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High-intensity running and energy restriction reduces postprandial lipemia in girls

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Running title: Exercise, energy restriction and lipemia

Abstract

Purpose: This study examined the potency of combining acute high-intensity exercise and energy-intake restriction on postprandial triacylglycerol concentrations ([TAG]) in healthy girls. **Methods:** Sixteen 11- to 13-year-old girls (mean(SD): body mass 45.1(7.6) kg; peak oxygen uptake ($\dot{V}O_2$) 43(6) mL·kg⁻¹·min⁻¹) completed three, 2-day conditions in a counterbalanced, crossover design separated by 14 days. On day 1, participants completed 10×1 min interval runs (HIIR), 5×1 min interval runs combined with 0.82(0.19) MJ energy-intake restriction (HIIR-ER) or rested (CON). Exercise was completed at 100% maximal aerobic speed, determined from an incremental peak $\dot{V}O_2$ test, with 1 min recovery between intervals. On day 2, capillary blood samples were taken in the fasted state and at pre-determined intervals throughout the 6.5 h postprandial period. A standardised breakfast and lunch were consumed immediately and 4 h, respectively, after the fasting sample. **Results:** Based on ratios of the geometric means (95% confidence intervals (CI) for ratios), fasting [TAG] was 16% and 8% lower than CON in HIIR (-24 to -7%, effect size (ES) = 0.49, P = 0.002) and HIIR-ER (-17 to 1%, ES = 0.24, P = 0.09) respectively; HIIR was 8% lower than HIIR-ER (-17 to 1%, ES = 0.25, P = 0.08). The total area under the [TAG] versus time curve was 10% and 9% lower than CON in HIIR (-16 to -3%, ES = 0.30, P = 0.01) and HIIR-ER (-15 to -2%, ES = 0.28, P = 0.01) respectively; HIIR-ER and HIIR were similar (-1%; -8 to 6%, P = 0.80). **Conclusion:** Manipulations of HIIR and ER reduce postprandial [TAG] in girls. The magnitude of effect was marginally, though not meaningfully, greater following HIIR than HIIR-ER.

Keywords: cardiovascular disease risk, energy deficit, exercise intensity, triacylglycerol, young people

Introduction

Elevated postprandial plasma triacylglycerol concentrations ([TAG]) are implicated in atherogenic development and progression (41), and are established as an independent predictor of cardiovascular disease incidence in women (2). Although the clinical manifestations of atherosclerotic disease are not apparent until adulthood typically, the process of atherosclerosis originates in childhood and progresses over the lifespan (26). The majority of waking hours are postprandial resulting in extended periods of elevated postprandial [TAG]. Therefore, interventions that reduce postprandial [TAG] and delay precursors of atherosclerotic disease should be initiated early in life (26).

Adult studies have shown consistently that acute aerobic exercise (30 min to 3 h in duration) performed the day before a standardised meal reduces postprandial [TAG] (25), and increases resting whole-body fat oxidation (9, 38). Similar reductions in postprandial [TAG] have been reported following acute moderate- to vigorous-intensity exercise in young people (37). Several recent studies in adults highlight the potential efficacy of acute, intermittent high-intensity exercise to elicit reductions in postprandial [TAG] (e.g., 13, 38), in addition to improvements in insulin sensitivity and resting whole-body fat oxidation (38, 40). Similarly, reductions in postprandial [TAG] have been demonstrated in healthy boys following acute high-intensity interval running (HIIR) (33) and repeated maximal cycle sprints (28). Approximately 80% of young people in England (19) and globally (17) fail to meet the current international guidelines of 60 min of daily moderate- to vigorous-intensity exercise for health promotion. Nevertheless, young people typically spend more of their active time engaged in high-intensity activities compared with adults (20). Considering lack of time and enjoyment are frequently highlighted as barriers to exercise participation in adolescent girls (5), the effect of different strategies that reduce the total exercise commitment and promote enjoyment on metabolic health markers should be investigated in girls. Therefore, the first

aim of the present study was to examine the effect of a single session of HIIR on postprandial plasma [TAG] and resting whole-body fat oxidation in healthy girls.

A small number of studies have compared manipulations in exercise and dietary intake on postprandial [TAG] to determine whether the exercise-evoked reduction in postprandial [TAG] is a consequence of the associated energy deficit or skeletal muscle contraction *per se*. Acute moderate-intensity exercise appears more efficacious in reducing postprandial [TAG] than isoenergetic mild energy-intake restriction in healthy 11 to 13 year old girls (34) and pre- and post-menopausal women (15, 24). Although the combination of moderate-intensity exercise and energy-intake restriction did not exceed the reduction seen for exercise alone in healthy pre-menopausal women, it did at least match it (24). To the author's knowledge, however, no study has examined whether combining exercise with energy-intake restriction to augment the total energy deficit reduces postprandial [TAG] in young people. Therefore, the second aim of the present study was to compare the effect of a smaller dose of HIIR combined with energy-intake restriction (HIIR-ER) with the full HIIR protocol (undertaken previously in boys; 33) and a rest control condition on postprandial plasma [TAG] and whole-body fat oxidation in healthy, recreationally active girls.

Methods

Participants

A total of 19 recreationally active girls recruited from local schools volunteered to participate in this study, with results presented for 16 girls (age 12.1(0.7) years; body mass 45.1(7.6) kg; body mass index 18.7(2.1) kg·m⁻²; peak oxygen uptake ($\dot{V}O_2$) 43(6) mL·kg⁻¹·min⁻¹) as one girl did not adhere to the required dietary replication and two girls dropped out for personal reasons unrelated to the study. The study procedures were approved by the University Ethical Advisory Committee. Written assent from participants and written informed consent from a

parent or guardian was obtained before the study commenced. All participants indicated that they were in good general health, had no history of medical conditions that may compromise participation in the study and were not taking any medications or dietary supplements known to influence lipid or carbohydrate metabolism.

Anthropometry and physical maturation

Stature was measured to the nearest 0.01 m using a fixed stadiometer (Holtain Ltd, Crosswell, UK), body mass was quantified to the nearest 0.1 kg using a digital scale (Seca 770, Seca Ltd, Hamburg, Germany) and body mass index was calculated as body mass (kg) divided by stature (m) squared. Skinfold thickness was measured at the triceps and subscapular to the nearest 0.2 mm using Harpenden callipers (Baty International, West Sussex, UK). All measurements were taken on the right-hand side of the body by the same investigator, and the median of three measurements at each site was used to estimate percent body fat (30).

Participants were asked to provide a self-assessment of their level of physical maturity using drawings depicting the five stages of breast and pubic hair development, ranging from 1 indicating pre-pubescence to 5 indicating full sexual maturity (32). Participants identified the stage most closely resembling their current level of sexual development. The median (interquartile range) stage of breast development was 3(2) (stage 1: $n = 1$; stage 2: $n = 5$; stage 3: $n = 5$; stage 4: $n = 5$) and pubic hair development was 2(3) (stage 1: $n = 4$; stage 2: $n = 6$; stage 3: $n = 1$; stage 4: $n = 3$; stage 5: $n = 2$).

Preliminary exercise measurements

Participants were familiarised with walking and running on the treadmill (h/p/cosmos mercury med, Nussdorf-Traunstein, Germany) prior to completing an incremental speed-

based treadmill protocol to determine peak $\dot{V}O_2$ and maximal aerobic speed (MAS). The protocol started at 5.0 km·h⁻¹ with 0.5 km·h⁻¹ increments every 30 s until volitional exhaustion, with the treadmill gradient set at 1%. Heart rate was recorded using short-range telemetry (Polar PE 4000, Kempele, Finland), ratings of perceived exertion were recorded in the last 10 s of each 30 s stage, and expired air samples were monitored continuously using an online breath-by-breath gas analysis system (Metalyzer 3B, Cortex, Leipzig, Germany). The analyser was calibrated according to the manufacturer's instructions before the exercise protocol began. Attainment of maximal effort was confirmed based on the presence of a plateau in $\dot{V}O_2$ ($\leq 3\%$ with an increase in treadmill speed). In the absence of a plateau in $\dot{V}O_2$ (10 (63%) participants), an exhaustive effort was confirmed based on the following secondary criteria: a peak heart rate $\geq 95\%$ of age-predicted maximum (220-chronological age); a respiratory exchange ratio ≥ 1.00 ; and clear subjective signs of fatigue. An average of the breath-by-breath $\dot{V}O_2$ data was taken every 10 s, and peak $\dot{V}O_2$ was defined as the highest 30 s rolling average; the treadmill speed corresponding to peak $\dot{V}O_2$ was recorded as MAS.

Experimental design

Using a within measures, incomplete counterbalanced, crossover design, participants completed three, 2-day experimental conditions separated by a standardised period of 14 days: high-intensity interval running (HIIR), high-intensity interval running and energy-intake restriction (HIIR-ER) and rest control (CON). The study design is presented schematically in Figure 1.

Day 1: Intervention day

Participants reported to the laboratory at 15:30 and completed all measures by 17:30. Body mass was quantified upon arrival to standardise the meals provided on day 2 (described below). During HIIR and HIIR-ER, the girls completed a 5 min warm-up at 60% MAS

followed immediately by the acute high-intensity running intervals. The high-intensity running comprised either 10 (HIIR) or 5 (HIIR-ER) $\times$ 1 min treadmill runs at 100% MAS, with 1 min active recovery between each interval. Participants dismounted the treadmill during the active recovery periods and were encouraged to pace around the lab to avoid venous pooling and feeling light headed. Heart rate was monitored continuously and the participants provided a rating of perceived exertion (RPE) in the last 10 s of each running interval as described previously, and affective valence was quantified at the end of each running interval using a validated feeling scale (FS) (18). Within 5 min of exercise completion, participants completed the modified Physical Activity Enjoyment Scale (PACES; 27), and total enjoyment was calculated by summing the 16 responses after eight items were reverse scored. During CON, participants rested in the laboratory for the duration of the visit. Participants maintained and replicated their habitual dietary intake throughout the day in all three conditions, but with a controlled reduction in habitual food energy intake at the evening meal in HIIR-ER by 0.82(0.19) MJ (195(46) kcal).

Standardisation of dietary intake and physical activity

Participants weighed, recorded and replicated their habitual dietary intake during the 48 h period (pre-intervention and intervention day) before day 2 of the first condition. The girls replicated this diet before the subsequent conditions, but with a controlled reduction in energy intake on the intervention day of HIIR-ER. Participants completing HIIR-ER as the first condition were asked to record their usual dietary intake for two consecutive days at least one week in advance so that the prescribed energy-intake restriction could be calculated and standardised. Two-day diet records were analysed using dietary analysis software (CompEat Pro Version 5.8.0, Nutrition Systems, Banbury, UK).

Participants consumed a cereal snack bar at 19:45 on the intervention day to standardise the overnight fasting period which provided 1.1 g fat, 15.7 g carbohydrate, 1.0 g protein and 337 kJ energy. Participants were allowed to drink plain water, but no other drinks or food, before arriving at the laboratory on day 2.

An ActiGraph GT1M accelerometer (ActiGraph, Pensacola, Florida, USA) was worn on the pre-intervention and intervention day of each condition, and participants were asked to minimise and replicate their physical activity during this period. The accelerometer was worn on the right hip during waking hours (removed for water-based activities). During data processing, 5 s epoch data were re-integrated to 60 s epochs, 60 min of consecutive zeros, allowing for 2 min of non-zero interruptions was used to remove non-wear, and a minimum of 9 h of valid wear time was required for a valid day. Physical activity was expressed as average counts per minute (CPM), and intensity cut-points for 12 year olds were applied (39): sedentary ($< 100 \text{ counts} \cdot \text{min}^{-1}$), light ($100 - 1262 \text{ counts} \cdot \text{min}^{-1}$), moderate ($1262 - 4136 \text{ counts} \cdot \text{min}^{-1}$) and vigorous ($> 4136 \text{ counts} \cdot \text{min}^{-1}$) activities.

Day 2: Postprandial day

Following a 12 h overnight fast, participants arrived at the laboratory at ~07:45 and provided a fasting capillary blood sample after 10 min seated rest. A standardised breakfast meal was consumed within 15 minutes marking the start of the postprandial period (08:00) (Figure 1). Breakfast consisted of croissants, chocolate spread, whole milk, double cream and milkshake powder. The meal quantity was prescribed relative to body mass and provided 1.5 g fat (61.3% of meal total energy), 1.8 g carbohydrate (32.3%), 0.4 g protein (6.4%) and 94 kJ energy per kilogram body mass. Subsequent capillary blood samples were taken at 0.5, 1, 3, 4.5, 5 and 6.5 h following the start of the breakfast, and participants consumed a standardised lunch, within 20 min, at 4 h (Figure 1). Lunch consisted of white bread, butter, mild cheddar

cheese, potato crisps, whole milk and milkshake powder, and provided 1.3 g fat (53.5%), 1.9 g carbohydrate (35.5%), 0.6 g protein (11.0%) and 92 kJ energy per kilogram body mass. To ensure consistency across participants and experimental conditions, participants consumed either chocolate or strawberry flavour milkshake powder on all visits. Participants rested throughout the day and were able to read, watch DVD films and play non-active computer games. Participants consumed water *ad libitum* in the postprandial period of the first condition; the ingested volume was replicated in the subsequent conditions.

Resting expired air samples were collected in the semi-supine position for 5 min after each blood sample into 100 L Douglas bags (Cranlea and Company, Birmingham, UK). Oxygen uptake and carbon dioxide production were analysed using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Servomex 1400, East Sussex, UK), and the volume of expired air was quantified using a dry gas meter (Harvard Apparatus Ltd, Kent, UK). For each sample, $\dot{V}O_2$, expired carbon dioxide and respiratory exchange ratio were determined, and energy expenditure (EE) and the oxidation of fat and carbohydrate were estimated via indirect calorimetry (12) assuming that the urinary nitrogen excretion rate was negligible. The postprandial expired air data for one girl were spurious so results are presented for 15 girls.

Analytical methods

After the hand was pre-warmed for 5 min in water heated to 40°C, the fingertip was pierced (Unistik 3 Extra, Owen Mumford, Oxford, UK) and 600 μ L whole capillary blood was collected into potassium EDTA coated Microvette CB 300 tubes (Sarstedt Ltd, Leicester, UK). The whole blood samples were centrifuged immediately at 12,800 g for 15 min (Eppendorf 5415c, Hamburg, Germany) and the resulting plasma was stored at -80°C for up to two months before subsequent analyses. Plasma [TAG], glucose concentration ([glucose])

(HORIBA ABX Diagnostics, Montpellier, France) and non-esterified fatty acid concentrations ([NEFA]) (Randox Laboratories Ltd, County Antrim, UK) were analysed by enzymatic, colorimetric methods using a benchtop analyser (Pentra 400, HORIBA ABX Diagnostics, Montpellier, France). The within-batch coefficient of variation for [TAG], [NEFA] and [glucose] were 1.6, 1.5 and 0.8% respectively. Haemoglobin concentration and haematocrit were also quantified in duplicate in the fasting and final postprandial samples to estimate the acute change in plasma volume (10). Haemoglobin concentration was assessed using the cyanmethemoglobin method; 20 μ L whole blood was added to 5 mL Drabkin's solution and the absorbance was quantified photometrically at a wavelength of 546 nm (Cecil CE1011, Cecil instruments, Cambridge, UK). Haematocrit was quantified using a microhaematocrit centrifuge and reader (Haematospin 1300 Microcentrifuge, Hawksley and Sons Ltd, Sussex, UK).

Statistical analyses

Data were analysed using the IBM SPSS Statistics Software for Windows version 21 (IBM Corporation, New York, USA). The trapezium rule was used to calculate the total area under the variable versus time curve for TAG (TAUC-TAG), NEFA (TAUC-NEFA), glucose (TAUC-glucose) and postprandial whole-body EE and substrate oxidation. The TAUC values for substrate oxidation were divided by the total duration of the postprandial period (6.5 h). The incremental area under the plasma concentration versus time curve for TAG (iAUC-TAG), NEFA (iAUC-NEFA) and glucose (iAUC-glucose) was calculated using the same method after adjusting for fasting concentrations. The iAUC-NEFA is negative due to the decrease in postprandial [NEFA] from the fasting concentration.

Normality of the data was checked using Shapiro Wilk tests. Normally distributed data are presented as mean (SD). Data for free-living physical activity and sedentary time, and

concentrations of plasma TAG, NEFA and glucose were not normally distributed and were natural log transformed prior to analysis. These data are presented as geometric mean (95% confidence intervals (CI)) and analysis is based on the ratios of geometric means and 95% CI for ratios. Homogeneity of variances was confirmed by Mauchly's test of sphericity, and a Greenhouse Geisser correction was applied to the degrees of freedom if the sphericity assumption was violated.

Linear mixed models repeated for condition and interval were used to examine differences between HIIR and HIIR-ER exercise responses for running intervals 1 to 5, and temporal changes between the first and final running interval were modelled with running interval as the sole factor. Dietary intake, free living physical activity and sedentary time, resting whole-body EE and substrate oxidation, fasting concentrations and TAUC and iAUC responses were analysed using separate linear mixed models with condition analysed as a repeated measures factor in the model. Differences in postprandial [TAG], [NEFA] and [glucose] were examined using linear mixed models repeated for condition and time. Temporal changes in TAUC-TAG between experimental conditions were examined over sub-sections of the postprandial period (0 to 1 h, 1 to 4.5 h and 4.5 to 6.5 h) using separate linear mixed models with condition as the sole factor. All linear mixed models included a random effect for each participant and were adjusted appropriately for the period effect (29).

Bivariate correlations identifying possible determinants of the exercise-induced changes in TAUC-TAG were quantified using Pearson's product moment correlations. Statistical significance was accepted as $P < 0.05$ and absolute standardised effect sizes (ES) are included to supplement important findings. In the absence of a clinical anchor, an ES of 0.2 was considered the minimum important difference in all outcome measures, 0.5 moderate and 0.8 large (6).

Results

Dietary intake

Energy and macronutrient intakes were similar on the pre-intervention day across the three conditions ($P \geq 0.14$). Average daily energy intake was 7.0(1.8) MJ, and dietary intake of protein, carbohydrate and fat was 59.8(19.0) g, 231(70) g and 56.5(14.7) g respectively. Energy and macronutrient intakes during the intervention day are displayed in Table 1. Energy intake on the intervention day of HIIR-ER was lower compared with CON (effect size (ES) = 0.60, $P < 0.001$) and HIIR (ES = 0.54, $P < 0.001$); HIIR was significantly, but not meaningfully, lower than CON (ES = 0.06, $P = 0.05$). Absolute protein, carbohydrate and fat intake were lower in HIIR-ER compared with CON and HIIR (ES = 0.35 to 0.63, $P < 0.001$), but were not different between HIIR and CON ($P \geq 0.09$). The only statistical difference in the contribution of protein, carbohydrate and fat to total energy intake was a marginally lower contribution of carbohydrate in HIIR than HIIR-ER (ES = 0.31, $P = 0.02$), and a marginally lower contribution of fat in HIIR-ER than CON (ES = 0.21, $P = 0.03$) and HIIR (ES = 0.23, $P = 0.02$).

Free-living physical activity and sedentary time

On the pre-intervention day, no differences were seen in physical activity levels or sedentary time across the conditions ($P \geq 0.27$). Physical activity levels and sedentary time on the intervention day are displayed in Table 2. No significant differences were seen across the conditions for daily wear time ($P = 0.30$), sedentary time ($P = 0.47$) or time spent in light-intensity activities ($P = 0.15$). Average counts per minute (CPM) were higher than CON by 128 counts·min⁻¹ in HIIR (ES = 1.49, $P < 0.001$) and by 54 counts·min⁻¹ in HIIR-ER (ES = 0.69, $P = 0.01$); HIIR was 74 counts·min⁻¹ higher than HIIR-ER (ES = 0.80, $P = 0.005$). Time spent in moderate-intensity activities was higher in HIIR by 18 min and 15 min compared

280 with CON (ES = 1.06, $P = 0.001$) and HIIR-ER (ES = 0.85, $P = 0.01$) respectively; CON and
281 HIIR-ER were similar (3 min; $P = 0.43$). Time spent in vigorous-intensity activities was
282 higher than CON by 12 min in HIIR (ES = 1.59, $P < 0.001$) and by 7 min in HIIR-ER (ES =
283 1.21, $P < 0.001$); HIIR and HIIR-ER were similar ($P = 0.10$). No differences were observed
284 in free-living physical activity or sedentary time when accounting for the time spent resting
285 or exercising in the laboratory on the intervention day ($P \geq 0.13$).

286 *Responses to high-intensity interval running (HIIR)*

287 The interval running session was performed at an average MAS of 11.5(1.1) km·h⁻¹ and was
288 well tolerated by participants in HIIR and HIIR-ER. Linear mixed models revealed no
289 differences between HIIR-ER and HIIR over running intervals 1 to 5 for heart rate, RPE or
290 FS response ($P \geq 0.11$). During HIIR, there was a progressive increase from interval 1 to
291 interval 10 for RPE (10(3) to 18(2) respectively; 95% CI 6 to 10, ES = 2.82, $P < 0.001$) and
292 end interval heart rate (185(12) to 202(7) beats·min⁻¹ respectively; 95% CI 12 to 21
293 beats·min⁻¹, ES = 1.36, $P < 0.001$), corresponding to 91(4) and 99(2)% of peak heart rate
294 respectively (95% CI 6 to 10%, ES = 1.99, $P < 0.001$). The FS response declined from
295 interval 1 to interval 10 (3(2) to -2(3) respectively; 95% CI -6 to -3, ES = 2.99, $P < 0.001$).
296 During HIIR-ER, there was a progressive increase from interval 1 to interval 5 for RPE
297 (10(3) to 15(3) respectively; 95% CI 3 to 6, ES = 1.50, $P < 0.001$) and end interval heart rate
298 (184(12) to 196(9) beats·min⁻¹ respectively; 95% CI 8 to 16 beats·min⁻¹, ES = 0.99, $P <$
299 0.001), corresponding to 90(4) and 96(2)% of peak heart rate respectively (95% CI 4 to 8%,
300 ES = 1.51, $P < 0.001$), and a decline in the FS response (3(2) to -1(2) respectively; 95% CI -5
301 to -2, ES = 1.57, $P < 0.001$). The summed PACES score was similar between HIIR-ER and
302 HIIR (57(9) vs. 56(10) respectively; 95% CI -6 to 3, $P = 0.55$).

Resting whole-body energy expenditure (EE) and substrate oxidation

Total resting EE over the 6.5 h postprandial period was similar across the conditions (HIIR 2.3(0.3) MJ, HIIR-ER 2.2(0.3) MJ, CON 2.3(0.3) MJ; $P = 0.42$). The relative contribution of fat oxidation to total resting EE tended to be greater than CON (44(17)%) in HIIR (53(17)%; 95% CI -1 to 20%, ES = 0.50, $P = 0.09$), but HIIR-ER (51(13)%) was not significantly different to CON (95% CI -4 to 18%, ES = 0.39, $P = 0.18$) or HIIR (95% CI -13 to 9%, $P = 0.69$). Reciprocally, the relative contribution of carbohydrate oxidation to total resting EE tended to be lower compared with CON (56(17)%) in HIIR (47(17)%; 95% CI -20 to 1%, ES = 0.50, $P = 0.09$), but HIIR-ER (49(13)%) was not significantly different to CON (95% CI -18 to 4%, ES = 0.39, $P = 0.18$) or HIIR (95% CI -9 to 13%, $P = 0.69$).

Plasma volume changes and fasting [TAG], [NEFA] and [glucose]

Average changes in plasma volume between the fasting and 6.5 h postprandial samples were not different across the three conditions (HIIR -0.3%, HIIR-ER 0.4%, CON -0.4%; $P = 0.77$). Therefore, the raw plasma [TAG], [NEFA] and [glucose] were used in all statistical analyses without adjustment. The fasting plasma [TAG], [NEFA] and [glucose] for each condition are displayed in Table 3. Linear mixed models revealed differences across the conditions in fasting plasma [TAG] ($P = 0.01$) and [NEFA] ($P = 0.04$), but not [glucose] ($P = 0.41$). Specifically, fasting plasma [TAG] was 16% and 8% lower than CON in HIIR (ES = 0.49, $P = 0.002$) and HIIR-ER (ES = 0.24, $P = 0.09$) respectively; HIIR was 8% lower than HIIR-ER (ES = 0.25, $P = 0.08$). Fasting plasma [NEFA] was 22% and 20% lower than CON in HIIR (ES = 0.65, $P = 0.02$) and HIIR-ER (ES = 0.58, $P = 0.04$) respectively; HIIR-ER and HIIR were not significantly different (-3%; $P = 0.78$).

Plasma [TAG], [NEFA] and [glucose] in the postprandial period

Plasma TAG responses over the postprandial period for HIIR, HIIR-ER and CON are shown in Figure 2. Linear mixed models revealed differences in postprandial plasma [TAG] over time across conditions (main effect condition $P < 0.001$; main effect time $P < 0.001$; condition by time interaction $P = 0.71$). Mean postprandial plasma [TAG] was 11% and 8% lower than CON in HIIR (-14 to -7%, ES = 0.27, $P < 0.001$) and HIIR-ER (-12 to -4%, ES = 0.21, $P < 0.001$) respectively; HIIR-ER and HIIR were similar (-3%; -7 to 2%, $P = 0.24$). The TAUC-TAG was 10% and 9% lower than CON in HIIR (ES = 0.30, $P = 0.01$) and HIIR-ER (ES = 0.28, $P = 0.01$) respectively; HIIR-ER and HIIR were similar (-1%; $P = 0.80$) (Table 3). Specifically, TAUC-TAG was lower after HIIR than CON between 0 to 1 h by 16% (-22 to -9%, ES = 0.53, $P < 0.001$) and 1 to 4.5 h by 11% (-17 to -4%, ES = 0.31, $P = 0.003$); HIIR-ER was lower than CON between 0 to 1 h by 11% (-17 to -4%, ES = 0.37, $P = 0.003$) and 1 to 4.5 h by 10% (-16 to -4%, ES = 0.30, $P = 0.005$). No differences in TAUC-TAG over sub-sections of the total postprandial period were seen between HIIR-ER and HIIR ($P \geq 0.16$). No differences were seen in iAUC-TAG across the conditions ($P = 0.53$) (Table 3).

Individual changes (delta) in TAUC-TAG for HIIR and HIIR-ER relative to CON are shown in Figure 3. The reductions in TAUC-TAG following HIIR and HIIR-ER were greater than changes in CON for ten (63%) and eleven (69%) girls respectively. Meaningful positive correlations were identified between the intervention-induced change in fasting plasma [TAG] and the change in TAUC-TAG relative to CON for HIIR ($r = 0.52$, $P = 0.04$) and HIIR-ER ($r = 0.59$, $P = 0.02$). The measured physical and physiological characteristics, dietary intake (Table 1), free-living physical activity and sedentary time (Table 2), exercise responses, resting whole-body EE and substrate oxidation and fasting [NEFA] or [glucose] (Table 3) did not account for any of the inter-individual variability in delta TAUC-TAG for

HIIR or HIIR-ER. The Pearson's product moment correlation for the individual changes in TAUC-TAG between HIIR and HIIR-ER was small ($r = 0.31$, $P = 0.25$).

No differences were observed in postprandial plasma [NEFA] across the conditions over time (main effect condition $P = 0.58$; main effect time $P < 0.001$; condition by time interaction $P = 0.57$). No meaningful differences were evident for TAUC-NEFA across the conditions ($P = 0.45$) (Table 3). The iAUC-NEFA was 56% and 55% higher than CON in HIIR (ES = 0.67, $P = 0.01$) and HIIR-ER (ES = 0.65, $P = 0.01$) respectively; HIIR-ER and HIIR were not different (1%; $P = 0.95$) (Table 3).

Linear mixed models revealed a trend for differences in postprandial plasma [glucose] over time (main effect condition $P = 0.06$; main effect time $P < 0.001$; condition by time interaction $P = 0.77$). The TAUC-glucose was 4% higher in HIIR compared with CON (ES = 0.58, $P = 0.01$), but HIIR-ER was not significantly different to HIIR (-1%; $P = 0.27$) or CON (2%; $P = 0.08$) (Table 3). The only significant difference in iAUC-glucose was a greater response in HIIR compared with HIIR-ER (39%; ES = 1.43, $P = 0.04$) (Table 3).

Discussion

The primary finding from the present study is that acute manipulations of low volume HIIR and ER completed the day before standardised meals reduced postprandial plasma [TAG] and increased whole-body fat oxidation in healthy, 11 to 13 year old girls. The magnitude of this effect was marginally, although not meaningfully, greater following HIIR than HIIR-ER. The exercise and diet interventions were well tolerated by all participants and, therefore, may have practical metabolic health benefits in similar cohorts.

The exercise and dietary restriction induced reductions in fasting plasma [TAG] support the majority of previous exercise postprandial studies in young people (e.g., 3, 28, 34, 35).

Although the lower fasting plasma [TAG] in HIIR and HIIR-ER are likely to influence the subsequent postprandial TAG response (7), substantial intra-individual variation is evident in childhood fasting [TAG] (36), and fasting [TAG] are less predictive of cardiovascular disease risk than postprandial [TAG] in women (2).

Several adult studies have reported reductions in postprandial [TAG] following a single session of intermittent, high-intensity exercise (e.g., 13, 38); however, this finding is not universal (1, 31). The contrasting results in these studies may reflect the variety of high-intensity exercise protocols adopted which, coupled with differences in participant characteristics, exercise timing, meal content and blood sampling, is likely to promote heterogeneity in the individual responses (1, 31). Nevertheless, we have demonstrated previously that a single session of HIIR promotes moderate reductions in postprandial plasma [TAG] in 11 to 12 year old boys (33). The current study extends this novel finding to 11 to 13 year old girls, and supports the commonly reported reductions in postprandial [TAG] following acute moderate- to vigorous-intensity exercise in boys and girls (37) and repeated maximal cycle sprints in boys (28).

An additional novel feature of the current study was the inclusion of a condition combining a lower volume of HIIR with a small reduction in energy intake (0.82(0.19) MJ, 195(46) kcal), which reduced postprandial plasma [TAG] to a similar extent as the full HIIR protocol (~10%; Table 3, Figure 2). Acute energy-intake restriction alone has been shown to elicit a small reduction in postprandial [TAG] previously in healthy girls (-10%, ES = 0.32; 34) and pre-menopausal women (-12%; 24). Although an exercise-induced energy deficit appears a more potent stimulus to reduce postprandial [TAG] than an isoenergetic diet-induced energy deficit in girls (34) and women (15, 24), the combination of light walking and energy-intake restriction did match the reduction seen for exercise alone in sedentary, pre-menopausal women (24). The similar reduction in postprandial plasma [TAG] following HIIR and HIIR-

397 ER is promising, and highlights the potential for metabolic health benefits following time-
398 efficient exercise combined with manageable dietary restriction in girls. A combination of
399 low volume, high-intensity exercise and mild dietary energy intake restriction may represent
400 a practical and attractive alternative in girls who struggle to accumulate sufficient physical
401 activity for health. It contributes to providing girls with a variety of lifestyle options that can
402 reduce postprandial plasma [TAG] and may have important long-term metabolic health
403 implications if employed regularly, but further work is required to support this in young
404 people. One limitation of the present study is that the girls recruited were healthy and
405 recreationally active. Therefore, further research is needed in overweight/obese girls who
406 may require appropriate exercise and dietary interventions for weight management and
407 improvements in the lipid profile.

408 The mechanisms underpinning the acute exercise- and diet-induced reductions in postprandial
409 plasma [TAG] in young people were not measured directly in the present study due to the
410 invasive nature of the methods required to do this accurately. In adults, two primary pathways
411 have been proposed involving the increased clearance of circulating TAG facilitated by
412 enhanced lipoprotein lipase (LPL) activity (16) and/or the secretion of fewer, TAG-rich
413 very low-density lipoproteins (VLDL) that have a higher affinity for LPL (23). A recent
414 stable isotope enrichment study in obese women suggested that the TAG-lowering effect of
415 acute exercise is mediated by a reduced abundance of endogenous fatty acids in plasma TAG
416 and not the enhanced clearance of dietary fat (9). The notion that endogenous, and not
417 exogenous, TAG metabolism exerts a stronger influence on the postprandial TAG response is
418 indirectly supported by the current study evidenced by the small differences in iAUC-TAG
419 between the conditions, and the meaningful relationship seen between the intervention-
420 induced changes in fasting plasma [TAG] and TAUC-TAG for HIIR ($r = 0.52$, $P = 0.04$) and
421 HIIR-ER ($r = 0.59$, $P = 0.02$).

422 Although whole-body fat oxidation was not statistically significant between the three
423 conditions, a thorough appraisal of the mean differences and absolute standardised ES
424 revealed that HIIR was 8% higher than CON (ES = 0.50) and HIIR-ER was 7% higher than
425 CON (ES = 0.39). Therefore, combinations of HIIR and ER appears to elevate resting whole-
426 body fat oxidation the following day, which represents a novel finding in young people and
427 supports exercise postprandial studies in adults employing acute high-intensity exercise
428 protocols (38, 40). The post-exercise shift in whole-body substrate utilisation towards fat
429 oxidation has been linked to a number of regulatory mechanisms promoting the resynthesis of
430 depleted skeletal muscle and/or hepatic glycogen stores (21). Circulating plasma fatty acids
431 and triacylglycerol-rich lipoproteins (TRL) are potential lipid sources utilised for oxidation,
432 which is in agreement with the lower postprandial plasma [TAG] after HIIR and HIIR-ER,
433 likely mediated by enhanced LPL activity (16, 21). However, the similar postprandial NEFA
434 response between the three experimental conditions suggests that plasma fatty acids did not
435 contribute to the greater whole-body fat oxidation in HIIR and HIIR-ER. Nevertheless, it is
436 possible that differences in plasma [NEFA] were evident before the commencement of the
437 postprandial period considering large increases in plasma free fatty acids have been shown in
438 the early post-exercise recovery period (21). The lack of association between whole-body fat
439 oxidation and indices of lipemia in the current study contrasts previous findings in adults
440 (38), suggesting that exercise- and diet-induced changes in postprandial plasma [TAG] and
441 whole-body fat oxidation may occur independently in girls. Nevertheless, elevated
442 postprandial [TAG] are associated independently with cardiovascular disease risk in women
443 (2), and low resting fat oxidation with an increased risk of weight gain (11) and Type 2
444 diabetes mellitus (4), highlighting the potential efficacy of acute high-intensity exercise and
445 dietary restriction to improve metabolic health outcomes early in life.

446 Although the clinical significance of our findings cannot be established, the majority (93%)
447 of the postprandial TAG samples were below the 2.3 mmol·L⁻¹ threshold considered a
448 desirable concentration in young people (22). Based on the physical activity data, nine (56%)
449 girls in the present study were achieving the current international physical activity
450 recommendations, although it should be noted that this is not a valid measure of habitual
451 physical activity as the girls were asked to minimise and replicate their physical activity
452 levels over a short measurement period. The majority of girls in England and globally
453 (approximately 80%) fall short of the current physical activity guidelines for health (17, 19),
454 and time and enjoyment are reported frequently as barriers to exercise participation in
455 adolescent girls (5). Therefore, the potential for HIIR and HIIR-ER, with a total exercise time
456 commitment of 24 and 14 min respectively (including warm-up and active recovery between
457 intervals), to reduce postprandial plasma [TAG] and increase resting whole-body fat
458 oxidation in girls is encouraging. The girls spent a greater amount of time engaged in
459 vigorous-intensity activities in HIIR and HIIR-ER, and a greater amount of time in moderate-
460 intensity activities in HIIR on the intervention day as a result of the prescribed exercise
461 intervention. There were no differences between conditions after accounting for the time
462 spent resting or exercising in the laboratory, suggesting that the implemented between
463 condition control of free-living physical activity and sedentary time was effective. The high-
464 intensity nature of the exercise adopted in the present study may better reflect the activity
465 patterns of young people who spend a greater proportion of time engaged in high-intensity
466 activities than adults (20). Furthermore, it has been demonstrated that children associate
467 moderate-intensity exercise interspersed with short high-intensity efforts with greater
468 perceived enjoyment than completing continuous moderate-intensity exercise alone (8). In
469 the present study, the similarly high PACES score between HIIR and HIIR-ER suggests

interval running performed at a high-intensity may be an attractive exercise model in girls independent of whether five or ten 1 min intervals are completed.

Previous high-intensity exercise postprandial studies highlight the substantial heterogeneity evident in postprandial TAG responses in young people (33) and adults (1, 31). We have shown previously in boys that exercising at a higher relative exercise intensity during HIIR is associated with a greater reduction in postprandial plasma [TAG] (33); however, this relationship was not apparent in the current study with girls, and the other measured variables in the study could not explain any of the heterogeneity present. A study with adults reported that exercise-induced changes in 3-OHB, a marker of hepatic fatty acid oxidation, was a strong predictor of the moderate-intensity exercise-induced reduction in fasting and postprandial [TAG] (14). Although this marker may explain some of the heterogeneity in the present study, we measured postprandial 3-OHB concentrations but the assay was unable to detect concentrations of 3-OHB in the majority of fasting and postprandial samples; therefore, further investigation is required in young people.

The higher postprandial plasma [glucose] after HIIR compared with CON supports a recent study in girls adopting a moderate-intensity exercise protocol (34); however, the majority of previous exercise postprandial studies in young people report no difference in postprandial [glucose] following acute exercise (e.g., 3, 28). The reason for this discrepant finding is not known; however, it is unlikely that the higher postprandial [glucose] in HIIR is implicated in the TAG-lowering effect of HIIR considering glucose has not been linked to the potential mechanistic pathways discussed above. Nevertheless, all participants in the present study demonstrated a healthy postprandial glucose profile independent of the experimental condition and the time of glucose measurement, suggesting the girls exhibited good glycemic control.

The present study is limited as the exercise protocol comprised running; therefore, the findings may not generalise to other exercise modalities such as cycling and game-based activities. Despite this limitation, the exercise protocol adopted in the present study is attainable for young people to achieve in a natural setting.

In conclusion, acute manipulations of low volume HIIR and ER completed the day before standardised meals reduced postprandial plasma [TAG] and increased resting whole-body fat oxidation in healthy, 11 to 13 year old girls. The magnitude of this effect was marginally, though not meaningfully, greater following HIIR than HIIR-ER. Low volume, HIIR performed alone or in combination with a mild reduction in habitual energy intake may represent time-efficient and enjoyable strategies to improve metabolic health in girls, but further work is required to examine this chronically and in overweight/obese girls for whom HIIR-ER may be an efficacious intervention.

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516 **Conflict of interest**

517 The authors declare that they have no conflict of interest. The results of the present study do
518 not constitute endorsement by ACSM.

519 **References**

- 520 1. Allen E, Gray P, Kollias-Pearson A, Oag E, Pratt K, Henderson J, Gray SR. The effect of
521 short-duration sprint interval exercise on plasma postprandial triacylglycerol levels in
522 young men. *J Sports Sci.* 2014;32(10):911–6.
- 523 2. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with
524 nonfasting triglycerides and risk of cardiovascular events in women. *JAMA.*
525 2007;298(3):309–16.
- 526 3. Barrett LA, Morris JG, Stensel DJ, Nevill ME. Exercise and postprandial plasma
527 triacylglycerol concentrations in healthy adolescent boys. *Med Sci Sports Exerc.*
528 2007;39(1):116–22.
- 529 4. Blaak EE, Wolffenbuttel BHR, Saris WHM, Pelsers MMAL, Wagenmakers AJM.
530 Weight reduction and the impaired plasma-derived free fatty acid oxidation in Type 2
531 diabetic subjects. *J Clin Endocrinol Metab.* 2001;86(4):1638–44.
- 532 5. Butt J, Weinberg RS, Breckon JD, Claytor RP. Adolescent physical activity participation
533 and motivational determinants across gender, age, and race. *J Phys Act Health.*
534 2011;8(8):1074–83.
- 535 6. Cohen J. *Statistical power analysis for the behavioural sciences.* 2nd ed. Hillsdale (NJ):
536 Lawrence Erlbaum Associates; 1988. pp. 22–5.
- 537 7. Couch SC, Isasi CR, Karmally W, Blaner WS, Starc TJ, Kaluski D, Deckelbaum RJ,
538 Ginsberg HN, Shea S, Berglund L. Predictors of postprandial triacylglycerol response in
539 children: the Columbia University Biomarkers Study. *Am J Clin Nutr.* 2000;72(5):1119–
540 27.
- 541 8. Crisp NA, Fournier PA, Licari MK, Braham R, Guelfi KJ. Adding sprints to continuous
542 exercise at the intensity that maximises fat oxidation: implications for acute energy
543 balance and enjoyment. *Metabolism.* 2012;61(9):1280–8.
- 544 9. Davitt PM, Arent SM, Tuazon MA, Golem DL, Henderson GC. Postprandial triglyceride
545 and free fatty acid metabolism in obese women after either endurance or resistance
546 exercise. *J Appl Physiol.* 2013;114(12):1743–54.

- 547 10. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and
548 red cells in dehydration. *J Appl Physiol*. 1974;37(2):247–8.
- 549 11. Ellis AC, Hyatt TC, Hunter GR, Gower BA. Respiratory quotient predicts fat mass gain
550 in premenopausal women. *Obesity (Silver Spring)*. 2010;18(12):2255–9.
- 551 12. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J*
552 *Appl Physiol*. 1983;55(2):628–34.
- 553 13. Gabriel B, Ratkevicius A, Gray P, Frenneaux MP, Gray SR. High-intensity exercise
554 attenuates postprandial lipaemia and markers of oxidative stress. *Clin Sci (Lond)*.
555 2012;123(5):313–21.
- 556 14. Gill JMR, Al-Mamari A, Ferrell WR, Cleland SJ, Perry CG, Sattar N, Packard CJ,
557 Caslake MJ, Petrie JR. Effect of prior moderate exercise on postprandial metabolism in
558 men with type 2 diabetes: heterogeneity of responses. *Atherosclerosis*. 2007;194(1):134–
559 43.
- 560 15. Gill JMR, Hardman AE. Postprandial lipemia: effects of exercise and restriction of
561 energy intake compared. *Am J Clin Nutr*. 2000;71(2):465–71.
- 562 16. Gill JMR, Herd SL, Vora V, Hardman AE. Effects of a brisk walk on lipoprotein lipase
563 activity and plasma triglyceride concentrations in the fasted and postprandial states. *Eur*
564 *J Appl Physiol*. 2003;89(2):184–90.
- 565 17. Hallal PC, Andersen LB, Bull FC, Guthold R, Haskell W, Ekelund U. Global physical
566 activity levels: surveillance progress, pitfalls, and prospects. *Lancet*.
567 2012;380(9838):247–57.
- 568 18. Hardy CJ, Rejeski WJ. Not what, but how one feels: the measurement of affect during
569 exercise. *J Sport Exerc Psychol*. 1989;11(3):304–17.
- 570 19. Health Survey for England 2012. *Volume 1: Health, social care and lifestyles. Chapter*
571 *3: Physical activity in children*. London: Health and Social Care Information Centre;
572 2012. p. 8. Available from Health and Social Care Information Centre.
- 573 20. Hoos MB, Kuipers H, Gerver WJM, Westerterp KR. Physical activity pattern of children
574 assessed by triaxial accelerometry. *Eur J Clin Nutr*. 2004;58(10):1425–8.

- 575 21. Kiens B, Richter EA. Utilization of skeletal muscle triacylglycerol during postexercise
576 recovery in humans. *Am J Physiol.* 1998;275(2 Pt 1):E332–7.
- 577 22. Kolovou GD, Biliannou H, Mikhailidis DP. Postprandial lipemia in children and
578 adolescents. *Curr Vasc Pharmacol.* 2011;9(3):318–20.
- 579 23. Magkos F, Wright DC, Patterson BW, Mohammed BS, Mittendorfer B. Lipid
580 metabolism response to a single, prolonged bout of endurance exercise in healthy young
581 men. *Am J Physiol Endocrinol Metab.* 2006;290(2):E355–62.
- 582 24. Maraki M, Magkos F, Christodoulou N, Aggelopoulou N, Skenderi KP, Panagiotakos D,
583 Kavouras SA, Sidossis LS. One day of moderate energy deficit reduces fasting and
584 postprandial triacylglycerolemia in women: the role of calorie restriction and exercise.
585 *Clin Nutr.* 2010;29(4):459–63.
- 586 25. Maraki MI, Sidossis LS. The latest on the effect of prior exercise on postprandial
587 lipaemia. *Sports Med.* 2013;43(6):463–81.
- 588 26. McGill HC, McMahan CA, Herderick EE, Malcom GT, Tracy RE, Strong JP. Origin of
589 atherosclerosis in childhood and adolescence. *Am J Clin Nutr.* 2000;72(5):1307S–15S.
- 590 27. Motl RW, Dishman RK, Saunders R, Dowda M, Felton G, Pate RR. Measuring
591 enjoyment of physical activity in adolescent girls. *Am J Prev Med.* 2001;21(2):110–7.
- 592 28. Sedgwick MJ, Morris JG, Nevill ME, Barrett LA. Effect of repeated sprints on
593 postprandial endothelial function and triacylglycerol concentrations in adolescent boys. *J*
594 *Sports Sci.* 2015;33(8):806–16.
- 595 29. Senn S. *Cross-over trials in clinical research.* Chichester, UK: Wiley; 1993. pp. 130–8.
- 596 30. Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD,
597 Bembien DA. Skinfold equations for estimation of body fatness in children and youth.
598 *Hum Biol.* 1988;60(5):709–23.
- 599 31. Tan MS, Mok A, Yap MC, Burns SF. Effect of sprint interval versus continuous cycling
600 on postprandial lipaemia. *J Sports Sci.* 2013;31(9):989–95.
- 601 32. Tanner JM. *Growth at adolescence.* 2nd ed. Oxford, UK: Blackwell Scientific
602 Publications; 1962. pp. 28–39.

- 603 33. Thackray AE, Barrett LA, Tolfrey K. Acute high-intensity interval running reduces
604 postprandial lipemia in boys. *Med Sci Sports Exerc.* 2013;45(7):1277–84.
- 605 34. Thackray AE, Barrett LA, Tolfrey K. Acute effects of energy deficit induced by
606 moderate-intensity exercise or energy-intake restriction on postprandial lipemia in
607 healthy girls. *Pediatr Exerc Sci.* 2015;27(2):192–202.
- 608 35. Tolfrey K, Bentley C, Goad M, Varley J, Willis S, Barrett L. Effect of energy
609 expenditure on postprandial triacylglycerol in adolescent boys. *Eur J Appl Physiol.*
610 2012;112(1):23–31.
- 611 36. Tolfrey K, Campbell IG, Jones AM. Intra-individual variation of plasma lipids and
612 lipoproteins in prepubescent children. *Eur J Appl Physiol.* 1999;79(5):449–56.
- 613 37. Tolfrey K, Thackray AE, Barrett LA. Acute exercise and postprandial lipemia in young
614 people. *Pediatr Exerc Sci.* 2014;26(2):127–37.
- 615 38. Trombold JR, Christmas KM, Machin DR, Kim IY, Coyle EF. Acute high-intensity
616 endurance exercise is more effective than moderate-intensity exercise for attenuation of
617 postprandial triglyceride elevation. *J Appl Physiol.* 2013;114(6):792–800.
- 618 39. Trost SG, Pate RR, Sallis JF, Freedson PS, Taylor WC, Dowda M, Sirard J. Age and
619 gender differences in objectively measured physical activity in youth. *Med Sci Sports*
620 *Exerc.* 2002;34(2):350–5.
- 621 40. Whyte LJ, Ferguson C, Wilson J, Scott RA, Gill JMR. Effects of single bout of very
622 high-intensity exercise on metabolic health biomarkers in overweight/obese sedentary
623 men. *Metabolism.* 2013;62(2):212–9.
- 624 41. Zilversmit DB. Atherogenesis: a postprandial phenomenon. *Circulation.*
625 1979;60(3):473–85.

626 **Figure legends**

627 **Figure 1** Diagram of the 2-day study protocol. TAG, triacylglycerol; NEFA, non-
628 esterified fatty acids. [†]Evening meal replicated from the first condition but with
629 a small reduction in energy intake in HIIR-ER.

630 **Figure 2** Fasting (F) and postprandial plasma triacylglycerol concentrations ([TAG]) in
631 the control (CON), high-intensity interval running and energy-intake restriction
632 (HIIR-ER) and high-intensity interval running (HIIR) conditions ($n = 16$).
633 Values are mean (SD). Black rectangles denote consumption of breakfast and
634 lunch meals at 08:00 and 12:00, respectively. Main effect condition $P < 0.001$;
635 main effect time $P < 0.001$; condition by time interaction $P = 0.71$.

636 **Figure 3** Individual changes (delta) in the total area under the plasma triacylglycerol
637 (TAG) concentration versus time curve (TAUC) between the high-intensity
638 interval running (HIIR) and high-intensity interval running and energy-intake
639 restriction (HIIR-ER) conditions compared with the control condition (CON):
640 A) HIIR minus CON; B) HIIR-ER minus CON. Participant data are organised
641 according to the size of the intervention-induced change in TAUC-TAG; thus,
642 the order of the individual participants is not identical in A and B. A negative
643 response indicates a reduction in TAUC-TAG in the intervention compared with
644 CON.

Table 1 Energy and macronutrient intakes during the intervention day of the high-intensity interval running (HIIR), high-intensity interval running and energy-intake restriction (HIIR-ER) and control (CON) conditions.

	HIIR	HIIR-ER	CON	CON vs. HIIR 95% CI*	CON vs. HIIR-ER 95% CI*	HIIR-ER vs. HIIR 95% CI*
Energy (MJ·day ⁻¹)	6.4 (1.4)	5.6 (1.5)	6.5 (1.4)	-0.19 to 0.00 ^a	-0.9 to -0.8 ^b	0.7 to 0.9 ^c
Protein (g·day ⁻¹)	54.4 (20.0)	47.1 (19.2)	54.1 (19.8)	-1.4 to 1.9	-8.2 to -4.9 ^b	5.2 to 8.4 ^c
CHO (g·day ⁻¹)	218 (42)	196 (47)	222 (42)	-9 to 1	-32 to -22 ^b	17 to 27 ^c
Fat (g·day ⁻¹)	48.5 (15.4)	41.1 (14.5)	49.1 (15.8)	-2.0 to 0.5	-9.1 to -6.6 ^b	5.9 to 8.4 ^c
% energy intake from protein	14 (4)	14 (4)	14 (4)	-0.1 to 0.7	-0.2 to 0.6	-0.3 to 0.5
% energy intake from CHO	58 (4)	59 (4)	58 (4)	-1.2 to 0.5	-0.2 to 1.5	-1.9 to -0.2 ^c
% energy intake from fat	28 (5)	27 (4)	28 (5)	-0.7 to 0.9	-1.7 to -0.1 ^b	0.2 to 1.8 ^c

Values are mean (SD) for $n = 16$. *95% confidence interval of the mean absolute difference between the experimental conditions.

CHO, carbohydrate.

^a Significant difference between HIIR and CON ($P < 0.05$)

^b Significant difference between HIIR-ER and CON ($P < 0.05$)

^c Significant difference between HIIR and HIIR-ER ($P < 0.05$)

Table 2 Physical activity levels and sedentary time during the intervention day in the high-intensity interval running (HIIR), high-intensity interval running and energy-intake restriction (HIIR-ER) and control (CON) conditions.

	HIIR	HIIR-ER	CON	CON vs. HIIR 95% CI*	CON vs. HIIR-ER 95% CI*	HIIR-ER vs. HIIR 95% CI*
Daily wear time (min)	838 (800 to 877)	810 (774 to 848)	808 (772 to 846)	-2 to 9%	-5 to 6%	-2 to 9%
Counts per minute	422 (375 to 476)	348 (309 to 393)	295 (261 to 332)	26 to 63% ^a	4 to 34% ^b	7 to 38% ^c
Sedentary activity (min)	494 (461 to 530)	502 (468 to 538)	521 (486 to 559)	-13 to 4%	-12 to 5%	-10 to 8%
Light activity (min)	248 (215 to 286)	228 (198 to 263)	224 (194 to 258)	-1 to 24%	-9 to 14%	-3 to 22%
Moderate activity (min)	68 (58 to 80)	54 (46 to 63)	50 (43 to 59)	15 to 59% ^a	-10 to 25%	8 to 50% ^c
Vigorous activity (min)	14 (9 to 23)	9 (6 to 15)	2 (1 to 4)	260 to 924% ^a	134 to 565% ^b	-9 to 160%

Values are geometric mean (95% confidence interval) for $n = 16$. Statistical analyses are based on natural log transformed data. *95% confidence interval for the ratio of geometric means.

^a Significant difference between HIIR and CON ($P < 0.05$)

^b Significant difference between HIIR-ER and CON ($P < 0.05$)

^c Significant difference between HIIR and HIIR-ER ($P < 0.05$)

Table 3 Fasting and postprandial plasma triacylglycerol, non-esterified fatty acids (NEFA) and glucose concentrations in the high-intensity interval running (HIIR), high-intensity interval running and energy-intake restriction (HIIR-ER) and control (CON) conditions.

	HIIR	HIIR-ER	CON	CON vs. HIIR 95% CI*	CON vs. HIIR-ER 95% CI*	HIIR-ER vs. HIIR 95% CI*
Triacylglycerol						
Fasting (mmol·L ⁻¹)	0.74 (0.63 to 0.87)	0.81 (0.69 to 0.95)	0.88 (0.75 to 1.03)	-24 to -7% ^a	-17 to 1%	-17 to 1%
TAUC (mmol·L ⁻¹ 6.5 h)	7.75 (6.36 to 9.43)	7.81 (6.41 to 9.51)	8.58 (7.05 to 10.45)	-16 to -3% ^a	-15 to -2% ^b	-8 to 6%
iAUC (mmol·L ⁻¹ 6.5 h)	3.18 (2.23 to 4.54)	2.80 (1.96 to 4.00)	2.76 (1.94 to 3.94)	-13 to 53%	-23 to 34%	-14 to 50%
NEFA						
Fasting (mmol·L ⁻¹)	0.68 (0.56 to 0.81)	0.70 (0.58 to 0.83)	0.87 (0.72 to 1.04)	-37 to -4% ^a	-35 to -1% ^b	-21 to 20%
TAUC (mmol·L ⁻¹ 6.5 h)	2.61 (2.24 to 3.03)	2.67 (2.30 to 3.11)	2.51 (2.16 to 2.92)	-6 to 15%	-4 to 17%	-12 to 8%
iAUC (mmol·L ⁻¹ 6.5 h)	-1.79 (-2.91 to -0.38)	-1.85 (-2.95 to -0.45)	-3.67 (-4.38 to -2.77)	14 to 115% ^a	13 to 113% ^b	-27 to 39%
Glucose						
Fasting (mmol·L ⁻¹)	5.65 (5.40 to 5.90)	5.80 (5.55 to 6.07)	5.70 (5.45 to 5.96)	-5 to 3%	-2 to 6%	-7 to 2%
TAUC (mmol·L ⁻¹ 6.5 h)	43.8 (41.9 to 45.8)	43.2 (41.3 to 45.2)	42.2 (40.4 to 44.1)	1 to 7% ^a	0 to 5%	-1 to 4%
iAUC (mmol·L ⁻¹ 6.5 h)	9.75 (7.24 to 12.94)	6.48 (4.67 to 8.78)	7.79 (5.70 to 10.45)	-12 to 63%	-36 to 18%	2 to 88% ^c

Values are geometric mean (95% confidence interval) for $n = 16$. Statistical analyses are based on natural log transformed data. *95% confidence interval for the ratio of geometric means.

TAUC, total area under the concentration versus time curve; iAUC, incremental area under the concentration versus time curve.

^a Significant difference between HIIR and CON ($P < 0.05$)

^b Significant difference between HIIR-ER and CON ($P < 0.05$)

^c Significant difference between HIIR and HIIR-ER ($P < 0.05$)

Figure 1

Figure 2

Figure 3