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Genetic determinants of cholesterol and energy metabolism : implications for cardiometabolic health

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Genetic variation in the obesity gene FTO is not associated with decreased fat oxidation: The NEO study

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Abstract

Introduction

The FTO gene harbours the strongest common genetic variant associated with obesity. Recently, rs1421085-T to -C substitution mapped in FTO was shown to induce a developmental shift of human adipocytes from an energy-combusting beige to an energy-storing white phenotype *in vitro*. As browning of adipocytes selectively enhances fat oxidation (FatOx), we hypothesized that rs1421085-C in FTO is associated with deceased FatOx compared with carbohydrate oxidation (CarbOx) and an increased respiratory quotient (RQ).

Methods

In the Netherlands Epidemiology of Obesity (NEO) study, a population-based cohort study of middle-aged individuals (45-65 years), anthropometry and genotyping was performed (n=5,744), in addition to indirect calorimetry (n=1,246). With linear regression analyses we examined associations of rs1421085 genotype with FatOx, CarbOx and RQ.

Results

In the total study population, 36.7% carried the rs1421085-TT genotype, 47.6% rs1421085-CT and 15.7% rs1421085-CC. Mean (SD) age was 56 (6) years, mean (SD) BMI was 26.3 (4.4) kg/m^2, and 56% of the total population were women. Measures of adiposity (difference, 95% CI) were higher in CC carriers than in rs1421085-TT carriers: BMI +0.56 (0.15, 0.98) kg/m^2; waist circumference +1.25 (0.02, 2.49) cm; total body fat mass +1.21 (0.28, 2.14) kg. However, no differences in mean FatOx (+2.5 (-2.4, 7.4) mg/min), CarbOx (-6.1 (-17.4, 5.2) mg/min) or RQ (-0.01 (-0.02, 0.01)) were observed between the two genotypes.

Conclusion

We observed no evidence for associations of rs1421085 in FTO with FatOx and RQ. This indicates that the rs1421085 C-allele in FTO induces obesity likely via other pathways than via reduced FatOx.

Introduction

With the global rise in the prevalence of obesity and accompanying increased risks of metabolic disorders, cardiovascular disease and cancer, the search for preventive strategies and therapies for obesity is of crucial importance.1 Environmental factors play a major role in the onset of obesity, and lifestyle interventions are therefore the keystone of treatment and prevention.[1] However, because 40-70% of the inter-individual differences in obesity risk in the general population is explained by genetics,[2,3] it is of great interest for future intervention strategies to unravel genetic factors and related biological pathways involved in obesity.

In the search for genetic factors contributing to inter-individual differences in obesity risk, genetic variation in the fat mass and obesity-associated gene (FTO) was identified through genome-wide association studies (GWAS) as being the top common genetic determinant of body mass index (BMI) and obesity risk.[4–6] Single nucleotide polymorphisms (SNPs) that cluster in the first intron of this gene are robustly associated with obesity. In addition, these SNPs associate with cardiovascular disease, metabolic syndrome and diabetes, mostly mediated by BMI.[7] Of the different common SNPs identified in relation to adiposity traits, rs1558902 showed the largest increase in BMI (0.39 kg/m^2) and obesity risk (1.2-fold) per additional copy of the risk allele.[6]

Although FTO was already identified in 2007 as an obesity susceptibility gene,[4,5] the biological explanation for the association of genetic variation in FTO with BMI and the risk of developing obesity remained largely unclear. Importantly, a potential mechanism was recently illuminated by Claussnitzer and colleagues.[8] Rs1421085-T to -C allele substitution, which is in perfect linkage disequilibrium with rs1558902, was shown to disrupt the binding of the transcriptional repressor ARID5B to IRX3 and IRX5, which increased their expression specifically in pre-adipocytes. This resulted in a developmental shift from energy-combusting beige adipocytes towards energy-storing white adipocytes *in vitro*, characterized by decreased mitochondrial thermogenesis and increased lipid storage. This study thereby provides evidence for a genetic basis of disturbed adipocyte beiging in humans. Previous studies have shown that decreased rates of fat oxidation are associated with obesity risk.[9–11] On the other hand, prolonged acclimation to cold, which selectively increases fat oxidation,[12] decreases body weight explained by a decrease in body fat mass.[13] Thus, a possible lower fat oxidation as a consequence of decreased browning in rs1421085 risk allele carriers may explain their increased obesity risk.

Previous studies that examined the association between FTO gene variants and resting energy expenditure (REE) obtained contradictory results.[14–19] Only one study observed

a lower REE in risk allele carriers independent of body size [14], while others found a higher REE in risk allele carriers which abolished after adjustment for fat free mass (FFM), [15–18] or observed no association. [19] Associations with specific components of substrate utilization have not been investigated to date. In light of the findings by Claussnitzer and colleagues, [8] it is of particular interest to study the association of rs1421085 with fat oxidation (FatOx), carbohydrate oxidation (CarbOx) and respiratory quotient (RQ) for several reasons: 1) brown adipose tissue activation selectively enhances the oxidation of fat over glucose, both in mice [20] and humans; [12] 2) long term exposure to cold that activates brown adipose tissue progressively increases lipid oxidation while reducing glucose oxidation; [21] 3) conversion of white adipocytes to beige adipocytes changes the substrate preference from glucose to lipid. [22] Because the rs1421085 variant in FTO was shown to be involved in adipocyte beiging in vitro, [8] we hypothesized that specifically the FTO rs1421085-C risk allele is associated with decreased whole-body FatOx compared with CarbOx, and a higher RQ in resting state in humans.

In the present study, we aimed to examine these associations in a large, population-based cohort of middle-aged participants with indirect calorimetry measurements performed in 1,246 participants. To the best of our knowledge, this is the largest study so far to determine the association of the FTO rs1421085 variant with REE, and the first to study the association of this SNP with substrate oxidation rates.

Materials and Methods

Study design and population

The study population was part of the Netherlands Epidemiology of Obesity (NEO) study, which is a population-based prospective cohort study including 6,671 participants recruited from September 2008 until September 2012. The NEO study was designed to study overweight and obesity-associated disease, and therefore persons with a BMI of ≥27 kg/m^2 were oversampled. Both men and women in the age of 45 to 65 years, living in the greater area of Leiden (The Netherlands), and with a self-reported BMI of ≥27 kg/m^2 were able to participate. To acquire a reference distribution for BMI, inhabitants from the nearby municipality Leiderdorp (The Netherlands) were asked to participate irrespective of their BMI. In the NEO study center extensive baseline measurement were performed after an overnight fasting period of at least ten hours. A random subgroup of participants underwent indirect calorimetry. Questionnaires on demographic and clinical characteristics were completed at home, preceding the study visit. The present study is a cross-sectional analysis of the baseline measurements of the NEO study. For this study we excluded 927 participants because of missing data on genotype, being a first-degree relative or being from non-

European ancestry. Therefore, the present study population comprised 5,744 NEO study participants, of which 1,246 participants underwent indirect calorimetry to measure REE and substrate oxidation rates (Supplementary figure 9.A.1).

The medical ethics committee of the Leiden University Medical Center approved the NEO study and written informed consent was given by all participants. The complete study design and detailed information on data collection can be found elsewhere.[23]

FTO genotype

DNA was extracted from venous blood samples obtained from the antecubital vein. Genotyping was performed using the Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, California, United States of America). From these data, we extracted the rs1421085 (C/T) polymorphism from the whole-genome data. The rs1421085 polymorphism was in Hardy-Weinberg equilibrium (p-value=0.97).

Resting energy expenditure and substrate oxidation

In a random subsample of NEO study participants REE was measured by indirect calorimetry (ventilated hood system, Oxycon Pro) for 30 minutes under standardized conditions. The measurement was performed between 8 and 9 a.m., after an overnight fast of at least ten hours and a resting period of 15 minutes. During the measurement, participants were awake while lying on a bed in a quiet room. The volumes of oxygen inspired (VO_2) and carbon dioxide exhaled (VCO_2) were measured every minute. REE was calculated using standard formulas.[24] RQ is defined as VCO_2/VO_2.[24] FatOx was calculated as $1.69*VO_2 – 1.69*VCO_2 – 2.03$*urinary nitrogen excretion (N) and CarbOx as $4.57*VCO_2 – 3.23*VO_2 – 2.6$*N, considering urinary nitrogen excretion negligible (zero).[25]

Anthropometry

We assessed anthropometry to be able to associate rs1421085 genotype with measures of adiposity (i.e. BMI, total body fat percentage, waist circumference, hip circumference, fat mass and FFM), in order to show that the NEO study population is a valid and representative cohort to study genetic variation in the obesity-associated gene FTO. Body weight and total body fat were measured with the Tanita bio-impedance balance (TBF-310, Tanita International Division, UK), without shoes and with a one kilogram (kg) subtraction to correct for weight of clothing. BMI was calculated as the weight in kilograms divided by the height in meters squared. Fat mass and FFM were calculated from body weight and body fat percentage as estimated with the Tanita bio-impedance balance (TBF-310, Tanita International Division, UK). Waist circumference was measured mid-way between the lower

costal margin and the iliac crest. Hip circumference was measured at the maximum circumference of the buttocks.

Covariates

Smoking status was reported on the questionnaire and grouped into never smoker, former smoker and current smoker. Long-term tobacco exposure was expressed in pack years of smoking, calculated as the number of packs of cigarettes smoked per day multiplied by the number of years the person smoked. A pack year is defined as smoking twenty cigarettes every day during one year. Pre-existing cardiovascular disease was defined as reporting a medical history of myocardial infarction, congestive heart failure, angina, peripheral vascular disease, or stroke. Diabetes was defined as self-reporting the disease via the questionnaire, using anti-diabetic medication, or having a fasting glucose measurement ≥7.0 mmol/L at baseline. With the short questionnaire to assess health-enhancing physical activity (SQUASH)[26] physical activity during leisure time was quantified and expressed as metabolic equivalents of task (MET)*hours/week, which indicates the intensity of an activity. Intake of alcohol was determined with a semi-quantitative food frequency questionnaire,[27] and calculated with the 2011 version of the Dutch food composition table (NEVO-2011).[28] At the study center, research nurses recorded current medication use.

Statistical analyses

represent baseline associations in the general population.[29] All participants were weighted towards the BMI distribution of the reference population from Leiderdorp (The Netherlands)[30], of which the BMI distribution corresponded to the BMI distribution of the Dutch general population aged 45 to 65 years.[31] As all analyses were weighted, the results apply to a population-based study without oversampling of persons with overweight or obesity. Descriptive characteristics of the participants were reported as mean (standard deviation, SD) or proportion and stratified by rs1421085 genotype, i.e. TT, CT and CC with C being the rs1421085 allele associated with a higher BMI.[5]

As an initial step, we attempted to replicate in the NEO study population the previously described associations between rs1421085-C and measures of increased adiposity. After verification, we determined the association of rs1421085 genotype with REE, RQ, FatOx and CarbOx. All associations were assessed using linear regression analysis assuming an additive model. Data were presented as weighted mean differences per additional rs1421085-C allele, with TT as the reference. Models were adjusted for age, sex, and four principal components to correct for population substructures. Importantly, the amount of metabolically active tissue is a major determinant of REE.[32–36] To study the association with REE irrespective of FFM we adjusted for FFM in two ways: 1) using the ratio of

REE and FFM (REE/FFM) as dependent variable and 2) adding FFM as a covariate in the linear regression models. Considering a power of 0.80 and an alpha of 0.05, we were able to detect a mean difference between rs1421085-CC and -TT carriers of 64 kcal/day REE, 0.012 unit RQ, 6 mg/min FatOx, and 13 mg/min CarbOx in our study population.

REE is usually higher in persons with obesity, probably because the absolute amount of FFM increases with body size.[37] As the FTO risk genotype is associated with obesity,[4–6] we expect it also to be associated with an increase in REE. On the other hand, disturbed adipocyte browning may play a role in the increased obesity risk of rs1421085 risk allele carriers via a decrease in energy combustion.[8] As these opposing associations may conceal the true association, we also studied effect modification by BMI by examining the associations of rs1421085 with measures of REE, RQ, FatOx and CarbOx stratified by BMI tertile.

All analyses were performed using STATA Statistical Software (Statacorp, College Station, Texas, USA), version 12.0.

Results

Population characteristics

The minor allele frequency of rs1421085 was 0.39, 47.6% of the population was heterozygous and 15.7% was homozygous for the rs1421085-C allele. Descriptive characteristics of the study population are shown in Table 9.1. Characteristics were similar for the three genotypes. Demographic and clinical characteristics of the random subsample in which indirect calorimetry was performed (n=1,246) were comparable to characteristics of the total study population (results not shown). In the random subsample in which indirect calorimetry was performed, mean (SD) REE was 1,476 (270) kcal/day. Mean FatOx was 59.8 (24.5) mg/min and mean CarbOx was 123.4 (55.4) mg/min. Mean RQ was 0.84 (0.05). RQ ranged from 0.69 to 1.11, with 8 participants having a RQ above 1.

Associations of rs1421085 genotype with measures of body fat

In the total study population (Figure 9.1 and Supplementary table 9.A.1), BMI was higher (+0.56 kg/m^2; 95% CI 0.15, 0.98) in participants that carried the rs1421085-CC genotype than in rs1421085-TT carriers. In addition, waist circumference (+1.25 cm; 95% CI 0.02, 2.49), hip circumference (+1.01 cm; 95% CI 0.10, 1.92), fat mass (+1.21 kg; 95% CI 0.28, 2.14), and FFM (+0.59 kg; 95% CI 0.05, 1.13) were higher in rs1421085-CC genotype carriers than in rs1421085-TT carriers. Associations were similar when we restricted to the subpopulation of participants in whom indirect calorimetry was performed (Supplementary table 9.A.2).

Table 9.1: Demographic and clinical characteristics of the genotyped Netherlands Epidemiology of Obesity study population (aged 45-65 years), stratified by rs1421085 genotype.

Characteristic	rs1421085 genotype			Total
	TT	CT	CC	
Proportion of participants (%)	36.7	47.6	15.7	100
Demographic				
Sex (% women)	55	57	56	56
Age (year)	56 (6)	56 (6)	56 (6)	56 (6)
Body mass index (kg/m^2)	26.1 (4.1)	26.3 (4.4)	26.7 (5.0)	26.3 (4.4)
Total body fat (%)	31.4 (8.5)	31.7 (8.6)	32.2 (9.1)	31.6 (8.7)
Educational level (% high)[a]	46	49	45	47
Tobacco smoking (% current and former smokers)	60	62	66	62
Quantity of smoking (pack years)	9.7 (16.5)	9.4 (14.2)	9.9 (14.5)	9.6 (15.2)
Physical activity during leisure time (MET hours/week)	37 (30)	38 (33)	36 (33)	38 (32)
Alcohol intake (g/day)	15 (16)	15 (16)	14 (15)	15 (16)
Comorbidity and medication				
Diabetes (%)	5	5	7	5
Cardiovascular disease (%)	5	6	8	6
Lipid lowering drug use (%)	11	10	12	11

Results are based on analyses weighted towards the normal BMI distribution and presented as percentage or mean (SD), with n=5,744[b].

[a] High educational level: higher secondary education (according to Dutch educational system), higher vocational education, university, PhD.

[b] Missing data: n=56 for educational level, n=5 for smoking status, n=104 for physical activity, n=3 for alcohol intake, n=30 for total body fat, n=32 for diabetes, n=22 for cardiovascular disease.

MET, metabolic equivalents of task.

Associations of rs1421085 genotype with measures of REE, RQ, fat oxidation and carbohydrate oxidation

In figure 9.2 the differences in measures of REE, RQ, FatOx and CarbOx are shown per additional rs1421085-C allele. In our study population, rs1421085 genotype was not associated with REE, REE/FFM, REE adjusted for FFM, RQ, FatOx and CarbOx (Supplementary table 9.A.3). The difference between CC and TT (reference) carriers was for REE +0.9 kcal/day (95% CI -38.6, 40.4), for REE adjusted for FFM -4.6 kcal/day (95% CI -33.4, 24.3), for REE/FFM -0.05 kcal/day/kg FFM (95% CI -0.58, 0.48), for RQ -0.007 (95% CI -0.018, 0.005), for FatOx +2.49 mg/min (95% CI -2.41, 7.38), and for CarbOx -6.12 mg/min (95% CI -17.43, 5.19). Also, there was no effect modification by BMI, as associations were not different between BMI tertiles (Supplementary table 9.A.4). The BMI range per tertile was 18.21-28.14 kg/m^2 for tertile 1 (n=416); 28.15-31.16 kg/m^2 for tertile 2 (n=415); and 31.17-55.93 kg/m^2 for tertile 3 (n=415). Within each tertile the rs1421085 genotype was not associated with REE, REE adjusted for FFM, REE/FFM, RQ, FatOx and CarbOx.

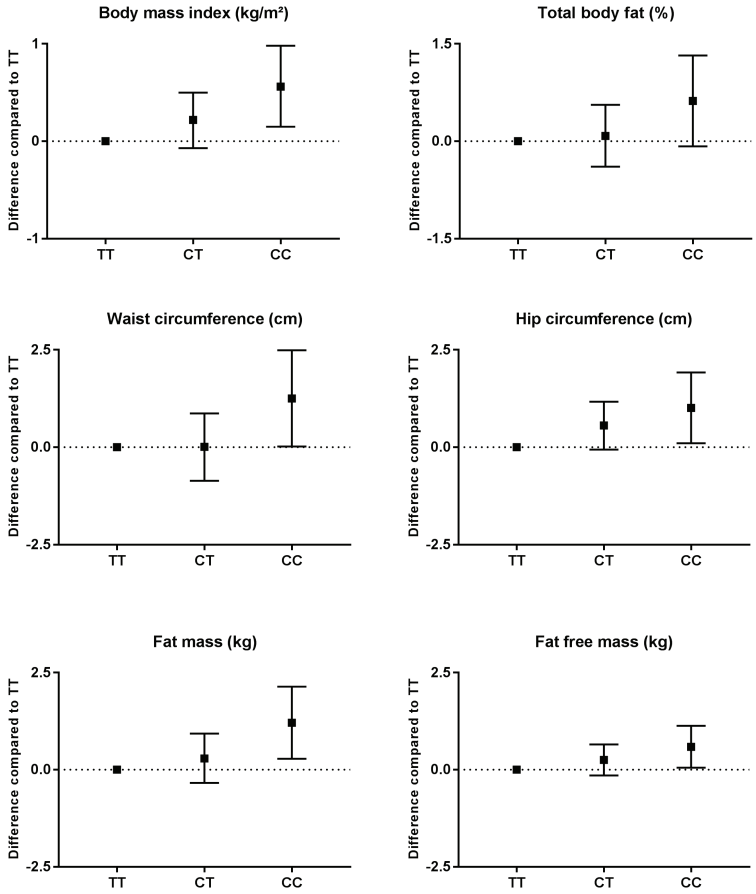

Figure 9.1: Difference in measures of body fat per additional rs1421085-C allele, with the TT genotype as reference. Results are based on analyses weighted towards the normal BMI distribution, with n=5,744[a]. Bars represent beta coefficients from linear regression calculated with respect to the adjusted mean of the reference group (TT genotype carriers; reference line y=0). Models were adjusted for age, sex and population structure. Error bars represent 95% confidence intervals.

[a] Missing data: n=30 for total body fat, n=5 for waist circumference, n=5 for hip circumference, n=30 for fat mass, n=30 for fat free mass.

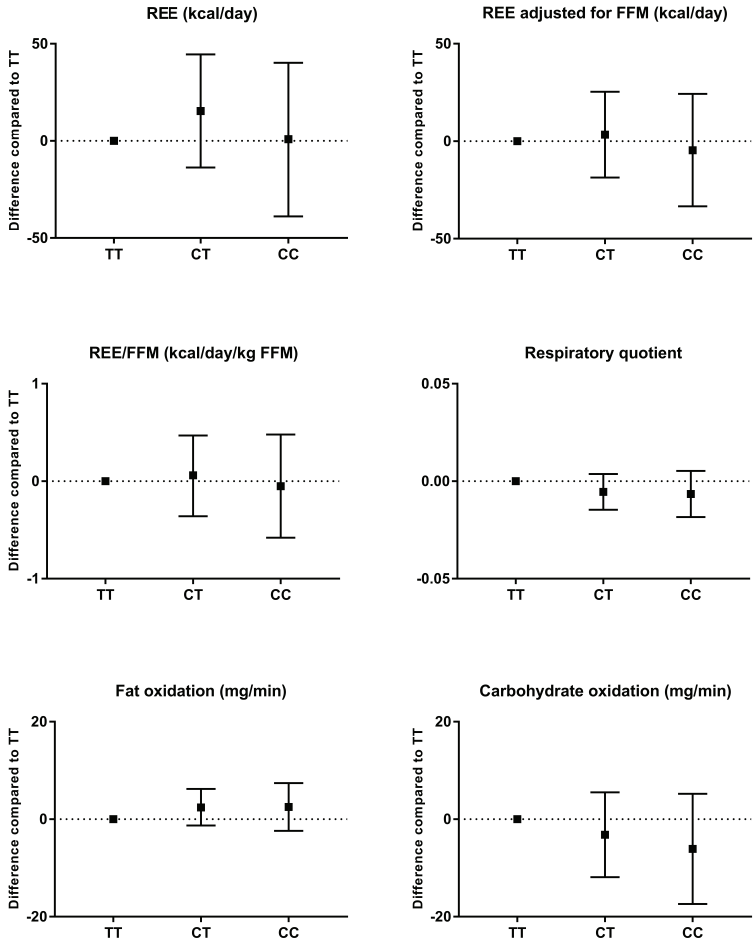

Figure 9.2: Difference in measures of REE, respiratory quotient, fat oxidation and carbohydrate oxidation per additional rs1421085-C allele, with the TT genotype as reference. Results are based on analyses weighted towards the normal BMI distribution, with n=1,246[a]. Bars represent beta coefficients from linear regression calculated with respect to the adjusted mean of the reference group (TT genotype carriers; reference line y=0). Models were adjusted for age, sex and population structure. Error bars represent 95% confidence intervals.

[a] Missing data: n=8 fat free mass.
FFM, fat free mass; REE, resting energy expenditure.

Discussion

Increased expression of IRX3 and IRX5 following rs1421085-T to -C substitution in the FTO gene was recently shown to skew human adipocyte browning *in vitro*, leading to reduced mitochondrial combustion of lipids for thermogenesis and increased intracellular lipid storage.[8] As decreased oxidation of lipids in rs1421085-C allele carriers may be an underlying mechanism explaining the association between FTO and obesity, we aimed to study the association between rs1421085 genotype and whole-body lipid oxidation. In the present study, we did not find evidence for an association between the rs1421085 polymorphism and whole-body FatOx, in a large population-based cohort of 1,246 middle-aged individuals.

In the NEO study population, we replicated the association between rs1421085 genotype and BMI that was previously shown in GWAS.[5] The effect sizes (+0.22 kg/m^2 for CT and +0.56 kg/m^2 for rs1421085-CC, relative to the rs1421085-TT genotype) were comparable with those determined in a large GWAS on BMI, in which BMI increased with 0.39 kg/m^2 per additional copy of the rs1558902 risk allele that is in perfect linkage disequilibrium with rs1421085.[6] For BMI and other markers of adiposity (i.e. total body fat, waist and hip circumference, fat mass and FFM) we observed an additive (i.e. per allele) effect of the rs1421085-C allele. These results imply that associations of genetic variation in the FTO gene with measures of REE and substrate oxidation rates can be validly studied in the NEO study population.

In the present study, there was no evidence for an association of rs1421085 with whole-body crude REE and FFM-adjusted REE. This is in line with several previous studies.[15–17,19,38] It has been shown before that obesity risk is associated with decreased rates of fat oxidation.[9–11] In contrast to our hypothesis, there was also no evidence for an association between rs1421085-C and FatOx. Claussnitzer and colleagues[8] showed a 5-fold reduction in mitochondrial thermogenesis in perirenal adipose tissue of rs1421085 risk allele carriers *in vitro*. Also, they showed a 4-fold increase in cellular metabolic rate after altering the causal nucleotide from rs1421085-C to -T with targeted genome editing technology (CRISPR-Cas9) in pre-adipocytes of risk allele carriers. Despite these considerable effects on a cellular level, the contribution of browning of white adipocytes to total energy metabolism is apparently too small to affect resting whole-body energy expenditure and fat oxidation, as implicated by the present study. Although our results indicate that genetic variation in FTO does not contribute to whole-body fat oxidation, there is evidence that other SNPs outside the FTO gene do affect fat oxidation, indicating that there is a genetic basis for whole-body fat oxidation in humans.[39]

Although we performed indirect calorimetry measurements in a very large population of 1,246 participants, based on power calculations it is possible that the effect size of rs1421085-C is too small to be detected in our study. In theory, small effect sizes could be of clinical relevance. An additive effect of small changes in FatOx over time could eventually contribute to a significant increase in adiposity, although no studies have yet explored this hypothesis. However, several factors argue against the existence and relevance of such a long-term additive effect. In the first place, the direction of the effect size of FatOx in CT and CC carriers was positive, which is opposite to what we hypothesized. The same holds for the effect sizes of the RQ, which was negative. Secondly, in the present study there was no evidence for an allele-dosage effect between rs1421085-C and measures of REE and FatOx, supporting the finding that there is no association of rs1421085 genotype with these measures. Thirdly, as a rule of thumb, a cumulative energy deficit of 3500 kcal is required to lose a pound (0.45 kg) of body weight.[40,41] By way of illustration, with an effect size of -4.6 kcal/day for the REE adjusted FFM in rs1421085-CC carriers, it would take over 760 days (2 years) to have an increase in body weight of 0.45 kg compared to rs1421085-TT carriers. The biological and clinical relevance of these effects should be further explored and GWAS meta-analyses on REE and substrate oxidation rates are eagerly awaited to further elucidate the genetic regulation of resting energy metabolism.

Our results do not provide further insight in the mechanistic basis for the association between FTO and obesity. However, they do indicate that FTO-associated obesity is induced via other mechanisms than reduced resting energy expenditure and whole-body fat oxidation in risk allele carriers. There is growing evidence that higher food intake via increased hunger and decreased satiety underlies the association between FTO and obesity.[38,42] The highest expression of FTO is in the brain4, and it was recently shown that the central nervous system plays a key role in genetic obesity susceptibility.[43] However, it is far from understood by which mechanisms the FTO gene regulates appetite and these pathways should be further explored.

The main strength of the present study is the large size of the genotyped population in which REE and substrate oxidation rates were measured with indirect calorimetry (n=1,246). Indirect calorimetry is a highly extensive and time-consuming measurement and was therefore previously performed in much smaller study populations. The RQ range resided in the physiological range of 0.67 to 1.2, which indicates a reliable measurement without technical errors.[44] However, it should be realized that the study population is from European-ancestry. The minor allele frequency of rs1421085 is high in populations from European ancestry compared with other ethnical groups.[45] Our results may therefore not be generalizable to populations of other ancestries.

In conclusion, in this large population-based study there was no evidence for an association between the FTO rs1421085 variant and whole-body FatOx measured with indirect calorimetry. Our findings imply that rs1421085-induced obesity is not attributable to decreased FatOx in rs1421085 risk allele carriers. Thus, the strong association between genetic variation in this FTO variant and obesity risk is probably induced via other pathways than via reduced whole-body FatOx. Further research is needed to elucidate the underlying mechanism of the strong association between the FTO region and obesity, in order to advance the search for new targets of anti-obesity intervention strategies.

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Appendix

9.A. Supplementary tables

Table 9.A.1: Association of rs1421085 genotype with measures of body fat.

Characteristic	rs1421085 genotype		
	TT	CT	CC
	Reference		
	Adjusted mean (95% CI)	Difference in measure of body fat compared with reference[a] (95% CI)	
Body mass index (kg/m^2)	26.2 (26.0, 26.4)	0.22 (-0.07, 0.50)	0.56 (0.15, 0.98)
Total body fat (%)	31.3 (31.0, 31.7)	0.08 (-0.39, 0.56)	0.62 (-0.08, 1.32)
Waist circumference (cm)	92.6 (92.0, 93.1)	0.01 (-0.86, 0.87)	1.25 (0.02, 2.49)
Hip circumference (cm)	103.6 (103.2, 104.0)	0.56 (-0.06, 1.17)	1.01 (0.10, 1.92)
Waist-to-hip ratio	0.89 (0.89, 0.90)	-0.005 (-0.011, 0.000)	0.003 (-0.005, 0.010)
Fat mass (kg)	25.2 (24.8, 25.6)	0.29 (-0.34, 0.93)	1.21 (0.28, 2.14)
Fat free mass (kg)	54.6 (54.3, 54.9)	0.25 (-0.15, 0.65)	0.59 (0.05, 1.13)

Results are based on analyses weighted towards the normal BMI distribution, with n=5,744[b]. Models were adjusted for age, sex and population structure.

[a] Beta coefficients from linear regression calculated with respect to the adjusted mean of the reference group (TT genotype carriers).

[b] Missing data: n=30 for total body fat, n=5 for waist circumference, n=5 for hip circumference, n=5 for waist-to-hip ratio, n=30 for fat mass, n=30 for fat free mass.

Table 9.A.2: Association of rs1421085 genotype with measures of body fat, in the random subsample of participants in which indirect calorimetry was performed.

Characteristic	rs1421085 genotype		
	TT	CT	CC
	Reference		
	Adjusted mean (95% CI)	Difference in measure of body fat compared with reference[a] (95% CI)	
Body mass index (kg/m^2)	26.5 (26.1, 27.0)	0.01 (-0.64, 0.66)	0.58 (-0.24, 1.40)
Total body fat (%)	31.3 (30.6, 32.0)	0.16 (-0.92, 1.25)	1.21 (-0.11, 2.54)
Waist circumference (cm)	93.0 (91.6, 94,3)	-0.51 (-2.44, 1.43)	0.97 (-1.64, 3.59)
Hip circumference (cm)	104.2 (103.3, 105.1)	-0.06 (-1.40, 1.27)	0.37 (-1.46, 2.21)
Waist-to-hip ratio	0.89 (0.88, 0.90)	-0.005 (-0.017, 0.007)	0.006 (-0.009, 0.021)
Fat mass (kg)	25.5 (24.5, 26.5)	0.22 (-1.24, 1.67)	1.44 (-0.49, 3.36)
Fat free mass (kg)	54.9 (54.4, 55.5)	0.49 (-0.28, 1.26)	0.29 (-0.90. 1.48)

Results are based on analyses weighted towards the normal BMI distribution, with n=1,246[b]. Models were adjusted for age, sex and population structure.

[a] Beta coefficients from linear regression calculated with respect to the adjusted mean of the reference group (TT genotype carriers).

[b] Missing data: n=8 for total body fat, n=1 for waist circumference, n=1 for hip circumference, n=1 for waist-to-hip ratio, n=8 for fat mass, n=8 for fat free mass.

Table 9.A.3: Association of rs1421085 genotype with measures of body fat, in the random subsample of participants in which indirect calorimetry was performed.

Characteristic	rs1421085 genotype		
	TT	CT	CC
	Reference		
	Adjusted mean (95% CI)	Difference in measure of body fat compared with reference[a](95% CI)	
REE (kcal/day)	1,494 (1,473, 1,516)	15.5 (-13.5, 44.6)	0.9 (-38.6, 40.4)
REE adjusted for FFM (kcal/day)	1,557 (1,541, 1,574)	3.4 (-18.6, 25.4)	-4.6 (-33.4, 24.3)
REE/FFM (kcal/day/kg FFM)	27.5 (27.2, 27.8)	0.06 (-0.36, 0.47)	-0.05 (-0.58, 0.48)
Respiratory quotient	0.84 (0.83, 0.84)	-0.005 (-0.015, 0.004)	-0.007 (-0.018, 0.005)
Fat oxidation (mg/min)	60.0 (57.4, 62.7)	2.41 (-1.33, 6.15)	2.49 (-2.41, 7.38)
Carbohydrate oxidation (mg/min)	126.2 (120.0, 132.5)	-3.21 (-11.90, 5.48)	-6.12 (-17.43, 5.19)

Results are based on analyses weighted towards the normal BMI distribution, with n=1,246[b]. Models were adjusted for age, sex and population structure.

Models were adjusted for age, sex and population structure.

[a] Beta coefficients from linear regression calculated with respect to the adjusted mean of the reference group (TT genotype carriers).

[b] Missing data: n=8 fat free mass.

FFM, fat free mass; REE, resting energy expenditure.

Table 9.A.4: Association of rs1421085 genotype with measures of REE, RQ, fat oxidation and carbohydrate oxidation, stratified by tertiles of BMI.

Characteristic	BMI tertile[a]	rs1421085 genotype		
		TT	CT	CC
		Reference		
		Adjusted mean (95% CI)	Difference in measure of REE compared to reference[b](95% CI)	
REE (kcal/day)	1	1,433 (1,409, 1,457)	12.7 (-19.8, 45.2)	-4.8 (-48.2, 38.6)
	2	1,569 (1,536, 1,601)	23.7 (-17.9, 65.3)	-3.9 (-62.4, 54.6)
	3	1,771 (1,730, 1,812)	-1.8 (-57.7, 54.0)	-15.3 (-85.9, 55.2)
REE adjusted for FFM (kcal/day)	1	1,517 (1,491, 1,543)	2.5 (-25.2, 30.3)	-6.9 (-42.0, 28.2)
	2	1,574 (1,544, 1,604)	5.5 (-33.0, 44.0)	-3.2 (-57.2, 50.8)
	3	1,667 (1,631, 1,703)	7.4 (-39.4, 54.2)	-18.8 (-79.3, 41.7)
REE/FFM (kcal/day/kg FFM)	1	27.3 (26.9, 27.6)	-0.01 (-0.55, 0.54)	-0.07 (-0.75, 0.60)
	2	27.8 (27.2, 28.5)	-0.07 (-0.85, 0.70)	-0.06 (-1.09, 0.98)
	3	28.7 (28.1, 29.2)	0.17 (-0.61, 0.94)	-0.30 (-1.29, 0.70)
Respiratory quotient	1	0.84 (0.83, 0.85)	-0.006 (-0.019, 0.006)	-0.009 (-0.024, 0.007)
	2	0.84 (0.83, 0.85)	-0.003 (-0.016, 0.011)	-0.006 (-0.024, 0.013)
	3	0.83 (0.82, 0.84)	-0.006 (-0.020, 0.008)	0.002 (-0.015, 0.019)
Fat oxidation (mg/min)	1	57.4 (53.8, 60.9)	2.75 (-2.12, 7.63)	2.93 (-3.46, 9.32)
	2	61.5 (57.4, 65.7)	0.89 (-4.89, 6.67)	2.07 (-6.32, 10.45)
	3	73.4 (68.2, 78.7)	2.31 (-4.89, 9.52)	-0.87 (-9.62, 7.87)
Carbohydrate oxidation (mg/min)	1	121.6 (113.4, 129.8)	-4.60 (15.91, 6.70)	-8.31 (-23.05, 6.43)
	2	136.2 (127.6, 144.9)	2.16 (-10.70, 15.03)	-5.96 (-23.67, 11.76)
	3	143.9 (132.6, 155.2)	-6.19 (-21.72, 9.33)	-0.64 (-19.23, 17.94)

Results are based on analyses weighted towards the normal BMI distribution, with n=1,246[c].Models were adjusted for age, sex and population structure.

[a] BMI range: tertile 1 (n=416) 18.21-28.14 kg/m^2; tertile 2 (n=415) 28.15-31.16 kg/m^2, tertile 3 (n=415) 31.17-55.93 kg/m^2.

[b] Beta coefficients from linear regression calculated with respect to the adjusted mean of the reference group (TT genotype carriers).

[c] Missing data: n=8 fat free mass.

BMI, body mass index; FFM, fat free mass; REE, resting energy expenditure.