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CLINICAL STUDY

FABP 4 is associated with inflammatory markers and metabolic syndrome in morbidly obese women

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Abstract

Objective: The adipocyte/macrophage fatty acid-binding protein 4 (FABP4) has been described as a biomarker for adiposity and metabolic syndrome (MS). The aims of this study were to assess the relationship between FABP4 and inflammatory cytokines related to obesity, and to evaluate *FABP4* mRNA expression in visceral and subcutaneous adipose tissue in non-diabetic morbidly obese women versus healthy lean women.

Methods: We analyzed circulating levels of FABP4 in 81 Spanish women: 38 lean (body mass index (BMI) <25 kg/m^2) and 43 morbidly obese (BMI >40 kg/m^2). We took 30 follow-up blood samples at 6 and 12 months after bariatric surgery. We assessed *FABP4* gene expression in samples of subcutaneous abdominal and visceral adipose tissue. Adipose tissue mRNA expression was determined by real-time RT-PCR.

Results: In morbidly obese women, plasma FABP4 levels were significantly higher than in non-obese patients. These levels positively correlated with BMI, homeostasis model assessment of insulin resistance (HOMA2-IR), and plasma glucose and insulin levels. Post-operative FABP4 levels decreased by a maximum of 30% after 12 months. We also found an inverse association between FABP4 and adiponectin levels, and positive correlations between FABP4 and circulating leptin, tumor necrosis factor (TNF) receptors, C-reactive protein (CRP) and interleukin 6 levels. Linear regression analysis revealed that FABP4 was more closely related to HOMA2-IR than adiponectin, CRP, TNF-RI, or leptin. Furthermore, high circulating FABP4 levels were associated with the presence of MS. *FABP4* mRNA expression in visceral adipose tissue was related to its circulating levels in morbidly obese women.

Conclusions: Our results indicate that serum FABP4 is associated with inflammatory factors related to obesity and MS in non-diabetic morbidly obese women.

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Introduction

Metabolic syndrome (MS) comprises a combination of characteristics and symptoms such as dyslipidemia, hypertension, impaired glucose tolerance, insulin resistance, central adiposity, and a generalized proinflammatory condition (1). MS is associated with an increased risk for the development of both type 2 diabetes and cardiovascular disease. Most obese patients have an impaired adipose tissue function caused by the interaction of genetic and environmental factors, which lead to adipocyte hypertrophy, hypoxia, and a variety of stresses and inflammatory processes within adipose tissue. Ectopic fat accumulation including visceral obesity is characterized by changes in cellular composition, increased lipid storage and impaired insulin sensitivity in adipocytes, and a proinflammatory, atherogenic, and diabetogenic adipokine pattern (2). Therefore, key factors associated with the development of MS might include an increase in the production of leptin, tumor necrosis factor-α (TNF-α), interleukin 6 (IL6), free fatty acids, and plasminogen activator inhibitor-1, and a decrease in the level of adiponectin secreted by the adipocyte tissue (3, 4).

Adipocyte fatty acid-binding protein (A-FABP, FABP4) is a member of the FABP superfamily and is highly expressed in adipose tissue by means of adipocytes and macrophages (5). FABP4 was traditionally thought to be a cytoplasmic protein, but Xu *et al.* (6) found that it was also released from adipocytes into the bloodstream.

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The regulatory functions of FABP4 in lipid and glucose metabolism have recently been described (7, 8). Functions of cytoplasmic FABPs include the enhancement of free fatty acid (FFA) solubility and transport to specific enzymes and cellular compartments (to the mitochondria and peroxisomes for oxidation, to the endoplasmic reticulum for reesterification, to the lipid droplet for storage, or to the nucleus for regulation of gene expression) (9, 10). Preclinical studies indicate that mice deficient in aP2 (FABP4 mouse homolog) are protected from the development of hyperinsulinemia, hyperglycemia, and insulin resistance in the context of both dietary and genetic obesity (11–13). Adipocytes obtained from aP2-deficient mice were found to have markedly reduced lipolysis efficiency both *in vivo* and *in vitro* (14, 15) and exhibited a two- to threefold decrease in fatty acid release, suggesting that FABP4 mediates the efflux of fatty acids in normal physiology (16). Furthermore, the acute insulin secretory response to β-adrenergic stimulation was profoundly suppressed in aP2 (−/−) mice compared with their wild-type littermates (15), suggesting that this protein might modulate systemic insulin sensitivity through its actions on other distal target tissues.

Other studies have shown the presence of FABP4 in macrophages, which possess striking overlapping biology and functions with adipocytes. In these cells, FABP4 modulates inflammatory cytokine production and cholesterol ester accumulation (7, 17). FABP4 expression in macrophages can be induced by oxidized low-density lipoprotein (LDL) and suppressed by statin therapy (17).

There is increasing evidence based on population studies supporting the predictive role of increased serum FABP4 for MS and cardiometabolic risk. In cross-sectional studies including overweight or mildly obese patients, FABP4 was closely associated with obesity and MS (6, 18). In prospective studies, FABP4 levels predicted the development of MS and type 2 diabetes in a diabetic lean Asian cohort (19, 20). Furthermore, Yeung *et al.* (21) reported that FABP4 levels were independently associated with carotid atherosclerosis. Tuncman *et al.* (22) reported that individuals with an aP2 variant had lower triglycerides and a reduced risk of coronary heart disease and obesity-induced type 2 diabetes. These findings suggest that FABP4 is closely associated with insulin resistance, MS, type 2 diabetes, and atherosclerosis. However, at the molecular level, no data explaining a causal relationship are available at present. Although the physiological functions of circulating FABP4 remain to be determined, some researchers speculate that circulating FABP4 might function as a lipid hormone transporter or in a hormone-like fashion to modulate systemic insulin sensitivity and energy metabolism (9, 23, 24).

Our objective in this study was to further evaluate whether FABP4 is an independent risk factor for the cluster of metabolic risk factors, such as low-grade inflammation and insulin resistance, which predispose morbidly obese women to cardiovascular disease and type 2 diabetes mellitus. Furthermore, because of the lack of human data on the relationship between FABP4 expression in adipose tissue and its serum concentration, we wanted to evaluate these parameters in parallel.

Patients and methods

Patients

The study was approved by the institutional review board. All participants gave written informed consent for participation in medical research. In this study, we analyzed circulating FABP4 levels in 81 Spanish women of European descent: 38 lean (body mass index (BMI) <25 kg/m^2) and 43 morbidly obese (BMI >40 kg/m^2). We also analyzed *FABP4* gene expression in paired samples of subcutaneous and visceral adipose tissue from 30 patients: 9 lean (BMI <25 kg/m^2) and 21 morbidly obese (BMI >40 kg/m^2). Adipose tissue samples were obtained from morbidly obese women who underwent bariatric surgery by laparoscopic gastric bypass and from lean patients who underwent laparoscopic cholecystectomy for benign gall bladder disease or laparoscopic hiatal hernia repair. Subcutaneous adipose tissue biopsies were taken from the right hypocondrion region, and visceral adipose tissue biopsies were taken from the greater epiploon region. Samples were obtained by the same specialist in each surgical case. Morbidly obese women and controls were age matched. The weight of all subjects was stable for at least 3 months before surgery. Follow-up samples were selected from morbidly obese patients who underwent bariatric surgery. We obtained blood samples ($n=30$) at 6 and 12 months after surgery. Patients who had an acute illness, acute or chronic inflammatory or infective diseases, or end-stage malignant disease were excluded from this study. Menopausal women, women receiving contraceptive treatment, and control or morbidly obese patients diagnosed with type 2 diabetes mellitus or receiving hypolipidemic treatment were also excluded from the study.

Diagnosis of MS

Morbidly obese patients were further subclassified according to the presence or absence of MS. MS and metabolic risks are defined according to the US National Cholesterol Education Program Adult Treatment Panel III guidelines and modified as recommended in the latest American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement (25) by adopting a lower cut-off value for fasting glucose (≥ 5.6 mmol/l). MS was defined as having ≥ 3 of the following metabolic risk factors: i) central obesity (waist circumference (WC) ≥ 88 cm in women), ii) hypertriglyceridemia (fasting

triglycerides $\geq$1.69 mmol/l (150 mg/dl)), iii) low high-density lipoprotein (HDL) cholesterol (fasting HDL $<$1.29 mmol/l (50 mg/dl) in women), iv) glucose intolerance (fasting glucose $\geq$5.6 mmol/l (100 mg/dl)), and v) hypertension (sitting blood pressure $\geq$130/85 mmHg obtained as a mean of two readings taken after resting for at least 10 min or on regular antihypertensive medications).

Hormonal and biochemical analysis

We determined the anthropometrical and metabolic characteristics of the subjects. The anthropometric evaluation included measures of BMI and WC. Laboratory studies included glucose, insulin, cholesterol, HDL, LDL, triglyceride, transaminases, and HbA1c performed using a conventional automated analyzer and measured after overnight fasting. The homeostasis model assessment of insulin resistance (HOMA2-IR) was completed using the HOMA calculator version 2.2.2 (26) (http://www.dtu.ox.ac.uk, accessed May 2010).

We determined circulating levels of different inflammatory-related molecules including adipokines (adiponectin, leptin, and resistin), IL6, acute phase proteins (C-reactive protein (CRP)), proinflammatory cytokines (TNF-RI and TNF-RII), and FABP4, a member of the lipid chaperone FABP family. Circulating levels of FABP4 (Biovendor, Modrice, Czech Republic), TNF-RI, TNF-RII (AssayPro, St Charles, MO, USA), CRP (Dade Behring, Marburg, Germany), adiponectin (Millipore, St Charles, MO, USA), leptin, resistin (Biovendor), and IL6 (Quantikine, R&D Systems, Minneapolis, MN, USA) were measured in duplicate using ELISAs following the manufacturer's instructions. Adiponectin assay sensitivity was 0.78 ng/ml, and inter-assay and intra-assay coefficients of variation (CV) were $<$8.4 and 7.4%. Leptin assay sensitivity was 0.2 ng/ml, and inter-assay and intra-assay CV were $<$7.6 and 4.4%. Resistin assay sensitivity was 33 pg/ml, and inter-assay and intra-assay CV were $<$6.9 and 3.4%. IL6 assay sensitivity was 0.039 pg/ml, and inter-assay and intra-assay CV were $<$9.6 and 6.9% respectively. CRP assay sensitivity was 0.2 ng/ml, and inter-assay and intra-assay CV were $<$4.8 and 3.8%. TNF-RI assay sensitivity was 50 pg/ml, and inter-assay and intra-assay CV were $<$5.7 and 1.7%. TNF-RII assay sensitivity was 0.1 ng/ml, and inter-assay and intra-assay CV were $<$3.2 and 3.3%. FABP4 assay sensitivity was 0.1 ng/ml, and inter- and intra-assay CV were $<$2.6 and 6.6% respectively.

RNA isolation and real-time PCR

Total RNA was isolated from adipose tissues according to the manufacturer's protocol for the RNeasy midi kit (Qiagen) and was digested with DNase I (RNase-Free DNase set, Qiagen). RNA quality was evaluated by measuring the 260/280 nm absorbance ratio ($\geq$1.8) and by electrophoresis. First-strand cDNA was synthesized using an equal amount of total RNA with a High Capacity RNA-to-cDNA Kit (Applied Biosystems, Madrid, Spain). Real-time quantitative PCR was performed in a final volume of 20 µl, which contained 10 ng of reverse-transcribed cDNA, 10 µl of 2X TaqMan Fast Universal PCR Master Mix (Applied Biosystems), and 1 µl TaqMan Assay predesigned by Applied Biosystems for the detection of FABP4 and GAPDH, which was used as housekeeping gene. All reactions were performed in triplicate and were carried out in 96-well plates using the 7900HT Fast Real-Time PCR systems (Applied Biosystems).

Statistical analysis

All the values reported are expressed as mean$\pm$S.E.M. and were analyzed using the SPSS/PC+ statistical package for Windows (v.15.0 Chicago, IL, USA). Differences between groups were calculated using either the Student's *t*-test or the one-way ANOVA analysis. The strength of association between variables was calculated using Pearson's method for parametric variables and the Spearman's ρ correlation test for non-parametric contrasts. Multiple linear regression analysis with backward variable selection was performed to identify independent predictors of HOMA2-IR. The validity of the regression model and its assumptions was assessed with the plot of residual versus predicted values. Not normally distributed variables were logarithmically transformed. Logistic regression analysis was performed to identify independent predictors of MS. Circulating FABP4 levels were age and BMI adjusted in some analysis. P values $<$0.05 were considered to be statistically significant.

Results

Patient characteristics

The baseline patient characteristics given in Table 1 show the mean and S.E.M. of the variables of interest. Patients were separated into control subjects (BMI$<$25 kg/m^2) and morbidly obese subjects (BMI$>$40 kg/m^2).

Biochemical analyses indicated that obese patients had significantly higher levels of glucose, insulin, HOMA2-IR, and HbA1c than the control group. Blood pressure was also higher in morbidly obese women. Lipidemic profiles differed significantly between groups. Obese patients showed higher triglyceride levels and lower HDL cholesterol. ALT and AST activity was higher in the obese group than in the control group (Table 1).

After bariatric surgery, patients lost more than 20% of their body weight, and consequently experienced a reduction in BMI at 6 and 12 months (Table 1). Postoperative systolic blood pressure was also lower, but not diastolic blood pressure. Fasting glucose, insulin, HbA1c, and insulin resistance index (HOMA2-IR)

Table 1 Baseline characteristics of the study cohort. Data are the mean±S.E.M. Differences between groups were calculated using the Student's *t*-test.

	Lean control ($n=38$)	Morbid obese ($n=59$)		6 months AS ($n=30$)		12 months AS ($n=30$)	
	Mean	Mean	*P* value†	Mean	*P* value‡	Mean	*P* value§
Age (years)	40.72±2.39	43.05±1.57	0.068				
Weight (kg)	57.56±1.09	123.37±2.59	<0.001*	90.52±2.09	<0.001*	83.82±2.34	<0.001*
WC (cm)	75.47±1.49	132.82±2.65	<0.001*	101.59±1.91	<0.001*	98.63±1.96	<0.001*
BMI (kg/m^2)	22.04±0.30	48.72±1.09	<0.001*	35.01±0.68	<0.001*	32.48±0.95	<0.001*
Systolic BP (mmHg)	120.91±2.87	137.49±2.39	<0.001*	114.54±2.74	<0.001*	110.50±2.99	<0.001*
Diastolic BP (mmHg)	70.62±1.62	77.35±2.07	0.013*	69.96±2.05	0.384	67.63±1.71	0.088
Glucose (mg/dl)	93.63±3.48	110.76±4.81	0.005*	86.76±2.02	<0.001*	84.47±2.17	<0.001*
Insulin (mU/l)	8.01±0.63	18.13±1.70	<0.001*	10.49±1.06	<0.001*	8.43±0.84	<0.001*
HbA1c (%)	4.51±0.05	4.97±0.10	<0.001*	4.66±0.11	<0.001*	4.79±0.10	0.006*
HOMA2-IR	1.23±0.12	2.45±0.26	<0.001*	1.35±0.14	<0.001*	1.09±0.11	<0.001*
Cholesterol (mg/dl)	185.63±5.87	178.30±4.88	0.125	184.94±6.18	0.976	190.80±7.16	0.748
HDL (mg/dl)	63.92±2.42	40.56±1.45	<0.001*	48.15±1.68	0.216	52.13±2.41	0.009*
LDL (mg/dl)	114.13±4.71	104.09±4.26	0.117	114.27±4.92	0.817	120.21±6.01	0.385
Triglycerides (mg/dl)	98.08±8.78	173.15±12.29	<0.001*	96.15±5.68	<0.001*	92.66±5.23	<0.001*
AST (U/l)	23.70±2.59	39.12±3.62	0.001*	19.88±1.01	<0.001*	20.69±1.68	<0.001*
ALT (U/l)	21.84±2.78	41.60±4.11	<0.001*	18.61±1.27	<0.001*	19.50±2.09	<0.001*
GGT (U/l)	20.62±4.87	26.47±2.98	0.295	18.76±2.99	0.217	15.59±3.42	0.066
ALP (U/l)	61.33±3.56	68.78±2.41	0.079	83.91±3.46	0.029*	83.19±3.25	0.046*

*Indicates significant differences between groups ($P<0.05$). *P* value†: comparisons between controls and morbid obese patients. *P* value‡: comparisons between 6 months after surgery (AS) and morbid obese patients. *P* value§: comparisons between 12 months after surgery (AS) and morbid obese patients.

decreased in parallel with weight loss. Finally, triglyceride, ALP, AST, and ALT levels had decreased at 6 and 12 months after surgery compared to the basal state (Table 1).

Circulating cytokine levels also varied between lean and obese patients (Table 2). Adiponectin levels decreased in morbidly obese women, whereas IL6, TNF-RI, leptin, and CRP showed significant increases compared to the control women.

We further subclassified the morbidly obese cohort according to the presence or absence of MS (Table 3). As expected, patients with MS had higher systolic blood pressure, and elevated levels of fasting glucose, total cholesterol, and triglycerides. However, adiponectin, resistin, IL6, TNF-RI and RII, leptin, and CRP levels were unchanged.

Circulating FABP4 levels

Serum levels of FABP4 were significantly higher in the morbidly obese group compared to the control group ($P<0.001$). A decrease in circulating FABP4 levels was observed after weight loss at 12 months after surgery but not after 6 months, compared to levels of the same patients at the basal state (preoperative) (Fig. 1).

We also investigated the relationship between circulating FABP4 levels and variables related to MS (Table 4). We found that plasma levels were strongly correlated with BMI, HOMA2-IR, fasting glucose and insulin, HbA1c, and systolic blood pressure. Circulating FABP4 levels were also positively correlated with triglyceride levels but negatively with HDL cholesterol. We found positive relationships between FABP4 and transaminase activity. The correlations found before (model 1) and after (model 2) FABP4 correction for BMI and age were very similar (Table 4).

In addition, we studied the relationship between FABP4 and circulating cytokine levels (Table 5). FABP4 correlated positively with leptin, IL6, TNF-RI, and CRP. Furthermore, a strong negative relationship between FABP4 and adiponectin levels was found. No association between resistin and circulating FABP4 levels was found in any model (Table 5).

The relationship between circulating FABP4 levels and HOMA2-IR

Figure 2 shows the highly significant relationship between FABP4 and HOMA2-IR by quartiles. We also analyzed the relationship between HOMA2-IR and other inflammatory-related molecules. Our results

Table 2 Adipo/cytokine circulating levels in lean and morbidly obese individuals. Data are the mean±S.E.M. Differences between groups were calculated using the Student's *t*-test.

Variables	Control ($n=38$)	Morbid obese ($n=43$)	*P* value
Adiponectin (μg/ml)	17.03±1.34	8.13±0.45	<0.001*
Resistin (ng/ml)	3.96±0.43	4.68±0.29	0.206
Leptin (ng/ml)	43.84±5.35	271.86±31.47	<0.001*
IL6 (pg/ml)	1.61±0.34	2.99±0.36	0.007*
TNF-RI (ng/ml)	2.34±0.10	2.95±0.10	<0.001*
TNF-RII (ng/ml)	4.44±0.29	5.05±0.31	0.153
CRP (mg/dl)	0.23±0.09	0.97±0.13	<0.001*

*Indicates significant differences between both groups ($P<0.05$).

Table 3 Baseline characteristics of morbid obese women according to the presence or absence of the metabolic syndrome. Data are the mean±S.E.M. Differences between groups were calculated using the Student's *t*-test.

	Metabolic syndrome		
Variables	No (n=17)	Yes (n=26)	***P* value**
BMI (kg/m^2)	50.45±2.33	47.59±0.95	0.268
Weight (kg)	128.04±4.73	120.31±2.90	0.148
WC (cm)	136.07±4.81	130.55±3.02	0.322
Age (years)	45.00±2.79	46.72±1.90	0.600
Systolic BP (mmHg)	131.29±3.41	141.54±3.07	0.035*
Diastolic BP (mmHg)	75.29±2.97	78.69±2.84	0.430
Glucose (mg/dl)	95.41±5.24	121.20±6.54	0.007*
Insulin (mU/l)	16.30±2.59	19.41±2.26	0.375
HbA1c (%)	4.86±0.10	5.06±0.16	0.329
HOMA2-IR	2.09±0.33	2.64±0.31	0.240
Cholesterol (mg/dl)	162.34±5.78	188.73±6.42	0.007*
HDL (mg/dl)	41.81±2.49	39.78±1.79	0.502
LDL (mg/dl)	95.99±4.02	109.48±6.41	0.083
Triglycerides (mg/dl)	123.82±9.46	205.40±16.64	<0.001*
AST (U/l)	32.47±4.32	43.83±5.23	0.123
ALT (U/l)	35.41±5.49	45.80±5.74	0.219
GGT (U/l)	27.24±6.54	25.96±2.60	0.837
ALP (U/l)	71.50±4.66	67.04±2.62	0.373
FABP4 (ng/ml)	51.12±5.82	66.92±4.69	0.042*
Adiponectin (μg/ml)	8.71±0.84	7.74±0.50	0.335
Resistin (ng/ml)	4.99±0.37	4.49±0.40	0.405
Leptin (ng/ml)	274.30±53.19	270.27±39.59	0.951
IL6 (pg/ml)	2.31±0.27	3.41±0.54	0.076
TNF-RI (ng/ml)	3.01±0.18	2.91±0.12	0.637
TNF-RII (ng/ml)	4.91±0.53	5.14±0.37	0.723
CRP (mg/dl)	1.06±0.14	0.90±0.20	0.539

*Indicates significant differences between both groups (P<0.05).

indicate that only adiponectin (r=−0.525, P<0.001), leptin (r=0.503, P<0.001), CRP (r=0.349, P=0.005), TNF-RI (r=0.347, P=0.002), and, as previously mentioned, FABP4 (r=0.540, P<0.001) are associated with HOMA2-IR. We then conducted a linear regression analysis including these molecules in order to determine whether FABP4 showed the strongest association with HOMA2-IR compared with the other variables tested. Linear regression analyses with a backward exclusion method revealed that FABP4 was the strongest cytokine associated with HOMA2-IR variability in our cohort, even after adjustment for age and BMI (Table 6).

After bariatric surgery, FABP4 was still positively related to HOMA2-IR, regardless of other cytokine levels. We also found a positive relationship between FABP4 and HOMA2-IR at 12 months after surgery regardless of age and BMI (Table 7).

The relationship between FABP4 and MS

We investigated the relationship between circulating FABP4 levels and the presence of MS. In the morbidly obese group, FABP4 concentration was the only cytokine significantly higher in patients with MS than in patients without it (Table 2). Through a logistic regression analysis, we found that FABP4 was strongly associated with the presence of MS (Table 8, model 1). This model predicted 46% of the variability of the incidence of MS (Nagelkerke Corrected R Square=0.462, P=0.001) and a percentage of being correctly diagnosed for MS of 78% in this cohort. Subjects who had serum FABP4 levels above the median were 30 times more likely to have MS than those below the median (Table 8, model 1). After adjustments for BMI and age, subjects who had serum FABP4 levels above the median were 17 times more likely to have MS than those below the median (Table 8, model 2). Finally, we found that high levels of FABP4 predicted the presence of MS independently of other adipo/cytokines (Table 8, model 3).

FABP4 *mRNA expression in visceral and subcutaneous adipose tissues*

We determined *FABP4* gene expression in human adipose tissue depots (Fig. 3). Our results showed that FABP4 expression in visceral AT was not affected by obesity. In contrast, its expression levels in subcutaneous AT increased significantly in morbid obesity. After comparing the expression of both tissues, we found that in lean subjects, FABP4 expression in visceral and subcutaneous adipose depots was similar. The expression of FABP4 in subcutaneous adipose tissue from morbidly obese women, however, was higher than its visceral expression (Fig. 3).

FABP4 gene expression is associated with BMI in visceral (r=0.414; P=0.048) but not in subcutaneous adipose tissue expression (r=0.375; P=0.060). The expression of FABP4 in adipose tissues was not related to HOMA2-IR or to insulin levels. Interestingly, circulating FABP4 levels in morbidly obese subjects and its visceral adipose tissue expression were strongly correlated (r=0.467; P=0.028), but not with FABP4 subcutaneous expression (r=−0.079; P=0.748).

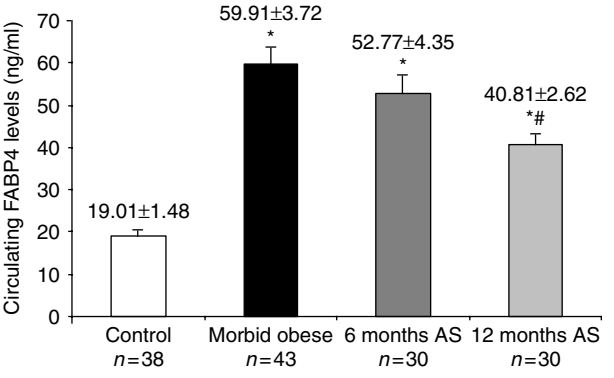

Figure 1 Circulating FABP4 levels. Values on bars indicate mean ±S.E.M. Differences between groups were calculated using the Student's *t*-test. *Indicates statistically significant differences between obese groups versus lean controls (P<0.001). #Indicates statistically significant differences between 6 and 12 months after surgery (AS) versus morbidly obese group (P<0.05).

Table 4 Correlations between FABP4 circulating levels and different parameters.

FABP4 serum levels	Model 1		Model 2	
Variables	*r*	*P* value	*r*	*P* value
BMI (kg/m^2)	0.771	<**0.001**		
WC (cm)	0.749	<**0.001**	0.777	<**0.001**
Glucose (mg/dl)	0.377	**0.001**	0.423	<**0.001**
Insulin (mU/l)	0.541	<**0.001**	0.503	<**0.001**
HbA1c (%)	0.433	**0.001**	0.455	<**0.001**
HOMA2-IR	0.540	<**0.001**	0.513	<**0.001**
Cholesterol (mg/dl)	−0.158	0.161	−0.251	0.125
HDL (mg/dl)	−0.615	<**0.001**	−0.695	<**0.001**
LDL (mg/dl)	−0.082	0.477	−0.172	0.134
Triglycerides (mg/dl)	0.540	<**0.001**	0.497	<**0.001**
AST (U/l)	0.619	<**0.001**	0.482	<**0.001**
ALT (U/l)	0.643	<**0.001**	0.530	<**0.001**
GGT (U/l)	0.528	<**0.001**	0.398	<**0.001**
ALP (U/l)	0.281	**0.015**	0.395	<**0.001**
Systolic BP (mmHg)	0.437	<**0.001**	0.567	<**0.001**
Diastolic BP (mmHg)	0.175	0.131	0.270	**0.018**

P values in boldface indicate statistically significant correlations ($P<0.05$). Model 1: Spearman's correlation test with uncorrected FABP4 serum levels. Model 2: Spearman's correlation test with corrected FABP4 for age and BMI.

Discussion

Accumulating evidence from animal experiments suggests that FABP4 is involved in the regulation of systemic insulin sensitivity, lipid metabolism, and inflammation, although its functional mechanisms remain poorly understood in humans (7, 8, 27). In this study, we found a relationship between FABP4 and proinflammatory cytokines related to obesity and MS. Furthermore, we provide clinical evidence demonstrating that FABP4 is a significant risk factor for the diagnosis of MS in a non-diabetic morbidly obese cohort. Other authors have reported similar results in other overweight (20, 28), mild-obesity populations (6), or diabetic patients (19), but it has never been addressed in morbid obesity.

The study has demonstrated an association between circulating FABP4 levels and all the metabolic risk factors: obesity, insulin resistance, dyslipidemia, hyperglycemia, and hypertension. Notably, a logistic regression analysis demonstrated that high FABP4 levels were strongly associated with the presence of MS in this cohort. These results are in accordance with those previously reported by Xu *et al.* (20) in an Asian overweight cohort.

Table 5 Correlations between FABP4 circulating levels and different inflammatory-related factors.

FABP4 serum levels	Model 1		Model 2	
Circulating levels	*r*	*P* value	*r*	*P* value
Adiponectin	−0.576	<**0.001**	−0.506	<**0.001**
Resistin	0.039	0.781	0.233	0.090
Leptin	0.657	<**0.001**	0.620	<**0.001**
IL6	0.439	<**0.001**	0.533	<**0.001**
TNF-RI	0.457	<**0.001**	0.451	<**0.001**
TNF-RII	0.240	**0.031**	0.164	0.143
CRP	0.333	**0.005**	0.525	<**0.001**

P values in boldface indicate statistically significant correlations ($P<0.05$). Model 1: Spearman's correlation test with uncorrected FABP4 serum levels. Model 2: Spearman's correlation test with corrected FABP4 for age and BMI.

After comparing the relationship between FABP4 and MS and other cytokines and MS, we demonstrated that FABP4 was the only cytokine that increased in a morbidly obese cohort in relation to the presence of MS. In addition, circulating FABP4 levels increased correspondingly with the increase in HOMA2-IR levels, even when the analysis was performed 6 and 12 months after bariatric surgery and despite the consequent weight loss. These results are consistent with those of Simón *et al.* (29) and Milner *et al.* (30), who found similar results in overweight men and women. Furthermore, we demonstrated that FABP4 was the cytokine most closely related to HOMA2-IR compared with adiponectin, TNF-RI, CRP, or leptin, even after adjustment for age and BMI.

Data obtained from animal models suggest that FABP4, one of the most abundant cytoplasmic proteins in adipocytes (5), acts at the interface of metabolic and inflammatory pathways and is involved in the development of key pathologies associated with MS (24, 31). Xu *et al.* (20) found a positive correlation between CRP and FABP4 and a negative relation to adiponectin in an Asian overweight population, suggesting that FABP4 plays a role in systemic inflammation. In accordance with various population-based studies (18, 20), we found strong positive relationships between FABP4 and proinflammatory factors such as CRP, but also with IL6, TNF receptors, and leptin. However, there are discrepancies in the correlation between FABP4 and the anti-inflammatory adipocytokine adiponectin, probably due to differences in the adiposity of the populations.

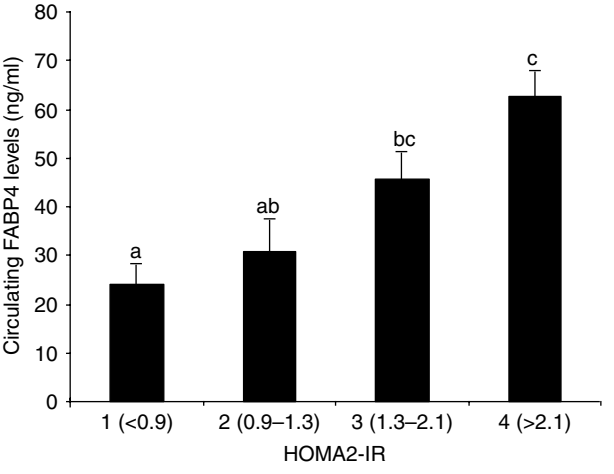

Figure 2 Relationship between HOMA2-IR by quartiles and circulating FABP4 levels. Differences between groups were calculated using the one-way ANOVA analysis. Different superscript letters indicate statistically significant differences between quartiles ($P<0.001$).

Table 6 Multiple stepwise linear regression analysis for factors associated with HOMA2-IR.

	Unstandardized coefficients		Standardized coefficients		
Dependent variable: HOMA2-IR*	β	S.E.M.	β	t	P value
Model 1					
FABP4* (ng/ml)	0.454	0.089	**0.554**	5.115	**<0.001**
Model 2					
FABP4* (BMI/age)	0.558	0.109	**0.517**	5.122	**<0.001**

Model 1: variables included in the original model are unadjusted leptin*, adiponectin*, TNFRI, CRP, and FABP4* circulating levels. Model 2: variables included in the original model are leptin*, adiponectin*, TNFRI, CRP, and FABP4 circulating levels, all adjusted for BMI and age. *Logarithmically transformed variables. P values in boldface indicate significant associations ($P<0.05$).

In addition, because there are sex-related differences in cytokine levels as suggested by Hyun Koh *et al.* (18), we only analyzed morbidly obese women in this work. In our cohort, FABP4 was negatively associated with adiponectin, which persisted after adjustment for BMI and age. Taken together, these results suggest that circulating FABP4 levels might be involved in the low-grade proinflammatory state present in MS, and strengthen the hypothesis that FABP4 is closely related to insulin resistance in obesity. In contrast to our results, Simón *et al.* (29) did not find a relationship between proinflammatory markers and FABP4 at basal level or after 1 year of follow-up, despite a clear improvement in these parameters after weight loss. The authors suggested that their results might indicate that FABP4 plays a predominant role in glucose homeostasis rather than in inflammatory pathways in diabetic morbidly obese women (29). However, our results indicate that when diabetes is not established, as was the case in our cohort because we excluded type 2 diabetic patients, FABP4 might play a different role. Further studies are needed in order to clarify these discrepancies.

Resistin, which is one of the most recently identified adipokines, has been proposed as an inflammatory marker involved in nutritional regulation in humans (32). Although serum resistin levels have been positively correlated with BMI in humans and rodent obesity models, in this work, we did not find any relationship between resistin and circulating FABP4 levels. Hertzel *et al.* (24) found that resistin levels were unchanged when FABP4 null mice were compared with the circulating levels of wild-type mice. These findings together with our results might indicate that resistin and FABP4 are not directly related, although resistin is an example of the new adipokines that appear to have contrasting roles when examined in mice versus humans (32).

After analyzing FABP4 expression in both adipose tissues, we found that there were depot-specific differences in the expression of FABP4. *FABP4* gene expression in subcutaneous adipose tissue from morbidly obese women was higher than in visceral adipose tissue, as other authors have previously reported (5). Furthermore, subcutaneous adipose tissue expression of FABP4 was significantly increased in obese women, whereas in visceral adipose tissue, it was unchanged in lean and morbidly obese subjects.

The mechanisms of FABP4 secretion from cells remain unclear (5). In this study, we have addressed circulating FABP4 levels and its expression in adipose tissue in parallel for the first time in humans in order to analyze the potential contribution of these FABP4 adipose tissues to circulation. Our results show a close positive correlation between serum FABP4 levels and the expression of FABP4 in visceral adipose tissue, although these results cannot be extrapolated to the lean group. Our results, along with *in vitro* findings that 3T3-L1 adipocytes release FABP4 to the extracellular medium (6), suggest that the production of FABP4 by visceral adipose tissue might be an important contributor to circulating levels of FABP4 in our morbidly obese cohort, but we cannot exclude the contribution of subcutaneous adipose tissue.

The association between visceral FABP expression and FABP plasma concentration, despite the lack of increase in expression in this tissue, might highlight the

Table 7 Correlations between FABP4 circulating levels and HOMA2-IR at 6 and 12 months after bariatric surgery. Spearman's correlation test between HOMA2-IR and FABP4 circulating levels is corrected for BMI and age (BMI–age), or FABP4 circulating levels are corrected for adiponectin, leptin, TNFR1, TNFR2, IL6, and CRP levels (cytokines).

	HOMA2-IR			
	6 months AS		12 months AS	
	r	P value	r	P value
FABP4 (BMI–age)	0.229	0.282	0.506	**0.008**
FABP4 (cytokines)	0.471	**0.018**	0.370	**0.048**

P values in boldface indicate statistically significant correlations ($P<0.05$). AS, after bariatric surgery.

Table 8 Simple logistic regression analysis showing FABP4 association with the presence of MS.

Presence of MS	OR	95% CI	P value
Model 1			
FABP4 (ng/ml)	30.47	6.5–144.0	**<0.001**
Model 2			
FABP4 (BMI/age)	17.88	4.7–67.6	**<0.001**
Model 3			
FABP4 (cytokines)	5.55	1.7–18.2	**0.005**

Model 1: unadjusted FABP4 circulating levels subclassified into values below the median (OR=1) and values above the median. Model 2: FABP4 adjusted for BMI and age subclassified into values below the median (OR=1) and values above the median. Model 3: FABP4 adjusted for adiponectin, leptin, TNFR1, TNFR2, IL6, and CRP subclassified into values below the median (OR=1) and values above the median. P values in boldface indicate significant associations ($P<0.05$).

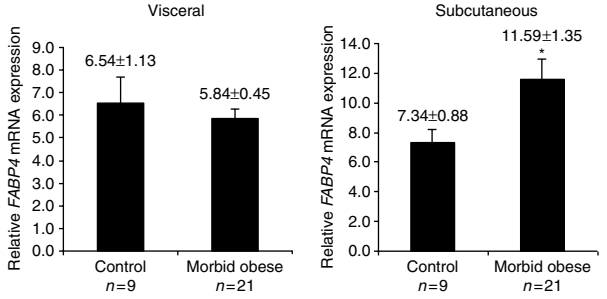

Figure 3 *FABP4* mRNA expression levels in visceral and subcutaneous adipose tissues. Values on bars indicate mean ±S.E.M. Differences between groups were calculated using the Student's *t*-test. *Indicates statistically significant differences between groups ($P<0.05$).

importance of considering the differences in the total production rate of adipose tissue-derived factors due to differences in the adiposity of the groups studied (33).

The major limitation of this study is the relatively small number of subjects included. Although our specific cohort of non-diabetic morbidly obese women revealed clear relationships between MS risk factors and FABP4 without the interference of confounding factors, these results cannot be extrapolated to other obese groups or men. Secondly, due to the difficulty in obtaining tissue samples, the relationship between adipose tissue FABP4 expression and circulating FABP4 levels needs to be confirmed through research with larger study populations.

We demonstrated that serum FABP4 is closely associated with dyslipidemia, insulin resistance, and low-grade inflammation. Taken together, these results indicate that in morbidly obese women, FABP4 plays a role in both the metabolic and inflammatory pathways involved in MS. In conclusion, our data suggest that FABP4 may be an independent marker of the presence of MS.

Large population-based prospective studies with the inclusion of different obesity grade patients and men are warranted to confirm whether FABP4 alone, or as a marker of FFA metabolic disruption, is an independent predictor of cardiometabolic risk and whether it plays a causative role in the development of MS.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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