Circulating soluble CD36 is associated with glucose metabolism and interleukin-6 in glucose-intolerant men

AASE HANDBERG, ABEL LO PEZ-BERMÉJO, JUDIT BASSOLS, JOAN VENDRELL, WIFREDO RICART, JOSE M FERNANDEZ-REAL

Abstract
Recently, soluble CD36 (sCD36) levels were reported to be elevated in type 2 diabetes, and to be tightly correlated with insulin resistance. Our aim was to obtain further insight into the relationship between insulin sensitivity, low-grade inflammation and sCD36.

We studied glucose-tolerant (n=90) and glucose-intolerant (n=57) moderately obese men. Insulin sensitivity was measured by the frequent sample intravenous glucose tolerance test, and sCD36 by an in-house ELISA assay.

In glucose-intolerant subjects, sCD36 was negatively associated with insulin sensitivity and positively with interleukin-6 (IL-6), fasting glucose, fasting triglycerides, fat-free mass and platelet count. On multiple linear regression analyses, insulin sensitivity contributed 22% of sCD36 variance, independent of age, body mass index (BMI) and IL-6, in glucose-intolerant subjects. The level of sCD36 in subjects with glycosylated haemoglobin (HbA1c) above the mean was higher than in those with HbA1c values below the mean.

Insulin sensitivity is a predictor of sCD36 in men with impaired glucose tolerance. IL-6 is related to sCD36 but does not predict sCD36 independent of insulin sensitivity and BMI.

Key words: diabetes, glucose intolerance, insulin resistance, low grade inflammation, obesity, risk marker, sCD36.

Introduction
CD36 is a multispecific, integral, 88 kDa membrane glycoprotein that is expressed on the surface of a wide variety of cell types, including adipocytes, skeletal muscle cells, platelets, endothelial cells and monocytes/macrophages. CD36 functions as a scavenger receptor for oxidised low-density lipoprotein (LDL) and apoptotic cells on macrophages, and as a fatty acid transporter in muscle and adipocytes. Furthermore, CD36 binds to collagen and probably serves as an adhesion protein. CD36 has also previously been shown to play a substantial role in the pathogenesis of atherosclerosis.

Premature atherosclerosis is the major cause of morbidity and mortality in type 2 diabetes. Indeed, even in a ‘pre-diabetic state’ of glucose intolerance there is an excess risk of atherosclerosis, which is disproportionate to risk factors such as lipid abnormalities. Insulin resistance, commonly seen in overweight individuals and in people genetically predisposed to the metabolic syndrome, and atherosclerosis have both been postulated to result from a state of chronic low-grade inflammation.

The inflammatory state has in part been attributed to secretion of cytokines from enlarged adipocytes or from macrophages that infiltrate the expanded fat depots. However, multiple organs seem to be involved in the elevation of surrogate markers such as high-sensitivity C-reactive protein (CRP) and interleukin-6 (IL-6). It has been proposed that CD36 may serve as a marker of both macrophage activation and inflammation. It is therefore a potentially important marker of macrophage activation in insulin-resistant conditions, as well as an early marker of cholesterol accumulation in the vessel wall.

We have recently identified a soluble form of CD36, soluble CD36 (sCD36), in cell-free plasma. We found increased plasma levels of sCD36 in diabetic patients, and furthermore, sCD36 was tightly correlated with insulin resistance. We hypothesised that sCD36 might be released into the circulation as part of the low-grade inflammatory state observed in insulin resistance, or during cell apoptosis such
The aim of this study was to obtain further insight into the relationship between sCD36, insulin sensitivity and low-grade inflammatory markers in glucose-tolerant and glucose-intolerant men.

**Research design and methods**

**Design of clinical studies**

One hundred and forty-seven male Caucasian subjects were recruited and studied, with assessment of glucose tolerance and insulin sensitivity, within an ongoing study dealing with non-classical cardiovascular risk factors. All subjects with normal glucose tolerance (n=90) had fasting plasma glucose < 7.0 mmol/L and two-hour post-load plasma glucose < 7.8 mmol/L after a 75 g oral glucose tolerance test. Glucose intolerance was diagnosed in 57 subjects according to the American Diabetes Association criteria (post-load glucose between 7.8 and 11.1 mmol/L). Inclusion criteria were: 1) body mass index (BMI) < 40 kg/m², 2) absence of systemic disease, and 3) absence of infection within the previous month. None of the control subjects were taking any medication or had evidence of metabolic disease other than obesity. Alcohol and caffeine were withheld for 12 hours before performing the insulin sensitivity test. Blood pressure was measured with the patient in the resting state. Liver disease and thyroid dysfunction were specifically excluded by biochemical evaluation.

All subjects gave their written informed consent after the purpose of the study had been explained to them. The institutional review boards of the participant institutions approved the protocol.

**Anthropometric and biochemical measurements**

BMI and waist-to-hip ratio (WHR) were measured as previously reported.14 Fat mass (FM), fat-free mass (FFM) and percentage fat mass were calculated using bioelectric impedance (Holtain BC Analyzer, UK).

**Table 1. Anthropometric and biochemical variables of the study participants**

<table>
<thead>
<tr>
<th></th>
<th>Men with normal glucose tolerance</th>
<th>Glucose-intolerant men</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>90</td>
<td>57</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.4±11.3</td>
<td>54.9±10.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.6±12.2</td>
<td>84.2±12.3</td>
<td>0.035</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.02±3.3</td>
<td>29±3.9</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.92±0.06</td>
<td>0.95±0.07</td>
<td>0.006</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>71.9±9.6</td>
<td>72±9.1</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>124.2±13.8</td>
<td>132.6±15.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79.3±9.5</td>
<td>83.6±9.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.2±0.4</td>
<td>5.9±1.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting insulin (mU/L)</td>
<td>8.7±4.7</td>
<td>12±5.9</td>
<td>0.001</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>4.73±0.3</td>
<td>5.1±0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>207±40</td>
<td>217±37</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>98.5±57</td>
<td>125.6±86</td>
<td>0.017</td>
</tr>
<tr>
<td>Insulin sensitivity (10^-4<em>mU/L)</em></td>
<td>1.76±4.11</td>
<td>0.77–2.36</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glucose effectiveness*</td>
<td>0.017–0.023</td>
<td>0.014–0.028</td>
<td>0.02</td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL)</td>
<td>1.01±0.5</td>
<td>1.3±0.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Soluble CD36 (arbitrary units)*</td>
<td>0.42–0.99</td>
<td>0.32–1.23</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Key:** HbA1C = glycosylated haemoglobin; *interquartile range. To convert cholesterol from mg/dL to mmol/L, multiply by 0.0259; to convert triglycerides from mg/dL to mmol/L, multiply by 0.01129

Serum glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Brea, CA, US). In the insulin sensitivity studies, plasma glucose was measured immediately on a YSI 2300 STAT Plus (YSI Incorporated, Yellow Springs, OH, US). Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase, and total serum triglycerides through the reaction of glycerol-phosphate-oxidase and peroxidase. Glycosylated haemoglobin (HbA1C) was measured by the high-performance liquid chromatography method (Bio-Rad, Munich, Germany, and autoanalyzer Jokoh HS-10, respectively). Intra-assay and inter-assay coefficients of variation were less than 4% for all these tests.

Serum insulin levels were measured in duplicate by
monoclonal immunoradiometric assay (IRMA) or enzyme-amplified sensitivity immunoassay (EASIA) (Medgenix Diagnostics, Fleunes, Belgium), and intra-assay and inter-assay coefficients of variation were similar to values previously reported.14

Serum interleukin-6 (IL-6) was measured using a commercial immunoassay (Medgenix IL-6 EASIATM, BioSource, Fleunes, Belgium), with coefficients of variation lower than 6%.

ELISA assays for determination of sCD36 in plasma
Soluble CD36 was measured by an in-house ELISA assay, essentially as previously reported.13 Catcher antibody Goat(sc5522)-CD36 and detection antibody sc9154 were from Santa Cruz Biotechnology, CA, US.

For determination of CD36 by ELISA, a pool of EDTA-plasma served as standard, and was applied in increasing dilutions in duplicate. Other pools of EDTA-plasma served as high and low controls, and PBS as background. Patient samples were applied in appropriate dilutions in duplicate. Absorptions were calculated relative to the standard EDTA-plasma pool, and were expressed as relative units. True values of controls and inter-assay SD were determined by double determinations of each control level on 15 different days. Runs were accepted if the controls were within ±2 SD from mean. Most were within 1 SD, in fact. The intra-assay coefficient of variation was 6%, and the total coefficient of variation was 16.4%.

EDTA-plasma from 100 subjects from the routine blood sampling was pooled, aliquoted and stored at -80˚C. This constituted the standard pool.

Statistical methods
Descriptive results of continuous variables are expressed as mean ± SD. Parameters that did not follow normal distribution (S, Sg, triglycerides and sCD36) were log-transformed. Differences between groups were assessed by Student’s t-test. The relationships between quantitative variables were analysed by simple correlation using log-transformed variables (Pearson’s test) and by stepwise multivariate linear regression analysis. Levels of statistical significance were set at p<0.05.

Results
Characteristics of the study participants are shown in table 1. Subjects with glucose intolerance were significantly older and heavier, and showed lower insulin sensitivity and glucose effectiveness, than subjects with normal glucose tolerance.

In all subjects studied, sCD36 was significantly correlated with HbA1C, triglycerides, Sg (glucose effectiveness), platelet count, IL-6 and FFM. Fasting glucose, glycosylated haemoglobin, plasma triglycerides, platelet count, and plasma IL-6 correlated positively while insulin sensitivity correlated negatively with sCD36 in subjects with glucose intolerance (table 2, figure 1a). In subjects with normal glucose tolerance, sCD36 correlated with HbA1C and Sg, and negatively with fasting glucose (table 2). Thus, the associations of sCD36 with triglycerides, platelet count and IL-6 (especially in non-obese subjects, figure 1b) were observed only in subjects with glucose intolerance but not in subjects with normal glucose tolerance. IL-6 correlated with fat mass (r=0.20, p=0.03).

We performed various multiple linear regression analyses to predict circulating soluble CD36. In the whole study population, and in subjects with normal glucose tolerance, fat-free mass was the only significant factor, contributing 12% of the variance in sCD36 even after controlling for BMI, age, insulin sensitivity and IL-6. In subjects with altered glucose tolerance, the results were clearly different, as shown in table 3. Insulin sensitivity contributed 22% of the variance in sCD36, independently of age, BMI and IL-6. When adding fat-free mass (FFM) to this model, however, both FFM and
IL-6 emerged as significant contributors to sCD36 variance. This finding suggests that fat-free mass and circulating IL-6 independently influence circulating sCD36 through decreased action of insulin. IL-6 independently contributed 18% to sCD36 variance after controlling for age, BMI, waist diameter and FFM (table 3).

We also performed a multiple linear regression analysis to predict insulin sensitivity. Age, BMI and sCD36 were considered as independent variables. In this model, BMI and sCD36 independently contributed 30% and 7%, respectively, to insulin sensitivity variance in subjects with altered glucose tolerance. In subjects with normal glucose tolerance,
only BMI (p<0.0001) contributed independently by 24% to insulin sensitivity variance.

It could be argued that the distinction between normal glucose tolerance and glucose intolerance is much more blurred than that between diabetes and non-diabetes, with many people likely to be re-classified on repeat testing. While the diabetic state may produce secondary changes in insulin sensitivity due to glucotoxicity, this does not occur in glucose intolerance. For that reason we re-classified the study participants according to integrated glucose values, as inferred from HbA1C levels. Subjects with HbA1C above the mean were heavier (BMI 28.5±3.9 vs. 27.1±3.4 kg/m², p=0.02), and had significantly decreased insulin sensitivity (log insulin sensitivity 0.43±0.21 vs. 0.54±0.21, p=0.002) and significantly higher plasma sCD36 (0.88±0.58 vs. 0.65±0.39 arbitrary units, p=0.02). When we tested the associations in these subgroups, they were very similar to those found in subjects with impaired and normal glucose tolerance, probably reflecting an inflammatory milieu underlying increased integrated glucose values and, in this case, increased plasma sCD36 levels.

Conclusions
Recently, we identified a soluble form of CD36 in human plasma, and found markedly increased levels of this substance in patients with type 2 diabetes. We proposed that sCD36 might represent a marker of the metabolic syndrome and a potential surrogate marker of atherosclerosis.13 In the present study, we looked for a relationship between sCD36, inflammatory markers and insulin resistance in a predisposed, non-diabetic population. Both inflammation and insulin action interacted with plasma sCD36 levels but only in patients with glucose intolerance. It should be noted that, although significant, the associations were relatively modest, and should therefore be interpreted with caution. According to our current findings, various scenarios are plausible.

Plasma sCD36 concentration may be linked to insulin resistance and obesity-driven low-grade inflammation. Here we demonstrated the relationship between insulin resistance and sCD36 in a large group of non-diabetic, glucose-intolerant males. Of importance is the novel association between sCD36 and IL-6. Elevated IL-6 has been attributed to macrophage activation and infiltration of fat tissue in obesity, and of liver tissue with fat accumulation in liver cells; both contribute to a systemic low-grade inflammatory state that promotes insulin resistance in skeletal muscle and other tissues.21 Despite the fact that IL-6 does not predict sCD36 independently of insulin resistance, hypothetically sCD36 may be released as a result of the low-grade inflammatory state and macrophage activation seen during the development of insulin resistance.

The insulin-resistant, glucose-intolerant subjects in the present study were more obese than subjects in the control group. Obesity and a high-fat diet activate IKKbeta/NFkappaB, leading to activation of the immune system and increased expression of numerous markers and potential mediators of inflammation that can cause insulin resistance.21 Elevated monocyte CD36 in ob/ob diabetic mice was proposed to result from a compromised insulin signal.16 We have previously reported negative associations between circulating sCD36 and insulin sensitivity in type 2 diabetic patients, and in obese subjects13 and women with polycystic ovary syndrome (PCOS).17 both groups having normal glucose tolerance. Our present finding that sCD36 is negatively associated with insulin sensitivity in glucose-intolerant subjects only indicates that when insulin sensitivity decreases it is reflected by higher sCD36 levels. Thus, it appears that IL-6 does not control sCD36 but the two parameters are interrelated and may both be inflammatory markers related to early changes in insulin sensitivity.

Our finding that FFM contributes to the sCD36 level in glucose-tolerant individuals may seem contradictory to the relationship between sCD36 and insulin resistance in glucose-intolerant subjects, and may be taken to indicate that a basal level of sCD36 originates from skeletal muscle as well as other tissues that express CD36. Once the inflammatory cascade is triggered by, for example, abdominal fat accumulation (in glucose-intolerant individuals), tissues might be infiltrated with macrophages, and CD36 expression may increase from the resulting insulin resistance and hyperglycaemia.13 In that case sCD36 represents a measure of insulin resistance and inflammation in addition to the basal contribution from muscle. Muscle tissue, which is one of the major components of FFM, is the major target for insulin-stimulated glucose disposal.18,19 FFM constitutes, among other factors, a determinant of insulin sensitivity.20 For instance, gender-related differences in insulin sensitivity have been well described.21-25 Women are more insulin-sensitive than men despite their higher fat mass, and this is thought to be related to differences in FFM metabolism.21-25 Indeed, sCD36 in healthy young non-obese humans may represent the whole body CD36 expression level, including a contribution from skeletal muscle, whereas the increased sCD36 observed in insulin resistance and type 2 diabetes is proposed to be part of the inflammatory response, with macrophage/monocyte activation and resulting elevated CD36 expression.

Plasma sCD36 concentration has been hypothesised to be linked to subclinical atherosclerosis-driven low-grade inflammation, based on the well-established excess atherosclerosis risk in diabetes and insulin resistance and the associated elevated sCD36 levels.31,32 Macrophage infiltration of the subendothelial space as a result of accumulation of oxidised cholesterol, among other factors,25,26,27 may create a systemic low-grade inflammatory state that promotes atherogenesis. Elevated sCD36 in insulin-resistant states might thus represent a marker of the accelerated fat accumulation in the vessel wall.

In conclusion, soluble CD36 in moderately obese, glucose-intolerant males is related to insulin resistance and IL-6. Circulating sCD36 might represent a marker of insulin resistance and inflammation, in addition to a possible basal contribution from muscle tissue, in men with decreased glucose tolerance.

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Conflicts of interest statement
None declared.

References