FTO Variant, Energy Intake, Physical Activity and Basal Metabolic Rate in Caucasians. The HAPIEE Study

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Summary

The *FTO* gene variants are the most important genetic determinants of body weight and obesity known so far, but the mechanism of their effect remains unclear. We have analyzed *FTO* rs17817449 variant (G>T in first intron) in 6024 adults aged 45-69 years to assess the potential mediating role of diet and physical activity. Diet was assessed by a 140-item food frequency questionnaire. Physical activity was measured by hours spent during a typical week by sport, walking and other activities outside of work requiring heavy and medium physical activity. Basal metabolic rate was calculated according Schofield formula. The *FTO* variant was significantly associated with body mass index (means in GG, GT and TT carriers were 28.7, 28.2 and 27.8 kg/m^2, $p<0.001$) and basal metabolic rate (BMR) (means in GG, GT and TT were 1603, 1588 and 1576 kcal per day, respectively, $p<0.008$) but it was not associated with physical activity, total energy intake or with energy intakes from fat, carbohydrates, proteins or alcohol. Results were essentially similar in men and women and the adjustment for physical activity or dietary energy intake did not reduce the effect of the *FTO* polymorphism. Means of BMR per kg of body weight was lowest in GG carriers (20.09, 20.21 for GT and 20.30 for TT, $p<0.006$) and this effect was more pronounced in females. These results suggest that the effect of the *FTO* rs17817449 variant on BMI in Caucasian adults is not mediated by energy intake or physical activity, but some effect on BMR per kg of body weight is possible.

Key words

FTO • Polymorphism • Body mass index • Energy expenditure • Physical activity • Basal metabolic rate

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Introduction

Obesity is one of the major global health problems. The prevalence of obesity increased dramatically over the last decades, and it is estimated that currently only one third of the population in the most affected countries has a normal (18-25 kg/m^2) body mass index (BMI).

Generally, obesity results from the interactions between unhealthy life style (abundant energy intake and low physical activity) and genetic predispositions, although some other factors such as non-exercise activity thermogenesis, sleeping behavior, higher inside temperature comfort, or different environmental chemicals and psychosocial factors play also an important role (Hubacek 2009, Adámková *et al.* 2009).

It has been estimated that genetic factors explain about 40-60 % of the variation in obesity (Wardle *et al.* 2008, Hainer *et al.* 2008). Recently, many new genes associated with obesity have been identified using genome wide association studies (for example see Thorleifsson *et al.* 2009). Among the newly detected genes, particular interest is focused on the *FTO* gene, the first locus unequivocally associated with BMI values. *FTO* ("fat mass and obesity related", gene ID 79068,

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OMIM accession number 610966) gene (and its SNPs) was detected by several research groups as a significant predictor of body mass index (Dina *et al.* 2007, Frayling *et al*. 2007, Tönjes *et al.* 2010). The *FTO* gene variants are associated with BMI in a wide range of populations, and the effect is not restricted to Caucasians (Dina *et al.* 2007, Frayling *et al.* 2007, Hunt *et al.* 2008, Hubacek *et al.* 2008, Hubacek *et al.* 2009); it affects BMI also in Asian (Ohashi *et al.* 2007, Tan *et al.* 2008) and African populations (Hennig *et al.* 2009, Liu *et al.* 2010).

FTO is widely expressed in almost all tissue, but the exact role of the FTO is unknown. The FTO exhibits slight DNA demethylase (Gerken *et al.* 2007) and non-heme dioxygenase activities (Sanchez-Pulido and Andrade-Navarro, 2007), but no direct function in energy homeostasis has been described. It has been shown that *Fto* knock-out mice develop postnatal growth retardation with a significant reduction of adipose tissue. The extreme leanness of *Fto* knock-out mice was a consequence of increased energy expenditure (despite the decreased activity of the animals and increased energy intake (Fischer *et al.* 2009). In humans, reports on the potential impacts of *FTO* variants on dietary intake and/or energy expenditure have been inconsistent; this inconsistency has in some cases been caused by a insufficient number of analyzed individuals and low statistical power of the studies (Speakman *et al.* 2008, Cecil *et al.* 2008, Berentzen *et al.* 2008, Timpson *et al.* 2008, Johnson *et al.* 2009, Hakanen *et al.* 2009, Haupt *et al.* 2009, Liu *et al.* 2010, Hasselbalch *et al.* 2010).

We have examined the hypothesis that BMI associated *FTO* variant is related to elevated energy intake, lower physical activity performed or with basal metabolic rate in a large population sample of middle-aged adults.

Material and Methods

Subjects

The study subjects come from the Czech part of the HAPIEE (Health, Alcohol and Psychosocial factors In Eastern Europe) project which examined random population samples of men and women aged 45-69 years in seven Czech towns: Jihlava, Havirov, Hradec Kralove, Karvina, Kromeriz, Liberec and Usti nad Labem. Details of the study have been described elsewhere (Peasey *et al.* 2006). Briefly, 8856 individuals have been recruited (response rate 55 %), 6681 (3079 males and 3602 females) of which provided a blood sample and have DNA material available. Participants completed an extensive questionnaire on their medical history, health status, life style, diet and socioeconomic and psychosocial factors, underwent a short examination, including anthropometry, and provided a blood sample. After validation of the dietary intake information (Boylan *et al.* 2009), 6024 individuals with complete data on all required variables were statistically analyzed in details. The study was approved by the local ethics committees at both Czech National Institute of Public health and University College London.

Genetic analysis

DNA was extracted using salting out method (Miller *et al.* 1988), and *FTO* SNP rs17817449 was genotyped using PCR – RFLP as described elsewhere (Hubacek *et al.* 2008). To ensure accuracy of genotyping, one plate (containing 94 DNA samples) was analyzed twice within one week with 100 % conformity. Call rate for genotyping was 96 %.

Dietary intake and BMR

Dietary intake was assessed by a 140-item food frequency questionnaire (FFQ) (Willett *et al.* 1985, Brunner *et al.* 2001). The intake of total energy, fats, carbohydrates, proteins and alcohol is estimated from the FFQ, using the McCance and Widdowson's *The Composition of Foods* (Food Standards Agency, 2002) and correcting for major differences in composition of principal foods and local foods and recipes (Boylan *et al.* 2009). Basal metabolic rate was calculated according Schofield (1985).

Physical activity

Physical activity has been assessed using questions on the number of hours spent by participants during a typical week in sports, walking and other activities outside of work requiring heavy or medium physical activity (2 questions). The potential confounding effect of physical disability was taken into account using the physical functioning module of the SF-36 questionnaire (Wagner *et al.* 1998).

Statistical analyses

Deviation of genotypes distributions from Hardy-Weinberg equilibrium were analyzed using the chi-square test (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls). One-way analysis of variance in STATA statistical

Table 1. Baseline characteristics for analyzed individuals (N=6024).

	All subjects	Males	Females
N	6024	2780	3244
Age (years)	58.1 ± 6.9	58.5 ± 6.9	57.7 ± 6.9
BMI (kg/m^2)	28.2 ± 4.6	28.2 ± 3.9	28.1 ± 5.1
WHR	0.885 ± 0.085	0.944 ± 0.059	0.834 ± 0.069
Diabetes	657 (10.9 %)	359 (12.9 %)	298 (9.2 %)
Current smoking	1531 (25.7 %)	793 (28.8 %)	738 (23.0 %)
Total energy intake	2038 ± 710	2103 ± 705	1982 ± 709
Total fat energy intake	742 ± 299	772 ± 308	717 ± 289
Total carbohydrate energy intake	886 ± 353	868 ± 330	901 ± 372
Total protein energy intake	354 ± 130	369 ± 134	342 ± 126
Total energy intake in alcohol	60 ± 99	101 ± 125	26 ± 49
*Physical activity (h/week)**	4.4 ± 5.3	4.4 ± 5.4	4.4 ± 5.2
Basal metabolic rate	1587 ± 237	1794 ± 164	1412 ± 118
BMR/kg of body weight	20.21 ± 2.18	21.01 ± 1.85	19.54 ± 2.21

* h/week in sports/games/walking/hiking. Data are given like numbers / percents or mean ± SD. Energy intakes are in kcal/day.

software (version 9; College Station, TX) was used for statistical analyses comparing dietary intake, BMR and physical activity between individuals with different genotypes. Mean ± SD are reported for each exposure and genotype group, and P-values less than 0.05 were considered to be significant.

Results

Study subjects

The basic characteristics of the groups under study are summarized in Table 1. The distribution of individual genotypes is within Hardy-Weinberg equilibrium (P=0.07) and is similar to the neighboring European samples reported previously (Dina *et al.* 2007, Frayling *et al.* 2007).

The total energy intake per day was higher in males than in females (2103±705 kcal vs 1982±709 kcal) and this difference was caused generally by higher fat and ethanol intake in males (Table 1), while physical activity was similar in males and females. As expected, basal metabolic rate and basal metabolic rate per kg of body weight were also higher in males.

Consistently with the results described in original articles (Dina *et al.* 2007, Frayling *et al.* 2007, for reviewed see Fawcett and Barroso 2010) rs17817449 G allele was significantly associated with obesity related phenotype (BMI) in a total combined sample (P=0.0001) as we have described before (Hubacek *et al.* 2008) and the presence of the G allele increase the BMI in linear trend both in males (P<0.005) and females (P<0.001). There were no significant associations of the *FTO* gene SNP with waist-hip ratio.

Association between FTO SNP and energy intake, physical activity and basal metabolic rate

Associations between *FTO* genotype and variables of interest are summarized in Table 2. *FTO* SNP rs17817449 was not associated with the mean values of the total energy intake. Similarly, total fat, carbohydrates, protein and alcohol energy intakes are independent on *FTO* genotypes in entire population or in males or females.

Basal metabolic rate was higher in GG homozygotes (P=0.008) and the effect was more pronounced in females (P=0.02) than in males (P=0.08). Interestingly, the basal metabolic rate per kilogram of body height was highest in carriers of the TT genotype, but this trends was observed only in females (P=0.006), not in males (P=0.42).

Mediating effect of physical activity, dietary intake and basal metabolic rate

Finally, we have examined whether the effect of the *FTO* genotype on BMI is statistically explained (attenuated) by energy intake, physical activity and basal metabolic rate. We did so by adjusting the effects of the *FTO* genotype on BMI for the potential mediators.

Table 2. Association between *FTO* rs17817449 variant, energy intake, physical activity and basal metabolic rate in HAPIEE Slavic Czech population.

	FTO			
	GG	**GT**	**TT**	**P-value**
N	1, 157	2, 886	1, 981	
ENTIRE POPULATION				
Total energy intake	2050 ± 712	2031 ± 702	2040 ± 721	0.75
Total fat energy intake	753 ± 300	739 ± 298	740 ± 301	0.38
Total carbohydrate energy intake	886 ± 352	881 ± 347	892 ± 363	0.59
Total protein energy intake	357 ± 13	355 ± 130	352 ± 131	0.61
Total energy intake in alcohol	59 ± 101	61 ± 101	60 ± 96	0.82
*Physical activity**	4.3 ± 5.1	4.4 ± 5.3	4.4 ± 5.4	0.94
Basal metabolic rate (BMR)	1603 ± 243	1588 ± 235	1576 ± 236	**0.008**
BMR/kg of body weight	20.09 ± 2.20	20.21 ± 2.16	20.30 ± 2.18	**0.03**
MALES				
Total energy intake	2078 ± 697	2104 ± 698	2117 ± 722	0.60
Total fat energy intake	771 ± 303	774 ± 312	769 ± 305	0.94
Total carbohydrate energy intake	855 ± 314	865 ± 322	880 ± 352	0.34
Total protein energy intake	363 ± 131	370 ± 135	371 ± 136	0.49
Total energy intake in alcohol	98 ± 128	101 ± 126	103 ± 121	0.73
*Physical activity**	4.3 ± 5.2	4.4 ± 5.5	4.3 ± 5.4	0.79
Basal metabolic rate	1808 ± 169	1792 ± 161	1788 ± 163	0.08
BMR/kg of body weight	20.92 ± 1.91	21.01 ± 1.84	21.06 ± 1.83	0.42
FEMALES				
Total energy intake	2024 ± 724	1968 ± 700	1977 ± 714	0.25
Total fat energy intake	738 ± 297	709 ± 281	716 ± 296	0.12
Total carbohydrate energy intake	914 ± 381	895 ± 368	901 ± 371	0.56
Total protein energy intake	352 ± 129	341 ± 125	337 ± 125	0.07
Total energy intake in alcohol	24 ± 44	26 ± 51	26 ± 48	0.54
*Physical activity**	4.4 ± 5.0	4.4 ± 5.2	4.4 ± 5.4	0.99
Basal metabolic rate	1419 ± 121	1414 ± 120	1404 ± 113	**0.02**
BMR/kg of body weight	19.33 ± 2.17	19.52 ± 2.19	19.69 ± 2.25	**0.006**

* h/week in sports/games/walking/hiking. Data are given like numbers / percents or mean ± SD. Energy intakes are in kcal/day.

The results are shown in Table 3. Adjustment for physical activity and energy intake did not attenuate the effect of the *FTO* genotype (neither did adjustments for energy from specific macro-nutrients, not shown in table).

Discussion

This large population-based study provides robust evidence that dietary intake of energy and physical activity are not associated with the *FTO* genotype, and they do not mediate the *FTO* gene effect on body mass index in Caucasian adults. We also found an association between the *FTO* gene variant and basal metabolic rate per kilogram of body weight, and basal metabolic rate also statistically "explained" part of the association between the *FTO* genotype and body mass index.

The major limitation of our data related to the self-reported dietary intake. Despite continuous quality control, self reported dietary intake assessment is likely to be inaccurate. Firstly, most people underestimate there

Table 3. Association between the *FTO* genotype and BMI after adjustment for physical activity, total energy intake and basal metabolic rate; the figures are linear regression coefficient (95 % confidence interval) for the differences between carriers of TT and GT genotypes, compared with GG (reference).*

	FTO			
	GG	**GT**	**TT**	**P-value**
ENTIRE POPULATION				
Crude effect	0.0	–0.42 (–0.72,–0.11)	–0.84 (–1.17,–0.51)	<0.001
Adj. for energy intake	0.0	–0.42 (–0.72,–0.11)	–0.84 (–1.17,–0.51)	<0.001
Adj. for physical activity	0.0	–0.42 (–0.73,–0.11)	–0.84 (–1.16,–0.51)	<0.001
Adj. for BMR	0.0	–0.12 (–0.30,0.06)	–0.37 (–0.56,–0.17)	<0.001
Adj. for BMR/kg	0.0	–0.18 (–0.35,–0.01)	–0.28 (–0.46,–0.10)	0.01
MALES				
*Crude effect**	0.0	–0.31 (–0.70,0.08)	–0.64 (–1.06,–0.22)	0.01
Adj. for energy intake	0.0	–0.31 (–0.70,0.08)	–0.64 (–1.05,–0.22)	0.01
Adj. for physical activity	0.0	–0.29 (–0.68,0.10)	–0.60 (–1.01,–0.18)	0.02
Adj. for BMR	0.0	–0.10 (–0.33,0.13)	–0.34 (–0.59,–0.09)	0.01
Adj. for BMR/kg	0.0	–0.20 (–0.44,0.03)	–0.34 (–0.60,–0.09)	0.03
FEMALES				
*Crude effect**	0.0	–0.48 (–0.95,–0.02)	–0.99 (–1.48,–0.50)	<0.001
Adj. for energy intake	0.0	–0.50 (–0.96,–0.03)	–1.00 (–1.49,–0.51)	<0.001
Adj. for physical activity	0.0	–0.51 (–0.98,–0.04)	–1.03 (–1.52,–0.53)	<0.001
Adj. for BMR	0.0	–0.16 (–0.39,0.07)	–0.33 (–0.57,–0.08)	0.03
Adj. for BMR/kg	0.0	–0.14 (–0.38,0.09)	–0.20 (–0.45,0.05)	0.29

* all models adjusted for age

dietary intakes (Schoeller 1995). And secondly, there is a considerable random error in reporting diet. Although underestimation is likely to be similar between carriers of different *FTO* genotypes, obese individuals are more prone to such underestimation (Westerterp and Goris 2002) and this may obscure differences in dietary intakes between individuals with different *FTO* genotypes. Random misclassification would further dilute any association. Similar argument may apply to physical activity. Although the large size of the study would, to some extent, counterbalance the influence of misclassification, it is nevertheless possible that the lack of association between *FTO* and energy intake and physical activity is due to imprecise measurement of diet and physical activity.

Our results are in contrast with the study performed by Speakman and colleagues (2008) who did not find a significant association between *FTO* and basal metabolic rate, however, they include 150 individuals only and the study is clearly underpowered to detect the effect which is according to our study, expected to be just about 20-25 kcal/kg/day between homozygotes.

The *FTO* gene variants were originally described to be associated with BMI values in Caucasians. The gene for the *FTO* codes for the protein with enzymatic activities that do not seem to have a direct effect on energy management; moreover, described enzymatic activities are detectable but low. In general, there are two easy possibilities suggested how the *FTO* gene can influence body weight: either through energy intake or through energy expenditure.

However, the possible associations of the *FTO* gene variants with the preference of energy dense food, higher fat intake or markers of physical activity in previous studies are inconsistent, and the exact mechanism of the effect of *FTO* on higher BMI/obesity development remains elusive.

The lack of the association between energy intake and physical activity performed in our study is in agreement with the previous findings of Hakanen *et al.*

(2009) who did not find an association between energy intake and physical activity at age 15, with data from Liu *et al.* (2010) who failed to detect an association between energy intake and physical activity in almost two thousands adults, and with a large study of 756 twin pairs which also failed to associate *FTO* variant with energy intake or physical activity (Hasselbalch *et al.* 2010). Dietary energy intake was also not associated with *FTO* variant in a group of about 2300 prepubertal children (Johnson *et al.* 2009). Finally, Berentzen *et al.* (2008) did not detect an association between energy expenditure or energy intake and *FTO* genotypes.

However, there have also been positive reports. The obesity associated allele was associated with increased energy intake (but not with the weight of food ingested) in a small sample of 97 children (Cecil *et al.* 2008), in 150 Germans who underwent an lifestyle intervention program (Haupt *et al.* 2009); finally, Timpson *et al.* (2008) have observed a significant effect of *FTO* variant on the both total energy and fat daily energy intake on much larger group of 3500 children. The *FTO* genotype was also found to be associated with lower satiety responsiveness and elevated enjoyment of food in children (Wardle *et al.* 2008).

Overall, the literature does not provide a strong support for an effect of the *FTO* alleles on physical activity and that the effect on dietary energy intake, if any, was detected only in pre-pubertal and pubertal children but not in adults. Not in all studies were analyzed identical SNPs but it is unlikely that this fact can explain the obtained discrepancies – all variants have been associated with mean BMI in a similar fashion and all SNPs within the *FTO* first intron locus are in almost complete linkage disequilibrium.

In our study, carriers of the GG genotype (rs 17817449), which is associated with elevated BMI, had slightly lower basal metabolic rate per kilogram of body weight. Previous studies, to our knowledge, did not report such association. A small effect on resting metabolic rate was suggested in the Quebeck Family study (Do *et al.* 2008), but it was in the opposite direction than in our study (i.e. higher RMR was observed in the obesity-associated genotype) and it disappeared after adjustment for fat composition. Haupt *et al.* (2009) also did not observe an association between *FTO* and basal metabolic rate. However, both studies mentioned above were very small, with 50 and 150 participants, respectively. Our observation may be a numerical artifact – basal metabolic rate was calculated from age, sex, height and weight (rather than measured directly); it is therefore possible that its strong association with body mass index, and its mediating effect on the *FTO*-body mass index relationship, is just due to the fact that it has been estimated from the same variables as body mass index. Our finding needs to be confirmed by other studies, preferably using directly measured basal metabolic rate.

In the light of the literature, and our study, the exact mechanisms of the *FTO* effect on body weight remain unknown. There is another potential mechanism how the *FTO* could affect body weight, namely increased lipolysis (Wåhlén *et al.* 2008) but empirical evidence on this hypothesis is not available. At present, the possibility should be considered that the variants within the first introns of *FTO* are in strong linkage disequilibrium not just with each other but also with variants in neighboring genes – for example, the gene for retinitis pigmentosa GTPase regulator-interacting protein like 1 was suggested (Fawcet and Barroso 2010), although the association of this gene with energy metabolism remains speculative.

In conclusion, our study confirms previous findings that the *FTO* genotype is not associated with energy intake (and extend this finding also on total fat, carbohydrates, protein and alcohol energy intakes) or physically activity. We have detected that it may have an effect on basal metabolic rate per kilogram of body weight but the issue of a numerical artifact needs to be clarified, and further studies should investigate whether the effect of *FTO* variants on basal metabolic rate per kilogram of body weight is a plausible explanation for the *FTO* effect on body weight.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

BMI, body mass index; CR, call rate; *FTO*, fat mass and obesity related; MAF, minor allele frequency; SNP, single nucleotide polymorphisms; WHR, waist hip ratio

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