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

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Abbreviations: DXA = dual-energy x-ray absorptiometry; PEA = proximity extension assay; NPX = normalized protein expression; LOD = limit of detection; ROI = region of interest; FMI = fat mass index; FDR = false discovery rate; FCR = false coverage rate

AT A Glance Commentary

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Background

Altered circulating levels of several cytokines and immune regulators have been observed in people with obesity. Despite major advances in the last 2 decades, the complex relationship between inflammation and obesity remains poorly understood.

Translational Significance

We describe novel associations between adiposity and the circulating level of DNER, SLAMF1, TRANCE, and CSF-1. The further characterization of these associations holds the potential to help us uncover new mechanisms that lead to disease development during obesity.

INTRODUCTION

Obesity is accompanied by adipose tissue dysfunction as well as abnormal cytokine production by immune cells and adipocytes.[1] Adipose tissue of individuals with obesity expresses increased amounts of inflammatory proteins such as TNF-α, IL-6, and MCP1.[2] Some of these proteins are released into the circulation and establish a state of systemic inflammation by eliciting a response from other organs, such as the liver.[3] Indeed, altered circulating levels of several cytokines and immune regulators have been observed in people with obesity, including IL-6, IL-1Ra, IL-8, FGF-21, and HGF.[4–8]

Differences in physiology and secretion patterns have been observed among different fat depots, namely subcutaneous and visceral adipose tissue.[9] This observation is in line with increasing evidence suggesting that poor health outcomes in obesity are not determined by total fat mass alone but are heavily influenced by the location and distribution of adipose tissue.[3]

Several lines of research suggest that low-grade systemic inflammation and the development of comorbidities, such as insulin resistance and cardiovascular disease, are inextricably connected.[3] Thus, shedding light on the molecular players involved in this inflammatory state is crucial to understanding this complex interaction. In this study, we investigated the association between measures of adiposity and the circulating level of 72 inflammation-related proteins (Inflammation panel, Olink Proteomics). This panel of proteins included a wide range of proteins known to participate in certain inflammatory processes as well as suitable candidates for exploratory analyses. We used 4 independent European studies: KORA-Fit (n = 805), BVSII (n = 277), ESTHER (n = 1667) and Bialystok-PLUS (n = 559). These studies are relatively large and well-characterized population-based studies with participants of mostly European descent. In addition, they used comparable questionnaires and methods to assess the characteristics of the participants. KORA-Fit was used for discovery since it was the study with the largest number of participants for which both BMI and waist circumference were available. The results were then replicated in BVSII, ESTHER, and Bialystok PLUS as well as further validated using DXA-derived fat mass measurements in the Bialystok PLUS study.

METHODS

Study samples. KORA (Cooperative Health Research in the Region of Augsburg, Germany) is a regional research platform for population-based studies.[10] The KORA S4 survey was conducted from 1999 to 2001 and included 4,261 participants aged 25-74 years. All participants who had completed the baseline survey were invited to take part in follow-up surveys and examinations at regular intervals. The present cross-sectional study included a sub-sample of KORA S4 subjects (n = 809) who participated in the KORA-Fit follow-up study (2017-2019) and for whom a blood sample was available. Blood collection was performed from fasting participants. Citrated plasma was stored at -80˚C until analysis with no freeze-thaw cycles.

The BVSII (Bavarian Food Consumption Survey II) is a cross-sectional study that aims at investigating the dietary and lifestyle habits of the Bavarian population (Germany).[11] Between 2002 and 2003, 1,050 participants aged 13-80 years were randomly recruited. All adults who had completed the interview and at least one dietary recall were invited for further examinations, which included anthropometric measurements and blood sample collection. Blood was collected during the day from fasting and non-fasting participants. EDTA plasma was stored at -80˚C until analysis with no freeze-thaw cycles. A sub-sample of 280 individuals of those who participated in the examination (n = 568) was included in the present study.

The ESTHER study (Epidemiological investigations on chances of preventing, recognizing early, and optimally treating chronic diseases in an elderly population) is an ongoing prospective cohort study in Saarland, Germany.[12] The initial assessment was conducted between 2000 and 2002 and included 9,949 participants aged 50-75 years, recruited during a routine health check-up. All participants were asked to complete a standardized questionnaire including lifestyle and medical history information. Serum samples of all participants were collected during the initial visit and frozen at -80˚C until analysis. A random sample of 1,939 baseline participants was used as replication sample in this study.

The Bialystok PLUS study is an ongoing longitudinal study that aims to provide a multidimensional picture of the health of the population of the city of Bialystok, Poland. In 2017-2018, 600 randomly selected residents aged 20-77 years were invited to be part of the test cohort of the study. 254 individuals responded and were examined. All study participants underwent a laboratory assessment and physical examination. Recruitment for the main cohort of the study, which will consist of 10,000 randomly selected residents aged 20-80, began in November 2018. As of August 2021, 3,000 participants have been recruited and approximately 1000 have been examined. Peripheral venous fasting blood was collected in the morning on a visit day and serum samples were stored at −80˚C until analyzed in both, the main and the test, cohorts. Protein measurements were performed in 248 samples of the test cohort and 401 samples of the main cohort (see Supplementary Material for a description of the normalization procedure).

The studies were approved by the Ethics Committees of the Bavarian Medical Association (KORA), the University of Heidelberg/Medical Association of Saarland (ESTHER), the Bavarian Ministry of Health (BVSII), and the Medical University of Bialystok (Bialystok PLUS). All study participants provided written informed consent.

Protein Measurement. Protein measurements were performed on plasma or serum samples using the Proseek Multiplex Inflammation panel, developed by Olink Proteomics (Uppsala, Sweden) and based on the Proximity Extension Assay (PEA). Briefly, this assay uses a pair of specific oligonucleotide-labeled antibodies targeting each protein. When two matching oligonucleotides are in close proximity, a new protein-specific sequence is formed by a proximity-dependent DNA polymerization event. The amount of each protein-specific sequence is then quantified by real-time quantitative Polymerase Chain Reaction (qPCR). This method allows the simultaneous quantification of 92 proteins in 96 samples at a time.

Protein levels are reported as Normalized Protein Expression (NPX) values, a relative unit proposed by Olink, which is calculated from Ct values (qPCR) and is on a log2 scale. A difference of 1 NPX is equivalent to a doubling of protein concentration. Normalization is performed to minimize intra- and inter-assay variation. The limit of detection (LOD) is calculated separately for each Olink assay and sample plate. The LOD is based on the background, estimated from negative controls included on every plate, plus 3 standard deviations. The standard deviation (SD) is assay-specific and estimated during product validation for every panel. For studies including more than 1 plate per panel, the maximum observed LOD for each assay is selected as study LOD. Consequently, all plates included in the study receive the same assay-specific LOD. Proteins for which more than 25% of the samples had values below the LOD were excluded from further analysis. For the rest of the proteins, values below the LOD were substituted with the respective LOD (KORA-Fit, BVSII, and Bialystok PLUS) or LOD/$\sqrt{2}$ (ESTHER). Protein levels and LODs are presented in Supplementary Table 1. 60 measurements in the ESTHER study and 18 in the Bialystok PLUS study did not pass the quality control test and were therefore excluded.

Measures of adiposity and body fat distribution. Body height, weight, and waist circumference were measured by trained personnel with comparable standardized methods across study samples except for ESTHER, in which height and weight were self-reported by study participants. Waist circumference was not measured in ESTHER. Weight was measured in light clothing to the nearest 0.1 kg (KORA-Fit), 0.5 kg (BVSII), and 0.1 kg (Bialystok PLUS). Height was measured to the nearest 0.1 cm (KORA-Fit), 0.5 cm (BVSII), and 0.1 cm (Bialystok). Waist circumference was measured using a flexible, inelastic tape measure midway between the lowest rib and the iliac crest to the nearest 0.1 cm. BMI was calculated as weight [kg]/height [m^2]).

In the Bialystok PLUS study, DXA measurements were taken using the Healthcare Lunar system (General Electric). Total body mass was divided into 3 compartments: bone, fat mass, and lean mass. By using the region of interest (ROI) program, total fat, gynoid fat, and android fat were measured automatically. The ranges for android ROI were: lower boundary at pelvis cut, upper boundary above pelvis cut by 20% of the distance between pelvis and neck cuts, lateral boundaries were the arm cuts. The ranges for gynoid ROI were: upper boundary below the pelvis cut line by 1.5 times the height of the android ROI, gynoid ROI height equal to 2 times the height of the android ROI,

lateral boundaries were the outer leg cuts. Visceral adipose tissue mass within the android region was estimated using the CoreScan software. Fat mass index (FMI, fat mass [kg]/height [m^2]), percentage of visceral fat in the android region (visceral fat mass/android fat mass * 100), android-gynoid ratio (android fat mass/gynoid fat mass), and trunk-to-limb ratio (trunk fat mass/total limb fat mass) were calculated from scan measurements.

Statistical analysis. Cases with missing data (in exposures or covariates) were excluded from the analysis, leaving the following sample sizes for analysis: 805 (KORA-Fit), 277 (BVSII), 1667 (ESTHER), and 559 (Bialystock PLUS). The associations between the different measures of adiposity and blood protein levels were examined using linear regression models with each protein level (NPX) as the dependent variable. Models were adjusted for sex, age, physical activity, smoking status, and alcohol intake (potential confounders). Measures of adiposity were scaled to mean 0 and 1-SD before data analysis to facilitate comparisons. The percentage of protein variance explained by each measure of adiposity was defined as the change in adjusted R^2 when the respective measure was added to a model including only covariates. P values were corrected for multiple testing using the Benjamini & Hochberg procedure (False Discovery Rate, FDR). Confidence intervals adjusted for multiple testing were calculated using the algorithm by Jung et al.[13] based on the Benjamini & Yekutieli procedure (False Coverage Rate, FCR). KORA-Fit was used as discovery sample. Significant associations (FDR-adjusted P values < 0.05) were then replicated in BVSII, ESTHER, and Bialystok PLUS. We additionally performed regression analyses for the association of DXA-derived measures of body fat distribution and protein levels in Bialystok PLUS. In the Bialystok PLUS study, regression models were further adjusted for the fat mass index to assess the effect of visceral fat, android-gynoid ratio, and trunk-to-limb ratio on protein levels independently of whole-body fat. Effect-measure modification by sex was examined by testing multiplicative interaction terms using Wald tests. Mediation analyses were carried out using the R package *mediation*.[14] FDR-adjusted P values were calculated to correct for multiple testing. Analyses were performed using R software R-3.6.3.

RESULTS

Study participant characteristics are summarized in Table I and the study design is shown in Fig 1. During discovery analyses in the KORA-Fit study, we found that 48 proteins were associated with BMI and/or waist circumference (Fig 2 and Supplementary Tables 2 and 3). The majority of the associations were positive, while 9 proteins showed an inverse association: CCL11, CCL28, CST5, CX3CL1, DNER, FGF-19, MMP-10, SCF, and TWEAK. Fourteen associations were successfully replicated in the BVSII, ESTHER, and Bialystok PLUS studies: DNER, CSF-1, CCL19, CCL28, FGF-21, HGF, IL-10RB, IL-18, IL-18R1, IL-6, RANKL, SCF, SLAMF1, and VEGF-A. These proteins are classified as cytokines, receptors, receptor subunits, or growth factors, with the majority belonging to the first category (Fig 3). The direction of the association was consistent in all cases and effect sizes were similar across studies with the exception of FGF-21, for which the effect was considerably larger in BVSII compared to the other study samples. The largest effect sizes were observed for the associations with FGF-21, IL-6, HGF, CCL19, and IL-18R1 (Supplementary Tables 2-8).

In addition, we explored the association between systemic levels of inflammation-related proteins and direct measurements of body fat mass obtained by DXA in the Bialystok PLUS study. Forty-two proteins showed an association with at least 1 DXA-derived variable (fat mass index, android-to-gynoid ratio, trunk-to-limb ratio, or visceral fat). Fig 4 illustrates the proportion of variance in protein levels explained by each variable. All associations were positive except for CCL11, CCL28, CX3CL1, DNER, FGF-19, IL-17A, SCF, and TWEAK (Supplementary Tables 9-12). All of the proteins for which an association with BMI and/or waist circumference was found and replicated, were also associated with at least 1 DXA-derived variable. The majority of these proteins were associated with all DXA-derived variables: CCL19, FGF-21, HGF, IL-10RB, IL-18, IL-18R1, IL-6, RANKL, SCF, SLAMF-1, and VEGFA. CSF-1 was associated with all variables except for visceral fat, while CCL28 and DNER were exclusively associated with whole-body fat. We also assessed whether the observed associations with fat accumulation in the android, truncal, and visceral regions were independent of whole-body fat. Eight associations with the android-gynoid ratio (CDCP1, CX3CL1, FGF-21, HGF, IL-18, IL-18R1, OSM, and SCF), six with the trunk-to-limb ratio (CX3CL1, FGF-21, HGF, IL-18, IL-18R1, and SLAMF1) and one with visceral fat (FGF-21) survived further adjustment for whole-body fat (Supplementary Tables 10-12).

We observed no sex differences in the association between measures of adiposity and protein levels that were consistent across all studies (not shown). Finally, we investigated the possible contribution of impaired glucose homeostasis to the association between adiposity and altered levels of inflammation-related proteins.

Table I. Characteristics of study participants

	KORA-Fit	BVSII	ESTHER	Bialystok PLUS
n	805	277	1667	559
Age	63 (58, 67)	46 (36,61)	62 (57,67)	48 (37,63)
Sex (F)	424 (53%)	162 (59%)	895 (54%)	324 (58%)
BMI (kg/m^2)	27.5 (24.4, 31.1)	25.8 (22.9, 29.2)	27.2 (24.8, 30.1)	26.4 (23.0, 29.9)
Waist circumference (cm)	94.3 (83.8, 104.2)	92.0 (83.0, 104.0)	-	86.0 (76.0, 97.0)
Visceral fat (%)	-	-	-	3.8 (2.1, 6.1)
Fat mass index (kg/m^2)	-	-	-	8.8 (6.8, 11.2)
Trunk-to-limb fat mass ratio	-	-	-	1.3 (1.1, 1.7)
Android-to-gynoid fat mass ratio	-	-	-	0.6 (0.4, 0.8)
HbA1c (%)	5.5 (5.1, 5.7)	-	5.6 (5.4, 6.0)	5.4 (5.1, 5.7)
Diabetes	69 (9%)	19 (7%)	240 (15%)	47 (13%)
Systolic blood pressure (mm Hg)	123.0 (113.5, 134.2)	-	140.0 (130.0, 150.0)	123.0 (111.0, 135.5)
Diastolic blood pressure (mm Hg)	75.5 (67.5, 80.5)	-	80.0 (80.0, 90.0)	81.3 (75.0, 88.5)
Hypertension	380 (47%)	46 (17%)	904 (54%)	173 (39%)
Cholesterol (mg/dl)	210.7 (183.0, 237.0)	206.0 (186.0, 235.0)	226.4 (195.9, 257.2)	185.0 (162.0, 213.0)
LDL-C (mg/dl)	127 (99.9, 151.0)	-	-	119.5 (96.9, 142.3)
Education[a]				
I	20 (3%)	112 (42%)	1218 (74%)	17 (3%)
II	389 (48%)	96 (36%)	245 (15%)	259 (46%)
III	396 (49%)	57 (22%)	175 (11%)	282 (51%)
Alcohol consumption[b]				
Abstainer	216 (27%)	45 (16%)	558 (34%)	74 (13%)
I	437 (54%)	199 (72%)	906 (54%)	240 (43%)
II	100 (12%)	27 (10%)	139 (8%)	171 (31%)
III	52 (7%)	6 (2%)	64 (4%)	74 (13%)
Smoking				
Never	339 (42%)	151 (55%)	817 (49%)	228 (40%)
Former	354 (44%)	55 (20%)	542 (33%)	215 (39%)
Current	112 (14%)	71 (26%)	308 (19%)	116 (21%)
Physical activity per week[c]				
Low	138 (17%)	139 (50%)	279 (17%)	56 (10%)
Medium	367 (46%)	76 (27%)	806 (48%)	267 (48%)
High	300 (37%)	62 (22%)	582 (35%)	236 (42%)

KORA, Cooperative Health Research in the Region of Augsburg; BVSII, Bavarian Food Consumption Survey II; ESTHER, Epidemiological Study on the Chances of Prevention, Early Detection and Optimized Therapy of Chronic Diseases in the Elderly Population; Bialystok PLUS, Polish Longitudinal University Study; F, female; LDL-C, low-density lipoprotein cholesterol. Data are given as median (25th, 75th percentiles) or count (percentage).

[a]I: ≤ 9 years, II: 10-11 years, III: ≥ 12 years.

[b]KORA-Fit, BVSII, ESTHER: I: 0-19.9 g/day (females), 0-39.9 g/day (males), II: 20-39.9 g/day (females), 40-59.9 g/day (males), III: ≥ 40 g/day (females), ≥ 60 g/day (males); Bialystok PLUS: I: monthly, II: weekly, III: > 2 days/week.

[c]KORA-Fit: 0h, 1h, ≥ 2h; BVSII: 0h, 1-3h, > 3h; ESTHER: 0h, 1h, ≥ 2h; Bialystok PLUS: Low, medium, high.

We conducted a mediation analysis in the replicated associations using HbA1C measurements as well as self-reported diabetes diagnosis. The effect of adiposity on the circulating level of FGF-21, HGF, IL-18R1, IL-6, SCF, and SLAMF1 was partially mediated by HbA1C in the KORA-Fit, ESTHER, and Bialystok PLUS studies (mediation effect FDR-adjusted P value < 0.05, Supplementary Table 13). The proportions of mediated effects were between 0.01 and 0.32, with the highest proportions corresponding to IL18R1 and IL-6. Diabetes partially mediated the associations with FGF-21, IL-6, and VEGFA in the Bialystok PLUS study and the associations with HGF, IL-18R1, and SLAMF1 in the KORA-Fit and ESTHER studies (Supplementary Table 14).

DISCUSSION

In this study, we explored the relationship between different measures of adiposity and the circulating level of 72 inflammation-related proteins in 3,308 participants of four independent population-based studies. We identified four novel associations with DNER, RANKL, CSF-1, and SLAMF1, and confirmed several associations that have been reported in previous studies. Furthermore, we validated our results using accurate measurements of body fat mass obtained by DXA.

In addition to confirming well-known associations such as IL-6, HGF, and FGF-21,[3,15] we identified associations with some chemokines of the CC family, which are a particular class of chemoattractants mainly

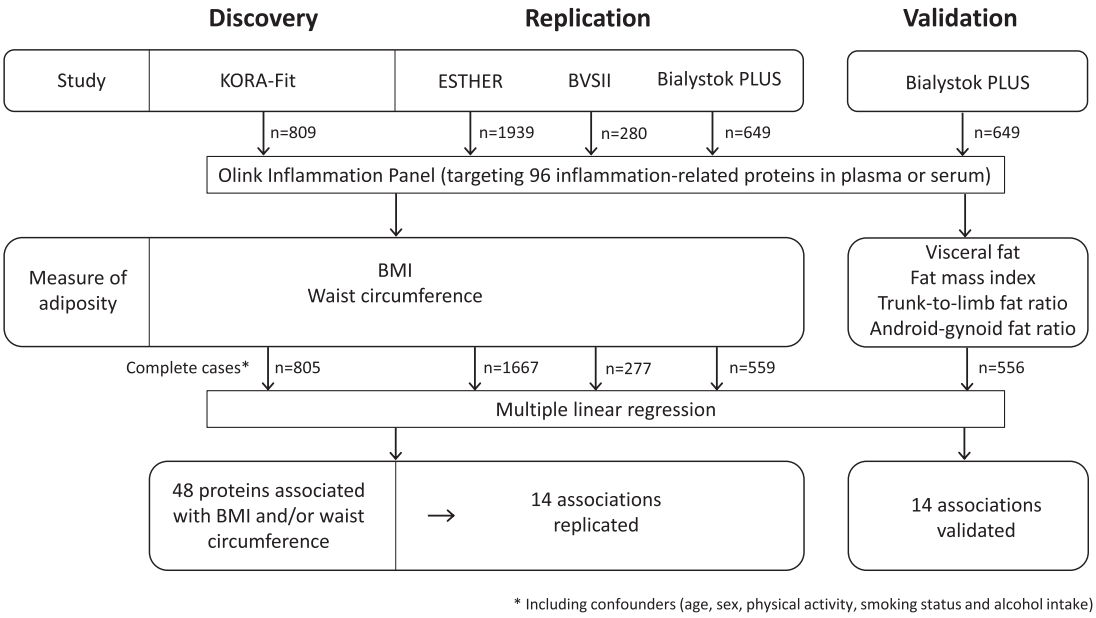

Fig 1. Flowchart showing the study design. KORA, Cooperative Health Research in the Region of Augsburg; BVSII, Bavarian Food Consumption Survey II; ESTHER, Epidemiological Study on the Chances of Prevention, Early Detection and Optimized Therapy of Chronic Diseases in the Elderly Population; Bialystok PLUS, Polish Longitudinal University Study

involved in the migration of immune cells. CCL28 is a chemokine constitutively expressed in various tissues and primarily involved in mucosal and epithelial immunity.[16] A recent study involving a sample of Japanese adults (n = 774) was the first to report an association between obesity and decreased plasma levels of CCL28.[17] The authors investigated 110 circulating biomarkers in relation to the metabolic syndrome and its components. CCL28 was uniquely associated with BMI. To our knowledge, our study is the first to confirm this association in a large European population. In addition, CCL28 was one of only 2 proteins in our study that was not associated with fat accumulation in the trunk or the abdomen, a phenotype frequently associated with the metabolic syndrome.

We also found associations with CCL2, CCL3, CCL20, and CCL19, although only the association with CC19 was consistent across all study samples and with previous literature.[17,18] It is worth noting that CCL2/MCP-1 expression is known to be increased in adipose tissue of people with obesity;[19] however, it is not clear to which extent this contributes to its systemic levels.[20–22] Consequently, there are conflicting results regarding the effect of obesity on circulating levels of this chemokine.[5,23–29] In our study, MCP-1 levels were associated with adiposity in only three of the four study samples.

In agreement with previous reports, we found associations with IL-10RB, IL-18, IL-18R1, VEGF-A, and SCF. IL-10RB is a subunit of the IL-10, IL-22, and IL-26 receptor complexes and is constitutively expressed in most cells and tissues.[30] Although there is scarce information about circulating levels of IL-10RB, animal studies have shown that IL-10RB is expressed in adipose tissue and that its expression is increased in obesity.[31] IL-18 is a pro-inflammatory cytokine and IL-18R1/IL-18RA is a subunit of its receptor, existing in membrane-bound and soluble forms. VEGF-A is an angiogenic factor with key functions in tissue remodeling and is implicated in the expansion of adipose tissue.[32] Circulating levels of IL-18, IL-18R1, and VEGF-A have been reported to be positively associated with obesity and the metabolic syndrome.[17,33–35]

SCF, also known as kit ligand, is an important cytokine participating in the regulation of several stem cell and progenitor lineages,[36] Björkbacka et al. recently reported an inverse association between plasma levels of SCF and the risk of cardiovascular events and death in participants of the Malmö Diet and Cancer Study. Moreover, they also reported a negative association with BMI.[37] In conflict with this report and our results, Huang, Z. et al. reported a positive correlation between SCF serum levels and BMI in mice and humans.[38] This discrepancy in the direction of the association is most likely due to the small sample size (n = 12) of the study conducted by Huang, Z. et al.

Novel associations between adiposity and inflammation-related proteins. In the present study, we

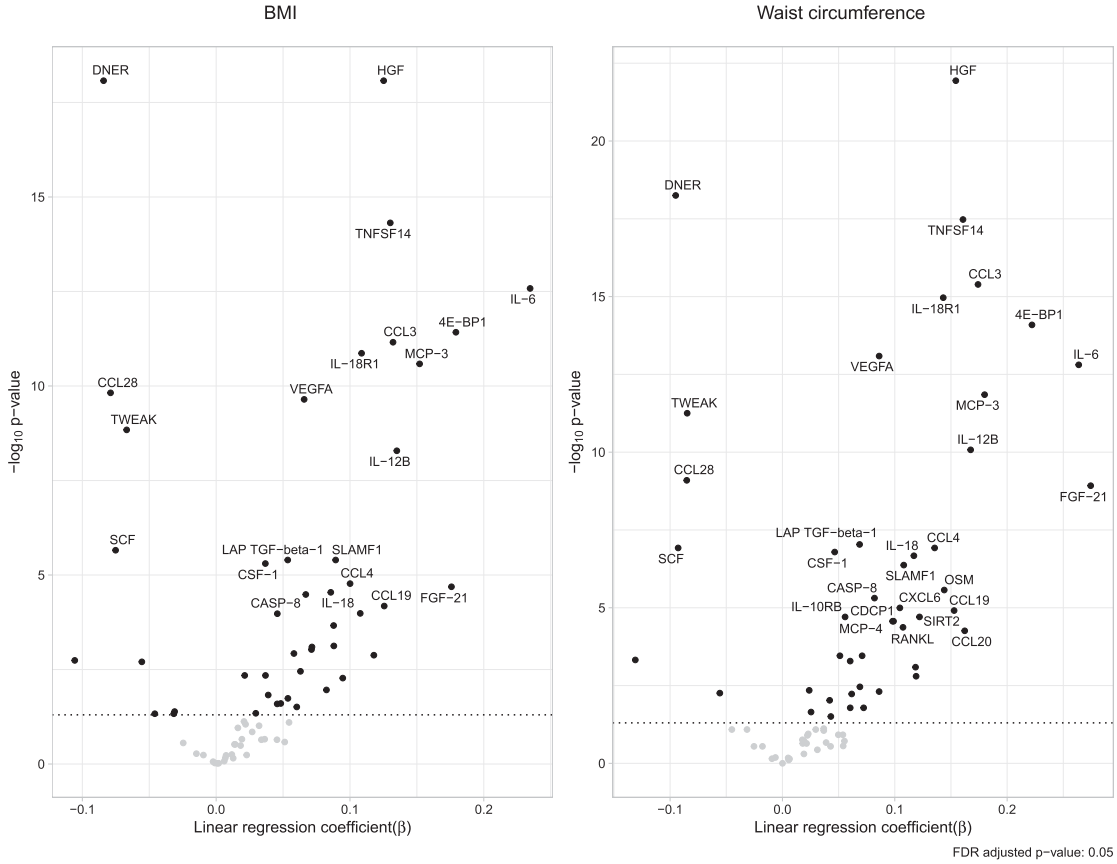

Fig 2. Volcano plots showing results from multiple linear regression analyses for BMI and waist circumference in the KORA-Fit study. Grey, non-significant association; black, significant association; FDR, false discovery rate. Protein names are shown when P value < 0.0001. Linear regression models were adjusted for age, sex, physical activity, smoking, and alcohol intake. β-coefficients are interpreted as the change in normalized protein expression (NPX) per one standard deviation change in waist circumference or BMI. Complete protein names can be found in Supplementary Table 1

identified novel associations between measures of adiposity and DNER, SLAMF1, RANKL, and CSF-1. RANKL, also known as TRANCE, and CSF-1 are both cytokines essential for osteoclast differentiation, produced mainly by osteoblasts in membrane-bound and soluble forms.[39–41] Interestingly, it has been suggested that obesity may affect bone resorption through adipocyte-derived cytokines, such as leptin, via the RANKL-RANK-OPG pathway.[42–44] Animal studies have shown that increased RANKL expression, induced by adipocytes, leads to increased osteoclastogenesis and bone density abnormalities.[45,46] Our study is the first to report an association between adiposity and circulating levels of RANKL in a large population-based study. Recently, a small study using weight discordant twins (n = 43) reported higher values of RANKL in the twin with higher adiposity, which corroborates our finding.[47]

CSF-1, in addition to its role in osteoclast differentiation, has been reported to participate in adipocyte metabolism and hyperplasia, and is essential for the proliferation, differentiation, and survival of macrophages.[48,49] Circulating CSF-1 is produced by endothelial cells in blood vessels.[50] In mice, CSF-1 is selectively cleared from circulation through receptor-mediated endocytosis by mature macrophages, which is thought to be a mechanism to control macrophage production.[51] High circulating CSF-1 levels could, therefore, reflect an impairment of this mechanism. CSF-1 circulating levels correlate with insulin resistance and its activity has been proposed as a therapeutic target.[52,53] An increased systemic concentration of CSF-1 has also been described in several types of cancer.[54,55] but not in obesity.

DNER is a transmembrane protein mainly expressed in the central nervous system (CNS), particularly in the cerebellum.[56] DNER functions outside the CNS are not

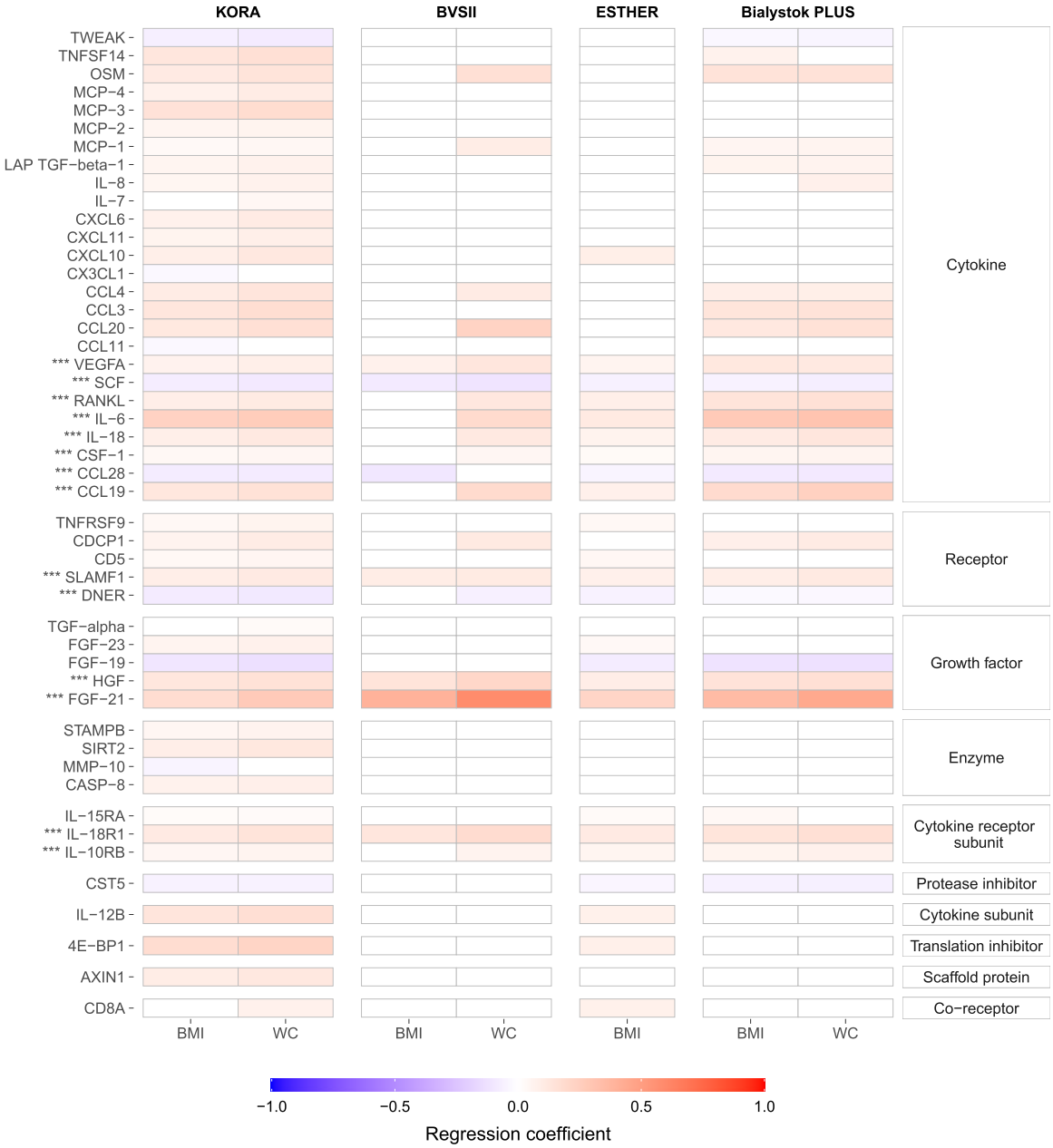

Fig 3. Heatmap showing significant associations between BMI and/or waist circumference and protein levels in the discovery (KORA-Fit) and the replication (BVSII, ESTHER and Bialystok PLUS) study samples. WC, waist circumference. *** Proteins for which an association with BMI and/or waist circumference was found across all study samples. Associations were considered significant when FDR-adjusted P values < 0.05. Regression coefficients, from multiple linear regression analyses, are displayed as colors ranging from red (positive) to blue (negative). Linear regression models were adjusted for age, sex, physical activity, smoking, and alcohol intake. Regression coefficients are interpreted as the change in normalized protein expression (NPX) per one standard deviation change in waist circumference or BMI. Proteins are grouped by molecular function

known. Circulating levels of DNER were recently identified as an independent risk marker for incident distal sensorimotor polyneuropathy in type 2 diabetes, although the finding has not been replicated.[57] No other reports have linked circulating DNER levels to adiposity. SLAMF1 is a member of the SLAM family of receptors and exists in transmembrane and soluble forms. It is involved in the innate inflammatory response and, in the membrane-bound form, serves as the measles virus receptor.[58] Elevated circulating

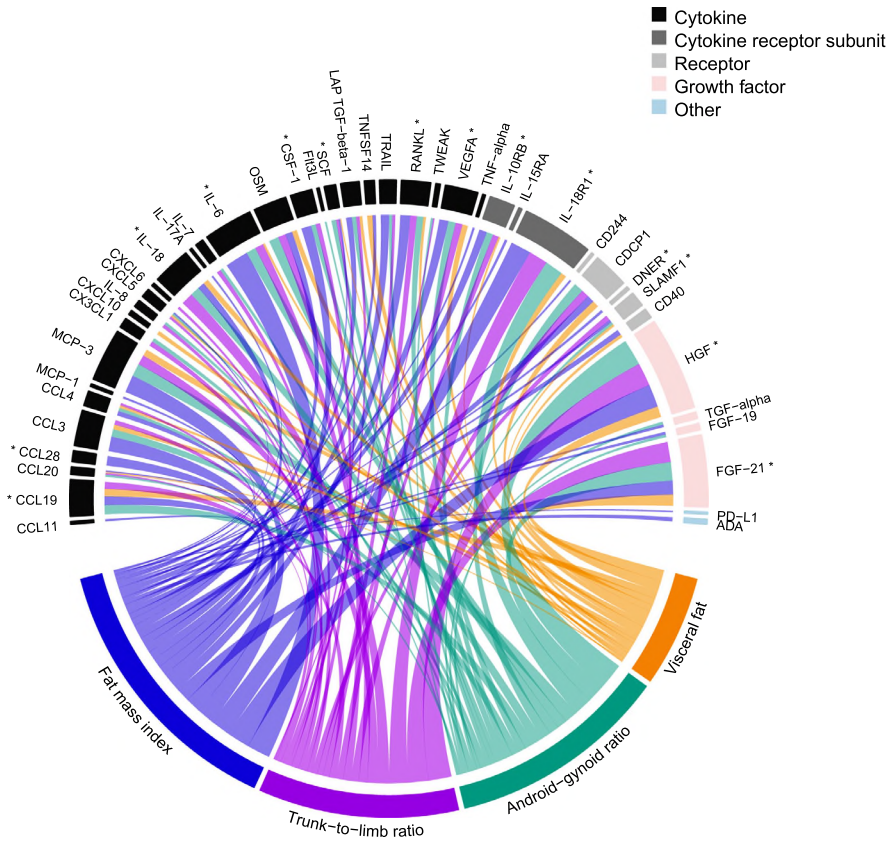

Fig 4. Chord diagram showing significant associations between DXA-derived variables and circulating protein levels. All associations are positive except for CCL11, CCL28, CX3CL1, DNER; FGF-19, IL-17A, SCF, and TWEAK. Connection width illustrates the proportion of variance in protein levels explained by the corresponding variable. Proteins marked with an asterisk were associated with anthropometric measures of adiposity in the KORA-Fit, BVSII, ESTHER, and Bialystok-PLUS studies

levels of the soluble form have not been reported in relation to obesity, although there are studies linking it to type 2 diabetes and several autoimmune diseases.[59,60]

It should be noted that cytokine activity is tightly regulated at different levels. After production, their activity can be modified by the presence of decoy receptors, receptor antagonists, and intracellular signaling inhibitors.[61] Thus, altered levels of cytokines in circulation do not necessarily translate into altered activity.

Inflammation related proteins and body fat distribution. Fat accumulation in the abdominal area is known to be associated with higher cardiometabolic risk compared to fat deposition in the lower body and limbs. Likewise, visceral adipose tissue has been linked to metabolic disease and low-grade inflammation.[3] In line with these observations, we found that the majority of the investigated proteins were associated with fat mass in the trunk and abdominal areas, as well as with visceral adipose tissue.

Strengths and limitations. Previous studies have investigated a limited number of inflammation-related proteins in smaller samples. In this relatively large population-based study, we explored an extensive set of proteins with a broad range of molecular functions. Furthermore, we replicated our results in 3 independent studies and used a highly sensitive and specific PEA-based technique for protein measurement. In addition, we used DXA-derived fat mass measurements that allowed us to carry out a comprehensive assessment of body fat distribution and explore its relationship with inflammation. The limitations of our study include the cross-sectional design and the use of single time-point (non-)fasting plasma samples. There is evidence of diurnal variation in the concentration of certain cytokines and inflammatory proteins.[62] and therefore protein measurements may reflect biased protein levels of study participants. This study was conducted in populations that include a majority of participants of European descent and it is possible that the findings are not

applicable to other populations of different ethnic compositions.

Conclusions. We have identified and replicated several associations, of which four are novel, between adiposity and the circulating level of inflammation-related proteins in four independent European population-based studies. Our results provide new insights into the immune dysregulation that accompanies obesity. The further characterization of the novel associations described in this study holds the potential to help us uncover new mechanisms that lead to disease development during obesity.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The studies were approved by the Ethics Committees of the Bavarian Medical Association (KORA), the University of Heidelberg/Medical Association of Saarland (ESTHER), the Bavarian Ministry of Health (BVSII), and the Medical University of Bialystok (Bialystok PLUS). All study participants provided written informed consent.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR'S CONTRIBUTIONS

Conceptualization: CM, SB, MP; formal analysis: MP; writing - original Draft: MP; writing - review & editing: all authors; resources and data curation: JL, AP, HG, HB, BS, KK, MP, IK, BL, MH, KT, MB, XG.

AUTHORSHIP AGREEMENT

All authors have read the journal's authorship agreement. The manuscript has been read and approved by all named authors.

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The authors declare that they have no competing interests. All authors have read the journal's policy on disclosure of potential conflicts of interest.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.trsl.2021.11.004.

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