Regulation and Function of *FTO* mRNA Expression in Human Skeletal Muscle and Subcutaneous Adipose Tissue

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OBJECTIVE—Common variants in *FTO* (the fat mass– and obesity-associated gene) associate with obesity and type 2 diabetes. The regulation and biological function of *FTO* mRNA expression in target tissue is unknown. We investigated the genetic and nongenetic regulation of *FTO* mRNA in skeletal muscle and adipose tissue and their influence on in vivo glucose and fat metabolism.

RESEARCH DESIGN AND METHODS—The *FTO* rs9939609 polymorphism was genotyped in two twin cohorts: *1*) 298 elderly twins aged 62–83 years with glucose tolerance ranging from normal to type 2 diabetes and *2*) 196 young (25–32 years) and elderly (58–66 years) nondiabetic twins examined by a hyperinsulinemic-euglycemic clamp including indirect calorimetry. *FTO* mRNA expression was determined in subcutaneous adipose tissue (n = 226) and skeletal muscle biopsies (n = 158).

RESULTS—Heritability of *FTO* expression in both tissues was low, and *FTO* expression was not influenced by *FTO* rs9939609 genotype. *FTO* mRNA expression in skeletal muscle was regulated by age and sex, whereas age and BMI were predictors of adipose tissue *FTO* mRNA expression. *FTO* mRNA expression in adipose tissue was associated with an atherogenic lipid profile. In skeletal muscle, *FTO* mRNA expression was negatively associated to fat and positively to glucose oxidation rates as well as positively correlated with expression of genes involved in oxidative phosphorylation including *PGC1α*.

CONCLUSIONS—The heritability of *FTO* expression in adipose tissue and skeletal muscle is low and not influenced by obesity-associated *FTO* genotype. The age-dependent decline in *FTO* expression is associated with peripheral defects of glucose and fat metabolism. ***Diabetes*** **58:2402–2408, 2009**

Obesity and type 2 diabetes are heterogenous disorders caused by both nongenetic and genetic components. Recent studies identified common variants in *FTO* (the fat mass– and obesity-associated gene) to be associated with obesity and type 2 diabetes in humans (1–5). The *FTO* genotype is suggested to be involved in the regulation of appetite and body weight (2,6–8) and may regulate transcription of genes involved in metabolism by nucleic acid demethylation (6). In addition, we demonstrated an association between the *FTO* genotype on one hand and increased energy efficiency and elevated blood glucose on the other, suggesting peripheral effects of *FTO* (9). A recent paper demonstrated that inactivation of the *FTO* gene protects from obesity in mice and supported the idea of *FTO* being involved in peripheral energy homeostasis, mitochondrial coupling, and/or substrate cycling (10). Thus, the association between *FTO* and obesity may not only involve control of appetite. Indeed, skeletal muscle and adipose tissue are important organs in the pathogenesis of obesity, insulin resistance, and type 2 diabetes.

Few studies have investigated the mRNA expression levels and biological function of *FTO* in human adipose tissue. In one study, expression levels of *FTO* mRNA was threefold higher in human subcutaneous adipose tissue (SAT) than in visceral adipose tissue (VAT) (11), whereas *FTO* expression decreased during adipocyte differentiation and was similar in SAT and VAT in another study (12). In our recent study, we were unable to document any impact of the *FTO* genotype on the expression of *FTO* in skeletal muscle (9). However, the number of subjects in this study was limited, and we are unaware of other studies that have investigated the *FTO* expression in human skeletal muscle and its putative peripheral role in glucose metabolism. The aim of the present study was to investigate the genetic versus nongenetic regulation of *FTO* mRNA expression in skeletal muscle and SAT and to study the impact of tissue *FTO* mRNA levels on obesity and insulin resistance in two twin cohorts.

RESEARCH DESIGN AND METHODS

The *FTO* rs9939609 polymorphism was genotyped in two cohorts of Danish twins: *1*) a population-based cohort of 298 elderly twins (mean age 74 ± 5 years) and *2*) a cohort of 110 young (age 28 ± 2 years) and 86 elderly (age 62 ± 2 years) twins without known type 2 diabetes. The subjects were identified through the Danish Twin Register, and all procedures were performed according to the Declaration of Helsinki and approved by the scientific ethics committees for the counties of Funen and Vejle. The recruitment and details of selection of cohorts 1 and 2 are previously described (13–16). In cohort 1, 57% were normal glucose tolerant (NGT), 27% had impaired glucose tolerance (IGT), and 16% had type 2 diabetes. In cohort 2, 74% had NGT, 22.0% had IGT, and 4% had previously unknown type 2 diabetes among elderly twins. Among the young twins, 98% had NGT and 2% had IGT.

Clinical examinations. In cohort 1 the subjects were examined with a standardized 75-g oral glucose tolerance test (OGTT) and BMI and waist-to-hip ratio (WHR) were measured as previously described (15,16). In addition, a SAT biopsy was taken from the abdomen under local anesthesia using a Bergstrom needle, and the tissue was immediately frozen in liquid nitrogen and stored at −80°C. We were able to obtain adipose tissue biopsies and measure mRNA *FTO* expression in a subgroup of 226 twins.

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TABLE 1
Subject characteristics

	Cohort 1	Cohort 2	
	Elderly	Young	Elderly
n (men/women)	226 (103/123)	110 (60/50)	86 (38/48)
n (MZ/DZ)	226 (92/134)	110 (66/44)	86 (42/44)
Age (years)	73.4 ± 5.3	28 ± 2	62 ± 2
BMI (kg/m^2)	26.1 ± 3.7	24.1 ± 3.1	26.1 ± 4.5
WHR	0.90 ± 0.1	0.84 ± 0.08	0.89 ± 0.1
Total fat (%)	NA	22.1 ± 7.0	27.9 ± 9.4
Vo_{2max} (ml · kg^{-1} · min^{-1})	NA	39.6 ± 7.8	26.3 ± 7.0
Basal GOX (mg · kg FFM^{-1} · min^{-1})	NA	114 ± 55	90 ± 52
Insulin-stimulated GOX (mg · kg FFM^{-1} · min^{-1})	NA	246 ± 24	200 ± 28
Basal FOX (mg · kg FFM^{-1} · min^{-1})	NA	69 ± 26	70 ± 24
Insulin-stimulated FOX (mg · kg FFM^{-1} · min^{-1})	NA	20 ± 24	62 ± 24
Insulin sensitivity (Rd) (mg · kg FFM^{-1} · min^{-1})	NA	11.7 ± 1.9	9.9 ± 3.3

Data are expressed as means ± SD. NA, not appilicable; FFM, fat-free mass; FOX, fat oxidation; GOX, glucose oxidation.

In cohort 2 the subjects underwent a 2-day clinical examination as described previously (13,17). Day 1 included an OGTT, anthropometric measures, and a dual-energy X-ray absorptiometry (DEXA) scan to determine body composition, and physical fitness was estimated as VO_{2max} calculated from the maximal load on ergometer bicycle (18). On day 2, a 2-h hyperinsulinemic-euglycemic clamp (40 mU/m^2 per min) with tritiated glucose preceded by a 30-min intravenous glucose tolerance test was performed. Indirect calorimetry was performed during both the basal and insulin-stimulated steady-state period using a computerized flow-through canopy gas analyzer system (Deltarac; Datex, Helsinki, Finland). After an equilibrium period of 10 min, the average gas exchange rates were used to calculate the basal and insulin-stimulated fat and glucose oxidation rates (19,20). The respiratory coefficient, which is the quotient of CO_2 production and O_2 consumption, reflecting the macronutrient being oxidized, was obtained from the calorimetry data. Before and after the hyperinsulinemic-euglycemic clamp, skeletal muscle biopsies were excised from the vastus lateralis muscle under local anesthesia using a Bergstrom needle, and the tissue was immediately frozen in liquid nitrogen and stored at −80°C. We were able to measure *FTO* mRNA expression in 159 basal and 158 insulin-stimulated skeletal muscle biopsies.

Analytical methods and calculations. Plasma glucose and insulin concentrations during the OGTT and the hyperinsulinemic-euglycemic clamp were analyzed as previously described (15,21). The fasting blood samples were analyzed for serum triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol as previously described (22). Homeostasis model assessment–insulin resistance was calculated as [(fasting plasma insulin × fasting plasma glucose)/22.5] × 0.144 (23). Calculation of hepatic glucose production, glucose disposal, and insulin secretion has been described previously (13,17). Glucose and fat oxidation rates were calculated with data obtained from the indirect calorimetry using the following equations: glucose; 4.55 V_{CO2} (L/min) – 3.21V_{O2} (L/min) – 2.87 n (g/min) and fat; 1.67 Vo_2 (L/min) – 1.67 Vco_2 (L/min) – 1.92 n (g/min), where n is nitrogen secreted in the urine (19). Glucose and lipid oxidation data are expressed as milligrams per kilograms of fat-free mass per minute.

Measurement of gene expression using real-time RT-PCR. Total RNA was extracted from frozen skeletal muscle and adipose tissue samples using the Tri Reagent kit according to the manufacturer's instructions (Sigma-Aldrich). cDNA was synthesized using the QuantiTect Reverse Transcription kit (Qiagen). *FTO* mRNA levels were quantified using TaqMan Real-Time PCR with an ABI 7900 system (Applied Biosystems) and an Assay-on-demand (Hs01057145_m1) covering exon 8–9. All samples were run in duplicates and data were calculated using the standard curve method and normalized to the mRNA level of cyclophilin A (4326316E, Applied Biosystems). An additional endogenous control gene, hypoxanthine-guanine phosphoribosyltransferase (4333768E, Applied Biosystems), was initially analyzed in the skeletal muscle biopsies. The expression of cyclophilin A as well as the ratio between cyclophilin A and hypoxanthine-guanine phosphoribosyltransferase were compared, and there was no effect of a number of variables tested (i.e., age or insulin), which is why only one control gene (cyclophilin A) was subsequently used for the statistical analysis of genetic and q-traits. Expression of three *OXPHOX* genes, *PGC1-α*, and *GLUT4* in vastus lateralis were previously performed with assays from Applied Biosystems: *NDUFB6* (Hs00159583), *UQCRB* (Hs00559884), ATP50 (Hs00426889), *PGC-1α* (Hs00173304), and GLUT4 (Hs00168966) (24,25).

Genotyping. The *FTO* polymorphism rs9939609 was genotyped using Taqman allelic discrimination (KBioscience, Hertz, U.K.) and obeyed Hardy-Weinberg equilibrium in both cohort 1 ($P = 0.44$) and cohort 2 ($P = 0.99$). The overall genotyping success rate was 97.4%. The minor allele frequency for *FTO* rs9939609 was 37.3% (33.3;41.3) and 39.1% (34.2;43.9) in cohorts 1 and 2, respectively.

Statistical methods. Comparison of *FTO* mRNA expression between young and elderly twins and comparison between *FTO* genotype and *FTO* mRNA expression were done by a mixed-effects regression model. Correlation analyses were made using the Pearson's correlation coefficient test with ln-transformed data. Multiple regression analyses were performed to test the influence of potential factors on *FTO* mRNA expression in adipose tissue and skeletal muscle and to test the impact of *FTO* mRNA expression on in vivo metabolism. In all the multiple regression analyses, the outcome variable was ln transformed. We tested the influence of the following variables on *FTO* mRNA expression in skeletal muscle: age (elderly [1] or young [2]), sex (men [1] or women [2]), zygosity status (MZ [monozygotic] [1] or DZ [dizygotic] [2]), total fat percentages (continuous percent), and total body aerobic capacity Vo_{2max} (continuous [ml · kg^{-1} · min^{-1}]) and in adipose tissue: age (continuous years), sex (men [1] or women [2]), zygosity status (MZ [1] or DZ [2]), and BMI (continuous [kg/m^2]). Both models took into account that the observations within twins cannot be assumed to be independent and that the dependency effects are different for MZ and DZ twin pairs. Thus, the full models include a random-effects term for twin pair membership and a fixed-effects term for zygosity. Because MZ twins have identical genotypes, any differences are theoretically because of environmental factors, whereas DZ twins, on average, share 50% of their genes. The extent to which MZ twins are more alike than DZ twins is presumed to reflect a genetic influence on the phenotype in question. Heritability (expressed as h^2) gives the proportion of the total variation of a trait attributable to genetic variation and can be estimated by comparing the similarity (i.e., intraclass correlations) of a given phenotype between MZ and DZ twin pairs. Statistical comparisons of intraclass correlations were made after transformation using the Fisher z transformation. The heritability is expressed as twice the difference of the intraclass correlation of MZ and DZ twins $h^2 = 2(r\text{MZ} - r\text{DZ})$ (26). The designation of a member in a twin pair is arbitrary; i.e., there is no consistency in which of the twins in a pair is assigned A and which is assigned B. The correlation coefficient may differ dependent on the assignment of the twins, and randomization is required. To avoid the randomization procedure, intrapair correlations were calculated using $2n$, as previously recommended (27). All analyses were carried out in SAS (version 9.1; SAS Institute); $P \leq 0.05$ was considered significant.

RESULTS

The clinical characteristics of the two cohorts are shown in Table 1. The A-allele of *FTO* rs9939609 was associated to measures of obesity, namely BMI (TT: 25.2 ± 0.3, AT: 26.1 ± 0.1, AA: 27.1 ± 0.6; $P = 0.03$) in cohort 1 and WHR in cohort 1 (TT: 0.87 ± 0.01, AT: 0.87 ± 0.01, AA: 0.91 ± 0.01; $P = 0.004$) and in the younger twins in cohort 2 (TT: 0.84 ± 0.02, AT: 0.81 ± 0.01, AA: 0.91 ± 0.02; $P = 0.004$). No associations between rs9939609 and either BMI ($P = 0.93$) or WHR ($P = 0.92$) were observed in the elderly twins in cohort 2. Furthermore, the A-allele of *FTO* rs9939609 was associated with reduced insulin-stimulated

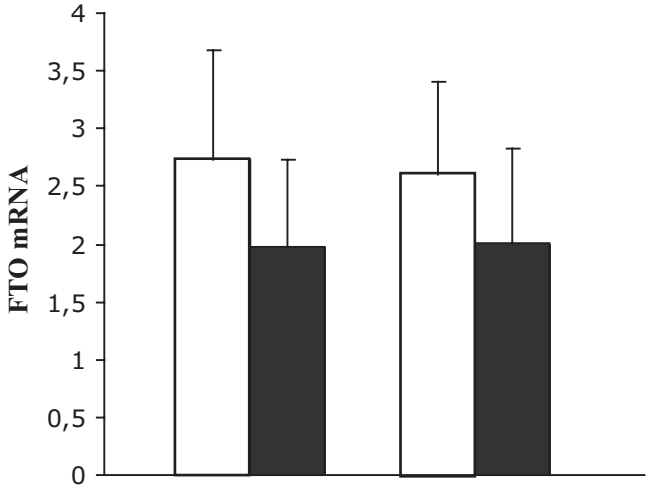

FIG. 1. *FTO* mRNA expression in skeletal muscle obtained before and after insulin stimulation in young (□) and elderly (■) twins. Results are expressed as means ± SD; mRNA levels were quantified with real-time PCR and normalized to the level of *Cyclophilin A*. After adjusting for intratwin pair relationship, $P < 0.0001$ for basal expression (*left panel*) and $P = 0.0004$ for post-clamp expression (*right panel*).

glucose uptake (TT: 12.0 ± 0.6, AT: 11.9 ± 0.5, AA: 9.9 ± 0.8; $P = 0.07$) and nonoxidative glucose metabolism (TT: 8.0 ± 0.6, AT: 7.3 ± 0.5, AA: 5.3 ± 0.7; $P = 0.01$) in the young (and not elderly) twins of cohort 2. However, these associations disappeared after adjustment for BMI. No association between rs9939609 and basal or insulin-stimulated glucose or fat oxidation was observed in either the young or elderly twins.

Young twins had significantly higher muscle *FTO* mRNA levels compared with elderly twins both in the basal state (2.73 ± 0.95 vs. 1.97 ± 0.77, $P < 0.0001$) and after insulin stimulation (2.61 ± 0.80 vs. 2.01 ± 0.82, $P = 0.0004$) (Fig. 1). The expression level of *FTO* mRNA did not change significantly by insulin stimulation in the group of all twins ($P = 0.27$) or in the subgroups of young ($P = 0.12$) and elderly ($P = 0.86$) twins, which is why only the mRNA expression levels during the basal state were used in the subsequent analyses.

Genetic influence on *FTO* expression in skeletal muscle and adipose tissue. We studied the association between *FTO* rs9939609 and *FTO* mRNA expression and found no significant association in either skeletal muscle or in adipose tissue (Table 2). Upon adjustment for age and sex in the analysis the association remained nonsignificant ($P = 0.90$ and $P = 0.28$, respectively). The intraclass correlations for *FTO* expression in skeletal muscle were similar in MZ ($r = 0.72$) and DZ twins ($r = 0.57$) ($P = 0.28$), with a heritability estimate of 0.30. In adipose tissue, the intraclass correlations for *FTO* expression in MZ were $r = 0.30$ and in DZ $r = 0.25$ ($P = 0.83$) with a heritability estimate of 0.10.

Nongenetic predictors of *FTO* mRNA expression

Skeletal muscle. This study aimed to identify nongenetic determinants of *FTO* mRNA expression including obesity and physical fitness, and correlation analyses were performed between *FTO* mRNA in skeletal muscle and total fat percentages ($r = -0.35$, $P < 0.0001$) and Vo_{2max} ($r = 0.41$, $P < 0.0001$). To quantify the independent effect of these variables on *FTO* mRNA expression, multiple regression analyses with the additional inclusion of age, sex, and zygosity were performed. Age ($\exp[\beta] = 0.72$, $P < 0.0001$) and sex ($\exp[\beta] = 1.27$, $P = 0.002$) were the only significant independent predictors of *FTO* mRNA expression in skeletal muscle (Table 3). Thus, being an elderly twin is associated with a 28% decrease in *FTO* mRNA expression and being a male is associated with a 27% increase in *FTO* mRNA expression.

Adipose tissue. Similarly, multiple regression analyses were performed to quantify the effect of age, sex, zygosity, and BMI on the level of *FTO* mRNA expression in adipose tissue. Age ($\exp[\beta] = 0.92$, $P = 0.002$) and BMI ($\exp[\beta] = 1.13$, $P < 0.0001$) were significant independent predictors of *FTO* mRNA expression in adipose tissue. A 1 SD increase in age (5.1 years) results in an 8% decline in *FTO* mRNA and a 1 SD increase in BMI (4.3 kg/m^2) results in a 13% increase in *FTO* mRNA level (Table 4).

Impact of *FTO* mRNA expression on in vivo metabolism

Skeletal muscle. To investigate the relationships between *FTO* mRNA expression and in vivo metabolism, regression analyses were made with *FTO* mRNA expression levels as the explanatory variable. Based on the above findings the analyses were adjusted for age and sex. The expression of *FTO* mRNA had a significant impact on both the basal and insulin-stimulated fat oxidation rate. An increase in *FTO* mRNA level of 1 SD (0.78 AU) was associated with a 12% ($P = 0.04$) decrease in basal fat oxidation and 18% ($P = 0.03$) decrease in insulin-stimulated fat oxidation (Table 5). In addition, an increase of 1 SD in *FTO* expression was associated with a borderline significant increase of 10% ($P = 0.07$) in basal glucose oxidation and with a 1% increase in basal respiratory coefficient ($P = 0.03$) and insulin-stimulated respiratory coefficient ($P = 0.03$) (Table 5). The associations between *FTO* mRNA and fat oxidation remained significant after adjustment for total fat mass and percentage. No influence of skeletal muscle *FTO* mRNA expression on peripheral insulin sensitivity, hepatic glucose production, insulin secretion, energy expenditure, or Vo_{2max} was observed (data not shown). Because of the putative effects on lipid and/or glucose oxidation, we investigated associations between *FTO* mRNA expression and expression of genes involved in mitochondrial function including *OXPHOS* genes and their key regulator *PGC-1α*. Significantly positive correlations between *FTO* expression and the *OXPHOS* genes *NDUFB6* ($r = 0.48$, $P < 0.0001$), *UQCRB* ($r = 0.48$, $P < 0.0001$), *ATP50* ($r = 0.62$, $P < 0.0001$), and the

TABLE 2
Association between *FTO* rs9939608 genotype and *FTO* mRNA expression in skeletal muscle and adipose tissue

Skeletal muscle	*n*	TT	*n*	AT	*n*	AA	*P*
Basal *FTO* expression	60	2.28 ± 0.81	76	2.26 ± 0.82	22	2.51 ± 1.31	0.46
Basal adipose tissue *FTO* expression	77	0.96 ± 0.38	108	0.89 ± 0.38	26	0.96 ± 0.39	0.37

Data are expressed as means ± SD.

TABLE 3
Variables with possible influence on *FTO* mRNA expression in vastus lateralis muscle

	Estimate	95% CI	Percent change	Effect of 1 SD	Percent change	*P*
Basal *FTO* mRNA level						
Age (elderly vs. young)	0.72	(0.64–0.82)	↓ 28%	—	—	<0.0001
Sex (men vs. women)	1.27	(1,12–1.43)	↑ 27%	—	—	0.002
Zygosity (MZ vs. DZ)	1.08	(0.98–1.20)	↑ 8%	—	—	0.11
Total fat (%)	1.00	(0.99–1.01)	—	0.99	↓ 1%	0.82
Vo_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	0.99	(0.99–1.00)	↓ 1%	0.97	↓ 3%	0.42

Model: ln *FTO* = adjusted for age, sex, zygosity, total fat percentages, and Vo_{2max}. SD: adjusted for total fat percentage = 8.65, Vo_{2max} = 9.62. *P* values are adjusted for twin pair and zygosity status.

PPAR-γ coactivator 1-α (*PGC-1α*) ($r = 0.44$, $P < 0.0001$) (Fig. 2) in skeletal muscle were found. Despite no effect of *FTO* expression on whole-body glucose uptake, *FTO* mRNA was significantly and positively correlated to GLUT4 mRNA ($r = 0.46$, $P < 0.0001$).

Adipose tissue. Using univariate analyses we found that *FTO* mRNA was correlated with fasting serum levels of total LDL ($r = 0.17$, $P = 0.009$) and HDL ($r = -0.21$, $P = 0.001$) cholesterol. When adjusted for age and BMI, the observed independent predictors of *FTO* mRNA in adipose tissue in the present study, the association with HDL and LDL cholesterol remained significant. A 1-SD increase in *FTO* mRNA (0.35 AU) was associated with a 12% ($P = 0.02$) decrease in HDL cholesterol levels and a 5% ($P = 0.02$) increase in LDL cholesterol levels. No impact of *FTO* expression in adipose tissue was seen on either glucose tolerance (2-h post-OGTT plasma glucose) or indirect measures of insulin sensitivity (homeostasis model assessment–insulin resistance) (data not shown). Likewise, *FTO* mRNA expression did not differ among twins stratified according to glucose tolerance: NGT ($n = 125$) 0.94 ± 0.36; IGT ($n = 62$) 0.89 ± 0.38; type 2 diabetes ($n = 37$) 0.94 ± 0.30.

DISCUSSION

Variations in *FTO* have been associated with obesity and type 2 diabetes, but the biological functions and regulation of *FTO* mRNA expression in human tissues still remain to be clarified. Contributing to the sparse knowledge of human *FTO* mRNA expression, our key findings of the present study were that *1*) the obesity-associated *FTO* rs9939609 does not influence tissue *FTO* mRNA expression in human skeletal muscle and adipose tissue in the basal state; *2*) heritability of *FTO* mRNA expression is low; *3*) accordingly, *FTO* mRNA expression in the basal state is mainly regulated by nongenetic factors in both skeletal muscle (age and sex) and adipose tissue (age and BMI); and *4*) *FTO* mRNA expression in skeletal muscle correlates with whole-body substrate oxidation rates.

We found no associations between the examined *FTO* genotype and tissue *FTO* mRNA expression, indicating that the *FTO* genotype has no major or direct role in the regulation of *FTO* mRNA in either adipose tissue or skeletal muscle in the present study samples. The lack of association between the *FTO* polymorphism and level of mRNA expression is supported by our observation of a low heritability estimate of *FTO* mRNA expression in both adipose and skeletal muscle tissue. These findings do not, however, exclude that *FTO* genotype may influence the biological function of *FTO* in terms of activity. Previous findings (11,12) are in line with the present, although a recent study indeed has shown an association between *FTO* genotype and mRNA expression in SAT in very obese (BMI ≥40 kg/m^2) subjects (28). Therefore, it cannot be excluded that a potential effect of genotype on tissue *FTO* mRNA expression levels may be unmasked in extreme states of metabolic disease such as severe obesity or upon certain challenges including exercise or dietary interventions. In fact, this was the case in a recent study demonstrating that insulin resistance per se was associated with a markedly reduced expression of *PGC-1α* in skeletal muscle only after an acute exercise bout (29). Moreover, it is possible that the polymorphism analyzed in this study, located in intron 1 of the *FTO* gene, may affect the expression pattern of the splice variants of *FTO*, which may vary in different tissues. The assay used in this study covers exon 8–9 and can detect three different splice variants. However, it does not distinguish between these variants. This question should be addressed in future ad hoc studies.

We showed that *FTO* expression in SAT decreased by 8% for every 5 years increase in age. Opposed to this finding, a study has shown that *FTO* expression in subcutaneous and visceral fat increased with age (11); this

TABLE 4
Variables with possible influence on *FTO* mRNA expression in adipose tissue

	Estimate	95% CI	Percent change	Effect of 1 SD	Percent change	*P*
Basal *FTO* mRNA level						
Age (years)	0.98	(0.97–0.99)	↓ 2%	0.92	↓ 8%	0.002
Sex (men vs. women)	1.09	(0.97–1.21)	↑ 9%	—	—	0.13
Zygosity (MZ vs. DZ)	0.96	(0.85–1.09)	↓ 4%	—	—	0.56
BMI (kg/m^2)	1.03	(1.02–1.04)	↑ 3%	1.13	↑ 13%	<0.0001

Model: ln *FTO* = adjusted for age, sex, zygosity, and BMI. SD: adjusted for age = 5.14, BMI = 4.26. *P* values are adjusted for twin pair and zygosity status.

TABLE 5
The relationship between *FTO* mRNA expression in skeletal muscle and substrate oxidation

	Estimate	95% CI	Percent change	Effect of 1 SD	Percent change	*P*
Basal FOX (mg · kg FFM^{-1} · min^{-1})	0.85	(0.73–1.00)	↓ 15%	0.88	↓ 12%	0.04
Clamp FOX (mg · kg FFM^{-1} · min^{-1})	0.77	(0.61–0.97)	↓ 23%	0.82	↓ 18%	0.03
Basal GOX (mg · kg FFM^{-1} · min^{-1})	1.12	(0.99–1.28)	↑ 12%	1.10	↑ 10%	0.07
Clamp GOX (mg · kg FFM^{-1} · min^{-1})	1.05	(0.97–1.13)	↑ 5%	1.04	↑ 4%	0.21
Basal respiratory coefficient	1.02	(1.00–1.03)	↑ 2%	1.01	↑ 1%	0.03
Clamp respiratory coefficient	1.02	(1.00–1.03)	↑ 2%	1.01	↑ 1%	0.03

Model: Ln dependent variable = *FTO* mRNA adjusted for age and sex. SD: Basal *FTO* mRNA = 0.78. *P* values are adjusted for twin pair and zygosity status. FOX, fat oxidation; GOX, glucose oxidation.

association disappeared, however, after adjustment for BMI. This discrepancy may be because of the narrow age range within the cohort of elderly twins in our study that in addition may limit generalization to younger individuals. Nevertheless, the findings are in line with those in skeletal muscle *FTO* expression, providing some support to a decrease in adipose tissue *FTO* expression with age. The few human studies of *FTO* expression in adipose tissue performed so far have been inconsistent concerning the relationship between obesity and *FTO* tissue expression. We found that the expression of *FTO* in adipose tissue was positively associated with obesity as measured by BMI, supporting some (12,28,30) but not all studies (11). A study in mice showed that *FTO* mRNA expression was 50% lower in adipocytes from *db/db* mice (a genetic model of obesity and type 2 diabetes) compared with wild-type mice (31). Independent of obesity, we demonstrated that increased *FTO* mRNA expression in adipose tissue was associated with an atherogenic serum lipid profile including an increase in circulating levels of LDL cholesterol and a decrease in HDL cholesterol levels. A previous study has demonstrated a borderline correlation between SAT *FTO* mRNA abundance and plasma glycerol, although no association to lipolysis was evident (12). *FTO* expression in adipose tissue was not influenced by glucose tolerance status and was not related to OGTT-derived measures of insulin sensitivity or insulin release. The lack of association between adipose tissue *FTO* expression and glucose metabolism is in accordance with a previous study of 55 Europeans (11).

FTO mRNA abundance in skeletal muscle was reduced by 28% in elderly compared with younger twins and significantly associated with in vivo measures of basal and insulin-stimulated fat oxidation and respiratory coefficient, in addition to a borderline significant association with glucose oxidation rate. These metabolic associations were independent of total fat mass and percentage, and collectively they point toward a shift from glucose to fat oxidation with an age-related decrease in *FTO* muscle expression. Because we previously demonstrated an age-related reduction in the mRNA expression of genes involved in oxidative phosphorylation (24), present expression data were adjusted for *PGC-1α* expression levels. Interestingly, the association between *FTO* mRNA expression in skeletal muscle and fat oxidation was persistent and independent of *PGC-1α* expression levels during both the basal ($P = 0.08$) state and after insulin stimulation ($P = 0.05$). Therefore, the age-related decline in skeletal muscle *FTO* expression may at least to some extent explain the higher fat oxidation rate among elderly twins.

The association between fat mass and fat oxidation is complex, with low fat oxidation potentially being causally related to risk of accumulating fat on one side and with fat accumulation in obesity potentially leading to increased fat oxidation as a consequence of excess tissue release of fatty acids on the other side. Thus, caution is warranted in the interpretation of cause and effect in these cross-sectional associations. We have recently reported that the obesity-associated A-allele of *FTO* rs9939609 was associated with increased mitochondrial energy efficiency in

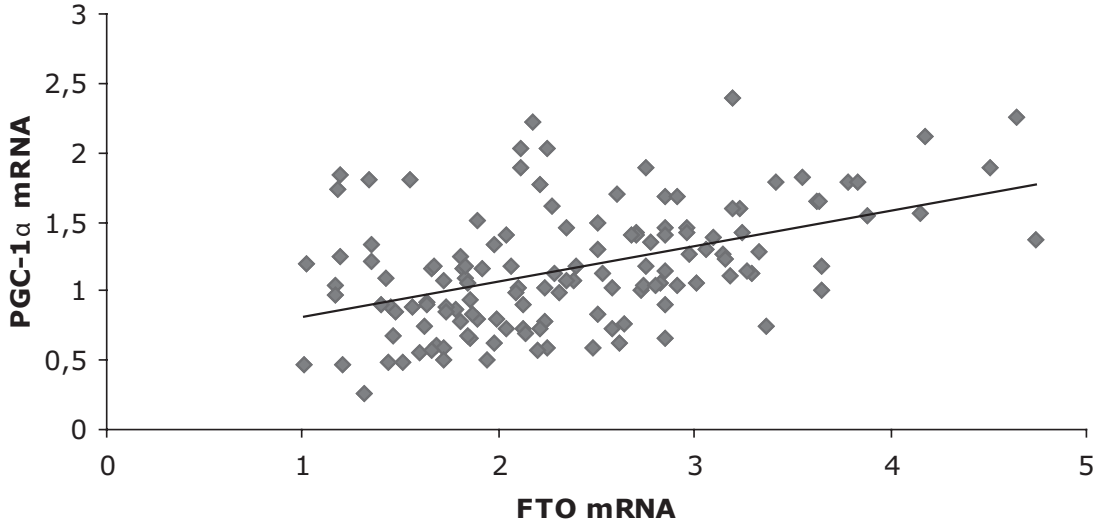

FIG. 2. Correlation between *FTO* mRNA and *PGC-1α* mRNA in skeletal muscle. $r = 0.44$, $P < 0.0001$.

skeletal muscle from young healthy men (9). Furthermore, in accordance with our previous study, we here demonstrate in a large cohort of young and elderly twins that the *FTO* mRNA transcript was associated with the expression of *PGC-1α* and mitochondrial OXPHOS genes (9). The molecular mechanisms and sequences of events linking mitochondrial (dys)function and lipid oxidation to insulin resistance, obesity, and type 2 diabetes are incompletely understood and may involve both accumulation of lipid per se in muscle and liver as well as accumulation of by-products because of incomplete fat oxidation (32,33). Lipid intermediates such as fatty acyl CoA and diacylglycerol can inhibit insulin signaling and reduce glucose uptake, leading to insulin resistance (32,34).

In conclusion, the age-dependent decline in *FTO* expression in adipose tissue and skeletal muscle is associated with an atherogenic serum lipid profile, as well as with a shift in in vivo fat and glucose oxidation independent of obesity and gene expression levels of key metabolic regulators including *PGC-1α*. *FTO* expression in adipose tissue and skeletal muscle may therefore play a role in the development of some components of the metabolic syndrome, including dyslipidemia and peripheral metabolic regulation.

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