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The impact of obesity-related SNP on appetite and energy intake

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Abstract

An increasing number of studies have reported a heritable component for the regulation of energy intake and eating behaviour, although the individual polymorphisms and their 'effect size' are not fully elucidated. The aim of the present study was to examine the relationship between specific SNP and appetite responses and energy intake in overweight men. In a randomised cross-over trial, forty overweight men (age 32 (SD 09) years; BMI 27 (SD 2) kg/m^2) attended four sessions 1 week apart and received three isoenergetic and isovolumetric servings of dairy snacks or water (control) in random order. Appetite ratings were determined using visual analogue scales and energy intake at an *ad libitum* lunch was assessed 90 min after the dairy snacks. Individuals were genotyped for SNP in the fat mass and obesity-associated (*FTO*), leptin (*LEP*), leptin receptor (*LEPR*) genes and a variant near the melanocortin-4 receptor (*MC4R*) locus. The postprandial fullness rating over the full experiment following intake of the different snacks was 17·2 % (P=0·026) lower in A carriers compared with TT homozygotes for rs9939609 (*FTO*, dominant) and 18·6 % (P=0·020) lower in G carriers compared with AA homozygotes for rs7799039 (*LEP*, dominant). These observations indicate that *FTO* and *LEP* polymorphisms are related to the variation in the feeling of fullness and may play a role in the regulation of food intake. Further studies are required to confirm these initial observations and investigate the 'penetrance' of these genotypes in additional population subgroups.

Key words: **Appetite: Genotype: Fat mass and obesity-associated gene: Leptin: Leptin receptor: Melanocortin-4 receptor**

Although obesity is generally associated with lifestyle factors, degree of adiposity is thought to have a significant heritable component[1]. SNP in several genes encoding for proteins involved in the hypothalamic control of food intake, energy balance and consequently management of body weight[2] have been associated with common (non-Mendelian) obesity[3]. The fat mass and obesity-associated (*FTO*), melanocortin-4 receptor (*MC4R*), leptin (*LEP*) and leptin receptor (*LEPR*) genes regulate food intake and energy homeostasis[4] through their actions on the leptin–melanocortin pathway in the hypothalamus[5], and variants in these loci regions have been identified as genetic risk factors for common obesity.

Genetic variation in *FTO* was the first common SNP related to BMI, with AA homozygotes for a SNP (rs9939609) in the first intron of the *FTO* gene having a 1·7-fold increased risk of obesity compared with TT individuals[6]. Consistent associations between identified SNP located 188 kb near *MC4R* and obesity have been found in genome-wide association studies[7]. Each copy of the rs17782313 C allele in the *MC4R* gene was associated with a 0·2 kg/m^2 increase in BMI. Furthermore, although genetic variation in the *LEP* gene and that of its receptor *LEPR* was not identified to be associated with obesity-related traits in genome-wide association studies, a link to obesity has been reported in several candidate gene studies[8–11].

However, the physiological basis for these genotype–adiposity associations is poorly understood. Given the fact that obesity is a disorder of energy imbalance between energy intake and expenditure, several studies have demonstrated that the SNP (rs9939609) in the *FTO* gene contributed to variations in energy intake[12–17]. Yet, most of the studies showing a greater energy intake in individuals carrying the risk allele were conducted in children. In addition, it has been proposed that particular genetic polymorphism in the *MC4R*, *LEP* and *LEPR* genes influences obesity by affecting eating patterns, snacking[18,19] and energy intake[20]. However, there is a paucity of evidence on whether they affect appetite responses (hunger, desire to eat and prospective food consumption)

Abbreviations: *FTO*, fat mass and obesity-associated gene; *LEP*, leptin gene; *LEPR*, leptin receptor gene; *MC4R*, melanocortin-4 receptor gene.

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including satiety (the feeling of fullness that influences the time interval between meals). The aim of the present study was to assess the effect of SNP in the *FTO*, *LEP*, *LEPR* and *MC4R* genes on postprandial appetite responses and *ad libitum* energy intake from a lunchtime meal in overweight men. The present analysis was conducted using data from a previously published acute appetite study, the primary aim of which was to examine the effect of consumption of individual dairy products as snacks on appetite[21].

Subjects and methods

Study population

A total of forty healthy, non-smoking overweight men, aged 18–50 years with a BMI of 25·0–29·9 kg/m^2, were recruited from the local Reading area. Subjects were excluded if they: had food allergies or irregular eating patterns; were athletes who trained >10 h/week; were cognitively dietary restrained eaters (Three-Factor Eating Questionnaire[22], factor 1 > 11), non-breakfast consumers or non-snack consumers; had any dislike of the 'study' foods or had blood pressure and biochemical measurements outside the 'normal' range (blood pressure >160/100 mmHg, and plasma total cholesterol >8·0 mmol/l, glucose <5 or >7 mmol/l, TAG >1·8 mmol/l, alanine transaminase >45 U/l (0.75 μkat/l) or γ-glutamyltransferase >55 U/l (0.92 μkat/l)) after a 12 h overnight fast. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the University of Reading Research and Ethics Committee. Subjects gave written informed consent before commencing the study.

Postprandial study design

A randomised within-subject experimental design was performed, with each subject returning for four separate test sessions in the Hugh Sinclair Nutrition Unit at least 1 week apart. After a 12 h overnight fast, appetite profile was assessed (baseline) using a visual analogue scale rating of hunger (how hungry do you feel?), desire to eat (how strong is your desire to eat?), fullness (how full do you feel?) and prospective food consumption (how much do you think you could eat right now?) anchored by the terms 'not at all' and 'extremely'. These four questions were also reflected in an average appetite score, which was calculated at each time point for each treatment as appetite (mm) = (desire to eat + hunger + (100 − fullness) + prospective consumption)/4[23]. A standardised light breakfast was provided at 09·00 h (0 min) and appetite was assessed at 10, 60, 115, 125, 145, 165, 185, 205 and 230 min. The light breakfast consisted of two oat cereal bars with strawberry filling (Nutrigrain soft baked bars; Kellogg's) and orange juice (250 ml; Sainsbury's), which together had an energy content of 1456 kJ and provided 60·5 g carbohydrate, 3 g protein and 7 g fat. The breakfast provided 15 % of the energy intake of an average UK male[24]. The dairy snacks were semi-skimmed milk (Cravendale; Arla Foods), a natural set biopot yogurt (Dr Oetker) or a mild Cheddar cheese (Sainsbury's), and provided the same energy (841 kJ) and volume (410 ml). Full details of the macronutrient composition of the dairy snacks have been previously reported[21]. Briefly, dairy composition was as follows: milk (28, 41 and 31 % of energy from protein, carbohydrate and fat, respectively); yogurt (22, 32 and 46 % of energy from protein, carbohydrate and fat, respectively); cheese (25 and 75 % of energy from protein and fat, respectively). The fourth treatment was an isovolumetric serving (compared with milk) of non-carbonated water. Non-carbonated water was ingested separately with cheese and yogurt in order to equate the volume of milk. Energy intake was assessed by an *ad libitum* lunch provided 90 min after the dairy snacks or water control. The lunch consisted of one main course composed of pasta, tomato and basil sauce and Parmesan cheese (2811·9 kJ/476·5 g of serving portion). Subjects were instructed to eat only until they felt comfortably satisfied and were given 20 min to consume the meal. Subjects ate individually in the dining room of the Unit for the entire length of time, and *ad libitum* food intake was monitored by determining total food consumed (g) and energy consumed (kJ).

DNA isolation and SNP genotyping

Genomic DNA was isolated from the leucocyte buffy coat layer, which was taken at screening, using the QIAamp DNA Mini Kit (Qiagen Limited). Genotyping was performed on four SNP (rs9939609 (*FTO*) in chromosome 16q12.2, rs7799039 (*LEP*) in 7q31.3, rs1137101 (*LEPR*) in 1p31 and rs17782313 (*MC4R*) in 18q21) by conducting allelic discrimination using a TaqMan Genotyping 'Assay-on-Demand' (Applied Biosystems). The percentage of replicate quality-control samples used for genotyping was 8 % with a >99 % concordance rate. The call rate was higher than 99·5 % and the genotype distribution obeyed Hardy–Weinberg equilibrium (P>0·05).

Statistical analyses

The agreement of allele frequency with Hardy–Weinberg equilibrium was assessed for all SNP using a χ^2 analysis. The genetic model of inheritance was based on the number of copies of an allele needed for increased susceptibility and was described as follows: (1) additive, the susceptibility is increased having 0, 1 and 2 copies of the risk allele with the risk of 0 alleles <1 allele <2 alleles; (2) recessive, 2 copies of the risk allele are needed; (3) dominant, 1 or 2 copies of the risk allele are equally related to the likelihood of possessing a trait. The model of inheritance for each SNP that fits the data best was selected when comparisons between the additive, recessive and dominant models for each SNP were made. The genotypic effect of the four SNP on the baseline-adjusted, self-reported appetite scores or energy intake was evaluated by the PROC MIXED procedure (SAS Institute, Inc. 1992). Random effects of subject and subject × time interactions and fixed effects of treatment (dairy snacks or water), visit and SNP on appetite score and energy intake were tested and adjusted for BMI and age. The first-order

autoregressive (AR (1)) covariance structure was selected for the appetite scores and variance components were selected for the energy intake based on goodness-of-fit criteria. The number of subjects was insufficient to test SNP interactions for the appetite scores and energy intake. Further backward stepwise analysis was conducted by checking the significance of the fixed effects or their interactions and including in the models only the significant effects. All models were tested for the normality of residuals. Standard diagnostics were used to ensure that all variables meet the normal distribution assumption. Statistical analyses were performed using SAS (release 9.2; SAS Institute, Inc.). Differences were considered statistically significant at $P<0{\cdot}05$ (two-tailed). Data are presented as means with their standard errors, unless otherwise indicated.

Results

All forty men completed the study. The mean age and BMI of the study participants was 32·1 (SD 9·1) years and 26·8 (SD 1·6) kg/m^2. The mean average appetite rating of 51·9 (SD 24·9) mm (out of a possible 100 mm) and an *ad libitum* lunch intake of 3978·7 (SD 1444·0) kJ were evident for the group as a whole (data not shown). The genotype distribution of the four examined SNP all obeyed Hardy–Weinberg equilibrium ($P>0{\cdot}05$) and their allele frequencies are provided in Table 1.

Effect of SNP on responses of hunger, desire to eat, fullness, prospective food consumption and energy intake at lunch

Mean postprandial responses of appetite according to SNP using backward stepwise analyses are presented in Table 2. There was no detectable difference between the genotype groups for the *LEPR* or *MC4R* SNP with respect to postprandial appetite responses. The mean ratings of hunger and desire to eat over the full experiment following intake of the different snacks were 23·9 % ($P=0{\cdot}019$) and 21·8 % ($P=0{\cdot}046$) higher in A carriers compared with TT homozygotes for *FTO* (dominant model), respectively. The fullness score was 17·2 % ($P=0{\cdot}026$) lower in A carriers compared with TT homozygotes for *FTO* (dominant model) and 18·6 % ($P=0{\cdot}020$) lower in G carriers compared with AA homozygotes for *LEP* (dominant model). The most notable effect was a genotype difference in prospective food consumption, with A and G carriers displaying 26·0 % ($P=0{\cdot}008$) and 19·1 % ($P=0{\cdot}028$) higher prospective food consumption compared with TT individuals in *FTO* and AA individuals in *LEP*, respectively. The average appetite, as a summary measure of the four specific appetite responses, was 11·0 (SE 4·5) mm ($P=0{\cdot}015$) higher in A carriers compared with TT homozygotes for *FTO* (dominant model) and 8·0 (SE 4·2) mm lower in G carriers compared with AA homozygotes for *LEP* (dominant model) without reaching significance ($P=0{\cdot}057$). The four individual SNP did not have an effect on *ad libitum* energy intake at lunch (Table 2).

Similar results on the phenotypes were observed in the backward stepwise analysis by checking the significance of the fixed effects or their interactions and including in the models only the significant effects (data not shown).

Discussion

A heritable component for the regulation of appetite and eating behaviour has been reported, although the individual polymorphisms, their 'effect size' and the molecular mechanisms underlying genotype–phenotype associations are not fully elucidated[4]. The present study investigated for the first time the effect of *FTO*, *LEP*, *LEPR* and *MC4R* variants on measurements of appetite and energy intake at an *ad libitum* lunch, in a 'fit for purpose' appetite research laboratory setting. The main finding was that primarily a *FTO* and to a lesser extent *LEP* polymorphisms were associated with an overall reduced appetite (based on the four phenotypes) and more specifically with perceptions of fullness and prospective food consumption.

While numerous studies, largely conducted in children, have examined the effect of the *FTO* rs9939609 SNP on food intake, dietary energy density and macronutrient selection[12,14,25], very few have examined its impact on appetite[16,26]. Wardle *et al.*[16] showed that children homozygous for the purported risk allele (AA) had reduced satiety scores, and these results have recently been replicated in adults by den Hoed *et al.*[26]. Carriers of the risk allele (TA/AA) had increased hunger (OR 3·02, 95 % CI 1·26, 7·24) and decreased satiety (OR 2·02, 95 % CI 1·26, 7·24) relative to TT homozygotes[26]. This is in agreement with the present results, since TT homozygotes had higher fullness and lower hunger and prospective food consumption scores compared with A carriers.

Table 1. Distribution of genotypes and alleles
(Number of subjects and percentages)

		Genotype frequency									Allele frequency						
Genes	SNP		*n*	%		*n*	%		*n*	%		*n*	%		*n*	%	*P**
FTO	rs9939609 T>A	TT†	11	27·5	TA	17	42·5	AA‡	12	30·0	T	39	48·7	A	41	51·3	0·35
LEP	rs7799039 -2548 G > A	AA†	12	30·0	GA	16	40·0	GG‡	12	30·0	G	40	50	A	40	50	0·21
LEPR	rs1137101 668A > G	AA†	9	22·5	AG	21	52·5	GG‡	10	25·0	A	39	48·7	G	41	51·3	0·75
MC4R	rs17782313 T > C	TT†	18	45·0	TC	20	50·0	CC‡	2	5·0	T	56	70·0	C	24	30·0	0·95

FTO, fat mass and obesity-associated gene; *LEP*, leptin gene; *LEPR*, leptin receptor gene; *MC4R*, melanocortin-4 receptor gene.
* *P* value for the χ^2 analysis of Hardy–Weinberg equilibrium.
† Wild type.
‡ Homozygote for the risk allele.

Table 2. Mean appetite responses, using repeated 100 mm visual analogue scale ratings, over the whole study and mean energy intake (kJ) following intake of dairy snacks or water according to gene variants
(Mean values with their standard errors)

			Genotypes						
Phenotype*	SNP	Model†		Mean	SE		Mean	SE	*P*‡
Hunger§	*FTO*	Dominant	TT	40·2	4·4	TA/AA	51·1	2·7	0·019
	LEP	Dominant	AA	43·0	4·2	GG/GA	48·3	2·7	0·215
	LEPR	Dominant	AA	48·4	4·5	AG/GG	42·9	2·6	0·245
	MC4R	Dominant	TT	42·7	3·6	TC/CC	48·6	3·3	0·149
Desire to eat‖	*FTO*	Dominant	TT	43·0	5·0	TA/AA	53·5	3·1	0·046
	LEP	Dominant	AA	44·5	4·8	GG/GA	52·0	3·1	0·129
	LEPR	Dominant	AA	50·2	5·1	AG/GG	46·3	3·0	0·473
	MC4R	Dominant	TT	45·3	4·1	TC/CC	51·2	3·7	0·199
Fullness¶	*FTO*	Dominant	TT	54·3	3·7	TA/AA	45·7	2·1	0·026
	LEP	Dominant	AA	54·0	3·7	GG/GA	44·8	2·4	0·020
	LEPR	Recessive	AA/AG	57·6	2·6	GG	47·2	3·7	0·300
	MC4R	Dominant	TT	50·4	3·3	TC/CC	48·4	2·7	0·583
Prospective consumption**	*FTO*	Dominant	TT	43·3	4·2	TA/AA	56·2	2·9	0·008
	LEP	Dominant	AA	45·0	4·0	GG/GA	54·5	2·7	0·028
	LEPR	Recessive	AA/AG	49·3	2·9	GG	50·3	4·1	0·838
	MC4R	Dominant	TT	47·5	3·6	TC/CC	52·0	3·0	0·273
Average appetite††	*FTO*	Dominant	TT	42·8	4·3	TA/AA	53·8	2·6	0·015
	LEP	Dominant	AA	44·3	4·1	GG/GA	52·3	2·7	0·057
	LEPR	Dominant	AA	49·8	4·4	AG/GG	46·9	2·5	0·533
	MC4R	Dominant	TT	45·8	3·5	TC/CC	50·8	3·2	0·205
Energy intake (kJ)	*FTO*	Recessive	TT/TA	4200	307	AA	3766	374	0·335
	LEP	Dominant	AA	3744	391	GG/GA	4222	285	0·290
	LEPR	Dominant	AA	4297	433	AG/GG	3669	250	0·195
	MC4R	Dominant	TT	4131	343	TC/CC	3836	317	0·473

FTO, fat mass and obesity-associated gene; *LEP*, leptin gene; *LEPR*, leptin receptor gene; *MC4R*, melanocortin-4 receptor gene.
* Appetite responses (hunger, desire to eat, fullness and prospective consumption) were assessed using a visual analogue scale and energy intake at the lunchtime meal.
† The genetic model of inheritance that fits the data best.
‡ *P* value for the difference between the genotypes after adjustment for baseline appetite scores, visit, treatment, BMI, age, and *FTO*, *LEP*, *LEPR* and *MC4R* SNP.
§ How hungry do you feel?
‖ How strong is your desire to eat?
¶ How full do you feel?
** How much do you think you could eat right now?
†† Average appetite calculated as (desire to eat + hunger + (100 − fullness) + prospective consumption)/4.

The role of leptin and its receptor in the regulation of energy homeostasis is well established, with studies demonstrating that functional mutations in the *LEP* and *LEPR* genes underlie a proportion of severe early-onset obesity cases[27,28]. However, the current epidemic of obesity cannot be explained by these rare monogenic mutations, and data in the literature considering the associations between common polymorphisms in the rs7799039 (*LEP*) or rs1137101 (*LEPR*) genes and polygenic obesity phenotypes are inconsistent[11,29–31]. The present study is one of the few that have examined the impact of leptin and its receptor gene variants on appetite responsiveness. Ratings of fullness and prospective food consumption were found to be different between the genotype groups for *LEP* with a significant increase in feelings of fullness and suppressed prospective food consumption in AA homozygotes relative to G carriers. This is in contrast with the two studies by den Hoed *et al.*[26,32], in which *LEP* was not associated with hunger and satiety[26] and where GG homozygotes felt more hungry compared with GA, but not AA individuals[32]. Additionally, den Hoed *et al.*[26] showed that AA individuals had lower hunger ratings compared with carriers of the risk allele in *LEPR*, while in the present study, there was no effect of *LEPR* on appetite. It has been proposed that *LEPR* variants may affect the transcription of leptin depending on the circulating concentration of leptin[10]. However, circulating leptin concentration is influenced by BMI, body fat mass, sex and hormonal status[10,33]. Thus, this discrepancy among the studies, although hard to explain, might be due to the relatively small homogeneous sample size in the present study (overweight men) compared with the larger more heterogeneous populations with respect to BMI distribution (from 19 to 31 kg/m^2), sex (both sexes) and body fat mass in the other two studies.

Both *LEP* and *MC4R* genes are involved in the regulation of appetite and food intake through the leptin–melanocortin pathway[5]. Despite the role of the *MC4R* in the regulation of energy intake and its association with obesity[34], there are controversial results regarding the association between the rs17782313 (*MC4R*) variant and intakes of total dietary energy and fat in human subjects[20,35,36]. In most studies, dietary energy intake was assessed using self-reported FFQ, which are prone to a number of limitations and errors. However, even with more valid and accurate measurement of intake in the present study, the *MC4R* genotype did not emerge as a significant determinant of *ad libitum* energy consumption.

There are a limited number of studies examining the impact of genotype on appetite responsiveness, with no data available to base valid power calculations. Although the strength of the present study is the careful phenotypic characterisation of our volunteers with respect to appetite regulation, a limitation is the relatively small number of subjects, which may result in limited power to detect more subtle effects of the minor allele relative to the wild-type genotype.

In conclusion, the present preliminary investigations have shown that *FTO* and *LEP* polymorphisms are related to a feeling of fullness and decreased prospective food consumption. Further research is warranted to validate these novel findings, to investigate the interactions between individual gene variants and the interactions between variants and dietary nutrients, to identify the best-fitting model of inheritance, to provide an insight into the underlying physiological mechanisms for the genotype–phenotype associations and to investigate the 'penetrance' of these genotypes in additional population subgroups.

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