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Research Article

Prediction of Circulating Adipokine Levels Based on Body Fat Compartments and Adipose Tissue Gene Expression

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Abstract
Background: Adipokines are hormones secreted from adipose tissue (AT), and a number of them have been established as risk factors for chronic diseases. However, it is not clear whether and to what extent adiposity, gene expression, and other factors determine their circulating levels. ***Objectives:*** To assess to what extent adiposity, as measured by the amount of subcutaneous AT (SAT) and visceral AT (VAT) using magnetic resonance imaging, and gene expression levels in SAT determine plasma concentrations of the adipokines adiponectin, leptin, soluble leptin receptor, resistin, interleukin 6, and fatty acid-binding protein 4 (FABP4). ***Methods:*** We performed a cross-sectional analysis of 156 participants from the EPIC Potsdam cohort study and analyzed multiple regression models and partial correlation coefficients. ***Results:*** For leptin and FABP4 concentrations, 81 and 45% variance were explained by SAT mass, VAT mass, and gene expression in SAT in multivariable regression models. For the remaining adipokines, AT mass and gene expression explained <16% variance of plasma concentrations. Gene expres-

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sion in SAT was a less important predictor compared to AT mass. SAT mass was a better predictor than VAT mass for leptin (partial correlation r = 0.81, 95% confidence interval 0.75–0.86, vs. r = 0.58, 95% confidence interval 0.46–0.67), while differences between AT compartments were small for the other adipokines. ***Conclusions:*** While plasma levels of leptin and FABP4 can be explained in a large and medium part by the amount of AT and SAT gene expression, surprisingly, these predictors explained only little variance for all other investigated adipokines.

Introduction

Obesity is an established risk factor for a number of chronic diseases. It is conceptually defined as a condition of abnormal or excessive body fat accumulation to the extent that health may be impaired [1]. In line with this, investigations into the underlying molecular processes have identified circulating adipose tissue (AT)-derived biomarkers, so-called adipokines, that are – among various pathways – involved in glucose and lipid metabolism, inflammation, insulin signaling, and energy homeostasis [2–4]. A number of these adipokines have also been associated with risk of cardiovascular disease, diabetes, hypertension, and cancer, and have been proposed as mediators for the association of obesity with chronic disease risk [3, 5–8]. Prominent examples are leptin [5, 9], the soluble leptin receptor (sOB-R) [5, 10], adiponectin with its different circulating molecular forms [4, 6], interleukin 6 (IL-6) [4, 11, 12], fatty acid-binding protein 4 (FABP4) [11], and resistin [13, 14], which are the focus of this study. Yet, the variance of their plasma levels explained by body mass index (BMI) or by waist circumference (WC) – which are currently used to classify obesity or abdominal obesity, respectively – was found to be relatively modest: around 36% for leptin and only up to 10% for most of the other adipokines [4, 5, 8]. One reason for this observation may be that BMI and WC are only crude measures of the amount of subcutaneous AT (SAT) and visceral AT (VAT), which are the major compartments of human AT [15] and purportedly the major source of most adipokines. SAT and VAT have established anatomical, cellular, molecular, physiological, metabolic, and endocrinological differences [15–17].

Magnetic resonance imaging (MRI) allows direct quantification of total AT (TAT), SAT, and VAT [18], but there is inconsistent empirical evidence as to what extent imaging-based assessment of VAT, SAT, or TAT determines adipokine levels [19–21]. Also, the gene expression in these tissues has often not been taken into account as a further predictor of plasma levels of these adipokines. Only few studies have reported on the association of imaging-based AT mass with gene expression and plasma or serum concentrations [22–37], and they have mostly investigated small study samples and focused on specific patient groups or the effect of interventions and the analysis of one or two candidate adipokines.

The aim of our study was therefore to investigate to which extent plasma levels of the above six adipokines are determined by the amount of SAT and VAT assessed by MRI and by gene expression measured in SAT. Our hypothesis was that direct quantification of SAT and VAT as well as gene expression in AT can explain a substantial amount of the variance in adipokine levels. In secondary analyses, we investigated additional predictors and compared the role of body fat compartments and gene expression in more detail.

Materials and Methods

Study Population

This study was conducted in a subcohort of EPIC Potsdam within the large European Prospective Investigation into Cancer and Nutrition (EPIC) study [38, 39]. EPIC Potsdam is

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an ongoing cohort study among 27,548 persons aged 35–65 years at recruitment between 1994 and 1998 from the general population of the city of Potsdam and the surrounding area in Germany. From 2010 to 2013, a random sample of 1,472 participants was reinvited to the study center. Inclusion criteria were the absence of severe disease within the previous year (e.g., cancer, myocardial infarction, stroke), not being a wheelchair user, and not having had surgery within 3 months prior to the examination. A total of 815 participants attended the reexamination and completed a detailed study protocol including the collection of blood samples and assessment of lifestyle factors, regular medication use, blood pressure, and standard anthropometric measures according to standardized protocols following the WHO guidelines [40] (online suppl. text S1; for all online suppl. material, see www.karger.com/doi/10.1159/000502117). MRI scans were obtained to assess body compartments from 594 participants on a separate visit (online suppl. text S2), scheduled within a maximum interval of 4 weeks from the initial visit (average difference 25 days). SAT biopsies were taken from 278 participants using a needle aspiration method, with sufficient material extracted from 200 participants (online suppl. text S3). For 156 of the 200 participants, MRI measurements and plasma samples are available, and they therefore constituted the sample for this study. In comparison to the full EPIC Potsdam cohort, these 156 probands were very similar regarding age and sex distribution, disease prevalence, and anthropometric measures (data not shown). Sex was set to equal the assessed gender.

Assessment of AT Mass

Body fat measures were obtained from the whole-body MRI scans using a fully automated segmentation approach, which provided very similar estimates compared to a manual expert segmentation and had high repeatability and reproducibility of the measurements (coefficient of variation = 0.4% for SAT and TAT and 3.5% for VAT [41, 42]; online suppl. text S2). Available measures include the amount of AT in the visceral compartment (in the abdominal cavity, i.e., around and between the organs in the abdomen), the subcutaneous compartment (fat tissue beneath the skin), and the coronary compartment (fat tissue around the heart and heart vessels in the thorax; coronary AT [CAT]) (see online suppl. text S2 for more details). TAT was calculated as the sum of VAT, SAT, and CAT. Additionally, the amount of skeletal muscle tissue (SMT) and total body volume were assessed. The AT/height [43], AT/height^2 (= fat mass index [44]), and AT/height^3 [43] ratios were computed for VAT, SAT, CAT, and TAT as measures of AT mass standardized by height, and AT/SMT was investigated to address the capacity-load model [45] to set AT mass in relation to SMT.

Assessment of Biomarker Plasma Levels and SAT Gene Expression

EDTA plasma levels of total, high molecular weight (HMW), and HMW + medium molecular weight (MMW) adiponectin were measured using ELISAs from ALPCO (Salem, NH, USA), and concentrations of MMW and low molecular weight (LMW) adiponectin computed by subtraction. Leptin, sOB-R, resistin, and FABP4 were measured using ELISAs from BioVendor (Brno, Czech Republic), and IL-6 was measured using an ELISA from R&D Systems (Minneapolis, MN, USA). All samples were measured in duplicate according to the standard protocol on the TECAN Infinite 200 PRO reader (Männedorf, Switzerland) and had small inter- and intra-assay coefficients of variation (online suppl. text S4).

Quantitative real-time polymerase chain reaction (PCR) was performed using the Applied Biosystems 7500 Fast Real-time PCR system with TaqMan technology (ABI, Darmstadt, Germany) to evaluate the gene expression in SAT of the target genes adiponectin, leptin, sOB-R, resistin, IL-6, and FABP4 (online suppl. text S5). For each sample and for each gene,

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gene expression was measured in triplicate and the three Ct values were averaged for each individual. As measure for gene expression in all analyses $2^{-\Delta Ct}$ values were used, assuming that the number of amplified target molecules at the threshold cycle is identical for the candidate genes and the measured housekeeper gene 18S [46]. All experiments had small inter- and intra-assay coefficients of variation.

Statistical Analysis

Statistical analyses were performed using R version 3.3.1 [47]. All gene expression, plasma concentration, and MRI-based measures of AT mass (including the AT/height, AT/height^2, AT/height^3, and AT/SMT ratios) were log-transformed to yield normally distributed measures for the analysis. All analyses are based on the sample of 156 participants, with the exception of analyses that include leptin, FABP4, and LMW adiponectin concentrations, which each have one missing value.

For our main aim, we fitted multiple linear regression models and calculated the adjusted R^2 to assess how much of the variance of plasma concentrations can be explained by AT compartments and gene expression. For each adipokine, these models included the following predictors in different combinations: SAT gene expression (of the respective gene), SAT mass, VAT mass, and the interaction of SAT mass with SAT gene expression (of the respective gene). The interaction can be interpreted as the amount of tissue (i.e., number and size of cells) multiplied by the cell-based measure of transcriptomic activity. The adjusted R^2 of each regression model is the total variance explained by all predictors in the model, adjusted for the number of included predictors.

We were also interested whether other predictors further increase the explained variance in adipokine concentrations. Men and women differ in their amount of AT, hence sex is expected to contribute to the variance in SAT and VAT mass. Therefore, we examined the amount of variance of adipokine concentrations explained by sex, as a single predictor as well as together with SAT gene expression of the corresponding gene, SAT mass, and VAT mass. In additional analyses we added the following predictors in different combinations to the regression model: age, occupational training, physical activity, employment status, partner status, smoking status, socioeconomic status, history of diabetes, BMI, waist-hip ratio, CAT, TAT, and gene expression and plasma concentrations of all other adipokines considered in our study (see Tables 2 and 3 for a detailed description of the regression models).

Although these models may not allow a valid separation of the effects of the different predictors, they provide a helpful quantification of the overall explained variance by body compartments, gene expression, and other predictors since any adjusted analysis also removes part of the variance of adipokine levels or AT compartments. In addition to these variance analyses, we also examined partial Pearson correlation coefficients of the plasma concentrations with AT measures and gene expression, adjusted for sex, age, physical activity, and occupational training. These covariates were chosen based on conceptual reasoning that they might act as confounders in the correlation analyses. For example, AT mass varies between the sexes, AT mass and adipokine levels change over the life course and by physical activity, and occupational training seemed to be the best proxy for socioeconomic status in our analyses, which has been shown to be associated with obesity.

For sensitivity analyses, we performed sex-stratified analyses, repeated the main analyses using an estimate of the absolute quantity of adipokine molecules in plasma (instead of plasma concentrations), and also computed analyses based on AT/height, AT/height^2, AT/height^3, as well as AT/SMT, which all yielded almost identical results (online suppl. text S6).

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Table 1. Sex-stratified characteristics of the study population

Measures	Women	Men	*p* value
Sample size	86	70	–
Age, years	62.7 (8.4)	66.8 (8.3)	2.8×10^{-3}
Smoking[4]			2.3×10^{-3}
Never	54.7%	28.6%	
Former	31.4%	57.1%	
Current	14.0%	14.3%	
CPAI[4]			0.99
Inactive	8.1%	8.6%	
Moderately inactive	26.7%	28.6%	
Moderately active	34.9%	34.3%	
Active	30.2%	28.6%	
Occupational training[4]			8.8×10^{-3}
No vocational training/vocational training	40.7%	32.9%	
Technical college	25.6%	11.4%	
University	32.6%	55.7%	
Systolic blood pressure, mm Hg	132.6 (15.2)	137.5 (16.2)	0.05
Diastolic blood pressure, mm Hg	79.5 (9.7)	81.9 (10.1)	0.15
Myocardial infarction[5]	0%	1.4%	0.45
Stroke	0%	0%	–
Heart failure	0%	0%	–
Diabetes[5]	1.2%	10.0%	1.6×10^{-2}
Medication use			
Blood pressure-lowering drugs[4]	38.4%	47.1%	0.35
Cholesterol-/triglyceride-lowering drugs[4]	17.4%	21.4%	0.67
Anti-inflammatory drugs[5]	8.1%	7.1%	1
Antidiabetic drugs[5]	2.3%	10.0%	0.08
Standard anthropometric measures			
Height, cm	161.9 (5.5)	175.0 (6.1)	2.3×10^{-28}
Weight, kg	72.9 (11.4)	85.5 (11.1)	9.5×10^{-11}
Body mass index, kg/m^2	27.8 (4.3)	28.0 (3.9)	0.81
Waist circumference, cm	91.6 (10.3)	102.3 (10.0)	7.6×10^{-10}
Hip circumference, cm	106.2 (9.6)	102.3 (6.4)	3.4×10^{-3}
Waist-hip ratio	0.9 (0.1)	1.0 (0.1)	4.8×10^{-30}
MRI AT measures[1, 3]			
VAT, kg	2.9 (1.1)	5.2 (1.6)	9.3×10^{-14}
CAT, kg	0.3 (0.1)	0.4 (0.2)	3.2×10^{-13}
SAT, kg	20.1 (5.1)	14.7 (4.3)	1.2×10^{-12}
TAT, kg	23.7 (5.4)	20.7 (5.8)	1.0×10^{-4}
SMT, kg	17.0 (1.6)	24.6 (3.0)	2.5×10^{-42}
Plasma concentration[3]			
Leptin, ng/mL	45.2 (23.9)	18.8 (12.6)	1.1×10^{-13}
sOB-R, ng/mL	20.1 (8.2)	21.9 (7.2)	0.30
Resistin, ng/mL	4.0 (1.2)	4.2 (1.3)	0.10
FABP4, ng/mL	32.5 (11.7)	21.2 (7.5)	1.8×10^{-9}
IL-6, pg/mL	1.7 (0.8)	1.8 (0.8)	0.13
Total adiponectin, µg/mL	8.3 (3.6)	5.7 (2.0)	2.5×10^{-5}
HMW adiponectin, µg/mL	4.7 (2.2)	2.7 (1.5)	3.2×10^{-6}
MMW adiponectin, µg/mL	1.5 (0.9)	1.2 (0.6)	1.6×10^{-2}
LMW adiponectin, µg/mL	1.9 (1.0)	1.6 (0.5)	2.0×10^{-2}
SAT gene expression[2, 3]			
Leptin	1×10^{-3} (4×10^{-4})	7×10^{-4} (2×10^{-4})	3.7×10^{-17}
sOB-R	3×10^{-5} (1×10^{-5})	4×10^{-5} (1×10^{-5})	0.54
Resistin	2×10^{-7} (2×10^{-7})	7×10^{-8} (7×10^{-8})	0.06
FABP4	3×10^{-2} (6×10^{-3})	3×10^{-2} (7×10^{-3})	0.13
IL-6	5×10^{-6} (3×10^{-6})	4×10^{-6} (2×10^{-6})	0.14
Adiponectin	3×10^{-3} (9×10^{-4})	2×10^{-3} (9×10^{-4})	4.5×10^{-3}

Results

Description of the Participants' Characteristics

The group of participants consisted of slightly more women (55%) than men, had an average age of 64.5 years (SD = 8.6 years), a mean BMI of 27.9 (SD = 4.1), and a low prevalence of cardiovascular and cardiometabolic diseases (Table 1) despite elevated blood pressure (mean systolic blood pressure of 134.8 mm Hg [SD = 15.8 mm Hg] and mean diastolic blood pressure of 80.6 mm Hg [SD = 9.9 mm Hg]). The use of medication corresponded to these statistics: a high percentage of participants took blood pressure-reducing medication, and only a small percentage took anti-inflammatory or antidiabetic drugs. All AT measures, plasma levels of leptin, FABP4 and adiponectin, and gene expression of leptin and adiponectin differed among sex-specific subgroups (Table 1). For example, SAT was on average 20.1 kg (SD = 5.1 kg) for women and 14.7 kg (SD = 4.3 kg) for men, and median leptin plasma levels were 45.2 μg/mL (median absolute deviation = 23.9 μg/mL) in women and 18.8 μg/mL (median absolute deviation = 12.6 μg/mL) in men.

Variance of Adipokine Concentrations Explained by Body Fat Compartments and SAT Gene Expression

For the primary aim, we investigated how much of the variance in the biomarker plasma concentrations could be explained overall by VAT mass, SAT mass, SAT gene expression, and their interaction for the respective gene (Table 2). SAT gene expression of the respective adipokine (model 1) explained 48% of the variance in leptin, but <10% of the variance in the remaining adipokines. SAT mass (model 2) explained 76% of leptin concentrations, 42% of FABP4, 13% of sOB-R, and close to 0% for the remaining adipokines. VAT mass (model 3) explained 15% for total and HMW adiponectin, 12% for IL-6, 10% for MMW adiponectin, and <10% for the remaining adipokines.

SAT mass together with SAT gene expression (model 4) explained 81% for leptin, 45% for FABP4, 12% for sOB-R, and close to 0% for all other adipokines. The interaction between SAT mass and SAT gene expression of the respective gene (model 5) accounted for 30% of leptin, 22% of FABP4, and 10% of sOB-R levels, but it did not explain any variance on top of the main effects of SAT gene expression and SAT mass (i.e., in model 6 compared to model 4).

Overall, when SAT gene expression, SAT mass, and VAT mass were considered in combination (model 7), a substantial proportion of the plasma concentration was explained for leptin (adjusted R^2 = 81%) and, albeit to a somewhat smaller extent, for FABP4 (45%). In contrast, the variance explained by these predictors was low for the remaining adipokines (between 10 and 16% for sOB-R, IL-6, total, HMW, and MMW adiponectin, and close to 0% for resistin and LMW adiponectin).

Table 1 (Footnote)

Values are relative frequencies, mean and SD, or [3] median and median absolute deviation. AT, adipose tissue; CAT, coronary adipose tissue; CPAI, Cambridge Physical Activity Index; FABP4, fatty acid-binding protein 4; HMW, high molecular weight; IL-6, interleukin 6; LMW, low molecular weight; MMW, medium molecular weight; MRI, magnetic resonance imaging; SAT, subcutaneous adipose tissue; SMT, skeletal muscle tissue; sOB-R, soluble leptin receptor; TAT, total adipose tissue; VAT, visceral adipose tissue. [1] MRI measures do not include arms and head. [2] Gene expression is shown relative to the housekeeping gene expression in the unit $2^{-\Delta Ct}$, with higher values representing higher expression levels. *p* values from hypothesis tests of the null hypothesis that there is no difference between sexes are computed from two-sample *t* tests of the untransformed variables or of the [3] log-transformed variables, from [4] Pearson's asymptotic χ^2 tests, or [5] Fisher's exact tests.

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Table 2. Variance (adjusted R^2) of the plasma adipokine concentrations explained by body fat compartments and SAT gene expression

Model	Predictors	Leptin	sOB-R	Resistin	FABP4	Total adiponectin	HMW adiponectin	MMW adiponectin	LMW adiponectin	IL-6
1	GE_{same}	0.48	0	0	0	0.04	0.03	0.03	0	0.02
2	SAT	0.76	0.13	0.01	0.42	0	0	0	0	0.01
3	VAT	0	0.06	0	0	0.15	0.15	0.10	0.02	0.12
4	GE_{same}, SAT	0.81	0.12	0.01	0.45	0.03	0.03	0.03	0	0.02
5	GE_{same} × SAT	0.30	0.10	0.02	0.22	0	0	0	0	0
6	GE_{same}, SAT, GE_{same} × SAT	0.81	0.12	0.01	0.45	0.03	0.02	0.05	0	0.04
7	GE_{same}, SAT, VAT	0.81	0.16	0.01	0.45	0.15	0.14	0.10	0.02	0.12

Explained variance (adjusted R^2) of the plasma adipokine concentrations from separate linear regression models for each adipokine and each model, with plasma adipokine concentrations as outcome and the above variables as predictors. Plasma adipokine concentrations, SAT gene expression, VAT, and SAT were log-transformed for analysis. FABP4, fatty acid-binding protein 4; GE_{same}, gene expression of the same gene as the respective predicted adipokine; HMW, high molecular weight; IL-6, interleukin 6; LMW, low molecular weight; MMW, medium molecular weight; SAT, subcutaneous adipose tissue; sOB-R, soluble leptin receptor; VAT, visceral adipose tissue.

For a description of the results from sex-stratified analyses, see online supplementary Table S1 and online supplementary text S6.

Variance of Adipokine Concentrations Explained by Additional Personal and Environmental Determinants

Next, we examined additional determinants (Table 3). Sex alone (model 8) explained 32% of the variance for leptin levels, 10–20% for FABP4, total, and HMW adiponectin levels, and close to 0% for the remaining adipokines. When sex was added to gene expression, SAT, and VAT (model 9), it did not or only slightly increase the explained variance in plasma levels (compared to model 7), suggesting that differences between sexes were accounted for by differences in AT mass and gene expression (Table 1).

Regarding the contribution of other factors, the largest increases in the explained variance in adipokine levels in comparison to model 9 were observed as follows: For sOB-R, resistin, FABP4, and HMW adiponectin, the plasma concentration of other adipokines (model 13) increased the explained variance from 16 to 24%, from 1 to 17%, from 49 to 54%, and from 18 to 24%, respectively. For MMW adiponectin and IL-6, the gene expression of other adipokines (model 12) increased the explained variance from 10 to 23% and from 12 to 21%, respectively. For leptin, total adiponectin, and LMW adiponectin, <5% additional variance was explained.

Partial Correlations of Adipokine Concentrations with Body Fat Compartments and SAT Gene Expression

Next, we investigated partial correlations between the different body fat compartments and gene expression with adipokine concentrations (Tables 4, 5), and provide here a descriptive comparison of these effect sizes.

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Table 3. Variance (adjusted R^2) of the plasma adipokine concentrations explained by additional personal and environmental determinants

Model	Predictors	Leptin	sOB-R	Resistin	FABP4	Total adiponectin	HMW adiponectin	MMW adiponectin	LMW adiponectin	IL-6
8	sex	0.32	0	0.01	0.21	0.10	0.13	0.03	0.03	0.01
9	GE_{same}, SAT, VAT, sex	0.82	0.16	0.01	0.49	0.16	0.18	0.10	0.02	0.12
10	GE_{same}, SAT, VAT, sex, age, occupational training, physical activity	0.81	0.16	0.06	0.49	0.15	0.18	0.16	0.02	0.20
11	GE_{same}, SAT, VAT, sex, age, occupational training, physical activity, BMI, WHR, CAT, TAT	0.82	0.16	0.04	0.50	0.16	0.17	0.14	0.01	0.20
12	GE_{same}, SAT, VAT, sex, age, occupational training, physical activity, GE_{other_1}, GE_{other_2}, GE_{other_3}, GE_{other_4}, GE_{other_5}	0.83	0.21	0.06	0.47	0.20	0.21	0.23	0.02	0.21
13	GE_{same}, SAT, VAT, sex, age, occupational training, physical activity, PL_{other_1}, PL_{other_2}, PL_{other_3}, PL_{other_4}, PL_{other_5}	0.82	0.24	0.17	0.54	0.18	0.24	0.17	0.03	0.20
14	GE_{same}, SAT, VAT, sex, age, occupational training, physical activity, employment status, partner status, smoking status, SES, diabetes status	0.82	0.15	0.03	0.46	0.17	0.23	0.13	0	0.19

Explained variance (adjusted R^2) of the plasma adipokine concentrations from separate linear regression models for each adipokine and each model, with plasma adipokine concentrations as outcome and the above variables as predictors. Plasma adipokine concentrations, SAT gene expression, VAT, and SAT were log-transformed for analysis. BMI, body mass index; CAT, coronary adipose tissue; FABP4, fatty acid-binding protein 4; $GE_{other\_1...5}$, gene expression of all five genes other than the predicted adipokine; GE_{same}, gene expression of the same gene as the respective predicted adipokine; HMW, high molecular weight; IL-6, interleukin 6; LMW, low molecular weight; MMW, medium molecular weight; $PL_{other\_1...5}$, circulating concentrations of all 5 adipokines other than the predicted adipokine; SAT, subcutaneous adipose tissue; SES, socioeconomic status; sOB-R, soluble leptin receptor; TAT, total adipose tissue; VAT, visceral adipose tissue; WHR, waist-hip ratio.

Regarding a comparison of AT compartments (Table 4), for leptin and FABP4 plasma levels, the highest partial correlations were observed with TAT and SAT, which were larger compared to the correlations with VAT. For sOB-R, resistin, IL-6, total, HMW, and LMW adiponectin, the correlations of each adipokine with SAT, VAT, and TAT were of similar size. For MMW adiponectin, the correlation with VAT was slightly larger compared to SAT and TAT. CAT showed smaller or equal correlations compared to VAT, SAT, and TAT for all investigated adipokines.

Regarding SAT gene expression (Table 5), leptin gene expression was moderately correlated with leptin concentration. Correlations between SAT gene expression and plasma levels were weak for all other adipokines.

Regarding a comparison of AT mass and SAT gene expression, AT mass showed stronger or similar associations for all adipokines. Analyses stratifying by sex instead of adjusting for sex indicated some differences for some AT measures and some adipokines (e.g., partial correlation of VAT with circulating leptin was $r = 0.48$ in women and $r = 0.71$ in men); however, none of these sex differences were statistically significant (online suppl. Tables S2 and S3).

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Table 4. Partial Pearson correlation coefficients (adjusted for sex, age, physical activity, and occupational training) and 95% CIs between anthropometric/MRI-based measures and plasma adipokine concentrations

	Leptin		sOB-R		Resistin		FABP4		Total adiponectin		HMW adiponectin		MMW adiponectin		LMW adiponectin		IL-6	
	r	95% CI	*r*	95% CI	*r*	95% CI	*r*	95% CI	*r*	95% CI	*r*	95% CI	*r*	95% CI	*r*	95% CI	*r*	95% CI
Weight	0.62	(0.52; 0.71)	−0.32	(−0.46; −0.17)	−0.12	(−0.27; 0.04)	0.45	(0.32; 0.57)	−0.31	(−0.45; −0.16)	−0.23	(−0.38; −0.08)	−0.13	(−0.28; 0.03)	−0.07	(−0.23; 0.09)	0.28	(0.13; 0.42)
BMI	0.67	(0.57; 0.75)	−0.38	(−0.51; −0.24)	−0.08	(−0.23; 0.08)	0.54	(0.42; 0.64)	−0.32	(−0.45; −0.17)	−0.25	(−0.40; −0.10)	−0.09	(−0.25; 0.07)	−0.10	(−0.26; 0.06)	0.27	(0.12; 0.41)
WC	0.66	(0.56; 0.74)	−0.41	(−0.54; −0.27)	−0.06	(−0.21; 0.10)	0.51	(0.38; 0.62)	−0.29	(−0.43; −0.14)	−0.26	(−0.40; −0.10)	−0.15	(−0.30; 0.01)	−0.14	(−0.29; 0.03)	0.30	(0.15; 0.44)
HC	0.64	(0.54; 0.72)	−0.38	(−0.51; −0.24)	−0.03	(−0.18; 0.13)	0.44	(0.31; 0.56)	−0.22	(−0.36; −0.06)	−0.19	(−0.33; −0.03)	−0.06	(−0.22; 0.10)	−0.10	(−0.25; 0.06)	0.25	(0.10; 0.39)
WHR	0.32	(0.17; 0.45)	−0.22	(−0.37; −0.07)	−0.06	(−0.21; 0.10)	0.30	(0.15; 0.44)	−0.22	(−0.36; −0.06)	−0.18	(−0.33; −0.03)	−0.17	(−0.32; −0.01)	−0.11	(−0.27; 0.05)	0.20	(0.04; 0.34)
VAT	0.58	(0.46; 0.67)	−0.40	(−0.53; −0.26)	−0.06	(−0.22; 0.10)	0.45	(0.32; 0.57)	−0.32	(−0.45; −0.17)	−0.30	(−0.43; −0.15)	−0.32	(−0.46; −0.17)	−0.10	(−0.26; 0.06)	0.31	(0.16; 0.44)
CAT	0.51	(0.38; 0.61)	−0.30	(−0.44; −0.15)	−0.01	(−0.17; 0.15)	0.50	(0.37; 0.61)	−0.24	(−0.38; −0.09)	−0.30	(−0.43; −0.15)	−0.24	(−0.38; −0.08)	−0.06	(−0.22; 0.10)	0.33	(0.19; 0.47)
SAT	0.81	(0.75; 0.86)	−0.37	(−0.50; −0.23)	−0.04	(−0.20; 0.12)	0.57	(0.45; 0.67)	−0.23	(−0.37; −0.07)	−0.24	(−0.38; −0.08)	−0.08	(−0.23; 0.08)	−0.10	(−0.26; 0.06)	0.27	(0.12; 0.41)
TAT	0.82	(0.76; 0.86)	−0.40	(−0.52; −0.25)	−0.05	(−0.20; 0.11)	0.59	(0.48; 0.68)	−0.26	(−0.41; −0.11)	−0.27	(−0.41; −0.12)	−0.14	(−0.29; 0.02)	−0.12	(−0.27; 0.04)	0.31	(0.16; 0.44)

Plasma adipokine concentrations, VAT, CAT, SAT, and TAT were log-transformed for analysis. Correlations with $r > |0.27|$ correspond to Bonferroni-adjusted p values <0.05 adjusted for 81 comparisons. BMI, body mass index; CAT, coronary adipose tissue; CI, confidence interval; FABP4, fatty acid-binding protein 4; HC, hip circumference; HMW, high molecular weight; IL-6, interleukin 6; LMW, low molecular weight; MMW, medium molecular weight; MRI, magnetic resonance imaging; SAT, subcutaneous adipose tissue; sOB-R, soluble leptin receptor; TAT, total adipose tissue; VAT, visceral adipose tissue; WC, waist circumference; WHR, waist-hip ratio.

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Table 5. Partial Pearson correlation coefficients *r* (adjusted for sex, age, physical activity, and occupational training) and 95% CIs between plasma adipokine concentrations and SAT gene expressions

Biomarker	*r*	95% CI
Leptin	0.52	0.40; 0.63
sOB-R	–0.05	–0.20; 0.11
Resistin	0.08	–0.08; 0.24
FABP4	0.02	–0.13; 0.18
Total adiponectin	0.15	–0.01; 0.30
HMW adiponectin	0.13	–0.03; 0.28
MMW adiponectin	0.16	0; 0.31
LMW adiponectin	–0.03	–0.19; 0.13
IL-6	0.19	0.03; 0.34

Plasma adipokine concentrations and SAT gene expressions were log-transformed for analysis. Correlations $r > |0.19|$ correspond to Bonferroni-adjusted p values <0.05 adjusted for 9 comparisons. CI, confidence interval; FABP4, fatty acid-binding protein 4; HMW, high molecular weight; IL-6, interleukin 6; LMW, low molecular weight; MMW, medium molecular weight; SAT, subcutaneous adipose tissue; sOB-R, soluble leptin receptor.

In comparisons of MRI quantification with traditional anthropometric measures (Table 4), for leptin and MMW adiponectin, MRI AT measures showed stronger correlations with plasma levels compared to the traditional measures, whereas for all remaining adipokines, correlations with MRI measures were in a similar range as their correlations with traditional measures. Among the traditional surrogate measures for adiposity and fat distribution, correlations of plasma levels with BMI and with WC were similar for all markers.

For background information regarding the correlations between gene expression, plasma levels, and AT measures and for results of the sensitivity analyses (which did not change any of the conclusions), see online supplementary text S6 and online supplementary Tables S4–S11.

Discussion

This study investigated to what extent plasma adipokine concentrations are determined by MRI-based quantification of AT compartments and by gene expression in SAT. For leptin and FABP4 concentrations, a large and moderate part of their variance was explained, respectively. However, surprisingly little variance was explained for adiponectin, sOB-R, resistin, and IL-6. While MRI-based measures yielded an improvement compared to standard anthropometric measures for the prediction of circulating levels for leptin [19] and MMW adiponectin, they yielded similar effect estimates for all other adipokines [20, 48, 49]. Since our effect estimates for the traditional anthropometric measures and, where available, for estimates of total body fat are similar to reports in the literature [4–7, 19, 21, 48–53], these results do not support our hypothesis that a large part of the variance of adipokine levels can be explained by MRI-derived measures of body fat and SAT gene expression, except for leptin and FABP4. In general, AT mass was the main contributor to the explained variance and played a much larger role than SAT gene expression [54]. Regarding the AT compartments – even though their correlation has to be considered in the interpretation of results – SAT seems to play a larger role for leptin [19, 55] compared to VAT, and interestingly little or no differences were observed for all other adipokines [48]. CAT seems to play a lesser role compared to VAT and SAT in explaining the plasma levels of all investigated adipokines,

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which might be explained by the small amount of CAT in comparison to the other compartments. For an interpretation of these descriptive comparisons, we want to stress that in line with our study aims, we focused on the estimation of effect sizes and deliberately did not statistically test for differences of effects between AT compartments (and gene expression measures), which would need to consider the multiple testing of the different compartments and adipokines (e.g., see legends of Tables 4 and 5) and could be performed in future studies.

Regarding leptin, a large proportion of the variance (76%) of its circulating levels was explained by SAT. While gene expression explained 48% when considered alone [24, 32], it contributed only little additional information when added to SAT mass. These data confirm that leptin is primarily a biomarker for SAT mass [19, 55]. However, SAT mass was also weakly associated with leptin gene expression (online suppl. Table S7), so that the estimated effect of SAT on circulating leptin might also contain part of the effect of leptin gene expression. The association of VAT mass with leptin levels was much smaller [19, 55]. Some of the remaining unexplained variance of leptin levels might be explained by VAT gene expression, which was not assessed in this study. Regarding FABP4, which acts as a carrier for fatty acids and is primarily expressed in adipocytes and macrophages, close to 50% of its variance was explained, with a slightly higher contribution of SAT compared to VAT. Gene expression did not contribute at all to the explained variance. Thus, somewhat similar as for leptin, these data suggest that FABP4 is a biomarker for AT mass although more than half of the variance remained unexplained [52]. sOB-R is the main leptin-binding protein whose expression has been reported in multiple other tissues besides AT [10]. In our analysis, SAT and VAT only explained 16% variance of its plasma levels, while gene expression did not contribute at all. For adiponectin, which is predominantly synthesized and secreted from adipocytes in VAT and SAT [4, 27], VAT explained slightly more of its variance compared to SAT [20]. However, the explained variance was very low and only about 10–15% for total, HMW, and MMW adiponectin and 2% for LMW adiponectin [6, 48, 56, 57]. This is surprising, given that adiponectin is often considered "the" classical adipokine. For resistin, which is expressed at lower levels in adipocytes but at higher levels in circulating blood monocytes [7], AT and gene expression did not predict its circulating levels in our study [14, 21]. This raises the question as to whether resistin can truly be considered an adipokine in humans. Finally, regarding IL-6, which is secreted by multiple cell types including monocytes, macrophages, and adipocytes [4], our results suggest that adiposity explains only a small proportion of its circulating levels in humans [8, 51, 53], in contrary to the established notion that obesity is associated with low-grade subclinical inflammation [4]. Including further inflammatory cytokines such as TNF-α as well as information on inflammatory processes and AT cell type composition in follow-up studies could enable a more detailed investigation of the obesity-inflammation link and separation of the effects of AT compartments on the population level. We anticipate that the cell type composition of AT and other measures of inflammation might primarily act as a mediator of the obesity-adipokine association, so that our estimates provide an overall estimate of how much variance of IL-6 levels can be explained by AT, of which parts might be further explained by inflammatory processes.

It is not clear why so little variance of plasma levels was explained by AT and SAT gene expression in our study for adiponectin, sOB-R, resistin, and IL-6. Measurement error as an explanation appears unlikely since well-established assays were used, quality measures were good, and the correlations between adipokine levels and traditional anthropometric measures such as BMI and WC were similar to those reported in other studies [4–7, 19, 21, 48–53] for all adipokines. Furthermore, we observed a substantial correlation of AT measures with leptin. Also, while protein degradation, different half-lives of the investigated adipokines, and fasting status may affect adipokine concentrations, previous studies have shown a good reliability of plasma concentration estimates over time, robustness against fasting status, and we confirmed the robustness of adipokine concentrations and SAT gene expression against fasting status by

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sensitivity checks in our study (online suppl. texts S4 and S5). Also, the observed associations between AT mass and adipokine plasma concentrations as well as gene expression were not affected by differences in blood pressure, and the partial correlations in Tables 4 and 5 were very similar when further adjusting for systolic and diastolic blood pressure (results not shown). Similarly, even if there was some indication in further sensitivity analyses that some adipokine levels differ between probands taking drugs of different medication classes (e.g., higher IL-6 levels in participants taking anti-inflammatory drugs), the partial correlations in Tables 4 and 5 were very similar when stratifying or adjusting for medication use (results not shown). Further, the results were similar when crude estimates of absolute adipokine quantities in plasma were analyzed. Hence, there is no indication that the weak observed associations between AT mass and plasma concentrations were due to the fact that plasma concentrations are relative to blood volume (which depends on height and also AT mass). Since our results were almost identical with and without adjusting for height, height^2, height^3, or SMT, there is no indication that the AT changes qualitatively in its metabolic activity with increasing relative AT mass, in contrast to what could be hypothesized from the results of previous small-scale studies [58] and even though we observed an association of AT mass and gene expression for some adipokines. Finally, the additional predictors investigated in our study added only little explained variance, and also when considering complex interaction effects between all measures in further exploratory analyses (online suppl. text S7; online suppl. Table S12), no or only little additional variance of adipokine levels could be explained.

We speculate whether other biological factors, e.g., posttranscriptional modifications and regulatory elements, may account for a large part of the variance in plasma levels of adipokines other than leptin and FABP4. Also, some adipokines such as IL-6 and FABP4 are also expressed and secreted into plasma from other tissues [59, 60]. Furthermore, other lifestyle factors such as diet [24], clinical parameters, other circulating proteins [61], and genetic markers that do not affect mRNA abundance but circulating levels through other processes might be interesting for future studies. Finally, more detailed sex-stratified analyses could be interesting for follow-up. In this study, we focused on sex-adjusted analyses with the underlying reasoning that sex may affect AT mass, but AT is the primary tissue from where the adipokines are secreted so that sex has only an indirect association with adipokine levels.

Without the assessment of VAT gene expression and secretion rates from SAT and VAT [62], parts of the overall molecular picture remain unclear. However, assessing VAT in a population-based study is rarely possible, and VAT gene expression measured after bariatric or other surgeries might not allow for a valid approximation of the metabolic activity in the general population. Since adipokine secretion as well as their biological function can be regulated on the transcriptional or posttranscriptional level [59, 60], gene expression might not be the best proxy for the metabolic activity of AT and differ from the secretion rate. As further limitations, we investigated probands from the general German population with exclusion of severe disease. Hence, our results might not generalize to other populations or patients. Furthermore, our results can only provide hints as to how much variance of other adipokines not investigated here can be explained by AT and gene expression. Since MRI protocols but not magnetic resonance spectroscopy were performed, further body compartments such as ectopic fat [63] could not be investigated. Finally, another limitation of our study is that obesity measures were only assessed cross-sectionally and longitudinal information such as recent weight changes could not be considered.

In conclusion, our study shows that while for leptin, most of the variance in plasma concentrations can be explained by AT mass (particularly SAT mass) and SAT gene expression, this is less so for FABP4. In contrast and counterintuitively, most of the variance in the plasma concentrations of the so-called adipokines sOB-R, resistin, adiponectin, and IL-6 cannot be explained by AT mass or SAT gene expression. Hence, imaging-based assessment of body

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compartments only yields improvements for the prediction of some adipokines. These data suggest that other factors or interactions with other factors are the main determinants of these plasma concentrations, and that there is not a straightforward path from obesity to chronic diseases through adipokine levels and the tissue secreting them. While our findings do not contradict a potential role of these adipokines in disease development, their circulating concentrations are unlikely to individually mediate the association between adiposity and disease risk observed in epidemiological studies to a large extent. Future studies on such mediation effects are warranted and should also consider the interplay of adipokines, their downstream products, and regulatory markers.

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Statement of Ethics

The study was approved by the ethics committee of the medical association of the state of Brandenburg (Germany), and all participants gave written informed consent.

Disclosure Statement

The authors have no conflicts of interest to declare.

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