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Effects of moderate to vigorous intensity cycling on appetite, *ad libitum* energy intake and appetite-related hormones in healthy South Asian and white European men

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ABSTRACT

Compensatory changes in appetite and energy intake do not appear to occur in the short-term after acute exercise; however, responses have not been compared in South Asians, a group at high risk of central obesity and type 2 diabetes, with white Europeans. This study examined appetite perceptions, energy intake and appetite-related hormones after moderate-to-vigorous intensity cycling in South Asian versus white European men. Fifteen South Asians (mean(SD) 29(8) years; 25.4(4.5) kg m^{-2}) and fifteen white Europeans (33(10) years; 26.1 (3.8) kg m^{-2}) matched for age and body mass index completed two 7 h trials (control and exercise). Participants rested throughout both trials apart from completing 60 min cycling at 2–3 h in the exercise trial. A standardised breakfast was consumed at 0 h and an *ad libitum* buffet meal at 4 h. Appetite perceptions and appetite-related hormones were measured at predetermined intervals. Exercise suppressed acylated ghrelin ($d = 0.19$, $P < 0.001$) and increased total peptide YY (PYY) ($d = 0.14$, $P = 0.004$), insulin ($d = 0.09$, $P = 0.046$) and glucose concentrations ($d = 0.31$, $P < 0.001$) (main effect of trial), without stimulating compensatory increases in energy intakes in either group (group-by-trial interactions). South Asians exhibited lower absolute energy intake and higher insulin concentrations than white Europeans (main effect group $d \geq 0.63$, $P \leq 0.003$), whereas group-by-time interactions revealed lower acylated ghrelin concentrations at 3 and 4 h ($d \geq 0.75$, $P \leq 0.038$) and higher glucose concentrations at 0.75 and 2 h ($d \geq 0.67$, $P \leq 0.008$) in South Asian than white European men. These findings demonstrate that acute exercise induces a short-term energy deficit and similar appetite responses in South Asian and white European men.

1. Introduction

South Asians are a heterogeneous group of individuals originating from the Indian subcontinent (India, Pakistan, Bangladesh, Nepal, Sri Lanka and Bhutan) with a large representation living in high-income countries including the United Kingdom (UK), United States of America (USA) and Canada (Sattar & Gill, 2015). Numerous studies have shown that South Asian individuals have a heightened risk of cardiovascular disease (CVD) and type 2 diabetes (T2D) compared to white Europeans, irrespective of their place of living, with both conditions manifesting typically 5–10 years earlier in the South Asian population (Gholap et al., 2011; Sattar & Gill, 2015; Tziomalos et al., 2008).

South Asians typically exhibit a greater level of total and central adiposity for a given body mass index (BMI) as well as a higher prevalence of insulin resistance than white European individuals, which contribute to the elevated CVD and T2D risk in this population (Ghouri

Abbreviations: CI, confidence intervals; RPE, ratings of perceived exertion; ES, effect size; VAS, visual analogue scales; PARQ, physical activity readiness questionnaire.

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et al., 2013; Lear et al., 2012). Appetite-related hormones play a key role in energy homeostasis and weight control, including the orexigenic hormone acylated ghrelin and the satiety hormone peptide YY (PYY), and these may be linked to the elevated adiposity in South Asians. Recent research from our laboratory demonstrated lower concentrations of fasting acylated ghrelin in South Asian compared with white European men at rest, which appeared to be associated with the greater adiposity in South Asians (Benedetti et al., 2019). These data are in agreement with previous findings showing lower fasting ghrelin concentrations in individuals with obesity compared with individuals who are lean (Cummings et al., 2003; Le Roux et al., 2005). However, other studies have shown the role of hyperinsulinemia and insulin resistance in the negative regulation of ghrelin concentrations, irrespective of adiposity (Becker et al., 2012; Flanagan et al., 2003; McLaughlin et al., 2004), although this finding remains inconsistent (Pagotto et al., 2002).

Lower levels of physical activity and cardiorespiratory fitness in South Asian than white European individuals have been suggested to contribute to exacerbating the excess adiposity, insulin resistance and CVD risk in this population (Ghouri et al., 2013; Williams et al., 2011; Yates et al., 2015). Given the important role of physical activity in the management of obesity and weight control, exercise may represent an effective strategy to prevent the greater central obesity and cardiometabolic disease risk in South Asian individuals. Research to date suggests single bouts of exercise transiently suppress appetite, suppress acylated ghrelin and increase PYY without provoking compensatory changes in energy intake on the day of exercise (Deighton & Stensel, 2014; Schubert et al., 2013, 2014), although a large variability in responses exists between individuals. In this regard, while differences in individual characteristics including adiposity and sex do not appear to modulate appetite responses to exercise (Dorling et al., 2018), there has been no research investigating ethnic differences in appetite perceptions, energy intake and appetite-related hormones in response to acute exercise between South Asian and white European individuals.

Therefore, the aim of this study was to investigate the effects of acute moderate-to-vigorous intensity exercise on appetite perceptions, appetite-related hormones and *ad libitum* energy intake in healthy South Asian and white European men. It was hypothesised that there would be no difference in appetite perceptions, appetite-related hormones and energy intake responses to a single bout of exercise between ethnic groups.

2. Methods

2.1. Participants

Following approval from Kingston University's Ethics Advisory Committee (1617/034), 15 South Asian and 15 white European men aged 19–50 years provided written informed consent to participate in this study. Based on previous data (Benedetti et al., 2019), it was estimated that a sample size of 15 participants per group would have 88% power at the 0.05 level to detect a between-group difference in fasting acylated ghrelin of 1.19 between-subject standard deviations (SDs). The sample size was calculated using G*Power (Faul et al., 2007). Groups were matched at recruitment for age and BMI, although body mass and fat free mass were lower in South Asian than white European men. The South Asian group comprised seven British Asians born in the UK (UK Indian n = 4; UK Pakistani n = 2; UK Bangladeshi n = 1) and eight individuals born in South Asia (India n = 4; Pakistan n = 2; Bangladesh n = 1; Nepal n = 1). Conversely, the white European group comprised nine British born participants and six individuals originating from European countries (Germany n = 3; Spain n = 1; Italy n = 1; France n = 1). Prior to participating in this study, all participants were asked to complete a questionnaire to identify their ethnic background and place of birth. All participants were non-smokers, had no personal history of cardiovascular/metabolic disease, were not taking any anticoagulant or anti-inflammatory medication and were not dieting. Before physical testing, the Physical Activity Readiness Questionnaire (PAR-Q) (Thomas et al., 1992) was completed by all participants to screen for possible contraindications to exercise. Table 1 shows the key participant characteristics.

2.2. Preliminary testing

Before the main trials, participants visited the laboratory to undergo preliminary assessments and to be familiarised with the laboratory environment and study procedures. Participants also completed questionnaires assessing general health status and contraindications for blood sampling. At this visit, the participants confirmed acceptability of the standardised breakfast and *ad libitum* buffet meal subsequently provided during the main experimental trials. Body mass, stature, body mass index, waist circumference, body composition (using air displacement plethysmography) and resting arterial blood pressure were measured as described in our previous work (Benedetti et al., 2019).

Participants then completed an incremental cycling test on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) for the determination of peak oxygen uptake (VO2 peak) at a self-selected pedal rate between 70 and 90 revolutions per min for 3 min at 80 W (warm up), followed by increments of 30 W every 3 min until volitional fatigue. Expired air samples were monitored continuously using an online breath-by-breath gas analysis system (Oxycon Pro, Viasys Healthcare Gmbh, Höchberg, Germany). An average of the breath-by-breath VO2 data was calculated every 15 s, and VO2 peak was recorded as the highest 15 s average. Oxygen consumption, heart rate and peak watts were used to determine the exercise intensity of the main trial.

Table 1
Participant characteristics.

	South Asians (n = 15)	White Europeans (n = 15)	White Europeans vs. South Asians Mean difference (95% CI[a])	Effect size
Age (years)	29 (8)	33 (10)	−4 (−11, 3)	0.44
Stature (cm)	173.7 (6.8)	181.5 (6.6)	−7.8 (−12.9, −2.8)[b]	1.16
Body mass (kg)	76.0 (12.5)	85.5 (14.4)	−9.5 (−19.5, 0.6)	0.73
Body mass index ($kg \cdot m^{-2}$)	25.4 (4.5)	26.1 (3.8)	−0.6 (−3.7, 2.4)	0.17
Fat free mass (kg)	59.6 (8.8)	68.5 (7.5)	−8.9 (−14.9, −2.8)[b]	1.09
Fat mass (kg)	17.6 (8.0)	17.5 (10.7)	0.1 (−7.0, 7.1)	0.01
Body fat (%)	22.4 (8.3)	19.3 (8.3)	3.2 (−3.0, 9.4)	0.37
Waist circumference (cm)	84.8 (9.8)	87.6 (11.5)	−2.8 (−10.8, 5.1)	0.26
Resting sBP (mmHg)	116 (12)	125 (8)	−9 (−16, −1)[b]	0.90
Resting dBP (mmHg)	77 (9)	75 (7)	2 (−4, 8)	0.26
VO_2 peak ($L \cdot min^{-1}$)	3.10 (0.61)	4.12 (0.59)	−1.02 (−1.47, −0.57)[b]	1.70
VO_2 peak ($mL \cdot kg^{-1} \cdot min^{-1}$)	41 (7)	49 (9)	−8 (−14, −2)[b]	0.99

All values are mean (SD). Data were analysed using linear mixed models with group (South Asian vs. white European) included as a fixed factor.
sBP, systolic blood pressure; *dBP*, diastolic blood pressure; *VO_2 peak*, peak oxygen uptake.
[a] 95% confidence interval for the mean absolute difference between the groups.
[b] Main effect of group ($P \leq 0.020$).

2.3. Experimental procedure

Participants completed two, 7 h trials (control and exercise) in a randomised crossover design with at least 7 days between each trial. Fig. 1 shows the trial protocol. Participants were asked to refrain from consuming alcohol, caffeinated drinks and from participating in strenuous exercise during the 24 h prior to each trial. Participants were also asked to consume 500 mL of plain water the night before the exercise trial to ensure euhydration. A food diary was completed in the 24 h prior to the first trial, with participants required to replicate food and drink intake as closely as possible for the 24 h prior to the subsequent trial.

On the morning of each trial, participants arrived at the laboratory at approximately 08:30 after a 9 h overnight fast and exerted themselves minimally when travelling to the laboratory, using motorised transport where possible. Upon arrival, participants rested in a semi-supine position whilst a cannula was inserted into the antecubital vein by a trained phlebotomist, and a standardised breakfast meal was then consumed within 15 min. The 7 h trial commenced at the start of the breakfast (0 h). In the exercise trial, participants rested throughout apart from completing 60 min of continuous cycling at 70% of VO_2 peak between 2 and 3 h. Samples of expired air were collected at 15, 30, 45 and 60 min during exercise using an online breath-by-breath gas analysis system to monitor the exercise work-load. Heart rate and ratings of perceived exertion (RPE) (Borg, 1973) were also recorded at this time. Energy expenditure and non-protein respiratory exchange ratio (RER) for the estimation of substrate utilisation were calculated from oxygen uptake and carbon dioxide production (Weir, 1990) during the exercise bout. A buffet meal was provided at 4 h and participants were free to consume *ad libitum* for 30 min. Identical procedures were followed in the control trial except that no exercise was performed.

2.4. Appetite perceptions

During each trial (exercise and control) perceptions of appetite (hunger, satisfaction, fullness and prospective food consumption) were assessed in the fasted state (0 h) and at 30 min intervals thereafter using 100 mm visual analogue scales (VAS) (Flint et al., 2000). An overall appetite rating was calculated as the mean value of the four appetite perceptions after reversing the values for satisfaction and fullness (Stubbs et al., 2000).

2.5. Standardised breakfast and ad libitum buffet meal

The standardised breakfast consisted of a sandwich (55 g white bread, 23 g cheese, 10 g mayonnaise and 40 g ham), 60 g chocolate muffin and 250 mL orange juice. For five South Asian participants ham was replaced with an isocaloric portion of tuna due to religious beliefs. The energy and macronutrient content of this meal was 2853 kJ, 46% carbohydrate, 14% protein and 40% fat.

The *ad libitum* buffet meal was set up identically for each trial and consisted of granola, oats, corn flakes, white bread, semi skimmed milk, orange juice, cheese, ham/tuna, butter, margarine, mayonnaise, salted crisps, chocolate bars, cereal bars, cookies, muffins, apples, oranges and bananas. All food was pre-weighed and presented in excess of expected consumption. Participants were told to eat until satisfied and that additional food was available if required. The buffet meal was consumed in isolation with no distraction and the use of computers or mobile phones was prohibited to minimise any influence on food consumption. At the end of the buffet meal, leftover food was weighed, and absolute energy intake and macronutrient composition of the food consumed was determined by calculating the weighted difference of each food item before and after each meal. In the exercise trial, relative energy intake was calculated as follows: absolute energy intake minus the net energy expenditure of exercise. Water was available *ad libitum* throughout the trials.

2.6. Blood sampling

Venous blood samples were collected via a cannula (Vasofix® Safety, B. Braun, Melsungen, Germany) inserted into an antecubital vein. Blood samples were collected at 0, 0.75, 2, 3, 4, 4.75 and 7 h for the determination of acylated ghrelin, total PYY, insulin and glucose concentrations. At each sampling point, haemoglobin and haematocrit were measured to estimate changes in plasma volume (Dill & Costill, 1974). During each trial, all blood samples were collected with the participants rested in a semi-supine position with the exception of the sample at 3 h in the exercise trial, which was taken with the participant seated, but not pedalling, on the cycle ergometer. Samples were collected into four pre-cooled vacutainers: 6.0 mL heparin, 10.0 mL EDTA and two, 4.0 mL EDTA (BD Vacutainer®, Plymouth, UK). To prevent the degradation of acylated ghrelin, a 40 μL solution containing potassium phosphate buffer (PBS), *p*-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH) was added immediately after blood collection to one of

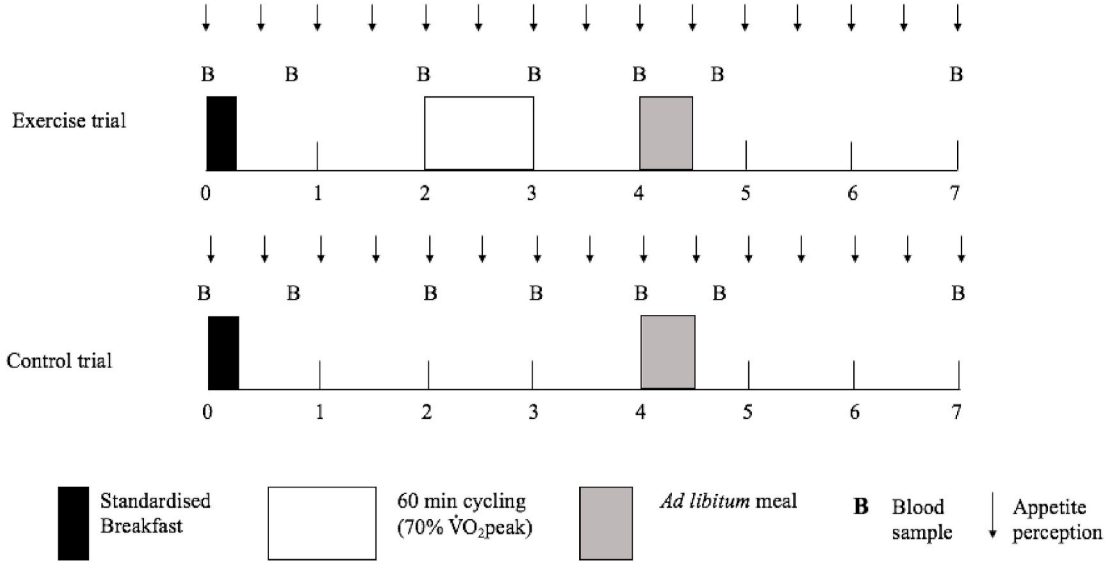

Fig. 1. Schematic illustration of the study protocol.

the 4.0 mL EDTA vacutainers which was then centrifuged at 1500×*g* for 10 min at 4 °C. The plasma supernatant was dispensed into a storage tube and 100 μL of 1 M hydrochloric acid was added per millilitre of plasma to preserve acylated ghrelin (Hosoda et al., 2004). Thereafter, samples were spun at 1500×*g* for 5 min at 4 °C prior to storage at −80 °C. The 6.0 mL heparin and 10 mL EDTA vacutainers were centrifuged immediately at 1500×*g* for 10 min at 4 °C prior to storage at −80 °C. The other 4.0 mL EDTA vacutainer was immediately used for determination of haemoglobin and haematocrit.

2.7. Analytical methods

Plasma concentrations of acylated ghrelin (Sceti K.K., Tokyo, Japan), total PYY (Millipore, Billerica, USA) and insulin (Mercodia, Uppsala, Sweden) were analysed in duplicate using commercially available enzyme-linked immunosorbent assays. Plasma glucose concentrations were analysed immediately in singular using a glucose analyser (Biosen C-Line Clinic, EKF Diagnostic, Germany). Haemoglobin and haematocrit were analysed immediately in duplicate using a haematology blood counter (Yuminez H500-CT, HORIBA ABX Diagnostic, Northampton, UK). The within batch coefficients of variation for the assays were as follows: 7.3% acylated ghrelin, 3.2% total PYY, 2.4% insulin and 3.3% glucose.

2.8. Statistical analyses

Statistical analyses were conducted using the analytical software SPSS version 23.0 for Windows (SPSS 23.0, IBM Corp, Armonk, NY, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated (Matthews et al., 1985). Normality of the data was checked using Shapiro-Wilk tests. Normally distributed data are presented as mean (SD). Data for plasma concentrations (excluding time averaged total under the curve (tAUC) values) and HOMA-IR were not normally distributed and were natural log-transformed before analysis. These variables are presented as geometric mean (95% confidence interval) and analysis is based on ratios of the geometric mean and 95% confidence intervals (CI) for ratios. The trapezium rule was used to calculate the time averaged tAUC. Correction of plasma values for plasma volume changes did not alter the interpretation of the results and the unadjusted data are reported for simplicity.

Participant characteristics and exercise responses were compared between the South Asian and white European men using linear mixed models with ethnic group included as a fixed factor. Differences in fasting plasma concentrations, baseline appetite perceptions, time-averaged tAUC, fasting HOMA-IR and energy/macronutrient intakes were examined using linear mixed models with ethnic group (South Asian, white European) and trial (exercise, control) modelled as fixed factors. Differences in appetite perceptions, and concentrations of acylated ghrelin, total PYY, glucose and insulin over time were examined using linear mixed models with ethnic group, trial and time as fixed factors. Where significant trial and interaction effects were found, post hoc analysis was performed using Fisher's Least Significant Difference (LSD) test, equivalent to no adjustment. Bivariate Pearson's correlations were also used to determine associations between total carbohydrate oxidation during exercise and absolute energy intake in South Asian and white European men. Absolute standardised effect sizes (ES) (Cohen's *d*) were calculated for each variable by dividing the difference between the mean values (South Asian versus white European and exercise versus control) with the pooled SD. An ES of 0.2 was considered the minimum important difference, 0.5 moderate and 0.8 large (Cohen, 1988). Statistical significance was accepted as $P < 0.05$.

3. Results

3.1. Participant characteristics

The physical and physiological characteristics of the South Asian and white European men are shown in Table 1. The 95% CI for age, body mass, BMI, fat mass, body fat percentage, waist circumference and resting diastolic blood pressure overlapped zero (all $P \geq 0.064$). However, standardised ESs were small-to-moderate for age, body fat percentage, waist circumference and resting diastolic blood pressure ($d = 0.26$–0.44) and a moderate-to-large ES was observed for body mass ($d = 0.73$). Compared with white European men, South Asian men had lower stature ($d = 1.16$, $P = 0.003$), fat free mass ($d = 1.09$, $P = 0.006$), resting systolic blood pressure ($d = 0.90$, $P = 0.020$) and VO_2 peak expressed in absolute ($d = 1.70$, $P < 0.001$) and relative ($d = 0.99$, $P = 0.007$) terms.

3.2. Exercise responses

Exercise net energy expenditure ($d = 1.54$, $P < 0.001$), total fat oxidation ($d = 0.93$, $P = 0.018$), total carbohydrate oxidation ($d = 1.07$, $P = 0.007$) and absolute exercise work rate ($d = 1.69$, $P < 0.001$) were lower in South Asian than white European men (Table 2). All other exercise responses were similar between ethnic groups (all $P \geq 0.128$) (Table 2).

3.3. Ad libitum energy and macronutrient intakes

Energy and macronutrient intakes during the *ad libitum* meal are shown in Table 3. Main effects of group revealed lower absolute energy intake ($d = 1.03$, $P = 0.003$), relative energy intake ($d = 0.65$, $P =$

Table 2
Cycling exercise responses in South Asian and white European men.

	South Asians (n = 15)	White Europeans (n = 15)	White Europeans vs. South Asians Mean difference (95% CI[a])	Effect size
Heart rate (beats·min^{-1})	159 (12)	154 (11)	5 (−4, 13)	0.43
RPE (6–20)	13.7 (2.1)	13.9 (1.9)	−0.3 (−1.8, 1.2)	0.20
Respiratory exchange ratio	0.94 (0.02)	0.92 (0.04)	0.02 (−0.01, 0.04)	0.63
Exercise intensity (% of VO_2 peak)	67 (4)	69 (5)	−3 (−6, 1)	0.56
Work rate (Watts)	115 (21)	161 (32)	−46 (−66, −25)[b]	1.69
Work rate (% max power)	59 (6)	58 (7)	2 (−3, 6)	0.23
Total fat oxidation (g)	13.7 (7.5)	21.9 (9.9)	−8.2 (−14.9, −1.5)[b]	0.93
Total carbohydrate oxidation (g)	106.7 (19.2)	136.5 (34.6)	−29.8 (−50.9, −8.7)[b]	1.07
Fat oxidation (% total energy expenditure)	20 (9)	22 (10)	−2 (−9, 5)	0.21
Carbohydrate oxidation (% total energy expenditure)	73 (8)	69 (10)	4 (−3, 11)	0.44
Net energy expenditure (kJ)	2475 (469)	3324 (624)	−849 (−1268, -430)[b]	1.54

All values are mean (SD). Data were analysed using linear mixed models with group (South Asian vs. white European) included as a fixed factor.
RPE rating of perceived exertion; *VO_2 peak,* peak oxygen uptake.
[a] 95% confidence interval for the mean absolute difference between the groups.
[b] Main effect of group ($P \leq 0.038$).

Table 3
Ad libitum energy and macronutrient intakes in South Asian and white European men.

	South Asians (n = 15)		White Europeans (n = 15)		White Europeans vs. South Asians Mean difference (95% CI[a])	Control vs. Exercise Mean difference (95% CI[a])
	Control	Exercise	Control	Exercise		
Absolute energy intake (kJ)	5902 (1564)	5773 (1244)	7441 (1901)	8010 (2478)	−1888 (−3088, −687)[b]	220 (−474, 914)
Relative energy intake (kJ)	5902 (1564)	3298 (1103)	7441 (1901)	4686 (2485)	−1464 (−2649, −278)[b]	−2679 (−3369, −1989)[c]
Carbohydrate intake (g)	166.4 (55.3)	146.8 (47)	190.7 (54.2)	224.8 (80.7)	−51.1 (−91.4, −10.8)[b,d]	7.2 (−13.7, 28.1)[d]
Fat intake (g)	58.9 (15.5)	60.2 (14.2)	83.5 (30.5)	82.3 (28.1)	−23.3 (−37.8, −8.8)[b]	0.1 (−9.5, 9.6)
Protein intake (g)	53.7 (18.8)	62.7 (35.1)	65.9 (20.3)	68.7 (20.5)	−9.1 (−24.1, 5.8)	5.9 (−4.8, 16.6)

All values are mean (SD). Data were analysed using linear mixed models with group (South Asian vs. white European) and trial (exercise vs. control) included as fixed factors.
[a] 95% confidence interval for the mean absolute difference between the groups or trials.
[b] Main effect of group ($P \leq 0.017$).
[c] Main effect of trial ($P < 0.001$).
[d] Group-by trial interaction ($P = 0.014$).

0.017), carbohydrate intake ($d = 0.83$, $P = 0.015$) and fat intake ($d = 1.02$, $P = 0.003$) in South Asian compared with white European men. Absolute energy intake was similar between trials ($d = 0.11$, $P = 0.521$) whereas relative energy intake was lower in the exercise than the control trial ($d = 1.37$, $P < 0.001$). A group-by-trial interaction for carbohydrate intake ($P = 0.014$) revealed higher intake in white Europeans after exercise (mean difference (95% CI) 34 (5, 64) g, $d = 0.50$, $P = 0.025$); the 95% CI for the mean between-trial difference overlapped zero in South Asians (mean difference (95% CI) −20 (−49, 10) g, $d = 0.38$, $P = 0.184$). No other main effects or group-by-trial interactions were identified for *ad libitum* energy and macronutrient intakes (all $P \geq 0.063$).

3.4. Appetite perceptions

Fasting overall appetite ratings at baseline were not significantly different across groups and trials (main effect group $P = 0.156$; main effect trial $P = 0.871$; group-by-trial interaction $P = 0.323$) (Fig. 2). Linear mixed models for overall appetite identified a main effect of time ($P < 0.001$), group-by-time interaction ($P = 0.013$) and trial-by-time interaction ($P = 0.014$), but not a main effect of group (mean difference (95% CI) −2 (−7, 4) mm, $d = 0.07$, $P = 0.521$) or trial (mean difference (95% CI) −1 (−2, 1) mm, $d = 0.02$, $P = 0.590$) (Fig. 2). Post hoc analysis of the group-by-time interaction revealed lower overall appetite in South Asian than white European participants at 6.5 h (mean difference (95% CI) −8 (−18, −0.1) mm, $d = 0.58$, $P = 0.047$). Post hoc analysis of the trial-by-time interaction revealed that overall appetite ratings were lower at 4.5 h (mean difference (95% CI) −11 (−18, −3) mm, $d = 0.51$, $P = 0.004$) and higher at 6.5 h (mean difference (95% CI) 8 (1, 15) mm, $d = 0.53$, $P = 0.024$) in the exercise than in the control trial. Time-averaged total area under the curve values for overall appetite ratings were similar across groups and trials (main effect group $P = 0.483$; main effect trial $P = 0.637$; group-by-trial interaction $P = 0.452$) (Table 4).

3.5. Plasma concentrations of appetite-related hormones and glucose

3.5.1. Acylated ghrelin

Fasting acylated ghrelin concentrations were not significantly different between groups (ratio difference (95% CI) −22.4 (−48.2, 16.4)%, $d = 0.46$; $P = 0.211$) but were lower in the exercise than the control trial (ratio difference (95% CI) −14.0 (−22.5, −4.6)%, $d = 0.27$, $P = 0.006$) (Fig. 3). Linear mixed model for acylated ghrelin concentrations identified a main effect of trial ($P < 0.001$), time ($P < 0.001$) and group-by-time interaction ($P = 0.006$) (Fig. 3). Acylated ghrelin concentrations were lower in the exercise than in the control trial (ratio difference (95% CI) −10.1 (−14.6, −5.3)%, $d = 0.19$, $P < 0.001$) (Fig. 3) and meaningfully, albeit not significantly, lower in South Asian than white European men (ratio difference (95% CI) −25.5 (−46.3, 3.4)%, d

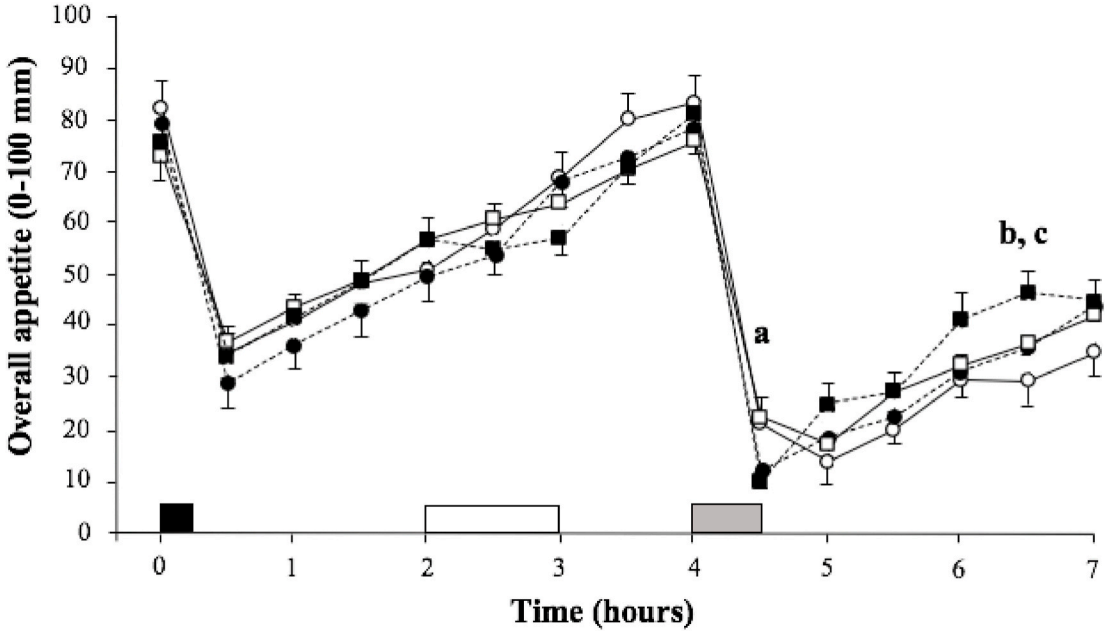

Fig. 2. Overall appetite perceptions.

Table 4
Time averaged total area under the curve for overall appetite, appetite-related hormone, glucose concentrations and fasting HOMA-IR in South Asian and white European men.

	South Asians (n = 15)		White Europeans (n = 15)		White Europeans vs. South Asians Mean difference (95% CI[a])	Control vs. Exercise Mean difference (95% CI[a])
	Control	Exercise	Control	Exercise		
Overall appetite	22.8 (4.5)	21.9 (3.9)	23.2 (4.3)	23.4 (4.6)	−0.9 (−3.8, 1.9)	−0.4 (−1.8, 1.2)
Acylated ghrelin (pg·mL^{-1} h)	31.1 (13.2)	28.0 (9.9)	44.4 (20.7)	39.8 (19.2)	−12.5 (−24.6, −0.5)[b]	−3.79 (−5.9, −1.6)[c]
Total peptide YY (pg·mL^{-1} h)	117.0 (26.1)	128.0 (37.0)	116.7 (40.0)	118.8 (28.0)	4.7 (−19.3, 28.6)	6.5 (−0.3, 13.4)
Insulin (μU·L^{-1} h)	48.0 (23.8)	50.9 (36.4)	21.4 (9.2)	25.6 (13.4)	25.9 (9.9, 42.0)[b]	3.6 (−3.2, 10.3)
Glucose (mmol·L^{-1} h)	6.3 (0.7)	6.6 (0.7)	5.8 (0.7)	6.4 (0.8)	0.3 (−0.1, 0.8)	0.5 (0.2, 0.7)[c]
HOMA-IR	1.6 (1.2, 2.1)	2.1 (1.6, 2.7)	1.2 (0.9, 1.6)	1.1 (0.8, 1.4)	58.2% (10.2, 127.3%)[b,d]	7.6% (−9.2, 27.4%)[d]

Values for overall appetite ratings, appetite-related hormones and glucose are mean (SD). Values for HOMA-IR are geometric mean (95% confidence interval) and statistical analyses are based on log-transformed data.
Data were analysed using linear mixed models with group (South Asian vs white European) and trial (exercise vs control) included as fixed factors.
[a] Normally distributed data: 95% confidence interval for the mean absolute difference between the groups or trials; and log transformed data: 95% confidence interval for the ratio difference of geometric means between the groups or trials.
[b] Main effect of group ($P \leq 0.042$).
[c] Main effect of trial ($P = 0.001$).
[d] Group-by trial interaction ($P = 0.036$).

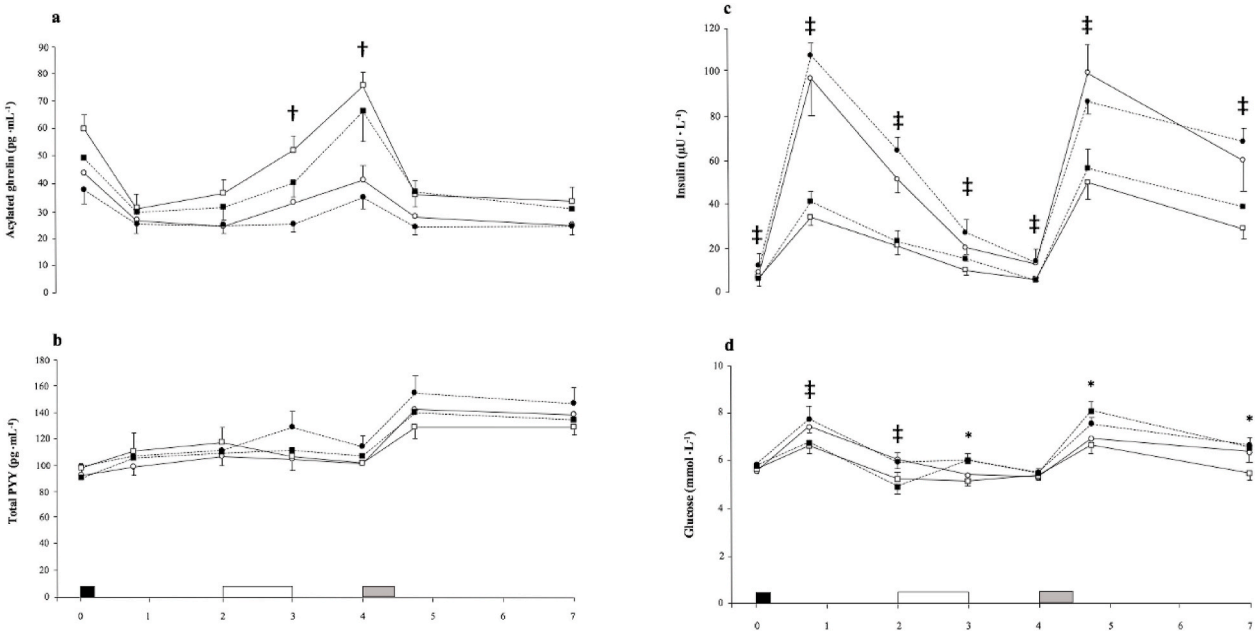

Fig. 3. Plasma concentrations of (a) acylated ghrelin, (b) total PYY, (c) insulin and (d) glucose.

= 0.54; $P = 0.076$) (Fig. 3). Post hoc analysis of the group-by-time interaction for acylated ghrelin revealed lower values in the South Asian compared with the white European men at 3 h (ratio difference (95% CI) −31.0 (−51.3, −2.1)%, $d = 0.75$; $P = 0.038$), at 4 h (ratio difference (95% CI) −40.8 (−58.3, −16.0)%, $d = 0.95$; $P = 0.004$) and meaningfully, albeit not significantly, at 4.75 h (ratio difference (95% CI) −26.6 (−48.2, 4.2)%, d = 0.68; P = 0.082) (Fig. 3). Time-averaged tAUC for acylated ghrelin was lower in the South Asian than in the white European men ($d = 0.77$, $P = 0.042$) and in the exercise than in the control trial ($d = 0.22$, $P = 0.001$) (Table 4) the magnitude of change in response to exercise was similar between groups ($P = 0.503$).

3.5.2. Total PYY

Fasting total PYY concentrations were similar across trials and groups (main effect group $P = 0.760$, main effect trial $P = 0.681$, group-by-trial interaction $P = 0.749$) (Fig. 3). Linear mixed model for total PYY identified a main effect of trial ($P = 0.004$), time ($P < 0.001$) but not group or any interaction effects ($P \geq 0.230$) (Fig. 3). The main effect of trial revealed higher total PYY concentrations in the exercise than in the control trial (ratio difference (95% CI) 5.0 (1.5, 8.7)%, $d = 0.14$, $P = 0.004$) (Fig. 3). The 95% CI for the mean between-trial difference for the time-averaged tAUC for total PYY overlapped zero and the standardised ES was small ($d = 0.20$, $P = 0.060$) while no main effect of group or interaction effects were identified ($P \geq 0.196$) (Table 4).

3.5.3. Insulin

Fasting insulin concentrations were similar between trials (ratio difference (95% CI) 3.8 (−10.7, 20.7)%, $d = 0.07$, $P = 0.614$) but were higher in the South Asian than in the white European men (ratio difference (95% CI) 59.0 (12.4, 125.2), $d = 0.92$, $P = 0.011$) (Fig. 3). A group-by-trial interaction ($P = 0.030$) for fasting insulin revealed higher concentrations in South Asians (ratio difference (95% CI) 23 (−1, 52)%, $d = 0.40$, $P = 0.058$) and lower values in white Europeans (ratio difference (95% CI) −12 (−29, 9)%, $d = 0.26$, $P = 0.220$) in the exercise compared with the control trial (Fig. 3). Linear mixed model for insulin identified a main effect of group ($P < 0.001$), trial ($P = 0.046$), time ($P < 0.001$), and group-by-time interaction ($P = 0.006$) (Fig. 3). Insulin concentrations were higher in South Asian than white European men at

all time-points (group-by-time interaction; all $d \geq 0.65$, $P \leq 0.038$) and were higher in the exercise than in the control trial (trial effect; ratio difference (95% CI) 10.2 (0.2, 21.2)%, $d = 0.09$; $P = 0.046$) (Fig. 3). Time averaged tAUC for insulin was higher in South Asian than white European men ($d = 1.13$, $P = 0.003$) while no main effect of trial or interactions were identified ($P \geq 0.290$) (Table 4).

The fasting HOMA-IR was higher in the South Asian than white European men ($d = 0.86$, $P = 0.015$) but similar between trials ($d = 0.13$, $P = 0.383$). A group-by-trial interaction ($P = 0.036$) for HOMA-IR revealed higher values in South Asians (ratio difference (95% CI) 29 (2, 64)%, $d = 0.47$, $P = 0.037$) and lower values in white Europeans (ratio difference (95% CI) −10 (−29, 14)%, $d = 0.21$, $P = 0.359$) in the exercise compared with the control trial (Table 4).

3.5.4. Glucose

Fasting glucose concentrations were similar between groups (ratio difference (95% CI) −0.5 (−6.6, 5.9)%, $d = 0.05$; $P = 0.869$) but were higher in the exercise than in the control trial (ratio difference (95% CI), 3.6 (0.1, 7.3)%, $d = 0.38$, $P = 0.043$) (Fig. 3). Linear mixed model for glucose identified a main effect trial ($P < 0.001$), time ($P < 0.001$), group-by-time ($P = 0.001$) and trial-by-time ($P = 0.002$) interactions (Fig. 3), but no group effect ($d = 0.27$, $P = 0.123$). Glucose concentrations were higher in the exercise than in the control trial (trial effect; ratio difference (95% CI) 6.6 (3.7, 9.5)%, $d = 0.31$, $P < 0.001$) particularly at 3 h, 4.75 h and 7 h (trial-by-time interaction; all $d \geq 0.55$, $P \leq 0.038$) (Fig. 3). The group-by-time interaction revealed higher glucose concentrations in the South Asian than white European men at 0.75 h and 2 h (both $d \geq 0.67$, $P \leq 0.008$) (Fig. 3). Time-averaged tAUC for glucose was higher in the exercise than in the control trial ($d = 0.61$, $P = 0.001$) while no main effect of group or interactions were identified ($P \geq 0.170$) (Table 4).

3.6. Correlations

Total carbohydrate oxidation during exercise was positively associated with absolute energy intake at subsequent the buffet meal in South Asian ($r = 0.60$, $P = 0.018$) but not in white European men ($r = 0.10$, $P = 0.723$).

4. Discussion

The primary findings of this study were that acute exercise suppressed acylated ghrelin and increased total PYY, insulin and glucose concentrations to a similar extent in South Asian and white European men (main effect of trial) without provoking compensatory increases in *ad libitum* absolute energy intake at the next meal in either group (group-by-trial interactions). South Asian men exhibited lower *ad libitum* absolute energy intake and higher insulin concentrations than white Europeans across both trials (main effect group), whereas group-by-time interactions revealed lower acylated ghrelin concentrations at 3 and 4 h and higher glucose concentrations at 0.75 and 2 h in South Asians than white Europeans. These findings demonstrate that exercise induces a short-term energy deficit in both ethnic groups and highlights the similarity of appetite responses to exercise in South Asian and white European men.

The present study is the first to compare acute exercise effects on appetite responses between South Asian and white European men. Our results demonstrate that appetite perceptions were largely similar between the ethnic groups in response to 60 min of acute moderate-to-vigorous intensity cycling, although subtle differences were detected at 6.5 h (2 h after the *ad libitum* buffet meal). Particularly at this time point appetite perceptions were lower in South Asian than white European men and higher in the exercise than in the control trial which appear to be driven by the greater values in white Europeans in the exercise trial. This may reflect the greater net energy expenditure during exercise observed in the white European group reflecting their higher body/lean mass (Hopkins & Blundell, 2017). The transient appetite suppression at 4.5 h (1.5 h post-exercise) in the exercise compared with the control trial differs with most previous studies demonstrating that appetite is suppressed during and immediately after acute exercise with values typically returning to resting control values within 30–60 min of exercise cessation (Becker et al., 2012; Broom et al., 2007; Douglas et al., 2017). This disparity, however, may reflect differences in the exercise protocol employed. The majority of previous research was performed with exercise occurring after an overnight fast, whereas hunger suppression may be prolonged for approximately 2 h post-exercise when exercise is performed 2 h after breakfast (Cheng et al., 2009; Deighton et al., 2012) as in the present study.

Previous studies suggest that suppression of circulating acylated ghrelin concentrations during exercise, with perturbations typically returning to control values within 30 min, may be associated with acute exercise-induced appetite suppression (Deighton & Stensel, 2014). Consistent with previous research, concentrations of acylated ghrelin were lower in the exercise than in the control trial over the 7 h protocol in both groups (Broom et al., 2007; Deighton et al., 2013; Wasse et al., 2013). However, values were not decreased during or immediately after exercise. This may be explained by the timing of exercise which occurred after the standardised breakfast was consumed, given that at 2 h (immediately prior to exercise) acylated ghrelin was still lower than baseline at this time point, which may have influenced the response of this gut hormone during exercise. Despite the similar exercise-induced responses between ethnic groups, acylated ghrelin concentrations were lower in South Asians over the 7 h protocol across both trials (effect size moderate to large in magnitude), as well as before (at 3 h and 4 h) and after (at 4.75 h, effect size moderate to large in magnitude) the *ad libitum* buffet meal. In our previous study, significant lower fasting acylated ghrelin concentrations at rest were observed in South Asian compared with white European men, (Benedetti et al., 2019). In the present study, fasting acylated ghrelin concentrations were visibly, albeit not significantly, lower in South Asian than white European men, with this difference displaying an effect size small to moderate in magnitude. One reason for the small divergence between findings, may be that the ethnic difference in fasting acylated ghrelin concentrations reported in the previous study was linked to a greater body fat percentage in South Asians (Benedetti et al., 2019), whereas in the current study, adiposity was not statistically different between groups. Moreover, hyperinsulinemia and/or insulin resistance have been inversely associated with ghrelin concentrations, independent of adiposity status (Becker et al., 2012; Flanagan et al., 2003; McLaughlin et al., 2004). This appears to support the findings of the present study where South Asians exhibited lower concentrations of acylated ghrelin (across the 7 h trials) but greater insulin concentrations (across the 7 h trials) and HOMA-IR than white Europeans with similar adiposity. Therefore, it is possible that the greater insulin resistance typically observed in South Asians (Sattar & Gill, 2015) may also contribute to the downregulation of ghrelin as part of a complex feedback system that regulates appetite and body weight, but further work is required to investigate this. The present study also demonstrated lower fat free mass, lower cardiorespiratory fitness and lower fat oxidation during exercise in the South Asian compared with white European men, which have been previously described as key features of the insulin resistance phenotype in this population (Ghouri et al., 2013; Hall et al., 2010; Sattar & Gill, 2015).

In agreement with previous investigations, concentrations of total PYY were greater in the exercise than in the control trial in both groups over the 7 h protocol (Douglas et al., 2017; Kawano et al., 2013; Martins et al., 2007). Although we did not identify an exercise by time interaction, the greater total PYY in the exercise trial visually becomes pronounced after the exercise bout and persists in the hours after the *ad libitum* buffet meal. This exercise-induced elevation in total PYY may contribute to the transient suppression in appetite perceptions observed immediately after the buffet meal, supporting the concomitant total PYY elevation and hunger suppression previously reported (Broom et al.,

2009; Douglas et al., 2017; Martins et al., 2007). Concentrations of fasting and postprandial total PYY were similar between ethnic groups supporting previous data (Benedetti et al., 2019), which appears to suggest no effect of ethnicity on this parameter.

Despite exercise-induced transient changes in appetite perceptions and hormones, absolute *ad libitum* energy intake was unchanged and relative energy intake was substantially lower after exercise in both ethnic groups supporting previous findings (Deighton et al., 2014; Alajmi et al., 2016; Douglas et al., 2017). Thus, these data suggest that a single bout of exercise can induce a negative energy deficit without stimulating compensatory changes in energy intake irrespective of ethnicity. Furthermore, our finding that carbohydrate intake after exercise was higher in white Europeans but not in South Asians is intriguing. Although this difference may be merely related to the lower net energy expenditure over the 60 min cycling in South Asian men, previous evidence demonstrated positive associations between carbohydrate oxidation during exercise and energy intake at a test meal provided 60 min after cycling at 70% of maximum heart rate (Hopkins et al., 2014). In agreement with this, we identified a positive association in South Asians but not in white Europeans, between total carbohydrate oxidation during exercise and absolute energy intake at the subsequent buffet meal. Therefore, the lower carbohydrate oxidation in South Asian men during exercise may be speculatively linked with lower glycogen depletion which elicited a lower compensatory drive to ingest food and restore glycogen stores at the buffet meal. However, future exercise studies examining substrate oxidation and energy intake in South Asians are required before definitive conclusions can be drawn. The present study also revealed lower absolute energy intake across both trials in South Asians which may be linked to the lower acylated ghrelin concentrations apparent in the South Asian men. However, this may be also explained by their lower fat free mass, and would support previous findings indicating fat free mass as an important determinant of resting metabolic rate and day-to-day food intake (Hopkins & Blundell, 2017; Hall et al., 2010; Ghouri et al., 2013; Sattar & Gill, 2015).

Although this research provides novel findings on the effects of exercise on appetite perceptions, appetite-related hormones and food intake in South Asian compared with white European men, this study has a few limitations. Firstly, the interpretation of our findings should be tempered by the fact that the number of participants in the study was small and the population sample was mostly limited to South Asian men originating from India and Pakistan; therefore, further investigations are required using a larger sample size and in other South Asian groups and in South Asian and white European women. Furthermore, a limitation of this study is that acylated ghrelin and total PYY were the only episodic gut hormones related to appetite examined. Lastly, the timing of the exercise bout 2 h after the standardised breakfast may have influenced the appetite-related outcomes, as the appetite perceptions and acylated ghrelin concentrations remained below and total PYY concentrations remained above the respective values at baseline at the start of exercise.

In conclusion, a single bout of moderate-to-vigorous intensity exercise suppressed acylated ghrelin and increased total PYY, insulin and glucose concentrations to a similar extent in South Asian and white European men, without stimulating changes in *ad* libitum energy intake on the day of exercise in either groups. Furthermore, South Asian men exhibited lower acylated ghrelin concentrations and *ad* libitum energy intake, but higher concentrations of insulin and glucose than white European men. These findings provide evidence that acute exercise induces a short-term energy deficit in both ethnic groups and highlights similar exercise-induced responses in appetite measures between South Asian and white European men.

Author contributions

DJS, SB and JA designed the study; SB and HJM conducted the experimental procedures; SB and JEA conducted the biochemical analysis; SB, AET, DJS and JEA analysed and interpreted the data; SB wrote the manuscript; DJS, AET, HJM, DN, and JEA provided critical revisions to the manuscript; all authors read and approved the final manuscript.

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Data and code availability

All data will be openly available via request to the lead author and when possible via the university repository.

Fig. 1. Schematic of the study protocol. Black rectangle indicates standardised breakfast, open rectangle indicates exercise, and grey rectangle indicates *ad libitum* buffet meal.

Fig. 2. Overall appetite perceptions in South Asian (n = 15) and white European (n = 15) men during the control (South Asian -○-; white European -□-) and exercise (South Asian -●-; white European -■-) trials. Values are mean (s.e.m.). Black rectangle indicates standardised breakfast, open rectangle indicates exercise, and grey rectangle indicates *ad libitum* buffet meal. [a]Lower in exercise than control trial (trial-by-time interaction, $P = 0.004$), [b]Higher in exercise than control trial (trial-by-time interaction, $P = 0.024$), [c]Lower in South Asian than white European men (group-by-time interaction, $P = 0.047$). Error bars are omitted from some trials for clarity.

Fig. 3. Plasma concentrations of (a) acylated ghrelin, (b) total PYY, (c) insulin and (d) glucose in South Asian (n = 15) and white European (n = 15) men during the control (South Asian -○-; white European -□-) and exercise (South Asian -●-; white European -■-) trials. Values are mean (s.e.m.). Black rectangle indicates standardized breakfast, open rectangle indicates exercise, and grey rectangle indicates *ad libitum* buffet meal. Linear mixed models identified main effect of group for insulin ($P < 0.001$) and main effects of time ($P < 0.001$) and trial ($P \leq 0.046$) for all outcomes. [†]Lower in South Asian than white European men (group-by-time interaction, all $P \leq 0.038$), [‡]Higher in South Asian than white European men (group-by-time interaction, all $P \leq 0.038$), *Higher in exercise than control trial (trial-by-time interaction, $P < 0.001$). Error bars are omitted from some trials for clarity.

Declaration of competing interest

None.

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