Plasma sCD36 is associated with markers of atherosclerosis, insulin resistance and fatty liver in a nondiabetic healthy population

Abstract. Handberg A, Højlund K, Gastaldelli A, Flyvbjerg A, Dekker JM, Petrie J, Piatti P, Beck-Nielsen H (Aarhus Hospital and Aalborg Hospital, Aarhus University Hospital, Aarhus, Denmark; Odense University Hospital, Odense, Denmark; Institute of Clinical Physiology, CNR Pisa, Pisa, Italy; Department of Endocrinology, Aarhus University Hospital, Aarhus, Denmark; EMGO Institute, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands; BHF Glasgow Cardiovascular Research Centre, Glasgow, UK; and Department of Metabolic Diseases, San Raffaele Institute, Milan, Italy). Plasma sCD36 is associated with markers of atherosclerosis, insulin resistance and fatty liver in a nondiabetic healthy population. J Intern Med 2011; doi: 10.1111/j.1365-2796.2011.02442.x.

Objectives. Insulin resistance is associated with increased CD36 expression in a number of tissues. Moreover, excess macrophage CD36 may initiate atherosclerotic lesions. The aim of this study was to determine whether plasma soluble CD36 (sCD36) was associated with insulin resistance, fatty liver and carotid atherosclerosis in nondiabetic subjects.

Methods. In 1296 healthy subjects without diabetes or hypertension recruited from 19 centres in 14 European countries (RISC study), we determined the levels of sCD36, adiponectin, lipids and liver enzymes, insulin sensitivity (M/I) by euglycaemic–hyperinsulinaemic clamp, carotid atherosclerosis as intima–media thickness (IMT) and two estimates of fatty liver, the fatty liver index (FLI) and liver fat percentage (LF%).

Results. IMT, FLI, LF%, presence of the metabolic syndrome, impaired glucose regulation, insulin and triglycerides increased across sCD36 quartiles (Q2–Q4), whereas adiponectin and M/I decreased (P ≤ 0.01). sCD36 was lower in women than in men (P = 0.045). Log sCD36 showed a bimodal distribution, and amongst subjects with sCD36 within the log-normal distribution (log-normal population, n = 1029), sCD36 was increased in subjects with impaired glucose regulation (P = 0.045), metabolic syndrome (P = 0.006) or increased likelihood of fatty liver (P < 0.001). sCD36 correlated significantly with insulin, triglycerides, M/I and FLI (P < 0.05) after adjustment for study centre, gender, age, glucose tolerance status, smoking habits and alcohol consumption. In the log-normal population, these relationships were stronger than in the total study population and, additionally, sCD36 was significantly associated with LF% and IMT (P < 0.05).

Conclusions. In this cross-sectional study of nondiabetic subjects, sCD36 was significantly associated with indices of insulin resistance, carotid atherosclerosis and fatty liver. Prospective studies are needed to further evaluate the role of sCD36 in the inter-relationship between atherosclerosis, fatty liver and insulin resistance.

Keywords: low-grade inflammation, metabolic syndrome, nonalcoholic fatty liver disease, sCD36, type 2 diabetes.

Abbreviations: sCD36, soluble CD36; MetSy, metabolic syndrome; T2D, type 2 diabetes; CVD, cardiovascular disease; oxLDL, oxidized low-density lipoprotein; BP, blood pressure; FPG, fasting plasma glucose; FFM, fat-free mass; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; FFA, free fatty acids; GGT, gamma-glutamyltransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; M/I, insulin sensitivity; FLI, fatty liver index; MRS, magnetic resonance spectroscopy; BMI, body mass index; LF%, liver fat percentage;
IMT, intima–media thickness; NGT, normal glucose tolerance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; +MetSy, presence of the metabolic syndrome; −MetSy, absence of the metabolic syndrome; Log-normal population, subjects with sCD36 within the log-normal distribution

Introduction

The metabolic syndrome (MetSy) describes a cluster of factors, including obesity, that are associated with an increased risk of type 2 diabetes (T2D) and cardiovascular disease (CVD). In addition, fat accumulation in the liver correlates with all components of the MetSy and has been proposed to be a novel component of the MetSy [1, 2]. From the public health perspective, there is a strong incentive to identify people at risk of future T2D and CVD. Thus, it is important to identify and evaluate new biomarkers linked to the MetSy and to use these biomarkers to increase our understanding of its pathophysiology.

CD36 is expressed in a variety of tissues and has a number of tissue-specific functions [3–5]. CD36 on the surface of muscle, liver and fat tissue is involved in uptake of fatty acids, and a number of studies have indicated that there is a relation between elevated CD36 expression in these tissues and insulin resistance [6–8]. CD36 on the surface of macrophages may initiate foam cell formation eventually leading to atherosclerotic lesions and thus may be an important risk factor for CVD. The process of foam cell formation is initiated and enhanced by the binding of oxidized low-density lipoprotein (oxLDL) to CD36 receptors with subsequent accumulation of cholesterol in macrophages [3–5]. In mice, increased liver CD36 expression in response to diet-induced obesity was associated with liver fat accumulation and insulin resistance [6], and defective insulin signalling led to increased CD36 expression on macrophages in ob/ob mice [9]. Together, these results suggested important correlations between decreased insulin sensitivity, accumulation of ectopic fat, inflammation and possible future atherosclerosis.

The recently identified soluble CD36 (sCD36) was proposed to reflect tissue CD36 expression and may thus be a potential marker integrating insulin resistance and atherosclerosis [10]. Indeed, we have reported consistent associations between sCD36, obesity and insulin resistance in insulin-insensitive conditions [10–13]. Elevated sCD36 was present in both overt T2D and prediabetic conditions such as obesity and polycystic ovary syndrome, indicating that sCD36 possesses the potential to reflect early changes in CD36 expression involved in the pathogenesis of diabetes or the development of components of the MetSy. The purpose of this study was to evaluate the relationships between plasma sCD36 and measures of insulin resistance, fatty liver and carotid atherosclerosis in a large population of nondiabetic healthy subjects.

Setting and methods

Study subjects

The RISC study is a prospective, observational, cohort study, and details of the rationale and methodology have been published previously [14]. In brief, clinically healthy Caucasian men and women, aged between 30 and 60 years, were recruited from 19 centres in 14 European countries. Initial exclusion criteria were as follows: treatment for obesity, hypertension, lipid disorders or diabetes; pregnancy; cardiovascular or chronic lung disease; weight change of ≥5 kg in the previous 6 months; cancer in the previous 5 years; and renal failure. Exclusion criteria after screening were as follows: systolic/diastolic blood pressure (BP) ≥140/90 mmHg; fasting plasma glucose (FPG) ≥7.0 mmol L⁻¹; 2-h plasma glucose ≥11.0 mmol L⁻¹; total serum cholesterol ≥7.8 mmol L⁻¹; serum triglycerides ≥4.6 mmol L⁻¹; ECG abnormalities; and carotid artery plaques. This study included 1296 subjects (712 women and 584 men) who satisfied all criteria, had available data for plasma sCD36 level and for whom the clamp study (see below) passed the quality control check. Local ethics committee approval was obtained by each recruiting centre.

Physical examination and lifestyle factors

Height, body weight, percentage body fat, fat-free mass (FFM), waist circumference, sitting BP and heart rate were measured as previously described [15]. A lifestyle questionnaire was used to collect information about smoking habits and alcohol consumption [14].
Analytical determinations

Blood collected during the studies was separated into plasma and serum, divided into aliquots and stored until required for measurement at −20 °C for glucose and at −80 °C for lipids, insulin and sCD36. Plasma levels of glucose and adiponectin and serum levels of insulin, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglycerides, free fatty acids (FFAs), gamma-glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were all measured in a central laboratory, as previously described [15].

Plasma sCD36

sCD36 was measured using an in-house enzyme-linked immunoassay (ELISA) in Li-heparin plasma [10]. A pool of EDTA plasma was applied in seven dilutions and used to produce a standard concentration curve. Internal controls were run in quadruplicate on each plate. Runs were accepted if the controls were within ±2 SD from mean, and most were within 1 SD. The intra-assay coefficient of variation (CV) was 6%, and total day-to-day assay CV was 16.4%. Log-transformed standard curves were linear. A few measurements were outside the standard curve range and were calculated by extrapolation. sCD36 was measured in fasting samples. The detection limit, established using absorbance of the zero calibrator plus 2 SD of the absorbance of the lowest calibrator (n = 48), was 0.027 arbitrary units in this study.

Insulin clamp

A euglycaemic–hyperinsulinaemic clamp was performed in all subjects as described previously [15]. Insulin sensitivity was expressed as the ratio of the M value – averaged over the final 40 min of the 2-h clamp and normalized with respect to FFM – to the mean plasma insulin concentration (I) during the same interval (M/I, in units of μmol min⁻¹ kg FFM⁻¹ (nmol L⁻¹)⁻¹).

Fatty liver index

The fatty liver index (FLI) is an algorithm based on body mass index (BMI), waist circumference, triglycerides and GGT with an accuracy of 0.84 (95% confidence interval, 0.81–0.87) in detecting fatty liver [16]. In the present study, we have used more restrictive cut-off values for FLI (<20 and >60), for which we have previously estimated the likelihood of having or not having fatty liver using the Bayes’ theorem in the RISC study cohort [1]; when the index value (FLI) is >60, the likelihood of having fatty liver is >78%, and if FLI is <20, the likelihood of not having fatty liver is >91%. Subjects were then categorized into three groups according to FLI: <20, 20–60 and >60. To validate the use of FLI in the RISC cohort, we have evaluated the hepatic fat content in a separate group of subjects (n = 37) by magnetic resonance spectroscopy (MRS) and compared the results with our estimated cut-off points for the likelihood of having fatty liver on the basis of FLI values. Subjects with FLI <20 had no hepatic fat content (range, 0.4–4.2%; n = 6), whereas those with FLI >60 had steatosis with hepatic fat content >5% (range, 8.6–24.0%; n = 10). Moreover, FLI was significantly correlated with hepatic fat content determined by MRS (r = 0.61, P = 0.0001) [17].

Liver fat percentage

Liver fat percentage (LF%) is an algorithm based on the presence of the MetSy and T2DM as well as fasting insulin, AST and ALT to predict the degree of liver fat accumulation. The algorithm was developed and validated using 1H-MRS, and moreover, the algorithm was validated in a separate study group [18]. The R² of the LF% algorithm against 1H-MRS was 0.49 [18].

Carotid intima–media thickness analysis

We followed a validated scanning and reading protocol for ultrasound measurement of carotid artery intima–media thickness (IMT) [19] as previously described [15]. The IMT value was computed as an average of all available carotid walls (up to four walls). Intra-observer variability of IMT measures was tested in 100 randomly chosen scans to calculate the average percentage difference between the first and second reading relative to the first reading; the mean difference was 4.6 ± 3.0%.

Glucose tolerance and the MetSy

Participants underwent a 2-h standard 75-g oral glucose tolerance test and were classified into three groups according to the American Diabetes Association criteria (proposed in 2000): normal (NGT), impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT). Subjects with IFG and/or IGT were grouped together and classified as impaired glucose regulation (n = 182 or 14% of the total cohort). The presence (+MetSy) or absence (−MetSy) of the MetSy was defined according to the criteria of the International Diabetes Federation [20].
Statistical analysis

All data analyses were performed with spss 11.0 for Windows (SPSS, Inc., Chicago, IL, USA). The results for continuous variables are reported as the mean ± SD unless otherwise stated and for class variables as percentages. Variables with skewed distributions (sCD36, insulin, triglycerides, FFA, adiponectin, ALT, GGT, FLI, LDH, HDL, M/I and IMT) were logarithmically transformed for statistical analyses. One-way anova and Tukey’s post hoc analysis were used to analyse the markers of insulin resistance, carotid atherosclerosis and fatty liver by quartiles (Q1–Q4) of sCD36. Chi-square tests were used for binominal variables (MetSy and IGT/IFG). Partial correlation analyses were used to test the relationship between sCD36 and the same markers when adjusted for centre, gender, glucose tolerance status, smoking habits and alcohol consumption. \( P \leq 0.05 \) was considered statistically significant.

Results

Effect of gender, glucose tolerance MetSy and FLI on sCD36

Plasma sCD36 was significantly lower in women than in men, similar to other indices of insulin resistance, atherosclerosis and fatty liver by quartiles (Q1–Q4) of sCD36. Chi-square tests were used for binominal variables (MetSy and IGT/IFG). Partial correlation analyses were used to test the relationship between sCD36 and the same markers when adjusted for centre, gender, glucose tolerance status, smoking habits and alcohol consumption. \( P \leq 0.05 \) was considered statistically significant.

Quartiles of plasma sCD36

HDL and M/I decreased across quartiles (Q2, Q3 and Q4) of plasma log sCD36, whereas FPG, insulin, triglycerides, LDL, clamp FFA, age, waist circumference, percentage body fat, ALT, GGT, FLI, LF%, BMI, systolic BP, IMT, IGT/IFG and +MetSy increased across the same quartiles (anova; \( P < 0.01 \) for all) (Table 2, Fig. 1). Plasma adiponectin decreased across all four quartiles. For all these parameters, the largest difference was observed between Q2 and Q4 values of plasma sCD36, and for all variables, except ALT, this difference was significant (Table 2). Most variables exhibited a J-shaped (or inverse J-shaped) relationship with sCD36, with Q1 values showing the opposite trend to that observed from Q2 to Q4. Waist circumference, percentage body fat, BMI, ALT, GGT, LF%, +MetSy and IGT/IFG showed significant differences between Q1 and Q2 (Table 2, Fig. 1), whereas LF, M/I, IMT and the all other variables shown in Table 2 did not reach statistical significance. In both men and women, fasting insulin, waist circumference, FLI, LF%, +MetSy and percentage body fat increased across sex-specific quartiles (Q2, Q3 and Q4) of sCD36, whereas adiponectin decreased across all four quartiles (anova; \( P < 0.05 \) for all; data not shown). In women, significant differences across quartiles of plasma sCD36 were seen for age, BMI, fasting glucose, triglycerides, HDL, systolic BP and IGT/IFG (\( P < 0.02 \) for all), whereas changes across quartiles of sCD36 were seen for GGT (\( P = 0.021 \)) and LDL (\( P = 0.005 \)) in men.

Bimodal distribution of sCD36: two populations

The J-shaped relationship between most variables and quartiles of sCD36 suggested the possible existence of two populations of sCD36 values. Closer examination of the log-transformed distribution of sCD36 revealed that part of the total population had extremely low values of sCD36 (Fig. 2). It was evident that log values of sCD36 \(< 0.38 \) do not belong to the log-normal distribution of sCD36. Excluding those individuals with log sCD36 \(< 0.38 \), we observed significantly higher plasma sCD36 levels in the resulting log-normal population in men versus women (4.1 ± 3.5 vs. 3.7 ± 3.1; \( P = 0.001 \)), in subjects with IGT/IFG versus NGT (4.5 ± 4.0 vs. 3.8 ± 3.2; \( P = 0.045 \)) and in subjects +MetSy versus −MetSy (4.6 ± 3.9 vs. 3.8 ± 3.2; \( P = 0.006 \)). The differences in glucose tolerance and MetSy status (+MetSy/−MetSy) were significant in women (\( P < 0.05 \)) but not in men. Moreover, plasma sCD36 levels increased significantly with increasing likelihood of fatty liver (FLI < 20 → 20–60 → >60: 3.5 ± 3.1 vs. 4.2 ± 3.5 vs. 4.6 ± 3.4; \( P < 0.001 \)). After post hoc analysis, plasma sCD36 was significantly lower in subjects with FLI < 20 than in subjects with FLI 20–60 (\( P = 0.009 \)) and FLI > 60 (\( P = 0.009 \)).

We also investigated whether the characteristics of the two populations of sCD36 distribution differed from each other, but observed no differences in the parameters shown in Table 1 except for significantly higher levels of plasma adiponectin (9.00 ± 3.89 vs. 8.22 ± 3.70 mg L\(^{-1} \); \( P < 0.001 \)), FFA clamp (0.062 ± 0.179 vs. 0.052 ± 0.084 mmol L\(^{-1} \); \( P < 0.028 \)) and increased +MetSy (18.3 ± 38.8 vs. 12.5 ± 33.1; \( P < 0.027 \)) in the population with log sCD36 below –0.38.
Of note, the higher plasma adiponectin levels were not caused by a higher ratio of women to men in the two populations ($P = 0.75$).

**Correlation analyses**

In the total population, univariate analysis revealed significant correlations between plasma sCD36 and fasting insulin, triglycerides, adiponectin, clamp FFA and FLI (Table 3). These associations remained significant after adjustment for centre, gender, age, glucose tolerance status, smoking habits and alcohol consumption using multivariate analysis. In addition, adjustment for these covariates introduced an inverse relationship between $M/I$ and sCD36. The sCD36 log-normal population revealed stronger or similar associations between sCD36 and fasting insulin, adiponectin, triglycerides, clamp FFA, GGT and FLI, and significant associations were introduced between sCD36 and fasting glucose, LDL, HDL, +MetSy, LF% and carotid IMT (Table 3). Except for fasting glucose, adiponectin and +MetSy, these associations persisted after adjusting for centre, gender, age, glucose tolerance status, smoking habits and alcohol consumption. Univariate or multivariate analysis of sCD36 and the variables shown in Table 3 within the population with log sCD36 < $-0.38$ showed no significant associations.

A strong positive correlation between the two estimates of fatty liver, FLI and LF%, was observed both in the total population ($r = 0.66; P < 0.001$) and in the sCD36 log-normal population ($r = 0.64; P < 0.001$).

**Discussion**

In this cross-sectional study of nondiabetic healthy subjects, plasma sCD36 showed significant associations with indices of insulin resistance, carotid...
Atherosclerosis and fatty liver. The association between sCD36 and a large range of risk factors for insulin resistance and CVD was consistently significant. Closer inspection showed that these associations tended to be J-shaped (or inverse J-shaped) for the majority of risk factors. Thus, both high and very low sCD36 levels in a general healthy population seem to carry an increased risk of disease. Several studies have demonstrated an association between high CD36 expression and increased foam cell formation and unstable atherosclerosis [3–5, 21, 22]. On the other hand, two studies of the general phenotypic appearance of CD36-deficient patients showed that CD36 deficiency was associated with hyperlipidaemia and an atherogenic lipid profile, insulin resistance and mild hypertension [23, 24]. Although case-control studies of larger cohorts of individuals with CD36 deficiency are necessary [25], it could be speculated that both too much and too little sCD36 may be associated with an increased disease risk, at least in clinically healthy people. Low CD36 expression [26] was associated with increased fatty acid flux to the liver, which may result in dyslipidaemia and insulin resistance. High sCD36 was associated with insulin resistance [10, 12, 13], and in diabetic mouse and human monocytes, CD36 expression was upregulated as a consequence of impaired insulin signalling [9]. In addition, impaired insulin signalling was associated with elevated CD36 expression in circulating leucocytes in a mouse model of increased atherosclerosis risk [27]. Furthermore, high oxLDL, as a consequence of increased oxidative stress and dyslipidaemia, upregulates monocyte and macrophage CD36 expression and may lead to accelerated development of atherosclerosis [3, 5]. Therefore, our findings that both high and low sCD36 may be associated with risk factors for insulin resistance and CVD are not in conflict with previously published data. Other possible explanations for our findings include a mutation in the coding region of the CD36 gene, which could result in altered immunoreactivity with the antibodies used in the sCD36 ELISA, and lead to falsely low sCD36 levels. A CD36 promotor mutation in Caucasians linked to diabetes has been reported, and muta-

### Table 2 Relationship between sCD36 and anthropometric measures and biochemical markers of insulin resistance and fatty liver in the RISC cohort

<table>
<thead>
<tr>
<th>Plasma sCD36</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Pall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.3 ± 8.2*</td>
<td>43.4 ± 8.3*</td>
<td>43.2 ± 8.1*</td>
<td>45.4 ± 8.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist</td>
<td>323</td>
<td>325</td>
<td>325</td>
<td>323</td>
<td>0.002</td>
</tr>
<tr>
<td>Percentage body fat (%)</td>
<td>86 ± 13**</td>
<td>83 ± 12***</td>
<td>88 ± 12</td>
<td>89 ± 12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg m⁻²)</td>
<td>25.8 ± 4.4**</td>
<td>24.6 ± 3.7***</td>
<td>25.5 ± 3.9</td>
<td>26.2 ± 4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (BP) (mmHg)</td>
<td>74 ± 8</td>
<td>74 ± 8</td>
<td>75 ± 8</td>
<td>75 ± 8</td>
<td>0.143</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>117 ± 13</td>
<td>116 ± 12*</td>
<td>117 ± 13</td>
<td>119 ± 12</td>
<td>0.034</td>
</tr>
<tr>
<td>Heart rate (beats min⁻¹)</td>
<td>67 ± 10</td>
<td>68 ± 11</td>
<td>69 ± 10</td>
<td>69 ± 10</td>
<td>0.085</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol (mmol L⁻¹)</td>
<td>2.9 ± 0.8*</td>
<td>2.8 ± 0.8*</td>
<td>2.9 ± 0.8*</td>
<td>3.1 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol (mmol L⁻¹)</td>
<td>1.4 ± 0.4</td>
<td>1.5 ± 0.4****</td>
<td>1.4 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol L⁻¹)</td>
<td>1.1 ± 0.9*</td>
<td>1.0 ± 0.6*</td>
<td>1.1 ± 0.6</td>
<td>1.2 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting free fatty acids (FFA) (mmol L⁻¹)</td>
<td>0.53 ± 0.21</td>
<td>0.55 ± 0.22</td>
<td>0.54 ± 0.27</td>
<td>0.53 ± 0.22</td>
<td>0.528</td>
</tr>
<tr>
<td>Clamp FFA (mmol L⁻¹)</td>
<td>60 ± 164*</td>
<td>48 ± 70</td>
<td>52 ± 81</td>
<td>58 ± 99</td>
<td>0.022</td>
</tr>
<tr>
<td>Fasting glucose (mmol L⁻¹)</td>
<td>5.1 ± 0.5</td>
<td>5.0 ± 0.5*</td>
<td>5.1 ± 0.6</td>
<td>5.1 ± 0.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Fasting insulin (pmol L⁻¹)</td>
<td>32 ± 25*</td>
<td>28 ± 17*</td>
<td>31 ± 19*</td>
<td>38 ± 22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alanine aminotransferase (U L⁻¹)</td>
<td>21.4 ± 12.8**</td>
<td>18.8 ± 12.4</td>
<td>20.2 ± 10.2</td>
<td>20.5 ± 10.6</td>
<td>0.016</td>
</tr>
<tr>
<td>Gamma-glutamyltransferase (U L⁻¹)</td>
<td>27.4 ± 23.0**</td>
<td>24.5 ± 24.7*</td>
<td>25.2 ± 16.4*</td>
<td>30.0 ± 25.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma adiponectin (mg L⁻¹)</td>
<td>9.2 ± 3.9****</td>
<td>8.7 ± 3.9****</td>
<td>7.9 ± 3.5</td>
<td>7.8 ± 3.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD. *P-values based on statistical analyses of log-normalized values. **P < 0.05 vs. Q4. ***P < 0.05 vs. Q2 and ****P < 0.05 vs. Q3 and in post hoc analysis.
tions in the coding area of the CD36 gene leading to CD36 deficiency in platelets and monocytes are rare in Caucasians [24, 28]. Another methodological explanation could be a matrix effect as low concentrations were mostly run undiluted in contrast to the remaining samples. Whatever the cause of the distribution of sCD36, the log-normal sCD36 population exhibited higher sCD36 in participants with disturbed glucose tolerance, with the MetSy and with an increased likelihood of fatty liver disease, in accordance with previous findings [10–13, 29].

Targeted over-expression of CD36 in the liver causes liver fat accumulation in mice. In addition, diet-induced obesity is associated with higher liver CD36 expression, liver fat accumulation and dyslipidaemia [6]. To date, there have been few studies on human liver CD36. In liver biopsies from patients with non-alcoholic fatty liver disease (NAFLD), expression of fatty acid transporting proteins, including CD36, correlated with liver fat content, and it was concluded that multiple genes related to lipid metabolism were involved in liver steatosis [30]. In a very recent study by Miquilena-Colina and co-workers [31], subjects with fatty liver and chronic hepatitis C virus infection were shown to have increased CD36 expression in liver and significant associations between hepatic CD36 expression and both insulin resistance and the degree of liver steatosis. Circulating sCD36 is associated with indicators of liver function, such as ALT, in insulin-resistant subjects with IGT or T2D but not in subjects with normal glucose homoeostasis [11], and it has been hypothesized that sCD36 may be a marker of NAFLD. Here, we find that sCD36 increases with increased likelihood of fatty liver and, furthermore, that this association between sCD36 and fatty liver was significant even after corrections for several confounders. The fact that we also find that sCD36 is

\[ \text{IGT/IFG} = \text{ANOVA} P = 0.012 \]

\[ \text{Metabolic syndrome} = \text{ANOVA} P < 0.001 \]

\[ \text{M/I} = \text{ANOVA} P = 0.002 \]

\[ \text{Carotid IMT} = \text{ANOVA} P = 0.006 \]

\[ \text{Liver fat index} = \text{ANOVA} P < 0.001 \]

\[ \text{Liver fat percentage} = \text{ANOVA} P < 0.001 \]
associated with LF% in the sCD36 log-normal population and that the variables used for the estimation of the LF% are different from those used for the FLI supports this hypothesis. One major limitation is that the derived measure FLI was developed in a general population using ultrasonography rather than MRS, which is the gold-standard technique for the diagnosis of NAFLD. However, LF% has been validated by MRS. As there is a strong correlation between the two algorithms of fatty liver despite the fact that they are derived from different variables, and because sCD36, being a fatty acid transporter of the liver, is correlated with both, it is reasonable to speculate that sCD36 may be a new marker of liver fat. Additional studies of sCD36 and direct measures of liver fat, and ideally also liver CD36 expression, would improve our understanding of the involvement of CD36 in liver steatosis and inflammation.

Numerous studies support the role of CD36 in foam cell formation and the development of unstable atherosclerotic plaques [3, 21, 32, 33]. CD36 expression levels are tissue specific and regulated by a variety of factors. Some of these factors are inter-related, such as plasma lipids and insulin resistance as well as inflammation, oxidative stress and insulin resistance. Most factors that influence CD36 expression also predispose to atherosclerosis. Previous studies have indicated that the same factors influence both plasma sCD36 level and tissue CD36 expression. The present finding of a positive relationship between sCD36 and IMT in a large healthy non-diabetic population indicates that sCD36 may be a marker of atherosclerosis. The persistent relationship between sCD36 and IMT after correction for confounders such as gender, age, glucose tolerance status, smoking habits and alcohol consumption supports this hypothesis, and although the correlation is weak, we propose that sCD36 has the potential to be an important marker of atherosclerosis. Previous analysis of the RISC cohort revealed that fasting insulin may be a stronger contributor to cardiometabolic risk and atherosclerosis than insulin sensitivity ($M/I$) in a healthy population [34], and consistent with this, we find that fasting insulin is better correlated with sCD36 than $M/I$.

In our study population, there were generally higher levels of MetSy risk factors in men as expected from a number of studies, and accordingly, sCD36 was higher in men than in women. We have previously found higher sCD36 levels in healthy men (A. Handberg, unpublished data), but to our knowledge gender-dependent expression of cell-bound CD36 has not been investigated. Elevated sCD36 may be linked to higher FLI and LF% in men, reflecting increased levels of liver enzymes, BMI, waist circumference and triglycerides and thus increased expression of liver CD36. IMT was also greater in men indicating increased atherosclerosis, which also potentially contributes to the elevated plasma CD36. Thus, at present, the cause of the observed sCD36 gender difference is unclear. A major limitation of the present study is that no data were available regarding CD36

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**Fig. 2** Distribution of sCD36 in the study populations. Distribution of log sCD36 amongst all study participants ($n = 1296$; upper panel) and in the log-normal study population ($n = 1029$; lower panel). The dashed line indicates the mean level of log sCD36 (~0.38) at the lower end of the distribution at which frequencies starts to increase again.
expression in cells and tissues key to the development of the MetSy, such as vessel macrophages, skeletal muscle, liver, fat and monocytes, to compare circulating sCD36 with tissue CD36 expression. Such studies should probably be conducted in an animal model of the MetSy and on a small scale.

In summary, the results of this study show that in the context of a cross-sectional study of healthy non-diabetic subjects, sCD36 represents a new biomarker of a phenotype of insulin resistance, carotid atherosclerosis and fatty liver. Prospective studies are needed to further evaluate the role of sCD36 as a marker of the inter-relationship between atherosclerosis and fatty liver in insulin resistance.

Conflict of interest statement
No conflicts of interest to declare.

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| Table 3 Univariate and multivariate analyses of the relationship between sCD36 and markers of insulin resistance, carotid atherosclerosis and fatty liver in the RISC cohort |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Total population | Population with log sCD36 ≥ −0.38 (n = 1029) |      |
|                                | Univariate analysis | Multivariate analysis | Univariate analysis | Multivariate analysis |
| Low-density lipoprotein cholesterol (mmol L⁻¹) a | 0.049 | 0.040 | 0.131** | 0.087** |
| High-density lipoprotein cholesterol (mmol L⁻¹) a | -0.013 | -0.019 | -0.103** | -0.102** |
| Triglycerides (mmol L⁻¹) a | 0.066* | 0.067* | 0.149** | 0.112** |
| Clamp free fatty acids (mmol L⁻¹) a | 0.086** | 0.075** | 0.066* | 0.094** |
| Fasting glucose (mmol L⁻¹) | -0.007 | -0.016 | 0.113** | 0.015 |
| Fasting insulin (pmol L⁻¹) a | 0.091** | 0.086** | 0.180** | 0.126** |
| Alanine aminotransferase (U L⁻¹) a | -0.020 | 0.019 | 0.049 | 0.023 |
| Gamma-glutamyltransferase (U L⁻¹) a | 0.025 | 0.027 | 0.152** | 0.101** |
| Fatty liver index a | 0.095** | 0.094** | 0.184** | 0.114** |
| Liver fat percentage a | 0.031 | 0.041 | 0.151** | 0.102** |
| Plasma adiponectin (mg L⁻¹) a | -0.118** | -0.067* | -0.115** | -0.048 |
| M/I (µmol min⁻¹ kg FFM⁻¹ nmol L⁻¹) a | -0.041 | -0.069* | -0.051 | -0.072* |
| No. of factors in the MetSy | 0.003 | 0.023 | 0.104** | 0.059 |
| Carotid intima–media thickness (mm) a | 0.022 | 0.047 | 0.104** | 0.085* |

In multivariate analysis, partial correlation coefficients are given after adjustment for centre, age, gender, glucose tolerance status, smoking habits and alcohol consumption. *P-values based on statistical analyses of log-normalized values. **P < 0.05, ***P < 0.01.
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References


Appendix: RISC investigators

RISC recruiting centres


Core laboratories and reading centres