

Acute effects of energy deficit induced by moderate-intensity exercise or energy-intake restriction on postprandial lipemia in healthy girls

Running head: Energy deficit and postprandial lipemia

Abstract

Eleven healthy girls (mean(SD): age 12.1(0.6) years) completed three, 2-day conditions in a counterbalanced, crossover design. On day 1, participants either walked at 60(2)% peak oxygen uptake (energy deficit 1.55(0.20) MJ) (EX), restricted food energy intake (energy deficit 1.51(0.25) MJ) (ER) or rested (CON). On day 2, capillary blood samples were taken at pre-determined intervals throughout the 6.5 h postprandial period prior to, and following, the ingestion of standardised breakfast and lunch meals. Fasting plasma [TAG] was 29% and 13% lower than CON in EX (effect size (ES) = 1.39, $P = 0.01$) and ER (ES = 0.57, $P = 0.02$) respectively; EX was 19% lower than ER (ES = 0.82, $P = 0.06$). The EX total area under the [TAG] versus time curve was 21% and 13% lower than CON (ES = 0.71, $P = 0.004$) and ER (ES = 0.39, $P = 0.06$) respectively; ER was marginally lower than CON (-10%; ES = 0.32, $P = 0.12$). An exercise-induced energy deficit elicited a greater reduction in fasting plasma [TAG] with a trend for a larger attenuation in postprandial plasma [TAG] than an isoenergetic diet-induced energy deficit in healthy girls.

Keywords: Cardiovascular disease risk; energy deficit; triacylglycerol; young people

Introduction

Elevated postprandial triacylglycerol concentrations ([TAG]) have been implicated in the development of atherosclerosis (45), and are established as an independent risk factor for future cardiovascular disease in adults (1, 32). Although the metabolic perturbations present following the ingestion of a meal appear short-lived, most people spend the majority of the daytime in a postprandial state typically. Therefore, repeated episodes of exaggerated postprandial [TAG] contribute to the atherogenic lipid phenotype of TAG-rich lipoprotein remnants, small, dense low-density lipoprotein and low concentrations of high-density lipoprotein (8). The process of atherosclerosis is initiated during childhood and progresses over the lifespan, prompting preventive lifestyle interventions, such as exercise and diet that may delay precursors of atherosclerotic progression early in life (31).

Acute aerobic exercise (30 min to 3 h in duration) performed up to 18 h before a test meal reduces postprandial [TAG] in adults (29). Furthermore, accumulating evidence in boys and girls demonstrates the postprandial TAG lowering effect of moderate- to vigorous-intensity exercise interventions (2, 24, 25, 36, 37, 41, 43, 44). It is possible that the energy deficit associated with a single session of exercise may be responsible for the attenuation in postprandial [TAG]. Replacing the exercise-induced energy deficit has been shown to diminish or even eliminate the reduction in postprandial [TAG] in men and women (6, 16, 21). Studies manipulating the origin of the energy deficit through exercise energy expenditure (EE) and dietary energy intake suggest that an exercise-induced energy deficit attenuates postprandial [TAG] to a greater extent than a diet-induced deficit in women (18, 28). Nevertheless, reductions in postprandial [TAG] have been reported following a single day of energy-intake restriction in young, healthy women (28). However, we are not aware of studies that have examined the acute effect of energy-intake restriction on postprandial [TAG]

in young people, highlighting an important gap in our current knowledge which may have important implications in terms of providing opportunities to improve metabolic health early in life.

Current international guidelines recommend that children and adolescents accumulate at least 60 min of moderate-intensity daily physical activity for health promotion (11, 22); however, many young people fail to meet these guidelines, and girls are less active typically than their male peers (34). To our knowledge, only one study has examined the acute effect of exercise on postprandial plasma [TAG] in girls, reporting that 60, but not 30 min of moderate-intensity exercise attenuates postprandial plasma [TAG] in 10 to 14 year old girls (44). Consequently, additional work is required to expand the evidence base in girls to identify engaging and sustainable lifestyle interventions that improve this cardiovascular disease risk factor from a young age. Therefore, the aim of the present study was to compare the effect of an isoenergetic energy deficit, induced by acute moderate-intensity exercise or energy-intake restriction on postprandial plasma [TAG] in healthy, recreationally active girls.

Methods

Participants

Thirteen girls volunteered to participate in this study. Results are presented for 11 girls (age 12.1(0.6) years; body mass 42.1(5.8) kg; body mass index 18.1(1.8) kg·m⁻²; peak oxygen uptake ($\dot{V}O_2$) 47(6) mL·kg⁻¹·min⁻¹) as one girl's habitual daily energy intake was too low to justify energy-intake restriction and another dropped out for personal reasons unrelated to the study. The study was approved by the University Ethical Advisory Committee. Written assent was obtained from each participant and written informed consent by a parent or guardian. A health screen questionnaire confirmed all participants 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), and body mass was quantified to the nearest 0.1 kg using a digital scale (Seca 770, Seca Ltd, Hamburg, Germany). Body mass index was calculated as body mass (kg) divided by stature (m) squared. Triceps and subscapular skinfold thickness was measured to the nearest 0.2 mm on the right-hand side of the body using Harpenden callipers (Baty International, West Sussex, England). The median of three measurements at each site was used to estimate percent body fat (39).

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 (40). Participants identified the stage most closely resembling their current level of sexual development. The scale ranges from 1 indicating pre-pubescence to 5 indicating full sexual maturity. The median(interquartile range) stage of breast development was 3(1) and pubic hair development was 3(2).

Preliminary exercise measurements

During the first visit to the laboratory, participants were familiarised with walking and running on the treadmill (h/p/cosmos mercury med, Nussdorf-Traunstein, Germany) before completing two preliminary exercise tests. The first test involved a 16 min submaximal incremental treadmill protocol divided into 4 x 4 min stages starting at a speed of 4 km·h⁻¹ and increasing 1 km·h⁻¹ at the start of each subsequent stage, with the gradient set at 1%

throughout. This enabled the individual steady-state relationship between treadmill speed, $\dot{V}O_2$ and heart rate to be established. Heart rate was monitored continuously via short-range telemetry (Polar PE 4000, Kempele, Finland) and ratings of perceived exertion (RPE) were recorded in the last 10 s of each minute. Expired air samples were collected during the final minute of each 4 minute stage 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.

Participants were given a standardised 10 min rest period before completing the second test involving an incremental uphill treadmill protocol to determine peak $\dot{V}O_2$. The girls ran at a fixed individual speed (7.0 to 8.5 km·h⁻¹), identified from the submaximal incremental treadmill protocol, while the treadmill gradient was increased 1% each minute until volitional exhaustion. In the absence of a plateau in $\dot{V}O_2$, attainment of maximal effort was confirmed based on the following criteria: a peak heart rate \geq 95% of age-predicted maximum (220-chronological age); a respiratory exchange ratio \geq 1.10; and clear subjective signs of fatigue. Data from the 16 min submaximal incremental and peak $\dot{V}O_2$ tests were used to determine the treadmill speed required to elicit 60% peak $\dot{V}O_2$ during the experimental exercise condition.

Experimental design

Using a within-measures, counterbalanced, crossover design, participants completed three, 2-day experimental conditions separated by a standardised period of 14 days: moderate-

intensity exercise (EX), energy-intake restriction (ER) and rest control (CON). The study design is presented schematically in Figure 1.

Day 1: Intervention day

Participants arrived at the laboratory at 15:30 and all measures were completed by 17:30. Body mass was recorded at the start of each condition to standardise the meals provided on day 2 (described below). During EX, participants exercised on the treadmill at 60% peak $\dot{V}O_2$ in 20 min intervals, with a standardised 5 min period of seated rest between each interval. Expired air samples were collected and analysed as described previously during the third, tenth and seventeenth minute to calculate the relative exercise intensity. The exercise EE and the oxidation of carbohydrate and fat were estimated via indirect calorimetry (14), assuming that participants reached a physiological steady state and that the urinary nitrogen excretion rate was negligible. The net EE of exercise was calculated as the exercise gross EE minus resting EE, where resting EE was estimated using age and sex-specific equations (13). The treadmill speed was adjusted occasionally to ensure the target exercise intensity was met. Heart rate was monitored throughout and RPE was recorded during the last 10 s of each expired air sampling period as described previously. Participants maintained their habitual dietary intake throughout the day. During ER, the girls rested in the laboratory for the duration of the visit and reduced their habitual food energy intake by the net EE of exercise, with the energy intakes at lunch and evening meal reduced by 43% and 57% of the total net EE of exercise respectively. During CON, participants rested in the laboratory for the duration of the visit and maintained their habitual dietary intake throughout the day.

Standardisation of diet and physical activity

Participants recorded their usual 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 ER. Participants completing 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 food records were analysed using dietary analysis software (CompEat Pro Version 5.8.0, Nutrition Systems, Banbury, UK). Energy and macronutrient intake during the intervention day is displayed in Table 1.

To standardise the overnight fasting period, participants were asked to consume a small cereal snack bar at 19:45 on the intervention day of each condition. The macronutrient content of the cereal snack bar was 1.3 g fat, 17.2 g carbohydrate and 1.0 g protein, which provided 357 kJ energy. After 20:00, the participants were allowed to drink plain water, but were asked not to consume any other drinks or food before arriving at the laboratory on day 2.

Participants recorded all physical activity categorised according to intensity level during the pre-intervention and intervention day of the first condition. Participants were asked to minimise their physical activity during this period, and the activity pattern was replicated before the remaining conditions. Free-living physical activity was not quantified objectively so a comparison between the conditions is not available.

Day 2: Postprandial day

On the postprandial day, participants arrived at the laboratory at ~07:45 following a 12 h overnight fast and provided a fasting capillary blood sample after 10 min seated rest. They

then consumed a standardised breakfast meal within 15 min, marking the start of the postprandial period (08:00). 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 meal, within 20 min, at 4 h (Figure 1). They 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.

Test meals

The test breakfast provided on the postprandial day 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 (60% of total energy), 1.8 g carbohydrate (33%), 0.4 g protein (7%) and 93 kJ energy per kilogram body mass. The test lunch consisted of white bread, mild cheddar cheese, butter, potato crisps, whole milk and milkshake powder, and provided 1.0 g fat (48%), 1.9 g carbohydrate (40%), 0.6 g protein (12%) and 79 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. The time taken to consume each meal during the first experimental condition was recorded and replicated in the remaining conditions.

Analytical methods

To collect the capillary blood samples, the hand was pre-warmed for 5 min in water heated to 40°C. The fingertip was pierced (Unistick 3 Extra, Owen Mumford, Oxford, UK) and 600 µL of whole capillary blood was collected into potassium-EDTA coated Microvette CB 300 tubes (Sarstedt Ltd, Leicester, UK). The whole blood samples were immediately centrifuged

at 12,800 g for 15 min (Eppendorf 5415c, Hamburg, Germany). An automatic pipette was used to dispense 200 μ L of plasma into a 0.5 ml Eppendorf tube (Eppendorf, Hamburg, Germany). The plasma samples were stored at -80°C for up to two months before subsequent analyses. Plasma [TAG] and glucose concentration ([glucose]) were analysed by enzymatic, colorimetric methods using a benchtop analyser (Pentra 400, HORIBA ABX Diagnostics, Montpellier, France). Plasma insulin concentration ([insulin]) was quantified using a commercially available enzyme-linked immunoassay (Mercodia Insulin ELISA, Mercodia AB, Uppsala, Sweden). The within-batch coefficients of variation for [TAG], [glucose] and [insulin] were 1.1, 0.5 and 6.2% 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 (12). 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 Ltd, Cambridge, England). Haematocrit was quantified using a microhaematocrit centrifuge and reader (Haematospin 1300 Microcentrifuge, Hawksley, Sussex, UK).

Statistical analyses

Data were analysed using the IBM SPSS Statistics software for Windows version 21.0 (IBM Corporation, New York, USA). Descriptive statistics illustrating the physical and physiological characteristics and exercise responses were calculated. The trapezium rule was used to calculate the total area under the plasma concentration versus time curves for TAG (TAUC-TAG), glucose (TAUC-glucose) and insulin (TAUC-insulin). The incremental area under the plasma concentration versus time curves for TAG (iAUC-TAG), glucose (iAUC-glucose) and insulin (iAUC-insulin) was calculated using the same method after adjusting for

fasting concentrations. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated (30). Normality of the data was checked by Shapiro Wilk tests. Normally distributed data are expressed as mean(SD). Concentrations of TAG, glucose and insulin and HOMA-IR were natural log transformed prior to analysis. These data are presented as median(interquartile range) and analysis is based on the ratios of the geometric means and 95% confidence intervals (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. Energy and macronutrient intake, estimated changes in plasma volume, fasting [TAG], [glucose] and [insulin], HOMA-IR and TAUC and iAUC responses were compared between each experimental condition using separate one-way within-measures analysis of variance (ANOVA), adjusting appropriately for the period effect (38). Temporal changes in TAUC-TAG between EX, ER and CON were examined over sub-sections of the total postprandial period (0 to 1 h, 1 to 4.5 h and 4.5 to 6.5 h) using separate one-way, within-measures ANOVA. *A priori* simple planned contrasts with CON as the reference category were conducted to follow up the effects of the omnibus ANOVAs. Differences in postprandial [TAG], [glucose] and [insulin] over time were modelled using Generalised Estimating Equations, adjusting appropriately for the period effect (38). We assumed a log-normal distribution and identity link function, with an exchangeable correlation matrix and a robust variance estimator. Model specification was checked using residuals plots. Bivariate correlations identifying possible determinants of the exercise-induced changes in TAUC-TAG were quantified using Pearson's product moment correlations. The 95% confidence intervals for mean absolute pairwise differences between experimental conditions were calculated using the t-distribution and degrees of freedom ($n - 1$). 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 (7).

Results

Dietary intake

Energy and macronutrient intake were not significantly different on the pre-intervention day across the three conditions ($P \geq 0.47$). Average daily energy intake was 6.9(2.1) MJ, and dietary intake of protein, carbohydrate and fat was 58.4(13.5) g, 248(92) g and 46.8(13.6) g respectively. As anticipated, two-way ANOVA identified differences in energy and macronutrient intake on the intervention day across the conditions ($P < 0.001$). Energy intake was considerably lower in ER compared with CON (ES = 1.12, $P < 0.001$) and EX (ES = 1.06, $P < 0.001$); CON and EX were not significantly different ($P = 0.27$) (Table 1). Absolute protein, carbohydrate and fat intake were considerably lower in ER compared with CON and EX (ES = 0.83 to 1.07, $P < 0.05$), with no significant difference between CON and EX (ES = 0.01 to 0.14, $P \geq 0.20$) (Table 1). Changes in the contribution of protein, carbohydrate and fat to total energy intake were not significantly different across the conditions ($P \geq 0.22$) (Table 1).

Exercise responses

Mean oxygen uptake during EX was 1.18(0.16) L·min⁻¹, corresponding to 60(2)% peak $\dot{V}O_2$, and the average respiratory exchange ratio was 0.85(0.03). Mean heart rate was 161(6) beats·min⁻¹, which represented 80(3)% of peak heart rate, and the average rating of perceived exertion was 13(1) ('*somewhat hard*' on the scale). The estimated exercise net EE was 1.46(0.01) MJ.

Exercise- and diet-induced energy deficits

Accounting for the exercise net EE and energy intake (Table 1) on the intervention day, the resulting energy deficit relative to CON was 1.55(0.20) MJ in EX and 1.51(0.25) MJ in ER. The exercise- and diet-induced energy deficits were not significantly different from each other (95% CI -0.07 to 0.14, $P = 0.49$).

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

Average changes in plasma volume between the fasting and 6.5 h postprandial samples were small and did not differ significantly between the three conditions (EX 0.6%; ER 1.0%; CON 1.2%; $P = 0.67$). Therefore, the raw plasma [TAG], [glucose] and [insulin] were used in all statistical analyses without adjustment. The fasting plasma [TAG], [glucose] and [insulin] for each condition are shown in Table 2. One-way ANOVA revealed differences across the conditions in fasting plasma [TAG] ($P = 0.002$). Specifically, fasting plasma [TAG] was 29% and 13% lower than CON in EX (ES = 1.39, $P = 0.01$) and ER (ES = 0.57, $P = 0.02$) respectively; EX was 19% lower than ER (ES = 0.82, $P = 0.06$). One-way ANOVA revealed a tendency for differences in fasting plasma [glucose] across the conditions ($P = 0.08$), with simple planned contrasts revealing a trend for lower fasting plasma [glucose] in EX than CON (-3%; ES = 0.67, $P = 0.07$). One-way ANOVA revealed a tendency for differences in fasting plasma [insulin] across the conditions ($P = 0.07$). Fasting plasma [insulin] was 26% and 18% lower than CON in EX (ES = 0.69, $P = 0.02$) and ER (ES = 0.46, $P = 0.10$) respectively; ER and EX were not significantly different (-10%; $P = 0.52$). One-way ANOVA revealed a tendency for differences in fasting HOMA-IR across the conditions ($P = 0.05$). Fasting HOMA-IR was lower compared with CON (2.35(1.71-2.97)) by 29% in EX (1.59(1.18-2.29); -46 to -6%, ES = 0.71, $P = 0.03$) and by 20% in ER (1.58(1.42-2.81); -39 to

3%, ES = 0.47, $P = 0.07$); ER and EX were not significantly different (-11%; -39 to 32%, $P = 0.49$).

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

Plasma [TAG] responses over the postprandial period for EX, ER and CON are shown in Figure 2. Inspection of the residuals plots revealed that the models were correctly specified. Mean postprandial [TAG] was 22% and 9% lower than CON in EX (-27 to -18%, ES = 0.66, $P < 0.001$) and ER (-15 to -4%, ES = 0.26, $P = 0.001$) respectively; EX was 14% lower than ER (-20 to -8%, ES = 0.40, $P < 0.001$). The TAUC-TAG was 21% lower after EX than CON (ES = 0.71, $P = 0.004$), with small, but statistically insignificant, differences seen between ER and CON (-10%; ES = 0.32, $P = 0.12$) and EX and ER (-13%; ES = 0.39, $P = 0.06$) (Table 2). The TAUC-TAG was lower after EX compared with CON between 0 to 1 h by 26% (-39 to -11%, ES = 1.12, $P = 0.01$), 1 to 4.5 h by 22% (-33 to -10%, ES = 0.67, $P = 0.01$) and 4.5 to 6.5 h by 19% (-26 to -10%, ES = 0.64, $P = 0.003$); ER was lower than CON between 0 to 1 h by 11% (-20 to -2%, ES = 0.43, $P = 0.03$) and 1 to 4.5 h by 13% (-23 to -1%, ES = 0.37, $P = 0.04$). The TAUC-TAG was lower following EX than ER between 0 to 1 h by 17% (-34 to 4%, ES = 0.69, $P = 0.08$) and 4.5 to 6.5 h by 14% (-25 to -1%, ES = 0.47, $P = 0.04$). No significant differences were observed in iAUC-TAG across the conditions ($P = 0.84$) (Table 2).

Meaningful positive correlations were identified between the intervention-induced change in fasting [TAG] and the change in TAUC-TAG relative to CON for EX ($r = 0.65$, $P = 0.03$) and ER ($r = 0.57$, $P = 0.07$). Individual changes (delta) in TAUC-TAG across the three conditions are shown in Figure 3. The reductions in TAUC-TAG following EX and ER were greater than changes in CON for ten (~91%) and eight (~73%) girls respectively. The measured physical and physiological characteristics, dietary intake (Table 1), exercise

responses and fasting [glucose] or [insulin] (Table 2) did not account for any of the inter-individual variability in delta TAUC-TAG for EX or ER. The Pearson's product moment correlation for the individual changes in TAUC-TAG between EX and ER was trivial ($r = 0.01$, $P = 0.98$).

Mean EX postprandial [glucose] was significantly, although not meaningfully, greater by 1% and 4% than CON (0 to 2%, $ES = 0.08$, $P = 0.05$) and ER (1 to 6%, $ES = 0.23$, $P = 0.001$) respectively; ER was 2% lower than CON (-4 to 0%, $ES = 0.15$, $P = 0.04$). No significant differences were observed in TAUC-glucose across the conditions ($P = 0.27$) (Table 2). The EX iAUC-glucose was higher by 17% and 26% than CON ($ES = 0.43$, $P = 0.05$) and ER ($ES = 0.62$, $P = 0.03$) respectively; CON and ER were not significantly different (-7%; $P = 0.55$).

Mean EX postprandial [insulin] was not significantly different compared with CON (-5%; -13 to 3%, $P = 0.24$) or ER (3%; -9 to 16%, $P = 0.64$); ER tended to be lower than CON (-8%; -15 to 0%, $ES = 0.11$, $P = 0.06$). No significant differences were evident in TAUC-insulin ($P = 0.56$) or iAUC-insulin ($P = 0.74$) across the conditions (Table 2).

Discussion

The main novel finding from the present study was that an acute exercise-induced energy deficit elicits a greater attenuation in fasting plasma [TAG] with a trend for a larger attenuation in postprandial plasma [TAG] than an isoenergetic diet-induced energy deficit in healthy, recreationally active girls. This suggests that the physiological origin of the energy deficit influences the magnitude of change in fasting and postprandial plasma [TAG] in girls.

The magnitude of reduction in fasting plasma [TAG] in ER (Table 2) supports the majority of previous findings in young people following moderate- to vigorous-intensity exercise (1.0 to 2.2 MJ) (2, 24, 41, 43, 44). However, the considerable attenuation seen after EX (Table 2) is

greater than the reductions reported previously (2, 24, 41, 43, 44). It is likely that the lower fasting plasma [TAG] in EX and ER contributed to the lower TAG response evident over the postprandial period (9). Nevertheless, fasting [TAG] vary considerably in children (42), and are typically less predictive of future cardiovascular events than postprandial [TAG] in women (1), suggesting that postprandial [TAG] may provide a better insight into metabolic health in young people.

The reduction in postprandial plasma [TAG] after EX supports previous studies with boys and girls demonstrating that acute moderate- to vigorous-intensity exercise (1.0 to 2.5 MJ) attenuates postprandial [TAG] (2, 24, 25, 36, 37, 41, 43, 44), but the small attenuation seen due to ER is a novel finding in this population (Figure 2, Table 2). Although the magnitude of reduction in postprandial [TAG] after ~60 min exercise varies in young people with estimated ES ranging from 0.18 to 0.86, on average, the changes are moderate (2, 24, 25, 36, 37, 41, 43, 44), and greater than the small attenuation seen in ER. However, the magnitude of reduction in postprandial plasma [TAG] after EX is greater than the reductions reported in the majority of exercise postprandial studies in young people (2, 24, 25, 36, 37, 41, 43, 44). While the clinical significance of our findings cannot be established, 96% of the postprandial TAG samples in the present study were below the $2.3 \text{ mmol}\cdot\text{L}^{-1}$ threshold suggested as a desirable concentration in young people (23); therefore, the potential for girls with healthy postprandial TAG metabolism to benefit from exercise and diet interventions is promising.

The mechanisms responsible for the reduction in postprandial [TAG] in young people following exercise and diet interventions are not known currently, and cannot be inferred from our findings. In adults, increased clearance of circulating TAG facilitated by enhanced skeletal muscle lipoprotein lipase has been implicated following acute exercise (20). Furthermore, acute exercise-induced reductions in the circulating concentration of fasting and

postprandial very-low density lipoprotein (VLDL)-TAG have been reported (27), possibly due to the secretion of fewer, TAG-rich VLDL particles which are likely to have a higher affinity for lipoprotein lipase (26). In the present study, the small differences identified in iAUC-TAG between EX, ER and CON suggests that changes in fasting [TAG], reflecting hepatic VLDL metabolism, contributes to the reduction in postprandial [TAG] after EX and ER. Indeed, the greatest difference in [TAG] between the three conditions was evident in the early postprandial period (0 to 1 h). This is further supported by the meaningful relationship seen between the intervention-induced changes in fasting [TAG] and TAUC-TAG for EX ($r = 0.65$, $P = 0.03$) and ER ($r = 0.57$, $P = 0.07$). A recent stable isotope enrichment study reported that the exercise-evoked reduction in postprandial [TAG] is achieved by a reduction in endogenous plasma [TAG] and not the concentration of meal-derived fatty acids in circulating TAG (10). Therefore, changes in exogenous TAG metabolism may elicit a smaller influence on the postprandial TAG response.

The present study demonstrates for the first time in 11 to 13 year old girls that an exercise-induced energy deficit tended to reduce postprandial plasma [TAG] to a greater extent than an equivalent diet-induced deficit, supporting the studies conducted to date in healthy women (18, 28). In the earliest of these studies, the attenuation in postprandial [TAG] following a single exercise session was treble that caused by a diet-induced energy deficit (18); although, it is worth noting that the energy deficit induced by intake restriction was approximately 17% lower than exercise. More recently, Maraki et al. (28) demonstrated that exercise was superior to an equivalent energy deficit from energy-intake restriction, reducing postprandial [TAG] by 23 and 12% respectively. Consequently, while reducing habitual energy intake may elicit a small reduction in postprandial [TAG], an exercise-induced energy deficit may be required to maximise the reduction in this important marker of atherogenic disease risk in

girls. Nevertheless, the present study contributes to providing young people with an array of lifestyle options that may attenuate postprandial plasma [TAG]. Mild, carefully managed reductions in dietary energy intake may be an attractive alternative in young people who find it difficult to accumulate sufficient physical activity for health.

The contrasting effect of EX and ER on postprandial plasma [TAG] may be attributable to the physiological origin of the energy deficit. Energy provision during moderate-intensity exercise is primarily met by the utilisation of skeletal muscle glycogen, intramuscular TAG, circulating free fatty acids and plasma glucose (35), although the contribution of lipid to the exercise EE is greater in children than adults at a given relative exercise intensity (33). In contrast, energy-intake restriction shifts the body towards the postabsorptive state leading to the breakdown of liver glycogen and the release of free fatty acids from adipose tissue (15). Therefore, it is possible that the effect of EX and ER on postprandial plasma [TAG] is mediated by a different mechanism. However, a series of basal VLDL kinetic studies have demonstrated recently that exercise- and diet-induced attenuations in fasting VLDL-[TAG] manifest through a reduction in hepatic VLDL-TAG secretion and increased plasma clearance of VLDL-TAG in healthy, young women (3, 4); although, a lower energy deficit from moderate-intensity exercise compared with energy-intake restriction (~2 vs. 3 MJ respectively) was required to reveal these effects (3, 4). Consequently, the mitigating effect of EX and ER on postprandial plasma [TAG] may not be mediated solely by the ensuing energy deficit, but further exercise postprandial studies are required to support this in young people.

In line with previous studies in young people (41, 44), substantial inter-individual variability is evident in the fasting and postprandial plasma [TAG] after EX and ER, which could not be accounted for by any of the measured variables included in the present study. Similar heterogeneity has been reported in adults previously, with exercise-induced changes in 3-

hydroxybutyrate, a marker of hepatic fatty acid oxidation, identified as a strong predictor of the exercise-induced reductions in fasting and postprandial [TAG] (17). This marker may explain some of the variance in the present study, but further work is required to examine this systematically. A range of self-assessed maturity ratings were identified in the present study. Although a possible maturational effect cannot be eliminated completely due to the relatively low sample size, we found no effect of maturity status on any of the outcome measures, including the inter-individual variability evident in fasting and postprandial [TAG].

The tendency for lower fasting plasma [glucose] in EX compared with CON is likely to contribute to the greater iAUC-glucose observed; however, a similar change in fasting plasma [glucose] was not seen between ER and EX despite the higher iAUC-glucose in EX (Table 2). The reason for these differences in glucose metabolism are unclear and appear inconsistent with the exercise postprandial studies in young people, with the majority reporting no exercise-induced changes in either fasting or postprandial [glucose] (2, 24, 25, 36, 37, 43, 44). In the absence of a change in postprandial [glucose], Sedgwick et al. (37) suggest the lower TAUC-insulin response following moderate-intensity exercise indicates an acute improvement in insulin sensitivity. Although we observed lower fasting [insulin] in EX and ER compared with CON, this difference did not persist into the postprandial period (Table 2). The lower fasting HOMA-IR in EX and ER compared with CON points to an acute improvement in insulin resistance. Nevertheless, the change in HOMA-IR was not associated with the change in postprandial plasma [TAG] in EX or ER and the TAG lowering effect of exercise has been shown to occur independent of changes in insulin sensitivity previously (19).

A limitation of the present study is that EE was not quantified during the short rest periods of EX and excess post-exercise oxygen consumption was not measured following the exercise

session. Although this omission may have underestimated the energy deficit in EX resulting in a higher energy deficit compared with ER, it is likely that the contribution to the total exercise EE was relatively small (5). Furthermore, this study investigated healthy, recreationally active girls. Further research should be conducted to examine if similar responses are observed in young people with cardiovascular disease risk factors, such as fasting hypertriglyceridemia, obesity and insulin resistance. Future studies investigating the effect of replacing the exercise EE on postprandial lipaemia in young people would also be timely.

In conclusion, this study shows for the first time in healthy, recreationally active girls that an exercise-induced energy deficit elicits a greater reduction in fasting plasma [TAG] with a trend for a larger attenuation in postprandial plasma [TAG] than an isoenergetic diet-induced energy deficit. Therefore, exercise prescription may promote greater acute benefits in TAG metabolism than dietary restriction alone in girls.

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Figure legends

Figure 1. Schematic representation of 2-day study protocol. *Lunch and evening meal replicated across conditions, but with a mild reduction in energy intake in ER. TAG, triacylglycerol.

Figure 2. Fasting and postprandial plasma triacylglycerol concentrations ([TAG]) in the moderate-intensity exercise (EX), energy-intake restriction (ER), and control (CON) conditions ($n = 11$). Black rectangle signifies consumption of breakfast and lunch meals at 08:00 and 12:00, respectively.

Figure 3. Individual changes (delta) in the total area under the plasma triacylglycerol (TAG) concentration versus time curve (TAUC) between the moderate-intensity exercise (EX) and energy-intake restriction (ER) conditions compared with the control condition (CON): a) EX minus CON; b) ER minus CON. Participant data are ordered according to the size of the intervention-induced change in TAUC-TAG; thus, the order of the individual participants is not identical in a and b. A negative response indicates a reduction in TAUC-TAG in the intervention compared with CON.

Table 1 Energy and macronutrient intake during the intervention day of the moderate-intensity exercise (EX), energy-intake restriction (ER) and control (CON) conditions ($n = 11$)

	EX	ER	CON	CON vs. EX 95% CI*	CON vs. ER 95% CI*	ER vs. EX 95% CI*
Energy (MJ·day ⁻¹)	7.2(1.3)	5.8(1.3)	7.3(1.3)	-0.2 to 0.1	-1.7 to -1.3 ^b	1.3 to 1.6 ^c
Protein (g·day ⁻¹)	59.1(11.5)	48.7(12.8)	60.5(12.5)	-3.9 to 1.2	-14.6 to -8.9 ^b	8.5 to 12.4 ^c
CHO (g·day ⁻¹)	249(51)	199(50)	249(53)	-6 to 6	-61 to -39 ^b	39 to 61 ^c
Fat (g·day ⁻¹)	55.4(12.7)	44.2(10.1)	57.1(12.1)	-4.2 to 1.1	-15.7 to -9.6 ^b	6.1 to 16.2 ^c
% energy intake from protein	14(2)	14(2)	14(2)	-0.4 to 0.1	-0.3 to 0.5	-0.7 to 0.1
% energy intake from CHO	57(5)	57(4)	57(4)	-0.6 to 2.0	-0.5 to 1.3	-1.8 to 2.4
% energy intake from fat	29(5)	29(4)	29(4)	-1.9 to 0.8	-1.5 to 0.4	-2.2 to 2.2

Values are mean(SD). *95% confidence interval of the mean absolute difference between the experimental conditions.

CHO, carbohydrate

^b Significant difference between ER and CON ($P < 0.001$)

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

Table 2 Fasting and postprandial plasma triacylglycerol, glucose and insulin concentrations in the moderate-intensity exercise (EX), energy-intake restriction (ER) and control (CON) conditions ($n = 11$)

	EX	ER	CON	CON vs. EX 95% CI*	CON vs. ER 95% CI*	ER vs. EX 95% CI*
Triacylglycerol						
Fasting ($\text{mmol}\cdot\text{L}^{-1}$)	0.69(0.53-0.82)	0.76(0.62-0.92)	0.92(0.87-1.20)	-42 to -13% ^a	-22 to -4% ^b	-35 to 1%
TAUC ($\text{mmol}\cdot\text{L}^{-1}$ 6.5 h)	6.97(6.35-9.16)	7.71(6.60-8.54)	8.89(6.77-12.23)	-30 to -11% ^a	-22 to 4%	-24 to 1%
iAUC ($\text{mmol}\cdot\text{L}^{-1}$ 6.5 h)	3.30(2.94-4.13)	2.98(2.26-3.62)	3.37(2.37-5.03)	-28 to 28%	-36 to 48%	-17 to 17%
Glucose						
Fasting ($\text{mmol}\cdot\text{L}^{-1}$)	5.36(5.18-5.52)	5.39(5.23-5.57)	5.55(5.33-5.63)	-7 to 0%	-5 to 1%	-4 to 2%
TAUC ($\text{mmol}\cdot\text{L}^{-1}$ 6.5 h)	44.1(42.1-47.3)	43.8(41.2-44.9)	43.8(41.7-47.2)	-1 to 3%	-7 to 3%	-2 to 8%
iAUC ($\text{mmol}\cdot\text{L}^{-1}$ 6.5 h)	13.07(9.77-15.48)	11.27(9.42-13.49)	10.55(7.78-12.70)	0 to 38% ^a	-30 to 24%	3 to 54% ^c
Insulin						
Fasting ($\text{pmol}\cdot\text{L}^{-1}$)	41.6(31.4-56.1)	39.2(36.0-72.4)	54.9(41.5-73.8)	-43 to -6% ^a	-37 to 6%	-38 to 32%
TAUC ($\text{pmol}\cdot\text{L}^{-1}$ 6.5 h)	1474(1208-1889)	1406(1224-1589)	1818(1341-1944)	-20 to 11%	-21 to 8%	-21 to 32%
iAUC ($\text{pmol}\cdot\text{L}^{-1}$ 6.5 h)	1262(952-1587)	1126(974-1371)	1503(967-1613)	-13 to 16%	-20 to 13%	-19 to 37%

Values are median(interquartile range). *95% confidence interval for the ratio of geometric means.

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

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

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

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

Figure 1

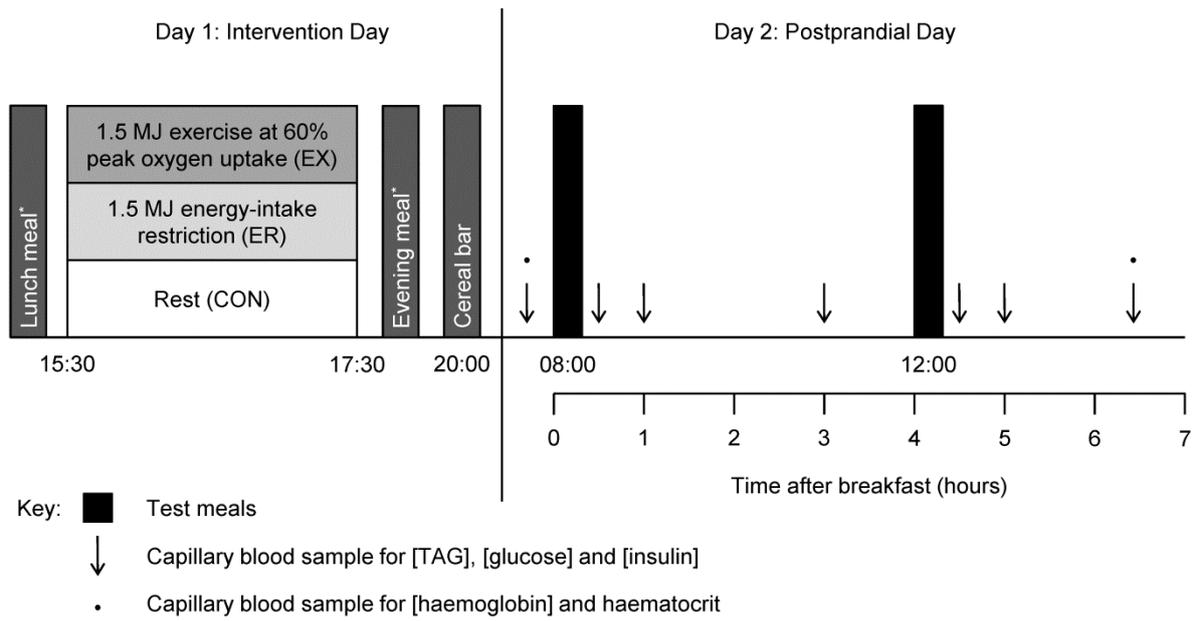


Figure 2

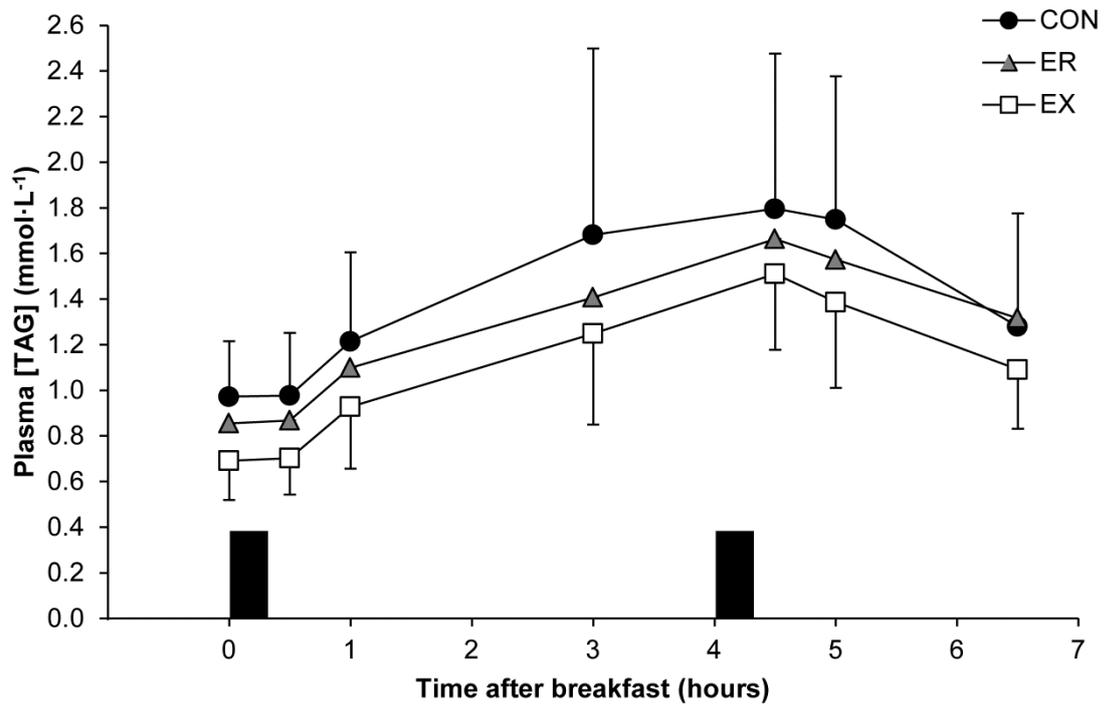


Figure 3

