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ABSTRACT

Background: The fat mass and obesity-associated gene (FTO) rs9939609 A-allele has been associated with obesity risk. Although the exact mechanisms involved remain unknown, the FTO rs9939609 A-allele has been associated with an impaired postprandial suppression of appetite. **Objectives:** To explore the influence of FTO rs9939609 genotype on fasting and postprandial appetite-related hormones and perceived appetite in a heterogeneous sample of men and women. **Design:** 112 healthy men and women aged 18-50-years-old completed three laboratory visits for the assessment of FTO rs9939609 genotype, body composition, aerobic fitness, resting metabolic rate, visceral adipose tissue, liver fat, fasting leptin, and fasting and postprandial acylated ghrelin, total PYY, insulin, glucose and perceived appetite. Participants wore accelerometers for seven consecutive days for the assessment of physical activity and sedentary behaviour. Multivariable general linear models quantified differences between FTO rs9939609 groups for fasting and postprandial appetite outcomes, with and without the addition of *a priori* selected physiological and behavioural covariates. Sex-specific univariable Pearson's correlation coefficients were quantified between the appetite-related outcomes and individual characteristics. **Results:** 95% confidence intervals for mean differences between FTO rs9939609 groups overlapped zero in unadjusted and adjusted general linear models for all fasting ($P \geq 0.28$) and postprandial ($P \geq 0.19$) appetite-related outcomes. Eta^2 values for explained variance attributable to FTO rs9939609 were <5% for all outcomes. An exploratory correlation matrix indicated that associations between fasting and postprandial acylated ghrelin, total PYY and general or abdominal adiposity were also small (r = -0.23 to 0.15, $P \geq 0.09$). Fasting leptin, glucose and insulin and postprandial insulin concentrations were associated with adiposity outcomes (r = 0.29 to 0.81, $P \leq 0.033$). **Conclusions:** Associations between the FTO rs9939609 genotype and fasting or postprandial appetite-related outcomes were weak in healthy men and women.

Keywords: FTO, appetite, ghrelin, PYY, hunger.

INTRODUCTION

The scientific understanding of appetite control has increased considerably in recent decades, which has been helpful in elucidating the complex nature of energy balance and weight control. Central components of the homeostatic control of appetite comprise signals from adipose tissue and peptide hormones secreted from the digestive tract, which act acutely and/or chronically on central neural pathways to influence hunger, satiety and subsequent energy intake (MacLean et al. 2017). These signals and hormones include the tonic signals leptin and insulin that regulate long-term changes in energy balance and adiposity status, as well as a variety of episodic gut signals, which mediate hunger and satiety on a meal-by-meal basis (Blundell et al. 2008, 2015a; MacLean et al. 2017). Notable among the episodic mediators of appetite and energy intake are acylated ghrelin and peptide YY (PYY) which exert orexigenic and anorexigenic effects, respectively, to facilitate meal initiation and termination (Neary and Batterham, 2009).

Over the last 16 years, our laboratory has measured circulating concentrations of appetite-related hormones in response to meal ingestion in many studies. A consistent observation from this body of work is the degree of variability in responses observed between participants studied under identical conditions. Furthermore, using the "gold standard" replicated crossover study design (Atkinson and Batterham, 2015; Senn, 2016), we have demonstrated recently the presence of true interindividual heterogeneity in appetite perceptions and circulating concentrations of acylated ghrelin, total PYY, insulin and glucose in response to a standardised meal, over and above any random within-subject variability and measurement error (Goltz et al. 2019). Similar findings were also observed in acylated ghrelin, total PYY and perceived appetite responses to replicated single bouts of aerobic exercise (Goltz et al. 2018).

The factors responsible for interindividual variability in appetite-related hormone concentrations are not fully understood, but it is plausible that differences in individual characteristics and behaviours may contribute to the variability observed. In this regard, the fat mass and obesity-associated gene (FTO) has been associated with obesity risk, with individuals homozygous for the A allele (AA) of FTO rs9939609 having a 1.7-fold higher obesity risk than individuals homozygous for the T allele (TT) (Frayling et al. 2007). Although the exact mechanisms through which FTO rs9939609 influences fat mass accumulation remain unknown, it has been suggested that it exerts its effect on food intake rather than on energy expenditure (Speakman et al. 2008). Furthermore, rs9939609 AA individuals have been shown to exhibit an attenuated postprandial suppression of hunger and acylated ghrelin compared with TT individuals, which may

predispose AA individuals to higher energy intake and, consequently, higher fat mass (Karra et al. 2013). However, the study by Karra and colleagues was performed in young healthy weight males and it is not known whether this influence of the FTO rs9939609 gene on postprandial appetite regulation is observed in a heterogenous sample of men and women.

Beyond genetic influence, it has been speculated that other individual factors may affect appetite regulation. Data from previous studies have indicated that women exhibit higher fasting concentrations of acylated ghrelin than men in those who were lean (Alajmi et al. 2016; Douglas et al. 2017) and in those who were overweight/obese (Douglas et al. 2017). Furthermore, an inverse relationship between general adiposity levels and fasting ghrelin levels has been suggested in study samples including individuals who were lean and individuals who were obese, possibly because of elevated insulin or leptin levels (Tschöp et al. 2001; Shiiya et al. 2002; Sondergaard et al. 2009). Individuals who are obese also exhibit a reduced postprandial suppression of ghrelin (Le Roux et al. 2005) and blunted postprandial increases in PYY (Le Roux et al. 2006). Limited evidence has also suggested an inverse association between visceral adipose tissue and fasting ghrelin levels in women who were lean and women who were obese, likely caused by substances secreted by visceral adipocytes, such as TNFα and leptin (Sondergaard et al. 2009). Moreover, fat-free mass, as the largest contributor to resting metabolic rate, has been identified as a key driver of appetite and energy intake in individuals who were lean and in individuals who were obese (Blundell et al. 2015b). In a systematic review including studies in individuals with normal weight, overweight or obesity, physical activity has also been suggested to alter the sensitivity of the appetite control system by enhancing meal-induced satiety which may facilitate energy balance over the long term (Beaulieu et al. 2016). Together, these findings highlight the importance of investigating the effect of the FTO rs9939609 gene on appetite parameters in a sample of males and females with a wide range of age, adiposity and physical activity levels, including physiological and behavioural characteristics as covariates in the analyses.

The primary aim of this study was to use objective assessment methods in order to explore the influence of the FTO rs9939609 genotype on fasting and postprandial appetite-related hormones and perceived appetite in a sample of healthy men and women. The secondary aim was to explore potential associations between fasting and postprandial appetite outcomes and physiological and behavioural characteristics.

METHODS

Participants

With the approval of the University Ethics Advisory Sub-Committee, a total of 121 participants (57 men, 64 women) aged 18 to 50 years provided written informed consent before taking part in the study. All participants were deemed to be stable in their body mass (≤ 3 kg change in the previous 3 months), non-smokers, habitual breakfast eaters, had no history of cardiovascular or metabolic disease, and were not dieting or taking any medications known to influence the outcome measures. Female participants were premenopausal and postmenopausal and not pregnant. Nine participants withdrew from the study before completing all study measurements due to time constraints. Therefore, data are presented for 112 participants (56 men, 56 women) in this manuscript. The study sample self-reported ethnicity distribution was as follows: 93% white Europeans, 6% Asians and 1% black.

Visit 1: Preliminary testing

Participants attended the laboratory for a preliminary visit to confirm eligibility, and to undergo familiarisation, anthropometric measurements and determination of peak oxygen uptake ($\dot{V}O_2$ peak). The eligibility assessment included screening questionnaires to assess health status and food preferences and/or restrictions. Stature was measured to the nearest 0.1 cm and body mass to the nearest 0.1 kg using an electronic measuring station (Seca, Hamburg, Germany), and body mass index (BMI) was calculated. The sum of three skinfolds (chest, abdomen and thigh for men, and triceps, suprailiac and thigh for women) was used to estimate body density (Jackson and Pollock 1978, 1980) and body fat percentage (Siri, 1961). All skinfold measurements were performed by the same experienced examiner throughout the study. Waist circumference was measured as the narrowest point between the lower rib margin and the iliac crest.

Participants were familiarised with walking and running on the treadmill (Technogym Excite Med, Cesena, Italy) before completing an incremental uphill treadmill protocol to determine $\dot{V}O_2$ peak. The participants ran at a fixed individualised speed (4.5 to 14.0 $km \cdot h^{-1}$), with the initial gradient of the treadmill set to 0%. The treadmill gradient was increased by 1% every minute until volitional exhaustion. Heart rate was monitored continuously using short-range telemetry (Polar A3, Kempele, Finland), and ratings of perceived exertion (Borg, 1973) were recorded at the end of each minute. Expired air samples were monitored continuously using a breath-by-breath gas analysis system (Cortex Metalyser 3B, Leipzig, Germany). An average of the breath-

147 by-breath oxygen uptake data was taken every 10 s, and $\dot{V}O_2$ peak was defined as the highest 30
148 s rolling average.

149 **Visit 2: Magnetic resonance imaging (MRI) scan**

150 Each participant underwent an MRI scan in the supine position using a dual-echo Dixon fat and
151 water sequence on a 3-T MRI scanner (MR750w, GE Healthcare, Chicago, USA). A detailed
152 description of the protocol has been reported previously (Borga et al. 2015; West et al. 2016).
153 Briefly seven overlapping image stacks were acquired from the neck to knee with stacks covering
154 the abdomen (stacks 2 to 5) acquired during breath-hold. Additional abdominal slices were
155 acquired with the IDEAL-IQ sequence to assess proton density fat fraction in the liver. Scans
156 were analysed to quantify visceral adipose tissue, abdominal subcutaneous adipose tissue and
157 liver fat fraction using the AMRA Profiler (AMRA Medical AB, Linköping, Sweden) (Borga et
158 al. 2015; West et al. 2016).

159 **Visit 3: Resting metabolic rate and test meal**

160 All premenopausal female participants completed the main trial during the follicular phase of
161 the menstrual cycle (days 6-12) to avoid potential hormonal influences on appetite parameters.
162 Participants were asked to refrain from caffeine, alcohol, and strenuous exercise during the 24 h
163 before the main trial. A standardised evening meal (3297 kJ, 40% fat, 39% carbohydrate, 21%
164 protein) was consumed the evening before the main trial and only plain water was permitted after
165 the meal until participants arrived at the laboratory the next day.

166 Participants reported to the laboratory at 08:00 after fasting overnight for 12 h. A cannula
167 (Venflon; Becton Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein for
168 venous blood sampling, and participants rested for 60 min to eliminate any stress effects in
169 response to the cannula (Chandarana et al. 2009). During this time, resting metabolic rate was
170 measured using an open circuit indirect calorimetry system (GEM Nutrition Ltd., Cheshire,
171 England). Participants were asked to lie in a comfortable supine position and were instructed not
172 to talk or sleep, and to move as little as possible during the measurement. The clear hood canopy
173 was placed over the head area, and plastic sheeting attached to the hood was placed around the
174 body to form a seal between the air inside and outside the hood. Oxygen uptake, carbon dioxide
175 production, respiratory exchange ratio and energy expenditure were determined at 30 s intervals
176 over a 30 min period. The first 10 min of data was discarded to account for any initial short-term
177 respiratory artefact.

A fasting venous blood sample and rating of perceived appetite were taken 60 min after the insertion of the cannula. Participants then consumed a standardised breakfast within 15 min marking the start of the postprandial assessment period (09:00; 0 h). Breakfast consisted of a ham and cheese sandwich, milkshake and chocolate biscuit which provided 4435 kJ of energy (41% carbohydrate, 18% protein, 41% fat). Subsequent venous blood samples and ratings of perceived appetite were taken at 0.5, 1 and 2 h after the start of the breakfast whilst the participants rested in a semi-supine position.

Appetite perceptions

Appetite perceptions (hunger, satisfaction, fullness, prospective food consumption) were assessed using 100 mm visual analogue scales (Flint et al. 2000). An overall appetite rating was calculated as the mean value of the four appetite ratings once satisfaction and fullness were reverse-scored (Stubbs et al. 2000).

Blood sampling and biochemical analysis

Venous blood samples were collected into pre-chilled EDTA monovettes (Sarstedt, Leicester, UK) for the determination of plasma acylated ghrelin, total PYY, leptin, insulin and glucose concentrations. Monovettes for acylated ghrelin also contained *p*-hydroxymercuribenzoic acid to prevent the degradation of acylated ghrelin by protease and were centrifuged at 2,383 *g* for 10 min at 4°C (Burkard, Hertfordhire, UK). The plasma supernatant was aliquoted into a storage tube and 100 µL of 1 M hydrochloric acid was added per millilitre of plasma. Samples were re-centrifuged at 2,383 *g* for 5 min at 4°C before being transferred into Eppendorf tubes and stored at -80°C for later analysis. Monovettes for total PYY, leptin, insulin and glucose were centrifuged immediately at 2,383 *g* for 10 min at 4°C prior to storage at -80°C. Haemoglobin concentration and haematocrit were quantified in duplicate at 0 and 2 h to estimate the acute change in plasma volume (Dill and Costill, 1974).

Commercially available enzyme-linked immunosorbent assays were used to determine the concentrations of plasma acylated ghrelin (Bertin Bioreagent, Montigney le Bretonneux, France), total PYY (Millipore, Billerica, MA, USA), leptin (R&D Systems, Minneapolis, MN, USA) and insulin (Mercodia, Uppsala, Sweden). Plasma glucose concentrations were determined by enzymatic, colorimetric methods using a benchtop analyser (Pentra 400, HORIBA Medical, Montpellier, France). The within-batch coefficient of variation for acylated ghrelin, total PYY, leptin, insulin and glucose concentrations were 4.3%, 5.1%, 8.3%, 4.7%, 0.4%, respectively.

An additional fasting venous blood sample was collected into a 2.7-mL EDTA monovette (Sarstedt, Leicester, UK) and the whole blood sample was stored at 4°C to undergo DNA extraction and genotyping. Genomic DNA was extracted from the whole blood samples using the QIAamp DNA Mini kit (QIAGEN, Hilden, Germany). The samples were genotyped for the rs9939609 allele within the FTO gene using the Applied Biosystems TaqMan® (Roche Molecular Systems, Pleasanton, California, USA) genotyping assay and real-time polymerase chain reaction system. Participants were assigned to one of three groups according to their genotype: homozygous major allele, TT (36%; males n = 23, females n = 17); heterozygous allele, AT (45%; males n = 22, females n = 29); or homozygous minor allele, AA (19%; males n = 11, females n = 10). Genotype frequency of FTO rs9939609 was assessed using a goodness-of-fit chi-square test and did not deviate from Hardy-Weinberg equilibrium (χ^2 = 0.435, P = 0.509).

Habitual physical activity and sedentary time

Participants wore an ActiGraph GT3X+ accelerometer (ActiGraph, Pensacola, USA) on an elasticated belt on the waist above the mid-line of the thigh on their non-dominant side of the body. The device was initialised at a frequency of 100HZ and downloaded using ActiLife software v6.11.8 and firmware version 2.0.0. ActiGraph data were downloaded in 60-seconds epochs and physical activity was classified as low, light and moderate-to-vigorous. Participants also wore an activPAL3 accelerometer (PAL Technologies Ltd., Glasgow, UK), attached directly to the skin on the midline of the anterior aspect of the thigh in line with the ActiGraph GT3X+ accelerometer. The activPAL3 determines posture using information derived from accelerations of the thigh, including the gravitational component, using a triaxial accelerometer (Atkin et al. 2012). The activPAL3 is a valid measure of time spent sitting/lying, standing, and walking in adults (Kozey-Keadle et al. 2011). ActivPAL3 sitting time data were retrieved and clustered into 60-seconds epochs using a customized spreadsheet. Participants were advised to wear both devices concurrently and continuously over a 7-day period. Non-wear time and sleep time were removed from the analysis and moderate-to-vigorous physical activity (MVPA) and sitting time data were averaged over the seven-day period.

Statistical analyses

We estimated the effect size detection sensitivity given our sample size using NQuery (version 3, Statistical Solutions, Cork, Ireland). For a total sample size of 110 and three study groups, we

estimated that a "medium" (Cohen, 1998) Eta^2 value of 0.18 would be detected in a univariable model as statistically significant ($P < 0.050$) with power of 90%.

Postprandial overall appetite and plasma concentrations of acylated ghrelin, total PYY, insulin and glucose are presented relative to baseline values (delta) to minimise the potential influence of day-to-day biological variability (Deighton et al. 2013, 2014). Total area under the curve (AUC) values were calculated using the trapezoidal method. Correction of blood parameter concentrations for acute changes in plasma volume had a negligible influence on our findings and, therefore, the unadjusted plasma concentrations are displayed for simplicity.

Multivariable general linear models were used to quantify the mean differences (and 95% confidence intervals) between FTO rs9939609 genotype groups for each fasting and postprandial appetite outcome. The eta-squared statistic (with associated 90% confidence interval) was also estimated for each model and each outcome (Kline, 2004; Steiger, 2004). This statistic is interpreted in a similar way as the coefficient of determination, where 100 x eta-squared gives the explained variance attributable to the FTO groups. A 90% rather than a 95% confidence interval is reported because the eta-squared statistic can only be positive in sign. The model residuals of the appetite outcome variables were explored for parity to a Gaussian distribution using histograms. The model residuals for fasting acylated ghrelin and insulin concentrations were observed to show a positively skewed distribution so these data were logarithmically-transformed prior to analysis (Bland and Altman, 1996). Three models were used for each of the fasting and postprandial appetite outcomes, as follows:

1. Model I: Univariable models with FTO rs9939609 genotype as single fixed effect;
2. Model II: A multivariable model based on the selection of matched covariates studied by Karra et al. (2013), i.e., age, fat mass and visceral adipose tissue. FTO rs9939609 genotype was entered as a fixed effect and sex, age, fat mass and visceral adipose tissue were entered as covariates;
3. Model III: A multivariable model, where FTO rs9939609 genotype was entered as a fixed effect and sex, age, BMI, $\dot{V}O_2$ peak, resting metabolic rate, visceral adipose tissue, abdominal subcutaneous adipose tissue, liver fat, sitting time and MVPA were entered as covariates. Rather than the now discouraged use of stepwise selection procedures, these covariates were included based on their hypothesised influence on the outcome variables, while considering the potential that some predictors were mathematically coupled (Flom and Cassell, 2007; Whittingham et al. 2006). For

example, total fat mass was excluded from this model because multiple specific adiposity parameters were considered.

The covariates in models II and III were each standardised prior to analysis by dividing each datum by twice the respective SD (Gelman and Pardoe, 2007). In sensitivity analyses, model III was also run with (i) waist circumference replacing BMI; (ii) percentage body fat replacing BMI; and (iii) with a sex-by-genotype interaction term.

Univariable general linear models with FTO rs9939609 genotype as a single fixed effect were used to quantify differences between genotype groups for body mass, BMI and fat mass. Between-sex differences in participant characteristics and appetite-related outcomes in the fasting and postprandial states were assessed using univariable general linear models with sex as a single fixed effect. Sex-specific univariable Pearson's correlation coefficients were quantified between appetite-related outcomes and individual characteristics, and between appetite-related blood parameters and perceived appetite.

95% confidence intervals (95% CI) were quantified for correlation coefficients. P-values are expressed in exact terms apart from very low values, which are expressed as $P < 0.001$. A threshold of statistical significance was accepted as $P < 0.050$, although we deemed a P value of < 0.005 as a stronger indication of potentially more reproducible results in line with recent advice (Benjamin et al. 2017). All statistical analyses were performed in SPSS (v.23, IBM Corporation, New York, USA).

RESULTS

Missing data

Due to technical issues with the equipment, resting metabolic rate is presented for 107 participants (53 males), sitting time for 96 participants (47 males) and MVPA for 100 participants (49 males). Eleven participants were unable to undertake the MRI scan for safety reasons and, therefore, visceral adipose tissue and abdominal subcutaneous adipose tissue are presented for 101 participants (50 males). Liver fat could not be quantified from some images due to motion artefacts and, therefore, data is presented for 97 participants (48 males).

Participant characteristics and appetite-related outcomes

Participant characteristics, perceived appetite and appetite-related blood parameters in the fasting and postprandial states are presented in Table 1. Postprandial delta values for acylated

304 ghrelin, total PYY, insulin and glucose concentrations and perceived overall appetite are
305 presented in Figure 1.

Table 1. Participant characteristics and appetite outcomes in the fasting and postprandial states.

	All (n = 112)	Range (min to max)	Men (n = 56)	Women (n = 56)	*P*	Mean difference 95% CI
Age (years)	34 (9)	18 to 50	35.3 (9.7)	33.5 (9.1)	0.303	-5.4 to 1.7
Stature (cm)	171.0 (9.2)	149.1 to 200.4	178.5 (6.6)	165.3 (6.2)	**< 0.001**	-15.6 to -10.8
Body mass (kg)	74.9 (14.7)	48.5 to 140.4	83.3 (12.9)	66.5 (11.1)	**< 0.001**	-21.2 to -12.2
Body mass index ($kg \cdot m^{-2}$)	25.2 (3.9)	18.4 to 40.3	26.1 (3.7)	24.4 (4.0)	**0.016**	-3.2 to -0.3
Waist circumference (cm)	82.7 (10.8)	62.4 to 125.0	88.4 (9.8)	77.0 (8.7)	**< 0.001**	-14.9 to -8.0
Fat mass (kg)	16.9 (8.4)	3.5 to 47.8	15.5 (9.1)	18.2 (7.4)	0.078	-0.3 to 5.9
Fat free mass (kg)	58.1 (12.2)	36.8 to 92.6	67.8 (8.8)	48.3 (5.5)	**< 0.001**	-22.2 to -16.8
$\dot{V}O_2$ peak ($mL \cdot kg \cdot min^{-1}$)	44.0 (9.3)	21.0 to 81.0	49.0 (9.3)	39.0 (6.1)	**< 0.001**	-13.0 to -7.1
Resting metabolic rate (kcal)*	1617 (322)	889 to 2567	1808 (290)	1430 (232)	**< 0.001**	-478 to -277
Visceral adipose tissue (L)*	1.70 (1.26)	0.11 to 6.22	2.27 (1.41)	1.14 (0.75)	**< 0.001**	-1.58 to -0.69
Abdominal subcutaneous adipose tissue (L)*	5.39 (3.02)	1.45 to 16.86	4.49 (2.39)	6.27 (3.33)	**0.003**	0.64 to 2.93
Liver fat (%)*	2.12 (1.81)	0.46 to 10.45	2.62 (2.19)	1.63 (1.16)	**0.006**	-1.69 to -0.28
Sitting time ($min \cdot day^{-1}$)*	509 (85)	256 to 737	513 (73)	504 (95)	0.630	-43 to 26
MVPA ($min \cdot day^{-1}$)*	55 (31)	11 to 163	57 (30)	54 (33)	0.706	-15 to 10
Fasting leptin ($ng \cdot mL^{-1}$)	8.62 (8.63)	1.34 to 43.85	4.07 (3.08)	13.16 (9.95)	**< 0.001**	6.33 to 11.84
Fasting acylated ghrelin ($pg \cdot mL^{-1}$)	173.6 (491.8)	12.0 to 4410.6	103.3 (108.8)	243.8 (682.9)	0.131	-42.6 to 323.6
Fasting total PYY ($pg \cdot mL^{-1}$)	117.5 (50.5)	13.6 to 270.0	121.9 (47.9)	113.0 (53.1)	0.353	-27.8 to 10.0
Fasting insulin ($pmol \cdot L^{-1}$)	23.3 (15.0)	2.9 to 97.1	22.9 (14.3)	23.6 (15.8)	0.825	-5.0 to 6.3
Fasting glucose ($mmol \cdot L^{-1}$)	5.24 (0.43)	4.29 to 6.56	5.37 (0.43)	5.12 (0.39)	**0.001**	-0.41 to -0.10
Fasting overall appetite (mm)	70.8 (15.3)	19 to 95	71.2 (13.4)	70.4 (17.1)	0.787	-6.5 to 5.0
Acylated ghrelin delta AUC (2 h, $pg \cdot mL^{-1}$)	-87.9 (126.6)	-1183.5 to 165.8	- 51.3 (56.3)	- 124.6 (162.6)	**0.002**	-118.9 to -27.8
Total PYY delta AUC (2 h, $pg \cdot mL^{-1}$)	101.6 (61.0)	-26.4 to 340.7	99.0 (62.4)	104.2 (59.9)	0.653	-17.7 to 28.1
Insulin delta AUC (2 h, $pg \cdot mL^{-1}$)	420.6 (236.8)	121.3 to 1485.8	403.9 (256.6)	437.3 (216.3)	0.458	-55.5 to 122.2
Glucose delta AUC (2 h, $pg \cdot mL^{-1}$)	0.77 (1.59)	-2.20 to 5.79	0.54 (1.37)	1.00 (1.77)	0.125	-0.13 to 1.05
Overall appetite delta AUC (2 h, $pg \cdot mL^{-1}$)	-77.4 (34.4)	-150.0 to -14.0	-65.7 (30.9)	-89.1 (34.0)	**< 0.001**	-35.5 to -11.1

Values are mean (SD). *P* values and 95% CI are from univariable general linear models with sex as a single fixed effect.
* n = 107 (53 males) for resting metabolic rate, 96 (47 males) for sitting time, 100 (49 males) for MVPA, 101 (50 males) for visceral adipose tissue and abdominal subcutaneous adipose tissue, and 97 (48 males) for liver fat.
AUC, area under the curve; CI, confidence interval; MVPA, moderate-to-vigorous physical activity, PYY, peptide YY; $\dot{V}O_2$ peak, peak oxygen uptake.

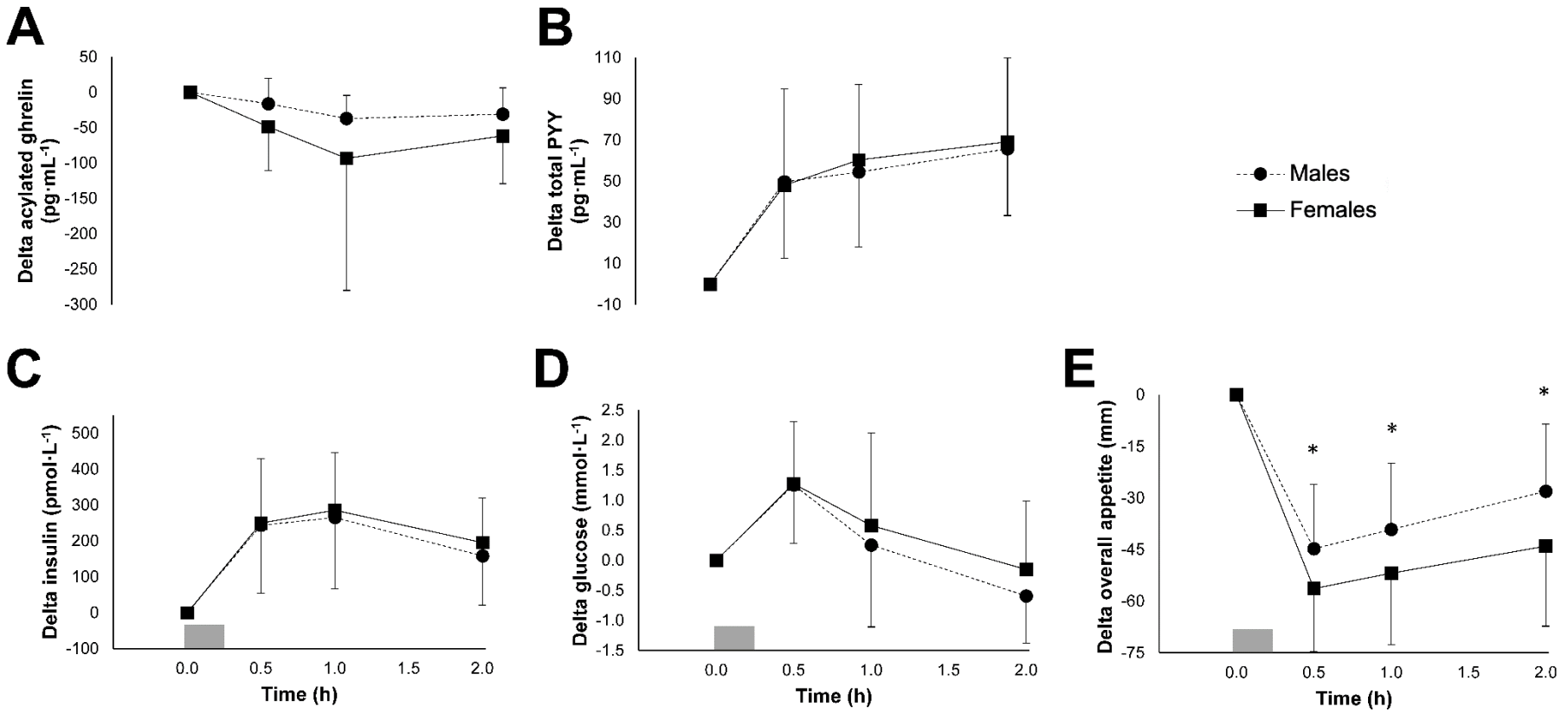

Figure 1. Delta postprandial values for acylated ghrelin (A), total peptide YY (PYY) (B), insulin (C), glucose (D) and overall perceived appetite (E) in 56 males and 56 females. Grey rectangles indicate meal consumed within 15 min. Values are presented as mean (SD). Linear mixed models identified main effects of sex for delta acylated ghrelin, delta glucose and delta overall appetite ($P \leq 0.045$), main effects of time for all outcomes ($P < 0.001$) and a sex-by-time interaction for delta appetite ($P = 0.004$). * $P < 0.001$ for post-hoc analysis of sex-by-time interaction between males and females.

Univariable and multivariable general linear models

No statistically significant influence of the FTO rs9939609 genotype was identified for body mass ($Eta^2 = 0.027$, $P = 0.234$), BMI ($Eta^2 = 0.003$, $P = 0.688$) or fat mass ($Eta^2 = 0.025$, $P = 0.259$).

Fasting appetite-related outcomes

Separate univariate modelling (model I) did not reveal any statistically significant influence of the FTO rs9939609 genotype on fasting acylated ghrelin, total PYY, insulin, glucose, leptin or overall appetite ($P \geq 0.501$) (Table 2). Similarly, no significant effect of the FTO rs9939609 genotype was detected on fasting appetite-related outcomes in model II ($P \geq 0.098$) or model III ($P \geq 0.453$) (Table 2). All eta-squared values were very low (< 0.05). Replacing BMI with waist circumference, replacing BMI with body fat percentage, and including a sex-by-genotype interaction term in the sensitivity analyses did not result in a significant effect of the FTO rs9939609 genotype on any of the fasting appetite-related outcomes ($P \geq 0.470$, $P \geq 0.437$, $P \geq 0.455$, respectively).

Postprandial appetite-related outcomes

Separate univariate modelling (model I) did not reveal any statistically significant influence of the FTO rs9939609 genotype on delta AUC for acylated ghrelin, total PYY, insulin, glucose, leptin or overall appetite ($P \geq 0.322$) (Table 3). Similarly, no significant effect of the FTO rs9939609 genotype was detected on delta AUC for any of the appetite-related outcomes in model II ($P \geq 0.271$) or model III ($P \geq 0.186$) (Table 3). Again, all eta-squared values were very low (< 0.05). Replacing BMI with waist circumference, replacing BMI with body fat percentage, and including a sex-by-genotype interaction term in the sensitivity analyses did not result in a significant effect of the FTO rs9939609 genotype on any of the postprandial appetite-related outcomes ($P \geq 0.133$, $P \geq 0.102$, $P \geq 0.206$, respectively). A sensitivity analysis was undertaken on all the postprandial outcomes AUC by adding the respective fasting measurement as a covariate to the model. Again, no statistically significant differences between FTO groups could be detected ($P > 0.200$) and mean differences were small.

Table 2. Estimated marginal means from the multivariable general linear models used to quantify the differences between FTO rs9939609 genotype groups in each fasting appetite outcome.

	Model I			Model II			Model III		
	AT (n = 49)	**AA (n = 21)**	**TT (n = 40)**	**AT (n = 45)**	**AA (n = 18)**	**TT (n = 37)**	**AT (n = 34)**	**AA (n = 17)**	**TT (n = 28)**
Fasting acylated ghrelin (log pg·mL^{-1})	4.47 (4.25 to 4.69)	4.59 (4.26 to 4.92)	4.51 (4.27 to 4.75)	4.42 (4.18 to 4.65)	4.57 (4.20 to 4.94)	4.57 (4.30 to 4.83)	4.42 (4.20 to 4.64)	4.56 (4.23 to 4.88)	4.29 (4.03 to 4.54)
	Eta^2 = 0.003 (90% CI: 0.000-0.023), *P* = 0.835			Eta^2 = 0.009 (90% CI: 0.000-0.047), *P* = 0.660			Eta^2 = 0.024 (90% CI: 0.000-0.091), *P* = 0.453		
Fasting total PYY (pg·mL^{-1})	110.3 (96.1 to 124.5)	123.5 (101.8 to 145.2)	120.4 (104.7 to 136.2)	109.2 (94.0 to 124.4)	123.6 (100.2 to 147.0)	122.4 (105.7 to 139.1)	114.3 (97.6 to 130.9)	117.2 (93.3 to 141.0)	114.1 (95.0 to 133.2)
	Eta^2 = 0.013 (90% CI: 0.000-0.055), *P* = 0.501			Eta^2 = 0.018 (90% CI: 0.000-0.069), *P* = 0.434			Eta^2 = 0.001 (90% CI: 0.000-0.014), *P* = 0.977		
Fasting insulin (log pmol·L^{-1})	3.00 (2.83 to 3.16)	2.87 (2.61 to 3.12)	2.97 (2.79 to 3.16)	3.03 (2.88 to 3.19)	2.93 (2.70 to 3.17)	2.96 (2.79 to 3.13)	3.01 (2.81 to 3.20)	2.98 (2.70 to 3.27)	2.95 (2.72 to 3.18)
	Eta^2 = 0.007 (90% CI: 0.000-0.038), *P* = 0.699			Eta^2 = 0.007 (90% CI: 0.000-0.041), *P* = 0.716			Eta^2 = 0.002 (90% CI: 0.000-0.028), *P* = 0.935		
Fasting glucose (mmol·L^{-1})	5.23 (5.11 to 5.36)	5.28 (5.09 to 5.47)	5.22 (5.09 to 5.36)	5.27 (5.15 to 5.38)	5.28 (5.11 to 5.46)	5.14 (5.02 to 5.27)	5.24 (5.10 to 5.38)	5.30 (5.10 to 5.51)	5.16 (5.00 to 5.32)
	Eta^2 = 0.002 (90% CI: 0.000-0.016), *P* = 0.882			Eta^2 = 0.027 (90% CI: 0.000-0.087), *P* = 0.278			Eta^2 = 0.018 (90% CI: 0.000-0.078), *P* = 0.553		
Fasting leptin (ng·mL^{-1})	9.17 (6.70 to 11.65)	8.06 (4.27 to 11.84)	7.95 (5.21 to 10.69)	9.77 (8.15 to 11.39)	6.67 (4.17 to 9.17)	7.93 (6.15 to 9.71)	9.76 (7.91 to 11.62)	8.71 (6.05 to 11.37)	8.72 (6.59 to 10.85)
	Eta^2 = 0.005 (90% CI: 0.000-0.030), *P* = 0.779			Eta^2 = 0.049 (90% CI: 0.000-0.122), *P* = 0.098			Eta^2 = 0.010 (90% CI: 0.000-0.057), *P* = 0.713		
Fasting overall appetite (mm)	70.0 (65.7 to 74.4)	69.6 (63.0 to 76.2)	72.2 (67.4 to 77.0)	67.6 (63.0 to 72.3)	70.2 (63.0 to 77.4)	72.4 (67.3 to 77.6)	66.8 (60.9 to 72.7)	68.9 (60.4 to 77.3)	69.3 (62.5 to 76.0)
	Eta^2 = 0.005 (90% CI: 0.000-0.033), *P* = 0.748			Eta^2 = 0.019 (90% CI: 0.000-0.072), *P* = 0.402			Eta^2 = 0.005 (90% CI: 0.000-0.034), *P* = 0.850		

Model I: Univariable model with FTO rs9939609 genotype as single fixed effect. Model II: Multivariable model with FTO rs9939609 genotype as a fixed effect and sex, age, fat mass and visceral adipose tissue as covariates. Model III: Multivariable model with FTO rs9939609 genotype as a fixed effect and sex, age, body mass index, peak oxygen uptake, resting metabolic rate, visceral adipose tissue, abdominal subcutaneous adipose tissue, liver fat, sitting time and moderate-to-vigorous physical activity as covariates.

Values are mean (95% confidence interval (CI)). Eta^2, 90% CI and *P*-values are from the fixed effect of the FTO rs9939609 genotype group.

Table 3. Estimated marginal means from the multivariable general linear models used to quantify the differences between FTO rs9939609 genotype groups in each postprandial appetite outcome.

	Model I			Model II			Model III		
	AT (n = 49)	**AA (n = 21)**	**TT (n = 40)**	**AT (n = 45)**	**AA (n = 18)**	**TT (n = 37)**	**AT (n = 34)**	**AA (n = 17)**	**TT (n = 28)**
Acylated ghrelin delta AUC (2 h pg·mL^{-1})	-76.0 (-110.8 to -41.2)	-86.3 (-139.5 to -33.1)	-96.3 (-134.9 to -57.8)	-69.5 (-107.1 to -32.0)	-93.1 (-151.1 to -35.0)	-103.2 (-144.5 to -61.8)	-87.4 (-106.9 to -67.9)	-87.0 (-114.9 to -59.0)	-67.8 (-90.2 to -45.4)
	Eta^2 = 0.006 (90% CI: 0.000-0.034), *P* = 0.740			Eta^2 = 0.015 (90% CI: 0.000-0.063), *P* = 0.494			Eta^2 = 0.026 (90% CI: 0.000-0.097), *P* = 0.414		
Total PYY delta AUC (2 h pg·mL^{-1})	101.1 (84.2 to 118.1)	89.7 (63.8 to 115.6)	113.4 (94.7 to 132.2)	98.5 (80.2 to 116.8)	86.5 (58.2 to 114.8)	113.7 (93.5 to 133.8)	103.5 (81.2 to 125.8)	80.4 (48.4 to 112.4)	120.1 (94.4 to 145.7)
	Eta^2 = 0.021 (90% CI: 0.000-0.072), *P* = 0.322			Eta^2 = 0.028 (90% CI: 0.000-0.088), *P* = 0.271			Eta^2 = 0.050 (90% CI: 0.000-0.137), *P* = 0.186		
Insulin delta AUC (2 h pmol·L^{-1})	411 (345 to 476)	404 (303 to 503)	432 (359 to 504)	409 (342 to 477)	415 (311 to 519)	430 (356 to 504)	411 (330 to 492)	429 (313 to 545)	463 (370 to 556)
	Eta^2 = 0.002 (90% CI: 0.000-0.017), *P* = 0.875			Eta^2 = 0.002 (90% CI: 0.000-0.022), *P* = 0.921			Eta^2 = 0.010 (90% CI: 0.000-0.055), *P* = 0.728		
Glucose delta AUC (2 h mmol·L^{-1})	0.66 (0.21 to 1.12)	0.60 (-0.10 to 1.30)	1.01 (0.51 to 1.52)	0.60 (0.19 to 1.02)	0.54 (-0.09 to 1.18)	0.79 (0.34 to 1.25)	0.68 (0.19 to 1.17)	0.44 (-0.26 to 1.14)	0.88 (0.32 to 1.44)
	Eta^2 = 0.012 (90% CI: 0.000-0.054), *P* = 0.511			Eta^2 = 0.006 (90% CI: 0.000-0.036), *P* = 0.766			Eta^2 = 0.013 (90% CI: 0.000-0.066), *P* = 0.642		
Overall appetite delta AUC (2 h mm)	-79.3 (-89.1 to -69.5)	-72.4 (-87.4 to -57.5)	-79.2 (-90.1 to -68.4)	-75.3 (-85.2 to -65.4)	-73.6 (-88.8 to -58.3)	-82.1 (-93.0 to -71.2)	-73.4 (-85.4 to -61.4)	-75.6 (-92.7 to -58.4)	-75.6 (-89.3 to -61.8)
	Eta^2 = 0.006 (90% CI: 0.000-0.036), *P* = 0.718			Eta^2 = 0.012 (90% CI: 0.000-0.056), *P* = 0.568			Eta^2 = 0.001 (90% CI: 0.000-0.021), *P* = 0.965		

Model I: Univariable model with FTO rs9939609 genotype as single fixed effect. Model II: Multivariable model with FTO rs9939609 genotype as a fixed effect and sex, age, fat mass and visceral adipose tissue as covariates. Model III: Multivariable model with FTO rs9939609 genotype as a fixed effect and sex, age, body mass index, peak oxygen uptake, resting metabolic rate, visceral adipose tissue, abdominal subcutaneous adipose tissue, liver fat, sitting time and moderate-to-vigorous physical activity as covariates.

Values are mean (95% confidence interval (CI)). Eta^2, 90% CI and *P*-values are from the fixed effect of the FTO rs9939609 genotype group.

Sex-specific Pearson's correlation coefficients

Appetite-related outcomes and individual characteristics

No significant correlations were observed between fasting acylated ghrelin and age, BMI, fat mass, $\dot{V}O_2$ peak, resting metabolic rate, visceral fat, abdominal subcutaneous adipose tissue, liver fat, average sitting or average MVPA in men (r = -0.18 to 0.07, P ≥ 0.185) or women (r = -0.19 to 0.06, P ≥ 0.175). Similarly, no significant correlations were observed between fasting total PYY and any of the individual characteristics in men (r = -0.13 to 0.14, P ≥ 0.330) or women (r = -0.14 to 0.10, P ≥ 0.323). Pearson's correlation coefficients between individual characteristics and fasting insulin, glucose and leptin are presented in Table 4. In summary, fasting insulin was positively correlated with general and abdominal adiposity parameters in both sexes and with liver fat in men (r = 0.32 to 0.53, P ≤ 0.010). Fasting insulin was negatively correlated with $\dot{V}O_2$ peak in both sexes and with MVPA in men (r = -0.35 to -0.47, P ≤ 0.004). Fasting glucose was positively correlated with total and abdominal adiposity parameters in both sexes, with age and liver fat in men, and with resting metabolic rate in women (r = 0.28 to 0.44, P ≤ 0.017). Fasting glucose was negatively correlated with $\dot{V}O_2$ peak in both sexes (r = -0.29 to -0.28, P ≤ 0.020). Fasting leptin was positively correlated with general and abdominal adiposity parameters in both sexes, and with age and liver fat in men (r = 0.24 to 0.83, P ≤ 0.040). Fasting leptin was negatively correlated with $\dot{V}O_2$ peak in both sexes and with MVPA in men (r = -0.35 to -0.64, P ≤ 0.006). In men, fasting overall appetite was negatively associated with fat mass (r = -0.31, P = 0.022, 95% CI = -0.53 to -0.05) and abdominal subcutaneous adipose tissue (r = -0.30, P = 0.032, 95% CI = -0.53 to -0.02). No significant correlations between fasting overall appetite and individual characteristics were observed in women (r = -0.12 to 0.09, P ≥ 0.391).

Delta AUC for acylated ghrelin was positively associated with sitting time (r = 0.29, *P* = 0.048, 95% CI = 0.00 to 0.53) and negatively associated with age (r = -0.32, *P* = 0.017, 95% CI = -0.54 to -0.06) in men. Insulin AUC was positively associated with visceral adipose tissue in men (r = 0.38, *P* = 0.007, 95% CI = 0.11 to 0.59) and women (r = 0.32, *P* = 0.021, 95% CI = 0.05 to 0.55), and with fat mass (r = 0.39, *P* = 0.003, 95% CI = 0.14 to 0.59), abdominal subcutaneous adipose tissue (r = 0.31, *P* = 0.026, 95% CI = 0.03 to 0.54) and liver fat (r = 0.47, *P* = 0.001, 95% CI = 0.21 to 0.66) in men. Insulin AUC was negatively associated with $\dot{V}O_2$ peak (r = -0.44, *P* = 0.001, 95% CI = -0.63 to -0.20) and MVPA (r = -0.38, *P* = 0.007, 95% CI = -0.60 to -0.11) in men. None of the correlations between AUC for total PYY, glucose and overall appetite and individual characteristics were statistically significant (r = -0.23 to 0.24, P ≥ 0.061).

Table 4. Sex-specific Pearson's correlation coefficients between fasting appetite-related blood markers and individual characteristics.

	Fasting insulin ($pmol \cdot L^{-1}$)	Fasting glucose ($mmol \cdot L^{-1}$)	Fasting leptin ($ng \cdot mL^{-1}$)
Age (years)	Men: r = -0.01, *P* = 0.457, 95% CI = -0.27 to 0.25 Women: r = -0.16, *P* = 0.123, 95% CI = -0.40 to 0.11	**Men: r = 0.34, *P* = 0.005, 95% CI = 0.08 to 0.55** Women: r = 0.08, *P* = 0.270, 95% CI = -0.19 to 0.33	**Men: r = 0.24, *P* = 0.040, 95% CI = -0.02 to 0.47** Women: r = -0.07, *P* = 0.298, 95% CI = -0.33 to 0.20
Body mass index ($kg \cdot m^{-2}$)	**Men: r = 0.39, P = 0.003, 95% CI = 0.14 to 0.59** **Women: r = 0.53, P < 0.001, 95% CI = 0.31 to 0.69**	**Men: r = 0.33, *P* = 0.013, 95% CI = 0.07 to 0.54** **Women: r = 0.35, *P* = 0.004, 95% CI = 0.10 to 0.56**	**Men: r = 0.62, *P* < 0.001, 95% CI = 0.43 to 0.76** **Women: r = 0.77, *P* < 0.001, 95% CI = 0.64 to 0.86**
Fat mass (kg)	**Men: r = 0.49, *P* < 0.001, 95% CI = 0.26 to 0.67** **Women: r = 0.32, *P* = 0.008, 95% CI = 0.06 to 0.54**	**Men: r = 0.44, *P* < 0.001, 95% CI = 0.20 to 0.63** **Women: r = 0.28, *P* = 0.017, 95% CI = 0.02 to 0.50**	**Men: r = 0.83, *P* < 0.001, 95% CI = 0.73 to 0.90** **Women: r = 0.75, *P* < 0.001, 95% CI = 0.61 to 0.85**
$\dot{V}O_2$ peak ($mL \cdot kg \cdot min^{-1}$)	**Men: r = -0.47, *P* < 0.001, 95% CI = -0.65 to -0.24** **Women: r = -0.35, *P* = 0.004, 95% CI = -0.56 to -0.10**	**Men: r = -0.29, *P* = 0.015, 95% CI = -0.51 to -0.03** **Women: r = -0.28, *P* = 0.020, 95% CI = -0.50 to -0.02**	**Men: r = -0.64, *P* < 0.001, 95% CI = -0.77 to -0.45** **Women: r = -0.58, *P* < 0.001, 95% CI = -0.73 to -0.37**
Resting metabolic rate (kcal)	Men: r = -0.04, *P* = 0.381, 95% CI = -0.31 to 0.23 Women: r = 0.03, *P* = 0.402, 95% CI = -0.24 to 0.29	Men: r = -0.12, *P* = 0.205, 95% CI = -0.38 to 0.15 **Women: r = 0.35, *P* = 0.005, 95% CI = 0.09 to 0.56**	Men: r = 0.05, *P* = 0.369, 95% CI = -0.22 to 0.32 Women: r = 0.05, *P* = 0.359, 95% CI = -0.22 to 0.31
Visceral adipose tissue (L)	**Men: r = 0.41, *P* = 0.002, 95% CI = 0.15 to 0.62** **Women: r = 0.33, *P* = 0.010, 95% CI = 0.06 to 0.55**	**Men: r = 0.42, *P* = 0.001, 95% CI = 0.15 to 0.63** **Women: r = 0.36, *P* = 0.005, 95% CI = 0.09 to 0.58**	**Men: r = 0.65, *P* < 0.001, 95% CI = 0.45 to 0.79** **Women: r = 0.62, *P* < 0.001, 95% CI = 0.42 to 0.76**
Abdominal subcutaneous adipose tissue (L)	**Men: r = 0.43, *P* = 0.002, 95% CI = 0.17 to 0.63** **Women: r = 0.44, *P* = 0.001, 95% CI = 0.19 to 0.64**	**Men: r = 0.39, *P* = 0.005, 95% CI = 0.13 to 0.60** **Women: r = 0.34, *P* = 0.013, 95% CI = 0.07 to 0.56**	**Men: r = 0.79, P < 0.001, 95% CI = 0.66 to 0.87** **Women: r = 0.79, P < 0.001, 95% CI = 0.66 to 0.87**
Liver fat (%)	**Men: r = 0.49, *P* < 0.001, 95% CI = 0.24 to 0.68** Women: r = 0.06, *P* = 0.338, 95% CI = -0.22 to 0.33	**Men: r = 0.33, *P* = 0.010, 95% CI = 0.05 to 0.56** Women: r = 0.07, *P* = 0.305, 95% CI = -0.21 to 0.34	**Men: r = 0.44, *P* = 0.001, 95% CI = 0.18 to 0.64** Women: r = 0.18, *P* = 0.112, 95% CI = -0.11 to 0.44
Average sitting time ($min \cdot day^{-1}$)	Men: r = -0.06, *P* = 0.340, 95% CI = -0.34 to 0.23 Women: r = 0.12, *P* = 0.196, 95% CI = -0.17 to 0.39	Men: r = -0.12, *P* = 0.210, 95% CI = -0.39 to 0.17 Women: r = 0.13, *P* = 0.190, 95% CI = -0.16 to 0.40	Men: r = -0.12, *P* = 0.207, 95% CI = -0.39 to 0.17 Women: r = 0.05, *P* = 0.353, 95% CI = -0.23 to 0.33
Average MVPA time ($min \cdot day^{-1}$)	**Men: r = -0.44, *P* = 0.001, 95% CI = -0.64 to -0.18** Women: r = -0.01, *P* = 0.493, 95% CI = -0.28 to 0.27	Men: r = -0.03, *P* = 0.420, 95% CI = -0.31 to 0.25 Women: r = 0.09, *P* = 0.274, 95% CI = -0.19 to 0.36	**Men: r = -0.35, *P* = 0.006, 95% CI = -0.57 to -0.08** Women: r = -0.10, *P* = 0.241, 95% CI = -0.36 to 0.18

AUC, area under the curve; FTO, fat mass and obesity associated gene; MVPA, moderate-to-vigorous physical activity, PYY, peptide YY; $\dot{V}O_2$ peak, peak oxygen uptake.

Perceived appetite and appetite-related blood parameters

Fasting overall appetite was negatively associated with fasting insulin (r = -0.32, P = 0.015, 95% CI = -0.54 to -0.06) and fasting leptin (r = -0.35, P = 0.008, 95% CI = -0.56 to -0.10) in men. Delta AUC for overall appetite was positively associated with insulin AUC (r = 0.35, P = 0.009, 95% CI = 0.10 to 0.56) in women. No other significant correlations between overall appetite and appetite-related blood parameters were evident in the fasted or postprandial state (r = -0.20 to 0.26, $P \geq 0.052$).

DISCUSSION

The primary finding of this study is that very little influence of the FTO rs9939609 genotype was identified for fasting and postprandial perceived appetite and appetite-related blood outcomes in healthy men and women. Explained variance for FTO group on all outcomes was small (< 5%) according to the thresholds suggested by Cohen (1998). Even the upper 90% confidence limits of the explained variance were low for each outcome (< 15%). In the context of precision medicine, we maintain that explained variance would need to be much larger than our observed values for the FTO rs9939609 gene to be a useful predictor of appetite-related outcomes. We also found that fasting and postprandial acylated ghrelin and total PYY were not associated with general or abdominal adiposity, while leptin, glucose and insulin concentrations were consistently associated with adiposity variables. Our study is the first to employ an integrative approach to investigate associations between a variety of genetic, physiological and lifestyle characteristics with appetite-related outcomes. Previous research has provided limited evidence on the influence of specific individual characteristics on appetite-related blood parameters and appetite perceptions.

The FTO gene represents the most extensively-studied gene that has been associated with a higher risk of obesity (Frayling et al. 2007), yet evidence on the physiological mechanisms involved is limited. The study undertaken by Karra et al. (2013) supported the hypothesis that satiety control differs between FTO rs9939609 genotype groups. Specifically, the group with higher obesity risk (AA) presented attenuated suppression of acylated ghrelin and perceived hunger after consumption of a meal, which can naturally lead to higher energy intake and, consequently, higher body mass (Karra et al. 2013). However, our results do not support this hypothesis as we found very little influence of genotype group on acylated ghrelin concentrations or perceived appetite ratings. Differences between study samples can possibly explain

discrepancies between findings, as Karra et al. (2013) recruited healthy young lean males, while our sample was composed of a heterogeneous group of males and females. Additionally, Karra et al. (2013) selectively sampled their participants in order to match groups for certain variables, whereas we adopted a multivariate-adjusted approach to our data analysis. Interestingly, recent studies have reported lower postprandial total ghrelin concentrations in AA compared to AT and TT individuals (Magno et al. 2018; Melhorn et al. 2018), and postprandial hunger ratings were either similar between genotype groups (Melhorn et al. 2018) or were lower in AA individuals (Magno et al. 2018). These findings were observed despite the AA individuals exhibiting higher energy intake during an *ad libitum* buffet (Melhorn et al. 2018). Of note, the active part of ghrelin (acylated ghrelin) only represents approximately 5 to 10% of total ghrelin (Hosoda et al, 2000; Yoshimoto et al. 2002) and, therefore, the assessment of total ghrelin in these studies could potentially explain the variability in findings.

Our research group has recently conducted a replicated crossover study to examine individual appetite responses to meal intake in healthy men recruited according to their FTO rs9939609 genotype (AA or TT) (Goltz et al. 2019). The findings from this study highlighted the existence of interindividual variability in perceived appetite and acylated ghrelin, total PYY, insulin and glucose responses to a standardised meal over and above any measurement errors and/or natural variance of the outcomes. However, the magnitude of postprandial appetite parameter responses after meal intake was not influenced by the FTO rs9939609 gene (Goltz et al. 2019). In line with our findings, previous studies have reported no differences between FTO rs9939609 genotype groups for fasting glucose and insulin (Speakman et al. 2008), fasting leptin (Speakman et al. 2008; Karra et al. 2013; Melhorn et al. 2018), fasting and postprandial PYY_{3-36} (Karra et al. 2013) and fasting and postprandial GLP-1 (Melhorn et al. 2018). Beyond the subjective appetite and appetite-related blood outcomes assessed in this study, AA and TT individuals have been shown to exhibit divergent neural responsiveness to food cues within homeostatic and reward brain regions in both fasted and postprandial states (Karra et al. 2013). Specifically, AA individuals rated high-energy food images as more appealing than TT individuals, and positive associations between circulating acylated ghrelin and central neural system responsiveness to food cues were observed only in TT individuals (Karra et al. 2013). Moreover, recent evidence suggests that AA individuals show higher total food cravings, compared to TT individuals, which correlated with BMI (Dang et al. 2018). Additional studies are needed to elucidate the precise role that FTO rs9939609 plays in moderating appetite control and energy intake which include both central and peripheral factors implicated in appetite regulation.

Although evidence to date suggests a negligible impact of FTO rs9939609 genotype on energy expenditure, higher levels of physical activity seem to exert a protective effect on the obesity risk associated with FTO (Sonestedt et al. 2009; Speakman, 2015). On the contrary, diets with higher fat content can exacerbate the susceptibility to obesity linked to the FTO rs9939609 high-risk genotype (Sonestedt et al. 2009; Speakman, 2015). Our study included objectively assessed sitting time, MVPA and cardiorespiratory fitness as covariates in the statistical analyses. However, only 20% of our participants accumulated, on average, less than 30 min of MVPA per day, indicating that most participants in our sample had relatively high levels of physical activity. Therefore, we cannot rule out the possibility of this hindering our ability to detect differences in appetite-related outcomes between the genotype groups (Speakman et al. 2008). Our study did not include any assessment of habitual dietary intake and, therefore, fat intake was not taken into consideration in our analyses. Nevertheless, it is well known that the currently available dietary intake assessment tools do not provide reliable data, and this currently represents a major challenge for those involved in nutrition-related research, clinical practice or policy development (Dhurandhar et al. 2015; Archer et al. 2018).

In contrast to previous studies (Alajmi et al. 2016; Douglas et al. 2017), we did not observe a statistically significant difference in fasting concentrations of acylated ghrelin between men and women. The reason for this disparity is unclear but it is worth noting that two female participants were identified as clear outliers within our sample, with fasting acylated ghrelin concentrations of 2,899 and 4,411 $pg \cdot mL^{-1}$. These extremely high concentrations of acylated ghrelin were observed consistently in all four samples collected for each participant, indicating these values represented physiological characteristics of these two individuals rather than merely one-off measurement errors. Further studies are needed to investigate potential causes and consequences of such extreme concentrations of acylated ghrelin, and care should be taken when interpreting group mean results, as group means can be greatly impacted by such outliers. Nevertheless, exclusion of the outliers did not influence any of the statistical models in this study and, therefore, data are presented with the outliers included. Higher concentrations of fasting glucose were observed in men than women in the current study, which may be indicative of a greater degree of insulin resistance resulting from the higher visceral adipose tissue and liver fat levels observed in men (Marchesini et al, 2001; Ibrahim, 2010). Higher levels of fasting leptin were observed in women, likely because of the higher fat mass values in relation to total body mass in women, compared to men (Marshall et al. 2000; Rosenbaum and Leibel, 2014).

After meal consumption, greater changes in acylated ghrelin and overall appetite were observed in women than men. It should be noted that all participants received an identical standardised meal and, as women had significantly lower body mass and fat free mass, and consequently lower resting metabolic rate, it was expected that the postprandial suppression of appetite would be stronger in women. However, it is interesting to observe that, apart from acylated ghrelin, no other statistically significant differences were observed between men and women in any of the remaining postprandial appetite-related blood parameters. Previous evidence has demonstrated a stronger suppression of acylated ghrelin in women than men after acute exercise and standardised meals (Douglas et al. 2017), but not after the consumption of a standardised liquid meal (Carroll et al. 2007).

Our exploratory analyses did not identify any statistically significant or meaningful association between adiposity parameters and fasting or postprandial concentrations of acylated ghrelin and total PYY. This is in contrast with findings from previous studies which demonstrated a lower postprandial suppression of total and acylated ghrelin (Le Roux et al. 2005; Carrol et al. 2007) and a blunted postprandial elevation in PYY (Le Roux et al. 2006) in individuals with obesity. However, as expected, fasting insulin, glucose and leptin and postprandial insulin were all positively associated with general and visceral adiposity, demonstrated by moderate to very large correlation coefficients, which is consistent with the well-established role of leptin in signalling adiposity levels (Rosenbaum and Leibel, 2014) and the impact of adiposity on insulin resistance (Ibrahim, 2010). Additionally, fat free mass, which represents the largest determinant of resting metabolic rate, has been identified as a primary determinant of appetite and energy intake (Blundell et al. 2015b). However, our findings did not reveal any significant associations of appetite-related hormones or perceived appetite with resting metabolic rate.

While acute bouts of exercise have been shown consistently to transiently suppress appetite (King et al. 2017), chronic exercise and high levels of physical activity have been suggested to increase the overall drive to eat and, concomitantly, to increase the satiating effect of a standardised meal (King et al. 2009; Beaulieu et al. 2016). We did not identify any significant associations between habitual physical activity levels and fasting or postprandial acylated ghrelin, total PYY, glucose or perceived appetite. However, a negative association was observed between MVPA and fasting leptin and insulin, and postprandial insulin in men. Additionally, negative associations between $\dot{V}O_2$ peak and fasting and postprandial insulin, fasting glucose and fasting leptin were observed. Acute and chronic exercise augments insulin sensitivity by increasing insulin-like growth factor

1, and individuals with higher cardiorespiratory fitness typically show higher insulin sensitivity (Borghouts and Keizer, 2000; Castro et el. 2016). Furthermore, a recent meta-analysis showed that leptin concentrations can be reduced by exercise in individuals who are overweight even in the absence of dietary interventions or major weight loss (BMI reduction of > 2.5%) (Rostás et al. 2017). Postprandial acylated ghrelin was positively associated with sitting time in men, but this correlation was small in magnitude and would not be considered significant if the stricter threshold of $P < 0.005$ was applied in line with recent recommendations (Benjamin et al. 2017).

Perceived fasting overall appetite was negatively associated with total fat mass in men supporting previous evidence suggesting the existence of negative feedback signals originating from fat mass in order to regulate appetite and maintain body weight (Weise et al. 2014; Blundell et al. 2015a). However, no association was observed between postprandial perceived appetite and any adiposity parameter in our study. Interestingly, no statistically significant associations between fasting or postprandial perceived overall appetite and acylated ghrelin or total PYY were identified. Even though circulating concentrations of acylated ghrelin and PYY vary on a meal-to-meal basis, concomitantly with perceived appetite, the magnitude and direction of the changes in hormone concentrations are not always mirrored by changes in perceived appetite (Goltz et al. 2018). In contrast, postprandial overall appetite AUC was positively associated with postprandial insulin AUC in women, which is consistent with previous findings showing that postprandial insulin concentrations are positively associated with postprandial satiety and negatively associated with postprandial hunger (Flint et al. 2007).

The strengths of our study include the use of an integrative approach and objective assessment methods to explore the associations of the FTO rs9939609 genotype with fasting and postprandial appetite-related hormones and perceived appetite, taking into consideration a variety of individual characteristics that have been previously suggested to influence appetite parameters. Furthermore, the recruitment of a highly heterogeneous sample for parameters such as age, adiposity and cardiorespiratory fitness levels adds strength to our analyses. Finally, the careful standardisation of diet and physical activity in the 24 h preceding the laboratory visit, as well as the inclusion of a cannula acclimatisation period, also contributed to the quality of the study outcome measurements obtained. However, it should be highlighted that our study employed an exploratory approach and the cross-sectional design makes it impossible to imply any causation in our results. Our results may have been compromised by the reduced sample size and by the loss of power in some of the statistical models due to missing data. Additionally, it is possible

that a study design where individuals are exposed to an obesigenic food environment, such as an *ad libitum* buffet meal rather than a standardised meal stimulus, may be more appropriate to elucidate the effect of FTO rs9939609 genotype on food choice and eating behaviour. Furthermore, participants were aware of the meal timing so it is possible that the higher preprandial ghrelin concentrations reflected an anticipatory response to impending meal intake (Cummings et al. 2001). Future studies should consider isolating meal provision from time-related cues and/or examining the influence of cephalic phase ghrelin release during meal anticipation on postprandial appetite responses.

In conclusion, the FTO rs9939609 genotype did not have any significant influence on fasting or postprandial perceived appetite or appetite-related blood parameters in healthy men and women. The associations between fasting and postprandial acylated ghrelin, total PYY and general or abdominal adiposity were also small, while fasting leptin, glucose and insulin and postprandial insulin concentrations were consistently and positively associated with adiposity outcomes. Further research is needed to clarify the precise role of the FTO rs9939609 genotype in moderating appetite control and energy intake, including both physiological and psychological factors that influence eating behaviour. Specifically, well-controlled long-term studies are needed to improve understanding of the effect of the FTO rs9939609 genotype on appetite and energy intake during and after interventions targeting weight loss and/or prevention of weight gain. Understanding the complex interaction between genetics and other individual characteristics, physiological appetite parameters and perceived appetite is of crucial importance for planning targeted strategies for weight control.

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