Lack of Association of Fatness-Related FTO Gene Variants with Energy Expenditure or Physical Activity

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Context: A common variant in the first intron of FTO (rs9939609, T/A) is associated with fatness in Caucasians.

Objective: FTO may regulate energy homeostasis through the hypothalamus, and we hypothesized that AA-genotypes of rs9939609 FTO have lower energy expenditure and/or a lower level of physical activity.

Methods: The study population included all obese young men (body mass index $\geq$ 31 kg/m^2) at the mandatory draft board examinations in the Copenhagen area from 1943 to 1977 and a randomly selected control group from this population. Subgroups of 234 obese and 323 controls were examined in 1998–2000 (median age 48 yr). Fat mass (FM), lean body mass (LBM), leisure-time physical activity (LTPA), maximum oxygen uptake (VO_2max), resting energy expenditure (REE), and glucose-induced thermogenesis (GIT) were measured. The *FTO* rs9939609 variant was genotyped. A recessive transmission mode fit the data best. Logistic regression was used to assess the odds ratios of the AA-genotype in relation to LTPA, VO_2max, REE, and GIT.

Results: The AA-genotype of *FTO rs9939609* had higher REE in the age-adjusted model, but the association was eliminated when adjusting for FM and LBM. The AA-genotype was not associated with LTPA, VO_2max, or GIT. This was not influenced by adjustment for age, FM, or LBM. The AA-genotype had increased FM, even with adjustment for age, LBM, REE, GIT, VO_2max, and LTPA. Results were similar for *FTO rs8050136* and *rs7193144.*

Conclusions: Homozygous carriers of the A-allele of *rs9939609 FTO* do not have lower REE, GIT, VO_2max, or LTPA but higher FM, irrespective of LBM, REE, GIT, VO_2max, and LTPA. **(*J Clin Endocrinol Metab* 93: 2904–2908, 2008)**

Recently genome-wide searches for type 2 diabetes susceptibility genes identified a common variant in *FTO* [*rs9939609* T/A; minor A-allele frequency, 0.45 in Caucasians], which predisposes to type 2 diabetes through an effect on body mass index (BMI) (1–4).

The function of *FTO* is unknown, but the gene is expressed particularly in the brain, skeletal muscle, and adipose tissue (1, 2). A recent study found that FTO mRNA was present in a wide range of murine tissues but most abundant in the brain and particularly in the hypothalamic nuclei, in which it may catalyze nucleic acid demethylation (5). *FTO* may thus be associated with fatness through effects on regulation of energy homeostasis in the hypothalamus (6, 7). Subjects with variants in *FTO* may have an altered adjustment or coordination of appetite regulation and energy expenditure, either by a somewhat greater appetite and energy intake or a reduced expenditure.

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Abbreviations: BMI, Body mass index; CI, confidence interval; FM, fat mass; GIT, glucose-induced thermogenesis; LBM, lean body mass; LTPA, leisure-time PA; OGTT, oral glucose tolerance test; OR, odds ratio; PA, physical activity; REE, resting energy expenditure; VO_2max, maximum oxygen uptake.

We hypothesize that the *FTO rs9939609* induces a lowering of the energy expenditure and/or the level of physical activity (PA).

Subjects and Methods

The study population consisted of Caucasian men identified at the mandatory draft board examination in the Copenhagen area from 1943 to 1977 (8, 9). Two cohorts were selected: an obese cohort, including all men with a BMI of 31 kg/m^2 or greater and a control cohort selected as a 1% random sample of all men at the draft board examination. All obese and half the controls who were alive and still residing within the same region were invited to participate in the examination program of the Copenhagen City Heart Study in 1981–1983 and again in 1991–1993. Details of these surveys are published elsewhere (10, 11).

A last examination was conducted in 1998–2000 among subjects who participated in the Copenhagen City Heart Study in 1991–1993, were younger than 65 yr of age and still residing in the same region. Details of the survey are published elsewhere (12, 13). In total, 234 of the obese and 323 of the controls were examined at a median age of 48 yr.

Height (without shoes) and weight (light clothes without shoes) were measured. BMI was calculated as weight per height squared. Fat mass (FM) and lean body mass (LBM) were assessed by dual-energy x-ray absorptiometry (DXA-IQ DEXA; Lunar, Madison, WI).

Energy expenditure was measured before and during an oral glucose tolerance test (OGTT) (13) with indirect calorimetry using a ventilated hood system. The first measurement period was before the OGTT and lasted 35 min. The first 10 min served as equilibration period, and measurements recorded between 10 and 30 min were used in the analysis (e1). The second measurement period started immediately after initiation of the OGTT and lasted 50 min. Measurements recorded between 10 and 45 min were used (e2). The third and fourth measurement periods both lasted 35 min with a break of 25 min in between. Analysis was performed on the measurements recorded between 10 and 30 min (e3, e4). The examination was kept quiet, calm, well ventilated, and at a comfortable temperature. The men rested on a couch but were not allowed to sleep.

The e1 was used as a measure of resting energy expenditure (REE). Glucose-induced thermogenesis (GIT) was estimated as the incremental area under the curve.

Cardiorespiratory fitness, measured as the maximal oxygen uptake (VO_2max), is influenced by the daily PA due to the correlation with high-intensity PA (14). VO_2max was estimated with a progressive bicycling test. After a 5-min conditioning period, the load was increased by 20 W/min until voluntary exhaustion. The men were instructed to pedal at a constant rate of 70 min^{-1}, but the ergometer (Jaeger ER 900; Ergoline Gmbh & Co., Bitz, Germany) adjusted the load to maintain external work effect irrespective of pedaling rate. The ergometer was connected to the indirect calorimetry equipment for breath-by-breath system measurement of CO_2 production and O_2 consumption.

Leisure time physical activity (LTPA) was assessed at this and previous follow-up examinations (13) with a self-administered questionnaire: sedentary, sedentary or light PA less than 2 h/wk; light PA, light PA 2–4 h/wk; moderate PA, more than 4 h/wk of light PA or 2–4 h/wk of more vigorous PA; high PA, more than 4 h/wk of moderate PA or regular heavy exercise/competitive sports several times per week.

Genotyping of the *FTO rs9939609* was performed using Taqman allelic discrimination (KBioscience, Herts, UK). Genotype data were obtained in more than 97% of the DNA samples with a genotype error rate of 0.27% based on 1464 duplicate samples. Genotyping was conducted in 231 obese and 320 controls. The minor A-allele frequency [95% confidence intervals (CIs)] was 0.51 (0.46:0.56) in the obese and 0.41 (0.37:0.45) in the controls (Table 1). All genotype groups were in Hardy-Weinberg equilibrium.

More detailed technical descriptions of the examination methods are available on request.

The *FTO* variants *rs8050136* and *rs7193144* were also genotyped in the cohort (Table 1). The three single-nucleotide polymorphisms were almost perfectly linked (compared with *rs9939609*, two subjects had another genotype according to *rs8050136* and *rs7193144*). The associations between *FTO* and REE, GIT, VO_2max, LTPA, FM, and LBM were similar for all three single-nucleotide polymorphisms, and results are therefore only reported for *FTO rs9939609*.

Statistical analysis

Previous work on *FTO rs9939609* and fatness in this cohort revealed that a recessive transmission mode fitted the data best (AA *vs.* TT and TA) (Kring, S. I. I., C. Holst, E. Zimmermann, T. Jess, T. Berentzen, S. Toubro, T. Hansen, A. Astrup, O. Pedersen, T. I. A. Sørensen, submitted for publication). Therefore, this transmission mode was used in the analyses.

The median (2.5 and 97.5 percentiles) was used to describe distributions of age, BMI, FM, LBM, REE, GIT, and VO_2max in the obese and control group. Distributions of LTPA and the *FTO rs9939609* genotypes were described by frequencies.

To obtain greater statistical power in the regression analyses, the obese and nonobese groups were pooled. Owing to the sampling design with massive enrichment of the right tail of the BMI distribution, we used logistic regression to assess the odds ratios (ORs) of the AA-genotype (*vs.* TT and TA) in relation to REE, GIT, VO_2max, LTPA, FM, and LBM, respectively. All models were first adjusted for age, and then FM and LBM were included in the models with REE, GIT, VO_2max, and LTPA. The statistical power of the study, given the data, is illustrated by the 95% CIs.

Models were tested for presence of first-order interactions between FM and each of the other variables, but none was found. Linearity of continuous variables was tested against a smoothing spline (5 *df*) estimated with general additive models in the logistic regression. Linearity was accepted for all variables.

Analyses were conducted in STATA (version 9.2; Stata Corp., College Station, TX). Significance level was accepted at $P < 0.05$.

Results

The median BMI, FM, LBM, REE, and VO_2max were significantly higher, whereas age, GIT, and level of LTPA were lower in the obese, compared with the controls (Table 1).

GIT, VO_2max, or LTPA was not significantly associated with the AA-genotype of *rs9939609 FTO* in the age-adjusted models. Adjustment for FM and LBM had no notable influence on these estimates (Table 2). Few subjects had a high level of LTPA, and analyses were also conducted with groups 3 and 4 merged, but this had no influence on the results (data not shown). A habitual LTPA index including information on LTPA from all three follow-up surveys was also analyzed, but there was no association with the AA-genotype (data not shown).

Subjects with the AA-genotype had a significantly higher REE in the age-adjusted model, but the association was eliminated when adjusting for FM, LBM, or both (Table 2).

A positive linear association between the AA-genotype and FM was present in the age-adjusted model [OR (95% CI) 1.26 (1.09:1.46) per 10 kg increase in FM, $P = 0.001$], and it persisted after adjustment for LBM [OR (95% CI) 1.26 (1.05:1.52), $P = 0.015$]. Furthermore, adjustment for REE, GIT, VO_2max, or LTPA had no influence on this association (data not shown). The AA-genotype and LBM was linearly associated in the age-adjusted model [OR (95% CI) 1.29 (1.01:1.64) per 10 kg increase in LBM, $P = 0.040$], but the association was eliminated after

TABLE 1. Description of the men according to age, body composition, REE, GIT, VO_2max, and LTPA

	Obese		Controls	
	n	Median (2.5–97.5)	n	Median (2.5–97.5)
Age (yr)[a]	231	47.0 (40.0:62.2)	320	49.5 (40.0:63.0)
BMI (kg/m^2)[a]	231	35.2 (26.9:50.2)	320	25.6 (20.1:34.9)
FM (kg)[a]	224	37.7 (17.3:63.9)	315	18.1 (5.8:41.5)
LBM (kg)[a]	224	71.4 (58.4:86.8)	315	60.8 (49.0:75.7)
REE (MJ)[a]	228	8.9 (6.7:12.3)	317	7.4 (5.8:9.7)
GIT (GJ)[ab]	224	0.7 (−0.4:2.3)	309	0.8 (−0.2:1.8)
VO_2max (liters/min)[a]	183	2.8 (1.7:4.2)	278	2.6 (1.6:3.8)
LTPA [c]	n	Percent	n	Percent
Less than 2 h light PA per week	30	13.3	24	7.7
2–4 h light PA per week	125	55.3	155	49.5
More than 4 h light or 2–4 h moderate PA per week	59	26.1	124	39.6
More than 4 h moderate PA per week	12	5.3	10	3.2
Total	226	100.0	313	100.0
FTO rs9939609[c]				
TT	61	26.4	114	35.6
TA	104	45.0	151	47.2
AA	66	28.6	55	17.2
Total	231	100.0	320	100.0
FTO rs7193144[c]				
TT	55	26.2	93	33.5
TC	92	43.8	136	48.9
CC	63	30.0	49	17.6
Total	210	100.0	278	100.0
FTO rs8050136[c]				
CC	55	26.6	94	33.7
CA	92	44.4	138	49.5
AA	60	29.0	47	16.9
Total	207	100.0	279	100.1

[a] Wald test obese *vs.* controls: $P < 0.001$ except for GIT ($P = 0.185$).

[b] GIT estimated as the incremental area under the curve = [27.5(e1+e2)/2 + (80 − 27.5)(e2+e3)/2 + (140 − 80)(e3+e4)/2] − (140e1).

[c] χ^2 test obese *vs.* controls: LTPA ($P = 0.004$), genotype distribution of *FTO rs9939609* ($P < 0.001$), genotype distribution of *FTO* rs7193144 ($P = 0.005$), genotype distribution of *FTO rs8050136* ($P = 0.005$).

adjustment for FM [OR (95% CI) 1.00 (0.73:1.37), $P = 0.992$]. Adjustment for GIT, REE, VO_2max, and LTPA had no influence on these estimates (data not shown).

Discussion

The present study shows that the AA-genotype of *FTO rs9939609* may not be associated with GIT, VO_2max, or LTPA but confirms that the AA-genotype of *FTO rs9939609* is linearly associated with FM. Adjustment for FM, LBM, or both in the analyses of GIT, VO_2max, and LTPA had no influence on the associations, and adjustment for GIT, VO_2max, LTPA, and LBM had no influence on the association between FM and *FTO rs9939609*. In contrast to our hypothesis, we found a higher REE in subjects with the AA-genotype. This association was, however, completely abolished when adjusting for FM, LBM, or both, which shows that the higher REE can be explained by the larger size of the LBM that is accompanying the increased fatness in the AA-genotypes of *FTO rs9939609*.

Our results on the AA-genotype of *FTO rs9939609* and FM are in accordance with other studies reporting a positive association between the *FTO rs9939609* and fatness in Caucasians (1–4).

Despite the limitations in interpreting the causal directions behind associations observed in a cross-sectional study like ours, our results indicate that the positive association between *FTO rs9939609* and fatness is not explained by an effect of *FTO rs9939609* on REE, GIT, VO_2max, or LTPA. It is, however, possible, as suggested by a recent Danish study (3), that PA modifies the effects of *FTO*, of which our study design does not allow a thorough analysis.

The primary strength of our study is the powerful case-cohort study design conducted in a population of Caucasian men, eliminating population stratification. Although the sample of 231 obese and 320 controls seems rather small, this apparent limitation is counterweighted by the unique sampling design, which implies that the controls represents 64,600 men, identified at the mandatory draft board examination of which the obese group represents the most extreme range of fatness in this population. The 95% CIs delineate the underlying possible true values, which with 95% confidence gave rise to the results. The study sample was sufficient to detect a highly significant association between *FTO*

TABLE 2. OR and 95% CIs for the AA-genotype (*vs.* TA and TT) according to REE, GIT, VO_2max, and LTPA

	OR (95% CI)	*P*
Resting energy expenditure (MJ)		
Adjusted for age	1.17 (1.01:1.35)	0.033
Adjusted for age, FM, and LBM	0.94 (0.70:1.27)	0.712
GIT (GJ)		
Adjusted for age	1.01 (0.71:1.44)	0.948
Adjusted for age, FM, and LBM	1.06 (0.74:1.51)	0.767
VO_2max (liters/min)		
Adjusted for age	1.01 (0.68:1.51)	0.954
Adjusted for age, FM, and LBM	0.90 (0.56:1.44)	0.662
LTPA		
Adjusted for age		
Less than 2 h light PA per week	1.00	0.859[a]
2–4 h light PA per week	0.69 (0.35:1.36)	0.285
More than 4 h light PA per week or 2–4 h moderate PA per week	1.00 (0.50:2.01)	0.991
More than 4 h moderate PA per week	0.45 (0.11:1.74)	0.244
Adjusted for age, FM, and LBM		
Less than 2 h light PA per week	1.00	0.319[a]
2–4 h light PA per week	0.79 (0.39:1.62)	0.525
More than 4 h light PA per week or 2–4 h moderate PA per week	1.32 (0.63:2.76)	0.468
More than 4 h moderate PA per week	0.65 (0.16:1.63)	0.545

[a] Test for trend.

rs9939609 and FM. The 95% CIs of Table 2 shows that we cannot exclude some possible functional associations between *FTO rs9939609* and REE, GIT, VO_2max, or LTPA but speaks for the consistency in finding no associations. A further strength of the study is that FM, LBM, REE, and GIT are assessed by high-quality methods, *i.e.* by dual-energy x-ray absorptiometry (15), ventilated hood systems, and indirect calorimetry. Accurate assessment of PA is a major challenge in free-living humans. Our measure of LTPA is a crude indicator of PA, but previous studies show that the used LTPA measure clearly predicts insulin resistance, impaired glucose tolerance, cardiovascular disease, and mortality (13, 16, 17). Thus, because there was no association between the AA-genotype of *FTO rs9939609* and self-reported LTPA as well as objectively measured VO_2max, we believe that no association between AA-genotype of *FTO rs9939609* and PA exist in our data.

In conclusion, homozygous carriers of the A-allele of *rs9939609 FTO* may not have low REE, GIT, VO_2max, or LTPA, but the variant is linearly associated with fatness, irrespective of REE, GIT, VO_2max, and LTPA in this cohort of Caucasian men.

Ethics

The Danish Data Protection Agency and the regional ethical committee approved the study, which was in accordance with the Helsinki Declaration II. Participants signed a written consent before participating.

Accession numbers

The National Center for Biotechnology Information GenBank (http://www.ncbi.nlm.nih.gov/) accession number for the gene discussed in this paper is *FTO* (NT_010498).

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