Soluble CD36 (sCD36) Clusters with Markers of Insulin Resistance, and High sCD36 Is Associated with Increased Type 2 Diabetes Risk

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Context and Objective: Soluble CD36 (sCD36) may be an early marker of insulin resistance and atherosclerosis. The objective of this prospective study was to evaluate sCD36 as a predictor of type 2 diabetes and to study its relationship with components of the metabolic syndrome (MetSy).

Design, Setting, Participants, and Outcome Measures: We conducted a case-referent study nested within a population-based health survey. Baseline variables included sCD36, body mass index, blood pressure, blood lipids, adipokines, inflammatory markers, and β-cell function. A total of 173 initially nondiabetic cohort members who developed type 2 diabetes during 10 yr of follow-up were matched (1:2) with referents. Exploratory factor analysis was applied to hypothesize affiliation of sCD36 to the MetSy components.

Results: Doubling of baseline sCD36 increases the odds ratio for diabetes development by 1.24 in the general study population and by 1.45 in the female population ($P < 0.025$). Comparing upper sCD36 quartiles with lower, odds ratio for diabetes was 4.6 in women ($P = 0.001$), 3.15 in men ($P = 0.011$), and 2.6 in obese individuals ($P < 0.025$). Multivariate analysis shows that sCD36 does not predict diabetes independent of fasting plasma glucose and insulin. Factor analysis of 15 variables generates a six-factor model explaining 66–69% of total variance, where sCD36, body mass index, insulin, proinsulin, and leptin were assigned to the obesity/insulin resistance cluster.

Conclusions: Upper quartile sCD36 is associated with elevated diabetes risk independent of age, gender, and obesity. Baseline sCD36 does not, however, predict diabetes independent of fasting glucose and insulin. sCD36 clusters with important markers of insulin resistance and MetSy that are key predictors of type 2 diabetes. ***(J Clin Endocrinol Metab* 95: 1939–1946, 2010)**

The transmembrane glycoprotein CD36 is expressed in a variety of tissues with tissue-specific function; it has been shown to be involved in angiogenesis, inflammation, lipid metabolism, and atherosclerosis, and has also recently been linked to platelet activation (1–5). Modification of low-density lipoprotein (LDL) to its oxidized form increases atherogeneity of this lipoprotein fraction and may even be mandatory for lipid accumulation in the subendothelial space. Membrane CD36 in monocytes and macrophages is up-regulated by oxidized LDL (1, 3),

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Abbreviations: 2-h glucose, 2 h after glucose administration; BMI, body mass index; BP, blood pressure; CI, confidence interval; CRP, C-reactive protein; CV, coefficient of variation; EFA, exploratory factor analysis; FFA, free fatty acid; FPG, fasting plasma glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MetSy, metabolic syndrome; NEFA, nonesterified fatty acids; OGTT, oral glucose tolerance test; OR, odds ratio; sCD36, soluble CD36.

which is elevated in type 2 diabetes (6, 7). Furthermore, hyperglycemia and insulin resistance up-regulate CD36 expression on the surface of monocytes (8–10). Finally, it has been proposed that CD36 is a marker of macrophage activation and inflammation (11). Thus, monocyte CD36 seems to be up-regulated under conditions associated with type 2 diabetes and the metabolic syndrome and provides an early step in the differentiation of macrophages into foam cells.

We have recently identified a soluble form of CD36, sCD36, in plasma. As in intact monocytes, we found increased plasma levels of sCD36 in diabetic and insulin-resistant patients, tightly correlated with insulin resistance (12, 13). We hypothesized that sCD36 was released to the circulation as part of the low-grade inflammatory state in insulin resistance, or during cell apoptosis such as that taking place after cholesterol accumulation in foam cells. Alternatively, sCD36 could be circulating in microparticles derived from monocytes, platelets, or endothelial cells.

The concept of the metabolic syndrome (MetSy) is based on its association with cardiovascular disease (14) and type 2 diabetes (15, 16). A previous study on the cohort presently studied supported the MetSy as a pathophysiological entity and proposed five main components: obesity/insulin resistance, hyperglycemia, dyslipidemia, hypertension, and an inflammation factor (17). Obesity/insulin resistance and the glycemia factors were the only composite factors that independently predicted the development of type 2 diabetes, and insulin resistance was a core perturbation associated with the metabolic syndrome (17). sCD36 is tightly related to risk factors of accelerated atherosclerosis in type 2 diabetes such as insulin resistance and glycemic control (12, 13), and we propose that sCD36 might represent a potential predictor/early marker of insulin resistance and atherosclerosis.

In the present study, there were two main objectives: 1) to investigate interplay and clustering of traditional MetSy variables and sCD36 to gain deeper insight into its possible pathophysiological role; and 2) to evaluate the impact of sCD36 as a predictor of type 2 diabetes.

Subjects and Methods

Study design and subjects

The Västerbotten Intervention Study (VIP) is a program for prevention of cardiovascular diseases and diabetes in the county of Västerbotten, Northern Sweden. Since 1985 all inhabitants have been invited to a health survey at ages 40, 50, and 60 yr. Participants were also asked to donate blood for future research purposes. No significant differences in social conditions have been found between participants and nonparticipants (18). Data from this case-referent study has previously been used in: 1) a study describing a simple prediction algorithm based on fasting plasma glucose (FPG), glycosylated hemoglobin, and body mass index (BMI) that identifies individuals at risk of developing type 2 diabetes (19); 2) a study evaluating associations between psychosocial stress variables and the risk of developing type 2 diabetes (20); 3) a study investigating the structure of MetSy and identifying prediction factors of type 2 diabetes (17); and 4) a study evaluating the fatty acid profile in the erythrocyte membrane preceding development of type 2 diabetes (21).

Our target population was the middle-aged population in the Umea Health Care District (total approximately 130,000 inhabitants) that was invited to a health survey in VIP at ages 40, 50, and 60 yr in the time period 1989–2000. Of these, 33,336 (representing 52% of the target population; 49% men, 56% women) participated in the VIP. We excluded persons who had prevalent or incident type 2 diabetes (n = 1038) at the time of the health survey or who did not have a complete oral glucose tolerance test (n = 3562). Our study base consists of the remaining 28,736 individuals.

The present study is a nested case-referent study. Among subjects in the study base, 277 subjects who were clinically diagnosed with type 2 diabetes after the health survey were identified from the registers of diagnoses from the Departments of Internal Medicine and Cardiology at the only local hospital, the Umeå University Hospital, and from the computerized patient records from public primary care in the health care district. It is likely that this ascertainment method will include essentially all diabetic individuals because 98.4% of the population is affiliated with public primary care centers, and in a few cases when clinical diabetes is not recognized in primary care, this is done at hospital clinics. For each case, two referents were randomly selected from the study base conditionally on sex, birth calendar year, and calendar year of health survey being the same as for the case. Case records from the study period January 1, 1989, to January 31, 2001, were evaluated for verification of correct type 2 diabetes diagnosis, according to World Health Organization (WHO) definitions. Forty individuals did not meet WHO definitions and were hence excluded, as well as corresponding referents. In addition, one referent was excluded by technical error.

Of the 237 cases, 34 were excluded because they had not donated any blood and 18 because the samples were consumed in prior studies; 11 cases with impaired glucose tolerance at health survey were excluded because they participated in an ongoing intervention study. Finally, one case was excluded because both referents were excluded. Of the 473 referents, 126 were excluded because corresponding matched cases were excluded, 13 were given priority to other studies, 22 were excluded because the samples were consumed in other studies, and seven had not donated any sample of blood. The study population thus consisted of 173 cases and 305 referents.

Mean duration from health survey until end of study for both cases and referents was 8.8 yr (range, 1.1–11.4), and duration from the health survey until type 2 diabetes diagnosis among cases was 5.4 yr (range, 0.1–10.4). The protocol was approved by the Research Ethics Committee of Umea University, and all participants gave informed consent.

Measurements

Obesity was measured by BMI. Blood pressure was measured once after 5 min of rest with a mercury sphygmomanometer with subjects in the supine position. Hypertension was defined as systolic blood pressure (BP) of at least 140 mm Hg or diastolic BP

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of at least 90 mm Hg or ongoing antihypertensive medication. Oral glucose tolerance test (OGTT) was performed with a 75-g glucose load, according to WHO standards. Glucose concentrations were measured in capillary plasma with a Reflotron benchtop analyzer (Roche Molecular Biochemicals GmbH, Mannheim, Germany) in the fasting state (FPG) and at 2 h after glucose administration (2-h glucose). Venous plasma samples were taken and were stored at −80 C, and analyses were performed after the study period. Insulin was analyzed with microparticle enzyme immunoassay (Axsym System; Abbott, Tokyo, Japan) with cross-reactivity to proinsulin 0.016%, no detectable cross-reactivity to C-peptide or glucagon, and intra- and interassay coefficients of variation (CVs) of 2.6 and 2.9% at insulin level 8.7 μU/ml. Proinsulin was analyzed with ELISA (Mercodia, Uppsala, Sweden), the four major proinsulin conversion intermediates reacting 84–95%, with cross-reactivity to insulin less than 0.03% and to C-peptide less than 0.006%. Intra- and interassay CVs were 3.2 and 6.1% at proinsulin level 20.7 pmol/liter. TNF-α and IL-6 were analyzed by ELISA (R&D Systems Ltd., Abingdon, UK): intraassay CV 8.8% at TNF-α 2.6 pg/ml and interassay CV 16.7% at 2.4 pg/ml; intraassay CV 5.9% at IL-6 level 2.73 pg/ml and interassay CV 16.5% at 3.575 pg/ml. Leptin was analyzed with RIA (Linco Research, St. Louis, MO), CVs were 3.9 and 4.7% at 10.4 ng/ml. Nonesterified fatty acids (NEFA) were analyzed with an enzymatic calorimetric method (Wako Chemicals, Richmond, VA). Interassay CV was 2.7% at level 0.33 mmol/liter. Serum lipids were analyzed with routine methods at the Department of Clinical Chemistry at the Umeå University Hospital.

Measurement of sCD36

sCD36 was measured using an in-house ELISA (13). A pool of EDTA plasma, aliquoted and stored at −80 C, was applied in seven dilutions in each run and used as a standard concentration curve. Two dilutions of another EDTA pool were used as internal controls, and each control was run in quadruplicate on each ELISA plate. Patient EDTA plasma samples, which were stored at −80 C, were analyzed in duplicate. Runs were accepted if the controls were within ± 2 SD from mean, and most were within 1 SD. Intraassay CV was 6%, and day-day-assay CV was 16.4%, estimated from internal controls in the study. Standard curves were log-transformed and thereby linear. A few measurements outside the range of the standard curve were calculated from the extrapolated standard curve.

Statistical analysis

Continuous characteristics of subjects are presented as means ± SD or median ± CV, depending on their distribution. Differences between cases and referents were tested by *t* test (continuous variables) or χ^2-test (categorical variables). Variables with a lognormal distribution were log transformed (natural logarithm) in all analyses. However, all resulting odds ratio estimates are presented in terms of the untransformed variables. All distributional assumptions were checked by graphical examinations of quantile-quantile plots. Because the study design involves matching on age and gender, these variables were not included in the statistical analyses.

Univariate analyses of the effect on diabetes development of sCD36, BMI, FPG, and 2-h postload glucose, insulin, triglyceride, C-reactive protein (CRP), IL-6, total cholesterol, TNF-α, and leptin with calculation of odds ratio (OR) for diabetes development were performed by conditional logistic regression. Multivariate analyses of the same variables were performed by conditional logistic regression with backward elimination. Finally, conditional logistic regression on diabetes development with backward elimination only including CRP, IL-6, and sCD36 was performed to investigate prediction of diabetes incidence by inflammatory variables. In supplementary analyses, sCD36 was stratified into gender-specific quartiles, and OR for diabetes development was estimated by univariate and multivariate analyses.

The referent and case populations were separately analyzed by exploratory factor analysis (EFA) to identify patterns in the diabetic and nondiabetic state. Fifteen variables were included: FPG, 2-h glucose, systolic and diastolic BP, triglycerides and high-density lipoprotein (HDL)-cholesterol, BMI, markers on β-cell function, *i.e.* insulin and proinsulin, and adipose tissue mediators, *i.e.* NEFA, leptin, IL-6, TNF-α, and CRP representing the variables previously analyzed with respect to diabetes prediction (17), and sCD36, whose affiliation with the MetSy components we wished to evaluate. EFA comprises several steps leading to reduction of variables into fewer factors, retaining as much as possible of the total variance of the variables. We used the rotated Varimax method that retains the composite factors statistically uncorrelated. The factor loadings express the associations between the single variable and the composite factor to which it is assigned. Only factors with eigenvalue greater than 1 were selected, indicating that a factor accounts for more total variance than any original standardized variable. Factor loadings of at least 0.40, which is a commonly used threshold, were used for further analytical interpretation (22).

For the statistical calculations, we used SAS 9.1 (SAS Institute Inc., Cary, NC) and SPSS 11.5 (SPSS Inc., Chicago, IL). Stata (StataCorp, College Station, TX) was used for receiver operating characteristic analyses.

Results

Study population

We included 478 study participants, 208 women and 270 men. Baseline characteristics are presented in Table 1. sCD36 was 33% higher in male referents compared with female referents ($P < 0.0001$) and 15% higher in male cases compared with female cases ($P = 0.01$). In females, sCD36 in cases tended to be higher (33%) than in referents ($P = 0.08$), whereas no difference was found in males ($P = 0.22$).

Univariate analyses

Univariate analysis with adjustment for age and gender (implicit in study design) showed that a 2-fold increase in baseline sCD36 significantly increased the risk of developing diabetes by 24% [OR = 1.24; 95% confidence interval (CI), 1.03–1.49; $P < 0.025$; Table 2]. In women and men, the OR was 1.45 (95% CI, 1.05–2.00; $P < 0.025$) and 1.14 (95% CI, 0.92–1.43; not significant), respectively (Table 2).

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TABLE 1. Characteristics for cases and referents at baseline

	Women			Men		
	Referents	**Cases**	***P***	**Referents**	**Cases**	***P***
n	133	75		172	98	
Age (median, yr)	50.4	50.3		50.1	50.1	
BMI (kg/m2)	25.3 ± 4.2	29.8 ± 5.1	<0.001	25.3 ± 3.0	29.2 ± 3.2	<0.001
Systolic BP (mm Hg)	130 ± 19	140 ± 17	<0.001	127 ± 16	138 ± 19	<0.001
Diastolic BP (mm Hg)	80 ± 11	85 ± 9	0.001	80 ± 10	88 ± 12	<0.001
FPG (mmol/liter)[a]	5.2 ± 0.8	5.9 ± 0.7	<0.001	5.3 ± 0.7	5.9 ± 0.8	<0.001
2-h glucose (mmol/liter)[a]	7.2 ± 1.6	8.4 ± 2.2	<0.001	6.1 ± 1.6	7.9 ± 2.1	<0.001
HDL-cholesterol (mmol/liter)[b,c]	1.4 ± 0.2	1.2 ± 0.2	<0.001	1.2 ± 0.2	1.0 ± 0.3	<0.001
Total cholesterol (mmol/liter)[b]	5.9 ± 1.1	6.0 ± 1.00	0.675	5.6 ± 1.0	6.0 ± 1.0	0.004
Triglyceride (mmol/liter)[b,c]	1.1 ± 0.5	1.6 ± 0.5	<0.001	1.2 ± 0.4	1.8 ± 0.5	<0.001
NEFA (mmol/liter)[b,c]	0.32 ± 0.74	0.33 ± 0.62	0.145	0.22 ± 0.63	0.27 ± 0.52	<0.001
Leptin (ng/ml)[b]	9.9 ± 0.7	17.0 ± 0.4	<0.001	3.5 ± 0.7	7.1 ± 0.5	<0.001
IL-6 (pg/ml)[b,c]	1.7 ± 0.7	2.4 ± 0.6	0.001	1.8 ± 0.7	2.1 ± 0.6	0.057
TNF-α (pg/ml)[b,c]	1.0 ± 0.9	1.3 ± 0.9	0.861	0.9 ± 0.8	1.0 ± 0.7	0.746
CRP (mg/liter)[b,c]	1.4 ± 0.8	2.7 ± 0.8	<0.001	1.2 ± 0.8	2.0 ± 0.8	<0.001
Insulin (mU/liter)[b,c]	7.0 ± 0.6	12.9 ± 0.6	<0.001	6.3 ± 0.5	12.8 ± 0.6	<0.001
Proinsulin (pmol/liter)[b,c]	11.6 ± 0.6	20.0 ± 0.5	<0.001	12.6 ± 0.5	24.6 ± 0.6	<0.001
sCD36 (arbitrary units)[b,c]	1.5 ± 0.8	2.0 ± 0.8	0.080	2.0 ± 0.9	2.3 ± 0.8	0.224

[a] Glucose determined on capillary plasma.

[b] Determined on samples stored at −80 C, plasma.

[c] Median ± CV. All other parameters are given as mean ± SD.

Comparing upper quartile with lower, baseline sCD36 in the upper quartile was associated with an OR of 4.6 (95% CI, 1.5–13.8; $P = 0.006$) for diabetes development in women and an OR of 3.2 (95% CI, 1.3–7.7; $P = 0.011$) in men (Table 3).

Multivariate analyses

Adjusting for age and gender (implicit in study design), a multivariate analysis of sCD36, BMI, FPG, 2-h glucose, insulin, triglyceride, CRP, IL-6, TNF-α, leptin, and cholesterol showed that only BMI, FPG, 2-h glucose in OGTT, and insulin were significant independent variables in predicting the risk of diabetes. Neither sCD36 nor triglyceride, CRP, IL-6, TNF-α, leptin, or cholesterol independently influenced diabetes risk (Table 4). When the contributions of insulin and glucose to risk of diabetes were omitted from the analysis, BMI (OR, 1.25; 95% CI, 1.16–1.35; $P < 0.001$) and fasting triglyceride (OR, 2.3; 95% CI, 1.5–3.5; $P < 0.0001$) were significant diabetes predictors, whereas sCD36, inflammatory markers, cholesterol, and leptin were not. Considering only the inflammatory variables CRP, IL-6, and sCD36, IL-6 was not significant ($P = 0.35$), whereas both CRP ($P < 0.0001$) and sCD36 ($P < 0.0075$) were significant diabetes predictors. A 2-fold increase in CRP and sCD36 increased

TABLE 2. Univariate analyses of continuous variables for diabetes prediction

Variable	n	OR	95% CI	*P*
sCD36	478	1.24	1.03–1.49	0.0227
sCD36, females	208	1.45	1.05–2.00	0.0246
sCD36, males	270	1.14	0.92–1.43	0.2327
BMI	477	1.32	1.23–1.41	<0.0001
BMI, females	207	1.21	1.12–1.31	<0.0001
BMI, males	270	1.51	1.33–1.72	<0.0001
FPG	478	4.97	3.34–7.40	<0.0001
2-h glucose	478	1.60	1.41–1.82	<0.0001
Insulin	477	3.27	2.43–4.40	<0.0001
Triglyceride	476	3.11	2.23–4.32	<0.0001
CRP	476	1.71	1.41–2.06	<0.0001
IL-6	472	1.49	1.20–1.85	0.0003
Cholesterol	476	1.25	1.02–1.52	0.0297
TNF-α	452	1.01	0.85–1.20	0.919
Leptin	473	3.19	2.34–4.34	<0.0001

CI is lower and upper values of the 95% CI. sCD36, insulin, triglyceride, CRP, IL-6, TNF-α, and leptin were analyzed on a log scale to obtain normal distribution. For the untransformed variables, OR represents an increase in one unit, whereas for the log-transformed variables, OR represents a doubling of the untransformed variable.

TABLE 3. Univariate analyses of sCD36 quartiles for diabetes prediction

	Gender	OR	95% CI	*P*
sCD36 (1)	F			
sCD36 (2)	F	1.95	0.77–4.95	0.16
sCD36 (3)	F	2.29	0.78–6.75	0.13
sCD36 (4)	F	4.60	1.54–13.77	0.006
sCD36 (1)	M			
sCD36 (2)	M	1.79	0.84–3.80	0.13
sCD36 (3)	M	1.43	0.60–3.44	0.42
sCD36 (4)	M	3.15	1.30–7.66	0.011

sCD36 was stratified into gender-specific quartiles: sCD36 (1) is the lower quartile and sCD36 (4) is the upper quartile. Risk of diabetes development compared to quartile 1 was calculated for quartiles 2–4, OR for females (F) and males (M). *P* values are for comparisons with the lowest quartile.

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TABLE 4. Multivariate analyses (conditional logistic regression) for diabetes prediction

	OR	95% CI	*P*
sCD36	1.13	0.77–1.64	0.54
BMI	1.15	1.04–1.27	0.007
FPG	3.63	2.08–6.31	<0.0001
2-h glucose	1.41	1.17–1.71	0.001
f-insulin	1.98	1.17–3.35	0.01
Triglyceride	1.45	0.82–2.59	0.21
CRP	1.16	0.81–1.64	0.43
IL-6	1.07	0.71–1.61	0.75
Cholesterol	0.97	0.66–1.41	0.85
TNF-α	0.84	0.60–1.17	0.30
Leptin	0.98	0.54–1.79	0.96

For the untransformed variables OR represents an increase in one unit, whereas for the log-transformed variables OR represents a doubling of the untransformed variable. sCD36, insulin, triglyceride, CRP, IL-6, TNF-α, and leptin were analyzed on a log scale to obtain normal distribution. CI is lower and upper values of the 95% CI.

diabetes risk by ORs of 1.69 (95% CI, 1.40–2.05) and 1.32 (95% CI, 1.09–1.62), respectively (results not shown). Among BMI, FPG, 2-h glucose, fasting insulin, triglycerides, CRP, and IL-6, multiple regression analysis with backward elimination showed that at a significance level of 0.05 only BMI and CRP were independent predictors of sCD36 (results not shown).

After adjusting for BMI [stratified into normal (BMI <25 kg/m^2), overweight (BMI 25–29.9 kg/m^2), and obese (BMI ≥ 30 kg/m^2)], sCD36 in the upper gender-specific quartile was associated with an increased risk of diabetes by 2.6 (95% CI, 1.2–5.9; $P \leq 0.02$) independent of obesity.

Factor analysis

EFA, applied on the case population, generated six factors explaining 69% of the total variance: all variables with varying loadings in all rotated factors (Table 5). In this model, sCD36 clusters with BMI, proinsulin, and insulin. Insulin and proinsulin also cluster with HDL and triglycerides, but with lower factor loadings. In addition, BMI clusters with leptin and NEFA. The referent population generated six factors explaining 66.3% of the total variance. In this model sCD36 was associated with proinsulin and free fatty acid (FFA) (results not shown). We applied the previously hypothesized model of MetSy based on EFA results from this population (17) on the present EFA results, and taking biological aspects into account, we assigned each variable to only one factor. This yielded a five-factor model, where sCD36 was assigned to the obesity/insulin resistance cluster together with BMI, insulin, proinsulin, and leptin.

Diabetes prediction model

In Ref. 17, we proposed multivariate models of diabetes prediction including FPG and proinsulin in women and FPG, proinsulin, and BMI in men. Adding sCD36 to the models had only a minor impact on diabetes prediction estimated by receiver operating characteristic analysis in men (0.9 *vs.* 0.85 without sCD36) and no change in women (0.84 *vs.* 0.84 without sCD36).

TABLE 5. EFA: rotated component matrix of cases

Component	1	2	3	4	5	6
Systolic BP				0.88		
Diastolic BP				0.91		
FPG						0.47
2-h glucose						0.83
BMI		0.49				
CRP[a]			0.8			
FFA[a]					0.8	
HDL	−0.81					
Triglyceride	0.81					
IL-6[a]			0.82			
f-insulin[a]	0.47	0.64				
Leptin[a]					0.71	
Proinsulin[a]	0.46	0.67				
TNF-α[a]						
sCD36[a]		0.7				

EFA comprises several steps leading to reduction of variables into fewer factors, retaining as much as possible of the total variance of the variables. The Varimax method retains the composite factors statistically uncorrelated. The factor loadings express the associations between the single variable and the composite factor to which it is assigned. Only factors with eigenvalue greater than 1 were selected, indicating that a factor accounts for more total variance than any original standardized variable. Factor loadings of at least 0.40, which is a commonly used threshold, are given in matrix.

[a] Parameters analyzed on a log-scale to obtain normal distribution.

Discussion

From the public health perspective, there is a strong incentive to identify people at risk of future type 2 diabetes and to increase our understanding of the pathophysiology of this disease because it carries a high risk for cardiovascular disease. Due to its many ligands and functions, CD36 could impact a variety of conditions such as insulin resistance, inflammation, and atherosclerosis (1–5, 23–25). The recently identified sCD36 has been proposed to reflect CD36 expression, particularly in monocytes and infiltrating macrophages, and may thus potentially be a marker integrating insulin resistance and atherosclerosis (13). We have reported consistent associations between sCD36 and insulin resistance in insulin-resistant conditions, but only weak associations in healthy populations (12, 13, 26). Elevated sCD36 was present not only in overt diabetes but also in prediabetic conditions such as polycystic ovary syndrome and impaired glucose tolerance, indicating that sCD36 may have the potential to reflect early changes in CD36 expression involved in the pathogenesis of diabetes or the development of components in

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the MetSy. Our present findings 1) of elevated diabetes risk particularly in women with high sCD36, independent of obesity; and 2) that sCD36 levels seem to be predicted by CRP and BMI, support this hypothesis. There are several potential underlying pathogenic mechanisms. Elevated FPG may be the consequence of liver insulin resistance secondary to fat accumulation in the liver. Liver fat accumulation may initially be an adaptive mechanism to elevated fatty acid levels, a result of increased lipolysis in the fat tissue, or diet-induced liver fat accumulation that eventually leads to insulin resistance and dyslipidemia. Liver CD36 expression plays an important role in this process, and liver fat accumulation, at least in animal models, is associated with increased liver CD36 expression (27, 28). In insulin-resistant humans, we previously found that (elevated) sCD36 and insulin resistance were independent predictors of enzymatic markers of liver injury (alanine aminotransferase, aspartate aminotransferase) (26). Liver fat accumulation may result in both local and systemic low-grade inflammation, each adding to risk of diabetes. Uptake of FFA in monocytes and macrophages may induce CD36 expression through a peroxisome proliferator-activated receptor γ-dependent mechanism, and CD36 expression may also be up-regulated by inflammation (23). In moderately obese males with impaired glucose tolerance, IL-6 is related to sCD36 but does not predict sCD36 independent of insulin sensitivity and BMI (29), whereas in women with polycystic ovary syndrome, sCD36 and IL-6 or CRP were not interrelated (12). Thus, in accordance with our present findings of sCD36 being associated with diabetes development independent of IL-6 and CRP, sCD36 seems not to be exclusively regulated by the inflammatory component of insulin resistance. That high sCD36 is associated with increased diabetes risk, independent of obesity, underscores the potential of sCD36 as an early marker of the liver component of insulin resistance during diabetes development.

Another potential pathogenic mechanism for the association of sCD36 and diabetes development is up-regulation of CD36 expression by insulin resistance *per se* (impaired insulin signaling cascade). Insulin resistance, well-established as an early predictor of diabetes, is associated with an increased transcription of CD36 in monocytes (9). In our study, no direct measurements of insulin resistance at baseline were included. Due to the complexity of MetSy, standard statistical methods may be insufficient to elucidate the nature of associations of different variables with disease. Factor analysis is a method to simplify the underlying structure by way of identifying composite factors, *i.e.* combinations of the simultaneously occurring components of MetSy (22). In a previous study of this population, five clusters of components in MetSy were proposed: BP, inflammation, obesity, lipids, and glycemia (17). In contrast to inflammation and dyslipidemia, obesity with accompanying insulin resistance and β-cell decompensation were core perturbations promoting and predicting progression to type 2 diabetes. Our current data suggest that circulating CD36 cluster with markers of insulin resistance. Recently, significant evidence for the association between common variants in the *CD36* gene and the MetSy and its components was presented (30). *CD36* polymorphisms contributed to individual and population variability in blood lipids and to susceptibility to the MetSy. Here, we present data at the protein level indicating that CD36 may be involved in the insulin resistance component of the MetSy. Our finding of a stronger diabetes prediction by sCD36 in females demands further investigations of the gender aspect. Also, the potential mechanisms linking CD36 to insulin resistance and the possible involvement of CD36 polymorphisms need further investigation.

In multivariate regression analysis only glucose, insulin, and BMI were independent predictors of diabetes. Inclusion of sCD36 in the diabetes prediction model that was proposed in Ref. 17 and generated from the same study cohort added very little to diabetes prediction. Of note is the large difference in assay performance for the glucose and insulin assays with CVs less than 3% compared with that of sCD36 which is around 5- to 6-fold higher. Circulating CD36 may well have a significant impact on one or several steps in diabetes pathogenesis despite lack of significance in statistical analyses that involves measures of diabetes (FPG) or consequences of increased FPG (increased f-insulin) that are measured at a much higher precision. When the contributions of insulin and glucose to risk of diabetes are ignored, BMI and fasting triglyceride are significant diabetes predictors, whereas sCD36, inflammatory markers, cholesterol, and leptin were not. We propose that the sensitivity of FPG and f-insulin for detecting diabetes under development in the baseline samples is higher than that of sCD36, because sCD36 is measured at a substantially lower precision, and because sCD36 most probably is not elevated before FPG and f-insulin starts to rise—even within their normal range.

From a pathophysiological perspective, our study has the strength of introducing a new biomarker, sCD36, in a population-based prospective setting. From a clinical point of view, sCD36, with its present assay-related limitations, does not add to the current test used for diabetes prediction. However, being involved in cholesterol accumulation in the arterial wall and related to insulin resistance, sCD36 has the potential of being a combined risk marker of diabetes and its associated atherosclerosis risk,

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and thus sCD36 has the potential of having an important impact from a screening and prevention perspective. The limitations are that this new biomarker is measured with a CV at a magnitude to be expected from a new immunological assay but that is also higher than that of glucose, which in addition is the measure used for outcome (*i.e.* diabetes). Furthermore, diabetes cases were identified by routine care examinations and not by standardized follow-up testing, which probably leads to underestimation of cases (17). Finally, neither abdominal obesity nor measures of atherosclerotic burden or liver fat were included in the original study design and thus are not available.

In conclusion, high baseline sCD36 is associated with elevated risk of diabetes independent of age and gender, and even in obese persons upper quartile sCD36 adds to their diabetes risk. sCD36 at the present assay performance does not, however, predict diabetes independent of FPG and insulin. sCD36 clusters with important markers of insulin resistance and MetSy that are key predictors of type 2 diabetes.

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