Associations of Plasma CD36 and Body Fat Distribution

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Context: CD36 is a class B scavenger-receptor involved in the uptake of fatty acids in liver and adipose tissue. It is unknown whether plasma CD36 levels are related to liver fat content or adipose tissue in the general population.

Methods: We measured plasma CD36 from 575 participants of the community-based PopGen cohort who underwent MRI to quantify visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and liver signal intensity (LSI), a proxy for liver fat content. Nonalcoholic fatty liver disease (NAFLD) was defined as LSI ≥3.0 in the absence of high alcohol intake. The relations between plasma CD36 and body mass index (BMI), VAT, SAT, LSI, and NAFLD were evaluated via multivariable-adjusted linear and logistic regression analysis.

Results: Plasma CD36 concentrations were correlated with BMI ($r = 0.11$; $P = 0.01$), SAT ($r = 0.16$; $P < 0.001$), and VAT ($r = 0.15$, $P < 0.001$) but not with LSI ($P = 0.44$). In multivariable-adjusted regression models, mean BMI values rose across CD36 quartiles [quartile 1 (Q1), 27.8 kg/m^2; Q4, 28.9 kg/m^2; P-trend = 0.013). Similarly, VAT (Q1, 4.13 dm^3; Q4, 4.71 dm^3; P-trend < 0.001), and SAT (Q1, 7.61 dm^3; Q4, 8.74 dm^3; P-trend < 0.001) rose across CD36 quartiles. Plasma CD36 concentrations were unrelated to LSI (P-trend = 0.36) and NAFLD (P-trend = 0.64). Participants with NAFLD and elevated alanine aminotransferase (ALT), a marker for liver damage, had higher CD36 compared with participants with NAFLD and normal ALT.

Conclusions: Higher plasma concentrations of CD36 were associated with greater general and abdominal adiposity but not with liver fat content or NAFLD in this community-based sample. However, plasma CD36 may reflect more severe liver damage in NAFLD. (***J Clin Endocrinol Metab* 104: 4016–4023, 2019**)

The global prevalence of obesity has tripled in the past two decades (1). In 2016, >650 million adults worldwide were obese, representing 13% of the total global population (1). Up to 90% of obese patients have nonalcoholic fatty liver disease (NAFLD) (2), which represents the most common chronic liver condition, characterized by liver fat accumulation (3). Both obesity and NAFLD are risk factors for increased morbidity and mortality (*e.g.*, from type 2 diabetes, cardiovascular disease, and cancer) (4, 5). Therefore, it is of scientific importance

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Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; CRP, C-reactive protein; FLD, fatty liver disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LSI, liver signal intensity; MET, metabolic equivalent task; MRS, magnetic resonance spectroscopy; NAFLD, nonalcoholic fatty liver disease; Q, quartile; ROC, receiver operating characteristic; SAT, subcutaneous adipose tissue; TC, total cholesterol; TG, triglycerides; VAT, visceral adipose tissue; WC, waist circumference.

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to deepen our understanding of the underlying etiology and look for effective targets for the prevention and treatment of obesity.

CD36 is a multifunctional glycosylated protein widely expressed in a variety of tissues (6). In metabolically active tissues such as liver and adipose tissue, CD36 functions as a lipid transporter that binds and uptakes long-chain fatty acids and thereby fosters fat accumulation in hepatocytes and adipocytes (6). In line with its biological function, CD36 expression has been reported to be upregulated in hepatocytes of patients with fatty liver (7, 8) and in adipose tissue of obese people (9). In addition, human genetic studies have identified several genetic variants in the *CD36* gene to be associated with traits reflecting lipid metabolism and obesity (10–12).

Traditionally, the measurement of CD36 required fresh blood or tissue samples and was therefore not feasible in large-scale population studies. Recent research efforts enabled the assessment of plasma CD36 in stored plasma samples (13). In subsequent studies, plasma CD36 levels were correlated with CD36 expression (14) and with body mass index (BMI) and other indices of body fat distribution (13, 15–19). In addition, positive correlations have been observed between plasma CD36 levels and liver fat in patients with higher liver fat content (NAFLD and chronic hepatitis C) (15, 20, 21) and obesity (17, 18). However, little is known about the association of plasma CD36 levels with liver fat and other fat depots in the general population. In 1296 healthy people free of diabetes and hypertension, a positive association of plasma CD36 levels with two indices of fatty liver has been reported (22). In that study, however, no direct measure of liver fat was available; instead, liver fat was estimated by using two algorithms based on anthropometric (BMI, waist circumference [WC]) and biochemical (alanine transaminase [ALT], levels of triglycerides [TG], and insulin) measures, as well as on the presence of metabolic syndrome and type 2 diabetes (22).

Noninvasive imaging techniques quantify abdominal fat depots and liver fat content more accurately than surrogate anthropometric measures, including WC, BMI, or liver fat algorithms (23). Therefore, in the present analysis we aimed to relate plasma CD36 concentrations in cross-sectional analyses to MRI traits of liver fat content and body fat distribution in a community-based sample from northern Germany (24). Because the expression of tissue CD36 is much higher in adipose tissue than liver (25), we hypothesized that plasma CD36 levels are more strongly related to visceral adipose tissue (VAT) than liver fat content in a general population.

Methods

Study sample

The PopGen control cohort is an ongoing prospective cohort study among a random sample of 1316 people living in northern Germany. The design has been described in detail previously (26). In brief, at baseline recruitment between 2005 and 2007, the PopGen biobank randomly identified 23,000 local residents through population registries in the city of Kiel and invited them to participate in the study. A total of 4267 participants agreed to take part, and among them, 747 subjects agreed to participate in the follow-up study. In addition, the PopGen biobank recruited 569 blood donors at the University Hospital Schleswig-Holstein in Kiel, and these two groups constituted the final PopGen control cohort (1316 subjects) (27). The first follow-up examination was conducted between 2010 and 2012, and 952 participants agreed to be reexamined at the study center. During this follow-up examination, trained study nurses performed a medical examination and drew blood. Furthermore, a whole-body MRI was part of this examination cycle, including an assessment of liver fat content and body fat distribution. Information on demographics, dietary intake, lifestyle factors, and medical history was collected via self-administered questionnaires (24, 26, 28).

For the present analysis, we used data from the first follow-up examination. A total of 575 participants underwent MRI to assess VAT, subcutaneous adipose tissue (SAT), and liver fat content. The flowchart of the current study design is shown in an online repository (29). The study procedures were approved by the ethical review board of the Medical Faculty of Kiel University, Germany. All study participants provided written informed consents.

Assessment of covariates

Information on participant characteristics such as age, sex, education, smoking status, alcohol consumption, physical activity, and medical history was collected via self-administered questionnaires. Alcohol consumption during the past year was calculated as the means of the reported values from a validated food frequency questionnaire for German populations (30). We quantified physical activity as weekly metabolic equivalent task (MET) hours by summing the reported weekly hours in different activities [walking, cycling, sports, gardening, do-it-yourself activities, and household tasks (28)] and multiplying them by their corresponding MET values averaged for winter and summer (31). Prevalent type 2 diabetes was determined by self-reported physician-diagnosed type 2 diabetes, antidiabetic medication usage, fasting blood glucose levels $\geq$126 mg/dL, or hemoglobin A1c levels $\geq$6.5% at the baseline or at the follow-up visit. In addition, prevalent hypertension was defined as self-reported hypertension diagnosed by a physician, use of hypertension medication, or diastolic blood pressure $\geq$90 mm Hg or systolic blood pressure $\geq$140 mm Hg (24, 28).

Trained study nurses measured WC, body weight, and height of participants wearing light clothing without shoes (28, 32). Study nurses subtracted 2 kg from the measured weight to correct for clothing. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. WC was measured at the midpoint between the spina iliaca crest and lower costal margin (32). Study nurses measured blood pressure three times by sphygmomanometry after the participant rested for $\geq$5 minutes. We calculated the arithmetic means of the last two blood pressure measurements (28).

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Biochemical measurements

All participants provided blood samples in a sitting position after overnight fasting. Most biosamples were stored at −80°C until biomarkers were assayed.

Standard laboratory parameters [including concentrations of total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)] were measured on the day of the examination at the Institute for Clinical Chemistry, University Hospital Schleswig-Holstein, Kiel, Germany (28). ALT was assessed by enzymatic colorimetry (Hitachi Modular; Roche Diagnostics, Mannheim, Germany), C-reactive protein (CRP) levels were determined by immunoturbidimetry. Fetuin-A levels were measured by a sandwich enzyme-linked immunosorbent assay (ELISA; BioVendor Laboratory Medicine, Brno, Czech Republic), blood glucose levels by enzymatic ultraviolet tests (Roche Diagnostics), and HbA1c levels by high-performance liquid chromatography and photometric detection (Bio-Rad Laboratories, Munich, Germany). Plasma CD36 levels were assayed (from samples stored at −80°C) at Aarhus University Hospital by an in-house ELISA assay (13), and the intra-assay coefficient of variation was 11%.

Outcome assessment

We used MRI to quantify the liver fat content and adipose tissue volumes on a Magnetom Avanto 1.5-T whole-body imager (Siemens Medical Solutions) (28, 33). Study nurses performed MRI with participants in the supine position with arms extended over the head (33). In-phase and out-of-phase images were acquired during a breath hold via axial T1-weighted gradient echo MRI with a 10.4-ms repetition time, echo time of 4.76 ms (in-phase) and 7.14 ms (opposed phase), 10° flip angle, 128 × 80 matrix, and 275- × 440-mm field of view (24). Liver fat content was quantified as the relative liver signal intensity (LSI) difference of the liver on out-of-phase compared with in-phase images in arbitrary units (24, 34). We validated the accuracy of LSI to quantify liver fat content via T2-corrected ^{1}H single-voxel proton magnetic resonance spectroscopy (MRS) on a 1.5-T whole-body imager (Siemens Medical Solutions) as the reference method in a sample of 40 subjects (55% men; median age, 55.6 years; median BMI, 27.7 kg/m^2) (24). Spearman correlation coefficient between LSI values and the MRS-determined liver fat content was $r = 0.609$ ($P < 0.001$) (24). Fatty liver disease (FLD) was determined as log-transformed LSI ≥3.0 according to a cutoff value determined by receiver operating characteristic (ROC) analysis with spectroscopic-determined FLD (liver fat ≥5.56%) used as the reference (area under ROC curve, 0.86; 95% CI, 0.74 to 0.98) (24, 35). The log-LSI cutoff of ≥3.0 was identified by Youden index, which corresponded to a sensitivity of 0.79 and a specificity of 0.81 (24). NAFLD was defined as having FLD in the absence of higher alcohol intake, defined as ≤7 drinks per week for women and ≤14 drinks per week for men (36, 37).

In addition, VAT was determined as the sum of VAT voxels from the top of the liver to the femoral heads inside the abdominal muscular wall, and SAT was determined as the sum of adipose tissue voxels underneath the skin layer surrounding the abdomen from the top of the liver to the femur heads (33). Both VAT and SAT were expressed in cubic decimeters cubed (28).

Statistical analysis

To describe the characteristics of the study participants, we divided the study sample into quartiles based on the distribution of plasma CD36 concentrations by using sex-specific cutoffs. Continuous variables with normal distribution were reported as means ± SD, continuous variables with skewed distribution were reported as medians (Q1, Q3), and categorical variables were presented as percentages.

Spearman partial correlations adjusted for age and sex were estimated between plasma CD36 concentrations and LSI, adiposity measures (BMI, WC, VAT, SAT), and blood biomarkers, including ALT, glucose, HbA1c, TC, TG, HDL-C, LDL-C, CRP, and fetuin-A. For participants (n = 229) whose CRP values were below the detection limit (0.9 mg/L), half of the detection limit (0.45 mg/L) was assigned as their CRP values.

Cubic spline analysis with four knots at the 5th, 35th, 65th, and 95th percentiles (50th percentile as referent) was used to assess potential nonlinearity of the relations between plasma CD36 and FLD as well as NAFLD. When assessing the association between plasma CD36 and NAFLD, we excluded participants with self-reported liver disease (hepatitis A, B, C, or D virus infection, hemochromatosis, autoimmune liver disease, or liver cirrhosis, n = 18) other than FLD. To analyze the associations between plasma CD36 and FLD as well as NAFLD, we used multivariable-adjusted logistic regression models and calculated ORs for FLD and NAFLD by using the lowest plasma CD36 quartile as the reference category. We adjusted for sex, age (continuous), years of education [≤9 years (no level of general education completed or secondary general school-leaving certificate), 10 years (intermediate school-leaving certificate), or ≥11 years (university of applied sciences or university entrance qualification)], smoking status [never (smoking period ≤3 months), former (smoking period >3 months), or current], physical activity (metabolic equivalent task hours per week; continuous), alcohol consumption (continuous), and history of diabetes (yes or no) as potential confounding factors. We additionally adjusted for BMI (Model 2) or VAT/SAT (Model 3) to assess whether the association of CD36 with FLD and NAFLD is independent of these adiposity measures. Adjusted means of LSI, BMI, VAT, and SAT according to CD36 quartiles were calculated by analysis of covariance.

Potential effect modification by sex, median age (<62.3 vs ≥62.3 years), prevalent diabetes, and prevalent hypertension were tested by including respective multiplicative interaction terms in the fully adjusted regression models.

Analyses were performed with SAS software, version 9.4 (SAS Institute, Cary, NC). All tests were two-sided, and P values < 0.05 were considered statistically significant.

Results

The median age of this sample was 62.3 (range: 25.1 to 82.8) years at the first follow-up examination. Characteristics of the study sample according to the sex-specific quartiles of plasma CD36 levels are shown in Table 1. In unadjusted statistical models, compared with people with lower plasma CD36 levels, subjects with higher CD36

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Table 1. Characteristics of the Study Sample (n = 575), Stratified by Sex-Specific Quartiles of Plasma CD36

	Quartiles of CD36			
	Q1 (n = 143)	Q2 (n = 143)	Q3 (n = 145)	Q4 (n = 144)
Median CD36, arbitrary unit (Q25, Q75) for men	0.57 (0.48, 0.70)	1.02 (0.89, 1.13)	1.53 (1.38, 1.64)	2.47 (1.99, 3.02)
Median CD36, arbitrary unit (Q25, Q75) for women	0.54 (0.43, 0.63)	0.89 (0.81, 1.02)	1.23 (1.14, 1.40)	1.95 (1.81, 2.34)
Men, %	57.3	58.0	56.6	57.6
Age, y	62.4 (53.7, 70.9)	64.0 (54.7, 72.4)	61.7 (55.5, 69.4)	61.3 (53.5, 69.0)
BMI, kg/m^2	26.4 ± 4.0	26.8 ± 4.4	27.1 ± 4.1	27.9 ± 4.6
Waist circumference for men, cm	97.3 ± 9.9	99.6 ± 10.3	99.9 ± 10.0	101.2 ± 11.2
Waist circumference for women, cm	88.1 ± 12.6	90.0 ± 12.6	89.9 ± 13.3	93.5 ± 14.1
Systolic blood pressure, mm Hg	138.3 ± 17.2	139.5 ± 18.5	140.8 ± 19.2	138.9 ± 18.8
Prevalent diabetes, %	8.4	13.3	8.28	13.2
Current smokers, %	11.9	11.9	6.90	9.72
Physical activity, MET-hr/wk	97.5 (56.8, 141.7)	92.8 (58.0, 123.5)	84.5 (61.8, 124.8)	88.0 (56.3, 128.8)
High education, %	39.2	34.3	34.5	32.6
Alcohol consumption, g/d	9.03 (2.89, 15.9)	10.1 (2.62, 21.2)	9.15 (3.68, 19.5)	9.93 (4.91, 17.0)
SAT, dm^3	5.40 (4.36, 7.53)	6.20 (4.46, 8.11)	6.12 (4.74, 8.18)	6.84 (5.28, 9.38)
VAT, dm^3	3.09 (2.12, 4.64)	4.10 (2.56, 5.44)	3.78 (2.41, 4.96)	4.21 (2.89, 5.62)
ALT, IU/L	21.0 (16.0, 27.0)	22.0 (17.0, 28.0)	23.0 (17.0, 31.0)	25.0 (18.0, 32.0)
LSI	2.89 ± 0.41	2.93 ± 0.39	2.98 ± 0.43	2.95 ± 0.54
FLD, %	37.8	37.8	42.8	43.8
NAFLD, %	32.6	32.1	37.1	37.7

Data are reported as mean ± SD for continuous normally distributed variables (BMI, WC, systolic blood pressure, and LSI) and as median (Q25, Q75) for continuous skewed distributed variables (CD36, age, physical activity, alcohol consumption, SAT, VAT, and ALT) and as percentage for categorical variables.

levels had higher mean values for different adiposity measures (BMI, WC, SAT, VAT), diastolic and systolic blood pressure, ALT, a higher prevalence of FLD and NAFLD, and lower education levels.

Plasma CD36 levels were positively correlated with BMI, WC, SAT, VAT, ALT, TC, and TG but not with LSI ($r = 0.03$; $P = 0.44$), HDL-C ($r = -0.01$; $P = 0.85$), LDL-C ($r = 0.07$; $P = 0.09$), glucose (r = 0.04; $P = 0.34$),

Table 2. Multivariable-Adjusted Means (95% CI) of Different MRI-Derived Adiposity Traits and Measures of Liver Fat, Stratified by Sex-Specific Quartiles of Plasma CD36 (n = 575)

	Multivariable-Adjusted Means (95% CI) by Quartiles of CD36				
	Q1 (n = 143)	Q2 (n = 147)	Q3 (n = 140)	Q4 (n = 146)	*P* for Trend[a]
LSI					
Model 1[b]	2.90 (2.83–2.98)	2.93 (2.86–3.00)	2.99 (2.91–3.06)	2.96 (2.89–3.03)	0.38
Model 2[c]	3.05 (2.96–3.14)	3.04 (2.95–3.13)	3.11 (3.02–3.20)	3.07 (2.98–3.15)	0.36
Mean difference (95% CI), Model 2	0 (Reference)	−0.01 (−0.12–0.11)	0.06 (−0.05–0.18)	0.02 (−0.10–0.14)	
BMI, kg/m^2					
Model 1[b]	26.4 (25.7–27.1)	26.8 (26.1–27.5)	27.1 (26.4–27.8)	27.9 (27.2–28.6)	0.022
Model 2[c]	27.8 (26.9–28.6)	27.9 (27.1–28.8)	28.2 (27.3–29.1)	28.9 (28.0–29.7)	0.013
Mean difference (95% CI), Model 2	0 (Reference)	0.16 (−0.99–1.31)	0.47 (−0.67–1.62)	1.13 (−0.03–2.28)	
VAT, dm^3					
Model 1[b]	3.38 (3.08–3.68)	4.07 (3.77–4.36)	3.86 (3.56–4.16)	4.22 (3.93–4.52)	<0.001
Model 2[c]	4.13 (3.77–4.48)	4.65 (4.31–5.00)	4.50 (4.14–4.86)	4.71 (4.36–5.06)	<0.001
Mean difference (95% CI), Model 2	0 (Reference)	0.53 (0.05–1.00)	0.38 (−0.10–0.86)	0.59 (0.11–1.07)	
SAT, dm^3					
Model 1[b]	6.40 (5.85–6.94)	6.99 (6.46–7.53)	7.10 (6.55–7.65)	7.87 (7.33–8.41)	0.002
Model 2[c]	7.61 (6.96–8.27)	7.99 (7.36–8.63)	8.21 (7.54–8.87)	8.74 (8.10–9.38)	<0.001
Mean difference (95% CI), Model 2	0 (Reference)	0.38 (−0.49–1.25)	0.59 (−0.29–1.47)	1.13 (0.25–2.01)	

[a]Linear trend was tested per SD increment of plasma CD36 levels.

[b]Model 1: Adjusted for age and sex.

[c]Model 2: Model 1 additionally adjusted for education levels (≤9, 10, or ≥11 years), smoking status (never, former, or current smoker), physical activity (continuous), prevalent diabetes (yes or no), and alcohol intake (continuous).

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HbA1c (r = 0.07; $P = 0.09$), CRP (r = -0.05; $P = 0.23$), or fetuin-A (r = 0.05; $P = 0.29$) in age- and sex-adjusted analyses (29).

In multivariable-adjusted least-square means analyses using the first CD36 quartile as referent, participants in the second, third, and fourth CD36 quartiles had significantly higher levels of BMI, SAT, and VAT with a graded increase across quartiles (all P-trend < 0.05; Table 2); however, such a graded association was not observed for LSI (P-trend > 0.10). In addition, BMI, SAT, and VAT explained 8%, 17%, and 13% of the variance in plasma CD36 concentrations, respectively, whereas LSI explained 1% of the variance of plasma CD36.

The association of plasma CD36 levels with FLD and NAFLD is shown in an online repository (29). Restricted cubic spline analysis did not suggest a nonlinear association of plasma CD36 levels with FLD and NAFLD (data not shown). After adjustment for age, sex, education level, smoking status, alcohol intake, physical activity, and prevalent diabetes, higher CD36 concentrations conferred slightly higher odds for FLD (highest vs lowest quartile, OR, 1.13; 95% CI, 0.69 to 1.87) and NAFLD (highest vs lowest quartile, OR, 1.28; 95% CI, 0.74 to 2.22), but the respective ORs were not statistically significant. The risk estimate of CD36 for liver fat was further attenuated toward the null after adjustment for BMI (highest vs lowest quartile, OR for FLD, 0.98; 95% CI, 0.58 to 1.64; OR for NAFLD, 1.09; 95% CI, 0.62 to 1.92) or adjustment for VAT/SAT (highest vs lowest quartile, OR for FLD, 1.02; 95% CI, 0.61 to 1.70; OR for NAFLD, 1.15; 95% CI, 0.66 to 2.01) (29).

We also compared mean CD36 levels in subjects with NAFLD and normal ALT concentrations (≤33 IU/L in men and ≤25 IU/L in women; n = 113) with CD36 levels of participants with NAFLD and elevated ALT concentrations (n = 70). CD36 levels were statistically significantly higher in subjects with NAFLD and elevated ALT (1.58 ± 0.82 units vs 1.21 ± 0.72 units; $P = 0.002$).

We found no evidence for effect modification by sex, age, prevalent diabetes, and prevalent hypertension (all P-interaction > 0.05). All supplemental materials were stored in the public data repository (29).

Discussion

Principal observations

In this cross-sectional analysis in a population-based sample, we observed statistically significant correlations between plasma CD36 levels and different markers of general and abdominal adiposity, including BMI, WC, VAT, and SAT. By contrast, no association of plasma CD36 concentrations with liver fat content, FLD, and NAFLD was observed.

In the context of the published literature

With the present analyses on MRI-derived traits of liver fat, SAT, and VAT in addition to BMI and WC, we expand on previous studies that have reported positive correlations of plasma CD36 with adiposity markers (mainly BMI and WC) (13, 15–19, 22) and liver fat content (15, 17, 18, 20–22) in other European samples, mainly in samples with defined disease conditions such as liver disease or obesity. Several previous studies have consistently observed a positive correlation between plasma CD36 levels and BMI (13, 16–18, 22). However, because BMI does not differentiate between body fat and muscle weight and because visceral fat may play a greater role in contributing to metabolic diseases than subcutaneous fat (38, 39), more detailed analyses regarding the association of CD36 levels with different fat depots were warranted. In 111 patients with NAFLD and 33 healthy controls, an inverse correlation between plasma CD36 levels and dual-energy X-ray absorptiometry VAT ($r = -0.21$; $P < 0.05$), but no correlation with SAT, was reported (15). Other indicators of body composition such as WC, fat mass, central fat mass, and truncal fat mass have also shown to be positively correlated with plasma CD36 levels in women with polycystic ovary syndrome (17), in obese children (18), and in morbidly obese people (19) undergoing lifestyle intervention programs and gastric bypass surgeries, respectively, for weight loss.

In our comprehensive analyses in a community-based sample, we related plasma CD36 levels to a broad panel of adiposity measures and observed a consistent and positive association of CD36 levels with VAT, SAT, and BMI in different multivariable-adjusted statistical models. Furthermore, higher plasma CD36 levels have been related to greater liver fat content in clinical samples (*e.g.*, in patients with NAFLD or chronic hepatitis C) (15, 20, 21), in patients with obesity (17, 18), and in general population samples (22). In contrast to these former studies, plasma CD36 levels were unrelated to liver fat content in our analyses in a community-dwelling sample, free of apparent liver disease. One potential explanation for the lack of association in our study is that the association of CD36 with liver fat might be much more pronounced (or only apparent) in clinical settings or in obese people but not in healthy people from the community. Indeed, clinical study groups with significantly elevated plasma CD36 as previously reported had liver fat content of 20% to 30% (15, 20, 21), whereas our sample included many normal-weight participants free of liver disease.

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Furthermore, in our sample CD36 levels were higher in people with NAFLD and elevated ALT concentrations as compared with people with NAFLD and normal ALT concentrations. Thus, CD36 concentrations might be more closely related to more severe liver cell damage (indicated by increased ALT levels) than to increased fat deposition in the liver.

In addition, differences in the definition of NAFLD and in the assessment of liver fat content must be considered when one is comparing the results across studies (15, 20–22). Previous studies among clinical samples used MRS (intrahepatic lipid content >5%) or liver biopsy in the absence of excessive alcohol intake to define NAFLD (15, 20, 21), whereas the current study defined NAFLD by using MRI-measured LSI (log LSI ≥3.0) in the absence of high alcohol intake (≤7 drinks per week for women and ≤14 drinks per week for men). In terms of liver fat content, a previous study conducted in a general population sample did not directly measure or visualize liver fat content but estimated liver fat based on algorithms including BMI, WC, TG, liver transaminases, fasting insulin, and presence of diabetes and metabolic syndrome (22). Of note, most variables included in the algorithm to predict liver fat in the previous study (22), such as BMI, WC, TG, and ALT, were also significantly correlated with plasma CD36 in our sample (29). By contrast, our study took advantage of MRI techniques to quantify liver fat content. However, liver fat content might not necessarily reflect NAFLD severity. A previous study suggested that liver fat content decreases in some patients with NAFLD progressing to cirrhosis (40). Long-term prospective studies assessing FLD severity including fibrosis are warranted to better understand the association between circulating CD36 concentrations, liver fat (as assessed by MRI), biochemical markers of liver function and damage, and NAFLD.

Potential mechanisms for the observed associations

Several experimental data support the observed association between plasma CD36 and adipose tissue traits. CD36 acts as a membrane fatty acid transporter (9) and has been shown to bind long-chain fatty acids and facilitate their uptake across the plasma membrane in adipose tissue (41). In line with this biological function, increased expression of CD36 has been observed on both mRNA and plasma membrane level in VAT and SAT of obese patients (42), and genetic variants in the *CD36* gene have been linked to lipid metabolism and obesity (10–12). Furthermore, CD36 expression in the liver and plasma CD36 are correlated (15, 20, 21). Although the mechanism of CD36 release into the circulation is currently unknown, it has been hypothesized that metabolic abnormalities such as inflammation in abdominal obesity could promote plasma CD36 release into the circulation (6).

Strengths and limitations

The strengths of the current study include its large sample size, the community-based design, and the comprehensive clinical examination of the study participants, including assessment of different adipose tissues [including assessments of liver fat content on a continuous scale, a method that has been validated against MRS (24)] via MRI and detailed assessment of potentially confounding factors. Thus, we were able to adjust for a variety of lifestyle factors in our statistical analyses.

The following limitations merit consideration. First, because this was a cross-sectional analysis, the causality and temporality of the relation of plasma CD36 levels with the different outcome measures could not be determined. Moreover, NAFLD was not diagnosed by liver biopsy in the current study, because this is not feasible and not ethically justifiable in a community-based setting. Therefore, we could not differentiate between different stages of NAFLD and their respective relations with plasma CD36 levels. Further studies are warranted that compare the accuracy of MRI-determined liver fat content to proton density fat fraction, which is less confounded by biological, physical, and technical factors (43) and has shown to accurately classify different grades of hepatic steatosis in patients with NAFLD (44, 45). Additionally, the current study was based on a community-based sample from northern Germany. Thus, our observations may not be generalizable to other populations.

Conclusions

In conclusion, higher plasma CD36 concentrations were statistically significantly associated with greater measures of general and abdominal adiposity, including higher values of MRI-determined SAT and VAT. However, CD36 levels were not related to MRI-determined liver fat content in our sample from the general population. These observations are consistent with the concept that plasma CD36 levels are relevant correlates of adipose tissue and provide initial evidence that plasma CD36 might be more closely related to SAT and VAT than liver fat content in the general population, a premise that warrants additional investigation. Patients with NAFLD and elevated ALT had higher plasma CD36 levels than those with NAFLD and normal ATL levels. Thus, plasma CD36 may reflect more advanced stages of NAFLD. Additional population-based studies (*e.g.,* with repeated measurements of plasma CD36) are warranted to describe trajectories of CD36 levels over time and their prospective association with metabolic and adiposity traits. Finally, studies of

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environmental, lifestyle, and genetic factors associated with circulating CD36 are warranted.

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Additional Information

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Disclosure Summary: The authors have nothing to disclose.

Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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