Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 20 (2009) 477–484
Journal of Nutritional Biochemistry

Circulating soluble CD36 is a novel marker of liver injury in subjects with altered glucose tolerance

Jose-Manuel Fernández-Real[a,*], Aase Handberg[b], Francisco Ortega[a], Kurt Højlund[b], Joan Vendrell[c], Wifredo Ricart[a]

[a]*Unit of Diabetes, Endocrinology and Nutrition, Hospital of Girona Dr. Josep Trueta and CIBER "Pathophysiology of Obesity" 06/03/0010, Girona, Spain*
[b]*Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark*
[c]*Unit of Diabetes, Endocrinology and Nutrition, Hospital of Tarragona, Tarragona, Spain*

Received 5 February 2008; received in revised form 21 April 2008; accepted 6 May 2008

Abstract

Liver injury linked to insulin resistance is characterized by mild to moderate increases in aminotransferase activity. A soluble form of CD36 (sCD36) was recently identified in human plasma. The aim of this study was to evaluate the relationships among plasma sCD36, insulin sensitivity (SI) and indicators of liver health. We evaluated a cohort of men from the general population (n=117). As expected, serum (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) were associated positively with body mass index (BMI) and age and negatively with SI (minimal model method). Circulating sCD36 was positively associated with ALT, AST and GGT in subjects with altered glucose tolerance, but not in those with normal glucose tolerance. The difference in the slope of the relationships was significant (P=.01). Age, BMI and triglycerides (but not sCD36) contributed independently to 29% of ALT variance in subjects with normal glucose tolerance. In contrast, SI and sCD36 contributed independently to 39% of ALT variance in subjects with altered glucose tolerance. The correlation between ALT activity and sCD36 was confirmed in an independent, replication study. In summary, circulating sCD36 could represent a novel marker of liver injury in subjects with altered glucose tolerance.

Keywords: Liver enzymes; Inflammation; Insulin resistance

1. Introduction

More than 20% of Americans have nonalcoholic fatty liver disease (NAFLD), and this is, by far, the leading cause of abnormal liver enzymes in the United States. Nonalcoholic steatohepatitis, a more serious form of NAFLD, can proceed to cirrhosis and even hepatocellular carcinoma. These liver diseases represent the hepatic component of the metabolic syndrome, and this spectrum of liver disease represents a major health problem both in the United States and worldwide. Hepatic steatosis is closely linked to nutrition, including obesity and consumption of certain types of fats. There are a variety of second insults or "hits" that appear to transform simple steatosis into nonalcoholic steatohepatitis, with some of these second hits including certain proinflammatory cytokines and oxidative stress. The interactions of cytokines with oxidative stress and lipid peroxidation have been postulated to play a key role in the induction of liver injury [1]. Cytokines can induce liver damage by different ways. In vivo and in vitro infusion of different cytokines increase fatty acid levels by stimulating adipose lipolysis [2–4]. This increased lipolysis results in a greater free fatty acid (FFA) flux to the liver leading to hepatic steatosis [21]. Inflammatory gene expression increases in liver with increasing adiposity suggesting that hepatocyte fat accumulation might induce a low-grade inflammatory response in liver similar to that induced in adipose tissue by fat accumulation [5–7].

Abbreviations: sCD36, soluble CD36; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, Gamma-glutamyltransferase; FFAs, free fatty acids.

* Corresponding author. Unit of Diabetes, Endocrinology and Nutrition, Hospital de Girona "Dr Josep Trueta," Ctra. França s/n, 17007 Girona, Spain. Tel.: +34 972 94 02 00; fax: +34 972 227 443.
E-mail address: uden.jmfernandezreal@htrueta.scs.es (J.-M. Fernández-Real).

0955-2863/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2008.05.009

CD36, also known as fatty acid translocase (FAT) [8] and platelet glycoprotein IV or IIIb, is a multispecific, integral, 88-kDa membrane glycoprotein expressed on the surface of a wide variety of cell types including adipocytes, skeletal muscle cells, platelets, endothelial cells and monocytes/macrophages [9,10]. Additionally, CD36 has been proposed to be a surrogate of macrophage activation and inflammation [10]. A soluble form of CD36 (sCD36), a marker of altered tissue CD36 expression, was recently identified in human plasma, and elevated levels were found in obesity and type 2 diabetes [11].

CD36 was identified as a facilitator of fatty acid (FA) uptake in muscle and adipose tissue [12,13]. Homozygous disruption of the CD36 locus led to hepatic insulin-resistance with high plasma FFAs and triglycerides [14,15]. The CD36-deficient mouse also exhibits greater than a 60% decrease of FA uptake and utilization by heart, oxidative skeletal muscle and adipose tissues [16,17]. In contrast, mice with muscle CD36 overexpression [13] have enhanced FA oxidation in response to contraction [14].

The liver was not believed to be a major site for FAT/CD36 expression. However, CD36 has been proposed to be involved in the hepatocyte uptake of fatty acids after the discovery of CD36 expression in the liver of animal models [18,19] and humans [20].

Given all this background, we aimed to explore circulating sCD36 in relation to markers of liver injury and insulin resistance in subjects with normal and altered glucose tolerance [21].

2. Subjects and methods

One hundred seventeen consecutive men fulfilling inclusion criteria and enrolled in a cross-sectional, population-based study on cardiovascular risk factors in healthy subjects in Northern Spain were studied. All subjects were of Caucasian origin and reported that their body weight had been stable for at least three months before the study. None of the patients were taking any medication or had any evidence of metabolic disease other than obesity. Of these subjects, 73 had strictly normal glucose tolerance, and 44 showed altered glucose tolerance during an oral glucose tolerance test, 7 of them with previously undiagnosed type 2 diabetes mellitus according to American Diabetes Association criteria. The study was approved by the Ethics Committee of the University Hospital of Girona. Enrolled subjects handed in their written consent after having been previously informed about the study.

Inclusion criteria were (1) body mass index (BMI) <40 kg/m^2, (2) absence of any systemic disease, (3) alcohol intake less than 40 g/day, (4) negative serologic markers for viral hepatitis and (5) absence of drugs that could induce liver disease.

2.1. Insulin sensitivity

Insulin sensitivity was measured using the frequently sampled intravenous glucose tolerance test on a different day. In brief, the experimental protocol started between 8 and 8:30 a.m. after an overnight fast. A butterfly needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at −30, −10 and −5 min, after which glucose (300 mg/kg body weight) was injected over 1 min starting at Time 0, and insulin (Actrapid, Novo, Denmark; 0.03 U/kg) was administered at time 20. Additional samples were obtained from a contra-lateral antecubital vein up to 180 min, as previously described [22].

2.2. Replication study

Ten healthy lean and 9 healthy obese control subjects carefully age- and gender-matched to 9 obese Type 2 diabetic patients participated in the replication study. Obese control subjects and Type 2 diabetic patients were matched for 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.

2.3. Analytical methods

Blood samples were taken after a 12-h fast. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) were analyzed by colorimetry using automated tests (Roche diagnostics, Manheim, Germany). Intraassay and interassay coefficients of variation were less than 4% for these tests. Plasma glucose level was measured in duplicate by the glucose oxidase method, with a coefficient of variation below 2%. Serum insulin was measured in duplicate by monoclonal immunoradiometric assay (Medgenix Diagnostics). Intraassay and interassay coefficients of variation have been previously reported to be less than 7% [22].

2.4. ELISA assay for determination of CD36 in plasma

Determination of sCD36 was determined by ELISA assay essentially as described in Ref. [11]. ELISA plates were coated with catcher antibody against CD36, sc-5522 (polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and stored at −20°C until use. Detection antibody sc-9154 (polyclonal, Santa Cruz Biotechnology) was biotinylated as described in Ref. [11] and stored at −80°C. 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 background was determined by applying phosphate-buffered saline in duplicates. Patient samples were applied in appropriate

dilutions in duplicates. Absorptions were calculated relative to the Standard EDTA plasma pool and expressed as relative units. The intraassay coefficient of variation (CV), which was estimated from double determinations of the high control on 15 different days, was 6%. The interassay CV, estimated from the controls in each run performed, was 16.4%. Runs were only accepted when controls were within the range of ±1.96×S.D. (interassay). An interassay CV of 16% on an immunological assay is slightly higher than the immunological, Food and Drug Administration-approved, CE-marked routine analyses run on automated equipment (5–10%) in our ISO 15189 standard-accredited laboratory. However, in new research analyses involving hand-pipetting and manual dilutions of both standards and internal controls, interassay CVs of 16% may be expected. Therefore, we find the interassay CV of 16% acceptable given the present status of the sCD36 ELISA as a research assay.

2.5. Statistical methods

Statistical analyses were performed using SPSS 12.0 software. Unless otherwise stated, descriptive results of continuous variables are expressed as mean and S.D. for Gaussian variables and median and interquartile range for non-Gaussian variables. Parameters that did not fulfill normal distribution (SI, AST, ALT, GGT and sCD36) were log-transformed to improve symmetry for subsequent analyses. The relation between variables was analyzed by simple correlation (Pearson's test) and multiple regression in a stepwise manner. Levels of statistical significance were set at $P<.05$.

For a given value of $P=.05$, the study had an 80% power to detect significant correlations between serum sCD36 and metabolic parameters (Pearson's coefficient of at least 0.26) in bilateral tests.

3. Results

Table 1 shows the anthropometrical and biochemical characteristics of the study subjects. Glucose-intolerant men were older, heavier and more insulin-resistant than subjects with normal oral glucose tolerance (Table 1). No significant differences in markers of liver injury were found.

When all subjects were considered as a whole, serum ALT activity correlated positively with BMI and plasma fasting triglycerides and negatively with insulin sensitivity (SI). Identical associations regarding serum GGT activity were found except in that the latter was also significantly linked to waist-to-hip ratio and alcohol intake (Table 2). All markers of liver injury were significantly associated with circulating sCD36.

When the analysis was performed separately in men with normal and altered glucose tolerance, significant differences emerged. In men with normal glucose tolerance, only the associations between serum ALT activity and insulin resistance phenotypes were maintained (Table 3A, Fig. 1). In contrast, in subjects with glucose intolerance, serum ALT activity was simultaneously associated with insulin resistance and circulating sCD36 concentration ($r=0.48$, $P=.001$, Table 3B and Fig. 1). The difference in the slope of the relationships (sCD36 and ALT) between subjects with normal and altered glucose tolerance was significant ($P=.01$). In these subjects, sCD36 was also associated with serum AST activity.

Table 1
Anthropometrical and biochemical variables of the study subjects

	Normotolerant men	Glucose-intolerant men	P
N	73	44	–
Age (years)	48.5±11.8	56.0±11.3	.001
Body mass index (kg/m^2)	26.62±3.2	28.7±3.8	.002
Waist-to-hip ratio	0.92±0.06	0.96±0.07	.004
Alcohol intake (g/week)*	63 (8.4–144)	64.3 (13.9–227)	NS
Fasting glucose (mmol/l)	5.17±0.45	5.85±0.6	.003
Fasting insulin (mU/l)	8.1±4.4	11.4±6.5	.003
Cholesterol (mg/dl)	206±42	217±35	NS
Triglycerides (mg/dl)*	78 (58.5–112)	100.5 (68.2–146.7)	.03
HDL-cholesterol (mg/dl)*	52 (45–57)	50 (41–58)	NS
ALT activity (U/L)*	22 (17.5–26.5)	22 (15.2–27)	NS
AST activity (U/L)*	22 (18–24)	21 (17.2–23)	NS
GGT activity (U/L)*	21 (16–33)	22 (19–32)	NS
Insulin sensitivity (10*min^{-1}* μU/ml)*	2.82 (2.05–4.4)	1.32 (0.78–2.39)	<.0001
Soluble CD36 (arbitrary units)*	0.58 (0.40–0.87)	0.54 (0.30–0.79)	NS

* Median and interquartile range.

Multiple regression analysis models were constructed to predict serum ALT, with age, BMI, alcohol intake, triglycerides, SI and sCD36 as independent variables (Table 4). Although SI and sCD36 contributed independently to 39% of ALT variance in glucose intolerant subjects, only BMI and triglycerides, in addition to age, contributed to ALT variance (29%) in subjects with normal oral glucose tolerance (Table 4).

3.1. Replication study

Serum ALT activity was significantly higher in type 2 diabetic patients (35.0±16.9 U/L) compared with obese subjects (20.0±7.79 U/L) ($P=.017$) and with lean subjects (16.5±5.84 U/L) ($P=.003$). Soluble sCD36 correlated significantly with ALT activity ($r=0.45$, $P=.014$) only when obese Type 2 diabetic patients were included in the analysis.

4. Discussion

The prevalence of obesity and insulin resistance has risen dramatically worldwide. This has led to a marked increase in the prevalence of liver injury [23–26]. The identification of early markers of liver dysfunction is very important. Traditionally, aminotransferase levels are directly associated with BMI, but the effect of overweight

Table 2
Correlations between circulating soluble CD36 and selected variables in all subjects as a whole.

n=117	Log AST	Log GGT	Age	BMI	WHR	Alcohol intake	Fasting glucose	Fasting insulin	Log triglyc	Log SI	Log sCD36
Log ALT	0.525	0.275	−0.267	0.222	0.113	0.011	0.175	0.274	0.228	−0.284	0.233
	0.000	0.003	0.004	0.016	0.226	0.906	0.059	0.004	0.013	0.002	0.012
Log AST	1	0.050	−0.129	−0.003	0.081	−0.112	0.038	−0.097	−0.073	−0.026	0.292
		0.596	0.166	0.977	0.411	0.246	0.688	0,318	0.436	0.783	0.001
Log GGT		1	0.063	0.228	0.230	0.247	0.096	0.259	0.278	−0.222	0.182
			0.498	0.013	0.013	0.009	0.302	0.007	0.002	0.016	0.050
Age			1	0.152	0.116	0.025	0.063	−0.005	−0.038	−0.105	0.048
				0.102	0.216	0.798	0.500	0.957	0.683	0.260	0.604
BMI				1	0.492	−0.047	0.195	0.494	0.300	−0.604	0.048
					0.000	0.624	0.035	0.000	0.001	0.000	0.607
WHR					1	0.005	0.259	0.400	0.287	−0.450	−0.101
						0.959	0.005	0.000	0.002	0.000	0.279
Alcohol intake						1	0.096	−0.081	0.023	0.099	−0.073
							0.319	0.411	0.815	0.306	0.45
Fasting glucose							1	0.321	0.002	−0.399	0.012
								0.001	0.986	0.000	0.898
Fasting insulin								1	0.401	−0.549	−0.028
									0.000	0.000	0.773
Log triglyc.									1	−0.345	0.180
										0.000	0.053
Log SI										1	−0.067
											0.473

The upper row of each correlation represents the correlation coefficient and the lower row reports *P* value.
WHR, waist-to-hip ratio; triglyc, fasting triglycerides.

on markers of liver function seems to be largely influenced by insulin action, inflammation and characteristics of the metabolic syndrome [27,28]. Aminotransferase levels, even at normal levels, correlate positively with proinflammatory cytokines (tumor necrosis factor α and interleukin 18) and negatively with anti-inflammatory cytokines [29–33].

The findings of our study suggest that circulating sCD36 may be a marker of liver injury in subjects with altered glucose tolerance, independent of SI. In more insulin-sensitive subjects (those with normal glucose tolerance), only BMI and triglycerides were significantly associated with markers of liver health. In the two-hit theory of liver injury, a baseline of liver steatosis induced by obesity

Table 3A
Correlations between circulating soluble CD36 and selected variables in men with normal oral glucose tolerance

n=73	Log AST	Log GGT	Age	BMI	WHR	Alcohol Intake	Fasting glucose	Fasting insulin	Log triglyc	Log SI	Log sCD36
Log ALT	0.478	0.189	−0.241	0.328	0.168	−0.042	0.112	0.363	0.323	−0.232	−0.005
	0.000	0.109	0.040	0.005	0.156	0.734	0.345	0.003	0.005	0.049	0.962
Log AST	1	0.077	−0.104	0.072	−0.103	−0.141	−0.097	−0.026	−0.077	0.010	0.180
		0.518	0.383	0.545	0.387	0.254	0.413	0.834	0.519	0.930	0.128
Log GGT		1	0.122	0.109	0.124	0.277	0.148	0.143	0.202	−0.115	0.154
			0.302	0.358	0.296	0.023	0.213	0.251	0.087	0.331	0.193
Age			1	−0.055	−0.011	0.066	0.034	0.000	−0.161	0.024	0.091
				0.646	0.927	0.598	0.777	0.999	0.174	0.842	0.444
BMI				1	0.400	−0.124	0.223	0.533	0.225	−0.591	0.004
					0.000	0.317	0.058	0.000	0.055	0.000	0.971
WHR					1	−0.095	0.346	0.447	0.239	−0.395	−0.195
						0.446	0.003	0.000	0.041	0.001	0.099
Alcohol intake						1	0.126	−0.035	−0.043	0.240	0.024
							0.308	0.786	0.732	0.051	0.847
Fasting glucose							1	0.395	0.037	−0.233	−0.026
								0.001	0.758	0.048	0.829
Fasting insulin								1	0.337	−0.488	−0.199
									0.006	0.000	0.110
Log triglyc.									1	−0.270	0.102
										0.021	0.390
Log SI										1	0.061
											0.607

The upper row of each correlation represents the correlation coefficient and the lower row reports *P* value.

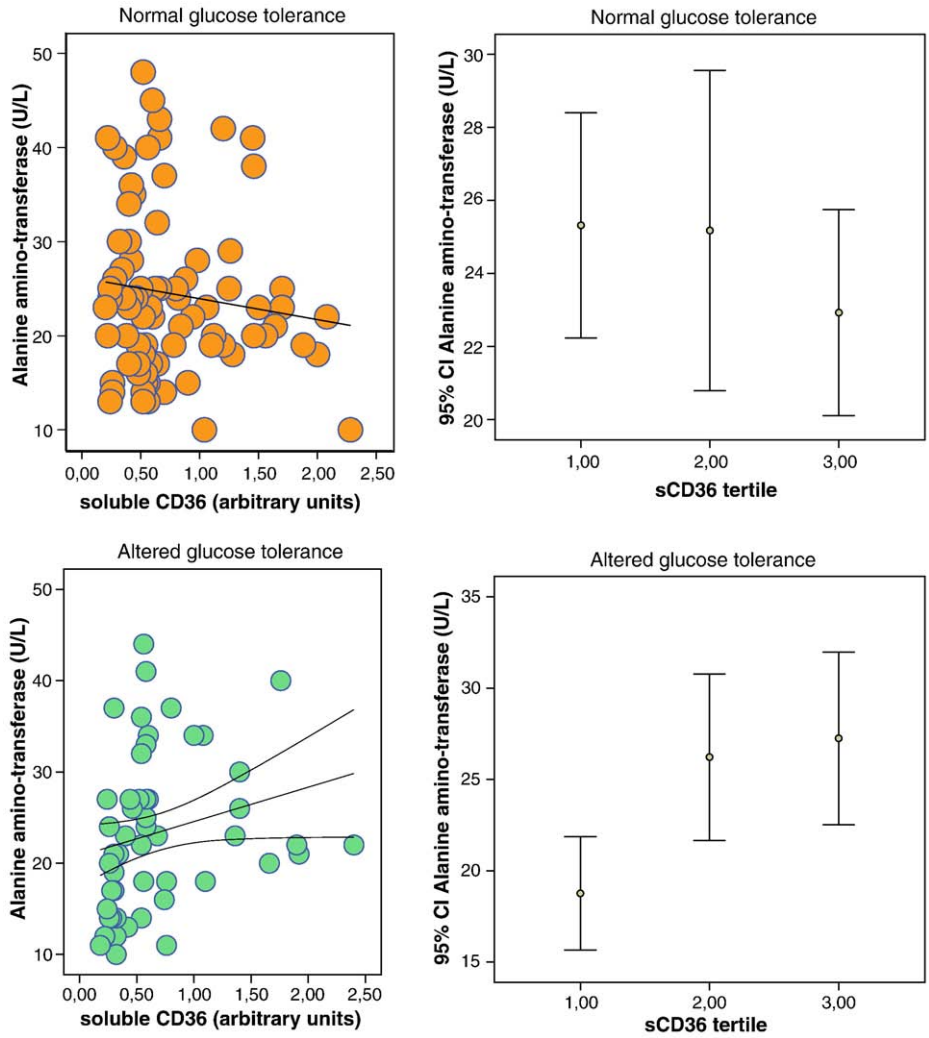

Fig. 1. Associations between sCD36 and serum ALT activity in subjects with normal (upper panels) or altered glucose tolerance (lower panels).

requires a second hit capable of inducing inflammation, fibrosis, or necrosis for significant liver injury to develop. The first hit is usually envisioned as insulin resistance, and the second hit, as "inflammation" [34]. We could hypothesize that sCD36 forms part of this "second hit" once established insulin resistance develops in subjects with altered glucose tolerance.

In the present study we have confirmed the inverse correlation between sCD36 and SI in glucose-intolerant men [11]. However, such a relationship was not found in glucose-tolerant males nor was it present when SI, and sCD36 was analysed in all study subjects. In the study of Handberg et al. [11], an inverse correlation between sCD36 and insulin-stimulated glucose disposal was found both in diabetic as well in all study subjects as a whole. There was no significant correlation between sCD36 and glucose infusion rate in neither glucose-tolerant lean subjects (r=0.16, P>.05, data unpublished) nor glucose-tolerant obese subjects (r=−.01, P>.05, data unpublished), and thus, there is no discrepancy between the SI and sCD36 relationships in our present study compared to the previous [11]. Furthermore, the measures of SI in the two studies cannot be readily compared.

sCD36 may be released from degrading cells just like ALT is increased with liver cell destruction associated with insulin resistance [7,35,36]. Interestingly, a recent article disclosed a lower expression of *CD36* gene in livers from Type 2 diabetic subjects [20]. With insulin resistance and obesity, plasma lipids are disturbed and accumulate in the liver, potentiating the "low-grade inflammation." This may induce release/secretion of CD36 by monocytes or macrophages (Kupffer cells) and may even release CD36 from liver cells.

It has been hypothesised that the lower expression of CD36 observed in liver might lead to a reduced capacity to repress hepatic lipogenesis when circulating levels of fatty acids increase. The livers of $CD36^{-/-}$ mice are severely insulin-resistant with regard to the suppression by insulin of endogenous glucose production. In $CD36^{-/-}$ mice, increased plasma FFA levels lead to an increased flux of fatty acids toward the liver [37]. The increased FA flux toward the liver

Table 3B
Correlations between circulating soluble CD36 and selected variables in men with altered glucose tolerance

n=44	Log AST	Log GGT	Age	BMI	WHR	Alcohol Intake	Fasting glucose	Fasting insulin	Log triglyc	Log SI	Log sCD36
Log ALT	0.580	0.460	−0.283	0.167	0.114	0.099	0.386	0.294	0.197	−0.565	0.481
	0.000	0.002	0.062	0.280	0.466	0.535	0.010	0.062	0.199	0.000	0.001
Log AST	1	0.027	−0.114	−0.038	0.113	−0.062	0.304	−0.114	−0.037	−0.220	0.419
		0.859	0.460	0.806	0.478	0.698	0.045	0.477	0.811	0.151	0.005
Log GGT		1	−0.146	0.388	0.388	0.212	−0.071	0.389	0.38	−0.386	0.246
			0.345	0.009	0.010	0.177	0.647	0.012	0.012	0.010	0.107
Age			1	0.263	0.107	−0.113	0.287	0.219	−0.065	0.066	0.023
				0.085	0.493	0.478	0.059	0.168	0.675	0.670	0.882
BMI				1	0.515	−0.040	−0.094	0.365	0.288	−0.514	0.117
					0.000	0.801	0.544	0.019	0.058	0.000	0.451
WHR					1	0.057	−0.098	0.265	0.257	−0.367	0.018
						0.719	0.530	0.095	0.096	0.015	0.909
Alcohol intake						1	−0.024	−0.198	0.039	0.078	−0.152
							0.880	0.220	0.806	0.625	0.338
Fasting glucose							1	0.048	−0.246	−0.203	0.082
								0.768	0.108	0.186	0.597
Fasting insulin								1	0.378	−0.527	0.142
									0.015	0.000	0.376
Log triglyc.									1	−0.325	0.259
										0.031	0.089
Log SI										1	−0.302
											0.046

The upper row of each correlation represents the correlation coefficient, and the lower row reports *P* values.

(+300%) in CD36$^{-/-}$ mice exceed the increased β-oxidation capacity (+50%). When fatty acid uptake exceeds utilization, the fatty acids are stored as TG in CD36$^{-/-}$ mice. Increased hepatic TG content correlates with impaired suppression of endogenous glucose production by insulin [38,39]. Epidemiological evidence has been presented that liver fat accumulation is associated with high circulating plasma FFA levels and hepatic insulin resistance in humans [40,41].

On the other hand, CD36 is regulated by the peroxisome proliferator-activated receptor (PPAR) gamma

Table 4

Independent variables	Log alanine aminotransferase (U/L)			*t*	Sig.
	Unstandardized	Coefficients	Standardized Coefficients		
	B	S.E.	Beta		
Linear Multiple regression of alanine aminotransferase as dependent variable in all subjects as a whole					
Age	−0.228	0.064	−.304	−3.539	.0006
BMI	0.1675	0.146	.579	1.507	.134
Alcohol intake	0.072	0.0826	.967	0.846	.399
Triglycerides	0.083	0.088	.856	0.907	.366
Insulin sensitivity	−14.477	3.6	−.3464	−4.021	.0001
Circulating sCD36	*t*7.022	2.812	.213	2.497	.0141
Linear Multiple regression of alanine aminotransferase as dependent variable in subjects with normal glucose tolerance					
Age	−0.167	0.080	−.223	−2.070	.042
BMI	0.9039	0.2941	.3357	3.073	.003
Alcohol intake	0.046	0.0545	.909	0.43	.668
Triglycerides	0.0378	0.017	.246	2.214	.030
Insulin sensitivity	−0.1296	−0.119	.598	−0.946	.348
Circulating sCD36	−0.034	−0.04	.892	−0.321	.749
Linear Multiple regression of alanine aminotransferase as dependent variable in subjects with altered glucose tolerance					
Age	−0.164	−0.211	.893	−1.334	.19
BMI	−0.03	−0.031	.606	−0.197	.844
Alcohol intake	0.174	0.221	.881	1.403	.168
Triglycerides	−0.039	−0.047	.858	−0.293	.77
Insulin sensitivity	−19.204	6.448	−.3915	−2.978	.005
Circulating sCD36	10.685	3.676	.382	2.907	.006

and is a gene target of thiazolidinediones, which are agonists of this nuclear receptor [42]. We could hypothesise that decreased sCD36 is the result of a lower action of endogenous PPAR. In fact, the presence of functional CD36 was shown to be essential for the actions of pioglitazone and roziglitazone to improve SI in general and that of muscle in particular [43,44].

Finally, increased sCD36 could also reflect increased shedding of CD36 receptor in muscle and adipose tissues, with decreased intracellular transport of fatty acids into these tissues and increased delivery to the liver leading to increased very low-density lipoprotein production. In humans, incidence of CD36 deficiency, mostly due to a Pro90Ser mutation [45], has been reported [46]. Insulin resistance has been evaluated in this situation of CD36 deficiency. However, the relationship between CD36 and insulin resistance is controversial in humans [47].

In conclusion, the circulating concentration of soluble CD36 is associated with markers of liver injury. Further studies with histological assessment are required to evaluate the significance of circulating sCD36 levels in subjects with different degrees of liver damage and to clarify whether sCD36 could be a specific and noninvasive marker of liver health.

Acknowledgments

This work was supported by research grants from the *Ministerio de Educación y Ciencia* (BFU2004-03654), from Instituto de Salud Carlos III (PI04-1383), and from Generalitat de Catalunya (2005SGR00467) to J.M. F-R, and grants from the Novo Nordisk Foundation and the Danish Research Agency to A. H. This collaboration was established through the EU Network Program COST-B17.

References

[1] Kugelmas M, Hill D, Vivian B, Marsano L, McClain CJ. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. Hepatology 2003;38:413–9.
[2] Esteve E, Ricart W, Fernandez-Real JM. Dyslipidemia and inflammation: an evolutionary mechanism. Clin Nutr 2005;24:16–31.
[3] Berson A, Beco VD, Letterson P, Robin M, Moreau C, Kahwaji JE, et al. Steatohepatitis-inducing drugs cause mitochondrial dysfunction and lipid peroxidation in rat hepatocytes. Gastroenterology 1998;114: 764–74.
[4] Medina J, Fernandez-Salazar LI, Garcia-Buey L, Moreno-Otero R. Approach to the pathogenesis and treatment of nonalcoholic steatohepatitis. Diabetes Care 2004;27:2057–66.
[5] Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006;116:1793–801.
[6] Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 2005;11:183–90.
[7] Cave M, Deaciuc I, Mendez C, Song Z, Joshi-Barve S, Barve S, et al. Nonalcoholic fatty liver disease: predisposing factors and the role of nutrition. J Nutr Biochem 2007;18:184–95.
[8] Abumrad NA, el-Maghrabi MR, Amri EZ, Lopez E, Grimaldi PA. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. J Biol Chem 1993;268: 17665–8.
[9] Febbraio M, Hajjar DP, Silverstein RL. CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. J Clin Invest 2001;108:785–91.
[10] Tuomisto TT, Riekkinen MS, Viita H, Levonen AL, Yla-Herttuala S. Analysis of gene and protein expression during monocyte-macrophage differentiation and cholesterol loading — cDNA and protein array study. Atherosclerosis 2005;180:283–91.
[11] Handberg A, Levin K, Højlund K, Beck-Nielsen H. Identification of the oxidized low-density lipoprotein scavenger receptor CD36 in plasma. A novel marker of insulin resistance. Circulation 2006;114: 1169–76.
[12] Tandon NN, Kralisz U, Jamieson GA. Identification of glycoprotein IV (CD36) as a primary receptor for platelet-collagen adhesion. J Biol Chem 1989;264:7576–83.
[13] Harmon CM, Abumrad NA. Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins: isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. J Membr Biol 1993;133:43–9.
[14] Febbraio M, Abumrad NA, Hajjar DP, Sharma K, Cheng W, Pearce SF, et al. A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. J Biol Chem 1999;274: 19055–62.
[15] Goudriaan JR, Dahlmans VE, Teusink B, Ouwens DM, Febbraio M, Maassen JA, et al. CD36 deficiency increases insulin sensitivity in muscle, but induces insulin resistance in the liver in mice. J Lipid Res 2003;44:2270–7.
[16] Coburn CT, Knapp Jr FF, Febbraio M, Beets AL, Silverstein RL, Abumrad NA. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. J Biol Chem 2000;275:32523–9.
[17] Ibrahimi A, Bonen A, Blinn WD, Hajri T, Li X, Zhong K, Cameron R, et al. Muscle-specific overexpression of FAT/CD36 enhances fatty acid oxidation by contracting muscle, reduces plasma triglycerides and fatty acids, and increases plasma glucose and insulin. J Biol Chem 1999;274:26761–6.
[18] Zhang X, Fitzsimmons R, Cleland L, Ey P, Zannettino A, Farmer E, et al. CD36/fatty acid translocase in rats: distribution, isolation from hepatocytes, and comparison with the scavenger receptor SR-B1. Lab Invest 2003;83:317–32.
[19] Stahlberg N, Rico-Bautista E, Fisher RM, Wu X, Cheung L, Flores-Morales A, et al. Female-predominant expression of fatty acid translocase/CD36 in rat and human liver. Endocrinology 2004;145: 1972–9.
[20] Takamura T, Sakurai M, Ota T, Ando H, Honda M, Kaneko S. Genes for systemic vascular complications are differentially expressed in the livers of type 2 diabetic patients. Diabetologia 2004;47:638–47.
[21] Fernandez-Real JM, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. Endocr Rev 2003;24: 278–301.
[22] Fernandez-Real JM, Broch M, Ricart W, Casamitjana R, Gutierrez C, Vendrell J, et al. Plasma levels of the soluble fraction of tumor necrosis factor receptor 2 and insulin resistance. Diabetes 1998;47: 1757–62.
[23] Bellentani S, Tribelli C, Saccoccio G, Sodde M, Fratti N, De Martin C, et al. Prevalence of chronic liver disease in the general population of northern Italy: the Dionysos Study. Hepatology 1994; 20:1442–9.
[24] Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, et al. Liver fibrosis in overweight patients. Gastroenterology 2000;118:1117–23.
[25] Garcia-Monzon C, Martin-Perez E, Iacono O, Fernandez-Bermejo M, Majano P, Apolinario A, et al. Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. J Hepatol 2000;33:716–24.

[26] Suzuki A, Angulo P, Lymp J, Sauver J, Muto A, Okada T, et al. Chronological development of elevated aminotransferases in nonalcoholic population. Hepatology 2005;41:64–71.
[27] Ruhl CE, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. Gastroenterology 2003;124:71–9.
[28] Jeong SK, Nam HS, Rhee JA, Shin JH, Kim JM, Cho KH. Metabolic syndrome and ALT: a community study in adult Koreans. In J Obes Relat Metab Disord 2004;28:1033–8.
[29] Bugianesi E, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, et al. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease. J Clin Endocrinol Metab 2005;90:3498–504.
[30] Lopez-Bermejo A, Botas P, Funahashi T, Delgado E, Kihara S, Ricart W, et al. Adiponectin, hepatocellular dysfunction and insulin sensitivity. Clin Endocrinol (Oxf) 2004;60:256–63.
[31] Targher G, Bertolini L, Scala L, Poli F, Zenari L, Falezza G. Decreased plasma adiponectin concentrations are closely associated with nonalcoholic hepatic steatosis in obese individuals. Clin Endocrinol (Oxf) 2004;61:700–3.
[32] Fernandez-Real JM, Lopez-Bermejo A, Broch M, Vendrell J, Richart C, Ricart W. Circulating soluble CD14 monocyte receptor is associated with increased alanine aminotransferase. Clin Chem 2004;50:1456–8.
[33] Lopez-Bemejo A, Bosch M, Recasens M, Biarnes J, Esteve E, Casamitjana R, et al. Potential role of inteleukin-18 in liver disease associated with insulin resistance. Obes Res 2005;13:1925–31.
[34] Day CP, James OFW. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842–5.
[35] Vozarova B, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardous C, et al. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 2002;51:1889–95.
[36] Festi D, Colecchia A, Sacco T, Bondi M, Roda E, Marchesini G. Hepatic steatosis in obese patients: clinical aspects and prognostic significance. Obes Rev 2004;5:27–42.
[37] Hajri T, Han XX, Bonen A, Abumrad NA. Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. J Clin Invest 2002;109:1381–9.
[38] Gupta G, Cases JA, She L, Ma XH, Yang XM, Hu M, et al. Ability of insulin to modulate hepatic glucose production in aging rats is impaired by fat accumulation. Am J Physiol Endocrinol Metab 2000;278: E985–91.
[39] Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci USA 2001;98: 7522–7.
[40] Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab 2002;87:3023–8.
[41] Tiikkainen M, Tamminen M, Hakkinen AM, Bergholm R, Vehkavaara S, Halavaara J, et al. Liver-fat accumulation and insulin resistance in obese women with previous gestational diabetes. Obes Res 2002;10: 859–67.
[42] Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 1998;93:241–52.
[43] Qi N, Kazdova L, Zidek V, Land V, Kren V, Pershadsingh HA, et al. Pharmacogenetic evidence that CD36 is a key determinant of the metabolic effects of pioglitazone. J Biol Chem 2002; 277:48501–7.
[44] Seda O, Kazdova L, Krenova D, Kren V. Rosiglitazone fails to improve hypertriglyceridemia and glucose tolerance in CD36-deficient BN. SHR4 congenic rat strain. Physiol Genomics 2003;12:73–8.
[45] Kashiwagi H, Tomiyama Y, Honda S, Kosugi S, Shiraga M, Nagao N, et al. Molecular basis of CD36 deficiency. Evidence that a 478CT substitution (proline90serine) in CD36 cDNA accounts for CD36 deficiency. J Clin Invest 1995;95:1040–6.
[46] Yamamoto N, Ikeda H, Tandon NN, Herman J, Tomiyama Y, Mitani T, et al. A platelet membrane glycoprotein (GP) deficiency in healthy blood donors: Naka-platelets lack detectable GPIV (CD36). Blood 1990;76:1698–703.
[47] Furuhashi M, Ura N, Nakata T, Shimamoto K. Insulin sensitivity and lipid metabolism in human CD36 deficiency. Diabetes Care 2003;26: 471–4.