

Low physical activity accentuates the effect of the *FTO* rs9939609 polymorphism on body fat accumulation

MSc Camilla H. Andreasen^{1*}, MSc Kirstine L. Stender-Petersen^{1*}, BSc Mette S. Mogensen¹, MSc Signe S. Torekov¹, MSc Lise Wegner¹, PhD Gitte Andersen¹, MSc Arne L. Nielsen¹, MSc Anders Albrechtsen², DMSc Knut Borch-Johnsen^{1,3,4}, MD Signe S. Rasmussen¹, DMSc Jesper O. Clausen¹, PhD Anneli Sandbæk⁵, DMSc Torsten Lauritzen⁵, DMSc Lars Hansen⁶, DMSc Torben Jørgensen³, DMSc Oluf Pedersen^{1,4}, PhD Torben Hansen¹

¹Steno Diabetes Center, Gentofte, Denmark; ²Department of biostatistics, University of Copenhagen, Denmark; ³Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark; ⁴Faculty of Health Science, University of Aarhus, Aarhus, Denmark; ⁵Department of General Practice, University of Aarhus, Aarhus, Denmark; ⁶Science and Medicine, Novo Nordisk A/S, Bagsværd, Denmark.

* , these authors contributed equally

Running title: The *FTO* rs9939609 variant and obesity

Corresponding author:

Camilla H. Andreasen, MSc
Steno Diabetes Center, Niels Steensens Vej 1, NLC2.13, DK-2820 Gentofte, Denmark
E-mail: cila@novonordisk.com

Received for publication 4 July 2007 and accepted in revised form 10 October 2007.

Additional information for this article can be found in an online appendix at <http://diabetes.diabetesjournals.org>.

ABSTRACT

Objective: Recently three independent studies have shown that variation in the fat mass and obesity associated gene (*FTO*) 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, comprising five different study groups.

Results: In studies of 3,856 type 2 diabetic cases and 4,861 normal glucose tolerant control subjects the minor A-allele of rs9939609 associated with type 2 diabetes ($p=9\cdot 10^{-5}$, OR 1.13 [1.06-1.20]). This association was abolished when adjusting for BMI ($p=0.2$, OR 1.06 [0.97-1.16]). Among 17,162 middle-aged Danes, the A-allele associated with overweight ($p=1\cdot 10^{-12}$, OR 1.19 [1.13-1.24]) and obesity ($p=2\cdot 10^{-16}$, OR 1.27 [1.20-1.34]). 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 an increase in BMI level by 1.95 ± 0.3 kg/m² 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.

KEYWORDS *FTO*, obesity, physical activity, insulin sensitivity, type 2 diabetes, genetics, association, fat mass.

ABBREVIATIONS Add, additive model; AF, allele frequency; ANOVA, analysis of variance; BIGTT-AIR, BIGTT acute insulin response; BIGTT-Si, BIGTT insulin sensitivity index; BMI, body mass index; CI, confidence interval; Dom, dominant model; *FTO*, fat mass and obesity associated; GD, genotype distribution; GWA, genome-wide association; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glycemia; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; LD, linkage disequilibrium; MAF, minor allele frequency; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; OR, odds ratio; Rec, recessive model; SDC, Steno Diabetes Center; SNP, single-nucleotide polymorphism; WHO, World Health Organization; WTCCC, Wellcome Trust Case Control Consortium

The world-wide 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, although difficult to validate (4,5,6). Recently, variation in the fat mass and obesity associated gene (*FTO*) was reported to associate with type 2 diabetes and increased fat mass. As a part of the Wellcome Trust Case Control Consortium (WTCCC) genome-wide association (GWA) study, which included 1,924 UK type 2 diabetic patients and 2,938 UK normoglycemic control subjects, a *FTO* variant (rs9939609) was found to associate with type 2 diabetes, which abolished following adjustment for body mass index (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 skin fold 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 ($BMI \geq 40$ kg/m²). By selecting HapMap tagSNPs this association was further replicated in four different European study samples comprising 2,081 obese and 2,783 non-obese 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 shows that *FTO* is expressed in several tissues, especially specific parts of the brain as well as 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

Subjects. The *FTO* rs9939609, rs8050136 and rs7193144 polymorphisms were genotyped in 17,508 Danes comprising five study groups 1) the population-based Inter99 study sample (ClinicalTrials.gov ID no: NCT00289237) ($n=6,104$), sampled at the Research Centre for Prevention and Health (10) 2) unrelated type 2 diabetic patients ($n=2,015$) sampled through the out-patient clinic at Steno Diabetes Center (SDC type 2 diabetes study group) 3) a population-based group of unrelated middle-aged glucose-tolerant subjects ($n=661$) examined at Steno Diabetes Center (SDC control group) 4) the ADDITION study cohort (ClinicalTrials.gov ID no: NCT00237548) ($n=8,382$) sampled by Department of General Practice at University of Aarhus (11) 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 study group 1 and 3 underwent a standard 75 g oral glucose tolerance test (OGTT) and all participants in study group 5 underwent a tolbutamide-modified intravenous glucose tolerance test (IVGTT) (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 Helsinki Declaration. Type 2 diabetes, impaired glucose tolerance (IGT), impaired

fasting glycemia (IFG) and normal glucose tolerance (NGT) were defined according to the World Health Organization (WHO) (13). Overweight and obesity was defined as $BMI \geq 25 \text{ kg/m}^2$ and $BMI \geq 30 \text{ kg/m}^2$, respectively. Interaction studies with physical activity and insulin sensitivity were performed in study group 1 including subjects with NGT, IFG, IGT and screen-detected diabetes. For further information on the study groups see Online-Only Appendix (available at <http://diabetes.diabetesjournals.org>).

Biochemical and anthropometrical measurements. Obesity-related measures, fasting serum lipids, plasma glucose and serum insulin were measured as described (10,11,12,15,16). For further information see Online-Only 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.

Genotyping. The *FTO* rs9939609, rs8050136 and rs7193144 polymorphisms were genotyped using Taqman allelic discrimination (KBioscience, Herts, UK). 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 (Add), dominant (Dom), and recessive (Rec) 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 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 analysis of variance (ANOVA) test, assuming an additive model. BIGTT-Si 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 where the variance of BMI in the subgroups of physical activity and insulin sensitivity were estimated from the residuals of a linear model where sex and age were included. All analyses were performed in RGui version 2.5.0 (available at <http://www.r-project.org>), p -values < 0.05 were considered significant.

RESULTS

Due to near-perfect linkage disequilibrium (LD) between the three genotyped SNPs (mean $r^2=0.99$), we excluded rs8050136 and rs7193144 from further analyses. The overall minor allele frequency (MAF) 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 ($p_{AF}=9 \cdot 10^{-5}$, OR 1.13 [1.06-1.20]) (Table 1). The association between rs9939609 and type 2 diabetes was abolished when adjusting for BMI ($p_{Add}=0.2$, OR 1.06 [0.97-1.16]). The A-allele was associated with overweight and obesity in the population-based Inter99 study sample, the ADDITION study cohort and the 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 ($p_{AF}=1 \cdot 10^{-12}$, OR 1.19 [1.13-1.24]), and obesity ($p_{AF}=2 \cdot 10^{-16}$, OR 1.27 [1.20-1.34]) (Table 1).

In the population-based Inter99 study sample, the *FTO* rs9939609 A-allele was highly associated with obesity-related measures including BMI ($p_{Add}=1 \cdot 10^{-9}$), body weight ($p_{Add}=2 \cdot 10^{-9}$) and waist circumference ($p_{Add}=1 \cdot 10^{-7}$). 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 insulin sensitivity index (BIGTT-Si) ($p_{Add}=0.004$), and increased BIGTT acute insulin response (BIGTT-AIR) ($p_{Add}=0.001$), 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 levels of BMI ($p_{Add}=0.002$), body weight ($p_{Add}=0.008$), fat mass ($p_{Add}=0.001$), body fat percent ($p_{Add}=3\cdot 10^{-4}$), and fasting serum leptin concentrations ($p_{Add}=0.003$), but not with height and 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 p -values remaining significant are marked with an asterisk, see Table 2 and 3.

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 figure 1. We found no interaction between glucose tolerance status and the *FTO* rs9939609 genotype effect on levels of 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 ($p_{Int}=0.007$). The *FTO* rs9939609 genotype effect between physically passive, light or medium physically active and hard or very hard physically active subjects was 0.38, 0.37, and -0.11 kg/m² respectively when comparing homozygous T-allele carriers and heterozygous carriers and 1.95, 0.69 and 0.47 kg/m² respectively when comparing homozygous T-allele carriers and homozygous A-allele carriers (Figure 1A).

Finally we found an interaction between the *FTO* rs9939609 genotype and measures of insulin sensitivity. The *FTO* rs9939609 genotype effect in the highest, medium and lowest insulin sensitivity groups, stratified by BIGTT-Si tertiles, was -0.14, 0.31 and 0.54 kg/m² respectively between homozygous T-allele carriers and heterozygous carriers, and 0.25, 0.60 and 1.34 kg/m² respectively when comparing homozygous T-allele carriers and homozygous A-allele carriers ($p_{Int}=2\cdot 10^{-4}$) (Figure 1B). Since the variance of BMI in the different physical activity and insulin sensitivity subgroups varies substantially, 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 ($p_{Int}=0.003$) and insulin sensitivity ($p_{Int}=0.03$).

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 (2.1-4.6) kg more than non-carriers, which is reflected in 1.1 (0.7-1.4) kg/m², and 2.3 (1.3-3.3) cm in waist circumference. The association with obesity-related measures was not affected by glucose tolerance status.

BMI is *per* 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 intra-abdominally fat accumulation.

Finally, the *FTO* rs9939609 genotype was associated with increased fasting serum leptin levels, which are considered to be 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 people from the population-based Inter99 study sample we found no differences in post-oral glucose load measures of serum insulin or plasma glucose. However, whole body insulin sensitivity, estimated by the BIGTT-Si, 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 mechanism 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 an increase of 1.95 ± 0.33 kg/m² in homozygous *FTO* rs9939609 A-allele carriers, whereas

no major effect of sedentary lifestyle was found comparing heterozygous- and non-carriers of the *FTO* rs9939609 A-allele. Obviously this finding needs replication in independent study populations in order 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 validate 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 people, the *FTO* rs9939609 genotype, may confer 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 when compared to heterozygous- and non-carriers.

ACKNOWLEDGEMENT

This study was supported by the Danish Medical Research Council, the Danish Diabetes Association, the Gerda and Aage Haensch Foundation, the A.P. Møller Foundation for the Advancement of Medical Science, University of Copenhagen, and the Velux Foundation. This work is part of the project "Hepatic and adipose tissue and functions in the metabolic syndrome" (HEPADIP www.hepadip.org), which is supported by the European Commission as an integrated project under the 6th Framework Programme (LSHM-CT-2005-018734). The study also received support from The Danish Obesity Research centre (DanORC; www.danorc.dk), which is supported by The Danish Council for Strategic Research (Grant No 2101-06-0005). The authors wish to thank Annemette Forman, Inge-Lise Wantzin and Marianne Stendal for technical assistance and Grete Lademann for secretarial support.

REFERENCES

1. Boutin P, Dina C, Vasseur F, Dubois S, Corset L, Séron K, Bekris L, Cabellon J, Neye B, Vasseur-Dalannoy V, Chikri M, Charles MA, Clément K, Lernmark A, Froguel P: GAD2 on chromosome 10p12 is a candidate gene for human obesity. *PLoS Biol* 1:361-371, 2003
2. Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoeur C, Vatin V, Ghossaini M, Wachter C, Herberg S, Charpentier G, Patch W, Pattou F, Charles MA, Tounian P, Clément K, Jouret B, Weill J, Maddux BA, Goldfine ID, Walley A, Bouttin P, Dina C, Froguel P: Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. *Nat Genet* 37:863-867, 2005
3. Herbert A, Gerry NP, McQueen MB, Heid IM, Pfeufer A, Illig T, Wichmann HE, Meitinger T, Hunter D, Hu FB, Colditz G, Hinney A, Hebebrand J, Koberwitz K, Zhu X, Cooper R, Ardlie K, Lyon H, Hirschhorn JN, Laird NM, Lenburg ME, Lange C, Christman MF: A Common Genetic Variant Is Associated with Adult and Childhood Obesity. *Science* 312:279-283, 2006
4. Swarbrick MM, Waldenmaier B, Pennacchio LA, Lind DL, Cavazos MM, Geller F, Merriman R, Ustaszewska A, Malloy M, Scherag A, Hsueh W-C, Rief W, Mauvais-Jarvis F, Pullinger CR, Kane JP, Dent R, McPherson R, Kwok P-Y, Hinney A, Hebebrand J, Vaisse C: Lack of Support for the Association between GAD2 Polymorphisms and Severe Human Obesity. *PLoS Biol* 3:1662-1671, 2005
5. Lyon HN, Florez JC, Bersaglieri T, Saxena R, Winckler W, Almgren P, Lindblad U, Tuomi T, Gaudet D, Zhu X, Cooper R, Ardlie KG, Daly MJ, Altshuler D, Groop L, Hirschhorn JN: Common Variants in the ENPP1 Gene Are Not Reproducibly Associated With Diabetes or Obesity. *Diabetes* 55:3180-3184, 2006
6. Hall DH, Rahman T, Avery PJ, Keavney B: INSIG-2 promoter polymorphism and obesity related phenotypes: association study in 1428 members of 248 families. *BMC medical genetics* 7:83-88, 2006
7. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JRB, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch A-M, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin M-R, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CNA, Doney ASF, Morris AD, Davey-Smith G, the Wellcome Trust Case Control Consortium, Hattersley AT, McCarthy MI: A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 316:889-894, 2007
8. Dina C, Meyre D, Gallina S, Durand E, Körner A, Jacobsen P, Carlsson LMS, Kiess W, Vatin V, Lecoeur C, Delplanque J, Valliant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Herberg S, Le Stunff C, Bougnères P, Kovacs P, Marre M, Balkau B, Cauchi S, Chèvre JC, Froguel P: Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* 39:724-726, 2007
9. Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orrú M, Usala G, Dei M, Lai S, Maschio A, Busonero F, Mulas A, Ehret GB, Fink AA, Weder AB, Cooper RS, Galan P, Chakravarti A, Schlessinger D, Cao A, Lakatta E, Abecasis GR: Genome-Wide Association Scan Shows Genetic Variants in the FTO gene Are Associated with Obesity-Related Traits. *PLoS Genetics* 3:1-11, 2007
10. Jørgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glümer C, Pisinger C: A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99 (1). *Eur J Cardiovasc Prevention Rehab* 10:377-386, 2003
11. Lauritzen T, Griffin S, Borch-Johnsen K, Wareham NJ, Wolfenbittel BHR, Rutten G, ADDITION study group: The ADDITION study: proposed trial of the cost-effectiveness of an intensive multifactorial intervention on morbidity and mortality among people with type 2 diabetes detected by screening. *Int J Obes Relat Metab Disord* 24 Suppl 3:S6-S, 2000

12. Clausen JO, Borch-Johnsen K, Ibsen H, Berman RN, Hougaard P, Winther K, Pedersen O: Insulin Sensitivity Index, Acute Insulin Response, and Glucose Effectiveness in a Population-based Sample of 380 Young Healthy Caucasians. *J Clin Invest* 98:1195-1209, 1996
13. World Health Organization Study Group: Definition, diagnosis and classification of diabetes mellitus and its complications; Part 1: Diagnosis and classification of diabetes mellitus. Tech Rep Ser WHO/NCD/NCS/99.2. Geneva, World Health Organization, 1999
14. Glümer C, Jørgensen T, Borch-Johnsen K: Prevalences of Diabetes and Impaired Glucose Regulation in a Danish Population. *Diabetes Care* 26: 2335-2340, 2003
15. Hansen T, Drivsholm T, Urhammer SA, Palacios RT, Vølund A, Borch-Johnsen K, Pedersen O: The BIGTT test. A novel test for simultaneous measurement of pancreatic β -cell function, insulin sensitivity, and glucose tolerance. *Diabetes Care* 30:257-262, 2007
16. Echwald SM, Clausen JO, Hansen T, Urhammer SA, Hansen L, Dinesen B, Borch-Johnsen K, Pedersen O: Analysis of the relationship between fasting serum leptin levels and estimates of beta-cell function and insulin sensitivity in a population sample of 380 healthy young Caucasians. *Eur J Endocrinol* 140:180-185, 1999
17. Clausen JO, Borch-Johnsen K, Pedersen O: Relation between Birth Weight and the Insulin Sensitivity Index in a Population Sample of 331 Young, Healthy Caucasian. *Am J Epidemiol* 146:23-31, 1997

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

	n (men/women)	TT	TA	AA	MAF (95%CI)	p_{GD}	p_{AF}	Odds Ratio
Stratified on glucose tolerance								
NGT	4,861 (2,259/2,602)	1,676 (35)	2,391 (49)	794 (16)	40.9 (39.9-41.9)			1.13
Type 2 diabetes	3,856 (2,286/1,567)	1,210 (31)	1,907 (50)	739 (19)	43.9 (42.8-45.0)	$3 \cdot 10^{-4}$	$9 \cdot 10^{-5}$	(1.06-1.20)
	$p_{(Add)}^1 = 1 \cdot 10^{-4}$ / OR 1.17 (1.08-1.26)				$p_{(Add)}^2 = 0.2$ / OR 1.06 (0.97-1.16)			
Stratified on BMI (kg/m²) level								
BMI <25	5,148 (2,155/2,993)	1,901 (37)	2,525 (49)	722 (14)	38.5 (37.6-39.5)			
BMI ≥25	12,014 (6,951/5,063)	3,945 (33)	5,888 (49)	2,181 (18)	42.7 (42.0-43.3)	$1 \cdot 10^{-12}$	$1 \cdot 10^{-12}$	1.19 (1.13-1.24)
BMI ≥30	4,867 (2,506/2,361)	1,510 (31)	2,406 (49)	951 (20)	44.3 (43.3-45.3)	$3 \cdot 10^{-16}$	$2 \cdot 10^{-16}$	1.27 (1.20-1.34)

Data are number of subjects, divided into genotype groups (% in each group), and frequencies of the minor A-allele (MAF) in percentages. 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 age¹, and sex, age and BMI². Association with overweight and obesity was determined comparing subjects with BMI<25 and BMI≥25, and subjects with BMI<25 and BMI≥30 respectively.

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

	TT	TA	AA	<i>P</i> _{Add}	<i>P</i> _{Dom}	<i>P</i> _{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-Si	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 (1261,2016)	1,639 (1288,2112)	1,663 (1320,2146)	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 ± standard deviation 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. Calculated *p*-values were adjusted for age and sex for obesity-related measures, for sex, age and BMI for serum lipids, serum insulin, plasma glucose and HOMA-IR, and for age for the BIGTT-Si and BIGTT-AIR indices. HOMA-IR was calculated as fasting plasma glucose (mmol/l) multiplied by fasting serum insulin (pmol/l) divided by 22.5. BIGTT-Si and BIGTT-AIR were calculated as described (15). * indicates that the *p*-value remains significant after Benjamini and Hochberg correction.

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

	TT	TA	AA	<i>P</i> _{Add}	<i>P</i> _{Dom}	<i>P</i> _{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 percent	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	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 x (min x 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)} (min x pmol/l))	1941 (1106,2620)	1993 (1305,2848)	1976 (1410,3120)	0.8	1	0.6

Data are means ± standard deviation or median (interquartile range). 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 to a Bergmann minimal model was determined upon a tolbutamide-modified intravenous glucose test as described (12). * indicates that the *p*-value remains significant after Benjamini and Hochberg correction.

FIGURE TITLE AND LEGEND

Figure 1: Effect of physical activity and insulin sensitivity, estimated by BIGTT-Si, 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. The bars indicate differences in BMI levels between heterozygous carriers 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 ($p_{\text{Int}}=0.007$). 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. **B:** Subjects were divided by insulin sensitivity tertiles, assessed by the BIGTT-Si (tertiles 7.4, 11.1 and 24.4) and stratified according to the *FTO* rs9939609 genotype. The bars indicate differences in BMI levels between heterozygous carriers 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-Si compared by an ANOVA test ($p_{\text{Int}}=2 \cdot 10^{-4}$). The number of subjects in each genotype group (TT/TA/AA) was (557/821/317) in the group with low BIGTT-Si, (568/833/294) in the group with middle BIGTT-Si and (609/834/253) in the group with high BIGTT-Si.

Figure 1

