Plasma fatty acid binding protein 4 is associated with atherogenic dyslipidemia in diabetes

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Abstract The aim of this study was to evaluate the impact of adipocyte fatty acid binding protein 4 (FABP4) on the lipid profile in type 2 diabetic subjects. Plasma levels of FABP4 and adiponectin and an extensive lipid profile were analyzed in 169 type 2 diabetic subjects and 105 controls. Type 2 diabetic subjects were categorized according the presence of atherogenic dyslipidemia. Univariate statistical analyses, partial correlation tests, and binary logistic regression models were applied. In type 2 diabetic subjects, FABP4 was positively correlated with plasma triglycerides ($P = 0.007$), apolipoprotein C-III (apoC-III) ($P = 0.009$), and all the components of triglyceride-rich lipoproteins, including VLDL triglycerides ($P = 0.002$), VLDL-cholesterol ($P = 0.001$), and VLDL apoB ($P = 0.001$). FABP4 was inversely correlated with apoA-I ($P = 0.038$), HDL-cholesterol ($P = 0.002$), and HDL apoA-I ($P = 0.010$) in type 2 diabetic subjects. These correlations are not significantly affected by age, gender, body mass index, adiponectin, insulin, or any pharmacological treatment. The associations are even stronger when the FABP4/adiponectin ratio is considered. None of these associations were observed in controls. High FABP4 and low adiponectin levels are independent predictors of atherogenic dyslipidemia. In conclusion, FABP4 plasma concentrations hold strong potential for development as a clinical biomarker for atherogenic dyslipidemia, independent of obesity and insulin resistance, in type 2 diabetic subjects.—Cabré, A., I. Lázaro, J. Girona, J. M. Manzanares, F. Marimón, N. Plana, M. Heras, and L. Masana. **Plasma fatty acid binding protein 4 is associated with atherogenic dyslipidemia in diabetes. *J. Lipid Res.* 2008.** 49: **1746–1751.**

Supplementary key words insulin • lipids • type 2 diabetes

The typical lipid profile for type 2 diabetic patients includes high triglycerides and low HDL-cholesterol, which leads to small and dense LDL known as atherogenic dyslipidemia (1). It is thought that a state of insulin resistance leads to this alteration in the lipid profile (1, 2). These mechanisms seem to be influenced by both environmental factors and genetic susceptibility. Once a patient becomes insulin resistant, there is a reduction in plasma triglyceride-rich lipoprotein (TRL) clearance and an increase in FFA delivery from the adipose tissue to the plasma. This change produces a higher lipid influx into the hepatocytes and leads to VLDL overproduction associated with vascular disease (3).

Adipose tissue is an actively metabolic organ that produces a variety of factors including adipokines, which are required for endocrine and paracrine metabolic actions (4). Adipokines are associated with increased inflammation, thrombogenicity, insulin resistance, and other metabolic effects (4). The increased likelihood that obese patients will develop glucose intolerance and diabetes has been linked to several adipokines (5). Adipose fatty acid binding protein 4 (FABP4) has similarly been proposed as a biochemical mediator of insulin resistance in obese patients, and patient plasma levels are predictors of metabolic syndrome development (6–9). Knock-out mice studies suggest that FABP4 may modulate fat mass and lipolysis through its action on hormone-sensitive lipase (HSL), perilipin, and LPL, among others (10, 11). FABP4 belongs to the intracellular lipid binding protein (iLBP) family. Both iLBPs and lipocalins, such as retinol binding protein (RBP4) and lipocalin-2, which are also linked to insulin resistance, are small, extracellular proteins sharing several common molecular recognition properties. These properties include the binding of small, principally hydrophobic molecules, which makes them ideal transporters of lipid molecules (12). Because of their involvement in lipid metabolism, they may play a role in the lipid profile alterations observed in individuals with adipose tissue derangements like diabetes, metabolic syndrome, and

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obesity. Adiponectin is also secreted by the adipose tissue, where it primarily functions as a counterbalance to other adipose tissue-derived signaling molecules. The balance between the insulin-sensitizer, adiponectin, and the insulin-resistance inducer molecules may be an important homeostatic controller of metabolic status. We hypothesize that adipose tissue-derived FABP4 induces a direct insulin resistance-independent effect on lipid metabolism in diabetes and that the plasma FABP4/adiponectin balance determines the presence of atherogenic dyslipidemia in type 2 diabetic patients. This study measures the statistical relationship between plasma FABP4 and atherogenic dyslipidemia and aims to address the clinical relevance of these plasma measurements in humans with diabetes, metabolic syndrome, and/or obesity to determine the usefulness of FABP4 as a disease progression biomarker.

MATERIALS AND METHODS

Clinical study

We studied 169 nonsmoking, type 2 diabetic subjects diagnosed using the American Diabetes Association criteria (13) and 105 gender-matched, nondiabetic controls (age range 36 to 79 years). Patients were recruited at Saint Joan University Hospital, Reus. Plasma samples for control populations were obtained from a Biobanc collection and are representative of the general population from the same geographic area. In type 2 diabetic patients, anamnesis and clinical examinations including anthropometrics were performed, and blood pressure and the presence of macro- or microvascular diseases were recorded. Measurements via carotid and femoral echo-Doppler as well as ankle-brachial index (ABI) were taken. Macrovascular disease was defined as a clinical history of at least one of the following criteria: coronary heart disease, stroke, peripheral vascular disease, $\geq$1 significant arteriosclerotic plaque (>40% stenosis), or an ABI index $\leq$0.9 or $\geq$1.3. Microvascular disease was defined in the clinical history as the presence of at least one of the following criteria: nephropathy, retinopathy, or neuropathy. Microalbuminuria was defined as albuminuria $\geq$30 mg/24 h. Atherogenic dyslipidemia was defined as triglycerides >1.69 mmol/l and either HDL <1.03 mmol/l (men) or HDL <1.29 mmol/l (women). Patients with albuminuria, type 1 diabetes mellitus, secondary diabetes mellitus, morbid obesity, body mass index (BMI) >40 kg/m^2), familial hypercholesterolemia, malignancy, liver disorder, or acute or chronic inflammation were excluded from this analysis. All subjects provided written informed consent for this study, which was approved by the hospital ethics committee.

Analytical methods

Lipoproteins (VLDL, IDL, LDL, and HDL) were subfractionated from EDTA-treated blood plasma by sequential preparative ultracentrifugation as described previously (14). Plasma, fraction lipids, and apolipoproteins were measured using enzymatic assays adapted for the Cobas-Mira autoanalyzer (Roche; Basel, Switzerland). In the control group, only the basic lipid profile (total cholesterol, HDL-cholesterol, total triglycerides, and Friedewald-calculated LDL-cholesterol) was available. HbA_{1c} was measured by HPLC on a Hi-auto A1c HA-8140 (Arkray KDR Corporation-Menarini Diagnostics; Florence, Italy). Glucose and insulin were measured on an automatic autoanalyzer Synchron LXi 725-Synchron Access Clinical Systems (Beckman Coulter; Fullerton, CA) using enzymatic assays or chemiluminescent immunoassays adapted to this system. Commercial ELISA kits (BioVendor Laboratory Medicine, Inc.; Brno, Czech Republic) were used to assess the plasma levels of adiponectin and FABP4 in both the type 2 diabetic group and the control group. Results are shown in "Système International" units.

Statistical analyses

Statistical analyses were performed using the SPSS platform (version 14.0, SPSS, Inc.; Chicago, IL). All data are presented

TABLE 1. Lipid profile of the type 2 diabetes group divided into gender-adjusted quartiles based on plasma FABP4 concentrations

	FABP4				
Variable	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*
n	42	42	43	42	
Plasma cholesterol (mmol/l)	4.72 ± 0.76	4.70 ± 0.84	4.63 ± 0.80	4.73 ± 0.69	ns
Plasma triglycerides (mmol/l)	1.66 ± 1.16	1.54 ± 0.87	1.66 ± 0.75	2.20 ± 1.10	0.001
Plasma apoA-I (mg/dl)	142.1 ± 21.8	139.2 ± 17.6	138.8 ± 23.3	135.2 ± 21.9	ns
Plasma apoB (mg/dl)	83.0 ± 13.5	87.4 ± 16.3	82.7 ± 18.7	85.7 ± 14.7	ns
Plasma apoC-III (mg/dl)	17.4 ± 4.8	16.5 ± 4.0	17.2 ± 3.5	20.7 ± 6.0	0.017
Plasma apoE (mg/dl)	3.8 ± 1.6	3.5 ± 0.9	3.6 ± 0.8	3.9 ± 1.0	ns
VLDL-cholesterol (mmol/l)	0.42 ± 0.50	0.37 ± 0.39	0.42 ± 0.33	0.66 ± 0.44	0.001
VLDL triglycerides (mmol/l)	0.93 ± 0.97	0.82 ± 0.68	0.92 ± 0.62	1.38 ± 0.97	0.001
VLDL apoB (mg/dl)	6.6 ± 4.4	6.1 ± 4.4	6.3 ± 3.7	10.2 ± 5.4	<0.001
IDL-cholesterol (mmol/l)	0.23 ± 0.15	0.22 ± 0.12	0.23 ± 0.09	0.27 ± 0.11	ns
IDL triglycerides (mmol/l)	0.17 ± 0.06	0.18 ± 0.07	0.18 ± 0.06	0.21 ± 0.06	ns
IDL apoB (mg/dl)	3.8 ± 2.1	4.3 ± 2.0	3.8 ± 1.8	4.6 ± 1.8	ns
LDL-cholesterol (mmol/l)	2.41 ± 0.55	2.51 ± 0.61	2.37 ± 0.57	2.24 ± 0.56	ns
LDL triglycerides (mmol/l)	0.23 ± 0.05	0.25 ± 0.07	0.23 ± 0.05	0.24 ± 0.06	ns
LDL apoB (mg/dl)	58.2 ± 12.4	63.9 ± 13.4	58.2 ± 14.2	58.1 ± 13.7	ns
HDL-cholesterol (mmol/l)	1.26 ± 0.28	1.25 ± 0.28	1.23 ± 0.30	1.15 ± 0.32	ns
HDL triglycerides (mmol/l)	0.15 ± 0.06	0.14 ± 0.04	0.14 ± 0.05	0.15 ± 0.04	ns
HDL apoA-I (mg/dl)	119.0 ± 20.4	116.5 ± 16.3	114.2 ± 22.1	110.5 ± 19.8	ns

ApoA-I, apolipoprotein AI; FABP4, fatty acid binding protein 4. Data are shown as mean ± SD. Gender-adjusted FABP4 quartiles were compared by one-way ANOVA. *P* values shown correspond to those variables with $P < 0.05$ adjusted for age, body mass index, plasma insulin and adiponectin concentrations, and thiazolidinedione and statin therapy from a univariate linear general model. ns, not significant.

as the mean ± SD, except where otherwise stated. A comparison of variables between FABP4 gender-adjusted quartiles was performed using a one-way ANOVA.

Univariate linear general models were used to adjust the results of continuous variables for age, pharmacological treatment, BMI, insulin, and adiponectin levels. Category distributions were compared between groups using the Fisher test. Binary logistic regression models were used to adjust the results of the categorical variables for age, diabetes duration and control, obesity, insulin concentrations, and pharmacological treatment. FABP4, adiponectin, and insulin concentrations were categorized and grouped as high and low concentrations by using the geometric mean of each variable for men and women. Partial Pearson correlation coefficients between FABP4 and other continuous variables were determined using a partial correlation test adjusted for age, gender, BMI, insulin, adiponectin levels, and pharmacological treatment. A binary logistic regression model was used to identify the predictive roles of high gender-adjusted FABP4 or adiponectin levels for the presence of atherogenic dyslipidemia. This model includes age, diabetes duration in years, diabetes control expressed as $HbA_{1c} \geq$ or $<7\%$, the presence of obesity, and categorized and gender-adjusted levels of FABP4, adiponectin, and insulin as independent variables. The adjusted odds ratios and their 95% confidence intervals were obtained and represented as a Forest plot. In all cases, a P value of less than 0.05 is considered statistically significant.

RESULTS

A total of 169 type 2 diabetic subjects (80 men and 89 women) participated in this study. The control group, which included 105 nondiabetic subjects (50 men and 55 women), has been previously described (9). For the diabetic group, the mean age was 63 ± 9 years (range from 36 to 79 years). The mean BMI was 30.2 ± 4.3 kg/m^2 (range from 21.0 to 40.0 kg/m^2), and 86 subjects were clinically obese. The mean diabetes history duration was 14 ± 8 years (range from 1 to 36 years), and the mean HbA_{1c} was 7.0 ± 1.1% (range from 4.1% to 10.4%). The mean glucose and insulin concentrations were 9.6 ± 2.9 mmol/l (range from 3.5 to 19.8 mmol/l) and 75.0 ± 87.1 pmol/l (range from 10.4 to 744.8 pmol/l), respectively. Eighty-eight subjects were on lipid-lowering drugs (78 on statins), 116 were on oral antidiabetic drugs (40 on thiazolidinediones), and 76 were on insulin treatment. In summary, 56 subjects fulfilled the criteria for atherogenic dyslipidemia and became the focus group for this analysis.

FABP4 plasma levels for men in the control group were strikingly lower than those in type 2 diabetic subjects with or without atherogenic dyslipidemia (16.00 ± 6.66 vs. 31.80 ± 19.54 and 24.10 ± 14.55 μg/l, respectively; overall $P < 0.001$). FABP4 plasma levels for women in the control group were also lower than those in type 2 diabetic subjects with or without atherogenic dyslipidemia (30.13 ± 12.29 vs. 46.56 ± 22.03 and 47.07 ± 21.81 μg/l, respectively; overall $P < 0.001$).

We next investigated the statistical relationship between FABP4 and atherogenic dyslipidemia in type 2 diabetics. **Table 1** shows the levels of lipids, apolipoproteins, and lipoprotein components divided by gender-adjusted quartiles of FABP4 serum levels from the type 2 diabetic subjects. The differences between quartiles were significant, even after adjustment for age, BMI, adiponectin, insulin concentrations, and pharmacological treatment with thiazolidinediones and statins. Plasma FABP4 concentrations, after adjustment for age, gender, BMI, adiponectin, insulin concentrations, and pharmacological treatment (thiazolidinediones and statins), were positively correlated with triglycerides ($r = 0.229$; $P = 0.007$), VLDL triglycerides ($r = 0.266$; $P = 0.002$), VLDL cholesterol ($r = 0.286$; $P = 0.001$), VLDL apolipoprotein B (apoB) ($r = 0.287$; $P = 0.001$), and apoC-III ($r = 0.225$; $P = 0.009$). Plasma FABP4 concentrations, after adjustment for the above parameters, were also inversely correlated with apoA-I ($r = -0.178$; $P = 0.038$), HDL-cholesterol ($r = -0.267$; $P = 0.002$), and HDL apoA-I ($r = -0.220$; $P = 0.010$). None of the differences listed above were observed in lipids from the control group, and the correlations were not significantly different between plasma FABP4 and lipid levels [triglycerides ($P =$

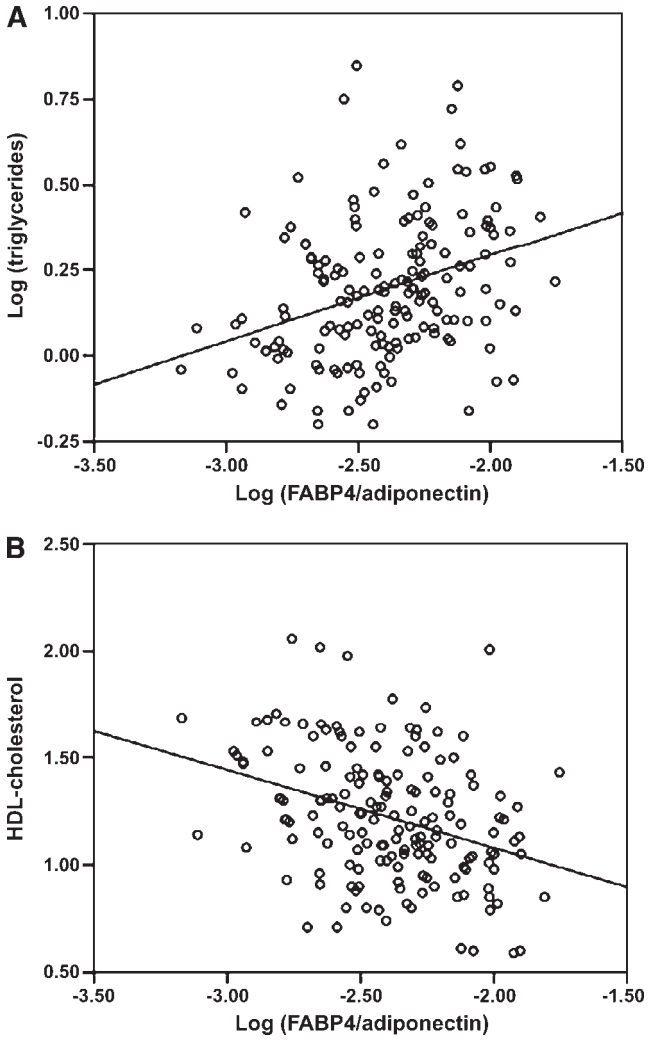

Fig. 1. Relationship of the fatty acid binding protein 4 (FABP4)/adiponectin ratio with triglycerides (A) and HDL-cholesterol (B) in type 2 diabetic subjects.

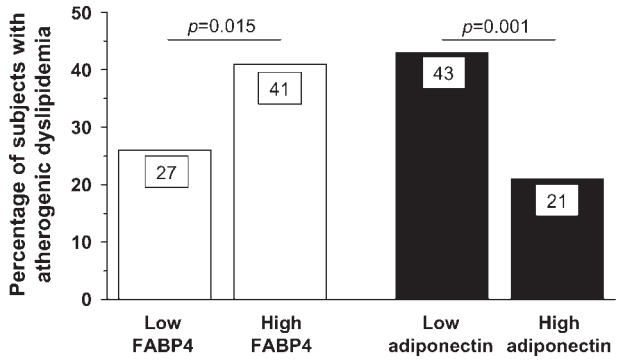

Fig. 2. Percentage of type 2 diabetic subjects with atherogenic dyslipidemia in groups with high and low gender-adjusted FABP4 or adiponectin plasma concentrations. *P* values shown for group comparisons are adjusted for age, obesity, diabetes duration and control, insulin concentrations, and thiazolidinedione and statin therapy from a binary regression model.

0.596), total cholesterol ($P = 0.940$), LDL-cholesterol ($P = 0.714$), and HDL-cholesterol ($P = 0.329$)].

The following analyses were performed only in type 2 diabetic subjects. Because adiponectin levels are a possible confounding variable, we also investigated the FABP4/adiponectin ratio to further study the effects on lipid metabolism. Correlations observed with FABP4 were stronger when the FABP4/adiponectin ratio was used for analyses (**Fig. 1**). Furthermore, using this ratio approach, we also found correlations to atherogenic dyslipidemia in subjects who were not obese or treated with lipid-lowering drugs. After adjustment for diabetes duration and control, age, obesity, high insulin, and pharmacological treatment, the prevalence of atherogenic dyslipidemia was higher in subjects with high gender-adjusted FABP4 ($P = 0.015$) and lower in subjects with high adiponectin concentrations ($P = 0.001$) (**Fig. 2**). To test the predictive value of plasma FABP4 concentrations on atherogenic dyslipidemia, we applied a logistic regression model. Independent of diabetes duration and control, age, obesity, high insulin, high adiponectin levels, or pharmacological treatment (thiazolidinediones, statins, or fibrates), high plasma concentrations of FABP4 [≥23.28 μg/l (men) and ≥42.43 μg/l (women)] were associated with an odds ratio of 2.72 (1.16–6.37; $P = 0.021$) for having atherogenic dyslipidemia. High adiponectin levels [≥6.74 mg/l (men) and ≥9.08 mg/l (women)] predicted protection from atherogenic dyslipidemia with an odds ratio of 0.17 (0.07–0.45; $P < 0.001$) (**Fig. 3**). FABP4 concentrations did not correlate with insulin levels.

DISCUSSION

We have found that FABP4, which is predominantly secreted by the adipose tissue, is correlated with lipid metabolism disturbances in diabetic patients. This protein was strongly associated with hypertriglyceridemia, which leads to atherogenic dyslipidemia, a condition that is clinically characterized by high triglycerides and low HDL-cholesterol levels. It is well accepted that the main mechanism associated with atherogenic dyslipidemia is insulin resistance (1). However, other molecular indicators of disease state should be considered for this complex disease. Some adipokine expression levels are linked with obesity and insulin resistance. Our results support the hypothesis that some metabolic effects observed in type 2 diabetic patients are directly mediated by molecules secreted from the adipose tissue in parallel to the insulin resistance. The direct relationship between adiponectin and serum lipids has already been described. High levels of adiponectin reduce triglycerides and VLDL formation and increase HDL levels (15–17). Several lipocalins have been proposed as biomarkers of several diseases (18). Lipocalin-2 has recently been associated with metabolic syndrome (19). RBP4 is produced both in fat cells and in the liver and it influences insulin resistance by modulating the expression of GLUT4 in the muscle, liver, and adipose tissue of mice (20). In humans, elevated RBP4 plasma levels have been linked to renal dysfunction (21).

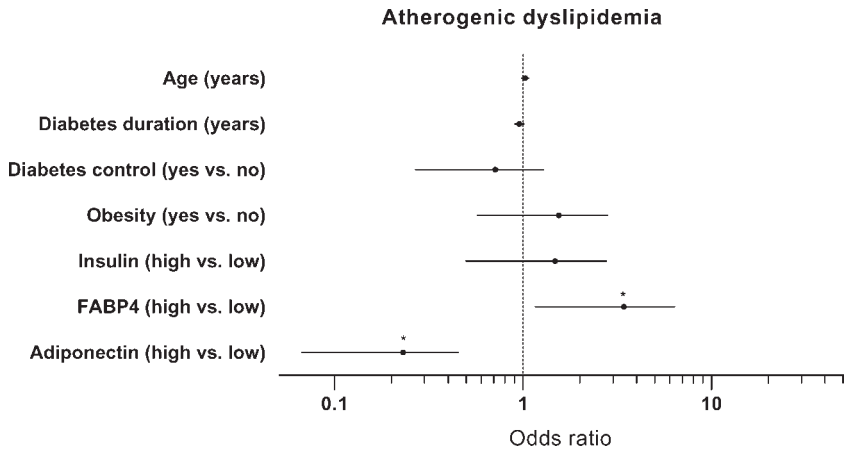

Fig. 3. Forest plot for atherogenic dyslipidemia in type 2 diabetic subjects. Horizontal lines represent the adjusted odds ratios and 95% confidence intervals obtained from a binary regression model. * $P < 0.05$.

FABP4 is expressed in adipose tissue (22) and macrophages when they become overloaded with fat (23–25). Its function is proposed to affect the transport of intracellular fatty acids (26). Elevated FABP4 plasma levels are associated with prediabetic states and obesity and they predict metabolic syndrome development (6–9). From studies in the same population, we have found that FABP4 is associated with metabolic syndrome components in type 2 diabetic subjects and that FABP4 plasma levels are increased by thiazolidinediones, which are peroxisome proliferator-activated receptor γ agonist drugs (9).

Because the association between FABP4 and lipids has potential as a predictive clinical biomarker, we have characterized the correlation between FABP4 and the complete lipid profile in diabetic patients. Our results show that high plasma FABP4 concentrations were consistently associated with hypertriglyceridemia, elevated apoC-III, and all the components of TRL, such as cholesterol, triglycerides and apoB in VLDL. Conversely, there was an inverse correlation with apoA-I and HDL-cholesterol. Although roughly 50% of subjects were being treated with statins, the correlations remained unchanged when we analyzed only those individuals who were not taking lipid-lowering drugs. Animal studies have shown that FABP4 influences lipid metabolism and that FABP4 seems to act on LPL, perilipin, and HSL to induce increases in plasma FFA concentrations, which promote hypertriglyceridemia (11). Interestingly, the observed relationships were reinforced when adiponectin concentrations were taken into account. Under these conditions, the correlations between the lipid profile and the FABP4/adiponectin ratio become stronger. Whereas lipocalins are thought to induce insulin resistance, adiponectin is the main insulin sensitizer adipokine, and the ratio between them may provide a more complete view of a patient's metabolic state. These observations are important because all of the correlations were maintained even after adjustment for BMI, insulin, and other confounding variables.

Because 46% of patients were treated with statins, any conclusion about the effect of FABP4 on LDL particles will be inaccurate; however, all of the correlations were maintained when only those patients without statin treatment were taken into consideration. Although our results demonstrate statistically significant correlations between FABP4 and lipid factors, our sample size is relatively small; therefore, additional studies using independent and larger sample groups are warranted. Regardless, FABP4 seems to modulate lipid metabolism in diabetic patients independent of its action on insulin resistance.

Our results contribute to a better understanding of the metabolic mechanisms involved in the development of atherogenic dyslipidemia in diabetic patients. We found that increased levels of FABP4 are associated with increased VLDL apoB levels. This association indicates that hypertriglyceridemia is associated with an increased number of TRL particles, which may suggest an increased synthesis. On the other hand, we observed a strong correlation between increased FABP4 and apoC-III levels. Although this may be explained by the fact that apoC-III is transported by TRL (27), it is also possible that FABP4 inhibits LPL and thereby promotes hypertriglyceridemia and low HDL-cholesterol levels. Therefore, FABP4 might modulate both anabolic and catabolic parts of the TRL metabolism independent of insulin action. In summary, our results suggest that the classic lipid profile alterations observed in type 2 diabetic patients might be mediated not only by insulin resistance but by a balance in the adipose tissue-derived molecules as well. In addition to influencing insulin sensitivity, these adipose tissue-derived molecules, such as FABP4, may directly modulate lipid metabolism.

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