Low Physical Activity Accentuates the Effect of the *FTO* rs9939609 Polymorphism on Body Fat Accumulation

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OBJECTIVE—Three independent studies have shown that variation in the fat mass and obesity-associated (*FTO*) gene associates with BMI and obesity. In the present study, the effect of *FTO* variation on metabolic traits including obesity, type 2 diabetes, and related quantitative phenotypes was examined.

RESEARCH DESIGN AND METHODS—The *FTO* rs9939609 polymorphism was genotyped in a total of 17,508 Danes from five different study groups.

RESULTS—In studies of 3,856 type 2 diabetic case subjects and 4,861 normal glucose-tolerant control subjects, the minor A-allele of rs9939609 associated with type 2 diabetes (odds ratio 1.13 [95% CI 1.06–1.20], $P = 9 \times 10^{-5}$). This association was abolished when adjusting for BMI (1.06 [0.97–1.16], $P = 0.2$). Among 17,162 middle-aged Danes, the A-allele associated with overweight (1.19 [1.13–1.24], $P = 1 \times 10^{-12}$) and obesity (1.27 [1.20–1.34], $P = 2 \times 10^{-16}$). Furthermore, obesity-related quantitative traits such as body weight, waist circumference, fat mass, and fasting serum leptin levels were significantly elevated in A-allele carriers. An interaction between the *FTO* rs9939609 genotype and physical activity ($P = 0.007$) was found, where physically inactive homozygous risk A-allele carriers had a 1.95 ± 0.3 kg/m^2 increase in BMI compared with homozygous T-allele carriers.

CONCLUSIONS—We validate that variation in *FTO* is associated with type 2 diabetes when not adjusted for BMI and with an overall increase in body fat mass. Furthermore, low physical activity seems to accentuate the effect of *FTO* rs9939609 on body fat accumulation. ***Diabetes* 57:95–101, 2008**

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BIGTT, β-cell function, insulin sensitivity, and glucose tolerance testing; BIGTT-AIR, BIGTT acute insulin response; BIGTT-S_I, BIGTT insulin sensitivity index; SDC, Steno Diabetes Center; SNP, single-nucleotide polymorphism.

Worldwide incidence of obesity has increased dramatically and is today one of the leading causes of lifestyle-related disorders such as type 2 diabetes and premature cardiovascular disease. Association between common forms of obesity and genes such as *GAD2* (1), *ENPP1* (2), and *INSIG2* (3) have been reported but difficult to validate (4–6). Recently, variation in the fat mass and obesity-associated (*FTO*) gene was reported to associate with type 2 diabetes and increased fat mass. As a part of the Wellcome Trust Case Control Consortium genome-wide association study, which included 1,924 U.K. type 2 diabetic patients and 2,938 U.K. normoglycemic control subjects, an *FTO* variant (rs9939609) was found to associate with type 2 diabetes; however, this association abolished following adjustment for BMI (7). Subsequently, an association with overweight and obesity was demonstrated in seven population-based study samples comprising a total of 19,424 white European adults and two birth cohorts including 10,172 white European children. Moreover, evidence was presented that the increase in BMI resulted from an overall increase in body fat, evaluated by waist circumference and fat mass estimates, including skinfold measures (7).

In another independent study, the effect of 48 neutral single-nucleotide polymorphisms (SNPs) on obesity was tested in 2,900 obese and 5,100 control subjects of European ancestry, and the *FTO* rs1121980 polymorphism, also located in the first intron of *FTO*, was strongly associated with morbid obesity (BMI ≥40 kg/m^2). By selecting HapMap tag SNPs, this association was further replicated in four different European study samples comprising 2,081 obese and 2,783 nonobese subjects of various ages (8). A third independent study showed that the *FTO* rs9930506 variant and a cluster of nearby SNPs, including rs9939609, were strongly associated with BMI, hip circumference, and body weight in 6,148 individuals from Sardinia. This finding was replicated in different study groups comprising a total of 3,467 individuals of different ethnicities (9).

The function of the *FTO* gene product and the involved biological pathways are as yet unknown, but gene expression profiles show that *FTO* is expressed in several tissues, especially specific parts of the brain, and in muscle (7,8). Here, we investigate the effect of *FTO* variation on obesity, type 2 diabetes, and related metabolic quantitative traits in large study samples of Danes.

RESEARCH DESIGN AND METHODS

The *FTO* rs9939609, rs8050136, and rs7193144 polymorphisms were genotyped in 17,508 Danes comprising five study groups: *1*) the population-based Inter99 study sample (clinical trial reg. no. NCT00289237, clinicaltrials.gov) (n =

6,104), sampled at the Research Centre for Prevention and Health (10); *2*) unrelated type 2 diabetic patients (n = 2,015), sampled through the outpatient clinic at Steno Diabetes Center (SDC) (hereafter referred to as the SDC type 2 diabetes study group); *3*) a population-based group of unrelated middle-aged glucose-tolerant subjects (n = 661) examined at SDC (the SDC control group); *4*) the ADDITION (Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen Detected Diabetes in Primary Care) study cohort (clinical trial reg. no. NCT00237548) (n = 8,382) sampled by the Department of General Practice at the University of Aarhus, Aarhus, Denmark (11); and *5*) a population-based sample of young healthy Danish Caucasians (n = 346) recruited from the Research Centre for Prevention and Health (12). All participants in the Inter99 and SDC control group study samples underwent a standard 75-g oral glucose tolerance test, and all participants in the young study group (group 5) underwent a tolbutamide-modified intravenous glucose tolerance test (12).

All study participants were Danes by self-report. Informed written consent was obtained from all subjects before participation. The studies were approved by the regional ethical committees and were in accordance with the principles of the Declaration of Helsinki. Type 2 diabetes, impaired glucose tolerance, impaired fasting glycemia, and normal glucose tolerance were defined according to the World Health Organization (13). Overweight and obesity were defined as BMI ≥25 and ≥30 kg/m^2, respectively. Interaction studies with physical activity and insulin sensitivity were performed in study group 1, including subjects with normal glucose tolerance, impaired fasting glycemia, impaired glucose tolerance, and screen-detected diabetes. Further information on the study groups can be found in an online appendix (available at http://dx.doi.org/10.2337/db07-0910).

Biochemical and anthropometrical measurements. Obesity-related measures, fasting serum lipids, plasma glucose, and serum insulin were measured as described (10–12,14–16). Further information can be found in the online appendix. The level of physical activity was self-reported by questionnaire (14) and divided into categories as physically passive, light or medium physically active, and hard or very hard physically active. The BIGTT insulin sensitivity index (BIGTT-S_i) and the BIGTT acute insulin response (BIGTT-AIR) were calculated as described (15).

Genotyping. The *FTO* rs9939609, rs8050136, and rs7193144 polymorphisms were genotyped using Taqman allelic discrimination (KBioscience, Herts, U.K.). Discordances between 1,464 random duplicate samples were 0.27, 0.14, and 0.14%, respectively, and the genotyping success rates were 97.4, 97.8, and 97.4%, respectively. All genotype groups obeyed Hardy-Weinberg equilibrium.

Statistical analyses. Fisher's exact test was applied to examine differences in genotype distributions and allele frequencies between affected and unaffected subjects, and logistic regression was used, assuming an additive model, when adjustments for sex, age, and BMI were introduced. A general linear model was used to test quantitative variables for differences between genotype groups, assuming an additive, dominant, or recessive model. Adjustment for sex, age, and BMI was applied when appropriate. The Benjamini and Hochberg method was used to correct for multiple testing, considering both the number of traits and the genetic models tested. Correction for multiple testing was performed separately in the two studies of quantitative traits. Linear models extended with environmental parameters were used to test for interaction using an ANOVA test, assuming an additive model. BIGTT-S_i was included as a covariate, while physical activity and glucose tolerance status were treated as categorical variables. A weighted analysis of the interactions was also performed, wherein variance of BMI in the subgroups of physical activity and insulin sensitivity was estimated from the residuals of a linear model with sex and age included. All analyses were performed with RGui, version 2.5.0 (http://www.r-project.org). P values <0.05 were considered significant.

RESULTS

Due to near-perfect linkage disequilibrium between the three genotyped SNPs (mean r^2 = 0.99), we excluded rs8050136 and rs7193144 from further analyses. The overall minor allele frequency for *FTO* rs9939609 was 41.6%.

We validated the previous observation of a strong unadjusted association between the *FTO* rs9939609 A-allele and type 2 diabetes (odds ratio 1.13 [95% CI 1.06–1.20]) (Table 1). The association between rs9939609 and type 2 diabetes was abolished when adjusting for BMI (1.06 [0.97–1.16], P_{additive} = 0.2). The A-allele was associated with overweight and obesity in the population-based Inter99 study sample, the ADDITION study cohort, and the

TABLE 1
Association study of type 2 diabetes, overweight, and obesity

	n (men/women)	TT	TA	AA	MAF (95% CI)	P_{GD}	P_{Add}*	P_{Add}†	P_{AF}	OR (95% CI)
NGT	4,861 (2,259/2,602)	1,676 (35)	2,391 (49)	794 (16)	40.9 (39.9–41.9)					
Type 2 diabetes	3,856 (2,286/1,567)	1,210 (31)	1,907 (50)	739 (19)	43.9 (42.8–45.0)	3×10^{-4}	1×10^{-4}	0.2	9×10^{-5}	1.13 (1.06–1.20)
BMI (kg/m^2)										
<25	5,148 (2,155/2,993)	1,901 (37)	2,525 (49)	722 (14)	38.5 (37.6–39.5)					
≥25	12,014 (6,951/5,063)	3,945 (33)	5,888 (49)	2,181 (18)	42.7 (42.0–43.3)	1×10^{-12}			1×10^{-12}	1.19 (1.13–1.24)
≥30	4,867 (2,506/2,361)	1,510 (31)	2,406 (49)	951 (20)	44.3 (43.3–45.3)	3×10^{-16}			2×10^{-16}	1.27 (1.20–1.34)

Data are number of subjects, divided into genotype groups (% in each group), and percentage frequency of the minor A-allele (major allele frequency [MAF]) (95% CI) unless otherwise indicated. Fisher's exact test was used to compare genotype distribution (P_{GD}) and allele frequency (P_{AF}). Logistic regression was used assuming a log-additive model (P_{Add}), with adjustments for *sex and age (OR 1.17 [95% CI 1.08–1.26] and for †sex, age, and BMI (1.06 [0.97–1.16]). Association with overweight and obesity was determined comparing subjects with BMI <25 and ≥25 kg/m^2 and subjects with BMI <25 and ≥30 kg/m^2, respectively.

TABLE 2
Anthropometric and metabolic characteristics of 5,722 treatment-naive Danish individuals from the population-based Inter99 study sample, stratified according to *FTO* rs9939609 genotype

	TT	TA	AA	P_{Add}	P_{Dom}	P_{Rec}
n (men/women)	1,977 (969/1,008)	2,783 (1,423/1,360)	962 (461/501)			
Age (years)	46.2 ± 8	45.9 ± 8	46.5 ± 8			
Obesity-related measures						
BMI (kg/m^2)	25.9 ± 7.9	26.2 ± 4.6	27.0 ± 4.9	1×10^{-9}*	2×10^{-5}*	4×10^{-9}*
Height (m)	1.72 ± 0.9	1.73 ± 0.9	1.72 ± 0.9	0.6	0.7	0.6
Body weight (kg)	76.9 ± 15.2	78.2 ± 16.0	80.3 ± 17.2	2×10^{-9}*	3×10^{-5}*	4×10^{-9}*
Waist circumference (cm)	85.6 ± 12.8	86.6 ± 13.3	87.9 ± 13.7	1×10^{-7}*	8×10^{-5}*	2×10^{-6}*
Waist-to-hip ratio	0.85 ± 0.09	0.86 ± 0.09	0.86 ± 0.09	0.03	0.03	0.2
Fasting serum lipids (mmol/l)						
Triglyceride	1.0 (0.8–1.5)	1.1 (0.8–1.5)	1.1 (0.8–1.6)	0.9	0.7	0.4
Total cholesterol	5.6 ± 1.1	5.5 ± 1.1	5.6 ± 1.0	0.2	0.1	0.6
HDL cholesterol	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.8	0.5	0.2
Plasma glucose (mmol/l)						
Fasting	5.5 ± 0.8	5.5 ± 0.8	5.6 ± 0.8	0.1	0.03	0.5
30 min	8.7 ± 1.9	8.7 ± 1.9	8.7 ± 1.9	0.2	0.3	0.4
120 min	6.2 ± 2.0	6.2 ± 2.2	6.4 ± 2.1	0.9	0.6	0.4
Serum insulin (pmol/l)						
Fasting	33 (23–49)	35 (24–52)	35 (24–52)	0.3	0.9	0.1
30 min	239 (172–340)	247 (177–358)	253 (179–368)	0.8	0.9	0.5
120 min	157 (101–254)	152 (91–249)	166 (101–277)	0.1	0.03	0.9
Derived indices						
BIGTT-S_i	9.3 (6.5–12.2)	9.2 (6.4–12.1)	8.8 (5.6–11.7)	0.004*	0.06	0.003*
BIGTT-AIR	1,585 (12.61–20.16)	1,639 (12.88–21.12)	1,663 (13.20–21.46)	0.001*	0.005*	0.02
HOMA-IR	8.1 (5.5–12.7)	8.4 (5.7–12.9)	8.6 (5.8–13.4)	0.2	0.8	0.1

Data are means ± SD or median (interquartile range). Values of serum insulin, values derived from insulin variables, and values of serum triglyceride were logarithmically transformed before statistical analysis. All analyses were made using additive (Add), dominant (Dom), and recessive (Rec) models. Plasma glucose and serum insulin were measured in the fasting state and after an OGTT. Calculated *P* values were adjusted for age and sex for obesity-related measures; sex, age, and BMI for serum lipids, serum insulin, plasma glucose, and homeostasis model assessment of insulin resistance (HOMA-IR); and age for the BIGTT-S_i and BIGTT-AIR indices. Homeostasis model assessment of insulin resistance was calculated as fasting plasma glucose (mmol/l) × fasting serum insulin (pmol/l)/22.5. BIGTT-S_i and BIGTT-AIR were calculated as previously described (ref. 15). *P value remained significant after Benjamini and Hochberg correction.

TABLE 3
Anthropometric and metabolic characteristics of 346 young healthy Danish Caucasians stratified according to *FTO* rs9939609 genotype

	TT	TA	AA	P_{Add}	P_{Dom}	P_{Rec}
n (men/women)	136 (67/69)	160 (79/81)	50 (19/31)			
Age (years)	24.7 ± 4	25.4 ± 4	25.4 ± 3			
Anthropometrics						
BMI (kg/m^2)	22.8 ± 3.7	23.9 ± 3.8	24.4 ± 3.4	0.002*	0.004*	0.05
Height (m)	1.75 ± 0.1	1.74 ± 0.1	1.72 ± 0.1	0.5	0.5	0.7
Body weight (kg)	69.8 ± 13.8	73.0 ± 15.0	73.1 ± 14.0	0.008*	0.01*	0.08
Waist circumference (cm)	75.7 ± 10.2	78.7 ± 10.9	79.4 ± 11.3	0.002*	0.003*	0.04
Fat mass (kg)	13.5 (10.1–18.3)	16.9 (11.4–23.1)	18.0 (14.2–24.3)	0.001*	0.004*	0.02
Lean body mass (kg)	54.5 ± 10.0	55.1 ± 10.4	53.7 ± 9.8	0.2	0.3	0.4
Fat percentage	21.4 ± 6.9	23.8 ± 8.3	26.0 ± 6.4	3×10^{-4}*	7×10^{-4}*	0.02
Birth weight (g)	3,318 ± 621	3,384 ± 509	3,408 ± 617	0.2	0.3	0.4
Birth length (cm)	51.3 ± 3.5	51.7 ± 2.2	51.7 ± 2.4	0.2	0.2	0.4
Ponderal index (kg/m^3)	2.4 ± 0.2	2.4 ± 0.2	2.4 ± 0.2	1	1	1
Fasting serum leptin (pmol/l)	6.5 (3.2–10.7)	6.9 (3.5–13.8)	9.7 (5.4–19.9)	0.003*	0.02	0.01*
Fasting serum lipids (mmol/l)						
Total cholesterol	4.4 ± 0.8	4.5 ± 0.9	4.6 ± 0.9	0.8	0.6	0.9
HDL cholesterol	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	0.1	0.1	0.3
Triglyceride	0.9 (0.7–1.1)	0.8 (0.7–1.2)	1.1 (0.8–1.4)	0.8	0.8	0.3
Insulin and glucose dynamics						
Insulin sensitivity index [$10^{-5} \times$ (min $\times$ pmol/l)$^{-1}$]	13.4 (9.2–19.2)	12.6 (9.0–20.1)	13.6 (7.2–19.7)	0.03	0.1	0.1
Fasting plasma glucose (mmol/l)	5.0 ± 0.5	5.0 ± 0.5	5.0 ± 0.5	1	0.7	0.6
Fasting serum insulin (pmol/l)	30.5 (22.8–46.3)	30.0 (25.0–42.3)	32.0 (25.0–51.5)	0.5	0.6	0.5
Acute serum insulin response [$AUC_{insulin\ (0–8min)} \times$ (min $\times$ pmol/l)]	19.41 (1,106–2,620)	19.93 (1,305–2,848)	19.76 (1,410–3,120)	0.8	1	0.6

Data are means ± SD or median (interquartile range) unless otherwise indicated. Metabolic traits were transformed logarithmically or cubically before statistical analysis. All analyses were made using additive (Add), dominant (Dom), and recessive (Rec) models. Calculated *P* values were adjusted for age and sex for obesity-related measures and for sex, age, and BMI for the remaining metabolic traits. Insulin sensitivity in accordance with a Bergmann minimal model was determined on a tolbutamide-modified intravenous glucose test as previously described (ref. 12). **P* value remained significant after Benjamini and Hochberg correction.

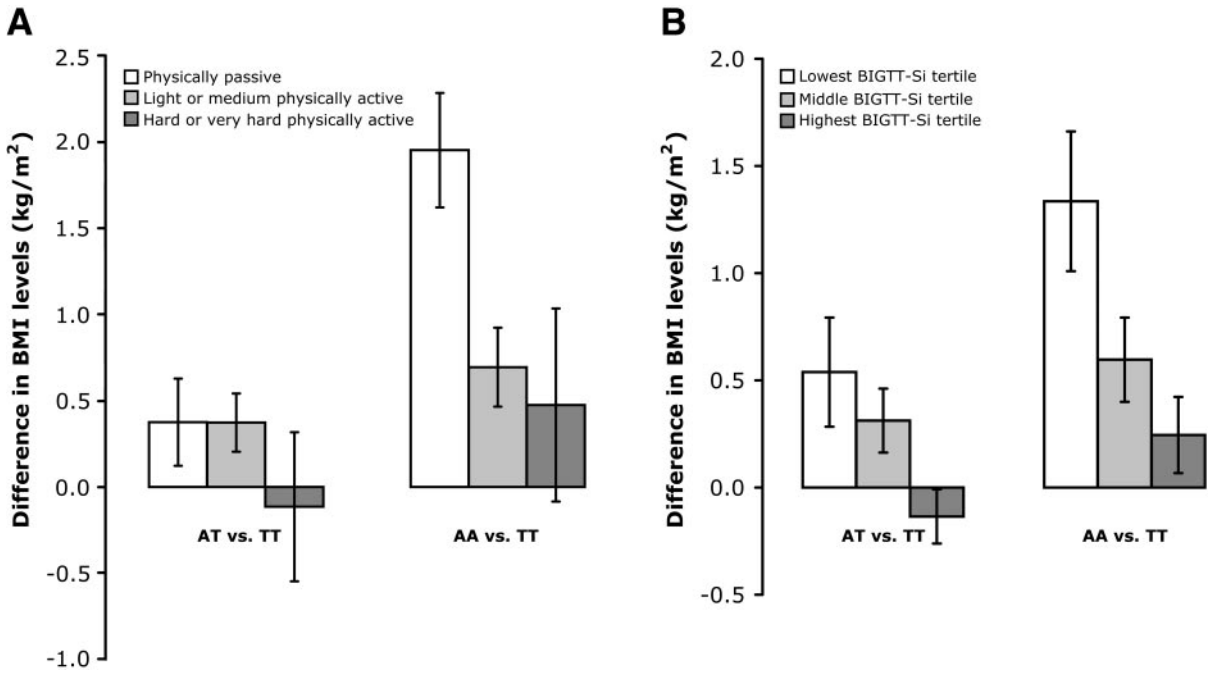

FIG. 1. Effect of physical activity and insulin sensitivity on the impact of the *FTO* rs9939609 genotype on BMI in the population-based Inter99 study sample. *A*: Subjects were divided according to self-reported physical activity and stratified according to the *FTO* rs9939609 genotype. Bars indicate differences in BMI levels between heterozygous and homozygous T-allele carriers and between homozygous A-allele carriers and homozygous T-allele carriers, respectively. We tested for interaction effects using linear models, with or without interaction parameters for physical activity, compared by an ANOVA test ($P_{\text{interaction}} = 0.007$). The number of subjects in each genotype group (TT/TA/AA) was (633/943/338) in the group of physically passive, (1,131/1,572/521) in the group of light or medium physically active, and (152/189/75) in the group of hard or very hard physically active, respectively. *B*: Subjects were divided by insulin sensitivity tertiles, assessed by the BIGTT-S_i (lowest tertile: BIGTT-S_i <7.4; middle tertile: 7.4 ≤ BIGTT-S_i < 11.1; highest tertile: BIGTT-S_i<11.1) and stratified according to the *FTO* rs9939609 genotype. Bars indicate differences in BMI levels between heterozygous and homozygous T-allele carriers and between homozygous A-allele carriers and homozygous T-allele carriers, respectively. We tested for interaction effects using linear models, with or without interaction parameters for BIGTT-S_i, compared by an ANOVA test ($P_{\text{interaction}} = 2 \times 10^{-4}$). The number of subjects in each genotype group (TT/TA/AA) was (557/821/317) in the group with low BIGTT-S_i, (568/833/294) in the group with middle BIGTT-S_i, and (609/834/253) in the group with high BIGTT-S_i.

SDC type 2 diabetes study group separately but not in the SDC control group (supplementary Table B). When combining these four study groups, we found a strong association with both overweight (1.19 [1.13–1.24], $P_{\text{allele frequency}} = 1 \times 10^{-12}$) and obesity (1.27 [1.20–1.34], $P_{\text{allele frequency}} = 2 \times 10^{-16}$) (Table 1).

In the population-based Inter99 study sample, the *FTO* rs9939609 A-allele was highly associated with obesity-related measures including BMI ($P_{\text{additive}} = 1 \times 10^{-9}$), body weight ($P_{\text{additive}} = 2 \times 10^{-9}$), and waist circumference ($P_{\text{additive}} = 1 \times 10^{-7}$). No convincing association with fasting levels of serum triglyceride or cholesterol or with post–oral glucose load levels of serum insulin or plasma glucose were shown. However, decreased BIGTT-S_i ($P_{\text{additive}} = 0.004$) and increased BIGTT-AIR ($P_{\text{additive}} = 0.001$) were observed in homozygous carriers of the *FTO* rs9939609 A-allele (Table 2).

In a study of 346 healthy young Danish whites, the *FTO* rs9939609 A-allele was associated with elevated BMI ($P_{\text{additive}} = 0.002$), body weight ($P_{\text{additive}} = 0.008$), fat mass ($P_{\text{additive}} = 0.001$), body fat percentage ($P_{\text{additive}} = 3 \times 10^{-4}$), and fasting serum leptin concentrations ($P_{\text{additive}} = 0.003$) but not with height or lean body mass. No association with birth weight, birth length, or the ponderal index at birth was observed (Table 3). To ensure the robustness of the quantitative trait analyses, we corrected for multiple testing using the Benjamini and Hochberg method.

The effect of the *FTO* rs9939609 genotype on BMI, body weight, and waist circumference in the population-based Inter99 study sample and the SDC type 2 diabetes study group, stratified according to glucose tolerance status, is shown in supplementary Fig. 1. We found no interaction between glucose tolerance status and the *FTO* rs9939609 genotype effect on BMI, body weight, or waist circumference (data not shown).

We found an interaction between the *FTO* rs9939609 genotype and self-reported physical activity on BMI levels in the population-based Inter99 study sample ($P_{\text{interaction}} = 0.007$). The *FTO* rs9939609 genotype effect on BMI for physically passive, light or medium physically active, and hard or very hard physically active subjects was 0.38, 0.37, and −0.11 kg/m^2, respectively, when comparing homozygous T-allele carriers and heterozygous carriers and 1.95, 0.69, and 0.47 kg/m^2, respectively, when comparing homozygous T-allele carriers and homozygous A-allele carriers (Fig. 1*A*).

Finally, we found an interaction between the *FTO* rs9939609 genotype and measures of insulin sensitivity. The *FTO* rs9939609 genotype effect on BMI in the highest, medium, and lowest insulin sensitivity groups, stratified by BIGTT-S_i tertiles, was −0.14, 0.31, and 0.54 kg/m^2, respectively, between homozygous T-allele carriers and heterozygous carriers, and 0.25, 0.60, and 1.34 kg/m^2, respectively, comparing homozygous T-allele carriers and homozygous A-allele carriers ($P_{\text{interaction}} = 2 \times 10^{-4}$) (Fig. 1*B*). Since the variance of BMI in the different physical activity and insulin sensitivity subgroups was substantial, we also performed a weighted analysis for the interactions. The subgroups were weighted by the reciprocal variance, and the interactions remained significant for both physical activity

($P_{interaction}$ = 0.003) and insulin sensitivity ($P_{interaction}$ = 0.03).

DISCUSSION

In the present study, we validated that *FTO* predisposes to type 2 diabetes. As previously observed (7), this association seems to be mediated by the effect of increased fat mass, since it abolishes when adjusting for BMI. The variant strongly associates with overweight and obesity and with quantitative traits such as BMI, body weight, and waist circumference. Homozygous carriers of the A-allele in the population-based Inter99 study sample weighed on average 3.3 kg (SE 2.1–4.6) more than noncarriers, which is reflected in a BMI increased by 1.1 kg/m^2 (0.7–1.4) and a waist circumference increased by 2.3 cm (1.3–3.3). The association with obesity-related measures was not affected by glucose tolerance status.

BMI is by definition influenced by measures of body weight and height, but in the present study, the *FTO* rs9939609 genotype only affected body weight. BMI is also influenced by lean body mass and fat mass, but in studies of 346 young healthy Danish whites, we only demonstrated an increase in fat mass. Analyses of BMI adjusted for waist circumference remained significant, whereas waist circumference adjusted for BMI did not (data not shown). This indicates that the observed increase in BMI is due to a global increase in fat mass rather than an intra-abdominal fat accumulation.

Finally, the *FTO* rs9939609 genotype was associated with increased fasting serum leptin levels, which are considered a result of increased adiposity. We observed an increase in body weight estimates at all ages except among newborns, which is in accordance with previous findings (7,8), suggesting that body fat accumulation takes place in early childhood.

In the study of 5,722 middle-aged individuals from the population-based Inter99 study sample, we found no differences in postoral glucose load measures of serum insulin or plasma glucose. However, whole-body insulin sensitivity, estimated by the BIGTT-S_i, was significantly decreased in homozygous carriers of the *FTO* rs9939609 risk A-allele. Furthermore, we found that the impact of the *FTO* rs9939609 genotype on BMI levels was highly influenced by insulin sensitivity. We only noticed a modest *FTO* rs9939609–induced increase in BMI levels among participants with a high insulin sensitivity index, whereas low insulin sensitivity index enhanced the genotype effect, particularly among homozygous A-allele carriers.

Thus, this is the first study implying interactions between the *FTO* rs9939609 genotype and insulin sensitivity. *FTO* is ubiquitously expressed, and numerous mechanisms leading to decreased insulin sensitivity exist. Since *FTO* is relatively abundantly expressed in muscle (7), it is feasible that the *FTO* rs9939609 genotype might affect insulin-mediated glucose uptake in muscle. Obviously, this hypothesis needs to be tested experimentally.

Interestingly, we showed that the impact of the *FTO* rs9939609 genotype is influenced by the habitual level of physical activity in the population-based Inter99 study sample. Physical inactivity was associated with a BMI increase of 1.95 ± 0.33 kg/m^2 in homozygous *FTO* rs9939609 A-allele carriers, whereas no major effect of sedentary lifestyle was found comparing noncarriers and those heterozygous for the *FTO* rs9939609 A-allele. Obviously, this finding needs replication in independent study populations to be used in a public health context. Also, since physical activity in our study has been assessed by questionnaire, it would be important to validate the finding with more direct measures of physical activity.

In conclusion, our study validates that variation in *FTO* associates with an overall increase in body fat accumulation as reflected by BMI, body weight, and waist circumference. Moreover, in middle-aged individuals, the *FTO* rs9939609 genotype may be associated with a decrease in estimates of whole-body insulin sensitivity, and in homozygous carriers of the *FTO* A-allele, physical inactivity associates with a relatively large increase in BMI compared with that in noncarriers and those heterozygous for the A-allele.

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