

## Anti-inflammatory Profile of *FTO* Gene Expression in Adipose Tissues From Morbidly Obese Women

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### Key Words

Adipokines • Adipose tissue *FTO* • Insulin resistance  
• Metabolic syndrome • Obesity

### Abstract

**Background:** The fat mass and obesity associated (*FTO*) gene has been found to contribute to the risk of obesity in humans, but the function and regulation of *FTO* mRNA expression in adipose tissues remain to be clarified. Our aims were to assess the *FTO* gene expression in subcutaneous and visceral adipose tissues from morbidly obese women and its relation with obesity, insulin resistance indices, and most importantly, to obesity-related inflammatory markers. **Methods:** Paired subcutaneous and visceral fat were excised from 33 morbidly obese women and 12 control women who underwent bariatric surgery by laparoscopic gastric by-pass and elective surgery respectively. Adipose tissue mRNA expression was determined by real time RT-PCR. **Results:** *FTO* mRNA expression in visceral adipose tissue (VAT) was significantly higher than in subcutaneous adipose tissue (SAT) from obese but not control patients. SAT *FTO* expression was

reduced in obese women compared to control subjects. It correlated negatively with BMI and insulin resistance indices. *FTO* expression in SAT was positively related to both circulating and mRNA levels of adiponectin, to adiponectin receptor and to PPAR- $\delta$  expression, but negatively with IL-6 gene expression and with circulating levels of leptin. *FTO* in VAT was also positively correlated with adiponectin, adiponectin receptor and PPAR- $\delta$  mRNA expression. **Conclusion:** *FTO* expression in subcutaneous adipose tissue negatively correlates with obesity and insulin resistance. On the other hand, *FTO* presents a positive association with the expression of adiponectin, an anti-inflammatory adipokine, and with PPAR- $\delta$  in both adipose tissues. Taken together, our results suggest that *FTO* is associated with an anti-inflammatory behaviour in morbid obesity.

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### Introduction

Obesity has become the most common human disorder and is considered an important risk factor for

the development of many metabolic disorders such as low-grade chronic inflammation, insulin resistance, dyslipidaemia, arterial hypertension, and atherosclerosis [1-4]. Recently a new candidate gene for obesity has been identified; the fat mass and obesity associated gene (FTO), after variants in the first intron of the FTO gene have been strongly associated with increased body mass index through independent studies in European populations of adults and children [5-8]. Frayling et al. [7] found the association between FTO variants (represented by *rs9939609*) and obesity in a genome-wide scan for type 2 diabetes. The association between SNPs in the FTO gene and type 2 diabetes was shown to be due to obesity. Other single nucleotide polymorphisms in the FTO gene were also associated with overweight and obesity [9, 10].

The function of the FTO gene product and the biological pathways involved are completely unknown. It is mapped on chromosome 16p12.2 where *de novo* duplication has been shown to be associated with obesity, dysmorphic facies, and other diseases [11, 12]. It has been shown that FTO localizes into the nucleus and functional studies with bioinformatics analysis revealed that the FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase which may have a potential role in regulating the transcription of genes involved in metabolism by nucleic acid demethylation of DNA [13, 14]. Studies are now being aimed towards determining the physiologically relevant FTO substrate and how nucleic acid methylation status is linked to increased fat mass [13].

FTO mRNA is expressed in different human tissues, but the highest levels of FTO gene were found in the brain [13], and specifically in the hypothalamus region, which has a key role on the control of energy homeostasis [7], in the pituitary, and the adrenal glands, although it is also detectable in a wide range of human tissues, including adipose tissue.

Based on these investigations, adipose tissue is an interesting candidate for investigating the basis for association between FTO and body fat regulation. Adipose tissue is now considered as an endocrine organ [15], that expresses a variety of genes related to energetic metabolism and involved in the control of energy balance [16], however, there are only a few studies that analyze the FTO expression in this tissue [17-20], with controversial results.

In our study, in order to investigate the associations of the FTO gene with obesity, we assessed the expression of FTO in paired samples of the subcutaneous and visceral adipose tissue of morbidly obese

and lean women, and investigated the relationships between its expression and different anthropometric and metabolic obesity-related measures: obesity markers, insulin resistance indices, and obesity-related inflammatory cytokines.

## Materials and Methods

### Subjects

The study was approved by the institutional review board. All participants gave written informed consent for participation in medical research. The study cohort was comprised of 45 Caucasian women: 12 lean (BMI < 25 kg/m<sup>2</sup>) and 33 morbidly obese women (BMI > 40 kg/m<sup>2</sup>). In this study we analysed visceral and subcutaneous adipose tissue FTO mRNA expression. Adipose tissue samples were obtained from morbidly obese women and from control women who underwent bariatric surgery by laparoscopic gastric by-pass and by elective surgery respectively. In each case of surgery samples were obtained by the same specialist. Subcutaneous adipose tissue biopsies were taken from the right hypocondrium region and visceral adipose tissue biopsies were taken from the greater epiploon region. Morbidly obese women and controls were age-matched. The weight of all subjects was stable for at least 3 months before surgery. Those patients who had an acute illness, acute or chronic inflammatory or infective diseases, or end-stage malignant disease were excluded from this study. Patients taking anti-diabetic medication, as PPAR- $\gamma$  agonists or insulin, were also excluded. We have not included the lipid profile in our results because our cohort is compressed by 20% of patients receiving a hypolipemiant treatment. We determined the anthropometric and metabolic features.

### Biochemical analyses

A complete physical examination was performed on each patient. Anthropometric evaluation included measures of body mass index (BMI). Laboratory studies included glucose and insulin, performed using a conventional automated analyser and measured after overnight fasting. The homeostasis model assessment of insulin resistance (HOMA2-IR) was completed using the HOMA Calculator version 2.2.2 (<http://www.dtu.ox.ac.uk>, accessed May 2010). Circulating levels of TNF- $\alpha$  (AssayPro, St. Charles, USA), TNF-RI, TNF-RII (Biosource Europe S.A., Nivelles, Belgium), adiponectin, HMW adiponectin (Linco Research, Inc., St. Charles, USA), C-reactive protein (CRP), resistin, leptin (Biovendor, Modrice, Czech Republic), and IL-6 (Quantikine, R&D Systems, Minneapolis, USA) were measured in duplicate using enzyme-linked immunosorbent assays (ELISA) following the manufacturer's instructions. RNA isolation and real time PCR.

Total RNA was isolated from adipose tissues according to the manufacturer's protocol RNeasy midi kit (Quiagen) and was digested with DNase I (RNase-Free DNase set, Quiagen). RNA quality was evaluated by measuring the 260/280nm ab-

	CONTROL (n=12)	MORBID OBESE (n=33)	<i>p</i> -value
General variables			
AGE (years)	49.50 ± 4.34	46.97 ± 1.42	0.589
BODY WEIGHT (Kg)	63.73 ± 5.75	117.10 ± 2.42 *	<0.001
BMI (Kg/m <sup>2</sup> )	23.24 ± 0.65	45.53 ± 0.97 *	<0.001
GLUCOSE (mg/dL)	86.80 ± 3.27	120.09 ± 5.22 *	<0.001
INSULIN (mU/L)	7.09 ± 1.66	17.58 ± 1.78 *	<0.001
HbA1c (%)	4.49 ± 0.62	5.05 ± 0.18 *	0.008
HOMA2-IR	0.99 ± 0.27	2.56 ± 0.29 *	0.001
Adipokine circulating levels			
HMW ADIPONECTIN (µg/mL)	8.83 ± 1.21	3.75 ± 0.40 *	<0.001
ADIPONECTIN (µg/mL)	20.39 ± 5.88	9.15 ± 2.23 *	0.047
RESISTIN (ng/mL)	3.96 ± 0.48	5.30 ± 0.32 *	0.042
LEPTIN (ng/mL)	34.91 ± 11.52	234.67 ± 23.66 *	<0.001
TNF-α (ng/mL)	0.16 ± 0.09	0.15 ± 0.03	0.933
TNF-RI (ng/mL)	2.12 ± 0.25	2.82 ± 0.13 *	0.013
TNF-RII (ng/mL)	3.37 ± 0.53	5.15 ± 0.35 *	0.011
IL-6 (pg/mL)	2.74 ± 0.81	2.81 ± 0.31	0.924
CRP (mg/dL)	0.10 ± 0.09	0.81 ± 0.13 *	<0.001

**Table 1.** Baseline characteristics of the study cohort. Data are the mean ± SEM. \* indicates significant differences vs. control group ( $p < 0.05$ ).

sorbance ratio (=1.8) and by electrophoresis. First-strand cDNA was synthesised using equal amount of total RNA with a High Capacity RNA-to-cDNA Kit (Applied Biosystems). Real-time quantitative PCR was performed in a final volume of 20 µL, which contained 10 ng of reverse-transcribed cDNA, 10µL of 2X Taq Man Fast Universal PCR Master Mix (Applied Biosystems) and 1µL Taq Man Assay predesigned by Applied Biosystems® for the detection of FTO, PPAR-δ, PPAR-γ, TNF-α, IL-6, adiponectin, adiponectin receptor 2, CRP, resistin and GAPDH, that 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 ± SEM (standard error of the mean). Analysis was performed with SPSS/PC+ for windows statistical package (v.15.0 Chicago, Illinois, USA). Intergroup differences were calculated using the Student's *t* test. The strength of association between variables was calculated using Pearson's method for parametric variables. Multivariable linear regression analysis was performed to identify independent factors affecting FTO expression, choosing the model with the best goodness-of-fit. The validity of the regression model and its assumptions was assessed with the plot of residuals vs predicted. *P* values <0.05 were considered statistically significant.

## Results

### Patient characteristics

The baseline patient characteristics given in Table 1 show the median and SEM of the variables of interest. Patients were separated into control (BMI<25 kg/m<sup>2</sup>), and morbidly obese patients (BMI>40 kg/m<sup>2</sup>). The groups were well matched for age as no differences between groups were detected.

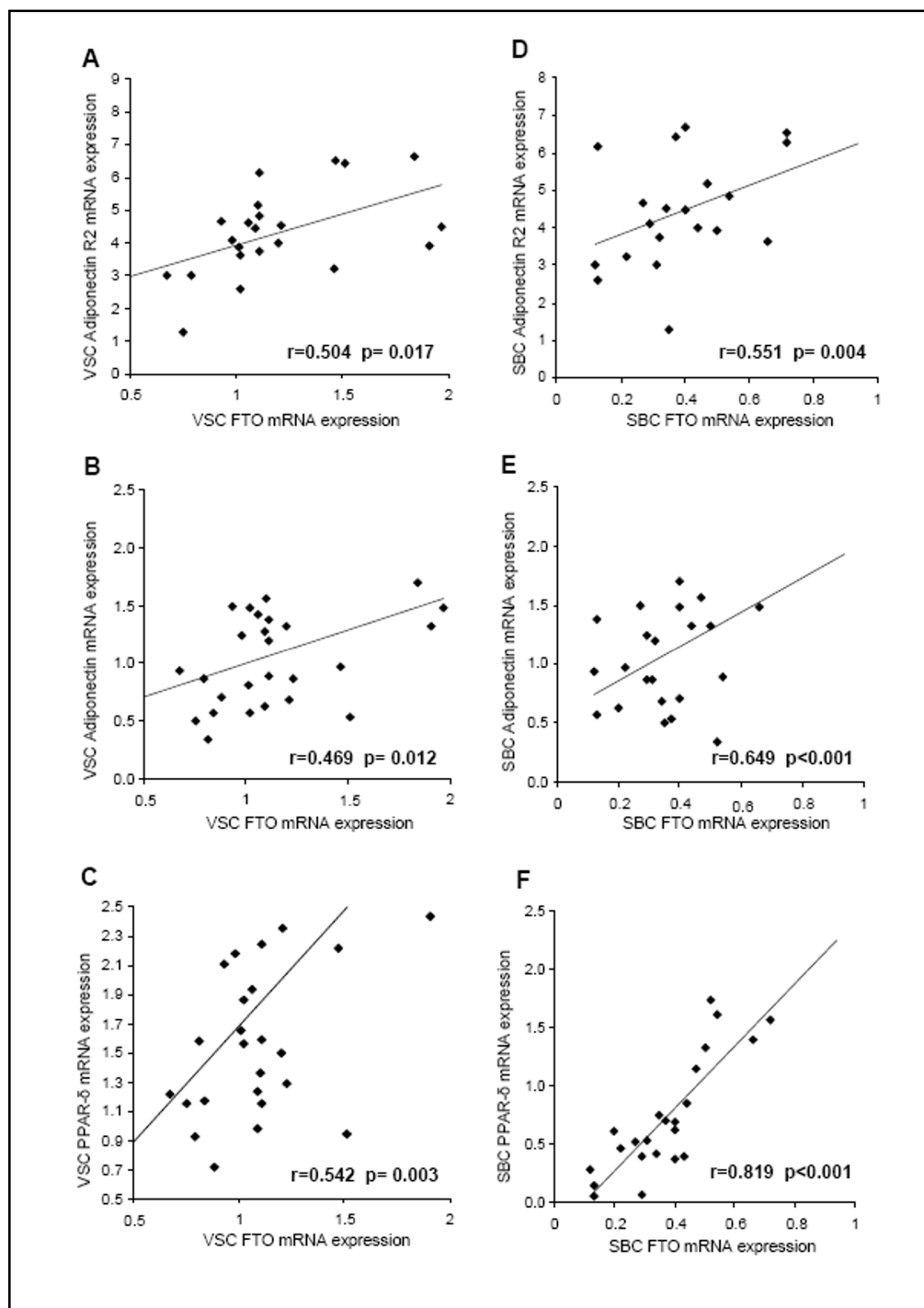
Biochemical analyses indicated that the morbidly obese women had significantly higher levels of glucose, insulin, HbA1c and HOMA2-IR than the control group.

We also measured the adipokine circulating levels of the patients studied. As expected, adiponectin levels were lower in obese patients, whereas circulating levels of TNF-RI, TNF-RII, CRP, resistin, and leptin were higher in the morbid obese group.

### Adipose tissues FTO gene expression

Real-time PCR analysis indicated that FTO mRNA expression levels in visceral adipose tissue were not significantly different between groups (Table 2). On the contrary, FTO subcutaneous mRNA expression was reduced

**Fig. 1.** Correlation between FTO mRNA expression and adiponectin, adiponectin R2 or PPAR- $\delta$  in visceral (VSC) and subcutaneous (SBC) adipose tissues from morbid obese women.



in morbid obese patients with respect to controls (Table 2). When comparing both adipose tissues we demonstrated that visceral FTO expression was more than three times higher in the morbid obese group, but we did not encounter any differences between the visceral and subcutaneous FTO expression in the control group (Table 2).

Together with FTO we also determined the expression of inflammatory related factors in visceral and subcutaneous adipose tissue (Table 2). As expected

adiponectin expression was higher than the expression of other cytokines in both adipose tissues. The visceral mRNA expression of resistin, TNF- $\alpha$ , IL-6, adiponectin R2 and PPAR- $\delta$  was increased in morbid obese compared to control subjects. In subcutaneous adipose tissue the expression of IL-6 was also higher in morbid obese than in controls. On the contrary SAT expression of adiponectin, ADIPOR2 and PPAR- $\delta$  was decreased in obese patients.

2 exp(-ΔCt)	VISCERAL ADIPOSE TISSUE			SUBCUTANEOUS ADIPOSE TISSUE				
	CONTROLS	MORBID OBESE	p-value <sup>1</sup>	CONTROLS	MORBID OBESE	p-value <sup>2</sup>	p-value <sup>3</sup>	p-value <sup>4</sup>
FTO	0.05±0.00	0.068±0.00	0.275	0.044±0.01	0.02±0.00	<0.001	0.321	<0.001
ADIPONECTIN	5.50±0.42	6.07±0.57	0.431	10.50±1.74	4.03±0.47	0.005	0.019	0.008
ADIPO-R2	0.71±0.29	1.75±0.14	0.002	0.04±0.01	0.02±0.00	0.042	0.008	<0.001
RESISTIN	3.0x10 <sup>-4</sup> ±2x10 <sup>-4</sup>	2.0x10 <sup>-3</sup> ±4.0x10 <sup>-4</sup>	0.001	1.2x10 <sup>-3</sup> ±3x10 <sup>-4</sup>	1.7x10 <sup>-3</sup> ±2x10 <sup>-4</sup>	0.226	0.039	0.776
TNF-α	3.5x10 <sup>-3</sup> ±8x10 <sup>-4</sup>	0.02±0.00	0.003	4.8x10 <sup>-3</sup> ±1.7x10 <sup>-3</sup>	3.9x10 <sup>-3</sup> ±1.1x10 <sup>-3</sup>	0.650	0.496	<0.001
IL-6	0.03±0.02	0.69±0.11	<0.001	0.29±0.16	1.50±0.39	0.008	0.129	0.049
PPAR-δ	0.02±0.00	0.03±0.00	0.002	1.0 x10 <sup>-2</sup> ±1.0 x10 <sup>-3</sup>	5.8 x10 <sup>-3</sup> ±1.0 x10 <sup>-3</sup>	0.027	0.018	<0.001
PPAR-γ	0.16±0.02	0.20±0.02	0.093	0.14±0.03	0.13±0.03	0.921	0.496	0.023

**Table 2.** Comparison between visceral and subcutaneous mRNA expression of the genes studied. Data are mean ± SEM. p-values considered statistically significant are p<0.05. p-value<sup>1</sup>: Comparisons between controls and morbid obese patients in visceral adipose tissue. p-value<sup>2</sup>: Comparisons between controls and morbid obese patients in subcutaneous adipose tissue. p-value<sup>3</sup>: Comparisons between controls in visceral and subcutaneous adipose tissue. p-value<sup>4</sup>: Comparisons between morbid obese in visceral and subcutaneous adipose tissue.

FTO	VAT mRNA levels		SAT mRNA levels	
	r	p-value	r	p-value
General variables				
AGE (years)	-0.056	0.740	-0.033	0.844
BODY WEIGHT (Kg)	0.079	0.655	-0.592	<0.001
BMI (Kg/m <sup>2</sup> )	0.111	0.512	-0.457	0.004
GLUCOSE (mg/dL)	0.194	0.263	-0.429	0.010
INSULIN (mU/L)	0.121	0.489	-0.351	0.049
HOMA2-IR	0.201	0.315	-0.392	0.035

**Table 3.** Correlations between FTO expression and metabolic or anthropometric variables in the morbid obese cohort. VAT; visceral adipose tissue, SAT; subcutaneous adipose tissue. p-values p<0.05 indicates significant differences vs. control group.

#### *Correlations of FTO expression with biochemical and anthropometric parameters*

Subcutaneous FTO expression revealed a strong negative correlation with anthropometric measures such as BMI and body weight. FTO subcutaneous expression correlated negatively with fasting glucose, insulin, and the HOMA2-IR index (Table 3). Visceral FTO expression did not correlate with any of the parameters tested (Table 3).

#### *Correlations of FTO expression with circulating cyto/adipokines*

We also assessed possible correlations of FTO expression with plasma cytokine levels. We found that subcutaneous FTO expression and diponectin levels were

strongly correlated. Furthermore, in subcutaneous adipose tissue we found negative correlations between FTO mRNA expression and circulating levels of the pro-inflammatory adipokine, leptin (Table 4). In visceral adipose tissue no significant correlations between FTO expression and circulating cyto/adipokines were found (Table 4).

#### *Relationship of FTO expression with different gene expression in subcutaneous and visceral adipose tissues*

In the morbid obese cohort, both visceral and subcutaneous FTO expression correlated with adiponectin, adiponectin receptor RII and the nuclear factor PPAR-δ expression in each tissue respectively (Fig. 1). In subcu-

FTO	VAT mRNA levels		SAT mRNA levels	
	r	p-value	r	p-value
Adipo/cytokine circulating levels				
HMW ADIPONECTIN	0.021	0.914	0.180	0.368
ADIPONECTIN	-0.094	0.621	0.401	0.038
RESISTIN	-0.063	0.742	0.100	0.621
LEPTIN	-0.159	0.468	-0.475	0.026
TNF- $\alpha$	-0.368	0.215	-0.298	0.402
TNF-RI	-0.135	0.521	0.117	0.586
TNF-RII	-0.115	0.585	-0.049	0.821
IL-6	0.050	0.812	0.142	0.507
CRP	-0.321	0.117	0.067	0.763

**Table 4.** Correlations between FTO expression and adipo/cytokine circulating levels in the morbid obese cohort. VAT; visceral adipose tissue, SAT; subcutaneous adipose tissue. *p*-values *p*<0.05 indicates significant differences vs. control group.

VAT		Unstandardized Coefficients		Standardized Coefficients	
Gene expression	B	Std. Error	Beta	t	p
PPAR- $\delta$	0.131	0.045	0.462	2.907	0.008
ADIPOR2	0.077	0.033	0.366	2.298	0.031

SAT		Unstandardized Coefficients		Standardized Coefficients	
Gene expression	B	Std. Error	Beta	t	p
ADIPONECTIN	0.550	0.089	0.802	6.154	0.000

**Table 5.** Linear regression analysis using FTO mRNA expression as the dependent variable. VAT; visceral adipose tissue, SAT; subcutaneous adipose tissue, PPAR- $\delta$ ; peroxisome proliferators, activated receptor-d, ADIPOR2; adiponectin receptor 2.

taneous adipose tissue FTO expression also correlated inversely with the expression of IL-6 ( $p=-0.405$ ,  $r=0.049$ ), but not in VAT ( $p=0.207$ ,  $r=0.361$ ). We did not find any association between FTO expression in VAT or SAT with TNF- $\alpha$  (VAT:  $p=0.198$ ,  $r=0.331$ ; SAT:  $p=0.027$ ,  $r=0.896$ ), resistin (VAT:  $p=0.064$ ,  $r=0.760$ ; SAT:  $p=0.313$ ,  $r=0.145$ ) or with PPAR- $\gamma$  expression (VAT:  $p=0.263$ ,  $r=0.175$ ; SAT:  $p=0.042$ ,  $r=0.842$ ). FTO expression between both tissues were not correlated ( $r=0.181$ ,  $p=0.398$ ).

#### Linear regression analysis

To determine whether FTO expression was independently associated with any of the parameters tested we created a linear regression analysis (backward exclusion method) including adipokine circulating levels and

adipose tissues mRNA expression which were the parameters with the highest correlation rates in the univariate analyses.

Linear regression analyses revealed that in visceral adipose tissue, adiponectin receptor RII (Beta= 0.366,  $p=0.031$ ) and PPAR- $\delta$  (Beta= 0.462,  $p=0.008$ ) mRNA expression were independently associated with FTO expression. Our results show that this model predicts a 37% (Adjusted R Square = 0.375,  $p=0.001$ ) of the variability of FTO visceral mRNA expression (Table 5).

In subcutaneous adipose tissue, the analysis revealed that among the variables included in the test (PPAR- $\delta$  and adiponectin SAT mRNA expression and circulating leptin and adiponectin) only adiponectin expression was independently associated with FTO expression (Beta=

0.802,  $p < 0.001$ ). This model predicts a 62% (Adjusted R Square = 0.626,  $p < 0.001$ ) of the variability of FTO subcutaneous mRNA expression.

## Discussion

FTO has been associated with obesity [7, 21, 22], and type 2 diabetes [23], but the biological functions and regulation of FTO mRNA expression in human tissues remain to be clarified. The aims of this study were to examine FTO gene expression in relation to obesity and insulin resistance and, for the first time, to analyse the relationship of FTO with adiponectin, leptin, resistin, TNF- $\alpha$ , CRP and IL-6, which are inflammatory markers associated with the aforementioned disorders [24, 25].

The adipose tissue has specific regional depots which have different cellular size, structural organization and biological functions. Furthermore, it has been reported that there are depot-specific differences in the lipid metabolism and inflammatory state of visceral and subcutaneous adipose tissues [26, 27]. In this work, we found that FTO expression in lean patients was similar in both adipose tissues. On the contrary, the FTO gene was differentially expressed in SAT and VAT from obese patients; VAT FTO expression was higher than in SAT. After comparing the FTO expression in lean and obese patients, we showed that FTO expression in SAT decreased in our morbid obese group with respect to controls, but no changes were detected in VAT FTO expression. The reduced FTO gene expression in SAT in a group of morbid obese women and its potential impact to the whole body, need to be studied in depth, because the subcutaneous adipose tissue is by far the body's largest fat depot [18].

To date, only a few studies have examined site-specific FTO gene expression in adipose tissues leading to discordant results. Lower levels of FTO mRNA expression in visceral than in subcutaneous adipose tissue have been recently reported in a study of 55 European men and women with BMI ranging from 27 to 47 kg/m<sup>2</sup> [17]. In contrast, other authors showed similar levels of FTO mRNA in both adipose tissue depots [18, 19, 28].

The few human studies of FTO expression in adipose tissue performed so far have also been inconsistent concerning the relationship between obesity and FTO tissue expression. A study in mice showed that FTO mRNA expression was 50% lower in adipocytes from a genetic

model of type 2 diabetes and obesity mice (*db/db*) [29]. Wåhlén et al. [18] found that the SAT level of FTO mRNA was increased in obesity in their study in 76 Swedish obese women with mean BMI < 36 kg/m<sup>2</sup>, but the authors did not report FTO expression levels in VAT. In a group of male and female individuals, Samaras et al. [28], showed that FTO mRNA expression in SAT and VAT in subjects with type 2 diabetes and BMI < 40 kg/m<sup>2</sup>, was similar to that of lean controls. In contrast to our morbidly obese cohort, these studies were performed in non-morbidly obese patients.

We also assessed the relationship of FTO expression and metabolic and anthropometric variables. We found significant negative correlations between FTO gene expression and BMI, glucose, insulin, and HOMA-index in SAT. In VAT we did not find any correlations with the parameters tested and FTO expression. Our data suggests that, at least in SAT, FTO gene expression might be down regulated in response to fat accumulation as other authors have previously hypothesized [17]. These results are in accordance with some but not all studies. Klöting et al. [17], found that FTO expression was negatively correlated with BMI in SAT, but also in VAT. They found that its expression was not related to measures of insulin sensitivity and glucose metabolism although they studied a different cohort in terms of age, sex, and BMI. In contrast to our results, it has been reported that FTO expression in VAT was positively related to waist circumference and fasting glucose in a cohort of 10 controls and 6 obese diabetic patients [28]. The discrepancies between these reports might be due to the wide range of BMI values included in their cohorts to perform this analysis. In order to discard the effect of the obesity grade in the correlations found, we analysed the morbid obese cohort alone.

The potential relationship between FTO and inflammatory factors has not been studied so far. In order to go further into depth on the local effect of inflammatory-related molecules on FTO expression, we analysed the associations between FTO and adipose tissue expressed cyto/adipokines [16, 30-33]. In accordance with our previously described results, we showed that FTO expression in SAT was positively correlated with the circulating levels of the anti-inflammatory cytokine adiponectin and, as previously reported [17], FTO expression negatively related to the pro-inflammatory molecule leptin [34]. Also in SAT, IL-6 and FTO expression were negatively related. Furthermore, we provide first descriptive information on

the strong positive associations between adiponectin and adiponectin receptor II expression with FTO in both adipose tissues. Interestingly, the highest correlation rates were found when we assessed the relationship between cytokine mRNA expression in each tissue and FTO. Also, the observation that FTO expression profile, in the two depots analysed, were different and not correlated, suggests that in a given individual up regulation or down regulation in one vs the other depot is controlled locally rather than in a coordinated fashion by systemic hormonal, neuronal, and/or nutritional signals [17].

Also in this work, we found that PPAR- $\delta$  mRNA levels in both adipose tissues were strongly correlated with FTO expression. In addition, the expression profile of PPAR- $\delta$  was characteristic for each AT depot, being higher in visceral AT from morbid obese but reduced in subcutaneous AT. Similar results were previously published by Bortolotto et al. [35], in a morbidly obese cohort compared to non-obese individuals. The activation of PPAR- $\delta$  in adipose tissue causes a marked decrease in fat mass which is mainly achieved by activation of fatty acid oxidative pathways [36]. Furthermore it has been speculated that inflammatory gene expression is under negative control of PPAR- $\delta$  in adipose tissue [36].

We then tested the hypothesis of whether cytokine expression predicts the variability of FTO expression using backward exclusion regression analyses. The fact that in SAT, the expression of adiponectin predicted 62% of FTO expression variability, and in VAT, adiponectin receptor and PPAR- $\delta$  expression accounted for 37% of the variations in FTO expression, is notable.

The major limitation of the present study is the number of subjects included due to the difficulty to obtain tissue samples from the same individual. The relationship between adipose tissue FTO expression and the expression of other cyto/adipokines needs to be confirmed involving larger study populations. These results are not extrapolable to other obesity groups or gender. In different reports, authors study groups of subjects with a wide range of obesity grades, and include patients of both genders, but in this work, in order to avoid heterogeneity of the group, we carefully selected the patients in terms of age, sex, and BMI. In this sense, our group of morbidly obese women permitted the obtainment of clear relationships between obesity related disorders and FTO, without the interference of confounding factors.

On the other hand, we have not analysed the FTO gene variants of the study population, although it has been reported that genotype variations of FTO does not appear to play a major role in regulating FTO transcription in adipose tissue [17]. FTO mRNA expression has been related to BMI, age, sex and physical activity, [5, 37, 38] so the selection and composition of the study population in each report might be involved in the discrepancies between these results.

To conclude, we found that FTO expression in subcutaneous adipose tissue is negatively correlated with obesity and measures of insulin resistance. Most importantly, we found for the first time in a morbid obese cohort, the strong positive relationship between FTO with the anti-inflammatory adipokine adiponectin and with the nuclear factor PPAR- $\delta$  expression. These associations between FTO and an anti-inflammatory profile of adipokines, suggests that FTO might have an anti-inflammatory role like adiponectin, but its possible regulatory mechanism has not been studied in this work. Further studies are needed in order to elucidate whether changes in adipose tissue FTO gene expression are secondary to the inflammatory state present in obesity, or causally related.

## Abbreviations

ADIPOR2 (adiponectin receptor 2); FTO (fat mass and obesity associated gene); PPAR (peroxisome proliferator activated receptor); IL-6 (interleukin 6); SAT (subcutaneous adipose tissue); VAT (visceral adipose tissue); HMW(adiponectin, high molecular weight adiponectin); CRP (c-reactive protein); TNF- $\alpha$ , (tumor necrosis factor); TNF-RI (tumour necrosis factor receptor I); TNF-RII (tumour necrosis factor receptor II).

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