Identification of the Oxidized Low-Density Lipoprotein Scavenger Receptor CD36 in Plasma
A Novel Marker of Insulin Resistance
Aase Handberg, MD, DMSc; Klaus Levin, MD, PhD; Kurt Højlund, MD, PhD; Henning Beck-Nielsen, MD, DMSc

Background—Macrophage CD36 scavenges oxidized low-density lipoprotein, leading to foam cell formation, and appears to be a key proatherogenic molecule. Increased expression of CD36 has been attributed to hyperglycemia and to defective macrophage insulin signaling in insulin resistance. Premature atherosclerosis is the major cause of morbidity and mortality in type 2 diabetes. Here, we report the identification of a soluble form of CD36 (sCD36) in plasma and hypothesize that sCD36 would be elevated in patients with type 2 diabetes and insulin resistance.

Methods and Results—sCD36 in plasma was demonstrated by immunopurification and Western blotting. We established ELISA assays to determine sCD36 in plasma and measured sCD36 in obese type 2 diabetic patients, obese nondiabetic relatives, and obese and lean control subjects. sCD36 was markedly elevated in type 2 diabetic patients compared with both lean (5-fold) and obese (2- to 3-fold) control subjects. There was a strong, inverse correlation between sCD36 and insulin-stimulated glucose disposal and a direct correlation with fasting plasma glucose, fasting insulin, and body mass index.

Conclusions—Our study demonstrates sCD36 in plasma for the first time. sCD36 is highly related to risk factors of accelerated atherosclerosis in type 2 diabetes such as insulin resistance and glycemic control, and we propose that sCD36 might represent a marker of the metabolic syndrome and a potential surrogate marker of atherosclerosis.

Key Words: antigens, CD36 ■ atherosclerosis ■ diabetes mellitus, type 2 ■ foam cells ■ inflammation

CD36 is a complex multifunctional protein that serves as a scavenger receptor for oxidized low-density lipoprotein (LDL) and apoptotic cells on macrophages and as a fatty acid transporter in muscle and adipocytes.1–3 Furthermore, CD36 binds to collagen and probably functions as an adhesion protein. CD36 has previously been shown to play a substantial role in the pathogenesis of atherosclerosis.2 Atherosclerosis, like insulin resistance, has been postulated to result from a chronically inflammatory state.4–7 In type 2 diabetes and insulin resistance, the inflammatory state has been attributed in part to secretion of cytokines from enlarged adipocytes or from macrophages infiltrating enlarged fat deposits.5,6,8,9 Multiple organs seem to be involved, however, in the elevation of surrogate markers such as high-sensitivity C-reactive protein and interleukin-6.5,6,8,9

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CD36 is believed to play a critical role in the initiation of atherosclerotic lesions through its ability to bind and internalize modified LDL trapped in the arterial wall, facilitating the formation of lipid-engorged macrophage foam cells.2,10 CD36 expression on the surface of monocytes and macrophages is upregulated by oxidized LDL,1–3,11 which is elevated in type 2 diabetes.12–14 Furthermore, hyperglycemia upregulates CD36 expression on the surface of monocytes from type 2 diabetic patients13 or monocytes incubated with high glucose levels in vitro.16 Additionally, it has been proposed that CD36 is a marker of macrophage activation and inflammation.17 Thus, monocyte CD36 seems to be upregulated under conditions that follow type 2 diabetes and the metabolic syndrome.15,16,18,19

Premature atherosclerosis is the major cause of morbidity and mortality in type 2 diabetes. The excess risk is disproportionate to lipid abnormalities. A direct association between cardiovascular disease and long-term glycemic control has been found.20–22 Insulin resistance, commonly seen in overweight individuals and in people genetically predisposed to the metabolic syndrome, is an important cause of diabetes, dyslipidemia, and increased atherosclerosis risk.5,12,14,23,24
CD36 is therefore a potentially important marker of macrophage activation in insulin-resistant conditions. So far, studies of CD36 have involved immunolabeling of vessels with atherosclerotic plaques and flow cytometric analyses of isolated monocytes and thus were not well suited for large population-screening studies.\(^{15,16,25-27}\) We hypothesized that CD36, or a truncated form thereof, could be released to the circulation as part of the low-grade inflammatory state in insulin resistance or during the cell apoptosis that occurs after cholesterol accumulation in foam cells, thus providing a marker of CD36 expression that can be measured in blood samples with simple techniques.

The aim of the present study was to look for a soluble form of CD36 (sCD36) in plasma and to establish an assay for easy measurements of sCD36. Subsequently, we wished to study sCD36 expression in patients with insulin resistance with and without accompanying diabetes to evaluate the possible upregulation of sCD36 with insulin resistance like that previously found on intact cells.

**Methods**

**Antibodies**

CD36 antibodies with different epitopes were from Santa Cruz Biotechnology, Inc (Santa Cruz, Calif; sc9154 [rabbit immunized with amino acid 1 to 300 of CD36 of human origin], sc5522 [goat immunized with peptide mapping near the N-terminal part of CD36 of human origin], and sc7309 [mouse monoclonal against CD36 purified from human tonsils and peripheral monocytes]) or Cell Sciences, Inc (Canton, Mass; mouse monoclonal anti-human CD36, clone FA6-152), and anti-rabbit antibody (sheep), anti-goat antibody (rabbit), and goat immunoglobulin G (IgG) were from Dakopats (Copenhagen, Denmark).

**Sample Preparation**

**Plasma**

Ethylenediaminetetraacetic acid (EDTA)– or heparin-stabilized blood was centrifuged 3000g for 15 minutes. The upper two thirds were placed in aliquots, immediately frozen, and stored at \(-80°C\).

**Platelet Membrane**

Platelets were lysed and centrifuged for 30 minutes at 4°C and 18 000g. The pellet was solubilized in 2% Triton X-100 overnight, and after a 40-minute spin at 20 000g and 4°C, the supernatant was stored at \(-80°C\).

**Immunoprecipitation and Immunoblotting of Human Plasma and Platelets**

Plasma or platelet membranes were incubated overnight at 4°C with CD36 antibody sc9154, sc5522, or sc7309. Immunocomplexes were bound to ImmunoPure-immobilized protein A, boiled in sample buffer, and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, Biorad, Hercules, Calif) essentially as described.\(^{28}\) After electrophoresis of proteins to Immobilon membranes, the membranes were blocked and incubated with rabbit(sc9154)-CD36 antibody, goat(sc5522)-CD36 antibody, or mouse(FA6-152) antibody.\(^{28}\) Immunoreactivity was visualized by enhanced chemiluminescence (Pierce Biotechnology, Rockford, Ill).

**Immunopurification of CD36 From Plasma on Dynabeads**

Goat(sc5522) anti-CD36 antibody or goat IgG was coupled to Dynabeads M-280 (Dynal Biotech, Oslo, Norway) according to the manufacturer’s instructions (0.25 mg antibody/10\(^7\) beads). Plasma (4 mL) was applied to each of the Dynabead preparations, and after washing, 100-μL fractions were eluted by 0.1 mol/L glycine-HCl (pH 2.7), immediately neutralized in 1 mol/L Tris(hydroxymethyl)aminomethane (pH 9.5), and stored at \(-20°C\).

**ELISA Assays for Determination of CD36 in Plasma**

ELISA plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with catcher antibody goat(sc5522)-CD36 (0.1 μg/mL) for ELISA-I or mouse(sc7309)-CD36 (0.1 μg/mL) for ELISA-II, followed by blocking with phosphate-buffered saline and 0.1% Tween (Sigma-Aldrich, St Louis, Mo) for 2 days at 4°C. The plates were stored at \(-20°C\).

**Biotinylation of Detection Antibody**

Detection antibody sc9154 was dialyzed overnight at 4°C against 10 mmol/L phosphate buffer (pH 7.4) and 145 mmol/L NaCl. Biotinylation was initiated by adding biotinamidocaproate-N-hydroxysuccinimide in dimethyl sulfoxide and stopped by lysin-HCl and rabbit and bovine γ-globulin. The biotinylated antibody was dialyzed against phosphate buffer for 4 days with buffer shift every 24 hours, placed in aliquots, and stored at \(-80°C\) with 0.1% NaAzid (Sigma-Aldrich, St Louis, Mo).

**Determination of CD36 by ELISA**

A pool of EDTA plasma served as standard and was applied in increasing dilutions in duplicates. Other pools of EDTA plasma served as high and low controls, and phosphate-buffered saline served as background. Patient samples were applied in appropriate dilutions in duplicates. After a 1-hour incubation at room temperature, the wells were rinsed, and biotinylated anti-CD36 (sc9154) was applied and incubated for 60 minutes. After rinsing, wells were incubated with POD-avidin (Dako, Copenhagen, Denmark) and rinsed, and TMB Microwell Peroxidase Substrate (Kirkegaard and Perry Laboratories, Inc, Gaithersburg, Md) was added. The reaction was terminated after \(\approx 10\) minutes by phosphorous acid, and extinctions were determined at 450 and 620 nm on a Multiscan apparatus (Labsystem, Helsinki, Finland). Absorptions were calculated relative to the standard EDTA plasma pool and expressed as relative units. Controls were allowed to deviate by 2 SD from the true value. True values of controls and interassay SD were determined by double determinations of each control level on 15 different days. Standard pool included EDTA plasma from 100 subjects from the routine blood sampling. CD36 in plasma from patients in this study was measured by ELISA-I.

**Design of Clinical Studies**

Screening blood tests of hepatic and renal function were within reference ranges. Informed consent was obtained from all subjects before participation. Both studies were approved by the local ethics committee and were performed in accordance with the Helsinki Declaration. The patients had no signs of any diabetic complications.

**Subjects: Study 1**

Ten healthy lean and 10 healthy obese control subjects carefully age and gender matched to 10 obese type 2 diabetic patients participated in study 1. Obese control subjects and type 2 diabetic patients were matched for body mass index (BMI). Diabetic patients, randomly recruited from the Endocrinology Unit of Odense University Hospital, were treated by either diet alone or diet combined with sulfonylurea, metformin, or insulin; these drugs were withdrawn 1 week before the study. Control subjects with normal glucose tolerance and no family history of diabetes were recruited by newspaper advertising.

**Subjects: Study 2**

Thirteen healthy obese control subjects, 22 first-degree relatives of patients with type 2 diabetes, and 21 obese type 2 diabetic patients matched for BMI participated. Diabetic patients were treated by either diet alone or diet combined with sulfonylurea, metformin, or acarbose, which, together with lipid-lowering drugs, were withdrawn 3 weeks before the study. The relatives had at least 1 first-degree and 1 second-degree relative with type 2 diabetes. Relatives and control
subjects underwent an oral glucose tolerance test to ensure normal glucose tolerance. Subjects were enrolled in study 2 to ensure the reproducibility of potential findings and to study the effect of genetic predisposition to type 2 diabetes.

**Study Design**

After an overnight fast, the study subjects underwent euglycemic-hyperinsulinemic clamp consisting of a 2-hour equilibration period followed by a 4-hour (study 1) or 3-hour (study 2) insulin infusion (40 mU · m⁻² · min⁻¹) as described previously.¹⁰ Physiological hyperinsulinemia (≈400 pmol/L) was obtained during the insulin-stimulated period. In study 1, plasma glucose was allowed to decline to ≈5.5 mmol/L in diabetic subjects before glucose infusion was initiated. In study 2, diabetic subjects had insulin infusion overnight to obtain a fasting glucose of ≈5.5 mmol/L before the euglycemic-hyperinsulinemic clamp was initiated. Glucose infusion rates (GIRs) during the insulin-stimulated steady-state period (last 30 minutes of the insulin infusion period) were calculated as the amount of glucose (in milligrams) infused per minute per 1 m² to maintain euglycemia at a plasma glucose concentration of ≈5.5 mmol/L. Plasma glucose was measured on a bedside Beckman glucose analyzer (Beckman Instruments, Fullerton, Calif), and serum insulin was measured by a 2-site, time-resolved immunofluorometric assay.¹¹

**Statistical Analysis**

Statistical analyses were performed with SPSS for Windows, version 10.0 (SPSS, Inc, Chicago, Ill). The Kolmogorov-Smirnov test was used to test for normality of data. Differences between the groups were assessed with 1-way ANOVA and Tukey post hoc testing. Relationships between plasma CD36 and continuous variables were examined by calculation of Pearson correlation coefficients. In study 1, a stepwise multiple linear regression analysis was performed to determine significant predictors of sCD36 concentrations. The 5 most relevant variables were entered into the model. Results are expressed as mean±SD. Values of *P*<0.05 were considered statistically significant.

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

**Results**

**Demonstration of CD36 in Human Plasma by Immunoprecipitation, SDS-PAGE, and Immunoblotting**

Immunoblotting of plasma separated by SDS-PAGE showed bands of 85 and 50 kDa. The 50-kDa band also was present in the background lane (Figure 1A). Platelet membranes expressed a positive control displayed an 85-kDa band (Figure 1B). Immunoprecipitation of EDTA plasma and heparin plasma showed identical bands of 85 kDa. The 50-kDa band also was present in the background lane (Figure 1A). Platelet membranes used for their integrity. All authors have read and agree to the manuscript as written.

**Characterization of CD36 ELISA Assay**

To study the specificity of the CD36 ELISA-I assay, plasma was purified on Dynabeads coated with goat (sc5522) anti-CD36 IgG or goat IgG. Elution fractions were applied to the CD36 ELISA-I, and only elution fractions from anti-CD36 Dynabeads were enriched in CD36 (15-fold); the goat IgG column elution fractions were devoid of CD36 (Figure 2A). When CD36 was measured by ELISA-II, enrichment of CD36 was 50-fold after purification of plasma on Dynabeads coated with goat anti-CD36 IgG (Figure 2B).

When plasma was applied to goat anti-CD36 antibody–coated Dynabeads and elution fractions (combined 1+2) and plasma were subjected to SDS-PAGE and Western blotting using monoclonal (FA6–152) anti-CD36 antibody, only the 85-kDa band was enriched (Figure 2C).

A nearly linear dilution curve of pooled EDTA plasma was obtained with dilutions from 1:256 to 1:8 on ELISA-I (Figure 2D). Parallel dilution curves were obtained by ELISA-I and -II assays. In ELISA-I, intra-assay and interassay coefficients of variation, which were estimated from double determinations of the high control on 15 different days, were 6% and 16.4%, respectively. Runs were accepted only when controls were within the range of ±2 SD (interassay). sCD36 in plasma expressed relative to the standard pool was linear over a 16-fold dilution range (results not shown).

**Study 1**

**CD36 in Plasma From Type 2 Diabetic Patients and Obese and Lean Control Subjects**

Fasting plasma glucose, serum insulin, and glycosylated hemoglobin (HbA₁c) were significantly higher and high-density lipoprotein (HDL) cholesterol was significantly lower in the diabetic group compared with the nondiabetic groups (Table 1). Fasting insulin was higher in the obese control compared with the nonobese control group (Table 1). CD36 in plasma was determined by ELISA-I, and the results were expressed relative to a pool of EDTA plasma, which was run as the standard curve. All samples were run in duplicates.
sCD36 was 4.5-fold higher in obese type 2 diabetic patients compared with lean control persons (0.71±0.35 versus 0.16±0.09 relative units; \( P<0.0005 \)) and 3 times higher in obese control compared with lean control subjects (0.46±0.25 versus 0.16±0.09 relative units; \( P=0.030 \)) (Figure 3). CD36 in obese diabetic patients was 1.6-fold that of obese controls (\( P=0.066 \)). There was no gender difference in sCD36 in either of the study groups or in all subjects studied (results not shown). In the diabetic group, sCD36 correlated significantly with fasting plasma glucose (\( r=0.69, P<0.03 \)) and HbA1c (\( r=0.76, P<0.02 \)) (Figure 4A and 4B). sCD36 in patients with type 2 diabetes was not significantly correlated with total, HDL, or LDL cholesterol, nor did we find any correlation with fasting plasma triglycerides. When all study participants were included, the correlations between plasma sCD36 and fasting plasma glucose (\( r=0.69, P<0.0001 \)) and sCD36 and HbA1c (\( r=0.69, P<0.0001 \)) were confirmed (Figure 4A and 4B). In addition, plasma sCD36 was correlated with fasting plasma insulin (\( r=0.58, P<0.001 \)), BMI (\( r=0.52, P<0.0025 \)), and triglycerides (\( r=0.44, P<0.05 \)) (Figure 4C and 4E). Correlations between sCD36 and fasting plasma glucose, HbA1c, and fasting insulin were not changed after adjustment for BMI.

### CD36 in Plasma and Insulin Sensitivity

The euglycemic-hyperinsulinemic clamp studies showed that obese type 2 diabetic patients were significantly more insulin-resistant than lean control subjects and obese control subjects. This conclusion was supported by the finding that sCD36 was higher in the plasma of obese type 2 diabetic patients compared with lean control subjects and obese controls. The increase in sCD36 was not significantly correlated with fasting plasma glucose, HbA1c, or fasting insulin levels. However, a significant correlation was observed between sCD36 and fasting plasma glucose (\( r=0.69, P<0.0001 \)) and HbA1c (\( r=0.69, P<0.0001 \)). These findings suggest that sCD36 may be a useful biomarker for identifying individuals at risk for type 2 diabetes.

### Table 1. Anthropometric Data and Laboratory Measurements on Participants in Study 1

<table>
<thead>
<tr>
<th></th>
<th>Lean Control Subjects</th>
<th>Obese Control Subjects</th>
<th>Obese Type 2 Diabetic Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, female/male, n</td>
<td>5/5</td>
<td>5/6</td>
<td>6/4</td>
</tr>
<tr>
<td>Age, y</td>
<td>50.8±3.3</td>
<td>48.6±4.9</td>
<td>49.9±4.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.2±1.7</td>
<td>33.7±4.7*</td>
<td>33.5±3.6*</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>5.7±0.4</td>
<td>5.7±0.6</td>
<td>10.0±2.0*‡</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.5±0.2</td>
<td>5.4±0.3</td>
<td>7.5±1.7*‡</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.3±0.7</td>
<td>5.4±1.6</td>
<td>5.4±1.2</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.9±0.7</td>
<td>3.3±1.1</td>
<td>3.2±0.8</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.85±0.46</td>
<td>1.48±0.46</td>
<td>1.36±0.10†</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.12±0.49</td>
<td>1.35±0.61</td>
<td>1.93±1.25</td>
</tr>
<tr>
<td>Serum insulin, pmol/L</td>
<td>24.3±18.2</td>
<td>52.7±16.6†</td>
<td>94.6±32.0*§</td>
</tr>
</tbody>
</table>

Values are mean±SD. *\( P<0.001 \) vs lean control subjects. **\( P<0.0005 \) vs lean control subjects. 
†\( P=0.05 \) vs lean control subjects. 
††\( P<0.001 \) vs obese control subjects. 
§\( P<0.01 \) vs obese control subjects.
resistant compared with both obese and lean control subjects (GIR, 118±59 versus 258±94 and 383±64 mg · m⁻² · min⁻¹; diabetic patients versus lean control subjects, P<0.0005; diabetics versus obese control subjects, P=0.001). Obese control subjects were more insulin resistant than lean control subjects (P=0.002). sCD36 was significantly inversely correlated with GIR when all participants were included (r=-0.67, P<0.0001) and when only the type 2 diabetic patients were included (r=-0.73, P<0.02) (Figure 5), thus demonstrating a significant relation between plasma CD36 and insulin resistance. sCD36 was not affected by 4 hours of hyperinsulinemia in any of the 3 study groups (P>0.28; results not shown).

Multiple Regression Analyses

Using multiple linear regression analysis, we examined the contribution of BMI, fasting plasma glucose, GIR, fasting serum insulin, and HbA₁c to sCD36 concentrations (Table 2). BMI and fasting plasma glucose level were the only significant independent predictors of plasma sCD36 level. The proportion of the variation in sCD36 concentration explained by these 2 variables was 61%.

<table>
<thead>
<tr>
<th>Table 2. Independent Predictors of Plasma CD36</th>
</tr>
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<tbody>
<tr>
<td>Model</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Constant</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
</tr>
</tbody>
</table>

R² for the model=0.61. Variables excluded from the model were HbA₁c (P=0.23), fasting serum insulin (P=0.98), and GIR (P=0.94).
TABLE 3. Anthropometric Data and Laboratory Measurements on Participants in Study 2

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>First-Degree Relatives of Type 2 Diabetic Patients</th>
<th>Type 2 Diabetic Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, female/male, n</td>
<td>2/11</td>
<td>9/13</td>
<td>4/17</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.7±9.7</td>
<td>30.6±6.4†</td>
<td>52.4±6.0†</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.4±4.4</td>
<td>30.4±3.6</td>
<td>30.5±4.0</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>6.0±0.3</td>
<td>5.9±0.5†</td>
<td>11.4±3.3*</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>...</td>
<td>7.3±1.5*</td>
<td>9.1±1.0</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.2±0.4</td>
<td>1.8±0.9</td>
<td>1.9±1.0</td>
</tr>
<tr>
<td>Serum insulin, pmol/L</td>
<td>49.6±31.6</td>
<td>73.1±31.8</td>
<td>161.8±75.2†</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*Fasting plasma glucose and HbA1c were measured 1 day before the euglycemic-hyperinsulinemic clamp in type 2 diabetic subjects. Insulin and triglycerides were measured in the basal state during the clamp studies.

†P<0.001 vs obese controls.
‡P<0.001 vs first-degree relatives.

Discussion

The present study reports for the first time the presence of CD36 in plasma. Our hypotheses were that CD36 is present in a soluble form in plasma and that plasma CD36 could serve as a biomarker of conditions with altered CD36 expression. The most prominent alterations of CD36 expression have been described in monocytes/macrophages in conditions with elevated levels of modified lipoproteins such as animal models of atherosclerosis and low-grade inflammation and diabetes.15,32–34 The increased CD36 expression on macrophages induces internalization of accumulated modified lipoproteins and phosphatidyl residues,1,35–38 and eventually the cells achieve the morphological characteristics of foam cells. Early atherosclerotic lesions are characterized by intimal foam cells.

Both the risk and the rate of atherosclerosis are increased in type 2 diabetes. During our evaluation of plasma CD36 as a biomarker, we looked for a putative covariation between plasma CD36 and glycosmia. Both study designs confirmed the relationship between glycosmia levels, measured as both HbA1c and fasting glucose, and plasma CD36. A recent study explored the expression of CD36 in vascular lesions in patients with severe atherosclerosis and demonstrated that CD36 was significantly more prevalent in patients with diabetes. Furthermore, human macrophages differentiated in the presence of high glucose showed increased expression of surface CD36, and it was proposed that this could be the link between diabetes and atherosclerosis.16 In vivo, CD36 expression on circulating monocytes also was increased, possibly as a consequence of chronic hyperglycemia,15 whereas there was no correlation with plasma glucose levels. Thus, the levels of sCD36 seem to parallel CD36 expression on intact monocytes under chronic hyperglycemic conditions. Elevated sCD36 in obese nondiabetic patients indicates that hyperglycemia and diabetes are not solely responsible for CD36 shedding to plasma.

The metabolic syndrome features a cluster of cardiovascular risk factors, including insulin resistance, central obesity, a low-grade inflammatory state, hypertension, and dyslipidemia.5,39–41 Insulin resistance is directly related to atherosclerosis.7,23,42 Indeed, insulin resistance measurements are markers of coronary atherosclerosis independently of hyperglycemia and inflammatory markers such as C-reactive protein.23 We measured plasma CD36 in various insulin-sensitive and -resistant study groups and demonstrated a strong, significant, and consistent relationship between insulin resistance and sCD36. We propose that sCD36 is derived from the surfaces of monocytes and macrophages with elevated CD36, possibly as a result of insulin resistance. Accordingly, a recent study on ob/ob mice showed increased macrophage binding and uptake of oxidized LDL due partly to a postrateational increase in CD36 protein.34 The study provides evidence that increased CD36 expression is caused by insulin resistance and concludes that defective macrophage insulin signaling predisposes to foam cell formation and atherosclerosis in insulin-resistant states.34 It was proposed that the relationship between chronic glucose levels and monocyte CD36 expression is caused by insulin resistance, leading to hyperglycemia more than glucose per se. Several studies demonstrated elevated monocyte CD36 levels resulting from hyperglycemia-induced oxidation of LDL32,43; thus, several factors possibly influence monocyte CD36 expression. In our study, fasting glucose and BMI were identified by multiple linear regression analysis to significantly predict levels of sCD36. Despite the lipid-transporting properties of CD36, we were unable to demonstrate correlations between sCD36 and HDL, LDL, or triglycerides. The correlations of sCD36 and fasting glucose and insulin resistance measurements, together with this lack of correlation with plasma lipids, emphasize that plasma CD36 is related to a specialized function. Literature on the regulation of CD36 expression points toward the role that CD36 plays as an oxidized LDL scavenger in foam cell formation. Studies in larger populations are needed to make solid conclusions from multiple regression analyses, however. Genetic predisposition to type 2 diabetes and obesity seems not to add to the expression of sCD36 besides obesity itself, but it cannot be excluded that, for example, fat distribution was different in the younger relatives of type 2 diabetic patients compared with an older group of obese persons without genetic predisposition.

CD36 has 2 predicted transmembrane domains, amino acid 7 to 28 and amino acid 441 to 463, and a large extracellular loop. Furthermore, the extracellular part is heavily glycosylated.44 We were unable to differentiate between the total CD36 protein and its extracellular part by migration length on SDS-PAGE and immunoblotting. Thus, we are unable to propose whether the plasma CD36 is derived from a proteolytic cleavage of the extracellular part of CD36 protein from CD36-expressing tissues or from microparticles from activated or apoptotic monocytes/macrophages or platelets. Trapping of LDL in the subendothelial space, the resulting liability to modification, the attraction of monocytes, and the upregulation of CD36 on macrophages are higher in insulin-resistant conditions, possibly because of low-grade inflammation.5,10,45 The resulting foam cells eventually become apoptotic, and thus microparticles containing CD36 may be released to the circulation.
Furthermore, concentrations of cell-derived microparticles are elevated in type 2 diabetic patients. Elevated levels of monocyte-derived microparticles in patients with oxidized LDL antibodies may even be a marker of atherosclerosis. Circulating CD36 may be present as the unbound protein or a peptide fraction thereof or may be present in microparticles shed from cells such as platelets, monocytes/macrophages, or adipocytes after triggering by various stimuli. Further dedicated studies are required to support this hypothesis. There is a need for a simple, cost-efficient method to predict at an early stage those at risk of developing atherosclerosis and/or athrotherbosis. This need could be met by measurements of sCD36 in plasma, which we propose may provide an integrated measurement of the influence of several risk factors in 1 analysis of a blood sample.

Conclusions

The present study demonstrates for the first time sCD36 in plasma. sCD36 is highly related to risk factors of accelerated atherosclerosis in type 2 diabetes such as insulin resistance and glycemic control, and we propose that sCD36 might represent a marker of the metabolic syndrome and a potential surrogate marker of atherosclerosis.

Acknowledgments

We thank Lene Dabelstein Petersen and Inge Marie Jensen for their excellent technical assistance.

Sources of Funding

This work was supported by the NovoNordisk Foundation and the Danish Research Agency. Dr Handberg is a member of the EU Network Program COST-B17.

Disclosures

None.

References

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CLINICAL PERSPECTIVE

CD36 is described in cell-free plasma for the first time in the present study, and a convenient and cost-efficient way to measure plasma CD36 is presented. CD36 is a membrane protein with various tissue-specific functions. In monocytes, CD36 plays a critical role in the initiation of atherosclerotic lesions through its ability to bind and internalize oxidized low-density lipoprotein in the arterial wall, eventually leading to the formation of lipid-engorged foam cells. Monocyte CD36 expression is upregulated by oxidized low-density lipoprotein, which is elevated in type 2 diabetes, hyperglycemia, and insulin resistance. Premature atherosclerosis is the major cause of morbidity and mortality in type 2 diabetes. The excess risk is disproportionate to lipid abnormalities. Insulin resistance is an important cause of diabetes, dyslipidemia, and increased atherosclerosis risk. Therefore, CD36 is a potentially important marker of atherosclerosis development in insulin-resistant conditions. We found substantially increased plasma CD36 levels in type 2 diabetic patients and high correlations between plasma CD36 and glucose levels, body mass index, and insulin resistance. There is a need for early-stage prediction of those at risk of developing atherosclerosis. This need could be met by measurements of CD36 in plasma, which we propose may provide an integrated measurement of the influence of several risk factors in 1 analysis of a blood sample. Plasma CD36 is highly related to risk factors of accelerated atherosclerosis in type 2 diabetes, and we propose that CD36 might represent a marker of the metabolic syndrome and a potential surrogate marker of atherosclerosis.
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Circulation. 2006;114:1169-1176; originally published online September 4, 2006; doi: 10.1161/CIRCULATIONAHA.106.626135

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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