

Associations of a Panel of Adipokines with Fat Deposits and Metabolic Phenotypes in a General Population

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Objective: This study provides a comprehensive overview of the associations of five adipokines (adiponectin, chemerin, galectin-3, leptin, and resistin) with fat deposits, behavioral risk factors, and metabolic phenotypes.

Methods: Using multivariable linear and logistic regression models, cross-sectional data from 4,116 participants of the population-based Study of Health in Pomerania were analyzed.

Results: Participants with obesity showed higher chemerin, galectin-3, and leptin but showed lower adiponectin concentrations. Independently of other fat compounds, liver fat content, visceral adipose tissue, and subcutaneous adipose tissue (SAT) were inversely associated with adiponectin. Independent positive associations of liver fat content and SAT with chemerin as well as of SAT with galectin-3 and leptin were observed. Physically inactive participants had higher chemerin and leptin concentrations. Smokers had higher chemerin and galectin-3 as well as lower leptin. Alcohol consumption was associated with adiponectin (positive) and resistin (inverse). All adipokines were associated with at least one lipid marker. Associations with glucose metabolism were seen for adiponectin, chemerin, galectin-3, and leptin.

Conclusions: High adiponectin concentrations were related to favorable metabolic conditions, whereas high chemerin, galectin-3, and leptin were associated with an unfavorable metabolic profile. High leptin seems to be primarily indicative of obesity, whereas high adiponectin and chemerin are associated with a broader range of metabolic phenotypes.

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Introduction

Adipose tissue is responsible for thermal insulation and mechanical organ protection and it acts as an important endocrine organ that secretes bioactive adipokines (1). These adipokines regulate biological processes in an autocrine, paracrine, or endocrine fashion in many different organs (1). Within the past several years, the number of discovered adipokines has steadily increased. Existing research studies in humans have shown that circulating adipokine concentrations are often associated with measures of body fat (e.g., BMI,

Study Importance

What is already known?

- ▶ Circulating adipokine concentrations are often correlated with measures of body fat and obesity, as well as associated parameters of metabolic diseases.
- ▶ However, the results of these previous studies are not consistent, and they often included only a small number of participants, just observed selected patient cohorts, or concentrated only on one or two specific adipokines.

What does this study add?

- ▶ By using data from a large population-based sample and appropriate multivariable regression models, we showed that the observed adipokines are differently associated with metabolic parameters.
- ▶ Interestingly, high leptin concentrations seem to be primarily indicative of obesity, whereas high chemerin and adiponectin concentrations are associated with a broader range of metabolic phenotypes. Galectin-3 and resistin concentrations appear to play only a subordinate role within metabolic conditions.

How might these results change the direction or the focus of clinical practice?

- ▶ This study provides a comprehensive overview of the associations of five adipokines with fat deposits as well as metabolic risk factors and phenotypes to clarify their meaning during metabolic abnormalities.

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fat mass, waist circumference) or obesity (2,3), as well as with markers of diabetes (4,5), dyslipidemia (2,4,5), and hypertension (6). Thus, it seems that the majority of adipokines are implicated in the pathogenesis of obesity and associated metabolic diseases. Moreover, a few randomized interventional trials have provided evidence that behavioral risk factors like smoking, alcohol consumption, and physical inactivity have an unfavorable influence on circulating adipokine concentrations (7-9). However, previous studies, which dealt with circulating adipokine concentrations in relation to behavioral risk factors and metabolic phenotypes in humans, mostly included only a small number of participants (2,3), just observed selected patient cohorts (2,3), or concentrated only on one or two specific adipokines (2,6,8). Furthermore, only a few human studies have investigated associations of circulating adipokine concentrations with fat deposits (2,4,10).

To our knowledge, a comprehensive investigation of the associations among different adipokines and a broad range of metabolic risk factors and phenotypes is still lacking. To improve the understanding of adipokines and their influence in metabolic health, data on large population-based cohorts are advantageous. Therefore, the aim of the present study is to provide a comprehensive overview of the cross-sectional associations of five different adipokines (adiponectin, chemerin, galectin-3, leptin, and resistin) with magnetic resonance imaging (MRI)-quantified fat deposits, behavioral risk factors, metabolic phenotypes, and blood pressure.

Methods

Study population

The Study of Health in Pomerania (SHIP) is a population-based study conducted in West Pomerania, a rural region in northeastern Germany (11). So far, the overall research project consists of two separate cohorts (SHIP and SHIP-TREND). The present study is based on data from SHIP-TREND. In SHIP-TREND, a stratified random sample of 8,826 persons, aged 20 to 79 years, was drawn from the central population registry of the German Federal State of Mecklenburg–West Pomerania. A total of 4,420 individuals participated in the baseline examinations, which were conducted between 2008 and 2012. The study followed the principles of the Declaration of Helsinki and it was approved by the ethics committee of the University of Greifswald. SHIP data are publicly available for scientific purposes, and those interested can apply for data usage.

From the 4,420 participants, pregnant women ($n=10$) and participants with missing values in adiponectin, chemerin, galectin-3, or resistin concentrations ($n=188$) were excluded. Furthermore, we excluded participants with missing values in confounding or outcome variables ($n=85$) or who had a daily alcohol consumption of more than 75 g ($n=21$). The final study population consisted of 4,116 participants. Although measurements of adiponectin, chemerin, galectin-3, and resistin were available in all of these participants, leptin concentrations were available only in 946 of these 4,116 participants. Data on MRI of subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and liver fat content (LFC) were available in a subsample consisting of 762 participants for leptin measurements and 1,733 participants for the other adipokines. The different study populations that were available for the analyses are presented in a flowchart (Figure 1).

Measurements

Data on age, sex, sociodemographic characteristics, and medical histories were obtained by standardized computer-assisted personal

interviews. Smoking status was categorized as current smoker or nonsmoker. Participants who participated in physical training during summer or winter for less than 1 h/wk were classified as being physically inactive. Mean daily alcohol consumption was calculated using beverage-specific pure-ethanol-volume proportions. Height, weight, and waist circumference of the individuals were quantified following a standardized protocol. Waist circumference was measured to the nearest 0.1 cm using an inelastic tape midway between the lower rib margin and the iliac crest in the horizontal plane, with the participant standing comfortably with weight distributed evenly on both feet. The measurement was taken at the level of the narrowest part of the waist. After a 5-minute resting period, blood pressure was measured three times on seated participants using a digital blood pressure monitor (HEM-705CP; Omron, Tokyo, Japan), with each reading being followed by a further resting period of 3 minutes. The mean of the second and third measurement was taken for these analyses.

MRI examinations were performed on a commercial 1.5-T magnetic resonance system (Magnetom Avanto, software version Syngo MR B15; Siemens Healthcare AG, Eschborn, Germany) using a body-phased array coil. The quantification of SAT and VAT was done using the in-house-developed Automatic Tissue and Labeling Analysis software from the University of Ulm, Germany (12). LFC was assessed using a three-echo chemical shift–encoded MRI of the liver. Postprocessing of MRI data was performed, and the proton-density fat fraction was acquired (13). At this time, the proton-density fat fraction is the noninvasive reference for assessment of liver fat. Proton-density fat fraction in the liver was measured with a region of interest placed in the center of the liver. Vessels and artifacts were excluded from the region of interest.

Blood samples were collected between 7 AM and 1 PM from the cubital vein in the supine position. The majority (62.1%, $n=2,554$) of the observed participants were fasting (without eating or drinking for at least 8 hours) at the time of the blood sampling. Samples were stored at -80°C in the Integrated Research Biobank of University Medicine, University of Greifswald, and were used in accordance with its regulations. Total triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and serum glucose concentrations were measured using the Dimension Vista analytical system (Siemens Healthcare AG). Glycated hemoglobin (HbA_{1c}) was determined by high-performance liquid chromatography (HPLC) with spectrophotometric detection (Diamat Analyzer; Bio-Rad, Munich, Germany). Diabetes mellitus was defined as self-reported diabetes, $\text{HbA}_{1c} \geq 6.5\%$, or use of hypoglycemic agents (classified according to anatomic, therapeutic, and chemical [ATC] code A10). Similarly, undiagnosed diabetes mellitus was defined as $\text{HbA}_{1c} \geq 6.5\%$, no self-reported diabetes, and no use of hypoglycemic agents.

Metabolic syndrome was defined by three or more of the following five components, as proposed by the National Cholesterol Education Program/Adult Treatment Panel III (14) and the International Diabetes Federation (15) and updated with minor modifications by the American Heart Association and the National Heart, Lung, and Blood Institute (16): (1) abdominal obesity (men: waist circumference ≥ 94 cm, women: waist circumference ≥ 80 cm); (2) elevated triglycerides (≥ 2.3 mmol/L for nonfasting participants or ≥ 1.7 mmol/L for fasting participants or use of lipid-modifying medication [ATC code C10AB or C10AD]); (3) reduced HDL-C (men: <1.03 mmol/L in nonfasting and fasting participants, women: <1.29 mmol/L in nonfasting participants or <1.3 mmol/L in fasting participants); (4) elevated blood pressure (systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg, or

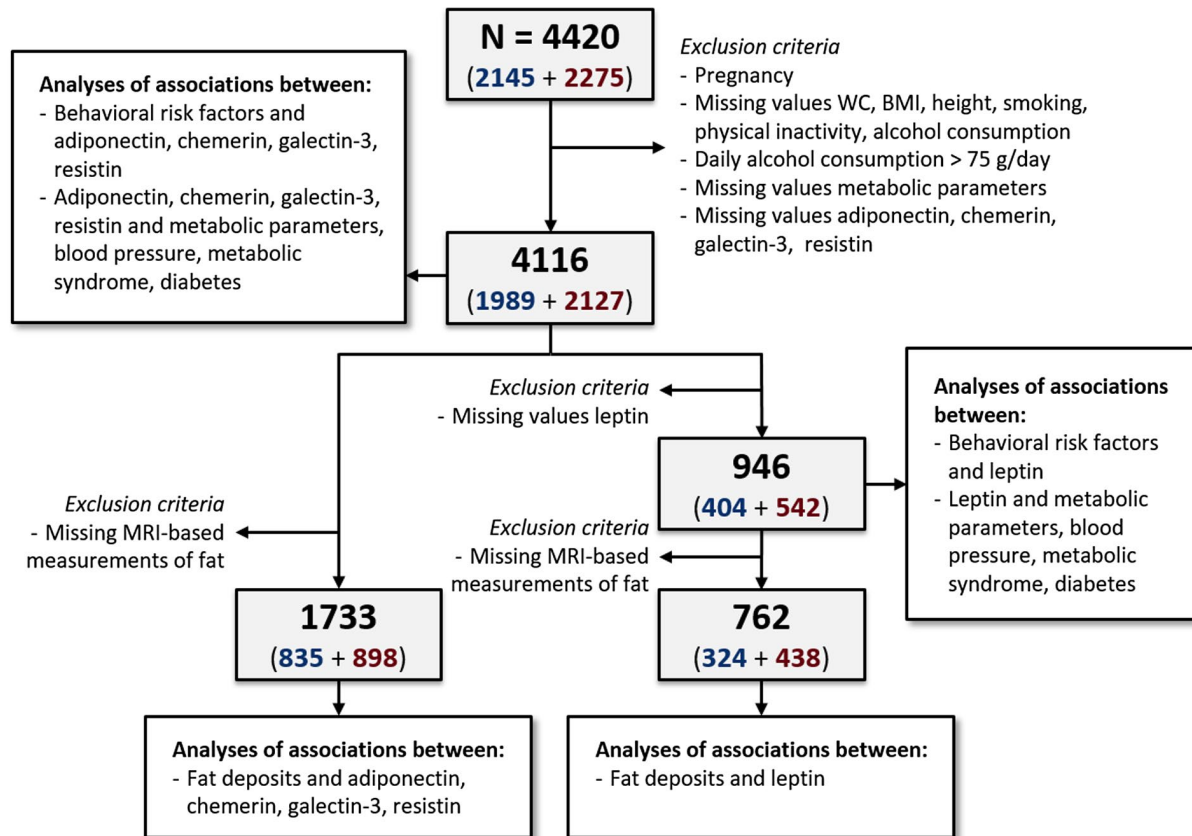


Figure 1 Flowchart presenting the selected study populations. Numbers printed in bold show number of participants available for the named analyses. Numbers of men (blue) and women (red) are also listed. MRI, magnetic resonance imaging; WC, waist circumference. [Color figure can be viewed at wileyonlinelibrary.com]

use of antihypertensive medication [ATC code C02]); and/or (5) elevated glucose (≥ 8 mmol/L for nonfasting participants, ≥ 6.1 mmol/L for fasting participants, or use of hypoglycemic agents [ATC code A10]). The number of participants categorized as having metabolic syndrome, its components, or diabetes mellitus in the different subpopulations is presented in Supporting Information Table S1.

Serum adiponectin, plasma chemerin, serum leptin (Mediagnost, Reutlingen, Germany), and serum resistin (AdipoGen LIFE SCIENCE, Liestal, Switzerland) concentrations were measured using commercially available enzyme-linked immunosorbent assays (ELISA). Plasma galectin-3 concentrations were determined using a quantitative sandwich enzyme immunoassay (R&D Systems, Abingdon, UK). The interassay coefficients of variation were 6.8% and 6.2% (adiponectin), 5.8% and 5.5% (chemerin), 8.5% and 6.4% (galectin-3), and 6.0% and 8.4% (leptin) for low and high concentrations, respectively. The interassay coefficient of variation for resistin was 10.1% for intermediate concentrations.

Statistical analyses

Continuous data were expressed as medians (25th and 75th quartiles), and nominal data were expressed as percentages. Concentrations of circulating adipokines were \log_2 -transformed to achieve a normal distribution. Scatterplots and Spearman correlation coefficients were used to visualize the correlations of the adipokines with each other as well

as with waist circumference. To facilitate comparisons among associations of different adipokines with fat deposits and metabolic parameters, the adipokines, fat deposits, and metabolic parameters were scaled according to their SDs (z scores).

We applied the residual method, an approach suitable to avoid multicollinearity, to examine the associations of fat deposits (LFC, SAT, and VAT) with the different adipokines. For this purpose, fat values were decomposed in independent parts (residuals/adjusted fat values) by applying sex-specific linear regression models to all fat values. This approach allows us to analyze, for example, the amount of SAT that is not explained by VAT or LFC. Linear regression models (adjusted for age, sex, smoking status, height, physical inactivity, daily alcohol consumption, and fasting status) were obtained to examine the associations between unadjusted or adjusted fat values and different adipokines. Furthermore, the associations of behavioral risk factors (physical inactivity, smoking status, and daily alcohol consumption) with adipokines were examined by applying linear regression models adjusted for age, sex, waist circumference, and fasting status. Multivariable linear regression models (adjusted for age, sex, waist circumference, smoking status, physical inactivity, daily alcohol consumption, and fasting status) were further used to assess the associations of adipokines with different metabolic parameters and blood pressure. Likewise, logistic regression models were obtained to analyze the associations of adipokines with metabolic syndrome as well as its components and (undiagnosed) diabetes mellitus.

Within all regression analyses, we tested possible interaction effects between sex and the respective exposure variable. As we did not see significant effect modifications for the vast majority of observed associations, we decided to present only the results for both sexes combined in the main part of this paper. However, all described analyses were also sex-specific and were, moreover, repeated separately in men and women ≤ 50 years of age as well as in men and women > 50 years of age. However, the number of cases in some subpopulations was comparatively small. Supporting Information Table S1 provides an overview of the numbers of participants and cases that were used in linear and logistic regression models analyzing the associations between adipokine concentrations and metabolic phenotypes. For reasons of space, the results of these sensitivity analyses are shown in the provided online Supporting Information (Supporting Information Figures S2-S7).

Statistical significance was assumed at $P < 0.05$ and at $P < 0.1$ for interactions. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina).

Results

General characteristics: correlations of adipokines with each other and with waist circumference

The study sample included 4,116 participants aged 20 to 84 years. Baseline characteristics of the study participants according to adiposity measurements, behavioral risk factors, metabolic parameters, blood pressure, and adipokines are presented in Table 1. In addition, in Supporting Information Table S2, these characteristics are shown in a sex-specific format.

The correlations of the observed adipokines with each other and with waist circumference are presented in Supporting Information Figure S1. We observed mostly poor correlations among the adipokines ($r = -0.06$ to $+0.13$). Exceptions with moderate correlations were detected between chemerin and leptin ($r = 0.43$), chemerin and galectin-3 ($r = 0.38$), and galectin-3 and leptin ($r = 0.26$). Adiponectin and chemerin showed moderate correlations with waist circumference ($r = \pm 0.38$). The poor correlations observed between leptin and waist circumference ($r = 0.16$) can be explained by sex differences, as sex-specific analyses revealed strong correlations between leptin and waist circumference for men ($r = 0.75$) and women ($r = 0.72$).

Associations between fat deposits and adipokines

Multivariable linear regression analyses showed inverse associations of LFC, SAT, and VAT with adiponectin (Figure 2 and Table 2, "original fat values"). In contrast, positive associations of these fat deposits were observed with chemerin, galectin-3, and leptin. Resistin was the only adipokine that did not show any association with SAT or VAT. Here, only a weak inverse association with LFC was seen. As the observed fat deposits are known to be highly correlated with each other, we further applied the residual method to assess the effect of the fat deposits independently of each other. Some of the previously detected associations with adipokine concentrations disappeared when using adjusted fat values (Figure 2 and Table 2, "adjusted fat values"). However, the inverse associations of all fat deposits with adiponectin persisted, but a decrease in the effect estimates was noticed. Furthermore, independent positive associations of LFC and SAT with chemerin as well as of SAT with galectin-3 were observed. The strongest independent association was detected between SAT and leptin.

TABLE 1 Descriptive statistics of study population

	Whole population (<i>n</i> = 4,116)
Men (%)	48.3
Age (y)	53 (40, 64)
Fasting (≥ 8 h, %)	62.1
Adiposity measurements	
BMI (kg/m ²)	27.5 (24.5, 31.0)
Waist circumference (cm)	90.5 (80.1, 101.0)
Subcutaneous fat (MRI-based, L) ^a	7.3 (5.4, 9.8)
Visceral fat (MRI-based, L) ^a	3.5 (1.9, 5.6)
LFC (MRI-based, %) ^a	3.9 (2.4, 8.0)
Behavioral risk factors	
Physical inactivity (%)	30.5
Current smokers (%)	26.6
Alcohol consumption (g/d)	3.4 (0.7, 10.4)
Metabolic parameters	
Serum glucose (mmol/L)	5.4 (5.0, 6.0)
HbA _{1c} (%)	5.3 (4.9, 5.7)
Total cholesterol (mmol/L)	5.4 (4.6, 6.1)
LDL-C (mmol/L)	3.3 (2.7, 4.0)
HDL-C (mmol/L)	1.4 (1.2, 1.7)
Total triglycerides (mmol/L)	1.4 (1.0, 2.0)
Systolic blood pressure (mmHg)	127.0 (114.5, 139.5)
Diastolic blood pressure (mmHg)	76.5 (70.0, 83.5)
Metabolic syndrome (%)	28.7
Abdominal obesity (%)	59.3
Elevated triglycerides (%)	28.0
Reduced HDL-C (%)	22.0
Elevated blood pressure (%)	46.2
Elevated glucose (%)	18.7
Diabetes (%)	12.1
Undiagnosed diabetes (%)	1.8
Adipokines	
Adiponectin (ng/mL)	6,969 (4,623, 10,319)
Chemerin (ng/mL)	98.0 (82.3, 117.9)
Galectin-3 (ng/mL)	7.5 (6.0, 9.2)
Leptin (ng/mL) ^b	11.1 (6.1, 21.0)
Resistin (ng/mL)	7.8 (5.5, 10.9)

Continuous data expressed as medians (first quartile, third quartile); nominal data given as percentages.

^aMRI examinations performed in subsample of 1,733 participants.

^bLeptin concentration measured in subsample of 946 participants.

HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LFC, liver fat content; LDL-C, low-density lipoprotein cholesterol; MRI, magnetic resonance imaging.

Associations of different behavioral risk factors with adipokines

Physical inactivity was associated with higher chemerin and leptin concentrations (Figure 3). Moreover, smokers had higher chemerin and galectin-3 as well as lower adiponectin and leptin concentrations than nonsmokers (Figure 3). Alcohol consumption was associated with adiponectin (positive) and resistin (inverse) concentrations (Figure 3).

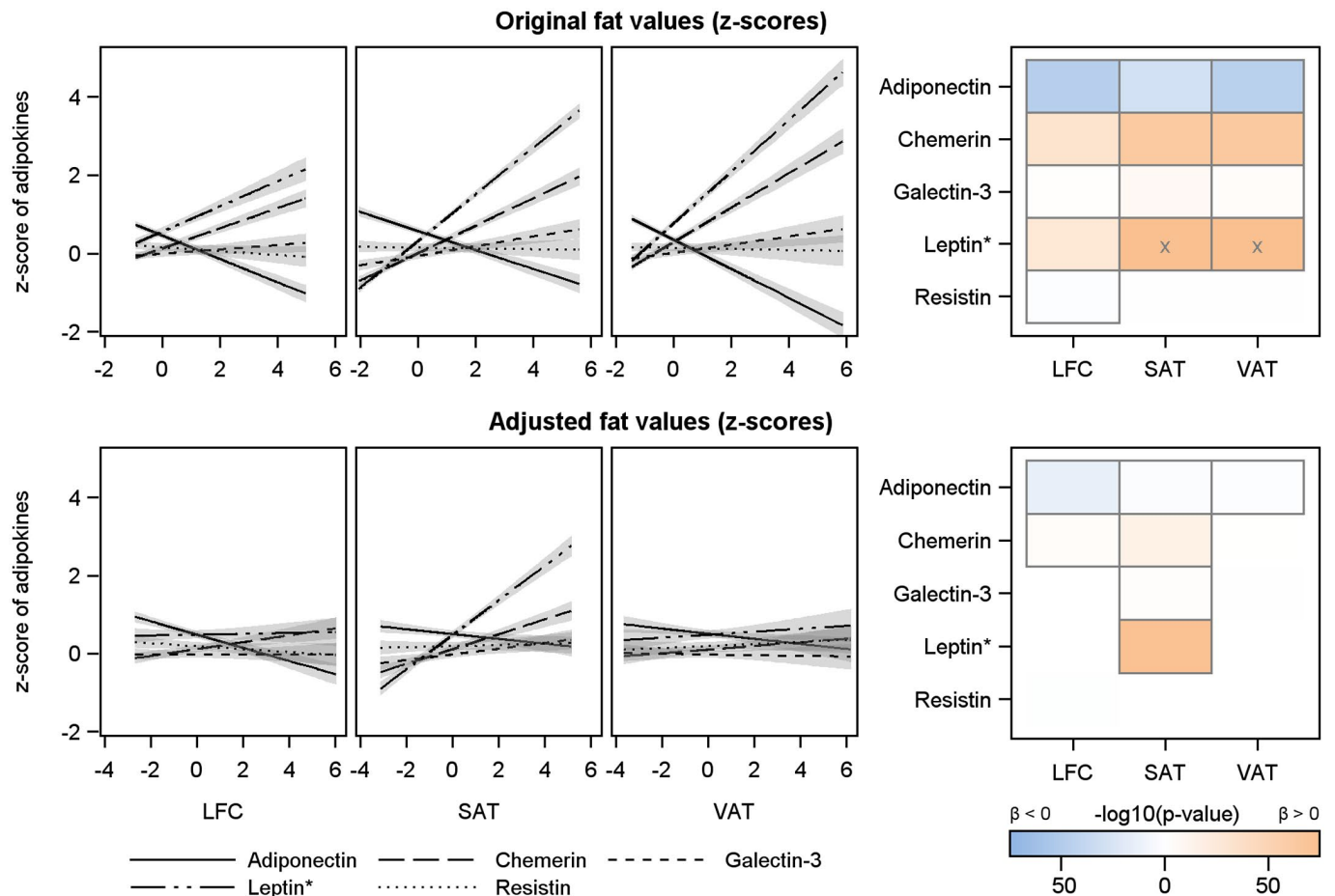


Figure 2 Associations between liver fat content (LFC), subcutaneous adipose tissue (SAT), or visceral adipose tissue (VAT) and different adipokines based on original (upper part) and adjusted (lower part) fat values ($n=1,733$). Left side of the figure shows predicted mean levels of adipokines. Right side of the figure shows color-coded P values based on linear regression analyses (the darker the color, the smaller the P value; significant associations are outlined, $P < 0.05$). Associations with $P < 10^{-75}$ are highlighted with an x . Color of the rectangle represents direction of the association (inverse associations [$\beta < 0$] are colored in blue shades; positive associations [$\beta > 0$] are colored in orange shades). All models were adjusted for age, sex, smoking status, height, physical inactivity, daily alcohol consumption, and fasting status. Adjusted fat values were calculated sex-specifically on the basis of the residuals method. Outcomes (adipokines): \log_2 -transformed, z score-standardized; exposures (fat compounds): z score-standardized. *Leptin concentration was measured in a subsample of 762 participants. [Color figure can be viewed at wileyonlinelibrary.com]

Associations of adipokines with metabolic phenotypes and blood pressure

Significant associations with parameters of glucose metabolism (HbA_{1c} and serum glucose) were seen for adiponectin, chemerin, galectin-3, and leptin (Figure 4A). Thereby, adiponectin (inverse) and chemerin (positive) showed the strongest associations, with similar strengths but in opposite directions. All of the observed adipokines were associated with at least one lipid marker (Figure 4A). In general, high chemerin, galectin-3, and leptin concentrations were related to an unfavorable lipid profile, whereas high adiponectin concentrations were related to a more favorable lipid profile. Remarkably, resistin was inversely associated with LDL-C, total cholesterol, and HDL-C. Furthermore, resistin was inversely associated with diastolic blood pressure (Figure 4A).

Associations of adipokines with metabolic syndrome, its components, and diabetes

Logistic regression models revealed that each 1-SD increase of leptin or chemerin concentrations was associated with 55% or 35% higher odds

of having metabolic syndrome, respectively (Figure 4B). In contrast, a 1-SD increase in adiponectin concentrations was associated with 44% lower odds of having metabolic syndrome. The analyses of the distinct components of metabolic syndrome have indicated that the associations between leptin and metabolic syndrome can be fully explained by its strong relation to abdominal obesity, whereas adiponectin and chemerin concentrations also showed associations with the other components of metabolic syndrome (Figure 4B). Significant associations with diabetes were observed for galectin-3 (positive), chemerin (positive), and adiponectin (inverse) concentrations (Figure 4B).

Sex-specific analyses of the presented associations

For the vast majority of the observed associations, the regression results were clearly comparable with those found in sex-specific analyses. In general, we saw that the sex-specific regression estimates ran in the same direction and had a similar strength compared with those detected in both sexes. However, in a few cases, associations lost their significance if only

TABLE 2 Results from linear regression analyses ($n = 1,733$): associations between LFC, SAT, or VAT and different adipokines based on original (upper part) and adjusted (lower part) fat values

	LFC			SAT			VAT		
	β	SE	P	β	SE	P	β	SE	P
Original fat values (z scores)									
Adiponectin	-0.299	0.021	<0.01	-0.243	0.021	<0.01	-0.371	0.026	<0.01
Chemerin	0.255	0.021	<0.01	0.351	0.021	<0.01	0.438	0.026	<0.01
Galectin-3	0.054	0.022	0.01	0.122	0.022	<0.01	0.103	0.028	<0.01
Leptin ^a	0.320	0.029	<0.01	0.597	0.018	<0.01	0.658	0.027	<0.01
Resistin	-0.050	0.024	0.04	-0.010	0.025	0.67	-0.013	0.030	0.66
Adjusted fat values (z scores)									
Adiponectin	-0.169	0.021	<0.01	-0.062	0.023	<0.01	-0.066	0.026	0.01
Chemerin	0.088	0.022	<0.01	0.189	0.022	<0.01	0.046	0.026	0.07
Galectin-3	0.001	0.022	0.95	0.073	0.023	<0.01	-0.010	0.026	0.71
Leptin ^a	0.011	0.029	0.71	0.441	0.024	<0.01	0.038	0.034	0.26
Resistin	-0.038	0.024	0.11	0.015	0.025	0.56	0.026	0.028	0.36

All models adjusted for age, sex, smoking status, height, physical inactivity, daily alcohol consumption, and fasting status. Adjusted fat values calculated sex-specifically on basis of residuals method. Outcomes (adipokines): \log_2 -transformed, z score-standardized; exposures (fat compounds): z score-standardized.

^aLeptin concentration measured in subsample of 762 participants.

LFC, liver fat content; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

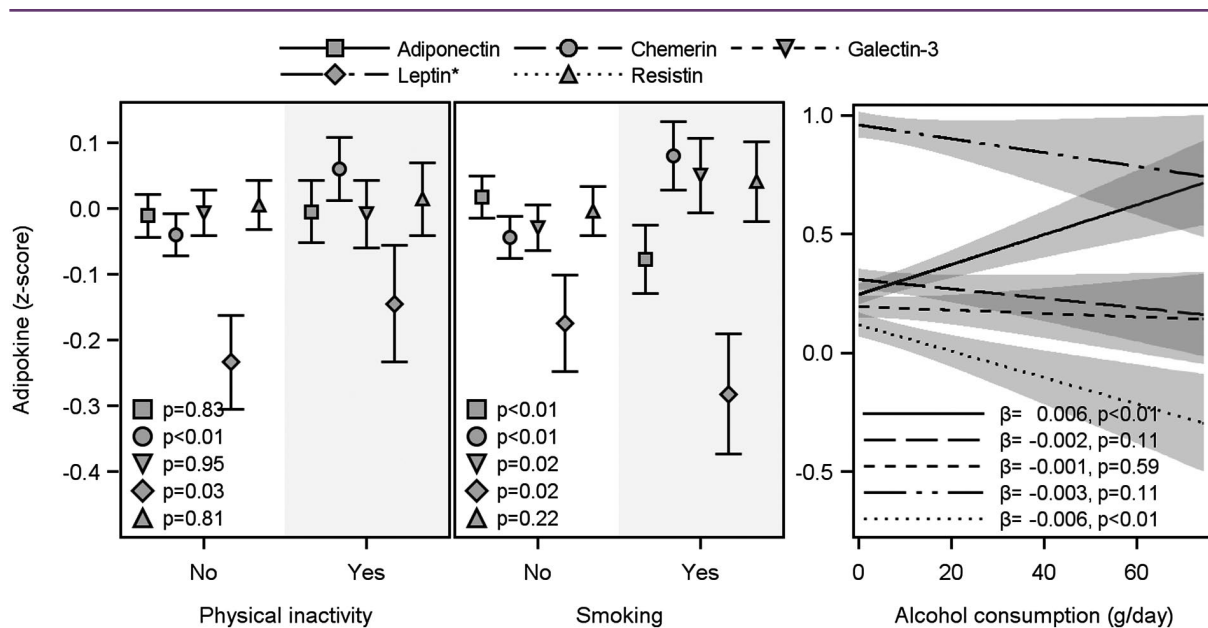


Figure 3 Associations of physical inactivity, smoking status, and daily alcohol consumption with different adipokines ($n = 4,116$). Two panels on the left show adjusted mean adipokine levels with 95% CIs. Panel on the right shows estimated linear regression lines with corresponding 95% CIs. All models were adjusted for age, sex, waist circumference, and fasting status. Outcomes (adipokines): \log_2 -transformed, z score-standardized. *Leptin concentration was measured in a subsample of 946 participants.

men or women were observed. This was especially true when the analyses were further divided according to the age of the participants (≤ 50 and > 50 years), possibly explained by the reduced number of cases in the different subpopulations. A detailed comparison of all regression results detected in men and women is presented in Supporting Information Figures S2-S7.

Discussion

In the present study, we investigated the associations of five adipokines with different fat deposits, behavioral risk factors, and metabolic phenotypes. In general, associations between the considered adipokines and

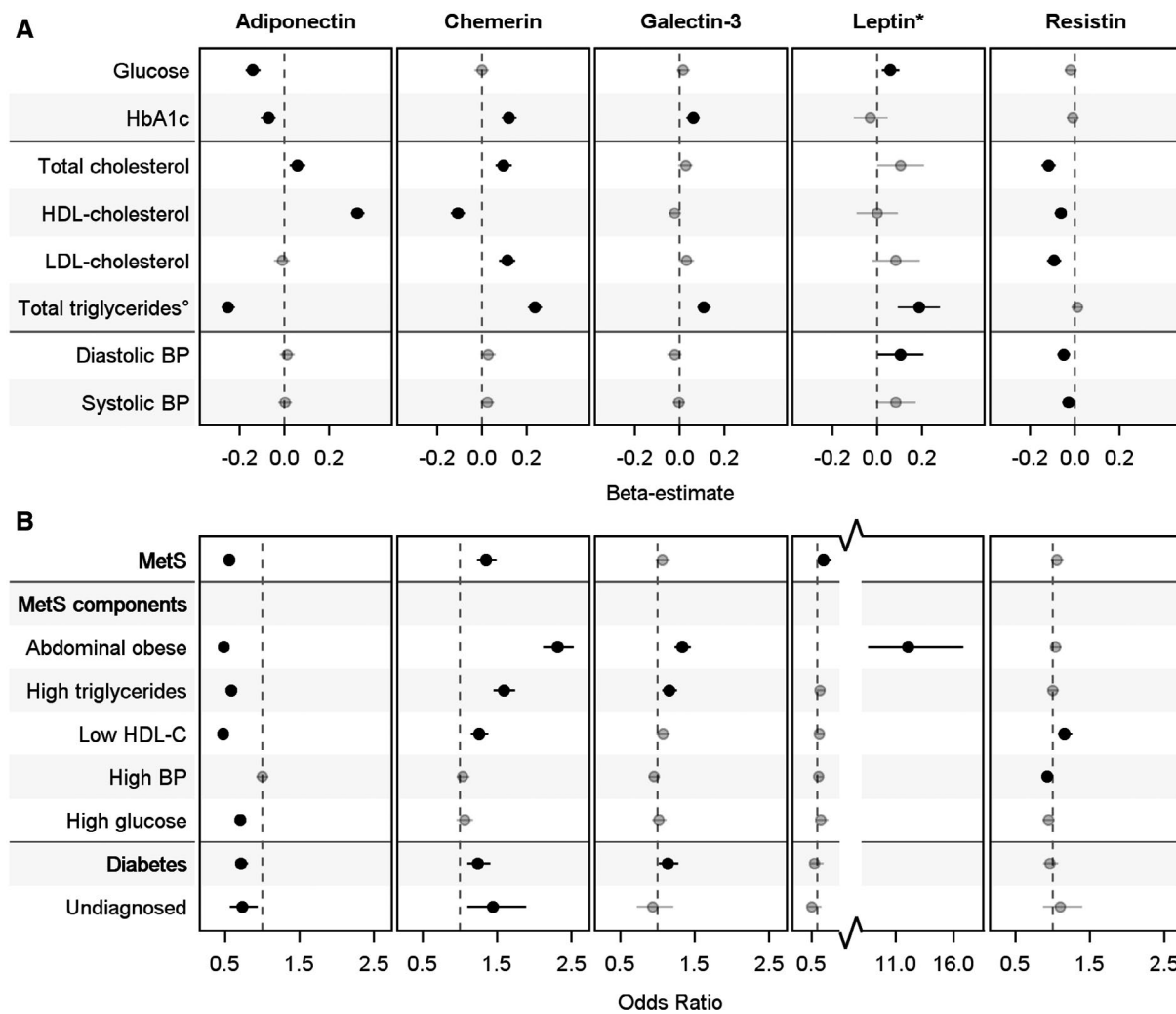


Figure 4 (A) Beta estimates from linear regression analyses ($n = 4,116$) for the associations of circulating adipokine concentrations with different metabolic parameters and blood pressure as well as (B) odds ratios from logistic regression analyses ($n = 4,116$) for the associations between circulating adipokine concentrations and metabolic syndrome (MetS) as well as its components. Significant associations stand out in bold ($P < 0.05$). All analyses were adjusted for age, sex, smoking status, physical inactivity, daily alcohol consumption, waist circumference, and fasting status (exception: the MetS component abdominal obesity had no adjustment for waist circumference). Outcomes for linear regression analyses: ($^{\circ}$) \log_2 -transformed) z score-standardized. Exposures (adipokines): \log_2 -transformed, z score-standardized. Diabetes was defined as self-reported diabetes, $HbA_{1c} \geq 6.5\%$, or use of hypoglycemic agents (classified according to anatomic, therapeutic, and chemical code A10). Undiagnosed diabetes was defined as $HbA_{1c} \geq 6.5\%$, no self-reported diabetes, and no use of hypoglycemic agents. *Leptin concentration was measured in a subsample of 946 participants. BP, blood pressure; HbA_{1c} , glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MetS, metabolic syndrome.

different phenotypes were quite different. High adiponectin concentrations were related to more favorable metabolic conditions, whereas high concentrations of chemerin, galectin-3, and leptin were associated with a less favorable metabolic profile. Interestingly, high concentrations of leptin were strongly related to abdominal obesity, suggesting that circulating leptin is primarily indicative of this phenotype. In contrast, adiponectin and chemerin concentrations were associated with a broader range of metabolic phenotypes. Furthermore, we observed that a disproportional amount of SAT was significantly associated with four out of five adipokines. Sex had only a minor influence on the examined associations. In general, the detected results were applicable to both sexes.

Fat deposits and adipokines

The present analyses showed inverse associations of LFC, SAT, and VAT with adiponectin concentrations as well as positive associations of these fat deposits with chemerin, galectin-3, and leptin concentrations. The majority of existing reports regarding the different contributions of body fat deposits to circulating adipokine concentrations have focused on leptin or adiponectin (10,17,18). In line with our results, these studies have mainly presented inverse associations of SAT and VAT with adiponectin (17,18), as well as positive associations with leptin (10,17,18) and chemerin concentrations (4). The contribution of LFC to circulating adipokine concentrations was addressed in only a few studies that reported conflicting results (10,18). Differences in

research design, study population, and methods applied to quantify the different fat deposits may explain the inconsistent results. The different fat deposits are known to be highly correlated with each other. But, with two exceptions that were both published by our research group (4,10), this multicollinearity was not taken into account in the previous studies (17,18). By using adjusted fat values, we observed that a disproportional amount of SAT was especially associated with the investigated adipokines. A high amount of SAT among those with low VAT values has been shown to be generally associated with an adverse cardiometabolic risk factor profile (19), which in turn is related to high chemerin, galectin-3, and leptin as well as low adiponectin concentrations in the blood (1) and therefore may explain the detected results.

The mechanistic link between body fat distribution and circulating adipokine concentrations remains unclear. It might be possible that circulating adipokine concentrations reflect the different secretory capacity of these adipokines in the specific fat depot (18), as correlations between adipokine gene expression and its circulating concentrations were reported (20). An experimental study showed clear differences in the adipokine secretion profiles among different fat deposits, especially between SAT and VAT (3).

Behavioral risk factors and adipokines

We observed that physically inactive participants had higher chemerin and leptin concentrations compared with those with a more active lifestyle. Epidemiological studies are often unable to measure physical activity appropriately and thus they use poor and imprecise characterizations (21,22). This might be a reason for the inconsistent findings reported in previous studies (21,22). However, a few cross-sectional studies have used an accelerometer as a more objective measurement (23,24) and have mainly revealed an inverse association between leptin and physical activity (23,24). However, the association between adiponectin and physical activity remains unclear (23,24). Several randomized trials have further reported beneficial changes in leptin and chemerin concentrations after exercise interventions, which correlated with improvements in body fat, dyslipidemia, and insulin sensitivity (9,25).

The present analyses showed that smokers had higher chemerin and galectin-3, as well as lower adiponectin and leptin concentrations, than nonsmokers. In line with our results, the majority of existing cross-sectional studies have reported lower concentrations of adiponectin in current smokers compared with nonsmokers (22). Data concerning leptin concentrations are much more conflicting (22,26,27). However, intervention studies have shown that both adiponectin and leptin concentrations significantly increase after smoking cessation (7,28). Only a few studies have examined the relation between smoking and the other three adipokines and reported ambiguous results (29-31). Consequently, the precise mechanisms by which smoking influences adipokine concentrations are largely unknown. Several human and rat studies have shown that tobacco use reduces appetite (32), stimulates energy expenditure and thermogenesis (33), and decreases body weight (32). Adipokines as endocrine signals that are strongly related with body fat may mediate these effects.

Our analyses revealed that alcohol consumption was significantly associated with adiponectin (positive) and resistin (inverse) concentrations. In line with our results, the majority of previous reports have detected positive relations with adiponectin (34,35), whereas only few and inconsistent data exist for the other adipokines (22,27,36). Several interventional trials in humans have further confirmed the observed

positive association with adiponectin by showing that moderate alcohol intake increases adiponectin concentrations (8,37). In rodent studies, this has also been demonstrated for resistin (38), leptin (38), and chemerin (36). Nevertheless, it remains unclear whether these findings can be translated to humans.

Adipokines and metabolic phenotypes

With respect to the investigated adipokines, different association patterns with metabolic phenotypes, including obesity, glucose and lipid metabolism, and blood pressure were observed. There is a substantial bulk of research on the relation between circulating adipokine concentrations and different metabolic phenotypes. In general, these findings are in line with our results, as they have shown that high adiponectin concentrations are associated with a favorable metabolic profile (5), whereas high chemerin, leptin, galectin-3, and resistin concentrations are associated with more unfavorable metabolic phenotypes (2,6,26,39). In the present analyses, higher odds for metabolic syndrome were actually seen only for high chemerin and leptin concentrations as well as low adiponectin concentrations. Thereby, metabolic disorders, including an unfavorable lipid profile or diabetes, actually seem not to matter for the observed association between circulating leptin and metabolic syndrome because this association can be fully explained by the strong relation of leptin to abdominal obesity. Leptin is primarily released by adipose tissue and it can cross the blood-brain barrier (40). Through binding to and activating its receptor in the brain, leptin has been reported to decrease food intake and increase energy expenditure (40). However, as individuals with obesity generally exhibit higher circulating leptin concentrations than individuals with normal weight, it has been claimed that a period of overeating results in leptin resistance, either through less hypothalamic leptin sensitivity or through a defect in the transport of leptin across the blood-brain barrier (40). In the present analyses, in contrast to leptin, adiponectin and chemerin were associated with a broader range of metabolic components, including diabetes and dyslipidemia. Several experimental studies have provided evidence that chemerin modulates insulin signaling in different cell types (41,42), but the exact molecular mechanisms are still ambiguous. Results from various clinical, experimental, and genetic studies support the association between decreased adiponectin concentrations and type 2 diabetes mellitus (43,44). Together, a major role of adiponectin and chemerin in insulin resistance can be assumed.

Interestingly, the present analyses showed that resistin was the only observed adipokine that was not associated with abdominal obesity. In humans, the exact role of resistin in obesity is still under debate. Resistin expression is known to be relatively low in human adipose tissue (45), and it has been assumed that the circulating resistin concentrations are mainly regulated by resistin's high expression levels in peripheral blood mononuclear cells and bone marrow cells (45). This would also explain why we did not observe any association of SAT or VAT with resistin. We assume that resistin probably does not play a crucial role during obesity or its metabolic consequences in humans.

Strengths and limitations

The main strengths of our study are the large, population-based sample size and the highly standardized data collection. We examined more than 4,000 participants by measuring their adipokine concentrations, SAT, VAT, LFC, and different metabolic and behavioral characteristics. Unlike previous researchers, we did not focus only on patients with a specific disease. Moreover, we considered the multicollinearity among the different

fat deposits. However, the study is limited by its cross-sectional design. Therefore, we were not able to describe any causality and could not evaluate the adipokines as risk factors for metabolic syndrome or diabetes mellitus.

Conclusion

Adipokines are differently associated with metabolic risk factors and phenotypes. Unlike high adiponectin concentrations, high chemerin, galectin-3, and leptin are associated with unfavorable metabolic conditions. Interestingly, high leptin concentrations seem to be primarily indicative of obesity, whereas high adiponectin and chemerin concentrations are associated with a broader range of metabolic phenotypes. Galectin-3 and resistin appear to play only a subordinate role within metabolic conditions. Overall, our study provides a better understanding of the associations of a panel of circulating adipokines with different fat deposits, behavioral risk factors, and metabolic phenotypes. However, very little is known about the full range of the complex physiology among adipokines. Thus, further studies are urgently needed to clarify some of their roles. **O**

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