Title: A randomized cross-over trial assessing the effects of acute exercise on appetite, circulating ghrelin concentrations and butyrylcholinesterase activity in normal weight males with variants of the obesity-linked *FTO* rs9939609 polymorphism.

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Short running title: Ghrelin, exercise and *FTO* rs9939609 genotype.

Abbreviations: AG, acyl-ghrelin; AUC, area under the curve; BChE, butyrylcholinesterase; BMI, body mass index; CI, confidence interval; DAG, des-acyl-ghrelin; ES, effect size; *FTO*, the fat mass and obesity-associated gene; GLP-1, glucagon-like peptide 1; PYY, peptide YY; SD, standard deviation; SEM, standard error of mean; SNP, single nucleotide polymorphism.

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Data described in the manuscript will be made available upon request pending application and approval.

Abstract

Background: The fat mass and obesity-associated gene (*FTO*) rs9939609 A-allele is associated with higher acyl-ghrelin (AG) concentrations, higher energy intake and obesity, though exercise may mitigate rs9939609 A-allele linked obesity risk. Butyrylcholinesterase (BChE) hydrolyses AG to des-acyl-ghrelin (DAG), potentially decreasing appetite. However, the effects of the *FTO* rs9939609 genotype and exercise on BChE activity, AG, DAG and energy intake are unknown.

Objective: We hypothesized that individuals homozygous for the obesity-risk A-allele (AAs) would exhibit higher postprandial AG and energy intake than individuals homozygous for the low obesity-risk T-allele (TTs), but that exercise would increase BChE activity and diminish these differences.

Methods: Twelve AA and 12 TT normal weight males completed a control (8 hours rest) and an exercise (1 hour of exercise at 70% peak oxygen uptake, 7 hours rest) trial in a randomized cross-over design. A fixed meal was consumed at 1.5 hours and an *ab libitum* buffet meal at 6.5 hours. Appetite, appetite-related hormones, BChE activity and energy intake were assessed.

Results: AAs displayed lower baseline BChE activity, higher baseline AG/DAG ratio, attenuated AG suppression after a fixed meal and higher *ad libitum* energy intake than TTs ($ES \geq 0.76$, $P \leq 0.049$). Exercise increased delta BChE activity in both genotypes ($ES = 0.41$, $P = 0.004$); however, exercise lowered AG and the AG/DAG ratio to a greater extent in AAs ($P \leq 0.041$), offsetting the higher AG ghrelin profile observed in AAs during the control trial ($ES \geq 0.88$, $P \leq 0.048$). Exercise did not elevate energy intake in either genotype ($P = 0.282$).

Conclusions: Exercise increases BChE activity, suppresses AG and the AG/DAG ratio and corrects the higher AG profile observed in obesity-risk AA individuals. These findings suggest that exercise or other methods targeting BChE activity may offer a preventative and/or therapeutic strategy for AA individuals.

Keywords: exercise; ghrelin; appetite; *FTO* gene; butyrylcholinesterase; obesity

INTRODUCTION

A cluster of single nucleotide polymorphisms (SNP) within intron one of the fat mass and obesity-associated gene (*FTO*) have been consistently associated with obesity (1–3). At the *FTO* rs9939609 SNP, homozygous obesity-risk A-allele carriers (AA) have a 1.7-fold higher risk for obesity compared to individuals homozygous for the T-allele (TT) (1). Compared with TTs, AA individuals exhibit lower postprandial satiety and higher energy intake (4–6). Karra et al. (7) also reported that AAs displayed an attenuated postprandial suppression of the orexigenic hormone acyl-ghrelin (AG) and appetite compared to TTs. These findings suggest the impaired postprandial suppression of AG might contribute to the higher energy intake and obesity risk in AAs.

Acute bouts of moderate- to vigorous-intensity exercise acutely suppress both subjective appetite perceptions and circulating AG concentrations (8,9). In addition, circulating concentrations of the anorectic hormones PYY and GLP-1 are increased by a single exercise bout (9,10). These gut hormone changes are suggested to provoke the acute anorectic effect of exercise (8,9,11). Further to changes during the exercise bout, circulating AG concentrations remain suppressed while PYY and GLP-1 are elevated in the hours after exercise (8,9,11). Importantly, the lack of compensatory changes in hunger and appetite-related hormones to an energy shortfall caused by exercise results in a short-term negative energy balance, which if sustained, could facilitate weight management (12).

The serine hydrolase butyrylcholinesterase (BChE) regulates circulating ghrelin concentrations by hydrolyzing AG to des-acyl-ghrelin (DAG), which is suggest to have an anorexigenic effect (13). Recent studies indicate that reduced BChE activity leads to a higher AG/DAG ratio, greater food consumption and weight gain (14,15). However, less is known about the interplay between BChE, *FTO* rs9939609 and exercise in humans. One study indicated that a single bout of light running increases BChE activity in humans (16), but further work is needed to examine if BChE activity is linked to *FTO* rs9939609 genotype and exercise-dependent changes in plasma ghrelin concentrations or appetite-related outcomes in humans.

Our primary aim was to investigate the effect of the *FTO* rs9939609 genotype and exercise on circulating AG and DAG concentrations, BChE activity, appetite and energy intake in a group of normal-weight AA males and a matched-group of TT males. As a secondary aim, we examined the effect of exercise and/or the *FTO* rs9939609 genotype on plasma concentrations of leptin, PYY and GLP-1. We hypothesized that AAs would exhibit higher AG, appetite and energy intake compared to TTs, but exercise would increase BChE activity and suppress these rs9939609-related differences.

PARTICIPANTS AND METHODS

Participants

The study was performed according to the principles set out in the Declaration of Helsinki and was approved by the Loughborough University ethical advisory committee. We recruited 202 healthy, non-smoking males aged 18-50 y of mixed European descent who provided written informed consent to take part in a database study. Exclusion criteria were history of cardio-metabolic disease, medical or psychiatric conditions, substance abuse and food allergies. Participants' height and body mass were measured, and waist circumference was assessed as the narrowest portion of the torso between the xiphoid process and the naval. Skinfold thickness was measured and body fat percentage was estimated (17). Habitual physical activity levels were assessed using the short form International Physical Activity Questionnaire (18) and eating behaviors and attitudes were assessed using the Three-Factor Eating Questionnaire (19). A venous blood sample was collected and DNA was extracted. All

DNA extractions from peripheral blood samples were performed using the QIAamp DNA Blood Midi Kit (Qiagen). Genotyping for rs9939609 was performed by LGC Limited (Hertfordshire, UK) using the KASP (KBioscience Competitive Allele-Specific PCR) SNP genotyping system (www.lgcgenomics.com/genotyping/kasp-genotyping-reagents/). Blind duplicates were used to detect possible DNA mix-up. From the database, we recruited a group of 12 AA and 12 TT participants (**Table 1**) for a randomized cross-over study (**Supplementary Figure 1**). Participants provided written informed consent if they were invited back and completed the study between January 2015 to February 2016. Further to the criteria mentioned, to be included in this trial, participants had to be weight stable (≤ 3 kg over previous 3 months) and habitually consumed breakfast on 5 or more days of the week in an attempt to reduce the influence of breakfast consumption on fasting ghrelin concentrations (20). Participants were also excluded if they presented any food allergies. Groups were matched for anthropometric indices, age and peak oxygen uptake (Table 1). The study is registered at clinicaltrials.gov as NCT03025347.

Main trials

Participants attended a preliminary measures and familiarization session prior to main trials. Body mass, height, body fat percentage, body mass index (BMI) and waist circumference were re-measured as described to confirm no substantial changes occurred from the database study. Participants performed submaximal incremental and peak oxygen uptake running tests on a motorized treadmill as described elsewhere (8). Individual running speed-oxygen uptake linear regression equations and peak oxygen uptake were used to calculate the running speed that corresponded to 70% of each participant’s peak oxygen uptake. Participants also completed a food preference questionnaire and were familiarized with the buffet meal, to reduce the risk of any changes in food intake due to novelty of the meal.

Next, in a randomized cross-over design stratified by rs9939609 genotype group, all participants completed two main trials separated by 7-14 days: exercise and control. Further to enrolling participants, the main investigator conducted the block randomization plan for each genotype from the website www.randomization.com and assigned participants to the order of trials completed. Participants were instructed to complete a weighed food diary in the 24 h before the first trial and replicate it in the 24 h before the second trial. Participants were also instructed to refrain from alcohol consumption and strenuous physical activity in this period. A pizza meal (5201 kJ) was consumed by participants between 19:00-20:00 the night before main trials to negate the influence of preceding food intake on morning appetite and appetite-related hormone concentrations (21). Adherence to these procedures was assessed by verbal confirmation.

A schematic representation of the main trial procedures is shown in **Figure 1**. Participants arrived at the laboratory at approximately 08:30 after an overnight fast. A cannula was inserted into an antecubital vein 60 min before blood sampling commenced to mitigate any stress response caused by anxiety with the cannula (21). In the control trial, participants rested for 8 h, while in the exercise trial, participants ran at 70% of peak oxygen uptake for 60 min and then rested for 7 h. Participants read, worked and watched TV through laptop and tablet devices while resting. Expired gas samples were collected into Douglas bags every 15 min throughout the first hour in both trials for calculation of energy expenditure (22).

Fixed test meal and buffet meal

Participants consumed a standardized 5623 kJ (52% carbohydrate, 25% fat, 23% protein) test meal consisting of white rolls, butter, cheese, chips, chocolate slices and milkshake at 1.5 h. Participants were instructed to consume the meal within 20 minutes.

At 6.5 h, participants were provided with a buffet meal in a booth and instructed to eat *ad libitum*. Food items of the buffet meal were presented identically on each trial and included white and brown bread, butter, chicken, ham, lettuce, tomato, yoghurts, cookies and apples. Participants were instructed to eat until “comfortably full and satisfied” before leaving the eating booth. To minimize distractions that may influence food consumption, the buffet was provided in isolation and participants were not permitted the use of mobile phones or electronic devices. Items were provided in excess of expected consumption and participants were provided with more food items if requested. The amount of each food item consumed was calculated by measuring the weighted difference of all the food items before and after the meal. Manufacturer details were used to determine energy and macronutrient consumption.

Appetite ratings

Visual analogue scales (VAS) were used to assess subjective feelings of hunger, fullness, prospective food consumption and hedonic wanting of food (23,24). Measures were taken every 30 min from baseline to 5.0 h, and then at 6.5, 7.0, 7.5 and 8.0 h.

Blood sampling

Blood samples were collected into chilled EDTA monovettes (Sarstedt, Leicester, UK) every 30 min from baseline to 4.0 h and subsequently at 5.0, 6.5 and 7.5 h to measure circulating concentrations of AG, DAG, total PYY and total GLP-1. Circulating leptin was measured from fasting samples only. Plasma BChE activity was determined from samples collected at 0, 0.5 and 1 h in the control and exercise trials. All collected samples were immediately centrifuged at 2383*g* for 10 min at 4°C. After centrifugation, 100 µL of 0.5 mol/L hydrochloric acid was added per 900 µL of plasma supernatant to preserve DAG. To preserve the stability of AG, one monovette was treated with a 50 µL solution of PBS, P-hydroxymercuribenzoic acid and sodium hydroxide. The plasma supernatant of this sample was dispensed into a storage tube and 100 µL of 1 mol/L hydrochloric acid was added per 1 ml of plasma. All samples were stored at -80°C until batch analysis.

Biochemical analysis

Enzyme-linked immunosorbent assays were used to measure circulating concentrations of AG, DAG (SCETI, Tokyo, Japan), total PYY, total GLP-1 (Millipore, Watford, UK) and leptin (R&D Systems, Abington, UK). The intra-assay variability was 4.3%, 3.5%, 1.9%, 3.6% and 1.8% for AG, DAG, total PYY, total GLP-1 and leptin, respectively.

Details of BChE analysis are documented in the Supplementary Methods. In short, BChE assays were performed based upon the cholinesterase assay method developed by Ellman (25), with butyrylthiocholine iodide as the enzymatic substrate.

Statistical analyses

A sample size of 24 was chosen based on data suggesting that a 10 pmol/L reduction in circulating AG during exercise could be detected with > 80% power using a two-tailed *t*-test whilst assuming a SD_{diff} of 16 pmol/L and adopting an alpha value of 0.05 (26). Primary outcomes measured in this trial were AG, DAG, BChE activity, appetite and *ad libitum* energy intake, and secondary outcomes were total GLP-1, total PYY and leptin. To reduce day-to-day variability, appetite-related hormone concentrations and BChE were analyzed and presented as delta values. Appetite ratings, appetite-related hormone concentrations and BChE activity were analyzed using linear mixed models with trial (exercise or control), genotype (AA or TT) and time included as fixed factors. Total area under the curve (AUC) was calculated using the trapezoidal rule. For blood parameters, AUC was calculated during the intervention (0.0-1.0 h), post-test meal (1.5-3.5 h), afternoon (3.5-6.5 h) and post-buffet meal (6.5-7.5 h) periods. AUC for subjective appetite ratings was calculated during the intervention (0.0-1.0 h), post-test meal (1.5-3.5 h), afternoon (3.5-6.5 h) and post-buffet meal (6.5-8.0 h) periods. Linear mixed models were used for trial and genotype comparisons of AUC values and food consumption at the buffet meal. Post-hoc analysis was conducted using Holm-Bonferroni correction for multiple comparisons. Absolute standardized effect sizes (ES) were calculated by dividing the difference between the mean values (exercise vs. control or AAs vs. TTs) with the pooled standard deviation. An ES of 0.2 was considered the minimum important difference for all outcome measures, 0.5 moderate and 0.8 large (27). The 95% confidence intervals (CI) for mean absolute pairwise differences between experimental trials or genotype groups were calculated. Statistical significance was accepted as $P < 0.05$. Linear mixed models were conducted with trial order as a fixed effect which revealed no main or interactive effects for any outcome ($P \geq 0.073$; data not shown). Unless stated otherwise, data presented in tables and figures are shown as mean ± SEM, while descriptive data are presented as mean ± SD. Data were analyzed using IBM SPSS Statistics for Windows software (version 23.0, IBM corporation, New York, USA).

RESULTS

Participant characteristics

There were no differences between AAs and TTs for age, height, body mass, BMI, body fat %, lean body mass, waist circumference, eating behaviors, habitual physical activity levels or peak oxygen uptake ($P \geq 0.121$) (Table 1). There were no differences in energy intake between AAs and TTs in the 24 h before the main trials (AA: 9516 ± 595 kJ vs TT: 9630 ± 891 kJ; $P = 0.716$).

Treadmill running responses

We observed no between-genotype differences in exercise responses for running speed (AA: 11.1 ± 1.5 vs. TT: 11.3 ± 1.6 km/h; P = 0.786), heart rate (AA: 178 ± 13 vs. TT: 177 ± 12 beats/min; P = 0.934), gross energy expenditure (AA: 3809 ± 366 vs. TT: 3568 ± 249 kJ; P = 0.117) or percentage of peak oxygen uptake (AA: 71 ± 2 vs. TT: 70 ± 2%; P = 0.283).

Circulating appetite-related hormones and BChE activity

Fasting concentrations of AG, DAG, total GLP-1, total PYY and leptin at baseline were not different between genotype groups (P ≥ 0.127) or between trials (P ≥ 0.259) (**Table 2**). The fasting AG/DAG ratio and BChE activity were similar between trials (P > 0.319), but the AG/DAG ratio and BChE were higher and lower, respectively, in AAs than TTs (ES ≥ 0.76, P ≤ 0.047) (Table 2).

Linear mixed models for delta AG identified a main effect of trial (P < 0.001) and time (P < 0.001) but not genotype (mean difference: -0.02 pmol/L, 95% CI -2, 2 pmol/L, P = 0.988) (**Figure 2A**). The main effect of trial revealed lower delta AG concentrations in the exercise than control trial (mean difference: -5 pmol/L, 95% CI -6, -5 pmol/L, ES = 0.79). Analysis also identified a genotype-by-time interaction (P = 0.007), but post-hoc analysis revealed no differences after Holm-Bonferroni adjustment (P ≥ 0.060). The AUC for delta AG was lower in the exercise than control trial during the intervention (0.0-1.0 h), post-test meal (1.5-3.5 h) and afternoon (3.5-6.5 h) periods (all ES ≥ 0.53, P ≤ 0.001) (**Table 3**). The magnitude of reduction in AUC for delta AG after exercise was greater in AAs than TTs during the post-test meal period (1.5-3.5 h; -23.98 pmol/L·h (ES = 2.49) vs. -14.3 pmol/L·h (ES = 1.62), respectively; genotype-by-trial interaction P = 0.041) (Table 3). Post-hoc analysis of the post-test meal period revealed higher AUC delta AG in AAs compared to TTs in the control trial

(ES = 1.25, P = 0.011), but no between-genotype differences were seen in the exercise trial (ES = 0.03, P = 0.951).

There was a main effect of trial (P < 0.001) and time (P < 0.001) but not genotype (mean difference: 9 pmol/L, 95% CI -5, 24 pmol/L, P = 0.197) for delta DAG (**Figure 2B**). The main effect of trial revealed lower delta DAG concentrations in the exercise than control trial (mean difference: -17 pmol/L, 95% CI -20, -14 pmol/L, ES = 0.44). The magnitude of reduction in delta DAG concentrations after exercise was greater in TTs than AAs (-25 pmol/L (ES = 0.58) vs. -9 pmol/L (ES = 0.26), respectively; genotype-by-trial interaction P < 0.001). The AUC for delta DAG was lower in the exercise than control trial during the intervention (0.0-1.0 h), post-test meal (1.5-3.5 h) and afternoon (3.5-6.5 h) periods (all ES ≥ 0.29, P ≤ 0.028) (Table 3). The magnitude of reduction in AUC for delta DAG after exercise was greater in TTs than AAs during the intervention period (0.0-1.0 h; -82.4 pmol/L·h (ES = 2.47) vs. -46.2 pmol/L·h (ES = 1.66), respectively; genotype-by-trial interaction P = 0.042) and post-test meal period (1.5-3.5 h; -100.8 pmol/L·h (ES = 1.66) vs. -39.0 (ES = 0.59), respectively; genotype-by-trial interaction P = 0.025) (Table 3).

Linear mixed models for the delta AG/DAG ratio identified a main effect of trial (P < 0.001) and time (P < 0.001) but not genotype (mean difference: -0.006, 95% CI -0.015, 0.003, P = 0.192) (**Figure 2C**). The main effect of trial revealed the delta AG/DAG ratio was lower in the exercise than control trial (mean difference: -0.025, 95% CI -0.029, -0.022, ES = 0.88). The magnitude of reduction in the delta AG/DAG ratio after exercise was greater in AAs than TTs at time points between 0.5 h to 2.5 h (genotype-by-trial-by-time interaction, P = 0.004). The AUC for the AG/DAG ratio was lower in the exercise than control trial during the intervention, post-test meal, and post-buffet meal periods (all ES ≥ 0.54, P ≤ 0.006) (Table 3). The magnitude of reduction in AUC for the delta AG/DAG ratio after exercise was greater in

AAs than TTs during the intervention period (0.0-1.0 h; -0.12 (ES = 5.18) vs. -0.07 (ES = 1.63), respectively; genotype-by-trial interaction P = 0.004) and post-test meal period (1.5-3.5 h; -0.16 (ES = 2.72) vs. -0.02 (ES = 0.28), respectively; genotype-by-trial interaction P = 0.001) (Table 3). Post-hoc analysis of the intervention period revealed a similar AUC delta AG/DAG ratio between groups in the control trial (ES = 0.27, P = 0.518), but the AG/DAG ratio was lower in AAs compared to TTs in the exercise trial (ES = 1.75, P < 0.001). Post-hoc analysis in the post-test meal period indicated that AAs exhibited higher AUC delta AG/DAG in the control trial (ES = 0.88, P = 0.048) but lower AUC delta AG/DAG in the exercise trial (ES = 1.17, P = 0.018) compared to TTs.

There was a main effect of trial (P < 0.001) and time (P < 0.001) but not genotype (mean difference: 2 pmol/L, 95% CI -2, 7 pmol/L, P = 0.335) for delta total GLP-1 (**Figure 3A**). The main effect of trial revealed higher delta total GLP-1 concentrations in the exercise than control trial (mean difference: 14 pmol/L, 95% CI 12, 15 pmol/L, ES = 1.14). Analysis also identified a genotype-by-time interaction (P = 0.002), but post hoc analysis showed no differences after Holm-Bonferroni adjustment (P ≥ 0.092). The AUC for delta total GLP-1 was higher in the exercise than control trial during all time periods (all ES ≥ 0.50, P ≤ 0.044), and higher in AAs than TTs during the post-buffet meal period (6.5-7.5 h; ES = 0.92, P = 0.011) (**Table 4**).

A main effect of trial (P < 0.001) and time (P < 0.001) but not genotype (mean difference: 10 pmol/L, 95% CI -9, 29 pg/mL, P = 0.278) was detected for delta total PYY (**Figure 3B**). The main effect of trial revealed higher delta total PYY concentrations in the exercise than control trial (mean difference: 25 pg/mL, 95% CI 20, 30 pmol/L, ES = 0.50). The AUC for delta total PYY was higher in the exercise than control trial during the intervention (0.0-1.0 h; ES =

3.08, P < 0.001) and post-test meal (1.5-3.5 h; ES = 1.56, P < 0.001) periods, and higher in AAs than TTs during the post-buffet meal period (6.5-7.5 h; ES = 0.78, P = 0.029) (Table 4).

Analysis for delta BChE identified a main effect of time (P < 0.001) and trial (P = 0.004), with elevated BChE activity in the exercise trial compared to the control trial (mean difference: 0.072 KU/L, 95% CI 0.024, 0.120 KU/L, ES = 0.41) (**Figure 4**). There was, conversely, no main effect of genotype (mean difference: -0.016 KU/L, 95% CI -0.095, 0.063, ES = 0.09, P = 0.681), and no two-way or three-way interactions for BChE activity (P ≥ 0.094) (Figure 4).

Appetite ratings

Linear mixed models for each appetite perception identified a main effect of trial (P = 0.002) and time (P < 0.001) but not genotype (P ≥ 0.072) (**Figure 5**). The main effect of trial for each perception revealed suppressed appetite in the exercise compared with the control trial (all ES ≥ 0.12). Analysis also identified a genotype-by-time interaction for each appetite perception (P < 0.001) (Figure 5). Post-hoc analysis of the genotype-by-time interaction revealed higher ratings of hunger and hedonic wanting of food and lower ratings of fullness in AAs than TTs at time points between 3.0 to 4.0 h (all ES ≥ 1.04, P ≤ 0.033). There were no between-genotype differences at any time point for prospective food consumption after Holm-Bonferroni correction (P ≥ 0.130). A main effect of trial for AUC values in the intervention period (0.0-1.0 h) revealed lower ratings of hunger, prospective food consumption and hedonic wanting of food and higher ratings of fullness in the exercise than control trial (all ES ≥ 1.14, P < 0.001) (**Table 5**). A main effect of genotype for AUC values in the post-test meal (1.5-3.5 h) and afternoon (3.5-6.5 h) periods revealed higher ratings of hunger, prospective food consumption and hedonic wanting of food but lower ratings of fullness in AAs than TTs (all ES ≥ 0.81, P ≤ 0.045) (Table 5).

Buffet meal

Absolute energy intake was greater in AAs than TTs (ES = 0.86, P = 0.049), but was similar between the exercise and control trials (P = 0.282) (**Table 6**). Relative energy intake was substantially lower in the exercise than control trial (ES = 1.84, P < 0.001), and tended to be greater in AAs than TTs (ES = 0.80, P = 0.081). Protein intake was higher in AAs than TTs (ES = 0.93, P = 0.033), and intakes of carbohydrate (ES = 0.73, P = 0.075) and fat (ES = 0.82, P = 0.072) were meaningfully, albeit not statistically, greater in AAs than TTs. Linear mixed models revealed no genotype-by-trial interactions for energy or macronutrient intakes (P ≥ 0.207).

DISCUSSION

The primary findings of this study are that normal weight males homozygous for the obesity-risk *FTO* rs9939609 A-allele displayed lower fasting BChE activity and higher postprandial AG and AG/DAG ratio which coincided with higher postprandial appetite and *ad libitum* energy intake compared to TTs. A single bout of exercise increased BChE activity and suppressed circulating AG. Importantly, the exercise-induced suppression of the AG/DAG ratio was greater in AA *versus* TT individuals, negating the differences in ghrelin seen in the control trial. Exercise transiently suppressed appetite and did not lead to compensatory increases in appetite or energy intake after the test meal in either genotype group.

Elevated AG and AG/total ghrelin ratio profiles in AAs have been implicated in their higher obesity risk (7,28). More recently, DAG has been shown to antagonize the orexigenic effects of AG, and the AG/DAG ratio has been suggested as a key determinant of appetite, energy intake and body weight (29,30). Thus, our novel finding of a higher AG/DAG ratio in AAs compared to TTs supports the concept that ghrelin may play an aetiopathogenic role in the higher energy intake and obesity-risk associated with the A-allele of rs9939609. However, we showed that exercise suppresses AG and the AG/DAG ratio and offsets these rs9939609 genotype differences. An acute reduction in AG during exercise has been shown before (8), but our study is the first to show differences between AA and TT individuals during exercise and immediately after the test meal. Specifically, in response to exercise, we found a greater reduction in the AG/DAG ratio during the exercise intervention period, and in AG and the AG/DAG ratio after provision of the test meal (1.5-3.5 h) in AAs compared with TTs. Physical activity attenuates the effect of rs9939609 A obesity-risk allele on adiposity (31), but our study may offer insights into the mechanisms of this genotype-lifestyle interaction (31). That is, the greater exercise-induced suppression of AG and the AG/DAG ratio in AAs could partly explain the greater weight loss seen in carriers of the risk genotype with exercise interventions (32,33).

The elevation in BChE activity in response to exercise supports previous findings suggesting that an acute bout of walking/running elevated plasma BChE activity (16). The mechanisms underlying this response require further study, though it may be that the transient increase in inflammatory markers could be implicated (34). It is possible that the elevation in BChE activity during exercise increased AG hydrolysis to DAG, providing a plausible mechanism for the exercise-induced reduction of plasma AG concentrations. However, we also showed that plasma DAG concentrations were suppressed during exercise, indicating that an attenuation of ghrelin release may also be implicated in response to exercise. Therefore, it is likely that several mechanisms are involved in the exercise-stimulated suppression of AG.

Another novel finding of lower fasting BChE activity in AA compared to TT individuals offers a potential explanation for the higher AG/DAG ratio and energy intake observed in AA versus TT individuals. BChE activity increases AG hydrolysis in plasma, leading to greater DAG and a lower AG/DAG ratio, which has been linked to lower energy consumption and lower adiposity in mice (14). In contrast to our findings, the *FTO* rs9939609 A-allele has previously been associated with higher BChE activity, yet this relationship was diminished when BMI was controlled (35). The careful matching of AAs and TTs in our study may have improved the sensitivity to detect differences in the *FTO* rs9939609 genotype, particularly as age, sex, substance abuse, physical activity and smoking have been shown to affect BChE activity (36,37).

Our findings may expound a complex set of mechanisms that link *FTO* and obesity. *FTO* encodes FTO protein, which demethylates the nucleoside N6-methyladenosine in RNA and, in turn, regulates mRNA export, RNA metabolism and RNA splicing (7,38). Ghrelin, ghrelin-O-acyltransferase and BChE mRNA have all been identified as targets for FTO demethylation and this could offer a mechanistic link between *FTO* rs9939609 and our findings (7). Indeed, AAs have been reported to exhibit higher FTO protein expression compared to TTs, indicating a potential direct mechanistic link between rs9939609 A-allele, the FTO protein, circulating ghrelin, lower BChE activity, higher energy intake and obesity. Taken together, this could suggest that therapeutic interventions augmenting BChE activity may offer a potential strategy that could assist with weight management in AA individuals.

Acute studies report that appetite is transiently suppressed during exercise and compensatory changes in these perceptions and energy intake do not occur (8–10). Our results are consonant with these findings, and we demonstrated that the appetite suppression during exercise was comparable in AAs and TTs and *ad libitum* energy intake was unaltered after exercise in both genotype groups. We also showed that AAs exhibited greater perceptions of appetite in the 4.5 hours after the test meal and consumed a higher energy intake and protein at the buffet meal. Our results are in agreement with studies indicating that individuals with the A-allele of rs9939609 exhibit reduced satiety (4,7,39), higher food intake (5,6) and elevated protein intake (40). It seems likely that the greater postprandial appetite displayed by AAs plays a role in the higher energy intake exhibited by this group. The *FTO*-linked change in protein consumption could be related to the role *FTO* plays in sensing amino acids (41). It is, nevertheless, noteworthy that there was a tendency for AA individuals to consume more carbohydrate and fat at the buffet meal. This indicates that the *FTO* rs9939609 A-allele is associated with a higher intake of all macronutrients and this may have been detected with a larger sample size.

In line with previous studies, total GLP-1 and total PYY concentrations were elevated during and immediately after exercise (9,11), and this rise was similar in AAs and TTs. At most periods of the day, concentrations of the satiety hormones, leptin, total GLP-1 and total PYY were not influenced by the *FTO* rs9939609 variant, supporting previous research (7). The only exception was after the buffet meal, where the elevations in total GLP-1 and total PYY were greater in AAs than TTs. However, rather than any effect of the *FTO* rs9939609 variant, this is likely to reflect the greater energy and protein intake seen in AAs at the buffet meal (42,43). Our data therefore bolster evidence suggesting that AAs and TTs exhibit no differences in circulating PYY and GLP-1 concentrations after standardized food intake (7).

Our study is not without limitations. First, we studied normal weight males who exhibited high peak oxygen uptake. It is unclear if the responses observed would be evident in other populations such as women, older adults, and in cohorts with overweight and obesity. It is also not known if the changes observed in response to exercise would be seen during exercise protocols lower in time and intensity. Hence, though our results may be important for obesity prevention, additional work is needed in other populations and in response to exercise regimens performed more frequently amongst the general population, especially in those who are overweight or obese. Second, we only examined BChE activity during the first hour of the main trials. Although this allowed us to evaluate the transient influence of exercise, further work is needed to elucidate the longer-term changes in BChE activity after exercise.

In conclusion, our study showed carriers of the *FTO* rs9939609 A-allele display lower fasting BChE activity, higher post-meal AG and AG/DAG ratio, and higher energy intake compared to TTs. However, a single bout of exercise enhances BChE activity, and corrects the attenuated meal-induced suppression of AG in AAs, while the energy cost of exercise did not engender an increase in energy intake in either genotype group. These findings suggest that exercise could be a strategy to ameliorate the adiposity-related traits mediated by the obesity-linked *FTO* rs9939609 SNP.

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Table 1. Characteristics of the AA and TT participants.

	AA (n = 12)	TT (n = 12)	Main effect genotype TT vs AA Mean difference (95% CI[1])
Age (years)	20.9 ± 3.5	21.3 ± 3.6	-0.4 (-3.4, 2.6)
Height (cm)	181.6 ± 5.8	177.5 ± 6.5	4.1 (-1.2, 9.3)
Body mass (kg)	77.6 ± 11.3	73.8 ± 7.0	3.9 (-4.1, 11.8)
BMI (kg/m^2)	23.5 ± 2.7	23.5 ± 2.3	0.1 (-2.1, 2.1)
Body fat (%)	15.6 ± 5.1	13.9 ± 4.7	1.7 (-2.4, 5.9)
Lean body mass (kg)	65.2 ± 7.4	63.3 ± 4.2	1.9 (-3.2, 7.0)
Waist circumference (cm)	80.3 ± 6.1	78.1 ± 4.1	2.1 (-2.2, 6.6)
Three-Factor Eating Questionnaire			
Dietary restraint	7.7 ± 4.5	7.6 ± 3.9	0.1 (-3.5, 3.6)
Dietary disinhibition	6.3 ± 2.3	6.6 ± 1.6	-0.3 (-1.9, 1.4)
Hunger	6.5 ± 2.1	6.9 ± 1.7	-0.4 (-2.0, 1.2)
Total physical activity (metabolic equivalent minutes/week)	4368 ± 1968	4790 ± 2728	-423 (-2436, 1591)
Peak oxygen uptake (mL/kg/min)	55.8 ± 5.8	56.6 ± 4.9	-0.8 (-5.4, 3.7)

Values are mean ± SD. Data were analyzed using linear mixed models with genotype (AA or TT) included as a fixed factor.

[1] 95% confidence interval of the mean absolute difference between the genotype groups. No differences were identified between genotype groups ($P \geq 0.121$).

Table 2. Fasting appetite-related hormone concentrations and butyrylcholinesterase activity at baseline for AAs and TTs in the control and exercise trials.

	AA (n = 12)		TT (n = 12)		Main effect trial Control vs exercise Mean difference (95% CI[1])	Main effect genotype TT vs AA Mean difference (95% CI[2])
	Control	Exercise	Control	Exercise		
Acyl-ghrelin (pmol/L)	22.4 ± 1.4	22.5 ± 1.3	20.9 ± 1.5	21.1 ± 1.5	0.1 (-0.4, 0.6)	1.4 (-2.7, 5.6)
Des-acyl-ghrelin (pmol/L)	135.0 ± 9.3	134.1 ± 8.7	156.3 ± 10.6	155.4 ± 10.0	-0.9 (-6.1, 4.3)	-21.3 (-49.1, 6.5)
Acyl-/des-acyl-ghrelin ratio	0.167 ± 0.005	0.169 ± 0.006	0.134 ± 0.004	0.135 ± 0.003	0.002 (-0.002, 0.006)	0.034 (0.021, 0.047)[3]
Total GLP-1 (pmol/L)	26.2 ± 2.2	25.3 ± 2.2	32.3 ± 3.3	31.7 ± 3.5	-0.8 (-2.1, 0.6)	-6.2 (-14.6, 2.1)
Total PYY (pg/mL)	156.2 ± 12.2	163.1 ± 12.7	187.4 ± 20.8	185.4 ± 17.8	2.5 (-11.3, 16.3)	-26.8 (-72.5, 18.9)
Leptin (pg/mL)	1216.5 ± 167.0	1358.1 ± 182.8	1343.3 ± 261.3	1267.0 ± 205.0	32.7 (-133.0, 198.3)	-17.9 (-657.9, 622.2)
Butyrylcholinesterase activity (KU/L)	1.481 ± 0.060	1.404 ± 0.062	1.613 ± 0.060	1.635 ± 0.062	-0.027 (-0.129, 0.074)	-0.181 (-0.360, -0.003)[3]

Values are mean ± SEM. Data were analyzed using linear mixed models with trial (exercise or control) and genotype (AA or TT) included as fixed factors.

[1] 95% confidence interval of the mean absolute difference between the experimental trials.
[2] 95% confidence interval of the mean absolute difference between the genotype groups.
[3] Significant main effect of genotype ($P < 0.05$).
Linear mixed models revealed no main effects of trial ($P \geq 0.127$) and no genotype-by-trial interactions ($P \geq 0.319$).

GLP-1, glucagon-like peptide-1; PYY, peptide YY.

Table 3. Time-averaged total area under the curve for delta acyl-ghrelin, des-acyl-ghrelin and the acyl-/des-acyl-ghrelin ratio for AAs and TTs in the exercise and control trials.

	AA (n = 12)		TT (n = 12)		Main effect trial Control vs exercise Mean difference (95% CI[1])	Main effect genotype TT vs AA Mean difference (95% CI[2])
	Control	Exercise	Control	Exercise		
Δ AG (pmol/L·h)						
Intervention period	3.8 ± 0.7	-17.4 ± 1.6	5.2 ± 0.9	-15.0 ± 1.5	-20.7 (-23.4, -18.0)[3]	-1.9 (-4.3, 0.5)
Post-test meal	-5.6 ± 1.9	-29.6 ± 3.5	-15.0 ± 2.4	-29.3 ± 2.7	-19.2 (-23.3, -15.1)[3,4]	4.5 (-2.1, 11.2)[4]
Afternoon	-38.9 ± 6.9	-52.1 ± 8.6	-40.2 ± 7.6	-53.9 ± 7.1	-13.4 (-19.8, -7.1)[3]	1.6 (-19.7, 22.9)
Post-buffet meal	-8.9 ± 2.4	-11.6 ± 2.7	-7.5 ± 2.7	-10.1 ± 1.8	-2.6 (-5.2, 0.1)	-1.4 (-8.1, 5.2)
Δ DAG (pmol/L·h)						
Intervention period	18.0 ± 3.5	-28.2 ± 10.8	27.0 ± 7.8	-55.4 ± 11.2	-64.3 (-81.7, -46.9)[3,4]	9.1 (-10.3, 28.5)[4]
Post-test meal	-62.4 ± 13.3	-101.4 ± 23.4	-66.6 ± 16.6	-167.4 ± 18.4	-67.9 (-96.2, -39.7)[3,4]	33.2 (-13.1, 79.4)[4]
Afternoon	-255.6 ± 49.1	-271.4 ± 48.5	-317.4 ± 54.5	-407.6 ± 61.2	-53.0 (-99.6, -6.4)[3]	99.0 (-51.1, 249.1)
Post-buffet meal	-73.2 ± 18.6	-46.3 ± 13.2	-76.7 ± 21.6	-74.7 ± 15.9	12.3 (-5.8, 30.5)	11. 8 (-37.1, 60.6)
Δ AG/DAG ratio						
Intervention period	0.01 ± 0.01	-0.11 ± 0.01	0.01 ± 0.01	-0.06 ± 0.01	-0.09 (-0.11, -0.08)[3,4]	-0.03 (-0.05, -0.02)[4,5]
Post-test meal	0.03 ± 0.02	-0.12 ± 0.02	-0.05 ± 0.02	-0.06 ± 0.01	-0.09 (-0.12 , -0.05)[3,4]	0.01 (-0.02, 0.04)[4]
Afternoon	0.04 ± 0.04	-0.09 ± 0.04	0.02 ± 0.04	0.02 ± 0.04	-0.7 (-0.14, 0.00)	-0.04 (-0.13, 0.05)
Post-buffet meal	0.04 ± 0.02	-0.04 ± 0.02	0.02 ± 0.01	0.00 ± 0.01	-0.05 (-0.09, -0.02)[3]	-0.01 (-0.04, 0.02)

Values are mean ± SEM. Intervention period covers 0.0-1.0 h; post-test meal covers 1.5-3.5 h; afternoon period covers 3.5-6.5 h; post-buffet meal covers 6.5-7.5 h. Data were analyzed using linear mixed models with trial (exercise or control) and genotype (AA or TT) included as fixed factors.

[1] 95% confidence interval of the mean absolute difference between the experimental trials.
[2] 95% confidence interval of the mean absolute difference between the genotype groups.
[3] Significant main effect of trial ($P < 0.05$).
[4] Significant genotype-by-trial interaction ($P < 0.05$).
[5] Significant main effect of genotype ($P < 0.05$).

AG, acyl-ghrelin; DAG, des-acyl-ghrelin.

Table 4. Time-averaged total area under the curve for delta concentrations of total glucagon-like peptide 1 and total peptide YY for AAs and TTs in the exercise and control trials.

	AA (n = 12)		TT (n = 12)		Main effect trial Control vs exercise Mean difference (95% CI[1])	Main effect genotype TT vs AA Mean difference (95% CI[2])
	Control	Exercise	Control	Exercise		
Δ Total GLP-1 (pmol/L·h)						
Intervention period	-3.8 ± 0.9	15.0 ± 1.8	-6.5 ± 1.2	10.7 ± 2.4	18.0 (14.7, 21.3)[3]	3.5 (-0.2, 7.2)
Post-test meal	34.2 ± 7.9	107.0 ± 12.1	21.4 ± 7.0	112.3 ± 8.0	81.6 (64.9, 98.3)[3]	3.9 (-16.8, 24.8)
Afternoon	97.0 ± 22.4	142.8 ± 15.2	80.0 ± 17.4	144.6 ± 15.4	55.2 (27.0, 83.4)[3]	7.6 (-36.5, 51.7)
Post-buffet meal	33.0 ± 7.5	44.6 ± 5.2	15.7 ± 4.8	25.0 ± 5.6	10.4 (0.3, 20.5)[3]	18.6 (4.7, 32.4)[4]
Δ Total PYY (pg/mL·h)						
Intervention period	-14.7 ± 8.3	51.5 ± 13.3	-18.3 ± 3.8	53.7 ± 13.3	69.1 (48.2, 90.0)[3]	0.7 (-21.8, 23.1)
Post-test meal	105.7 ± 22.9	215.2 ± 34.6	61.1 ± 24.7	207.3 ± 30.7	128.4 (74.3, 182.6)[3]	25.7 (-40.0, 91.3)
Afternoon	507.5 ± 82.9	536.4 ± 85.8	394.0 ± 85.4	458.7 ± 67.8	46.8 (-76.5, 170.0)	95.6 (-106.9, 298.1)
Post-buffet meal	198.4 ± 23.5	166.6 ± 21.7	108.9 ± 22.0	131.8 ± 23.7	-4.0 (-43.2, 35.3)	61.6 (7.1, 116.2)[4]

Values are mean ± SEM. Intervention period covers 0.0-1.0 h; post-test meal covers 1.5-3.5 h; afternoon period covers 3.5-6.5 h; post-buffet meal covers 6.5-7.5 h. Data were analyzed using linear mixed models with trial (exercise or control) and genotype (AA or TT) included as fixed factors.

[1] 95% confidence interval of the mean absolute difference between the experimental trials.
[2] 95% confidence interval of the mean absolute difference between the genotype groups.
[3] Significant main effect of trial ($P < 0.05$).
[4] Significant main effect of genotype ($P < 0.05$).
Linear mixed models revealed no genotype-by-trial interactions ($P \geq 0.169$).

GLP-1, glucagon-like peptide-1, PYY, peptide YY.

Table 5. Time-averaged total area under the curve for appetite perceptions for AAs and TTs in the control and exercise trials.

	AA (n = 12)		TT (n =12)		Main effect trial	Main effect genotype
	Control	Exercise	Control	Exercise	Control vs exercise Mean difference (95% CI[1])	TT vs AA Mean difference (95% CI[2])
Hunger (mm·h)						
Intervention	68 ± 4	39 ± 5	80 ± 3	53 ± 6	-27 (-37,-18)[3]	-10 (-20, 1)
Post-test meal	83 ± 8	87 ± 6	60 ± 6	60 ± 5	2 (-9, 13)	25 (10, 40)[4]
Afternoon	172 ± 14	192 ± 13	138 ± 10	144 ± 14	13 (-4, 30)	41 (7, 74)[4]
Post-buffet meal	35 ± 4	44 ± 4	32 ± 3	31 ± 3	-1.6 (-2, 9)	8.0 (-1, 17)
Fullness (mm·h)						
Intervention	21 ± 4	39 ± 5	13 ± 3	25 ± 5	15 (9, 21)[3]	11 (0, 23)
Post-test meal	112 ± 7	115 ± 7	132 ± 6	137 ± 5	4 (-6, 15)	-20 (-37, -3)[4]
Afternoon	108 ± 13	102 ± 13	142 ± 12	141 ± 12	-4 (-27, 19)	-37 (-66, -8)[4]
Post-buffet meal	99 ± 4	101 ± 3	112 ± 3	110 ± 3	0 (-4, 3)	-11 (-20, -2)[4]
Prospective food consumption (mm·h)						
Intervention	77 ± 4	51 ± 5	80 ± 4	58 ± 6	-24 (-32, -16)[3]	-6 (-17, 6)
Post-test meal	99 ± 8	102 ± 7	77 ± 8	71 ± 9	-2 (-11, 8)	26 (5, 48)[4]
Afternoon	186 ± 14	205 ± 11	163 ± 12	157 ± 16	6 (-10, 23)	(1, 71)[4]
Post-buffet meal	46 ± 5	52 ± 5	39 ± 3	43 ± 6	5 (-1, 11)	7 (-6, 20)
Hedonic wanting of food (mm·h)						
Intervention	78 ± 4	49 ± 6	83 ± 4	57 ± 6	-28 (-38, -19)[3]	-7 (-19, 6)
Post-test meal	107 ± 10	107 ± 6	80 ± 9	78 ± 10	-2 (-12, 8)	28 (4, 52)[4]
Afternoon	201 ± 12	219 ± 9	161 ± 13	158 ± 17	8 (-11, 26)	51 (17, 84)[4]
Post-buffet meal	55 ± 7	61 ± 5	52 ± 6	51 ± 7	2 (-5, 10)	7 (-10, 23)

Values are mean ± SEM. Intervention period covers 0.0-1.0 h; post-test meal covers 1.5-3.5 h; afternoon period covers 3.5-6.5 h; post-buffet meal covers 6.5-8.0 h. Data were analyzed using linear mixed models with trial (exercise or control) and genotype (AA or TT) included as fixed factors.

[1] 95% confidence interval of the mean absolute difference between the experimental trials.
[2] 95% confidence interval of the mean absolute difference between the genotype groups.
[3] Significant main effect of trial ($P < 0.05$).
[4] Significant main effect of genotype ($P < 0.05$).
Linear mixed models revealed no genotype-by-trial interactions ($P \geq 0.061$).

Table 6. Energy and macronutrient intakes at the buffet meal for AAs and TTs in the exercise and control trials.

	AA (n = 12)		TT (n = 12)		Main effect trial Control vs exercise Mean difference (95% CI[1])	Main effect genotype TT vs AA Mean difference (95% CI[2])
	Control	Exercise	Control	Exercise		
Absolute energy intake (kJ)	5229 ± 576	5554 ± 627	3788 ± 463	3897 ± 490	217 (-191, 625)	1549 (10, 3088)[3]
Relative energy intake (kJ)	5139 ± 571	1888 ± 642	3710 ± 429	532 ± 467	-3214 (-3674, -2755)[4]	1393 (-186, 2973)
Carbohydrate (g)	160 ± 18	162 ± 17	117 ± 16	119 ± 17	3 (-12,18)	43 (-5, 90)
Protein (g)	48 ± 4	52 ± 5	36 ± 4	37 ± 5	3 (-1, 7)	14 (1, 26)[3]
Fat (g)	47 ± 7	52 ± 8	33 ± 4	34 ± 4	3 (0, 7)	16 (-1, 34)

Values are mean ± SEM. Relative energy intake is energy intake at the buffet meal minus the gross energy expenditure of the intervention period (0.0-1.0 h). Data were analyzed using linear mixed models with trial (exercise or control) and genotype (AA or TT) included as fixed factors.

[1] 95% confidence interval of the mean absolute difference between the experimental trials.
[2] 95% confidence interval of the mean absolute difference between the genotype groups.
[3] Significant main effect of genotype ($P < 0.05$).
[4] Significant main effect of trial ($P < 0.05$).
Linear mixed models revealed no genotype-by-trial interactions ($P \geq 0.207$).

Figure legends

Figure 1. Schematic representation of the main trials.

Figure 2. Δ AG concentrations (A), DAG concentrations (B) and AG/DAG ratio (C) in AAs (n = 12) and TTs (n = 12) during the control and exercise trials. *Dotted rectangle* indicates exercise, *horizontally dashed rectangle* indicates standardized test meal, *vertically dashed rectangle* indicates buffet meal. Data are represented as mean ± SEM. Data were analyzed using linear mixed models with trial (exercise or control), genotype (AA or TT) and time included as fixed factors. Δ AG: main effect trial $P < 0.001$, main effect time $P < 0.001$, genotype-by-time interaction $P = 0.007$; Δ DAG: main effect trial $P < 0.001$, main effect time $P < 0.001$, genotype-by-trial interaction $P < 0.001$; Δ AG/DAG ratio: main effect trial $P < 0.001$, main effect time $P < 0.001$, genotype-by-trial interaction $P < 0.001$, genotype-by-time interaction $P = 0.001$, genotype-by-trial-by-time interaction $P = 0.004$. Linear mixed models for Δ AG, Δ DAG and Δ AG/DAG ratio revealed no main effect of genotype (all $P \geq 0.192$) or other interactive effects ($P \geq 0.083$). AG, acyl-ghrelin; DAG, des-acyl-ghrelin.

Figure 3. Δ Total GLP-1 (A) and total PYY (B) concentrations in AAs (n = 12) and TTs (n = 12) during the control and exercise trials. *Dotted rectangle* indicates exercise, *horizontally dashed rectangle* indicates standardized test meal, *vertically dashed rectangle* indicates buffet meal. Data are represented as mean ± SEM. Data were analyzed using linear mixed models with trial (exercise or control), genotype (AA or TT) and time included as fixed factors. Δ total GLP-1: main effect trial $P < 0.001$, main effect time $P < 0.001$, genotype-by-time interaction $P = 0.002$; Δ total PYY: main effect trial $P < 0.001$, main effect time $P < 0.001$. Linear mixed models for Δ total GLP-1 and Δ total PYY revealed no main effect of genotype (all $P \geq 0.278$) or other interactive effects ($P \geq 0.089$). GLP-1, glucagon-like peptide-1, PYY, peptide YY.

Figure 4. Δ Plasma BChE activity in AAs (n = 12) and TTs (n =12) during the control and exercise trials at 0.5 and 1.0 h. *Dotted rectangle* indicates exercise. * *P* = 0.004 for main effect of trial. Data are represented as mean ± SEM. Data were analyzed using linear mixed models with trial (exercise or control), genotype (AA or TT) and time included as fixed factors. Δ BChE activity: main effect trial P = 0.004, main effect time P < 0.001. Linear mixed models for Δ BChE activity revealed no main effect of genotype (P = 0.681) or interactive effects (P ≥ 0.094). BChE, butyrylcholinesterase.

Figure 5. Hunger (A), fullness (B), prospective food consumption (C) and hedonic wanting of food (D) in AAs (n = 12) and TTs (n = 12) during the control and exercise trials. *Dotted rectangle* indicates exercise, *horizontally dashed rectangle* indicates standardized test meal, *vertically dashed rectangle* indicates buffet meal. Data are represented as mean ± SEM. Data were analyzed using linear mixed models with trial (exercise or control), genotype (AA or TT) and time included as fixed factors. All appetite perceptions: main effect trial P = 0.002, main effect time P < 0.001, genotype-by-time interaction P < 0.001. Linear mixed models for each appetite perception revealed no main effect of genotype (P ≥ 0.072) or other interactive effects (P ≥ 0.094).