	3.47	0.41
2	0.67	0.21	0.14	0.36	0.66	0.12	1.43	0.08	3.08	0.16
3	0.72	0.20	-0.20	0.23	0.83	0.06	-0.20	0.81	-1.10	0.63
4	-0.63	0.39	-0.28	0.21	0.00	0.99	-1.88	0.10	-2.71	0.38
5	**1.62**	**0.03**	0.27	0.23	0.47	0.42	1.21	0.30	5.20	0.10
6	-0.16	0.96	0.39	0.63	-0.88	0.68	3.77	0.37	-3.98	0.73
7	1.99	0.49	1.51	0.08	-1.11	0.62	7.05	0.11	20.46	0.09
8	0.96	0.73	1.31	0.11	-1.44	0.51	1.16	0.79	3.37	0.77
Fasting participants										
1	0.86	0.73	0.37	0.59	0.89	0.93	2.70	0.70	0.06	0.60
2	-0.98	0.61	0.44	0.40	-1.01	0.14	-0.67	0.86	5.09	0.48
3	-0.73	0.84	0.29	0.77	-0.98	0.44	-4.72	0.53	-5.20	0.71

Supplementary Table 15 continued: Differences between phenotypes among clusters formed from urinary fingerprints in subgroup analyses.

	VAT		SAT		VSR		BMI		WC	
Cluster	ß	p	ß	p	ß	p	ß	p	ß	p
Non-fasting men										
1	-0.58	0.68	0.56	0.34	0.17	0.62	1.23	0.51	2.88	0.51
2	0.05	0.95	0.15	0.42	0.01	0.99	1.65	0.11	5.31	0.07
3	-1.25	0.19	0.09	0.71	-1.13	0.18	-0.33	0.80	-1.55	0.67
4	0.20	0.84	0.28	0.24	-0.73	0.38	1.22	0.35	3.93	0.29
5	-2.14	0.47	0.79	0.29	-2.53	0.33	1.95	0.63	1.75	0.88
6	0.25	0.93	1.47	0.05	-2.18	0.40	1.65	0.68	4.95	0.66
Non-fasting women										
1	0.05	0.62	0.36	0.51	-0.74	0.55	2.31	0.33	3.53	0.46
2	0.72	0.27	0.17	0.45	0.47	0.29	1.36	0.21	1.97	0.48
3	-0.70	0.34	-0.25	0.32	-0.15	0.76	-1.69	0.18	-2.56	0.43
4	0.16	0.87	-0.03	0.92	0.27	0.69	0.75	0.66	2.46	0.57
5	-0.68	0.80	0.39	0.67	-1.00	0.58	3.52	0.44	-4.73	0.69
6	0.75	0.82	1.50	0.17	-1.65	0.45	7.60	0.17	20.50	0.15
Subjects with no urinary glucose										
1	0.26	0.62	0.17	0.30	0.23	0.59	1.38	0.10	2.57	0.23
2	0.78	0.15	-0.15	0.37	0.77	0.07	-0.20	0.82	-0.66	0.76
3	-0.66	0.36	-0.33	0.15	0.01	0.98	-2.12	0.06	-3.23	0.27
4	0.58	0.68	-0.09	0.83	0.22	0.84	-0.56	0.80	2.03	0.72
5	-0.22	0.93	0.33	0.70	-0.82	0.70	3.41	0.42	-5.03	0.64
6	1.51	0.59	1.24	0.15	-0.79	0.71	5.38	0.22	17.73	0.11
7	0.91	0.73	1.33	0.12	-1.55	0.46	1.18	0.78	3.48	0.75
Glucose subjects										
1	-0.28	0.83	-0.21	0.49	-0.58	0.52	-0.60	0.79	-2.17	0.61
2	-0.01	0.99	0.10	0.77	-0.42	0.66	-0.80	0.73	0.03	0.99
3	-2.94	0.22	0.27	0.63	-1.74	0.30	-2.17	0.59	3.10	0.76

Linear regression adjusted for age and sex, grand mean=reference category, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, VSR=visceral-to-subcutaneous-fat-ratio, BMI=body mass index, WC=waist circumference, ß=regression coefficient, p=p-value. In each case, the stratification variable was excluded from the model.

Supplementary Table 16: Differences between phenotypes among clusters formed from serum fingerprints in subgroup analyses

	VAT		SAT		VSR		BMI		WC	
Cluster	ß	p	ß	p	ß	p	ß	p	ß	p
Men										
1	-0.31	0.64	0.12	0.81	0.47	0.62	0.26	0.39	1.23	0.25
2	-1.02	0.11	-0.02	0.91	-0.66	0.21	-0.92	0.34	-3.30	0.21
3	0.17	0.84	0.05	0.85	-0.26	0.71	-1.25	0.34	-4.34	0.23
4	-0.07	0.98	0.33	0.68	-1.43	0.53	2.95	0.48	11.32	0.32
Women										
1	-0.07	0.55	-0.57	0.01	-0.52	0.32	-0.51	0.55	-3.24	0.30
2	-0.45	0.46	**-0.46**	**0.02**	0.44	0.25	-1.26	0.26	-4.62	0.07
3	0.31	0.64	**-0.67**	**0.002**	1.50	0.06	0.25	0.84	-1.86	0.52
Non-fasting participants										
1	0.45	0.64	0.03	0.36	-0.98	0.64	0.35	0.63	-0.07	0.63
2	0.00	1.00	-0.11	0.48	0.05	0.89	-0.07	0.93	-0.29	0.89
3	0.17	0.78	**-0.42**	**0.04**	0.96	0.05	0.14	0.89	-0.85	0.75
4	0.62	0.33	0.03	0.87	-0.07	0.89	0.99	0.34	0.24	0.93
Fasting participants										
1	0.35	0.53	0.09	0.70	-0.54	0.73	0.26	0.61	-1.31	0.61
2	1.57	0.41	-0.11	0.82	0.83	0.19	2.32	0.56	2.65	0.72
3	-0.87	0.65	0.28	0.57	-0.62	0.32	-1.81	0.66	-5.26	0.49
Non-fasting men										
1	0.73	0.37	0.10	0.68	0.51	0.29	0.54	0.54	0.85	0.49
2	0.69	0.35	0.07	0.73	-0.20	0.75	1.30	0.21	3.12	0.29
3	0.77	0.39	0.12	0.62	-0.14	0.85	-0.22	0.86	-1.42	0.69
Non-fasting women										
1	0.31	0.45	-0.48	0.04	1.42	0.87	0.34	0.35	-1.54	0.52
2	-0.25	0.68	-0.39	0.06	0.37	0.38	-0.76	0.47	-3.66	0.18
3	0.86	0.21	**-0.57**	**0.02**	1.68	0.06	1.44	0.23	0.59	0.85

Supplementary Table 16 continued: Differences between phenotypes among clusters formed from serum fingerprints in subgroup analyses

	VAT		SAT		VSR		BMI		WC	
Cluster	ß	p	ß	p	ß	p	ß	p	ß	p
Subjects with no urinary glucose										
1	-0.26	0.73	-0.91	0.69	-0.58	0.92	-2.60	0.19	-0.17	0.59
2	0.43	0.96	0.14	0.72	1.86	0.36	-0.68	0.76	1.03	0.91
3	-2.94	0.25	0.28	0.18	-1.26	0.39	-0.17	0.83	1.10	0.86

Linear regression adjusted for age and sex, grand mean=reference category, VAT=visceral adipose tissue, SAT=subcutaneous adipose tissue, VSR=visceral-to-subcutaneous-fat-ratio BMI=body mass index, WC=waist circumference, ß=linear regression coefficient, p=p-value.

Supplementary Table 17: Significant[#] results from regression analyses to detect relations of VAT to urinary bins among subgroups.

		Model 1		Model 2		
Anthropometric variable	bin (ppm)	ß	p	ß	p	metabolite identification
Non-fasting subjects (n=198)						
VAT	9.135	-0.153	0.0029	-0.160	0.0020	unknown
	9.075	-0.126	0.0076	-0.128	0.0068	unknown
	8.455	-0.089	0.0013	-0.095	0.0005	Formic acid
	7.075	-0.083	0.0036	-0.083	0.0037	1-Methylhistidine, unknown
	6.965	-0.074	0.0016	-0.078	0.0009	4-Hydroxyhippuric acid
	6.955	-0.065	0.0022	-0.070	0.0008	4-Hydroxyhippuric acid
	6.585	-0.068	0.0005	-0.072	0.0002	unknown
	6.575	-0.056	0.0089	-0.061	0.0038	unknown
	6.555	-0.057	0.0093	-0.062	0.0046	unknown
	4.095	-0.036	0.0118	-0.039	0.0059	D-Saccharic acid
	4.075	-0.131	0.0011	-0.133	0.0010	Creatinine
	4.065	-0.118	0.0019	-0.119	0.0019	Choline/creatinine, others
	3.945	-0.044	0.0064	-0.047	0.0034	4-Hydroxyhippuric acid, others
	3.925	-0.047	0.0024	-0.050	0.0009	Tyrosine, unknown
	3.565	-0.086	0.0001	-0.088	0.00004	Glycine, unknown
	3.525	-0.056	0.0004	-0.058	0.0002	Choline, others
	3.365	-0.095	0.0001	-0.096	0.0001	scyllo-Inositol, unknown
	3.355	-0.052	0.0008	-0.053	0.0006	scyllo-Inositol
	3.345	-0.062	0.0017	-0.066	0.0007	3-Methylhistidine
	3.205	-0.055	0.0041	-0.055	0.0044	Choline, others
	3.155	-0.032	0.0073	-0.032	0.0063	1-Methylhistidine, unknown
	3.145	-0.032	0.0039	-0.033	0.0027	cis-Aconitic acid, unknown
	3.115	-0.070	0.0057	-0.070	0.0061	3-aminoisobutyric acid, unknown
	3.055	0.089	0.0069	0.093	0.0047	Tyrosine, creatinine
	2.985	-0.030	0.0137	-0.033	0.0064	unknown

Supplementary Table 17 continued: Significant[#] results from regression analyses to detect relations of VAT to urinary bins among subgroups.

		Model 1		Model 2		
Anthropometric variable	bin (ppm)	ß	p	ß	p	metabolite identification
VAT	2.975	-0.036	0.0047	-0.040	0.0018	Asparagine, unknown
	2.965	-0.037	0.0058	-0.041	0.0024	Asparagine, unknown
	2.835	-0.038	0.0003	-0.040	0.0001	Methylguanidine
	2.775	-0.025	0.0145	-0.028	0.0046	unknown
	2.735	0.068	0.0047	0.067	0.0054	Dimethylamine
	2.725	0.061	0.0004	0.063	0.0003	Dimethylamine/citric acid
	2.705	-0.102	0.0061	-0.103	0.0061	Citric acid
	2.675	-0.131	0.0011	-0.133	0.0010	Citric acid
	2.645	-0.040	0.0017	-0.043	0.0006	unknown
	2.605	-0.038	0.0010	-0.038	0.0009	Ketoleucine
	2.515	-0.051	0.0001	-0.053	0.00003	L-Pyroglutamic acid
	2.475	-0.041	0.0008	-0.043	0.0004	Pyridoxine, isocitric acid, glutamine, 3-hydroxy-3-methylglutaric acid, carnitine
	2.465	-0.031	0.0029	-0.033	0.0017	3-hydroxy-3-methylglutaric acid, carnitine
	2.455	-0.032	0.0016	-0.033	0.0012	Isocitric acid, glutamine, 3-hydroxy-3-methylglutaric acid, carnitine
	2.445	-0.040	0.0003	-0.041	0.0002	Isocitric acid, glutamine, 3-hydroxy-3-methylglutaric acid, carnitine
	2.435	-0.040	0.0001	-0.042	0.0001	Isocitric acid, glutamine, 3-hydroxy-3-methylglutaric acid, carnitine

Supplementary Table 17 continued: Significant[#] results from regression analyses to detect relations of VAT to urinary bins among subgroups.

		Model 1		Model 2		
Anthropometric variable	bin (ppm)	ß	p	ß	p	metabolite identification
VAT	2.425	-0.036	0.0027	-0.039	0.0008	Isocitric acid, glutamine, 3-hydroxy-3-methylglutaric acid, carnitine
	2.405	-0.051	0.0007	-0.054	0.0002	L-Pyroglutamic acid, glutamine, 3-hydroxy-3-methylglutaric acid, carnitine
	2.395	-0.029	0.0139	-0.033	0.0041	L-Pyroglutamic acid
	2.365	-0.040	0.0016	-0.042	0.0006	3-Hydroxyisovaleric acid
	2.355	-0.033	0.0050	-0.036	0.0028	unknown
	2.335	-0.028	0.0105	-0.031	0.0046	unknown
	2.325	-0.035	0.0016	-0.038	0.0005	unknown
	2.315	-0.036	0.0021	-0.039	0.0009	unknown
	2.305	-0.039	0.0055	-0.041	0.0034	unknown
	2.245	-0.030	0.0058	-0.033	0.0024	5-Aminopentanonic acid, unknown
	2.235	-0.084	0.0000	-0.086	0.00001	Acetone, 5-aminopentanonic acid, unknown
	2.225	-0.030	0.0119	-0.033	0.0058	5-Aminopentanonic acid, unknown
	2.215	-0.034	0.0067	-0.036	0.0036	unknown
	2.205	-0.036	0.0080	-0.038	0.0053	unknown
	2.195	-0.042	0.0045	-0.044	0.0030	Suberic acid, sebacic acid, unknown
	2.185	-0.058	0.0017	-0.061	0.0010	unknown
	2.175	-0.044	0.0076	-0.046	0.0047	unknown
	2.155	-0.032	0.0087	-0.034	0.0069	unknown, Hydroxyacetone

Supplementary Table 17 continued: Significant# results from regression analyses to detect relations of VAT to urinary bins among subgroups.

		Model 1		Model 2		
Anthropometric variable	bin (ppm)	ß	p	ß	p	metabolite identification
VAT	2.135	-0.035	0.0037	-0.036	0.0027	unknown
	2.125	-0.039	0.0051	-0.040	0.0052	Glutamine, N-acetyl-L-glutamine
	2.075	-0.030	0.0115	-0.032	0.0056	unknown
	2.025	-0.030	0.0046	-0.032	0.0014	L-Pyroglutamic acid, unknown
	2.015	-0.030	0.0074	-0.033	0.0026	L-Pyroglutamic acid, unknown
	2.005	-0.034	0.0059	-0.037	0.0020	L-Pyroglutamic acid, unknown
	1.815	-0.033	0.0149	-0.036	0.0062	4-Guanidinobutyric acid, unknown
	1.655	-0.035	0.0103	-0.038	0.0041	Arginine, 5-aminopentanoic acid, 2-aminoadipicacid
	1.645	-0.036	0.0028	-0.039	0.0012	Arginine, 5-aminopentanoic acid, glycyl-L-leucine, 2-aminoadipicacid
	1.635	-0.029	0.0089	-0.031	0.0038	Arginine, 5-aminopentanoic acid, glycyl-L-leucine, 2-aminoadipicacid
	1.625	-0.029	0.0118	-0.031	0.0055	Arginine, 5-aminopentanoic acid, glycyl-L-leucine, 2-aminoadipicacid
	1.615	-0.028	0.0146	-0.031	0.0071	Arginine, 5-aminopentanoic acid, glycyl-L-leucine, 2-aminoadipicacid
	1.555	-0.033	0.0046	-0.035	0.0026	Suberic acid, sebacic acid
	1.475	0.036	0.0067	0.037	0.0066	Alanine, lysone
	1.355	0.032	0.0136	0.035	0.0070	unknown
	1.235	-0.046	0.0021	-0.049	0.0009	unknown

Supplementary Table 17 continued: Significant# results from regression analyses to detect relations of VAT to urinary bins among subgroups.

		Model 1		Model 2		
Anthropometric variable	bin (ppm)	ß	p	ß	p	metabolite identification
VAT	1.225	-0.038	0.0095	-0.041	0.0047	unknown
	1.185	-0.043	0.0073	-0.046	0.0039	unknown
	1.145	-0.052	0.0104	-0.058	0.0039	Isobutyrylcarnitine, unknown
	1.095	-0.039	0.0103	-0.042	0.0050	unknown
	1.015	-0.032	0.0155	-0.035	0.0070	unknown
Subjects with no urinary glucose						
	2.405	-0.052	0.0017	-0.055	0.0448	L-Pyroglutamic acid, glutamine
	2.435	-0.033	0.0052	-0.036	0.0011	3-Hydroxy-3-methylglutaric acid, others
	2.605	-0.036	0.0039	-0.039	0.0010	unknown, ketoleucine
	2.615	-0.049	0.0043	-0.056	0.0006	unknown, ketoleucine
	2.465	-0.033	0.0052	-0.037	0.0009	3-Hydroxy-3-methylglutaric acid, others
	3.345	-0.057	0.0089	-0.070	0.0007	3-Methylhistidine, others
	3.355	-0.050	0.0030	-0.055	0.0007	scyllo-Inositol
	6.585	-0.056	0.0063	-0.064	0.0010	unknown
	6.965	-0.078	0.0037	-0.086	0.0008	4-Hydroxyhippuric acid
	8.455	-0.093	0.0014	-0.091	0.0009	unknown
	9.135	-0.154	0.0036	-0.162	0.0010	unknown

Model 1: linear regression model adjusted for study, age (non-linear) and sex.
Model 2: linear regression model adjusted for study, age and sex interaction (non-linear), eGFR, smoking status, menopausal status (women only), fasting status, urinary glucose, and physical activity. In each case, the stratification variable was excluded from the model. VAT=visceral adipose tissue, bin=spectra position (ppm), ß=beta-coefficient p=corrected p-value.
[#]p-values<0.05 after correction for multiple testing are considered significant.

Supplementary Table 18: Significant[#] results from regression analyses to detect relations of BMI to urinary bins.

		Model 1		Model 2	
Anthropometric variable	bin (ppm)	ß	p	ß	p
Non-fasting subjects (n=198)					
BMI	1.015	-0.028	0.010	-0.026	0.025
	1.095	-0.032	0.009	-0.027	0.038
	1.145	-0.046	0.007	-0.041	0.023
	1.185	-0.038	0.005	-0.037	0.013
	1.225	-0.033	0.006	-0.030	0.021
	1.235	-0.041	0.001	-0.038	0.005
	1.305	-0.030	0.006	-0.027	0.024
	1.315	-0.029	0.008	-0.026	0.025
	1.485	-0.026	0.015	-0.025	0.030
	1.545	-0.026	0.011	-0.024	0.029
	1.555	-0.028	0.005	-0.026	0.013
	1.565	-0.023	0.014	-0.020	0.049
	1.615	-0.024	0.011	-0.020	0.038
	1.625	-0.023	0.012	-0.019	0.047
	1.645	-0.027	0.007	-0.022	0.029
	1.815	-0.029	0.008	-0.023	0.036
	2.005	-0.028	0.006	-0.022	0.025
	2.015	-0.025	0.008	-0.020	0.037
	2.025	-0.025	0.004	-0.020	0.018
	2.135	-0.025	0.010	-0.023	0.029
	2.145	-0.025	0.008	-0.020	0.041
	2.165	-0.035	0.007	-0.031	0.026
	2.185	-0.041	0.008	-0.037	0.028
	2.205	-0.026	0.015	-0.025	0.037
	2.215	-0.024	0.015	-0.023	0.034
	2.235	-0.038	0.006	-0.037	0.013
	2.305	-0.030	0.008	-0.024	0.046
	2.315	-0.027	0.006	-0.023	0.025
	2.325	-0.028	0.003	-0.023	0.013
	2.335	-0.023	0.011	-0.018	0.028
	2.355	-0.026	0.007	-0.022	0.036
	2.365	-0.031	0.003	-0.029	0.011
	2.405	-0.042	0.001	-0.038	0.005

Supplementary Table 18 continued: Significant[#] results from regression analyses to detect relations of BMI to urinary bins.

		Model 1		Model 2	
Anthropometric variable	bin (ppm)	ß	p	ß	p
BMI	2.425	-0.028	0.005	-0.026	0.013
	2.435	-0.029	0.001	-0.026	0.007
	2.445	-0.030	0.002	-0.027	0.008
	2.455	-0.025	0.004	-0.022	0.013
	2.465	-0.022	0.009	-0.020	0.029
	2.475	-0.027	0.008	-0.024	0.025
	2.515	-0.035	0.002	-0.030	0.011
	2.525	-0.060	0.011	-0.061	0.021
	2.645	-0.035	0.002	-0.034	0.004
	2.675	-0.099	0.004	-0.111	0.005
	2.705	-0.078	0.010	-0.083	0.013
	2.725	0.058	0.000	0.062	0.000
	2.735	0.047	0.015	0.060	0.008
	2.755	-0.021	0.017	-0.020	0.029
	2.775	-0.021	0.010	-0.019	0.025
	2.835	-0.024	0.006	-0.023	0.013
	2.925	-0.024	0.032	-0.026	0.030
	2.935	0.021	0.065	0.028	0.029
	2.965	-0.028	0.011	-0.027	0.024
	2.975	-0.028	0.008	-0.028	0.013
	2.985	-0.026	0.008	-0.027	0.013
	2.995	-0.026	0.008	-0.026	0.013
	3.055	0.051	0.056	0.065	0.026
	3.145	-0.024	0.008	-0.024	0.015
	3.225	-0.036	0.020	-0.038	0.025
	3.245	-0.012	0.121	-0.017	0.044
	3.255	-0.012	0.192	-0.019	0.047
	3.265	-0.058	0.006	-0.066	0.007
	3.345	-0.051	0.002	-0.045	0.013
	3.355	-0.036	0.005	-0.034	0.013
	3.365	-0.048	0.008	-0.048	0.018
	3.375	-0.028	0.011	-0.024	0.039

Supplementary Table 18 continued: Significant[#] results from regression analyses to detect relations of BMI to urinary bins.

		Model 1		Model 2	
Anthropometric variable	bin (ppm)	ß	p	ß	p
BMI	3.405	-0.025	0.008	-0.024	0.025
	3.415	-0.031	0.008	-0.031	0.019
	3.535	-0.031	0.011	-0.025	0.049
	3.545	-0.032	0.011	-0.026	0.044
	3.565	-0.061	0.001	-0.063	0.003
	3.685	-0.026	0.026	-0.028	0.025
	3.695	-0.026	0.028	-0.027	0.029
	3.705	-0.025	0.055	-0.028	0.044
	3.715	-0.033	0.018	-0.031	0.049
	3.735	-0.019	0.085	-0.026	0.021
	3.745	-0.016	0.128	-0.022	0.034
	3.755	-0.026	0.035	-0.028	0.026
	3.795	-0.026	0.043	-0.030	0.037
	3.855	-0.020	0.011	-0.016	0.042
	3.925	-0.033	0.008	-0.030	0.024
	4.025	-0.020	0.048	-0.020	0.049
	4.035	-0.027	0.008	-0.023	0.018
	4.055	-0.079	0.011	-0.090	0.013
	4.095	-0.035	0.004	-0.031	0.010
	4.245	-0.027	0.005	-0.024	0.013
	4.275	-0.020	0.015	-0.018	0.039
	4.305	-0.016	0.015	-0.015	0.029
	4.425	-0.025	0.008	-0.023	0.024
	6.585	-0.049	0.003	-0.047	0.008
	6.685	-0.031	0.021	-0.033	0.026
	6.805	-0.038	0.021	-0.038	0.032
	7.075	-0.059	0.010	-0.059	0.020
	7.085	-0.062	0.021	-0.062	0.042
	7.675	-0.021	0.006	-0.019	0.015
	7.985	0.022	0.112	0.031	0.042
	8.455	-0.069	0.003	-0.070	0.008
	9.075	-0.092	0.015	-0.093	0.036

Supplementary Table 18 continued: Significant[#] results from regression analyses to detect relations of BMI to urinary bins.

		Model 1		Model 2	
Anthropometric variable	bin (ppm)	ß	p	ß	p
BMI	9.165	-0.148	0.023	-0.163	0.027
	9.385	-0.093	0.010	-0.086	0.024
Subjects with no urinary glucose					
	1.015	-0.023	0.0324	-0.031	0.0468
	1.445	-0.027	0.0198	-0.034	0.0468
	2.165	-0.030	0.0279	-0.046	0.0446
	2.185	-0.034	0.0349	-0.056	0.0387
	2.235	-0.037	0.0151	-0.049	0.0432
	2.355	-0.026	0.0177	-0.032	0.0387
	2.365	-0.027	0.0198	-0.038	0.0178
	2.405	-0.041	0.0071	-0.044	0.0248
	2.425	-0.028	0.0117	-0.030	0.0468
	2.645	-0.033	0.0090	-0.036	0.0372
	2.725	0.034	0.0139	0.049	0.0453
	2.735	0.030	0.1114	0.066	0.0468
	3.225	-0.030	0.0756	-0.057	0.0432
	3.265	-0.059	0.0117	-0.116	0.0055
	3.325	-0.036	0.0213	-0.052	0.0253
	3.365	-0.053	0.0119	-0.073	0.0156
	3.375	-0.024	0.0418	-0.038	0.0432
	3.415	-0.035	0.0117	-0.047	0.0258
	3.925	-0.038	0.0107	-0.043	0.0468
	4.025	-0.014	0.1479	-0.032	0.0468
	4.055	-0.077	0.0213	-0.113	0.0432
	4.095	-0.029	0.0218	-0.039	0.0432
	6.685	-0.030	0.0397	-0.047	0.0251
	7.985	0.026	0.0963	0.057	0.0203
	8.455	-0.069	0.0088	-0.091	0.0094
	8.685	-0.034	0.0567	-0.067	0.0083
	9.075	-0.092	0.0263	-0.155	0.0464
	9.135	-0.088	0.0363	-0.143	0.0182
	9.165	-0.120	0.0988	-0.289	0.0099

Model 1: linear regression model adjusted for study, age (non-linear) and sex.

Model 2: linear regression model adjusted for study, age and sex interaction (non-linear), eGFR, smoking status, menopausal status (women only), fasting status, urinary glucose, and physical activity. In each case, the stratification variable was excluded from the model. BMI=body mass index, bin=spectra position (ppm), ß=beta-coefficient p=corrected p-value.
#p-values<0.05 after correction for multiple testing are considered significant.

Supplementary Table 19: Significant[#] results from regression analyses to detect relations of WC to urinary bins.

Anthropometric variable	bin (ppm)	Model 1 ß	Model 1 p	Model 2 ß	Model 2 p
Non-Fasting subjects (n=198)					
WC	1.095	-0.012	0.013	-0.011	0.037
	1.145	-0.016	0.014	-0.016	0.021
	1.185	-0.014	0.006	-0.014	0.010
	1.235	-0.013	0.004	-0.013	0.009
	1.485	-0.009	0.022	-0.008	0.049
	1.645	-0.009	0.014	-0.008	0.046
	2.005	-0.009	0.014	-0.008	0.044
	2.025	-0.009	0.006	-0.007	0.025
	2.135	-0.010	0.012	-0.009	0.039
	2.165	-0.013	0.011	-0.011	0.045
	2.185	-0.015	0.011	-0.014	0.043
	2.235	-0.016	0.002	-0.016	0.004
	2.325	-0.009	0.014	-0.008	0.030
	2.365	-0.011	0.004	-0.011	0.005
	2.405	-0.014	0.002	-0.014	0.003
	2.425	-0.011	0.004	-0.010	0.006
	2.435	-0.011	0.002	-0.010	0.005
	2.445	-0.011	0.002	-0.010	0.005
	2.455	-0.009	0.004	-0.008	0.013
	2.465	-0.008	0.014	-0.007	0.046
	2.475	-0.010	0.011	-0.010	0.020
	2.515	-0.013	0.002	-0.012	0.006
	2.525	-0.023	0.014	-0.027	0.006
	2.555	-0.021	0.047	-0.023	0.047
	2.645	-0.011	0.011	-0.012	0.004
	2.665	-0.021	0.052	-0.025	0.047
	2.675	-0.032	0.011	-0.039	0.005
	2.705	-0.025	0.039	-0.029	0.026
	2.715	0.019	0.075	0.026	0.028
	2.725	0.018	0.002	0.020	0.002
	2.735	0.016	0.041	0.023	0.005

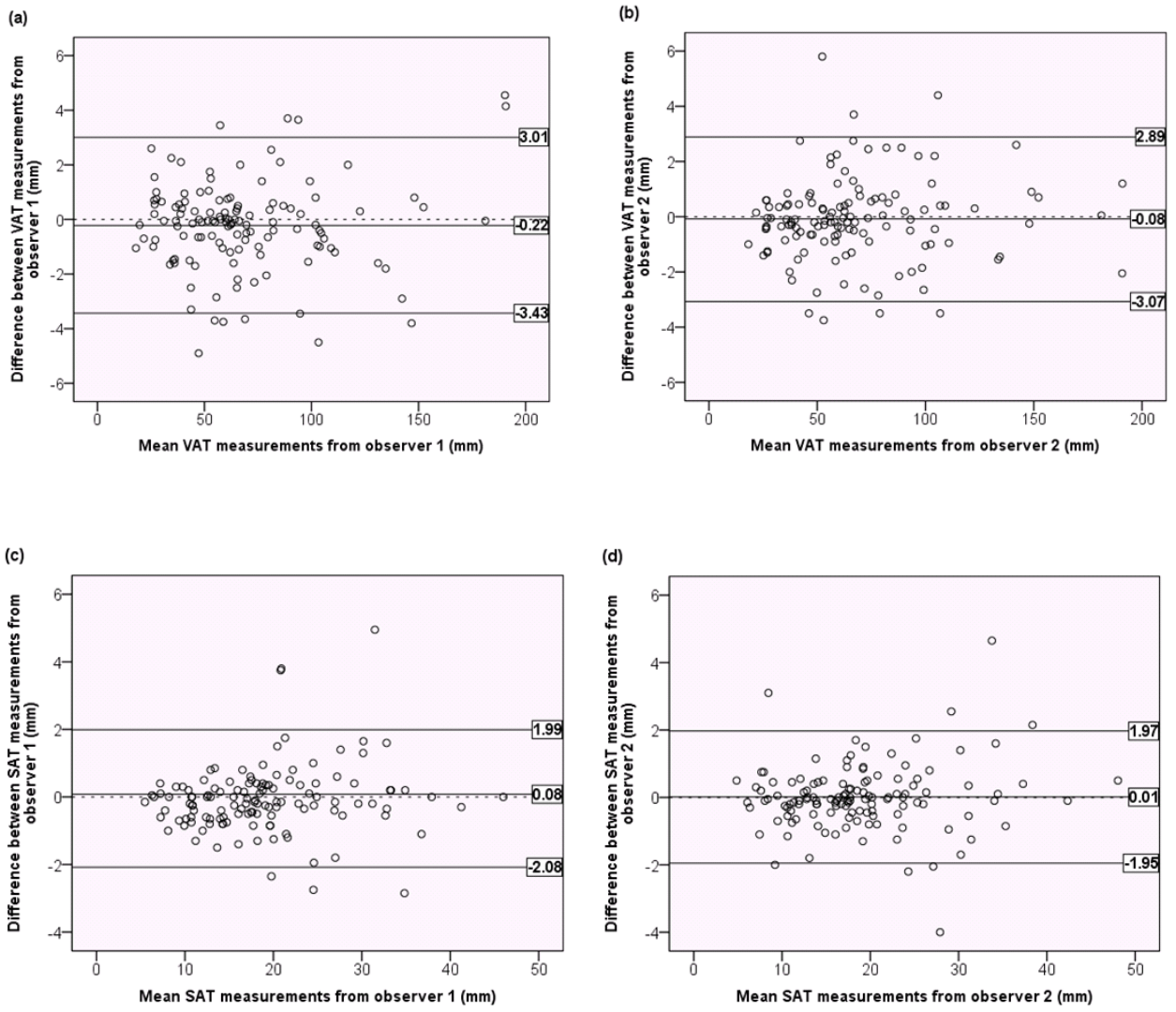
Supplementary Table 19 continued: Significant[#] results from regression analyses to detect relations of WC to urinary bins.

Anthropometric variable	bin (ppm)	Model 1 ß	Model 1 p	Model 2 ß	Model 2 p
WC	2.755	-0.008	0.017	-0.009	0.010
	2.775	-0.008	0.014	-0.008	0.012
	2.835	-0.011	0.002	-0.011	0.002
	2.925	-0.008	0.075	-0.010	0.044
	2.935	0.008	0.082	0.012	0.014
	2.965	-0.010	0.021	-0.011	0.018
	2.975	-0.009	0.025	-0.010	0.021
	2.985	-0.009	0.027	-0.010	0.015
	2.995	-0.009	0.012	-0.010	0.008
	3.055	0.017	0.111	0.025	0.028
	3.125	-0.010	0.026	-0.011	0.039
	3.145	-0.010	0.002	-0.011	0.003
	3.225	-0.014	0.021	-0.014	0.030
	3.265	-0.022	0.006	-0.027	0.002
	3.345	-0.019	0.002	-0.016	0.015
	3.355	-0.012	0.014	-0.011	0.040
	3.365	-0.022	0.002	-0.023	0.002
	3.415	-0.010	0.021	-0.011	0.020
	3.565	-0.021	0.002	-0.021	0.003
	3.685	-0.009	0.049	-0.011	0.027
	3.795	-0.009	0.061	-0.010	0.045
	4.055	-0.026	0.031	-0.032	0.015
	4.095	-0.012	0.009	-0.011	0.010
	4.245	-0.008	0.016	-0.008	0.028
	4.305	-0.006	0.039	-0.005	0.044
	4.375	-0.007	0.078	-0.008	0.046
	4.425	-0.009	0.023	-0.009	0.027
	6.585	-0.019	0.002	-0.020	0.002
	6.685	-0.014	0.009	-0.016	0.005
	6.805	-0.015	0.023	-0.016	0.020
	7.075	-0.022	0.012	-0.022	0.021

Supplementary Table 19 continued: Significant[#] results from regression analyses to detect relations of WC to urinary bins.

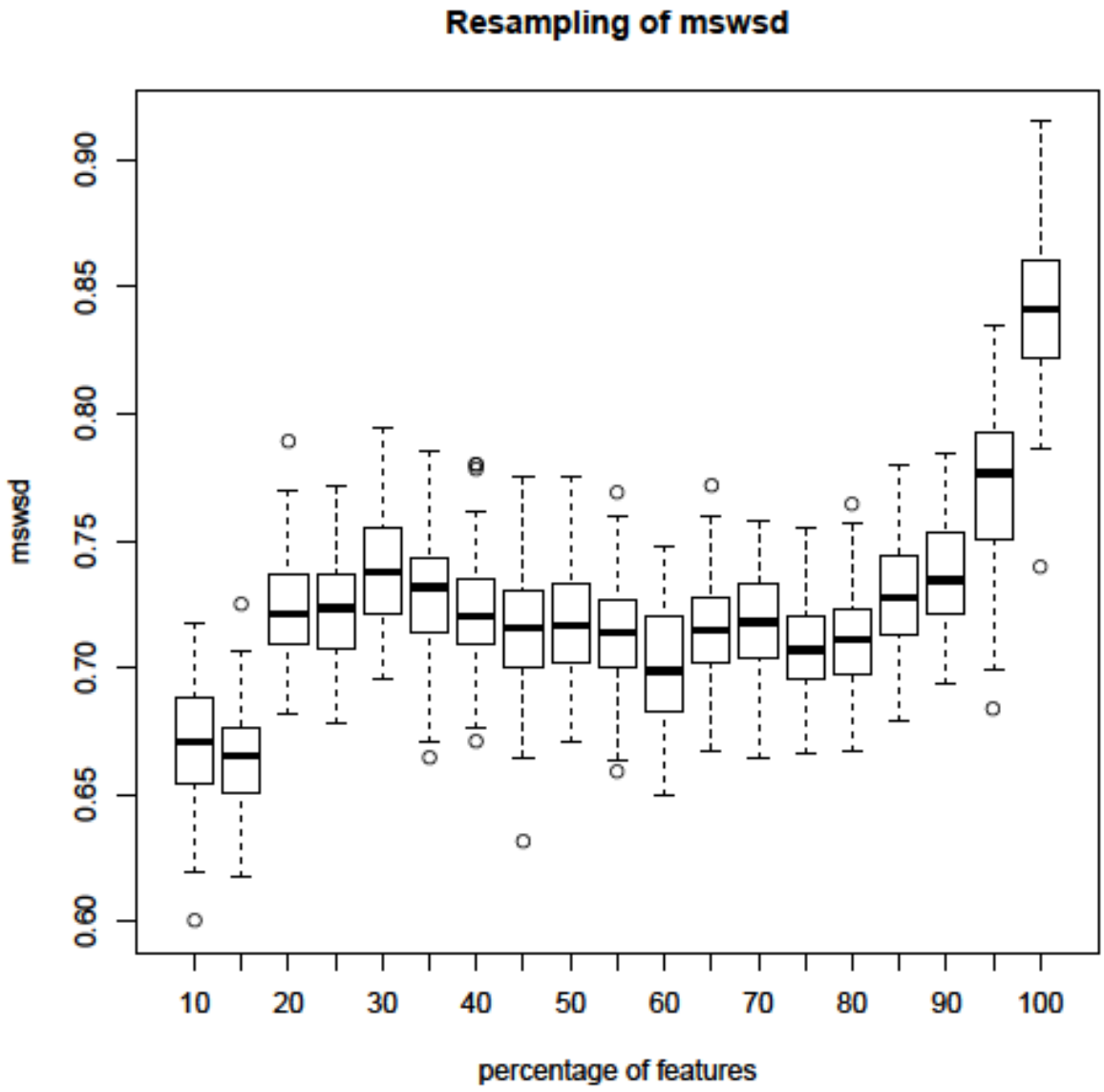
Anthropometric variable	bin (ppm)	Model 1 ß	Model 1 p	Model 2 ß	Model 2 p
WC	7.085	-0.027	0.010	-0.029	0.012
	7.675	-0.007	0.011	-0.007	0.015
	8.455	-0.026	0.002	-0.029	0.002
	8.685	-0.014	0.047	-0.016	0.040
	9.075	-0.033	0.028	-0.035	0.047
	9.135	-0.036	0.028	-0.038	0.043
	9.285	-0.022	0.015	-0.025	0.011
	9.385	-0.023	0.119	-0.029	0.047
Subjects with no urinary glucose					
	2.365	-0.011	0.024	-0.014	0.023
	2.555	-0.024	0.048	-0.036	0.043
	3.265	-0.025	0.005	-0.043	0.004
	3.365	-0.024	0.005	-0.028	0.012
	6.685	-0.014	0.019	-0.016	0.047
	7.985	0.009	0.194	0.022	0.013
	8.455	-0.025	0.008	-0.028	0.047
	8.685	-0.013	0.095	-0.023	0.013
	9.135	-0.035	0.049	-0.054	0.019
	9.165	-0.031	0.320	-0.095	0.031
	2.365	-0.011	0.024	-0.014	0.023
	2.555	-0.024	0.048	-0.036	0.043

Model 1: linear regression model adjusted for study, age (non-linear) and sex.
Model 2: linear regression model adjusted for study, age and sex interaction (non-linear), eGFR, smoking status, menopausal status (women only), fasting status, urinary glucose, and physical activity. In each case, the stratification variable was excluded from the model. WC=waist circumference, bin=spectra position (ppm), ß=beta-coefficient p=corrected p-value.
[#]p-values<0.05 after correction for multiple testing are considered significant.

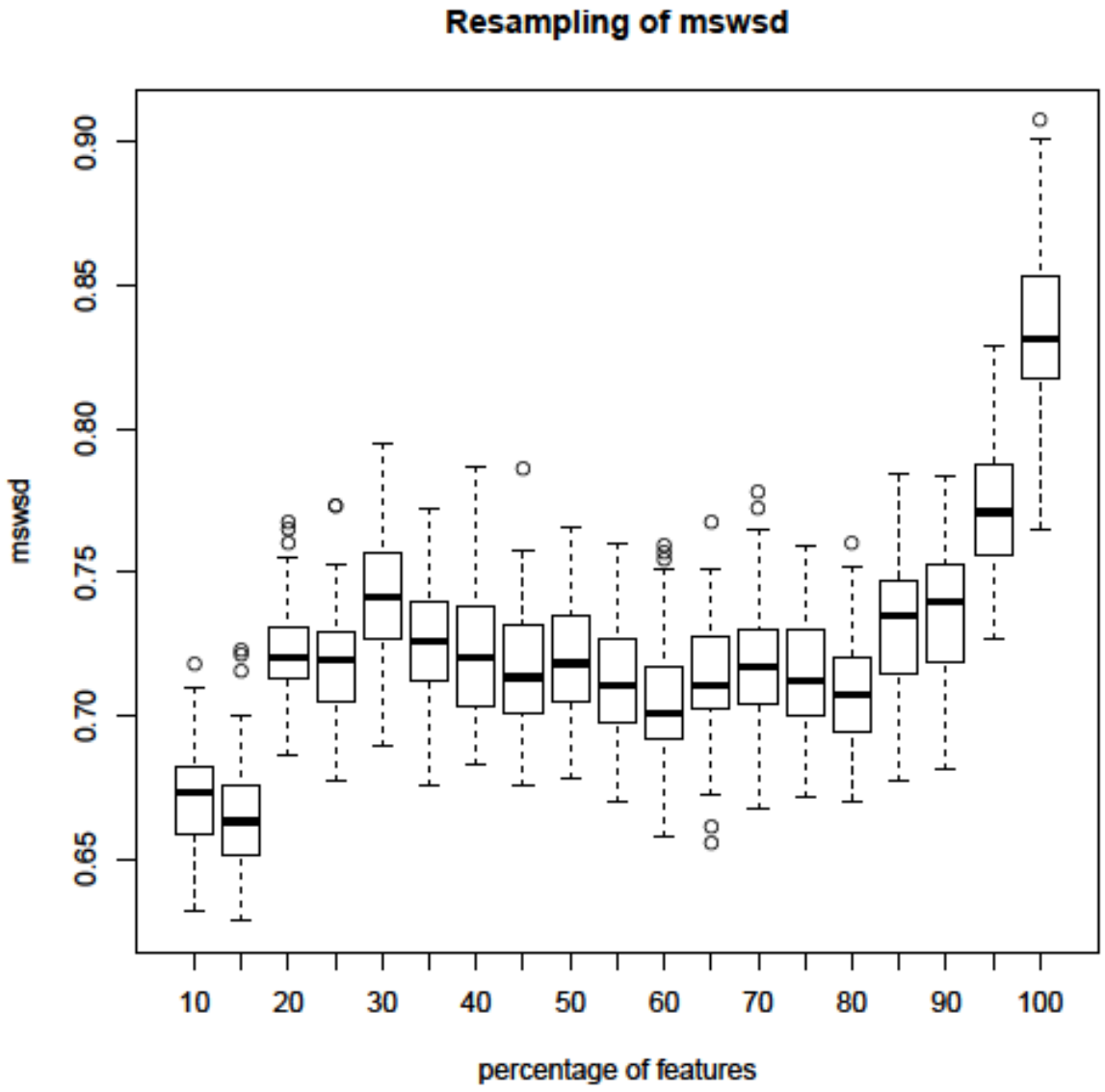


Supplementary Figure 1: Bland-Altman Plots for the intra-observer agreement between VAT respectively SAT measurements.

(a) Agreement between VAT measurements from observer 1; (b) Agreement between VAT measurements from observer 2;
(c) Agreement between SAT measurements from observer 1; (d) Agreement between SAT measurements from observer 2; n=127.



Supplementary Figure 2: Distribution of urine spectral area.
Mean standard deviation of the individual scaling factors of the selected reference features (mswsd).



Supplementary Figure 3: Distribution of serum spectral area.
Mean standard deviation of the individual scaling factors of the selected reference features (mswsd).

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Lebenslauf

Inga Schlecht

Geb. am 27.02.1983

In Herdecke (Ruhr)

Ledig

Berufstätigkeit:

Seit 03/2011	Wissenschaftliche Mitarbeiterin am Institut für Epidemiologie und Präventivmedizin der Universität Regensburg
08/2010-02/2011	Wissenschaftliche Mitarbeiterin am Institut für Ernährungsverhalten am Max Rubner-Institut (Bundesforschungsinstitut für Ernährung und Lebensmittel), Karlsruhe
08/2009-04/2010	Wissenschaftliche Mitarbeiterin am Fachbereich Oecotrophologie der Hochschule Fulda

Akademische Ausbildung:

Seit 10/2012	Postgraduales, berufsbegleitendes Studium zum European Master of Science in Epidemiology an der Johannes Gutenberg-Universität Mainz
Seit 07/2011	Promotion zum Dr. rer. physiol. (Biomedizin) an der medizinischen Fakultät der Universität Regensburg
10/2007-10/2009	Master of Science in Public Health Nutrition an der Hochschule Fulda
09/2005-06/2007	Bachelor of Science in Nutrition and Food Science mit Nebenfach Economics an der University of Rhode Island, USA
10/2003-08/2005	Studium der Ernährungswissenschaften (Oecotrophologie) an der Justus-Liebig Universität Gießen

Stipendien und Auszeichnungen:

09/2015	Reisestipendium der Deutschen Gesellschaft für Epidemiologie 2015
04/2015	Finalist of the 2015 Metabolism Award for Junior Investigators
04/2009-07/2009	Erasmus Placements Scholarship (student mobility for placements in Europe)
09/2006-06/2007	Deans list, University of Rhode Island, USA
09/2005-06/2007	Athletic Scholarship, University of Rhode Island, USA

Regensburg, Dezember 2015

Unterschrift

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Ich, Inga Schlecht, geboren am 27. Februar 1983 in Herdecke (Ruhr), erkläre hiermit, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe der Quelle gekennzeichnet. Insbesondere habe ich nicht die entgeltliche Hilfe von Vermittlungs- bzw. Beratungsdiensten (Promotionsberater oder andere Personen) in Anspruch genommen. Die Arbeit wurde bisher weder im In- noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

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Relevante Publikationen (Originalarbeiten):

1) **Schlecht, I** / Wiggermann, P / Behrens, G / Fischer, B / Koch, M / Freese, J / Rubin, D / Nöthlings, U / Stroszczynski, C / Leitzmann, MF: Reproducibility and validity of ultrasound for the measurement of visceral and subcutaneous adipose tissues, Metabolism (2014), http://dx.doi.org/10.1016/j.metabol.2014.07.012.

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