Journal of Exercise Science & Fitness 22 (2024) 39–50

Contents lists available at ScienceDirect

Journal of Exercise Science & Fitness

journal homepage: www.elsevier.com/locate/jesf

Acute effects of exercise intensity on butyrylcholinesterase and ghrelin in young men: A randomized controlled study

Yibin Li [a], Yusei Tataka [a], Miki Sakazaki [a], Kayoko Kamemoto [b], Chihiro Nagayama [a], Yoshie Yoshikawa [a], Yoshiki Yamada [a], Masashi Miyashita [c,d,e,*]

[a] Graduate School of Sport Sciences, Waseda University, Saitama, Japan
[b] Waseda Institute for Sport Sciences, Waseda University, Saitama, Japan
[c] Faculty of Sport Sciences, Waseda University, Saitama, Japan
[d] School of Sport, Exercise and Health Sciences, Loughborough University, Leicestershire, United Kingdom
[e] Department of Sports Science and Physical Education, The Chinese University of Hong Kong, Shatin, Hong Kong

ARTICLE INFO

Keywords:
Butyrylcholinesterase
Exercise intensity
Ghrelin
Running
Subjective appetite

ABSTRACT

Background/objectives: Butyrylcholinesterase (BChE), a liver-derived enzyme that hydrolyzes acylated ghrelin to des-acylated ghrelin, may trigger a potential mechanism responsible for the acute exercise-induced suppression of acylated ghrelin. However, studies examining the effects of an acute bout of high-intensity exercise on BChE and acylated ghrelin have yielded inconsistent findings. This study aimed to examine the acute effects of exercise intensity on BChE, acylated ghrelin and des-acylated ghrelin concentrations in humans.

Methods: Fifteen young men (aged 22.7 ± 1.8 years, mean ± standard deviation) completed three, half-day laboratory-based trials (*i.e.*, high-intensity exercise, low-intensity exercise and control), in a random order. In the exercise trials, the participants ran for 60 min (from 09:30 to 10:30) at a speed eliciting 70 % (high-intensity) or 40 % (low-intensity) of their maximum oxygen uptake and then rested for 90 min. In the control trial, participants sat on a chair for the entire trial (from 09:30 to 12:00). Venous blood samples were collected at 09:30, 10:00, 10:30, 11:00, 11:30 and 12:00.

Results: The BChE concentration was not altered over time among the three trials. Total acylated and des-acylated ghrelin area under the curve during the first 60 min (*i.e.*, from 0 min to 60 min) of the main trial were lower in the high-intensity exercise trial than in the control (acylated ghrelin, mean difference: 62.6 pg/mL, $p < 0.001$; des-acylated ghrelin, mean difference: 31.4 pg/mL, $p = 0.035$) and the low-intensity exercise trial (acylated ghrelin, mean difference: 87.7 pg/mL, $p < 0.001$; des-acylated ghrelin, mean difference: 43.0 pg/mL, $p = 0.042$).

Conclusion: The findings suggest that BChE may not be involved in the modulation of ghrelin even though lowered acylated ghrelin concentration was observed after high-intensity exercise.

1. Introduction

Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor, is a 28-amino-acid peptide hormone purified from the stomach with a unique acylated structure, in which the serine 3 residue is modified by n-octanoic acid.[1] The n-octanoyl modification is essential for the biological regulation of the activity of ghrelin.[1] Moreover, ghrelin, the only known orexigenic hormone that stimulates appetite, is also involved in the regulation of appetite and energy homeostasis.[2] Circulating ghrelin exists in two forms: n-octanoyl-modified (acylated) ghrelin and des-acyl (des-acylated) ghrelin.[3] Although it has been suggested that des-acylated ghrelin possibly has its receptors and some unique physiological functions,[4] it has limited, if any, biological action despite comprising approximately 88–94 % of the total ghrelin (acylated and des-acylated ghrelin combined).[5,6] In contrast, although acylated ghrelin disappears more rapidly from circulation than total ghrelin,[7] due to its rapid des-acylation in the circulation,[8] acylated ghrelin is largely responsible for stimulating appetite and energy intake.[9,10]

* Corresponding author. Waseda University, Faculty of Sport Sciences, 2-579-15 Mikajima, Tokorozawa, Saitama, 359-1192, Japan.
E-mail addresses: yibinli@toki.waseda.jp (Y. Li), y.tataka@ruri.waseda.jp (Y. Tataka), miki.sakazaki@ruri.waseda.jp (M. Sakazaki), k.kamemoto@aoni.waseda.jp (K. Kamemoto), c-nagayama@moegi.waseda.jp (C. Nagayama), y.yoshikawa@toki.waseda.jp (Y. Yoshikawa), yoshiki-yamada@akane.waseda.jp (Y. Yamada), m.miyashita@waseda.jp (M. Miyashita).

https://doi.org/10.1016/j.jesf.2023.11.001
Received 6 August 2023; Received in revised form 16 November 2023; Accepted 17 November 2023
Available online 20 November 2023

Circulating ghrelin concentrations are related to energy homeostasis as plasma ghrelin concentration exhibits a diurnal pattern with preprandial increases and postprandial decreases in humans,[11] indicating ghrelin plays an important role in short-term energy homeostasis. Given the role of ghrelin in the regulation of short-term energy homeostasis and evidence demonstrating that acute exercise influences energy balance, the acute effect of exercise on ghrelin has received significant attention.[12] A large body of evidence suggests that an acute bout of aerobic exercise performed at intensities above 60 % of peak oxygen uptake transiently suppresses circulating concentrations of acylated ghrelin,[13] with higher-intensity exercise associated with greater acylated ghrelin suppression in humans.[14] However, limited evidence is available regarding the acute effect of exercise on des-acylated ghrelin concentration,[14] and the findings are inconsistent whether it causes suppression[15,16] or no change.[17,18]

Although the mechanism by which exercise suppresses acylated ghrelin is still unclear, elevations in butyrylcholinesterase (BChE), a liver-derived enzyme that hydrolyzes acylated ghrelin to des-acylated ghrelin,[19] may be responsible. Only two laboratory-based studies have examined the effects of an acute bout of exercise on BChE and acylated ghrelin with disparate findings.[16,18] One study reported increased BChE activity and decreased acylated ghrelin concentrations measured immediately after 60 min of running at 70 % of maximum oxygen uptake in young men,[16] whereas another study reported no change in BChE concentrations despite decreased acylated ghrelin concentrations measured immediately after 30 min of running at 70 % of maximum oxygen uptake in young men.[18] The amount of energy expended during exercise, the types of assays used for measuring BChE and the lack of a resting control trial[18] are possible reasons for the inconsistent findings between studies. Dorling et al.[16] reported decreased des-acylated ghrelin concentrations after exercise whereas Li et al.[18] reported no change in des-acylated ghrelin concentrations after exercise. Therefore, additional research is needed to elucidate whether BChE is a key mediator of ghrelin modulation. Furthermore, since higher-intensity exercise is associated with greater acylated ghrelin suppression[14] while low-intensity exercise (*i.e.,* walking) often does not alter acylated ghrelin concentrations in humans,[20,21] a study design including both lower and higher exercise intensities will permit an evaluation of whether different energy flux statuses created by acute exercise affect changes in acylated ghrelin through possible changes in BChE in humans. Collectively, this ensures us to evaluate BChE along with both forms of ghrelin appropriately to see whether the exercise-induced suppression of acylated ghrelin concentrations after high-intensity exercise would be expected via the changes in BChE concentrations. In addition, we measured total peptide tyrosine-tyrosine (PYY), insulin and glucose since PYY is an anorexigenic gut hormone which has a close link to ghrelin in a reciprocal manner,[22] and insulin and glucose are modulated by ghrelin.[23]

The purpose of the present study was to examine the acute effects of exercise intensity on BChE, acylated ghrelin and des-acylated ghrelin concentrations in young men. We used plasma samples to evaluate circulating concentrations of BChE, acylated ghrelin and des-acylated ghrelin given that the rate limiting step in the ghrelin hydrolysis reaction is dependent on the concentration of BChE,[24] and ghrelin and the ghrelin-derived molecules are present in plasma.[25] We hypothesized that compared to low-intensity exercise and control trials, the circulating concentration of BChE would increase after high-intensity exercise, while the circulating concentration of acylated ghrelin, and not des-acylated ghrelin, would decrease. In addition, the magnitude of change in BChE correlates negatively with the magnitude of change in acylated ghrelin concentration. These findings shed light on the potential mechanisms by which acute exercise influences appetite and appetite-related hormones.

2. Methods

2.1. Participants

The study was conducted by the guidelines of the Declaration of Helsinki, and the protocol was reviewed and approved by the institutional ethics committee on human research (approval number 2021-436). The study was registered in advance with the University Hospital Medical Information Network Center (UMIN), a system for registering clinical trials (ID: UMIN00004811). Participants of the present study were recruited between May 2022 and March 2023 through advertisements placed within the campus. Following an explanation of the study protocol and disclosure of any potential risks that may arise, written informed consent was obtained from 15 Japanese (*i.e.*, self-reported ethnicity) healthy young men. The exclusion criteria were as follows: 1) aged <20 or >30 years, 2) regular consumption of any medication or supplementation, 3) diagnosed with a major illness, 4) current smoker, 5) unstable body mass for at least 3 months before the study, 5) to lose weight during the study or 6) already participating in other studies. The participant flow diagram is shown in Fig. 1 and described in detail as follows. The physical and descriptive characteristics of the participants are shown in Table 1.

2.2. Screening and preliminary exercise tests

Participants visited the laboratory at least 7 days before the first main experimental trial to collect baseline data and familiarize themselves with the study procedures. After obtaining consent to participate in the study, anthropometric and arterial blood pressure measurements were recorded under non-fasting conditions. Body mass and body fat percentage were measured to the nearest 0.1 kg and 0.1 % respectively using a digital scale (TANITA MC780, Tanita Corporation, Tokyo, Japan), meanwhile, height was measured to the nearest 0.1 cm using a stadiometer (YS-OA, AS One Corporation, Osaka, Japan). Body mass index was calculated as weight in kilograms divided by the square of height in meters. Arterial blood pressure was measured from the left arm after 5 min of seated rest using a standard mercury sphygmomanometer (605P, Yagami Co.Ltd., Yokohama, Japan). Two consecutive measurements were obtained 1 min apart, and the mean of these values was recorded.

Participants then underwent two preliminary exercise tests performed on a motorized treadmill (Jog Now 700, Technogym, Cesena, 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-min incremental runs starting at a speed of 4.0 km/h. The treadmill was level throughout the test period, and speed was increased by 1.0 or 1.5 km/h every 4 min. After a 20-min test (*i.e.*, following completion of the submaximal treadmill test), the participants were asked to complete a maximum oxygen uptake test using an incremental uphill protocol at a constant speed.[26] The initial inclination of the treadmill was set to 3.5 % for the test. Thereafter, the gradient was increased by 2.5 % every 3 min until participants reached volitional fatigue. Heart rate was monitored throughout these tests using short-range telemetry (Polar RCX3, Polar Electro, Kempele, Finland). Oxygen uptake, carbon dioxide production and respiratory exchange ratio were measured using a stationary gas analyzer (Quark RMR, COSMED, Rome, Italy). The ratings of perceived exertion (RPE) were recorded at the end of each stage during both exercise tests on a subjective scale of 6–20.[27] The participants were considered to have attained the maximum oxygen uptake 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) respiratory exchange ratio >1.15, 3) a plateau in oxygen consumption and 4) RPE ≥19. Data generated from these two tests were used to calculate the running intensity (*i.e.*, 70 % of maximum oxygen uptake and 40 % of maximum oxygen uptake) of the participants in the main trials.

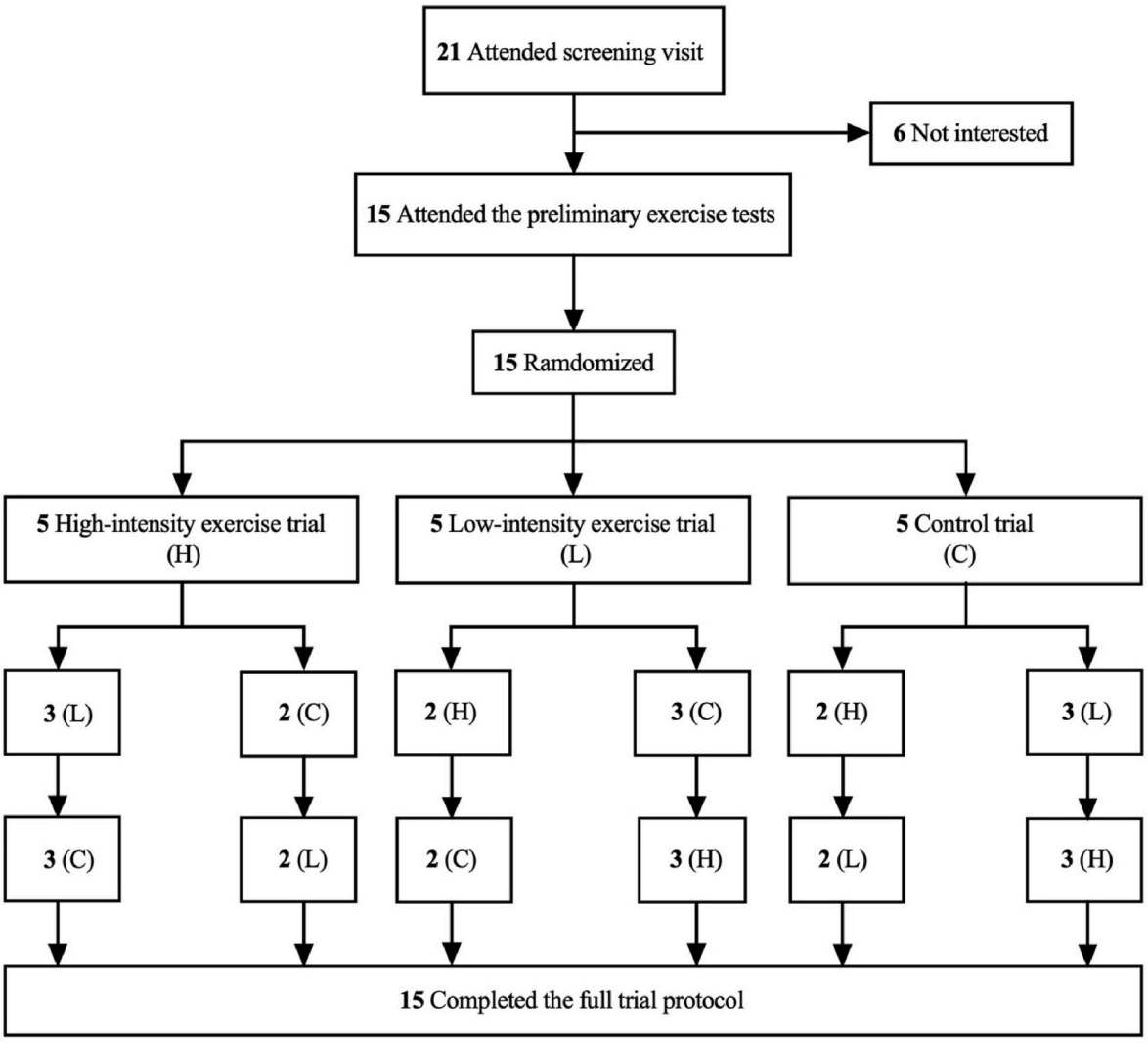

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) diagram showing participant flow. Numbers in bold indicate the number of individuals/participants.

Table 1
Physical and physiological characteristics of the participants (n = 15).

Characteristic	
Age (years)	22.7 ± 1.8
Body mass (kg)	66.6 ± 9.1
Height (m)	1.73 ± 0.66
Body mass index (kg/m^2)	22.2 ± 2.6
Waist circumference (cm)	76.9 ± 7.6
Body fat (%)	15.9 ± 5.1
Systolic blood pressure (mm Hg)	121 ± 10
Diastolic blood pressure (mm Hg)	72 ± 9
Maximum oxygen uptake (mL/kg/min)	56.3 ± 4.2

Values are mean ± standard deviation.

2.3. Standardization of energy intake and physical activity

The participants weighed and recorded all food and drinks consumed the day before each main trial and refrained from consuming alcohol during the prescribed period. They replicated their energy intake from the first to the subsequent trials to ensure that their energy intake was standardized across the trials. The food diaries were analyzed using nutrition analysis software (Excel Eiyoukun Ver 9.0, Kenpakusha, Tokyo, Japan) by a registered dietician to determine the energy intake of the participants and the macronutrient content of the foods. Moreover, the participants were instructed to avoid any strenuous exercise for 1 day before each main trial. They wore a uniaxial accelerometer (Lifecoder-EX; Suzuken Co. Ltd., Nagoya, Japan) on their hips to objectively monitor their daily activity during this period. The accelerometer defined 11 levels of activity intensity (0, 0.5 and 1–9), with 0 indicating the lowest intensity and 9 being the highest intensity. A level of 4 corresponds to an intensity of approximately three metabolic equivalents.[28] Levels 1–3 corresponded to light physical activity, levels 4–6 corresponded to moderate physical activity and levels 7–9 corresponded to vigorous physical activity. On the day before each main trial, the participants received text messages from a researcher asking them to replicate their energy intake and physical activity patterns. The compliance with replicating each main test condition was verbally confirmed upon arrival at the laboratory.

2.4. Study design and protocol

The lead investigator enrolled the participants in the research and randomly assigned the participants to each experiment using computer-generated random numbers. Eligible participants completed three half-day laboratory-based experimental trials (*i.e.*, high-intensity exercise, low-intensity exercise and control) in random order. The interval between trials was at least 7 days. A schematic illustration of the study protocol is presented in Fig. 2.

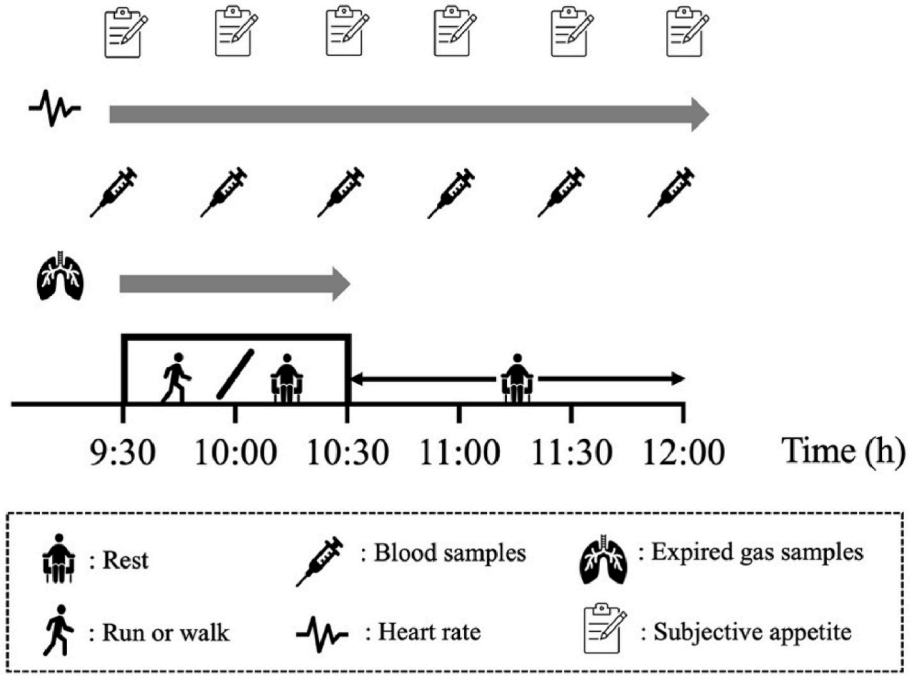

Fig. 2. Schematic representation of the study protocol.

On the day of each main trial, the participants were asked to drink 200 mL of water upon waking up at home and reported to the laboratory at 08:45 after a 10-h overnight fast (except for water). Upon arrival, each participant was asked to drink an additional 50 mL of water to make sure they were sufficiently hydrated before each main trial. Then, body mass and body fat percentage were measured to the nearest 0.1 kg and 0.1 % respectively using a digital scale (TANITA MC-780, Tanita Corporation, Tokyo, Japan). After a 10-min seated rest, the resting arterial blood pressure was measured using a digital monitor (OMRON HEM-907, Omron Cooperation, Kyoto, Japan) in a seated position. A heart rate monitor (Polar RCX3, Polar Electro, Kempele, Finland) was then fitted (*i.e.*, around 09:00) to measure the heart rate continuously throughout the trial. Thereafter, subjective appetite was evaluated using a paper-based questionnaire (details in "Subjective appetite") and a fasting venous blood sample was collected by venipuncture in a seated position (*i.e.,* prior to exercise or rest (just before 09:30)). Then, the participants performed a 60-min exercise (*i.e.*, from 09:30 to 10:30) on a treadmill at a speed eliciting 70 % of their maximum oxygen uptake (determined from the preliminary test) in the high-intensity exercise trial or at a speed eliciting 40 % of their maximum oxygen uptake (determined from the preliminary test) in the low-intensity exercise trial. An exercise duration of 60 min was chosen since this exercise duration was effective in increasing BChE activity while decreasing circulating concentrations of acylated ghrelin measured after exercise (*i.e.,* running at 70 % of maximum oxygen uptake) in young men.[16] Although the treadmill speed was adjusted occasionally to ensure that the target intensity was achieved in both exercise trials, it was decreased to ensure that the participants were able to perform the exercise for 60 min in the high-intensity exercise trial. In the control trial, participants were asked to sit on a chair in a comfortable position for 60 min from 09:30 to 10:30. During the 60-min period, oxygen uptake, respiratory exchange ratio, fat oxidation rate and carbohydrate oxidation rate were measured using a stationary gas analyzer (Quark RMR, COSMED Co. Ltd., Roma, Italy) and RPE was assessed periodically.[27] Further venous blood samples were collected by venipuncture for the measurement of circulating concentrations of BChE, acylated ghrelin, des-acylated ghrelin, total PYY, insulin and glucose, and further subjective appetite was evaluated at 10:00, 10:30, 11:00, 11:30 and 12:00 in all trials. Participants consumed water *ad libitum* (except for 50 mL of water consumed at the beginning of each main trial) during the first trial, and the volume ingested was replicated in subsequent trials. The average water intake was 290 ± 196 mL over 3 h (*i.e.*, from 09:00 to 12:00). The mean atmospheric temperature and relative humidity during the experimental trials were 23.2 ± 0.4 °C and 40.8 ± 1.9 % (mean ± standard deviation), respectively. There were no differences in the mean atmospheric temperature among trials (high-intensity exercise, 23.2 ± 2.1 °C; low-intensity exercise, 22.8 ± 2.7 °C; and control, 23.7 ± 1.9 °C, $p = 0.329$). There was a difference in the mean relative humidity among trials (high-intensity exercise, 42.6 ± 7.2 %; low-intensity exercise, 41.2 ± 6.6 %; and control, 38.7 ± 7.0 %, $p = 0.023$). The *post-hoc* analysis did not reveal where the between-trial differences were for relative humidity.

2.5. Blood collection and analysis

For plasma BChE, total PYY and insulin measurements, venous blood samples were collected in dipotassium salt-ethylenediaminetetraacetic acid (EDTA) tubes (Venoject 2, Terumo Corporation, Tokyo, Japan). For plasma glucose measurements, venous blood samples were collected in sodium fluoride-EDTA tubes (Venoject 2, Terumo Corporation, Tokyo, Japan). Both tubes were centrifuged immediately at 1861×*g* for 10 min at 4 °C. The plasma was removed, divided into three microtubes, and stored at −80 °C until further analysis. For plasma acylated ghrelin and des-acylated ghrelin measurements, blood samples were immediately transferred to EDTA tubes containing aprotinin (Neo tube, Nipro Corporation, Osaka, Japan) to prevent the degradation of ghrelin by protease. Both tubes were immediately centrifuged at 1861×*g* for 10 min at 4 °C. The samples were stored at −80 °C until further analysis. Enzyme-linked immunosorbent assays (ELISAs) were used to measure the plasma BChE (DBCHE0, R&D System, Minneapolis, USA), acylated ghrelin (A05306, Bertin Pharma, Montigny-le-Bretonneux, France), des-acylated ghrelin (A05319, Bertin Pharma, Montigny-le-Bretonneux, France), PYY (YK080, Yanaihara Institute Inc., Shizuoka, Japan) and insulin (Mercodia Insulin ELISA, Mercodia AB, Uppsala, Sweden) concentrations. Enzymatic colorimetric assays were used to measure plasma glucose (GLU-HK(M), Shino-Test Corporation, Kanagawa, Japan). The

intra-assay coefficients of variation were 10.2 % for BChE, 8.2 % for acylated ghrelin, 9.0 % for des-acylated ghrelin, 8.6 % for total PYY, 10.2 % for insulin and 0.6 % for glucose.

2.6. Subjective appetite

Subjective appetite (satiety, fullness, hunger and prospective food intake) was assessed on a 100-mm visual analog scale using a paper-based questionnaire (*i.e.*, each end of the line represents the most extreme sensation experienced by the participant) at 09:30, 10:00, 10:30, 11:00, 11:30 and 12:00.[29] From the results of the four appetite ratings assessed, an overall subjective appetite score was calculated using the following equation: Satiety + fullness + (100 – hunger) + (100 – prospective food intake)/4[30] in which 100 indicated less appetite and 0 indicated more appetite.

2.7. Calculations and statistical analysis

We calculated the required sample size based on data from our previous study[18] using G*Power 3.1.9.6.[31] The previous study reported no change in BChE concentration after performing 30 min of running exercise at 70 % of maximum oxygen uptake in healthy young men. This sample size calculation was estimated to detect an effect size of 0.74 (Cohen's *d*) using a paired *t*-test for comparison between trials and revealed that 17 participants would suffice to find a significant effect of acute exercise on BChE concentration. However, given that our study designed 60 min running exercise with 70 % of maximum oxygen uptake in healthy young men, we assumed that a larger effect size (*i.e.*, 0.80) would be attainable for the present study. For two trials with an alpha level set at 0.05, and a correlation of 0.5, an estimated total sample size of 15 would achieve 0.82 % power to detect between-trial differences. Data were analyzed with IBM SPSS Statistics for Windows version 28.0 (IBM Corp., New York, USA). The total area under the curve (AUC) was calculated using the trapezoidal rule. Generalized estimating equations were used to examine between-trial differences for all parameters. In case of difference in values at baseline (09:30), generalized estimating equations were used for adjustment of such values when examining differences over time among the three trials. Where a main effect of trial and/or a significant trial-by-time interaction was identified, *post-hoc* pairwise comparisons were performed using the Bonferroni method. The 95 % confidence interval (CIs) for the mean absolute pairwise differences between trials were calculated using the t-distribution and degrees of freedom (n − 1). Statistical significance was set at < 5 %. Results are reported as mean ± standard deviation. Graphical representations of the results are presented as mean ± standard error to avoid distortion of the figures. Boxplot analysis of acylated ghrelin total AUC values revealed three participants as outliers.[32] The mean acylated ghrelin concentrations of these participants were 10, 19 and 27 times higher than the mean standard deviation of the remaining participants (range: 69.2–451.5 pg/mL). Consequently, these three participants were removed from the data analysis for both ghrelin and BChE, and the results are presented for 12 participants.

3. Results

3.1. Dietary record data

The mean self-reported energy intake for the day before each trial was 8.1 ± 3.8 MJ (1943 ± 898 kcal). Energy intake equated to 9.4 ± 5.2 % (45.8 ± 43 g/day) from protein, 24.3 ± 17.7 % (52.4 ± 35 g/day) from fat and 66.3 ± 18.6 % (159.3 ± 156 g/day) from carbohydrate.

3.2. Physical activity data

The step counts recorded the day before the trials did not differ among trials (9120 ± 6363 versus 6223 ± 4462 versus 8833 ± 4253 steps/day for the high-intensity exercise, low-intensity exercise and control trials, respectively; $p = 0.130$). For the day before each main trial, the accelerometer recorded frequencies for light (levels 1–3; 45 ± 36 versus 40 ± 31 versus 60 ± 39 min/day for the high-intensity exercise, low-intensity exercise and control trials, respectively; $p = 0.096$), moderate (levels 4–6; 28 ± 22 versus 22 ± 16 versus 29 ± 16 min/day for the high-intensity exercise, low-intensity exercise and control trials, respectively; $p = 0.321$) and vigorous activity (levels 7–9; 4 ± 10 versus 2 ± 1 versus 4 ± 5 min/day for the high-intensity exercise, low-intensity exercise and control trials, respectively; $p < 0.001$). Vigorous activity differed among trials ($p < 0.001$) and was lower in the low-intensity exercise trial than in the control trial (mean difference: 2.9 min; 95 % CI: 0.24–5.54 min; $p = 0.027$). No other levels of activity exhibited significant differences among the trials.

3.3. Responses during exercise

Exercise characteristics and physiological responses to high- and low-intensity exercises are shown in Table 2. The treadmill speed, oxygen uptake, percent maximum oxygen uptake, gross energy expenditure, heart rate, RPE and respiratory exchange ratio were higher in the high-intensity exercise trial than in the low-intensity exercise trial (all $p < 0.005$). The relative contribution of fat to energy expenditure was lower in the high-intensity exercise trial than in the low-intensity exercise trial ($p = 0.001$). The relative contribution of carbohydrates to energy expenditure was higher in the high-intensity exercise trial than in the low-intensity exercise trial ($p < 0.001$).

3.4. Baseline blood parameters

The baseline blood parameters are shown in Table 3. Plasma acylated ghrelin concentrations differed among trials and were higher in the

Table 2
Physiological responses during a 60-min high-intensity exercise, low-intensity exercise and control trials (n = 15).

	High-intensity exercise	Low-intensity exercise	Control	*p* value[a]
Treadmill speed (km/h)	10.6 ± 1.4	6.2 ± 0.7	–	< 0.001
Oxygen uptake (mL/kg/min)	36.2 ± 2.7	21.5 ± 2.6	4.5 ± 0.9	< 0.001
Percent maximum oxygen uptake (%)	65.1 ± 4.1	38.4 ± 3.1	8.0 ± 1.5	< 0.001
Gross energy expenditure (MJ)	3.19 ± 0.47	1.81 ± 0.31	0.35 ± 0.07	< 0.001
Heart rate (beats/min)	160 ± 17	106 ± 13	59 ± 20	< 0.001
Rating of perceived exertion	14 ± 3	11 ± 2	6 ± 1	< 0.001
Respiratory exchange ratio	0.87 ± 0.07	0.78 ± 0.03	0.80 ± 0.01	< 0.001
Fat oxidation (%)	50.96 ± 22.78	73.14 ± 10.22	59.01 ± 20.94	0.001
Carbohydrate oxidation (%)	49.04 ± 22.78	26.86 ± 10.22	40.99 ± 20.94	< 0.001

Values are mean ± standard deviation.
[a] Values are compared using generalized estimating equations between the high-intensity exercise and low-intensity exercise trials (values in the control trial represent as reference values).

Table 3
Fasting concentrations of acylated ghrelin, des-acylated ghrelin, the ratio of acylated ghrelin to des-acylated ghrelin (AG:DAG ratio), butyrylcholinesterase, total peptide tyrosine-tyrosine (PYY), insulin and glucose at baseline for the high-intensity exercise, low-intensity exercise and control trials.

	High-intensity exercise	Low-intensity exercise	Control	*p* value[a]
Acylated ghrelin (pg/mL)	239.3 ± 143.5[b]	244.2 ± 165.4	262.5 ± 140.6	0.017
Des-acylated ghrelin (pg/mL)	274.2 ± 114.2	270.7 ± 87.3	288.2 ± 119.1	0.564
AG:DAG ratio	0.93 ± 0.57	0.94 ± 0.66	0.95 ± 0.48	0.922
Butyrylcholinesterase (ng/mL)	3634.4 ± 1195.9	3346.3 ± 1933.0	4203.6 ± 1716.6	0.401
Total PYY (ng/mL)	0.59 ± 0.29	0.59 ± 0.31	0.65 ± 0.33	0.224
Insulin (pmol/L)	22.0 ± 10.5	22.3 ± 20.1	21.1 ± 9.1	0.774
Glucose (mmol/L)	5.01 ± 0.28	5.02 ± 0.34	4.98 ± 0.32	0.862

Values are mean ± standard deviation. n = 12 for acylated ghrelin, des-acylated ghrelin and butyrylcholinesterase. n = 15 for all others.
[a] Values are compared using generalized estimating equations. *Post-hoc* analysis was adjusted for multiple comparisons using the Bonferroni method.
[b] *Post-hoc* analysis revealed a significant difference between the high-intensity exercise and control trials ($p = 0.007$).

control trial than the high-intensity exercise trial (mean difference 262.5 ± 140.6 versus 239.3 ± 143.5 pg/mL, 95 % CI: 4.98–41.47 pg/mL, $p = 0.007$). No other blood parameters displayed significant differences at baseline among the trials.

3.5. Circulating concentrations of BChE, acylated ghrelin, des-acylated ghrelin and the ratio of acylated ghrelin to des-acylated ghrelin

Plasma concentrations of BChE, acylated ghrelin, des-acylated ghrelin and the ratio of acylated ghrelin to des-acylated ghrelin (AG: DAG ratio) for each trial over 150 min are shown in Fig. 3 and Fig. 4A–C. There was no main effect of the trial ($p = 0.141$) on plasma BChE concentration (Fig. 3). There was a main effect of time ($p = 0.032$) and trial-by-time interaction ($p < 0.001$) on plasma BChE concentration (Fig. 3). *Post-hoc* analysis of the interaction effect did not reveal where the between-trial differences at each time point were for plasma BChE concentrations. The total AUC values during the first 60 min (*i.e.*, from 09:30 to 10:30) of the main trial for the total plasma BChE did not differ among trials ($p = 0.590$).

There was no significant effect of the trial ($p = 0.121$) on plasma acylated ghrelin concentration (Fig. 4A). There was a significant effect of time ($p = 0.046$) and a trial-by-time interaction ($p < 0.001$) on the plasma acylated ghrelin concentration (Fig. 4A). *Post-hoc* analysis of an interaction effect revealed that plasma acylated ghrelin concentration was lower in the high-intensity exercise trial than the control trial (mean difference: 74.8 pg/mL; 95 % CI: 9.02–140.62 pg/mL; $p = 0.007$) and the low-intensity exercise trial (mean difference: 119.4 pg/mL; 95 % CI: 33.40–205.55 pg/mL; $p < 0.001$) at 10:00. Further *post-hoc* analysis of an interaction effect revealed that plasma acylated ghrelin concentration was lower in the high-intensity exercise trial than the low-intensity exercise trial (mean difference: 107.0 pg/mL; 95 % CI: 30.04–183.86 pg/mL; $p < 0.001$) at 10:30. The total AUC values during the first 60 min (*i.e.*, from 09:30 to 10:30) of the main trial for plasma acylated ghrelin differed among trials ($p < 0.001$). *Post-hoc* analysis revealed that total plasma acylated ghrelin AUC was lower in the high-intensity exercise trial than the control trial (mean difference: 62.6 pg/mL; 95 % CI: 23.97–101.28 pg/mL; $p < 0.001$) and the low-intensity trial (mean difference: 87.7 pg/mL; 95 % CI: 47.69–127.73 pg/mL; $p < 0.001$).

There was a significant effect for trial ($p = 0.028$), time ($p < 0.001$) and trial-by-time interaction ($p < 0.001$) on plasma des-acylated ghrelin concentration (Fig. 4B). *Post-hoc* analysis of an interaction effect revealed that plasma des-acylated ghrelin concentration was lower in the high-intensity exercise trial than the low-intensity exercise trial (mean difference: 91.4 pg/mL; 95 % CI: 15.69–167.12 pg/mL; $p = 0.002$) at 10:30. The total AUC values during the first 60 min (*i.e.*, from 09:30 to 10:30) of the main trial for plasma des-acylated ghrelin differed among the trials ($p = 0.028$). *Post-hoc* analysis revealed that total plasma des-acylated ghrelin AUC was lower in the high-intensity exercise trial than the control trial (mean difference: 31.4 pg/mL; 95 % CI: 1.60–61.29 pg/mL; $p = 0.035$) and the low-intensity exercise trial (mean difference: 43.0 pg/mL; 95 % CI: 1.14–84.92 pg/mL; $p = 0.042$).

There was no significant effect for trial ($p = 0.659$) or time ($p = 0.056$) on the AG: DAG ratio (Fig. 4C). There was a significant effect for the trial-by-time interaction ($p < 0.001$) on the AG:DAG ratio (Fig. 4C). *Post-hoc* analysis of an interaction effect revealed that the AG: DAG ratio was lower in the high-intensity exercise trial than the low-intensity exercise trial (mean difference: 0.24; 95 % CI: 0.07–0.41; $p < 0.001$) at 10:00. The total AUC values during the first 60 min (*i.e.*, from 09:30 to 10:30) of the main trial for the AG:DAG ratio differed among trials ($p = 0.001$). *Post-hoc* analysis revealed that total the AG:DAG ratio AUC was lower in the high-intensity exercise trial than in the control trial (mean difference: 62.6; 95 % CI: 23.97–101.28; $p < 0.001$) and the low-

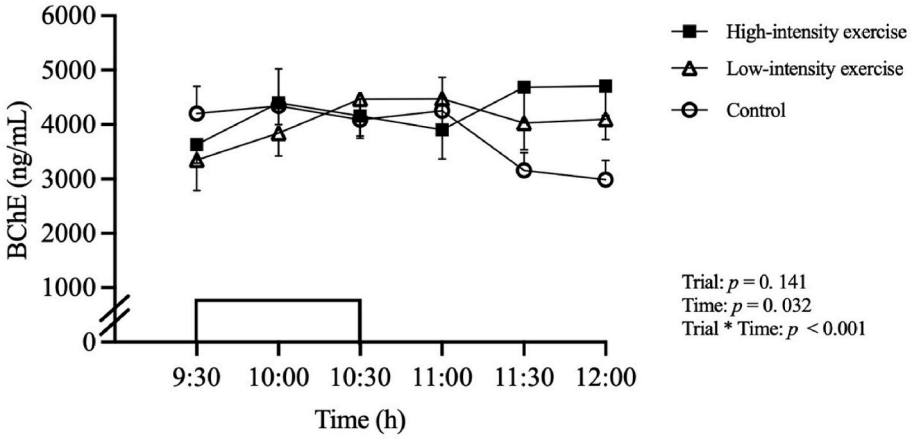

Fig. 3. Butyrylcholinesterase (BChE) during the high-intensity exercise, low-intensity exercise and control trials. Values are mean ± standard error represented by unidirectional bars. n = 12. Horizontally rectangle indicates a 60-min run/walk or rest. Values were compared using generalized estimating equations.

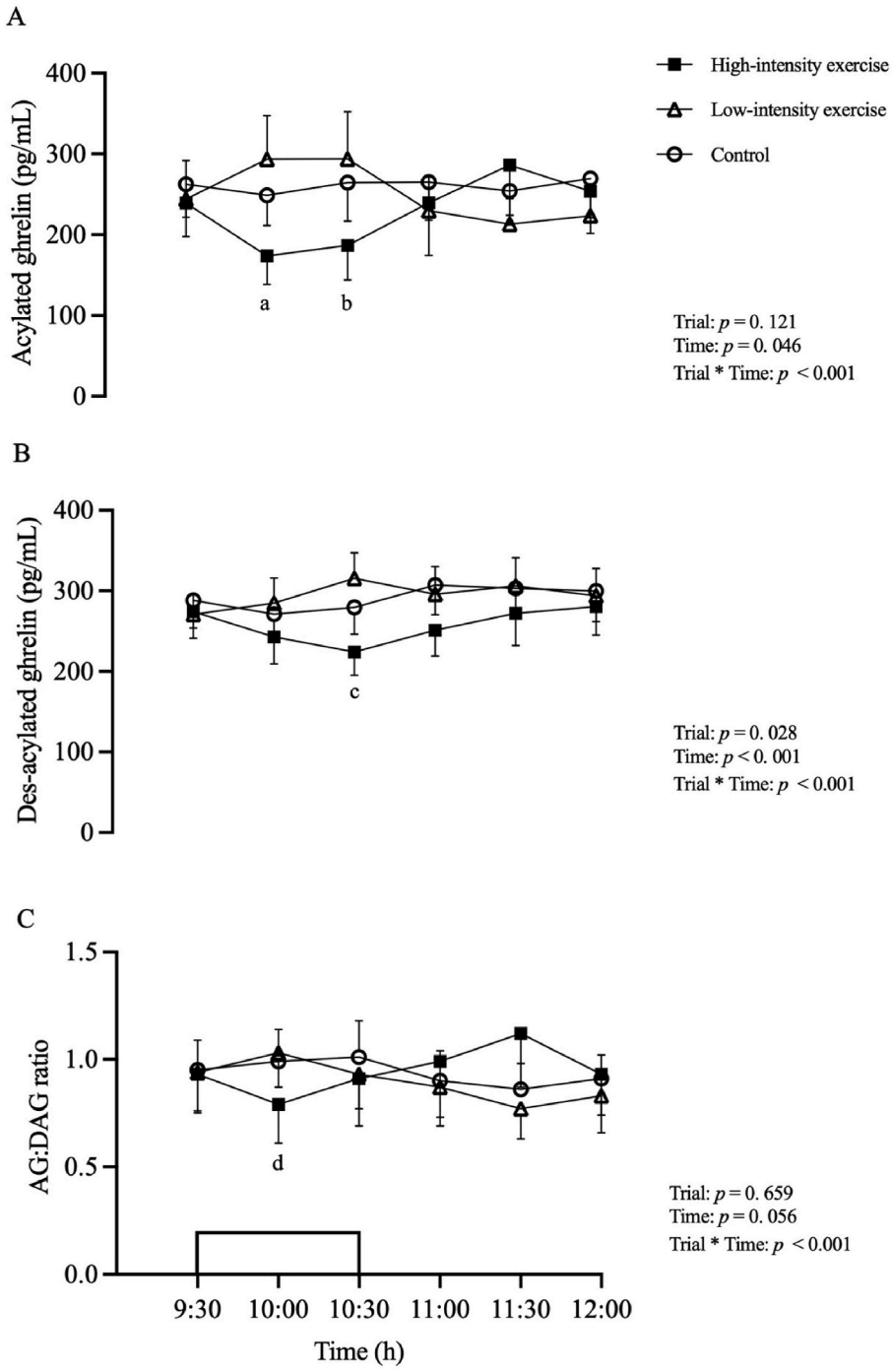

Fig. 4. Acylated ghrelin (AG), des-acylated ghrelin (DAG), and the ratio of acylated ghrelin to des-acylated ghrelin (AG:DAG ratio) during the high-intensity exercise, low-intensity exercise and control trials. Values are mean ± standard error represented by unidirectional bars. n = 12. Horizontally rectangle indicates a 60-min run/walk or rest. Values were compared using generalized estimating equations. *Post-hoc* analysis was adjusted for multiple comparisons using the Bonferroni method. [a]Significantly lower in the high-intensity exercise than the control ($p = 0.007$) and low-intensity exercise ($p < 0.001$) trials at 10:00. [b]Significantly lower in the high-intensity exercise trial than the low-intensity exercise trial ($p < 0.001$) at 10:30. [c]Significantly lower in the high-intensity exercise trial than the low-intensity exercise trial ($p = 0.002$) at 10:30. [d]Significantly lower in the high-intensity exercise trial than the low-intensity exercise trial ($p < 0.001$) at 10:00.

intensity exercise trial (mean difference: 87.7; 95 % CI: 47.69–127.73; $p < 0.001$).

3.6. Circulating concentrations of PYY, insulin and glucose

Circulating concentrations of plasma total PYY, plasma insulin and plasma glucose for each trial over 150 min are shown in Table 4. There was no main effect of the trial ($p = 0.077$) or time ($p = 0.278$) on the plasma total PYY concentration (Table 4). There was a main effect of the trial-by-time interaction ($p < 0.001$) (Table 4). *Post-hoc* analysis of the interaction effect did not reveal where the between-trial differences at each time point were for plasma total PYY. The total AUC values during the first 60 min (*i.e.*, from 09:30 to 10:30) of the main trial for plasma total PYY did not differ among trials ($p = 0.676$). There was no significant effect of the trial ($p = 0.282$) on plasma insulin concentration (Table 4). There was a significant effect of time ($p = 0.001$) and a trial-

Table 4
Circulating concentrations of total peptide tyrosine-tyrosine (PYY), insulin and glucose measured at each time-point in the high-intensity exercise, low-intensity exercise and control trials.

		09:30	10:00	10:30	11:00	11:30	12:00	*p* value[a]
Total PYY (ng/mL)	High-intensity exercise	0.59 ± 0.29	0.64 ± 0.32	0.66 ± 0.37	0.65 ± 0.36	0.66 ± 0.33	0.63 ± 0.33	
	Low-intensity exercise	0.59 ± 0.31	0.60 ± 0.31	0.63 ± 0.30	0.59 ± 0.33	0.55 ± 0.23	0.58 ± 0.28	< 0.001
	Control	0.65 ± 0.33	0.63 ± 0.31	0.65 ± 0.37	0.66 ± 0.31	0.63 ± 0.29	0.63 ± 0.28	
Insulin (pmol/L)	High-intensity exercise	22.0 ±10.5	14.8 ± 16.5	8.1 ± 6.8	21.2 ± 9.8	12.3 ± 5.3	16.0 ± 11.6	
	Low-intensity exercise	22.3 ± 20.1	13.3 ± 9.3	13.3 ± 11.2	19.5 ± 13.5	17.5 ± 12.3	17.4 ± 8.0	< 0.001
	Control	21.1 ± 9.1	20.1 ± 15.5	21.2 ± 16.2	18.9 ± 14.0	17.6 ± 11.8	17.2 ± 15.2	
Glucose (mmol/L)	High-intensity exercise	5.01 ± 0.28	5.11 ± 0.61	4.78 ± 0.65	4.59 ± 0.36	4.66 ± 0.43	4.59 ± 0.33[b]	
	Low-intensity exercise	5.02 ± 0.34	4.88 ± 0.31	4.84 ± 0.35	4.84 ± 0.37	4.82 ± 0.32	4.79 ± 0.35	< 0.001
	Control	4.98 ± 0.32	5.01 ± 0.30	4.98 ± 0.35	4.87 ± 0.32	4.91 ± 0.34	4.89 ± 0.32	

Values are mean ± standard deviation. n = 15.
[b]

[a] Values are compared using generalized estimating equations. *Post-hoc* analysis was adjusted for multiple comparisons using the Bonferroni method.
[b] *Post-hoc* analysis revealed a significant difference at the same time-point between the high-intensity exercise and control trials ($p = 0.028$).

by-time interaction ($p < 0.001$) on plasma insulin concentration (Table 4). *Post-hoc* analysis of the interaction effect did not reveal where the between-trial differences at each time point were for plasma insulin. The total AUC values during the first 60 min (*i.e.*, from 09:30 to 10:30) of the main trial for plasma insulin differed among trials ($p = 0.001$). *Post-hoc* analysis revealed that total insulin AUC was lower in the high-intensity exercise trial than in the control trial (mean difference: 5.7 pmol/L; 95 % CI: 0.1–11.3 pmol/L; $p = 0.044$). There was no significant effect of the trial ($p = 0.121$) on plasma glucose concentration (Table 4). There was a significant effect of time ($p < 0.001$) and trial-by-time interaction ($p < 0.001$) on plasma glucose concentration (Table 4). *Post-hoc* analysis of an interaction effect revealed that plasma glucose concentration was lower in the high-intensity exercise trial than in the control trial (mean difference: 0.30 mmol/L; 95 % CI: 0.01–0.58 mmol/L; $p = 0.028$) at 12:00. The total AUC values during the first 60 min (*i.e.*, from 09:30 to 10:30) of the main trial for plasma glucose did not differ among trials ($p = 0.310$).

3.7. Subjective appetite

The subjective appetite score for each trial over 150 min is shown in Fig. 5. There were no significant differences at baseline among the trials. There was a significant effect of the trial ($p = 0.005$), time ($p = 0.001$) and trial-by-time interaction ($p < 0.001$) on subjective appetite score (Fig. 5). *Post-hoc* analysis of the interaction effect did not reveal where the between-trial differences at each time point were for subjective appetite score. The total AUC values during the first 60 min (*i.e.*, from 09:30 to 10:30) of the main trial for the subjective appetite score differed among trials ($p = 0.008$). *Post-hoc* analysis revealed that the total subjective appetite score AUC was higher in the high-intensity trial than in the control trial (mean difference: 10.4; 95 % CI: 1.83–19.02; $p = 0.011$) and the low-intensity exercise trial (mean difference: 7.6; 95 % CI: 0.19–15.12; $p = 0.042$).

4. Discussion

The main findings of the present study were as follows: 1) BChE concentration was not altered by an acute session of high-intensity exercise or low-intensity exercise in healthy young men and 2) an acute session of high-intensity exercise transiently suppressed acylated ghrelin and des-acylated ghrelin concentrations compared with low-intensity exercise and sitting rest. These findings suggest that BChE does not mediate the suppression of acylated ghrelin after high-intensity exercise. The interpretation is also confirmed by the fact that an acute bout of high-intensity exercise, but not low-intensity exercise, transiently suppressed des-acylated ghrelin concentrations.

The key finding of the present study was that BChE concentration remained unchanged among different intensities of exercise and control conditions, despite high-intensity exercise inducing both acylated ghrelin and des-acylated ghrelin suppression in healthy young men. These findings also indicate that BChE may not be involved in the suppression of acylated ghrelin induced by high-intensity exercise. In addition, the present findings aligned closely with the previous study, which was a single-arm design (*i.e.*, no controlled trial) that reported BChE concentrations were unchanged while acylated ghrelin, but not des-acylated ghrelin, concentrations were reduced after a 30-min of run

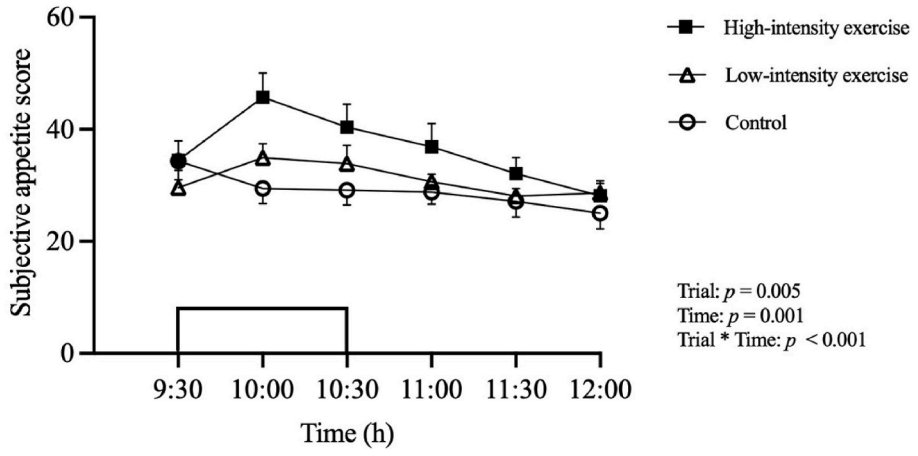

Fig. 5. Subjective appetite score during the high-intensity exercise, low-intensity exercise and control trials. Values are mean ± standard error represented by unidirectional bars. n = 15. Horizontally rectangle indicates a 60-min run/walk or rest. Values were compared using generalized estimating equations.

at 70 % of maximum oxygen uptake in healthy young men.[18] Conversely, increased BChE activity while reduced acylated ghrelin and des-acylated ghrelin concentrations were observed when acute running exercise was performed 60 min at 70 % of peak oxygen uptake in young men with and without obesity risk genes (*i.e.*, obesity-associated genes).[16] It is worth noting that the authors of this study addressed that several mechanisms are involved in exercise-induced suppression of acylated ghrelin.[16] This postulation is possibly derived from the notion that if BChE is the main mediator of ghrelin hydrolysis, it would be expected to observe increased hydrolysis of acylated ghrelin to des-acylated ghrelin (*i.e.*, decreased acylated ghrelin and increased des-acylated ghrelin, leading to a lower AG:DAG ratio). It is also worth mentioning that the exercise-induced suppression of the AG:DAG ratio (*i.e.,* the total AUC values, but not the trial-by-time interaction effect, possibly due to slightly increased acylated ghrelin concentrations measured at 10:30 compared with its corresponding value at 10:00) was greater in the high-intensity exercise trial than the low-intensity exercise and control trials in the present study, despite no changes in BChE concentrations among trials. Collectively, the response of the des-acylated ghrelin to an acute bout of exercise in the present and previous studies[16] implies that other factors may be related to ghrelin hydrolysis. It has been suggested that the primary enzyme responsible for ghrelin hydrolysis may differ depending on the species and specific tissue context.[33] Furthermore, previous studies have demonstrated that BChE was not the only enzyme that contributed to the hydrolysis of ghrelin,[25] but carboxylesterase[33] and lysophospholipase 1[34] may also be involved in ghrelin degradation. However, a limited number of relevant studies have made it difficult to thoroughly assess the association between BChE and both acylated and des-acylated forms of ghrelin in response to acute exercise. Further mechanistic research is required to determine the role of BChE, along with other potential enzymes for the hydrolysis of ghrelin, in exercise-induced acylated ghrelin suppression.

It is well documented that acylated ghrelin concentrations are transiently suppressed in response to acute moderate-to high-intensity exercise in humans.[13] This response pattern was also observed in the present study with a 60-min run at 65 % of maximum oxygen uptake in young men. In addition, des-acylated ghrelin concentration was also measured in the present study, as it has long been known that des-acylated ghrelin is an inert degradation product of acylated ghrelin.[4] The previous studies have demonstrated that acute exercise performed 60 min at 70 % or 59 % of peak oxygen uptake on a treadmill suppressed both acylated ghrelin and des-acylated ghrelin concentrations in young men with and without obesity risk genes (*i.e.*, obesity-associated genes),[16] and in middle-aged adults with lean and overweight/-obesity.[15] Another key finding of the present study was that it extended the findings of the previous findings[15,16] by demonstrating that des-acylated ghrelin decreased after acute high-intensity exercise, but not after low-intensity exercise. Collectively, these findings indicate that exercise intensity may be involved in the alteration of des-acylated ghrelin along with acylated ghrelin which has been established for a while. However, this speculation should be interpreted with caution given that des-acylated ghrelin is considered a degradation product of acylated ghrelin[4] and has a slower clearance rate than acylated ghrelin under resting conditions in systemic circulation.[35] Indeed, a previous study demonstrated that infusion of acylated ghrelin resulted in increased des-acylated ghrelin concentrations in healthy men and women.[35] Moreover, studies directly investigating both acylated ghrelin and des-acylated ghrelin in response to acute exercise in humans[17,18,36] reported no significant alterations in circulating concentrations of des-acylated ghrelin measured in the post-exercise period, whereas there was a reduction in acylated ghrelin. Although the reasons for the discrepancy are unclear among the acute exercise studies[15–18,36] and the present study, the potential explanation may be related to the combined factors, including key variations in the protocols applied, such as differences in exercise duration/intensity,[37] lack of a controlled trial[18] and inter-individual differences.[38] Although no research has extensively explored the effect of exercise on des-acylated ghrelin, an alternative view is that des-acylated ghrelin may have specific physiological roles. Indeed, it has been suggested that des-acylated ghrelin may have specific binding sites and act as an antagonist to acylated ghrelin,[4] although a previous infusion study reported no changes in acylated ghrelin after des-acylated ghrelin administration to humans.[35] More mechanistic studies are needed to further understand how the two forms of ghrelin interact with exercise to provide valuable insights for guiding future therapeutic approaches and developing personalized exercise prescriptions to optimize ghrelin concentrations in specific clinical populations.[14]

Despite the reduced acylated ghrelin, a circulating orexigenic appetite hormone, in the high-intensity exercise trial, both intensities of exercise did not alter total PYY, a circulating anorexigenic appetite hormone, concentrations although there was a tendency to elevate throughout the high-intensity exercise trial in the present study. This supports the previous findings in healthy young men with a similar study design,[39,40] but is not reported universally with evidence of elevated total PYY after exercise.[38] Although the findings are inconsistent, our measurement of total PYY may not be physiologically relevant to appetite regulation as $PYY_{3\text{-}36}$ is primarily responsible for appetite regulation.[41] Indeed, the previous review suggested that the exercise intensity may not influence total PYY secretion, but rather $PYY_{3\text{-}36}$.[42] Previous infusion and postprandial studies have demonstrated that there is a reciprocal pattern between insulin and acylated ghrelin concentrations in humans.[43,44] In the present study, decreased insulin and no changes in glucose concentrations were observed immediately after high-intensity exercise despite decreased acylated ghrelin concentrations, which is consistent with the findings of previous acute exercise studies.[15,36,37] In addition, no correlation was observed between the magnitude of change in insulin and acylated ghrelin concentrations following acute high-intensity exercise (data not exhibited). Therefore, our findings indicate that insulin and glucose are unlikely to contribute to the suppression of plasma acylated ghrelin during exercise. It is possible that the reduction in insulin concentration was a consequence of enhanced local and systemic fuel mobilization during exercise,[45] and this suppressive effect of insulin was often observed in higher-intensity exercise as a result of inhibition of insulin secretion rates.[46] Nonetheless, the temporal changes in insulin and ghrelin (both acylated ghrelin and des-acylated ghrelin) during exercise highlight important areas for further work, as an *in vitro* study reported that ghrelin-producing cells express insulin receptors.[47]

In the present study, the subjective ratings of appetite were transiently suppressed (*i.e.*, increased appetite score) following an acute bout of high-intensity exercise, but not after low-intensity exercise. These findings are in line with the previous findings of exercise-induced anorexia observed from land-based exercise performed at or greater than 60 % of peak oxygen uptake.[48] As the perceptions of individual appetite were evaluated in the present study, it is difficult to ascertain the mechanisms responsible for changes in subjective appetite. However, decreased acylated ghrelin during acute exercise, at least in part, has been proposed to explain the appetite-suppressive effect following exercise.[49] However, a large inter-individual variation in subjective appetite (*i.e.*, hunger) was observed in the pooled dataset of 17 studies.[48] Indeed, this was the case in the present study with appetite scores ranging from a marked suppression (+46.0 mm) to appetite stimulation (−26.5 mm) measured before and immediately after exercise in the high-intensity exercise trial. Given that appetite control is influenced by a complex interplay of physiological, psychological and environmental processes that merge in the brain to reflect human eating behavior,[50] future research needs to examine exercise-induced appetite suppression comprehensively using multi-dimensional approaches.[51] In addition, it has been reported that subjective appetite and energy intake show a dissociation response.[20,52] For instance, a temporary suppression of appetite was observed after 90 min of high-intensity running, yet exercise did not influence absolute short-term energy intake.[52] Additionally,

brisk walking for 60 min did not impact subjective appetite or absolute short-term energy intake.[20] Although we did not evaluate energy intake in the present study, the inclusion of energy intake evaluation greatly enhances the understanding of appetite-related human eating behavior in response to acute exercise. Further research is required to develop behavioral understanding of the exercise-induced suppression of appetite.

The mechanisms responsible for altering ghrelin, including redistribution of blood flow, sympathetic nervous system activity, gastrointestinal motility, and the production of lactate and interleukin-6 which are influenced by exercise intensity, have been addressed.[42] It is worth noting that carbohydrate oxidation was significantly higher in the high-intensity exercise trial than the low-intensity exercise trial in the present study. High-intensity exercise produces energy quickly, and therefore often use more of the glycolytic system which increase the production of the carbohydrate oxidation and lactic acid.[53–55] In the meantime, a recent review suggested that lactate inhibits ghrelin production from gastric cells, which is possibly one of the peripheral mechanisms of exercise-induced appetite suppression.[56] Indeed, a previous study has reported that vigorous intensity exercise (85 % of maximum oxygen uptake) suppressed acylated ghrelin concentrations and increased blood lactate concentrations, and postexercise changes in the AUC values of lactate concentrations correlated with acylated ghrelin concentrations.[57] These findings indicate that exercise-induced changes in metabolites and cytokine may be involved in the regulation of ghrelin. Also, a previous research study reported that plasma concentrations of interleukin-6 was increased after exercise in young men.[58] In the meantime, the high doses of interleukin-6 suppressing ghrelin mRNA and protein expression in pancreatic cell lines in a dose-dependent manner.[59,60] These findings suggest that exercise-induced changes in acylated ghrelin may be mediated by interleukin-6 in a dose-dependent manner suggesting it is possibly one of the peripheral mechanisms of the effects of exercise intensity on appetite suppression. Thus, to have a further understanding of potential mechanisms underline the high-intensity exercise-induced acylated ghrelin suppression, further research work also should be concentrated on the relationship between high-intensity exercise-induced appetite suppression and energy-related metabolic markers.

A key strength of the present study was the inclusion of a low-intensity exercise trial in the study design. The study design allowed a better insight into whether circulating concentrations of BChE truly influence acylated ghrelin responses to low-intensity exercise, as this exercise intensity does not often alter acylated ghrelin concentrations in humans.[13] Furthermore, the extension of the observation period allowed us to evaluate BChE responses over time with and without acute exercise, since previous studies have measured only BChE activity before and immediately after a 60-min high-intensity run[16] or have evaluated post-exercise BChE responses without the rest control trial.[18] It is important to note some limitations. The recruitment of a small sample of healthy young men was difficult to generalize from the present findings to larger, more diverse groups, including women, and individuals with overweight and obesity who typically blunted the suppression of postprandial appetite-related hormones.[61] It should be noted that there are well known methods to work around menstrual cycle in women by testing in the same phase of the early follicular cycle. Although this has been used in the previous studies examining post-exercise appetite-related hormones,[62,63] concerning potential body composition changes due to a long-testing period (i.e., at least three months to complete all three trials), which may affect our outcomes. Thus, due to this feasibility of the present study (*i.e.*, maintain lifestyle including diet and physical activity throughout the whole experimental period), it has not been feasible for us to conduct the study in women yet. Another limitation is that our blood parameters were not measured in duplicate or triplicate. Indeed, the intra-assay coefficients of variation for BChE, acylated ghrelin, des-acylated ghrelin, total PYY and insulin were higher than manufacture's stated values (BChE, 1.8–3.1 %; acylated ghrelin, 2.6 % and 2.8 % for 150 pg/mL and 200 pg/mL respectively; des-acylated ghrelin, 6.8 % and 4.2 % for 150 pg/mL and 200 pg/mL respectively; total PYY, 6.1–8.5 %; insulin, 2.8–4.0 % (depending on the concentration). Although we measured total PYY concentrations, other satiety hormones, including $PYY_{3\text{-}36}$ and glucagon-like peptide-1 (7–36 amide) which are more sensitive to appetite regulation[64,65] and energy intake, were not assessed in the present study. In addition, we did not measure the functional BChE activity in the microcirculation but we did measure BChE protein mass in the circulation. Although the previous study reported the rate limiting step in the ghrelin hydrolysis reaction is dependent on the concentration of BChE,[25] it is unlikely that the BChE protein mass we measured is directly involved in the hydrolysis of ghrelin in the circulation. Thus, future studies integrating these measures are required to provide a better overview of exercise and appetite regulation.

5. Conclusion

In conclusion, the present study demonstrated that a 60-min high-intensity run transiently suppressed circulating concentrations of both acylated ghrelin and des-acylated ghrelin in healthy young men. The present study also showed that circulating concentrations of total PYY, insulin and glucose were not altered (at least for the first 60-min of the experimental trial) in response to high- or low-intensity exercise whereas subjective appetite was suppressed only after high-intensity exercise. Despite the transient suppression of both forms of ghrelin, high-intensity running did not alter the circulating concentrations of BChE, an enzyme responsible for converting acylated ghrelin into des-acylated ghrelin. These findings indicate that the potential mechanisms underlying exercise-induced suppression of acylated ghrelin, which were typically observed following high-intensity exercise, may not be explained by BChE.

Author contributions

Yibin Li: lead data collection, formal analysis, investigation, writing – original draft, writing – review and editing, and visualization. **Yusei Tataka**, **Miki Sakazaki**, **Kayoko Kamemoto**, **Chihiro Nagayama**, **Yoshie Yoshikawa**, **Yoshiki Yamada**: assist data collection and data analysis. **Masashi Miyashita**: conceptualization, writing – review and editing, supervision and funding acquisition. All authors approved the final version of the manuscript.

Funding

This research was funded by the Faculty of Sport Sciences, Waseda University (Japan).

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgments

We thank all of the participants for their participation in this study. We are also sincerely grateful to the members of Exercise Metabolism Laboratory, Faculty of Sport Sciences, Waseda University who contributed to this research.

References

1. Kojima M, Hosoda H, Date Y, et al. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999;402(6762):656–660. https://doi.org/10.1038/45230.
2. Murphy KG, Bloom SR. Gut hormones and the regulation of energy homeostasis. *Nature*. 2006;444(7121):854–859. https://doi.org/10.1038/nature05484.

3. Hosoda H, Kojima M, Matsuo H, et al. Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. *Biochem Biophys Res Commun*. 2000; 279(3):909–913. https://doi.org/10.1006/bbrc.2000.4039.
4. Delhanty PJD, Neggers SJ, van der Lely AJ. Mechanisms in endocrinology: ghrelin: the differences between acyl-and des-acyl ghrelin. *Eur Endocrinol*. 2012;167(5): 601–608. https://doi.org/10.1530/EJE-12-0456.
5. Yoshimoto A, Mori K, Sugawara A, et al. Plasma ghrelin and desacyl ghrelin concentrations in renal failure. *J Am Soc Nephrol*. 2002;13(11):2748–2752. https://doi.org/10.1097/01.asn.0000032420.12455.74.
6. Nakai Y, Hosoda H, Nin K, et al. 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*. 2004;150(6):913–914. https://doi.org/10.1530/eje.0.1500913.
7. Akamizu T, Takaya K, Irako T, et al. Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. *Eur J Endocrinol*. 2004;150(4):447–455. https://doi.org/10.1530/eje.0.1500447.
8. Hosoda H, Doi K, Nagaya N, et al. Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. *Clin Chem*. 2004;50(6):1077–1080. https://doi.org/10.1373/clinchem.2003.025841.
9. Wren AM, Seal LJ, Cohen MA, et al. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab*. 2001;86(12):5992. https://doi.org/10.1210/jcem.86.12.8111.
10. Kojima M, Kangawa K. Ghrelin: structure and function. *Physiol Rev*. 2005;85(2): 495–522. https://doi.org/10.1152/physrev.00012.2004.
11. Cummings DE, Purnell JQ, Frayo RS, et al. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*. 2001;50(8):1714–1719. https://doi:10.2337/diabetes.50.8.1714.
12. King JA, Wasse LK, Stensel DJ, et al. Exercise and ghrelin. A narrative overview of research. *Appetite*. 2013;68:83–91. https://doi.org/10.1016/j.appet.2013.04.018.
13. Dorling J, Broom DR, Burns SF, et al. Acute and chronic effects of exercise on appetite, energy intake, and appetite-related hormones: the modulating effect of adiposity, sex, and habitual physical activity. *Nutrients*. 2018;10(9):1140. https://doi.org/10.3390/nu10091140.
14. Anderson KC, Zieff G, Paterson C, et al. The effect of acute exercise on pre-prandial ghrelin levels in healthy adults: a systematic review and meta-analysis. *Peptides*. 2021;145, 170625. https://doi.org/10.1016/j.peptides.2021.170625.
15. Douglas JA, King JA, Clayton DJ, et al. 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*. 2017;41(12):1737–1744. https://doi.org/10.1038/ijo.2017.181.
16. Dorling JL, Clayton DJ, Jones J, et al. 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*. 2019;110(5):1055–1066. https://doi.org/10.1093/ajcn/nqz188.
17. Tiryaki-Sonmez G, Ozen S, Bugdayci G, et al. Effect of exercise on appetite-regulating hormones in overweight women. *Biol Sport*. 2013;30(2):75–80. https://doi.org/10.5604/20831862.1044220.
18. Li G, Tataka Y, Kamemoto K, et al. Does butyrylcholinesterase mediate exercise-induced and meal-induced suppression in acylated ghrelin? *Endocr J*. 2022;69(12): 1395–1405. https://doi.org/10.1507/endocrj.EJ22-0150.
19. Darvesh S, Grantham DL, Hopkins DA. Distribution of butyrylcholinesterase in the human amygdala and hippocampal formation. *J Comp Neurol*. 1998;393(3): 374–390.
20. King JA, Wasse LK, Broom DR, et al. Influence of brisk walking on appetite, energy intake, and plasma acylated ghrelin. *Med Sci Sports Exerc*. 2010;42(3):485–492. https://doi:10.1249/MSS.0b013e3181ba10c4.
21. Unick JL, Otto AD, Goodpaster BH, et al. Acute effect of walking on energy intake in overweight/obese women. *Appetite*. 2010;55(3):413–419. https://doi:10.1016/j.appet.2010.07.012.
22. Batterham RL, Ffytche DH, Rosenthal JM, et al. PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans. *Nature*. 2007;450 (7166):106–109. https://doi:10.1038/nature06212.
23. Poher AL, Tschöp MH, Müller TD. Ghrelin regulation of glucose metabolism. *Peptides*. 2018;100:236–242. https://doi:10.1016/j.peptides.2017.12.015.
24. Hosoda H, Kojima M, Mizushima T, et al. Structural divergence of human ghrelin. Identification of multiple ghrelin-derived molecules produced by post-translational processing. *J Biol Chem*. 2003;278(1):64–70. https://doi:10.1074/jbc.M205366200.
25. Schopfer LM, Lockridge O, Brimijoin S. Pure human butyrylcholinesterase hydrolyzes octanoyl ghrelin to desacyl ghrelin. *Gen Comp Endocrinol*. 2015;224: 61–68. https://doi.org/10.1016/j.ygcen.2015.05.017.
26. Taylor HL, Buskirk E, Henschel A. Maximal oxygen intake as an objective measure of cardio-respiratory performance. *J Appl Physiol*. 1955;8(1):73–80. https://doi.org/10.1152/jappl.1955.8.1.73.
27. Borg GA. Perceived exertion: a note on "history" and methods. *Med Sci Sports*. 1973; 5(2):90–93.
28. Kumahara H, Schutz Y, Ayabe M, et al. The use of uniaxial accelerometry for the assessment of physical-activity-related energy expenditure: a validation study against whole-body indirect calorimetry. *Br J Nutr*. 2004;91(2):235–243. https://doi.org/10.1079/BJN20031033.
29. Flint A, Raben A, Blundell JE, et al. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensation in single test meal studies. *Int J Obes Relat Metab Disord*. 2000;24(1):38–48. https://doi.org/10.1038/sj.ijo.0801083.
30. Gibbons C, Hopkins M, Beaulieu K, et al. Issues in measuring and interpreting human appetite (satiety/satiation) and its contribution to obesity. *Curr Obes Rep*. 2019;8(2):77–87. https://doi.org/10.1007/s13679-019-00340-6.
31. Faul F, Erdfelder E, Lang AG, et al. G* Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;(2):175–191. https://doi.org/10.3758/bf03193146.
32. Field A, ed. *Discovering Statistics Using SPSS (3rd)*. London, UK: Sage Publications Ltd.; 2009.
33. De Vriese C, Hacquebard M, Gregoire F, et al. Ghrelin interacts with human plasma lipoproteins. *Endocrinology*. 2007;148(5):2355–2362. https://doi.org/10.1210/en.2006-1281.
34. Satou M, Nishi Y, Yoh J, et al. 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*. 2010;151(10):4765–4775. https://doi.org/10.1210/en.2010-0412.
35. Tong J, Dave N, Mugundu GM, et al. The pharmacokinetics of acyl, des-acyl, and total ghrelin in healthy human subjects. *Eur J Endocrinol*. 2013;168(6):821–828. https://doi.org/10.1530/EJE-13-0072.
36. Shiiya T, Ueno H, Toshinai K, et al. Significant lowering of plasma ghrelin but not des-acyl ghrelin in response to acute exercise in men. *Endocr J*. 2011;58(5):335–342. https://doi.org/10.1507/endocrj.k11e-021.
37. Broom D, Miyashita M, Wasse LK, et al. Acute effect of exercise intensity and duration on acylated ghrelin and hunger in men. *J Endocrinol*. 2017;232(3): 411–422. https://doi.org/10.1530/JOE-16-0561.
38. Goltz F, Thackray A, King J, et al. Interindividual responses of appetite to acute exercise: a replicated crossover study. *Med Sci Sports Exerc*. 2018;50(4):758–768. https://doi.org/10.1249/MSS.0000000000001504.
39. Deighton K, Barry R, Connon CE, et al. Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur J Appl Physiol*. 2013;113(5):1147–1156. https://doi:10.1007/s00421-012-2535-1.
40. McIver VJ, Mattin L, Evans GH, et al. The effect of brisk walking in the fasted versus fed state on metabolic responses, gastrointestinal function, and appetite in healthy men. *Int J Obes*. 2019;43(9):1691–1700. https://doi:10.1038/s41366-018-0215-x.
41. Batterham RL, Cowley MA, Small CJ, et al. Gut hormone PYY3-36 physiologically inhibits food intake. *Nature*. 2002;418(6898):650–654. https://doi.org/10.1038/nature00887.
42. Hazell TJ, Islam H, Townsend LK, et al. Effects of exercise intensity on plasma concentrations of appetite-regulating hormones: potential mechanisms. *Appetite*. 2016;98:80–88. https://doi.org/10.1016/j.appet.2015.12.016.
43. Broglio F, Arvat E, Benso A, et al. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. *J Clin Endocrinol Metab*. 2001;86(10):5083–5086. https://doi.org/10.1210/jcem.86.10.8098.
44. Writing team for the diabetes control and complications trial/epidemiology of diabetes interventions and complications research group. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the epidemiology of diabetes interventions and complications (EDIC) study. *JAMA*. 2003;290(16):2159–2167. https://doi.org/10.1001/jama.290.16.2159.
45. Richter EA, Sylow L, Hargreaves M. Interactions between insulin and exercise. *Biochem J*. 2021;478(21):3827–3846. https://doi.org/10.1042/BCJ20210185.
46. Malin SK, Rynders CA, Weltman JY, et al. Exercise intensity modulates glucose-stimulated insulin secretion when adjusted for adipose, liver and skeletal muscle insulin resistance. *PLoS One*. 2016;11(4), e0154063. https://doi.org/10.1371/journal.pone.0154063.
47. Iwakura H, Li Y, Ariyasu H, et al. Establishment of a novel ghrelin-producing cell line. *Endocrinology*. 2010;151(6):2940–2945. https://doi.org/10.1210/en.2010-0090.
48. King JA, Deighton K, Broom DR, et al. Individual variation in hunger, energy intake and ghrelin responses to acute exercise. *Med Sci Sports Exerc*. 2017;49(6): 1219–1228. https://doi.org/10.1249/MSS.0000000000001220.
49. Broom DR, Stensel DJ, Bishop NC, et al. Exercise-induced suppression of acylated ghrelin in humans. *J Appl Physiol*. 2007;102(6):2165–2171. https://doi.org/10.1152/japplphysiol.00759.2006.
50. Casanova N, Finlayson G, Blundell JE, et al. Biopsychology of human appetite—understanding the excitatory and inhibitory mechanisms of homeostatic control. *Curr Opin Physiol*. 2019;12:33–38. https://doi.org/10.1016/j.cophys.2019.06.007.
51. Thackray AE, Stensel DJ. The impact of acute exercise on appetite control: current insights and future perspectives. *Appetite*. 2023;186, 106557. https://doi.org/10.1016/j.appet.2023.106557.
52. King JA, Miyashita M, Wasse LK, et al. Influence of prolonged treadmill running on appetite, energy intake and circulating concentrations of acylated ghrelin. *Appetite*. 2010;54(3):492–498. https://doi:10.1016/j.appet.2010.02.002.
53. Hargreaves M, Richter EA. Regulation of skeletal muscle glycogenolysis during exercise. *Can J Sport Sci*. 1988;13(4):197–203.
54. Iaia FM, Perez-Gomez J, Thomassen M, et al. Relationship between performance at different exercise intensities and skeletal muscle characteristics. *J Appl Physiol*. 2011;110(6):1555–1563. https://doi.org/10.1152/japplphysiol.00420.2010, 1985.
55. Brouwer E. On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals, from gaseous exchange (Oxygen intake and carbonic acid output) and urine-N. *Acta Physiol Pharmacol Neerl*. 1957;6:795–802.
56. McCarthy SF, Islam H, Hazell TJ. The emerging role of lactate as a mediator of exercise-induced appetite suppression. *Am J Physiol Endocrinol Metab*. 2020;319(4): E814–E819. https://doi.org/10.1152/ajpendo.00256.2020.

57. Islam H, Townsend LK, McKie GL, et al. Potential involvement of lactate and interleukin-6 in the appetite-regulatory hormonal response to an acute exercise bout. *J Appl Physiol*. 2017;123(3):614–623. https://doi.org/10.1152/japplphysiol.00218.2017.
58. Bilski J, Mazur-Bialy AI, Surmiak M, et al. Effect of acute sprint exercise on myokines and food intake hormones in young healthy men. *Int J Mol Sci*. 2020;21(22):8848. https://doi.org/10.3390/ijms21228848.
59. Chew C, Choo Q, Lim W, et al. 27: IL-6 possibly modulates ghrelin expression through MEK1/p90RSK signaling cascade in pancreatic cell lines. *Cytokine*. 2014;70(1):34. https://doi.org/10.1016/j.cyto.2014.07.034.
60. Lao K, Lim W, Ng D, et al. Molecular regulation of ghrelin expression by pro-inflammatory cytokines TNF-α and IL-6 in rat pancreatic AR42J cell line. *J Biol Life Sci*. 2013;4(1):32–40. https://doi.org/10.5296/jbls.v4i1.2306.
61. Hernandez D, Mehta N, Geliebter A. Meal-related acyl and des-acyl ghrelin and other appetite-related hormones in people with obesity and binge eating. *Obesity*. 2019;27(4):629–635. https://doi.org/10.1002/oby.22431.
62. Alajmi N, Deighton K, King JA, et al. Appetite and energy intake responses to acute energy deficits in females versus males. *Med Sci Sports Exerc*. 2016;48(3):412–420. https://doi.org/10.1249/MSS.0000000000000793.
63. Hallworth JR, Copeland JL, Doan J, et al. The effect of exercise intensity on total PYY and GLP-1 in healthy females: a pilot study. *J Nutr Metab*. 2017;2017, 4823102. https://doi.org/10.1155/2017/4823102.
64. Batterham RL, Cowley MA, Small CJ, et al. Gut hormone PYY3-36 physiologically inhibits food intake. *Nature*. 2002;418(6898):650–654. https://doi.org/10.1038/nature00887.
65. Turton MD, O'shea D, Gunn I, et al. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature*. 1996;379(6560):69–72. https://doi.org/10.1038/379069a0.