Does butyrylcholinesterase mediate exercise-induced and meal-induced suppression in acylated ghrelin?

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Abstract. Ample evidence supports the notion that an acute bout of aerobic exercise and meal consumption reduces acylated ghrelin concentration. However, the mechanisms by which this exercise- and meal-induced suppression of acylated ghrelin occurs in humans is unknown. This study aimed to examine the concentration of butyrylcholinesterase (BChE), an enzyme responsible for hydrolysing ghrelin and other appetite-related hormones in response to a single bout of running and a standardised meal in young, healthy men. Thirty-three men (aged 23 ± 2 years, mean ± standard deviation) underwent two (exercise and meal conditions) 2-h laboratory-based experiments. In the exercise condition, all participants ran for 30 min at 70% of the maximum oxygen uptake (0930–1000) and rested until 1130. In the meal condition, participants reported to the laboratory at 0930 and rested until 1000. Subsequently, they consumed a standardised meal (1000–1015) and rested until 1130. Blood samples were collected at baseline (0930), 1000, 1030, 1100 and 1130. BChE concentration was not altered in both the exercise and meal conditions (p > 0.05). However, acylated ghrelin was suppressed after exercise (p < 0.05) and meal consumption (p < 0.05). There was no association between the change in BChE concentration and the change in acylated ghrelin before and after exercise (p = 0.571). Although des-acylated ghrelin concentration did not change during exercise (p > 0.05), it decreased after meal consumption (p < 0.05). These findings suggest that BChE may not be involved in the suppression of acylated ghrelin after exercise and meal consumption.

Key words: Butyrylcholinesterase, Acylated ghrelin, Des-acylated ghrelin, Exercise, Standardised meal

GHRELIN, an endogenous ligand that specifically binds to the growth hormone secretagogue receptor, was first purified from and identified in rat stomachs [1]. Ghrelin is involved in the short-term regulation of energy intake since its concentration rises prior to meals and decreases after meals [2, 3]. Ghrelin exists in two forms: acylated and des-acylated. Des-acylated ghrelin has limited biological functions, if any, despite comprising approximately 88–94% of the total ghrelin concentration (acylated and des-acylated ghrelin combined) [4-6]. Conversely, acylated ghrelin is largely responsible for stimulating appetite and energy intake [7, 8].

It is well known that an acute bout of high-intensity exercise can transiently suppress subjective hunger; this is known as “exercise-induced anorexia” [9]. This acute reduction in perceived hunger often coincides with exercise-induced fluctuations in ghrelin concentration. Ample evidence has revealed that an acute bout of aerobic exercise performed at or greater than 70% of the maximum oxygen uptake (VO2 max) suppresses subjective hunger and acylated ghrelin. However, limited data are available on the association between aerobic exercise and des-acylated ghrelin [10]. Previous studies have suggested that both acylated and des-acylated ghrelin after energy intake are suppressed in healthy humans [11] but not in obese individuals or individuals with binge eating behaviors [12, 13]. However, the mechanism(s) underlying the modulation of ghrelin concentration post-exercise and meal ingestion remains unclear.

A previous study reported that butyrylcholinesterase (BChE), an α-glycoprotein synthesised in the liver, hydrolyses acylated ghrelin into des-acylated ghrelin [14]. Only two studies have examined the acute effects of exercise on BChE activity in humans [15, 16], and no study has reported on its concentration during the
post-exercise recovery period. A recent study has demonstrated that BChE activity was increased and plasma acylated ghrelin concentration was decreased in young men immediately after one hour of running at 70% of VO$_{2\text{max}}$, leading to a lower acylated ghrelin to des-acylated ghrelin ratio [16]. It has also been reported that BChE activity increased in healthy young adults immediately after acute exercise [15]. These findings indicate that the increased activity of BChE after acute exercise might be one of the mechanisms underlying the suppression of acylated ghrelin during this time. A limited number of studies have evaluated BChE in response to meals [17, 18]. A previous study reported no difference in BChE activity in healthy young men, irrespective of whether they fasted or consumed food despite decreased acylated ghrelin concentration after consuming a standardised meal [17]. It is worth addressing the fact that exercise and meal consumption modulate appetite-related hormones and appetite differently when the energy deficit was matched [19, 20]. Therefore, to better understand how BChE links, if any, to ghrelin modulation, we evaluated the concentrations of BChE, acylated ghrelin and des-acylated ghrelin after both exercise and meal consumption, using a within-subjects study design.

The present study aimed to investigate the acute effects of exercise and meal consumption on circulating concentrations of BChE, acylated ghrelin, des-acylated ghrelin and subjective appetite in healthy young men. We hypothesised that the circulating concentration of BChE would increase after exercise and that the magnitude of change in the concentration of circulating acylated ghrelin would correlate negatively with the magnitude of change in BChE concentration.

### Methods

#### Participants

The study protocol was reviewed and approved by the Ethics Committee on Human Research of Waseda University (approval number: 2020-153). This study was registered in advance with the University Hospital Medical Information Network Center (UMIN), a system for registering clinical trials (ID: UMIN000045434). Thirty-three young Japanese men provided written informed consent to participate in the study. The participants were excluded from the study if: 1. they were a current smoker; 2. they were currently on any medication or supplement known to affect lipid and carbohydrate metabolism; 3. they had any major illnesses, including gastrointestinal disorders or physical problems (acute or chronic); or 4. they had limited ability to perform physical activity. The physical characteristics of the participants are presented in Table 1.

#### Study design

Eligible participants who provided informed consent were screened and performed preliminary testing. Subsequently, they were invited to participate in the two experiments (exercise condition and meal condition) in a random order. The lead investigator enrolled the participants in the research and randomly assigned the participants to each experiment in a counterbalanced manner using computer-generated random numbers. The interval between the two visits of each participant was at least six days to eliminate any potential carry-over effects. A schematic illustration of the study protocol is shown in Fig. 1.

| Table 1  | Physical characteristics of the 33 participants |
|----------|-----------------------------------------------|
|          | Mean ± SD                                      | Range  |
| Age (year) | 23 ± 2                                         | 20–28  |
| Height (cm) | 171.1 ± 6.0                                    | 161.3–186.2 |
| Body mass (kg) | 64.7 ± 8.4                                      | 49.1–84.7 |
| Body mass index (kg/m$^2$) | 22.1 ± 2.4                                      | 17.9–27.2 |
| Body fat percentage (%) | 16.9 ± 4.4                                      | 8.1–27.0 |
| Maximum oxygen uptake (mL/kg/min) | 51.5 ± 8.1                                      | 37.5–77.3 |
| Waist circumference (cm) | 75.7 ± 6.3                                      | 65.2–94.5 |
| Walking (MET-min/week)* | 818.0 ± 1,201.2                                 | 0–6,732 |
| Moderate-intensity physical activity (MET-min/week)* | 1,070.3 ± 913.0                                 | 0–3,150 |
| Vigorous-intensity physical activity (MET-min/week)* | 1,475.2 ± 2,264.5                               | 0–7,920 |
| Total physical activity (MET-min/week)* | 3,363.5 ± 3,042.7                               | 272–11,959 |

SD: standard deviation, MET: metabolic equivalent, * data were collected from the International Physical Activity Questionnaire.
Screening and preliminary tests

All participants were asked to complete the standard health screening form, which provided us with information on their medical history and medication use, and the International Physical Activity Questionnaire-Long Form. Thereafter, their anthropometric measurements were taken in the laboratory. Heights were measured using a stadiometre (YS-OA, AS ONE Corporation, Osaka, Japan). Body mass and body fat percentage were measured using a body composition analyser (MC-780A-N, Tanita Corporation, Tokyo, Japan). Waist circumference was measured at the level of the umbilicus using a flexible plastic tape. Resting arterial blood pressure was measured on the left arm after 10 minutes of seated rest using a digital monitor (OMRON HEM-907, Omron Healthcare Co., Kyoto, Japan). An average of two measurements were obtained for each time point, and the mean of these values was recorded.

After collecting anthropometric measurements, the participants underwent two preliminary exercise tests in which they were familiarised with exercising on a treadmill (JOG NOW 700, Technogym, Rome, Italy). The first test consisted of a 16-min submaximal incremental running test to determine the relationship between running speed and oxygen uptake. Participants performed four 4-minute incremental runs starting at a speed of 7 or 8 km/h, depending on their fitness level (as determined by the initial screening interview and questionnaire); the speed was increased by 1 or 1.5 km/h every 4 minutes. The treadmill gradient was set at 0% throughout the test. After a 20-min rest (i.e., following completion of the submaximal treadmill test), the participants were asked to complete a VO$_{2\text{max}}$ test using a treadmill protocol for uphill running at constant speed but with an incremental increase in inclination [21]. The initial gradient was set at 3.5% and was increased by 2.5% every 3 minutes until volitional exhaustion was achieved. The oxygen uptake, carbon dioxide production rate and respiratory exchange ratio (RER) were measured continuously throughout the tests using an online breath-by-breath gas analyser (Quark RMR, COSMED Co. Ltd., Roma, Italy). The heart rate was measured continuously throughout the test by short-range telemetry (Polar RCX3, Polar Electro, Kempele, Finland), and the values at the last minute of each stage were recorded at an interval of 15 seconds. The ratings of perceived exertion (RPE) was recorded at the end of each stage during both exercise tests on a subjective scale of 6–20 [22]. The participants were considered to have attained the VO$_{2\text{max}}$ if their measured parameters adhered to two or more of the following criteria: 1) heart rate >95% of the age-predicted maximum heart rate, 2) RER >1.15, 3) a plateau in oxygen consumption and 4) RPE ≥19. Data from the two preliminary tests were used to calculate the exercise intensity (i.e., 70% of VO$_{2\text{max}}$) of the participants in the exercise condition.

Standardisation of energy intake and physical activity

The participants weighed and recorded all food and drinks consumed the day before each test condition and refrained from drinking alcohol during this period. They replicated their energy intake from the first test condition to the second test condition to ensure that their energy intake was standardised across the test conditions. Their food diaries were analysed using Excel Eiyoukun Ver 9.0 software (Kenpakusha, Tokyo, Japan) by a registered dietician to determine their energy intake and the macronutrient content of the foods. The participants were asked to avoid any strenuous exercise for two days before each main test condition. They wore a uniaxial accelerometer (Lifecoder-EX; Suzuken Co Ltd, Nagoya, Japan) on their hip to monitor their daily activity objectively during this period. On the day before each main test condition, they received text messages from a researcher asking them to replicate their energy intake and physical activity patterns. Their compliance with replicating each main test condition was checked.
verbally upon their arrival at the laboratory.

**Exercise condition**

Participants reported to the laboratory at 0915 after an 11-h overnight fast (no food or drink except water). Their body mass was measured to the nearest 0.1 kg using a digital scale (MC-780A-N, Tanita Corporation, Tokyo, Japan). After a 10-minute rest, their resting arterial blood pressure was measured on their left arm using a digital monitor (OMRON HEM-907, Omron Healthcare Co., Kyoto, Japan). Then, baseline venous blood samples were collected in a seated position by venipuncture at 0930. Thereafter, the participants performed a 30-minute run (i.e., from 0930 to 1000) on a treadmill at a speed equivalent to 70% of their VO_{2max} (determined from the preliminary test). Oxygen uptake rate, carbon dioxide production rate, RER, heart rate and RPE were measured as described previously. The participants were asked to remain in a comfortable sitting position until 1130, during which time their heart rate was measured continuously, as described previously. During this rest period, blood samples were collected at 1000, 1030, 1100 and 1130 for the measurement of BChE, acylated ghrelin, des-acylated ghrelin, peptide YY (PYY), insulin, glucose and triglycerides (TG). In addition, the participants were asked to complete a 100-mm visual analogue scale (VAS) at 0930, 1000, 1015, 1030, 1100 and 1130, using a paper-based questionnaire [23] which assessed their subjective appetite (i.e., hunger and fullness).

**Meal condition**

The meal condition was identical to the exercise condition, except that participants were asked to sit on a chair in a comfortable position for 30 minutes from 0930 to 1000, following which they were asked to consume a standardised meal within 15 minutes from 1000–1015. The standardised meal consisted of rice, miso soup, fried green pepper, cucumber, broccoli, potato, mayonnaise, crackers and cream cheese. The standardised meal provided 45.6 kJ (10.9 kcal) of energy, 1.51 g of carbohydrate (which constituted 55% of the meal), 0.41 g of protein (15%) and 0.34 g of fat (30%) per kg body mass. Venous blood samples were collected, and the ratings of subjective appetite was assessed at the same time points used in the exercise condition. Water was not available during this period, as the volume of fluid intake would influence subjective satiety and gastric motility [24]. Thereafter, participants were asked to sit comfortably until 1130.

**Analytical methods for the measurement of blood parameters**

For plasma BChE, plasma PYY and insulin measurements, blood samples were collected in dipotassium salt-EDTA tubes (Venoject 2; Terumo Corporation, Tokyo, Japan), while for plasma glucose measurement, the blood samples were collected in sodium fluoride-EDTA tubes (Venoject 2; Terumo Corporation, Tokyo, Japan). The tubes were centrifuged immediately at 1,861 × g for 10 min at 4°C. The plasma was removed, divided into four tubes, and stored at –80°C until further analysis. For the measurement of plasma acylated ghrelin and des-acylated ghrelin, blood samples were collected in EDTA tubes containing aprotonin (Venoject 2; Terumo Corporation, Tokyo, Japan) to prevent the degradation of ghrelin by protease. The tube was immediately centrifuged at 1,861 × g for 10 min at 4°C. For plasma acylated ghrelin, 200 μL of the plasma was removed and transferred to a tube containing 20 μL of hydrochloric acid. The samples were stored at –80°C until further analysis. For the measurement of serum TG, blood samples were collected in tubes containing clotting activators for serum isolation (Venoject 2; Terumo Corporation, Tokyo, Japan). The samples were allowed to clot for 30 min at room temperature and then treated as described above. Enzyme-linked immunosorbent assays (ELISAs) were used to measure the plasma BChE (DBCHE0, R&D. System, Minneapolis, USA), acylated ghrelin (MM-401, SCETI K.K, Tokyo, Japan), des-acylated ghrelin (MM-402, SCETI K.K, Tokyo, Japan), PYY (YK080, Yanaihara Institute Inc., Shizuoka, Japan) and insulin (Merckodia Human ELISA Kit; Mercodia AB, Uppsala, Sweden) concentrations. Enzymatic colorimetric assays were used to measure the plasma glucose (GLU-HK(M); Shino-Test Corporation, Kanagawa, Japan) and serum TG (Pure Auto S TG-N; Sekisui Medical Co. Ltd., Tokyo, Japan) concentrations. The intra-assay coefficients of variation were 3.2% for BChE, 5.8% for acylated ghrelin, 2.4% for des-acylated ghrelin, 4.2% for PYY, 4.1% for insulin, 2.8% for glucose and 0.3% for TG.

**Statistical analyses**

Data were analysed using IBM SPSS Statistics for Mac, version 27.0 (IBM Corp., New York, USA). The normality of the distribution was assessed using the Shapiro-Wilk test. In case of non-normal distribution, a logarithmic transformation was performed. One-way analysis of variance (ANOVA) was used to examine the differences in the serum or plasma concentrations of the analytes and the subjective appetite of the participants in the exercise and meals conditions over a period of time. Any significant difference observed was subsequently analysed using post-hoc analysis and adjusted for multiple comparisons using the Bonferroni method. Pearson correlation analysis was used to examine the relationship between changes in the plasma concentrations of acylated ghrelin and BChE, measured before and immediately
after exercise or meal consumption.

The differences in the magnitude of change in BChE (ΔBChE), acylated ghrelin (Δacylated ghrelin), des-acylated ghrelin (Δdes-acylated ghrelin) and the ratio of acylated ghrelin to des-acylated ghrelin (ΔAG:DAG) between the two conditions (i.e., 0930–1000) were examined using paired t-tests; since the participants in the meal condition rested during this period, we compared this data between the exercise and non-exercise conditions. The 95% confidence interval (95% CI) for the means of all the absolute pairwise differences between the two conditions was calculated from the t-distribution table depending on the degrees of freedom (n-1). Data were reported as mean ± standard deviation (SD). The statistical significance was set at p < 0.05.

**Results**

**Exercise condition**

During exercise (0930–1000), the treadmill speed, heart rate and RPE were 10.1 ± 2.0 (km/h), 159.0 ± 11.9 (beats/min) and 12.5 ± 2.2, respectively. The baseline (0930) plasma concentrations of BChE, acylated ghrelin and des-acylated ghrelin were 3,161 ± 575 (ng/mL), 23.11 ± 9.18 (fmol/mL) and 95.15 ± 34.67 (fmol/mL), respectively. The magnitude of increase in BChE concentration observed in the exercise condition (mean difference: 367 ng/mL; 95% CI: 193–540 ng/mL; p < 0.001) was greater compared to that in the non-exercise period (0930–1000) in the meal condition. The magnitude of decrease in acylated ghrelin concentration observed in the exercise condition (mean difference: –5.76 fmol/mL; 95% CI: –8.57 to –2.93; p < 0.001) was greater compared to that in the non-exercise period (0930–1000) in the meal condition. No difference was found in the magnitude of change in des-acylated ghrelin concentration between the exercise and non-exercise periods (p = 0.665). The magnitude of decrease in the acylated ghrelin to des-acylated ghrelin (AG:DAG) ratio observed in the exercise condition (mean difference: –0.043; 95% CI: –0.09 to –0.00; p = 0.050) was greater compared to that in the non-exercise period (0930–1000) in the meal condition.

The baseline and post-exercise plasma concentrations of BChE, acylated ghrelin, des-acylated ghrelin and the AG:DAG ratio in the exercise condition are shown in Fig. 2A and Fig. 3A–C. One-way ANOVA revealed differences in the concentrations of plasma acylated ghrelin and AG:DAG ratio (p = 0.003 and p = 0.006, respectively). Post-hoc analysis revealed that the concentration of acylated ghrelin was lower immediately after exercise (1000) than at baseline (0930) or at 60-min post-exercise (1100) and 90-min post-exercise (1130). Post-hoc analysis revealed that the AG:DAG ratio was lower immediately after exercise (1000) compared to that at all other time points. One-way ANOVA revealed no differences in the concentrations of BChE and des-acylated ghrelin (all p > 0.05). Pearson correlation analysis revealed that there was no correlation between ΔBChE and Δacylated ghrelin concentrations measured at baseline (0930) and that measured immediately after exercise (1000) (r = 0.102; p = 0.571).

The subjective feelings of hunger and fullness of the participants in the exercise condition are presented in Table 2. One-way ANOVA revealed differences in the subjective feelings of hunger measured during the entire experimental period in the exercise condition (p < 0.001). No differences were found in the subjective feelings of fullness measured during the entire experimental period in the exercise condition (p = 0.954). The plasma concentrations of PYY, glucose, insulin and serum TG in the exercise condition are shown in Table 3. One-way ANOVA revealed differences in the concentration of PYY (p = 0.001) and glucose (p = 0.003) but not in the concentrations of insulin and serum TG (all p > 0.05). Pearson correlation analysis revealed that there was no correlation between ΔBChE and Δacylated ghrelin concentrations measured at baseline (0930) and that measured immediately after exercise (1000) (r = 0.102; p = 0.571).
ANOV A revealed differences in insulin and glucose concentrations measured during the entire experimental period in the exercise condition ($p < 0.001$ and $p = 0.007$, respectively). No differences were found in the plasma PYY and TG concentrations measured during the entire experimental period in the exercise condition ($p = 0.951$ and $p = 0.735$, respectively).

**Fig. 3** Acylated ghrelin (AG), des-acylated ghrelin (DAG) and the ratio of acylated ghrelin to des-acylated ghrelin (AG:DAG) during the exercise and meal conditions.

Black rectangle indicates a 30-min run, white rectangle indicates a 30-min rest and dotted rectangle indicates consumption of the standardised meal. Data are represented as means ± SD ($n = 33$). * Significantly different from 0930, $p < 0.05$. # Significantly different from 1000, $p < 0.05$.

**Table 2** The subjective feelings of hunger and fullness measured at each time-point in the exercise and meal conditions

| Time (h) | 0930 h | 1000 h | 1015 h | 1030 h | 1100 h | 1130 h | Whole experimental period |
|----------|--------|--------|--------|--------|--------|--------|--------------------------|
| **Hunger (mm)** | | | | | | | |
| Exercise* | 71 ± 24 | 62 ± 28 | 70 ± 23 | 74 ± 21 | 80 ± 18 | 85 ± 13 | † 1000 h vs. 1100, 1130 h |
| Meal* | 71 ± 24 | 78 ± 17 | 20 ± 15 | 24 ± 18 | 28 ± 20 | 31 ± 19 | § 0930 h vs. 1015, 1030, 1100, 1130 h |
| **Fullness (mm)** | | | | | | | |
| Exercise | 21 ± 22 | 26 ± 22 | 27 ± 24 | 23 ± 21 | 17 ± 18 | 17 ± 18 | § 0930 h vs. 1015, 1030, 1100, 1130 h |
| Meal* | 16 ± 15 | 14 ± 11 | 76 ± 18 | 75 ± 16 | 65 ± 25 | 65 ± 25 | § 1000 h vs. 1015, 1030, 1100, 1130 h |

Values are mean ± standard deviation for $n = 33$. Means were compared using the one-way ANOVA: * $p < 0.05$. Post-hoc analysis was adjusted for multiple comparisons using the Bonferroni method. Post-hoc analysis of the main effect of time: † exercise condition, § meal condition.
Regulation of ghrelin and its trigger

### Table 3  Peptide tyrosine tyrosine (PYY), insulin, glucose and triglycerides (TG) concentrations measured at each time-point in the exercise and meal conditions

|          | Exercise         | 0930 h | 1000 h | 1030 h | 1100 h | 1130 h | Whole experimental period |
|----------|------------------|--------|--------|--------|--------|--------|---------------------------|
| PYY (ng/mL) | Exercise         | 0.64 ± 0.25 | 0.68 ± 0.26 | 0.64 ± 0.26 | 0.62 ± 0.26 | 0.63 ± 0.25 |                      |
|          | Meal*            | 0.64 ± 0.26 | 0.62 ± 0.25 | 0.70 ± 0.27 | 0.67 ± 0.25 | 0.68 ± 0.25 |                      |
| Insulin (pmol/L) | Exercise*         | 25.99 ± 12.45 | 16.90 ± 13.37 | 26.15 ± 14.72 | 20.15 ± 11.60 | 22.01 ± 14.38 | 0930 h vs. 1000 h | 1000 h vs. 1030 h |
|          | Meal*            | 22.89 ± 11.56 | 21.62 ± 11.79 | 236.67 ± 117.87 | 243.00 ± 167.55 | 161.53 ± 86.12 | 0930 h vs. 1000 h | 1000 h vs. 1030 h |
| Glucose (mmol/L) | Exercise*         | 4.83 ± 0.41 | 5.04 ± 0.63 | 4.64 ± 0.40 | 4.75 ± 0.33 | 4.74 ± 0.33 |                      |
|          | Meal*            | 4.81 ± 0.32 | 4.86 ± 0.32 | 6.43 ± 0.68 | 5.45 ± 1.04 | 4.78 ± 0.84 |                      |
| TG (mmol/L) | Exercise         | 0.88 ± 0.38 | 0.89 ± 0.37 | 0.79 ± 0.31 | 0.79 ± 0.30 | 0.81 ± 0.30 | 0930 h vs. 1130 h |
|          | Meal*            | 0.82 ± 0.28 | 0.79 ± 0.26 | 0.82 ± 0.26 | 0.99 ± 0.31 | 1.12 ± 0.40 | 0930 h vs. 1130 h |

Values are mean ± standard deviation for n = 33. Means were compared using the one-way ANOVA: † p < 0.05. Post-hoc analysis was adjusted for multiple comparisons using the Bonferroni method. Post-hoc analysis of the main effect of time: † exercise condition, § meal condition.

### Meal condition

The baseline (0930) plasma concentrations of BChE, acylated ghrelin and des-acylated ghrelin were 3.235 ± 554 ng/mL, 24.62 ± 11.15 pmol/mL and 100.09 ± 38.15 pmol/mL, respectively. The baseline and post-exercise plasma concentrations of BChE, acylated ghrelin, des-acylated ghrelin and the AG:DAG ratio in the meal condition are shown in Fig. 2B and Fig. 3D–F. One-way ANOVA revealed no significant differences in the plasma concentration of BChE (p = 0.542). One-way ANOVA revealed that there were differences in the concentrations of acylated ghrelin and des-acylated ghrelin and the AG:DAG ratio measured during the entire experimental period (p < 0.001, p = 0.006 and p = 0.035, respectively). Post-hoc analysis revealed that the concentration of acylated ghrelin was lower after a meal (1100 and 1130) compared to that at baseline (0930) or before a meal (1000). Post-hoc analysis revealed that the concentration of des-acylated ghrelin was lower at 75-min after food intake (1130) compared to that before the meal (1000). Post-hoc analysis revealed no difference in the AG:DAG ratio across all time points.

The subjective feelings of hunger and fullness in the meal condition are presented in Table 2. One-way ANOVA revealed differences in the subjective feelings of hunger and fullness measured during the entire experimental period in the meal condition (all p < 0.001). The plasma concentrations of PYY, glucose, insulin and serum TG during meal consumption are shown in Table 3. One-way ANOVA revealed no differences in PYY concentrations (p = 0.653). One-way ANOVA revealed differences in the plasma concentrations of insulin, glucose and TG measured during the entire experimental period in the meal condition (all p < 0.001).

### Discussion

The present study demonstrated that an acute bout of high-intensity running suppressed acylated ghrelin concentration but not that of des-acylated ghrelin in healthy young men. We also found that exercise had no effect on the concentration of BChE. Our findings also showed no change in the BChE concentration but a reduction in the plasma concentrations of acylated ghrelin and des-acylated ghrelin after consuming a standardised meal. Furthermore, no correlation was observed between the magnitude of change in BChE and acylated ghrelin concentrations following acute exercise. These findings suggest that BChE may not mediate the suppression of acylated ghrelin after exercise and meal consumption.

The present findings suggest that BChE concentration is not altered by exercise; however, acylated ghrelin concentration was reduced after a 30-minute high-intensity run. The magnitude of change in BChE concentration was greater before and immediately after exercise than at the same time points in the meal condition (the participants in the meal condition were at rest during this period). This finding was consistent with that of a previous study in which young men performed a 60-minute high-intensity run [16]. In rats, the plasma BChE activity increased after a single bout of acute exercise [25], probably due to exercise-stimulated metabolic and enzymatic changes in this non-specific cholinesterase in the liver, where it is primarily synthesised [26, 27]. In addition, a previous study [15] on human subjects reported an increase in BChE activity after brisk walking at 7 km/h; they also observed inter-individual variation in the activity of BChE. However, the mechanism underlying this association between BChE activity and exercise...
remains unclear. BChE is also known for its role in the hydrolysis of ghrelin, both in central and peripheral sites [28, 29]; it can degrade ghrelin at $6.2 \times 10^4$ M/min [29, 30]. However, in the present study, the magnitude of change in BChE concentration observed in the exercise condition did not correlate with the magnitude of change in acylated ghrelin concentration. These findings suggest that the suppression of acylated ghrelin after acute exercise may not be due to the hydrolysis of BChE. Although the rate of ghrelin hydrolysis was linearly dependent on BChE concentration and BChE was sufficient to hydrolyze acylated ghrelin into des-acylated ghrelin under normal physiological conditions, this hydrolysis was physiologically slow when the substrate volume of ghrelin was large [30]. It is also possible that other factors, including the temperature and blood redistribution brought about by exercise, affect the hydrolysis of ghrelin by BChE. It has also been suggested that lyso-phospholipase I, a ghrelin deacetylase enzyme, exhibits potent ghrelin deacylase activity in circulation [30, 31]. Moreover, our findings extend that of a previous study [16] by reporting no change in BChE concentration after meal consumption, despite observing a decrease in acylated ghrelin concentration. Indeed, our findings are consistent with those of a previous study that reported no difference in BChE activity between the fasting and postprandial states of healthy young men, although a reduction in acylated ghrelin concentration was observed after the consumption of a standardized meal; however, no evidence exists on the assessment of the changes in BChE and ghrelin concentrations over time [17]. The findings of the present study also suggest that BChE may not acutely respond to meal consumption. Nonetheless, due to the heterogeneity of the study methodologies used and the lack of relevant studies, it is difficult to comprehensively evaluate the relationship between BChE and both forms of ghrelin in response to meal consumption. Collectively, more empirical studies are needed to examine whether BChE mediates changes in acylated ghrelin and des-acylated ghrelin concentrations after exercise and meal consumption.

The exercise-induced acylated ghrelin-lowering effect is consistent with previous studies in young men [10]. In addition, the present study found that circulating concentrations of des-acylated ghrelin were not altered before and after a 30-minute high-intensity run and that this effect persisted throughout the remaining experimental period in the exercise condition. These findings support those of a previous study that showed reduced acylated ghrelin concentrations, but not des-acylated ghrelin concentrations, in young men after acute exercise (i.e., 60 minutes of moderate-intensity cycling) [32]. Collectively, given that acylated ghrelin is removed rapidly from the circulation [33], these findings suggest that this removal of acylated ghrelin is more rapid than that of des-acylated ghrelin [34] although present study did not ascertain the synthesis of ghrelin during exercise. In contrast, other studies have reported that the synthesis of both acylated ghrelin and des-acylated ghrelin was suppressed after 60 minutes of high-intensity running [15, 35]. The inconsistency associated with the reduced des-acylated ghrelin concentrations post-exercise observed in other previous studies could be attributed to the difference in the exercise duration i.e., 30 minutes vs. 60 minutes) since exercise duration has been known to influence ghrelin response [36]; however, this needs more corroborating evidence. Much of the literature has revealed the following observations during or after acute exercise: blood distribution [36], up-and-down motion of the center of the body [37] and lactate accumulation [38]. This may explain the reduction in acylated ghrelin concentration during this period. However, little is known regarding the underlying physiological mechanism(s) involved in the des-acylated ghrelin response to acute exercise. Collectively, future studies involving the measurement of both acylated ghrelin and des-acylated ghrelin concentrations are required, as the changes in ghrelin secretion and degradation are triggered independently by exercise.

The present study found that acylated ghrelin and des-acylated ghrelin concentrations were lowered during the postprandial period. Our findings are consistent with those of previous studies demonstrating that acylated ghrelin [20, 39, 40] and des-acylated ghrelin [12, 16] concentrations in healthy adults are reduced after a mixed meal. Although another study reported that des-acylated ghrelin was not suppressed in middle-aged individuals after a mixed meal [13], the weight of evidence suggests that meal consumption may suppress the secretion of total ghrelin. Unlike exercise, the suppression of acylated ghrelin after meal consumption may involve other physiological factors, for example, slow gastric emptying to allow sufficient digestion in the small intestine [41]. It has also been reported that the meal-induced suppression of ghrelin may be triggered by gastric feedback after food or fluid intake [42]. Interestingly, in the present study, we observed that the effects of exercise and meal consumption on the glucose and insulin responses were different. Thus, despite gastric modulation, the change in ghrelin concentrations after food intake may be attributed to the marked increase in glucose and insulin concentrations during this period [43]. Therefore, the role of the difference in the glucose and insulin concentrations between the exercise and meal conditions on the regulation of appetite cannot be excluded.

In contrast to previous studies [38, 44, 45], the transient
suppression of subjective hunger after exercise, often termed exercise-induced anorexia [9], was not observed in the present study, although a suppressive effect was noted after meal consumption. Thus, it is difficult to address, at least in our study, whether exercise-induced anorexia, typically seen after high-intensity exercise, may be potentially linked to the decreased acylated ghrelin levels observed immediately after exercise. Initially, we speculated that the absence of exercise-induced anorexia in the present study could be attributed to the variation in the participant’s fitness level (i.e., obtained VO_{2max} ranged from 37.5 to 77.3 mL/kg/min) and/or the duration of exercise. In other words, individuals with high fitness levels may perceive less hunger as they are accustomed to exercising for such long durations, whereas individuals with a low fitness level may perceive more hunger as they are not accustomed to exercising while in a state of fasting. However, this speculation was not confirmed in the present study (data not shown). Since appetite control is regulated by the interaction between various physiological and psychological factors, further investigations are required on subjective appetite in response to acute exercise [46].

The unique strengths of the present study lie in its controlled experimental design, which examined the acute effects of a standardised meal and an acute bout of exercise on BChE concentration. This design allowed us to simultaneously evaluate the exercise-induced and meal-induced suppression of acylated ghrelin to determine whether BChE mediates this suppressive effect on acylated ghrelin in humans. In addition, the present study examined the response of des-acylated ghrelin to an acute bout of exercise in a large number of young men (only a limited number of studies have examined this aspect) [16, 35, 47]. This is important because measuring the concentration of both forms of ghrelin will help us estimate the magnitude of ghrelin removal from the circulation [32]. Although enlightening, our study has certain limitations that need to be addressed. First, the present study was limited to a single-arm design for each intervention; no control was available to directly compare the exercise and meal conditions. Second, the experimental protocol in the present study lasted only two hours. Further investigations involving longer observational periods are required since the circulating concentrations of appetite-related hormones, including ghrelin, fluctuate diurnally [3].

In conclusion, the present study demonstrated that a 30-minute high-intensity run suppressed the circulating concentration of acylated ghrelin but not des-acylated ghrelin in healthy young men. Furthermore, the concentration of BChE was not altered by exercise. There was no correlation between the magnitude of change in BChE and acylated ghrelin concentrations following an acute bout of running. The concentrations of both acylated and des-acylated ghrelin decreased after consuming a standardised meal, while there was no difference in the BChE concentration before and after meal consumption. These findings indicate that BChE may not be involved in acylated ghrelin suppression after exercise and meal consumption.

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Author Contributions

GL and YT supervised the data collection, and performed the blood and data analysis. KK, HW, KY and CN provided assistance to GL and YT for the data collection. GL drafted the manuscript, and GL and MM took the lead in writing the manuscript. MM conceived the study. All authors approved the final version of the manuscript.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

References

1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, et al. (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402: 656–660.
2. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, et al. (2002) Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab 87: 240–244.
3. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, et al. (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50: 1714–1719.
4. Yoshimoto A, Mori K, Sugawara A, Mukoyama M, Yahata K, et al. (2002) Plasma ghrelin and desacyl ghrelin concentrations in renal failure. J Am Soc Nephrol 13:
2748–2752.
5. Weickert MO, Loeffelholz CV, Arafat AM, Schöfl C, Otto B, et al. (2008) Euglycemic hyperinsulinemia differentially modulates circulating total and acylated-ghrelin in humans. J Endocrinol Invest 31: 119–124.
6. Nakai Y, Hosoda H, Nin K, Ooya C, Hayashi H, et al. (2004) Short-term secretory regulation of the active form of ghrelin and total ghrelin during an oral glucose tolerance test in patients with anorexia nervosa. Eur J Endocrinol 150: 913–914.
7. Kojima M, Kangawa K (2005) Ghrelin: structure and function. Physiol Rev 85: 495–522.
8. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, et al. (2001) Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 86: 5992.
9. King NA, Burley VJ, Blundell JE (1994) Exercise-induced suppression of appetite, effects on food intake and implications for energy balance. Eur J Clin Nutr 48: 715–724.
10. Anderson KC, Zieff G, Paterson C, Stoner L, Weltman A, et al. (2021) The effect of acute exercise on pre-prandial ghrelin levels in healthy adults: a systematic review and meta-analysis. PLoS One 14: 170625.
11. Foster-Schubert KE, Overduin J, Prudom CE, Liu J, Callahan HS, et al. (2008) Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. J Clin Endocrinol Metab 93: 1971–1979.
12. Hernandez D, Mehta N, Geliebter A (2019) Meal-related acyl and des-acyl ghrelin and other appetite-related hormones in people with obesity and binge eating. Obesity (Silver Spring) 27: 629–635.
13. Dardzińska JA, Małgorzewicz S, Kaska Ł, Proczko M, Stefaniak T, et al. (2014) Fasting and postprandial acyl and des-acyl ghrelin levels in obese and non-obese subjects. Endokrynol Pol 65: 377–381.
14. Darvesh S, Grantham DL, Hopkins DA (1998) Distribution of butyrylcholinesterase in the human amygdala and hippocampal formation. J Comp Neurol 393: 374–390.
15. Zimmer KR, Lencina CL, Zimmer AR, Thiesena FV (2012) Influence of physical exercise and gender on acetylcholinesterase and butyrylcholinesterase activity in human blood samples. Int J Environ Health Res 22: 279–286.
16. Dorling JL, Clayton DJ, Jones J, Carter WG, Thackray AE, et al. (2019) A randomized crossover 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. Am J Clin Nutr 110: 1055–1066.
17. Liu J, Prudom CE, Nass R, Pezzoli SS, Oliveri MC, et al. (2008) Novel ghrelin assays provide evidence for independent regulation of ghrelin acylation and secretion in healthy young men. J Clin Endocrinol Metab 93: 1980–1987.
18. Bestas A, Goksu H, Erhan OL (2013) The effect of pre-operative consumption of potatoes on succinylcholine-induced block and recovery from anesthesia. J Clin Monit Comput 27: 609–612.
19. Deighton K, Batterham RL, Stensel DJ (2014) Appetite and gut peptide responses to exercise and calorie restriction. The effect of modest energy deficits. Appetite 81: 52–59.
20. King JA, Wasse LK, Zeyun J, Crystallis K, Emmanuel J, et al. (2011) Differential acylated ghrelin, peptide YY3-36, appetite, and food intake responses to equivalent energy deficits created by exercise and food restriction. J Clin Endocrinol Metab 96: 1114–1121.
21. Taylor HL, Buskirk E, Henschel A (1955) Maximal oxygen intake as an objective measure of cardio-respiratory performance. J Appl Physiol 8: 73–80.
22. Borg GA (1973) Perceived exertion: a note on “history” and methods. Med Sci Sports 5: 90–93.
23. Flint A, Raben A, Blundell JE, Astrup A (2000) Reproducibility, power and validity of visual analogue scales in assessment of appetite sensation in single test meal studies. Int J Obes Relat Metab Disord 24: 38–48.
24. Fujihira K, Hamada Y, Suzuki K, Miyashita M (2020) The effects of pre-meal drink volume on gastric motility and energy intake in healthy men. Physiol Behav 213: 112726.
25. Husain K, Somani SM (1997) Influence of exercise and ethanol on cholinesterase activity and lipid peroxidation in blood and brain regions of rat. Prog Neuropsychopharmacol Biol Psychiatry 21: 659–670.
26. Pawlowska D, Moniuszko-Jakoniuk J, Soltys M (1985) The effect of chronic physical exercise on the activity of hydrolytic enzymes in acute poisoning with parathion-methyl in rats. Pol J Pharmacol Pharm 37: 639–646.
27. Davis L, Britten JJ, Morgan M (1997) Cholinesterase. Its significance in anaesthetic practice. Anaesthesia 52: 244–260.
28. Chen VP, Gao Y, Geng L, Brimijoin S (2017) Butyrylcholinesterase regulates central ghrelin signaling and has an impact on food intake and glucose homeostasis. Int J Obes (Lond) 41: 1413–1419.
29. De Vriese C, Greffe F, Lema-Kisoka R, Waelbroeck M, Robberecht P, et al. (2004) Ghrelin degradation by serum and tissue homogenates: identification of the cleavage sites. Endocrinology 145: 4997–5005.
30. Schoopfer LM, Lockridge O, Brimijoin S (2015) Pure human butyrylcholinesterase hydrolyzes octanoyl ghrelin to desacyl ghrelin. Gen Comp Endocrinol 224: 61–68.
31. Satou M, Nishi Y, Yoh J, Hattori Y, Sugimoto H (2010) Identification and characterization of acyl-protein thioesterase 1/lysophospholipase I as a ghrelin deacylation/lysophospholipid hydrolyzing enzyme in fetal bovine serum and conditioned medium. Endocrinology 151: 4765–4775.
32. Shiya T, Ueno H, Toshinai K, Kawagoe T, Naito S, et al. (2011) Significant lowering of plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men. Endocr J 58: 335–342.
33. Hosoda H, Doi K, Nagaya N, Okumura H, Nakagawa E,
et al. (2004) Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. Clin Chem 50: 1077–1080.

34. Tong J, Dave N, Mugundu GM, Davis HW, Gaylinn BD, et al. (2013) The pharmacokinetics of acyl, des-acyl, and total ghrelin in healthy human subjects. Eur J Endocrinol 168: 821–828.

35. Douglas JA, King JA, Clayton DJ, Jackson AP, Sargeant JA, et al. (2017) Acute effects of exercise on appetite, ad libitum energy intake and appetite-regulatory hormones in lean and overweight/obese men and women. Int J Obes (Lond) 41: 1727–1744.

36. Broom DR, Miyashita M, Wasse LK, Pulsford R, King JA, et al. (2017) Acute effect of exercise intensity and duration on acylated ghrelin and hunger in men. J Endocrinol 232: 411–422.

37. Kawano H, Mineta M, Asaka M, Miyashita M, Numao S, et al. (2013) Effects of different modes of exercise on appetite and appetite-regulating hormones. Appetite 66: 26–33.

38. Vanderheyden LW, McKie GL, Howe GJ, Hazell TJ (2020) Greater lactate accumulation following an acute bout of high-intensity exercise in males suppresses acylated ghrelin and appetite postexercise. J Appl Physiol (1985) 128: 1321–1328.

39. Slater T, Mode WJA, Hough J, James RM, Sale C, et al. (2022) Effect of the perception of breakfast consumption on subsequent appetite and energy intake in healthy males. Eur J Nutr 61: 1319–1330.

40. Callahan HS, Cummings DE, Pepe MS, Breen PA, Matthys CC, et al. (2004) Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. J Clin Endocrinol Metab 89: 1319–1324.

41. Levin F, Edholm T, Schmidt PT, Gryba P, Jacobsson H, et al. (2006) Ghrelin stimulates gastric emptying and hunger in normal-weight humans. J Clin Endocrinol Metab 91: 3296–3302.

42. Williams DL, Cummings DE, Grill HJ, Kaplan JM (2003) Meal-related ghrelin suppression requires postgastric feedback. Endocrinology 144: 2765–2767.

43. Flanagan DE, Evans ML, Monsod TP, Rife F, Heptulla RA, et al. (2003) The influence of insulin on circulating ghrelin. Am J Physiol Endocrinol Metab 284: E313–E316.

44. Broom DR, Stensel DJ, Bishop NC, Burns SF, Miyashita M (2007) Exercise-induced suppression of acylated ghrelin in humans. J Appl Physiol (1985) 102: 2165–2171.

45. Broom DR, Batterham RL, King JA, Stensel DJ (2009) Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. Am J Physiol Regul Integr Comp Physiol 296: R29–R35.

46. MacLean PS, Blundell JE, Mennella JA, Batterham RL (2017) Biological control of appetite: a daunting complexity. Obesity (Silver Spring) 25: S8–S16.

47. Tiryaki-Sonmez G, Ozan S, Bugdayci G, Karli U, Ozan G, et al. (2013) Effect of exercise on appetite-regulating hormones in overweight women. Biol Sport 30: 75–80.