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

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1. Introduction

In a healthy state, lipid storage occurs in defined adipose tissue depots throughout the body. During obesity, increased demands for energy storage lead to the expansion of adipose tissue depots and ectopic lipid accumulation in certain organs such as the pancreas and the liver. Fat accumulation in the liver, in the absence of significant alcohol consumption or secondary causes, has been widely studied and is recognized as a spectrum of diseases collectively called non-alcoholic fatty liver disease (NAFLD) [1]. Similarly, the term non-alcoholic fatty pancreas disease (NAFPD) has been recently adopted to describe obesity-associated pancreatic fat infiltration and its clinical implications [2].

Changes in adipose tissue function during obesity are characterized by local and systemic inflammation, which have profound effects on the overall metabolic state and the function of various organs. In the liver, fat accumulation can promote insulin resistance, local inflammation, hepatocyte damage, and fibrosis [3]. In addi-

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tion to local inflammation, NAFLD also causes systemic alterations of several markers of inflammation [4]. In contrast, the relationship between pancreatic fat accumulation and systemic inflammation remains largely unknown; only a few studies, focusing on a small set of inflammatory biomarkers, have been conducted [5,6].

In this study, we used data from the population-based Study of Health in Pomerania (SHIP) to investigate the association between fat accumulation and the circulating level of a panel of 37 inflammatory biomarkers. This panel included tumor necrosis factor (TNF) superfamily proteins, interferon (IFN) family proteins, regulatory T cell cytokines, and matrix metalloproteinases (MMPs). Pancreatic and hepatic fat as well as abdominal subcutaneous and visceral adipose tissue (SAT and VAT) were quantified by magnetic resonance imaging (MRI).

2. Materials and methods

2.1. Study sample

SHIP is an ongoing population-based project in Germany to assess the prevalence and the incidence of common risk factors, subclinical disorders, and clinical diseases and to investigate their associations [7]. The SHIP project consists of two population-based cohorts: SHIP and SHIP-Trend. For SHIP-Trend, a random stratified sample of 8 826 adults aged 20–79 years was drawn from population registries. In total, 4 420 subjects participated in the baseline data collection from 2008 to 2012 (response 50.1%). The baseline examinations included blood sampling and a computer-assisted interview.

A sample of 1000 subjects of those who had participated in the oral glucose tolerance test (OGTT, carried out on the examination day) was selected for molecular phenotyping. The molecular phenotyping included the measurement of biomarkers of inflammation (Bio-Plex Pro^{TM} Human Inflammation Panel) for which 503 subjects were selected. This subsample was selected based on high-sensitivity C-reactive protein (hs-CRP), leucocyte count, and fibrinogen concentrations, to obtain a maximally broad distribution of these parameters (Supplementary Fig. 1). Participants with excessive alcohol consumption were excluded from the analysis (men $>$ 30 g/day, women $>$ 20 g/day; $n = 34$). The exclusion criteria for the OGTT included known (self-reported) diabetes. Therefore, the study sample is composed of participants without known diabetes. Participants of the OGTT were required to fast for at least 8 h before blood sampling. Nighty one of the participants adhered to this requirement. None of the participants in the study sample reported having any type of hepatitis in the 12 months prior to the examination.

The study followed the recommendations of the Declaration of Helsinki and was approved by the ethics committee of the University of Greifswald. Written informed consent was obtained from all participants.

2.2. MRI measurements

The examination protocol of whole-body MRI for SHIP-Trend participants has been previously described in detail [8]. Briefly, examinations were performed in a 1.5-Tesla system (Magnetom Avanto, Siemens Healthcare AG, Erlangen, Germany). Abdominal visceral and subcutaneous adipose tissue volumes (VAT, SAT) were quantified using ATLAS (Automatic Tissue and Labeling Analysis Software) [9]. The upper and lower borders for the abdominal fat analysis were the left diaphragm and the bladder, respectively. The main examination protocol included a 3D gradient-echo chemical shift-encoded pulse sequence with water and/or fat separation, which covered all upper abdominal organs. Pancreatic and hepatic fat measurements are described in detail elsewhere [10,11]. In short, proton density fat fraction (PDFF) maps were reconstructed using Matlab (R2011a; Mathworks, Natick, Mass). In the case of the liver, R2* relaxation rate (a parameter used to assess liver iron concentration) maps were reconstructed simultaneously. PDFFs were corrected for all known confounders before the measurement of PDFF from the reconstructed images. In addition, because fat, water and R2* were estimated simultaneously in the liver, R2* was corrected for the presence of fat and fat was corrected for R2* signal decay. All measurements in the regions of interest were performed with Osirix (versions 3.9.1 [pancreas] and 4.6 [liver]; Pixmeo Sarl, Bernex, Switzerland). Regions of interest were placed in the head, body, and tail of the pancreas and on the entire liver. Pancreatic and hepatic fat fractions are reported in percentages.

2.3. Whole-body fat measurements

Whole-body fat was assessed using Body Impedance Analysis (BIA) with a Nutriguard M device (Data Input GmbH, Darmstadt, Germany). Source and sensor electrodes were placed on the dorsum of the hand and foot of the dominant body side. Body fat was automatically calculated using NutriPlus software (Data Input GmbH, Darmstadt, Germany) [7].

2.4. Protein measurements

Inflammatory biomarkers were measured in EDTA plasma using the Bio-Plex Pro^{TM} Human Inflammation Panel 1 magnetic bead-based assay (Bio-Rad, USA). Briefly, cytokine assay plates were prewet with assay buffer and washed twice with wash buffer. 50 µl of the coupled magnetic bead mixture, along with serial dilutions of the reconstituted standard, blanks, controls, and plasma samples (diluted 1:4) were added to each well of a 96-well plate. This mixture was incubated overnight in the dark, at room temperature on a shaker (850 rpm) and then washed three times. The detection antibody mixture was added to the wells for an additional 30-min incubation step (room temperature, 850 rpm) followed by washing three times. Streptavidin-PE was added and the plates were incubated for 10 min (room temperature, 850 rpm) and washed three times. Measurements were carried out on a FLEXMAP 3D® (Luminex Corp) instrument using xPONENT v4.2 software. The quantitative analysis was performed using a 5P-logistic regression model on the standard dilution curve data to calculate the absolute quantitative data of the samples. The median coefficient of variation was 7.7%. Measurements were done in six batches, differences between the plates were corrected using median normalization. After stringent quality control (including the number of missing values, stability along the plates, and the number of extrapolated values) data of 31 cytokines were kept for further analyses (Supplementary Table 1).

2.5. Covariates

The measurement of variables used as covariates in this study has been described previously [12]. Participants underwent an extensive computer-aided personal interview for the collection of socio-demographic characteristics and medical history. Participants were defined as physically inactive if they exercised for less than one hour per week during leisure time (summer and winter). Alcohol consumption was determined based on the drinking behavior in the previous month and is reported as grams of pure ethanol per day. Smoking status is reported as current, ex-, and never-smoker.

2.6. Statistical analysis

Multiple ordinary least square (OLS) regression models were used to assess the association between MRI-derived fat

measurements (exposures) and biomarker levels (outcome variables). Biomarker concentrations were log2-transformed and all fat measurements were scaled to a mean of 0 and SD of 1. All models were adjusted for the following potential confounders: sex, age, physical activity, alcohol intake, and smoking status. Position of the sample on the 96-well plate (for biomarker quantification) and platelet count were additionally included as covariates in all models since preliminary analyses showed an influence on biomarker concentrations. Biomarker variance explained by fat content in each depot was calculated as the change in adjusted R^2 when the respective fat measurement was added to a model including only covariates. Potential effect-measure modification by sex of significant associations was tested using multiplicative interaction terms. MRI-derived fat measurements were adjusted for whole-body fat by using the residual method [13]. For this purpose, MRI fat measurements were individually regressed on whole-body fat percentage, the residuals from each regression were extracted and used as exposures in the main regression models.

We performed three additional analyses. First, we used robust regression models using an M estimator [14] to overcome possible violations of the OLS-model assumptions. Second, we used multiple imputation by chained equations [15] to test possible violations of the missing completely at random assumption underlying the primary complete-case analysis. Finally, we excluded participants based on medication intake (platelet aggregation inhibitors, HMG-CoA reductase, lipid-modifying agents, anti-inflammatory and antirheumatic medication, sex hormones, and modulators of the genital system and antihypertensives; Supplementary Table 9). P values and confidence intervals were adjusted for multiple testing using the Benjamini & Hochberg (False Discovery Rate, FDR) and the Benjamini & Yekutieli procedures [16] (False Coverage Rate, FCR), respectively. Associations with P values ≤ 0.05 were considered significant.

Analyses were performed using R software R-4.0.4.

3. Results

The study sample included 203 men and 266 women with a median age of 49 years. Characteristics of participants by sex are presented in Table 1. Biomarker concentrations are presented in Supplementary Table 1. Hepatic fat was associated with MMP-2, PTX3, and TNFSF12; whereas pancreatic fat was associated with CHI3L1, sCD163, sTNFR1, and sTNFR2. VAT and SAT were associated with sCD163, MMP-2, OSTCN, sTNFR1, sTNFR2, TNFSF12, and TNFSF14. VAT was additionally associated with CHI3L1 and TNFSF13B (Fig. 1A, Supplementary Tables 2–5).

Although some biomarkers were associated with several fat depots, there were differences in the amount of biomarker variance explained by each (Supplementary Fig. 2). The association between VAT and sTNFR1 was significantly stronger in women compared to men (interaction P value < 0.05; Supplementary Fig. 3). No other sex differences were detected.

Additional analyses using regional pancreatic fat measurements revealed no associations between fat content in the head of the pancreas and inflammatory biomarkers, while fat content in both the body and the tail showed a positive association with sCD163, sTNFR1, and sTNFR2 (Fig. 1B, Supplementary Tables 6–8). To assess the effect of each fat depot independently of whole-body fat we calculated adjusted values for each fat depot using the residual method. Some associations with SAT (OSTCN, sTNFR1, and TNFSF14) and VAT (CHI3L1, TNFSF13B, and TNFSF14) were no longer significant after the adjustment. The associations between pancreatic fat with sCD163, sTNFR1, and sTNFR2, as well as those between hepatic fat with MMP-2 and PTX3 survived the adjustment (Supplementary Tables 2-8).

The analyses using robust regression and multiple imputation led to results similar to those of the primary analyses; no changes in effect direction and only minor changes in effect sizes were observed. When using robust regression, all of the previously described associations remained significant (data not shown). In the case of imputed data, the associations between pancreatic fat and CHI3L1 as well as those between pancreatic fat (body) and sCD163 and sTNFR2 were no longer significant. None of the associations involving fat in the tail of the pancreas remained significant (Supplementary Tables 2-8).

Finally, we performed a sensitivity analysis excluding participants taking medications that could potentially affect inflammatory biomarkers. Medication intake in the study sample is shown in Supplementary Table 9. Most of the results remained unaffected by the exclusions. However, the exclusion of participants taking antihypertensives (27%) led to several changes in the results (Supplementary Table 10). These changes could also be explained by the loss of statistical power.

4. Discussion

In this study, we explored the associations between abdominal fat depots (SAT, VAT), pancreatic, and hepatic fat and 31 key circulating biomarkers of inflammation in a non-diabetic population-based sample. We report novel associations for pancreatic fat: sTNFR1, sTNFR2, and sCD163. After accounting for whole-body fat, VAT and SAT were associated with the largest number of biomarkers.

The soluble forms of TNFR1 and TNFR2 are the two known receptors of TNF-α and result from shedding of their respective transmembrane forms or from alternative splicing. Although TNF-α exerts its main effects by binding the membrane-bound receptor forms, several studies suggest that soluble forms play an important role in the modulation of its activity by acting as inhibitors [17]. Here, we show that the circulating level of both receptors increases with the accumulation of VAT, SAT, and pancreatic fat. The association between soluble TNF-α receptors and obesity was first reported at least two decades ago [18]. Several studies have corroborated these early reports, although there are still inconsistencies concerning the relative contribution of VAT and SAT to the circulating levels of both receptors [19,20]. On the other hand, there are no previous reports on an association between pancreatic fat and sTNFR1 and sTNFR2. Further studies are needed to confirm both associations and to investigate the production and secretion of soluble forms of TNF-α receptors in the pancreas.

We found associations with three members of the TNF ligand superfamily: TNFSF12, TNFSF13B, and TNFSF14. TNFSF13B, also known as BAFF, is involved in adipocyte differentiation and function and seems to be a regulator of weight gain [21]. Reports about its circulating levels in obesity are scarce. However, our results showing a positive association with VAT are in agreement with a recent study that found increased plasma levels of TNFSF13B in obese patients without type 2 diabetes [22]. TNFSF14 and TNFSF12, also known as LIGHT and TWEAK, respectively, are cytokines that have been shown to inhibit human adipose tissue differentiation without altering metabolic functions, such as glucose uptake and lipolysis [23,24]. This distinguishes their activity from that of TNF-α, which is also implicated in adipocyte biology but has an important effect on metabolism, promoting insulin resistance. Interestingly, soluble TNFSF14 and TNFSF12 circulating levels show opposite trends in obesity. Several studies have shown reduced TNFSF12 levels in obesity, as well as in other diseases with increased cardiovascular risk [25], whereas TNFSF14 levels seem to be elevated [26,27]. It has been proposed that TNFSF14 may have a protective role and that increased levels in obesity

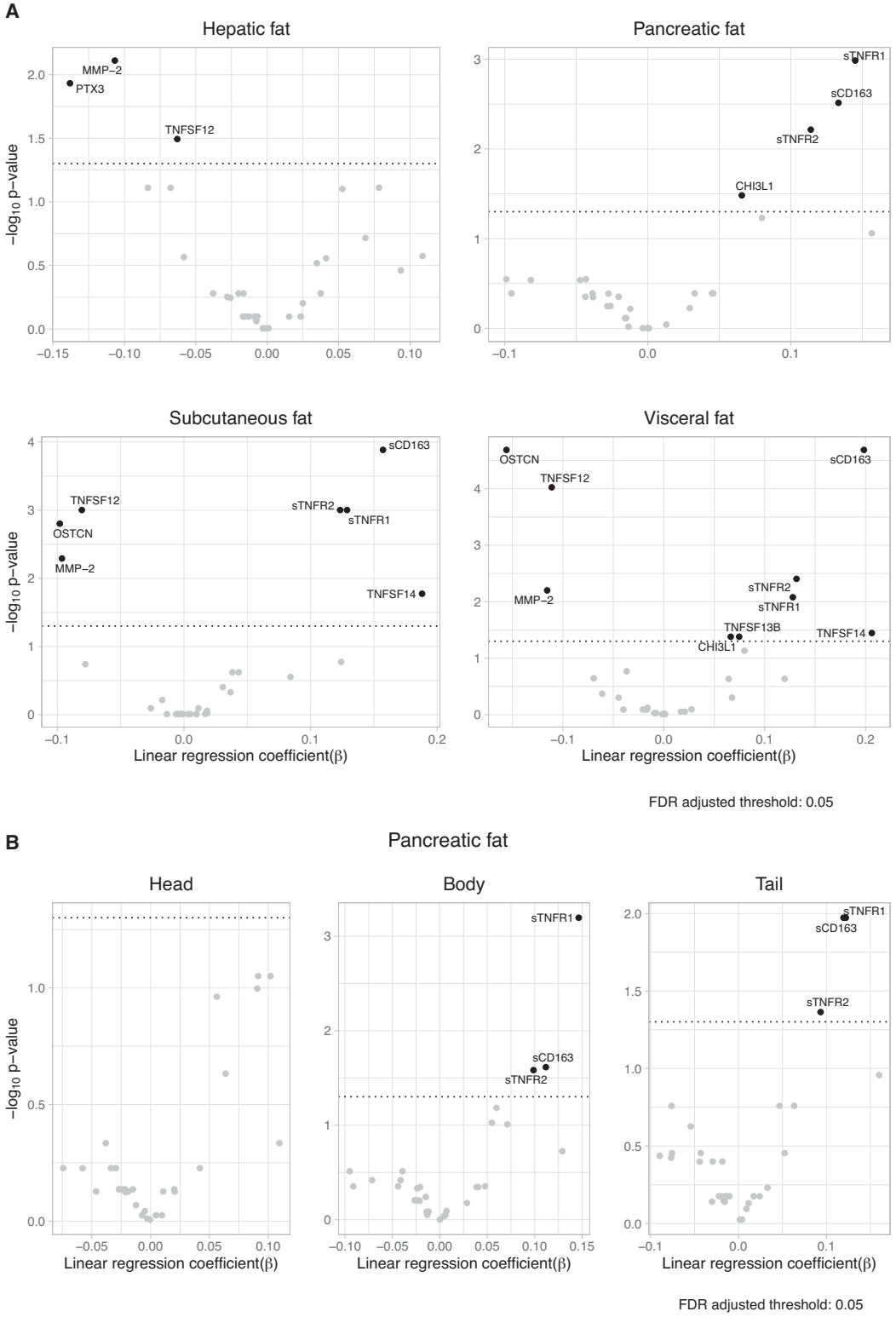

Fig. 1. Volcano plots showing results from multiple linear regression between fat content and biomarkers of inflammation for (A) liver, pancreas, subcutaneous and visceral fat, and (B) pancreatic regions. FDR, false discovery rate. Gray, non-significant association; black, significant association. All models were adjusted for age, sex, physical activity, smoking, alcohol, platelet count, and sample position in the Bio-plex plate. β-coefficients are interpreted as the change in biomarker concentration (log2 transformed) per 1 standard deviation increase in fat content.

Table 1
Study sample characteristics.

	Men (n = 203)	Women (n = 266)	Overall (n = 469)
Age (years)	49.0 [40.0, 61.5]	49.0 [40.0, 59.0]	49.0 [40.0, 60.0]
Alcohol (g/day)	6.93 [2.82, 13.2]	2.56 [0.701, 5.66]	3.92 [1.09, 8.68]
Physical activity			
Inactive	54 (26.6%)	68 (25.6%)	122 (26.0%)
Active	149 (73.4%)	198 (74.4%)	347 (74.0%)
Smoking status			
Never	71 (35.0%)	141 (53.0%)	212 (45.2%)
Former	89 (43.8%)	69 (25.9%)	158 (33.7%)
Current	43 (21.2%)	56 (21.1%)	99 (21.1%)
Total body fat (%)	22.9 [20.3, 27.2]	33.7 [28.7, 38.6]	28.8 [23.5, 35.1]
Missing	1 (0.5%)	5 (1.9%)	6 (1.3%)
HbA1c (%)	5.20 [4.90, 5.50]	5.10 [4.80, 5.40]	5.20 [4.80, 5.50]
eGFR (ml/min/1.73m^2)	107 [97.8, 116]	118 [109, 128]	114 [103, 124]
ALT (µmol/L·s)	0.450 [0.350, 0.625]	0.300 [0.240, 0.420]	0.360 [0.270, 0.510]
AST (µmol/L·s)	0.310 [0.255, 0.390]	0.270 [0.210, 0.320]	0.290 [0.220, 0.360]
Hepatic fat (%)	4.43 [2.38, 7.05]	2.95 [2.12, 5.82]	3.49 [2.20, 6.59]
Missing	45 (22.2%)	48 (18.0%)	93 (19.8%)
Pancreatic fat (%)	6.88 [5.38, 8.62]	6.27 [4.59, 7.83]	6.62 [4.96, 8.22]
Missing	49 (24.1%)	53 (19.9%)	102 (21.7%)
Abdominal subcutaneous fat (L)	5.88 [4.74, 8.05]	8.20 [6.18, 11.3]	7.37 [5.35, 9.79]
Missing	30 (14.8%)	35 (13.2%)	65 (13.9%)
Visceral fat (L)	4.72 [3.02, 6.46]	2.44 [1.29, 3.81]	3.33 [1.78, 5.16]
Missing	30 (14.8%)	35 (13.2%)	65 (13.9%)

HbA1c, glycated hemoglobin; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
Data are given as median [25th, 75th percentiles] or counts (percentage).

could be part of a compensatory mechanism [28]. Our results are in agreement with these observations. We found positive associations between SAT and VAT and TNFSF14 and inverse associations between SAT and VAT and TNFSF12. In addition, we also report an inverse association between hepatic fat and TNFSF12, which is in line with a recent study conducted in severely obese patients screened for NAFLD [29]. In that study, Lozano-Bartolomé et al. observed reduced TNFSF12 concentrations in NAFLD and showed that TNFSF12 reduces lipid accumulation in human liver cells *in vitro*. Other studies suggest that the TNFSF12 pathway is involved in the progression of NAFLD [30] and report increased expression of the TNFSF12 receptor *TNFRSF12A* in the liver in the more severe forms of the disease spectrum [31]. These paradoxical observations are not well understood and the role of TNFSF12 in metabolic diseases remains unclear. Several hypotheses have emerged trying to explain its inverse association with obesity and liver steatosis; one of these hypotheses suggests that circulating TNFSF12 is internalized by CD163-expressing macrophages, which would result in a decreased plasma concentration [25].

CD163 is a scavenger membrane receptor exclusively expressed by monocytes and macrophages. Its soluble form, sCD163, mainly originates from the constitutive shedding of the extracellular part of the receptor and is found in various fluids of healthy subjects. Soluble CD163 circulating levels vary depending on the membrane-bound CD163 expression as well as the shedding and clearance rates. Increased shedding has been observed following macrophage activation in response to inflammatory stimuli [32]. Although no specific function has been described, it is recognized as a specific marker of macrophage activation and can act as a decoy receptor for TNFSF12 [25]. In our study, sCD163 levels were positively associated with SAT, VAT, and pancreatic fat. Our results regarding abdominal fat depots are consistent with several previous studies [33–35] that report increased levels in obesity. However, several studies also report an association with hepatic fat [36,37], which we, and others [38,39], were unable to detect. Previously studied samples differed in the grade of obesity, the presence of comorbidities, and the degree of NAFLD, which could explain the discrepant results. It is, nevertheless, recognized that sCD163 levels correlate with fibrosis stage, the NAFLD activity score, and hepatic inflammation observed in other liver diseases [32,37,40]. sCD163 is a marker of acute pancreatitis [41] and a predictor of type 2 diabetes risk [42], but an association with pancreatic fat has not been reported. Given the close relationship between pancreatic fat and abdominal fat depots, further studies are needed to corroborate this finding.

Furthermore, we found that hepatic fat was inversely associated with PTX3. Previous studies concerning obesity and hepatic fat are inconsistent. Both inverse [43–45] and positive [46] associations with obesity have been reported, as well as an increase in PTX3 levels upon weight loss [43,46]. Given the increased frequency of hepatic steatosis in subjects with obesity and the fact that some of the studies have been conducted in small patient samples, it could be hypothesized that these associations are driven by hepatic fat rather than obesity. However, at least one large population-based study reported an inverse association with BMI [45], in addition to a community-based study which also found an inverse association with total and visceral fat mass [43]. These discrepant reports extend to the association with hepatic fat. Although the majority of the studies report higher levels of PTX3 in cases of steatohepatitis compared to simple steatosis, not all studies have found a difference between patients with simple steatosis and controls [47–50]. The reason for these inconsistent observations, and our contrasting findings, is not yet clear. PTX3 is a secreted acute-phase protein of the pentraxin family, which includes the well-known C-reactive protein (CRP) mainly produced by the liver. In contrast to CPR, PTX3 is produced by a variety of cell types [51]. It is therefore unknown how the different tissues producing PTX3 contribute to its circulating levels.

We also found an inverse association between VAT, SAT, and hepatic fat and MMP2. MMPs are a family of endopeptidases that participate in extracellular matrix (EMC) remodeling. Their activity is regulated at different levels and can be altered by specific tissue

inhibitors (TIMPSs). The lack of balance between MMPs and their inhibitors leads to an increased matrix deposition and has been linked to fibrosis [52]. Our findings contrast with earlier studies in which higher MMP-2 levels have been observed in NAFLD and obesity [53–55]. MMP-2 is secreted by human adipose tissue and several studies have suggested it regulates adipocyte differentiation [56]. However, it is not clear if adipose tissue contributes to circulating levels [57]. Moreover, reports on the effect of weight loss on MMP-2 levels are also inconsistent [58,59].

Finally, VAT and SAT were inversely associated with OSTCN; and VAT was positively associated with CHI3L1. Both findings are in agreement with published literature [60,61]. OSTCN is a protein secreted by osteoblasts that is important in bone remodeling and also participates in metabolism regulation [62]. Its beneficial metabolic effects in animal studies [63], including improved insulin sensitivity and lipid metabolism, indicate it might have therapeutic properties in metabolic diseases. CHI3L1, on the other hand, is a non-enzymatic member of the glycoside hydrolase 18 family produced by many cell types. It plays an important role in inflammation and tissue remodeling and its circulating levels are elevated in several diseases including several types of cancer, asthma, diabetes, and Alzheimer's disease [61].

4.1. Strengths and limitations

Strengths of our study include the use of MRI for the quantification of fat, which is currently one of the most accurate methods, and the measurement of a broad number of inflammatory biomarkers. The latter is particularly important in the case of the pancreas, given that the study of fat deposition in this organ is relatively new and that previous studies focusing on inflammation have used less than ten inflammatory markers [5]. Another strength of our study is the relatively large population-based sample, comprising participants without known diabetes. Earlier studies have used selected patient populations, especially for the study of hepatic fat in which study samples often included only NAFLD patients. Previous studies have measured fat content using liver biopsies; however, while this remains the gold standard for the diagnosis and classification of NAFLD, it is not accurate for the measurement of hepatic fat due to the uneven distribution throughout the organ. On the same note, the pancreas also shows differences in cell composition [64] as well as in patterns of fat infiltration [65] across its regions. Here, we used regional measurements of pancreatic fat in addition to total pancreatic fat, which is another strength of our study. Limitations of our study include the cross-sectional study design and the use of single-time biomarker measurements. Inflammatory biomarkers could be affected by several factors that were not investigated in this study such as stress levels and fluctuations throughout the day or the week. In addition, our study might be underpowered to investigate the effect of medication intake. Finally, the sample used for this study is a subpopulation of the SHIP-Trend study and, thus, selection bias cannot be ruled out.

5. Conclusions

Our findings revealed a complex, and sometimes paradoxical, relationship between the accumulation of fat and the circulating level of biomarkers of inflammation. The alterations observed might reflect the activation of compensatory mechanisms in addition to pro-inflammatory ones. Furthermore, our findings provide new insights into the involvement of hepatic and pancreatic fat on systemic inflammation. This is particularly important in the case of pancreatic fat, which has been poorly studied in the past.

Conflict of interest

None declared.

CRediT authorship contribution statement

Mariana Ponce-de-Leon: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Anke Hannemann:** Writing – review & editing, Resources, Data curation. **Jakob Linseisen:** Writing – review & editing. **Matthias Nauck:** Writing – review & editing, Resources, Data curation. **Markus M. Lerch:** Writing – review & editing, Resources, Data curation. **Robin Bülow:** Writing – review & editing, Resources, Data curation. **Henry Völzke:** Writing – review & editing, Resources, Data curation. **Nele Friedrich:** Writing – review & editing, Resources, Data curation. **Jan Kassubek:** Writing – review & editing, Software. **Hans-Peter Müller:** Writing – review & editing, Software. **Sebastian-Edgar Baumeister:** Conceptualization, Writing – review & editing. **Christa Meisinger:** Conceptualization, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2022.02.003.

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