

Energy replacement diminishes the effect of exercise on postprandial lipemia in boys

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Abstract

Purpose: Acute bouts of exercise reduce postprandial triacylglycerol concentrations ([TAG]) in healthy boys and girls; however, it is not known whether this effect is mediated by the energy deficit. This study examined whether the exercise-induced reduction in postprandial [TAG] persists after immediate dietary replacement of the exercise energy expenditure (EE).

Methods: Eighteen healthy 11- to 13-year-old boys (mean(SD): body mass 41.3(8.4) kg; peak oxygen uptake ($\dot{V}O_2$) 55(5) mL·kg⁻¹·min⁻¹) completed three, 2-day conditions in a within-measures, crossover design separated by 14 days. On day 1, participants rested (CON), exercised at 60% peak $\dot{V}O_2$ inducing a net EE of 32 kJ·kg⁻¹ body mass (EX-DEF) or completed the same exercise with the net EE replaced immediately (EX-REP). 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 meal 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), EX-DEF fasting [TAG] was 19% and 15% lower than CON (-32 to -4%, ES = 1.15, *P* = 0.02) and EX-REP (-29 to 0%, ES = 0.91, *P* = 0.05) respectively; CON and EX-REP were similar (-4%; *P* = 0.59). The EX-DEF total area under the [TAG] versus time curve was 15% and 16% lower than CON (-27 to 0%, ES = 0.55, *P* = 0.05) and EX-REP (-29 to -2%, ES = 0.62, *P* = 0.03) respectively; CON and EX-REP were not different (2%; -13 to 20%, *P* = 0.80). **Conclusion:** Immediate replacement of the exercise-induced energy deficit negates the reduction in postprandial [TAG] in boys; this highlights the importance of maintaining a negative energy balance immediately post-exercise to maximise the metabolic health benefits of exercise.

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

Abbreviations

CI	Confidence interval
CON	Control condition
CPM	Counts per minute
EE	Energy expenditure
ES	Effect size
EX-DEF	Exercise with energy deficit condition
EX-REP	Exercise with energy replacement condition
iAUC	Incremental area under the concentration versus time curve
RPE	Rating of perceived exertion
TAG	Triacylglycerol
TAUC	Total area under variable versus time curve
$\dot{V}O_2$	Oxygen uptake

1. Introduction

Elevated postprandial triacylglycerol concentrations ([TAG]) are predisposed to the development and progression of atherosclerosis [1], and independently predict future cardiovascular disease risk in adults [2]. Although the clinical manifestations of atherosclerotic disease emerge in adulthood typically, the paediatric origins of atherosclerosis are well established [3]. Furthermore, childhood fasting [TAG] predicts young adult cardiovascular disease risk [4]. Most people spend the majority of waking hours in a postprandial state, resulting in extended periods of elevated postprandial [TAG]. Considering cardiovascular disease remains the leading cause of mortality in the United Kingdom [5], prevention by targeting modifiable risk factors is a high priority on the public health agenda. Therefore, lifestyle modifications that reduce postprandial [TAG] from a young age may delay precursors of atherosclerotic disease leading to important long-term metabolic health benefits [3].

Previous research highlights the potency of acute moderate- to vigorous-intensity exercise interventions completed up to 18 h before a standardised meal to reduce postprandial [TAG] in adults [6] and young people [7]. Furthermore, acute exercise has been shown to increase resting fat oxidation in the postprandial period in adults [8,9]. Considering energy status can have profound effects on metabolism [10], the acute exercise-evoked changes in postprandial TAG metabolism may be mediated by the associated energy deficit. In this regard, an exercise-induced energy deficit appears more potent than an isoenergetic diet-induced deficit in reducing postprandial [TAG] in girls [11] and women [12,13]. Moreover, replacement of the exercise-induced energy deficit in adults diminishes or even eliminates the reduction in postprandial [TAG] [8,14–16], and concomitant increase in resting whole-body fat oxidation [8,16]. However, the effect of replacing the exercise-induced energy deficit on postprandial [TAG] and resting whole-body fat oxidation has not been investigated in young people.

Metabolic and hormonal responses to exercise differ considerably between men and boys [17,18], and hormonal changes occurring during pubertal development may influence [TAG] [19]. Consequently, it is important to address whether the acute exercise-induced reduction in postprandial [TAG] and increase in resting whole-body fat oxidation persist after replacing the exercise energy expenditure (EE) in boys.

Therefore, the aim of the present study was to examine the effect of acute moderate-intensity exercise, with and without immediate dietary replacement of the exercise-induced energy deficit, on postprandial [TAG] and resting whole-body fat oxidation in healthy, recreationally active boys.

2. Methods

2.1. Participants

Eighteen boys aged 11.4 to 13.2 years volunteered to participate in this 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. Participants completed a health screen questionnaire which confirmed they were all 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. Physical and physiological characteristics of participants are presented in Table 1.

2.2. 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. Skinfold thickness was measured at two sites (triceps and subscapular) to the nearest 0.2 mm on the right-hand side of the body using Harpenden callipers (Baty International, West Sussex, UK). The median of three measurements at each site was used to estimate percent body fat [20].

Participants undertook a self-assessment of their level of physical maturity using drawings depicting the five stages of genital and pubic hair development [21]. The median (interquartile range) stage of genital development was 3 (1) (stage 1: $n = 3$; stage 2: $n = 5$; stage 3: $n = 10$) and pubic hair development was 2 (1) (stage 1: $n = 2$; stage 2: $n = 11$; stage 3: $n = 5$).

2.3. 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. First, a submaximal incremental treadmill protocol was completed to establish the individual steady-state relationship between treadmill speed, oxygen uptake ($\dot{V}O_2$) and heart rate. Participants completed 4×4 min stages at a starting speed of $5 \text{ km}\cdot\text{h}^{-1}$ and increasing $1 \text{ km}\cdot\text{h}^{-1}$ at the start of each subsequent stage, with the gradient set at 1% throughout. 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.

After 10 min standardised rest, the boys completed an incremental uphill treadmill protocol and verification stage to determine peak $\dot{V}O_2$. The boys ran at a fixed individual speed (8.0 to 10.0 km·h⁻¹), identified from the submaximal incremental treadmill protocol, while the treadmill gradient was increased 1% each minute until volitional exhaustion. The verification stage was completed after a 10 min period of recovery. Participants ran at the same fixed individual speed, but the treadmill gradient was set 2% higher than the final gradient attained during the incremental protocol and the boys ran until volitional exhaustion (typically 2 to 3 min). Expired air samples were collected in one minute intervals using Douglas bags, heart rate was monitored continuously and RPE was recorded in the final 10 s of each minute. 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 gradient). In the absence of a plateau in $\dot{V}O_2$ (3 (17%) 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. 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 conditions.

2.4. Experimental design

Using a within-measures, crossover design, participants completed three, 2-day experimental conditions separated by 14 days: rest control (CON), exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP). The study design is presented schematically in Figure 1.

2.4.1. Day 1: Intervention day

Participants arrived 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 (section 2.4.3). During CON, participants rested in the laboratory for the duration of the visit. During EX-DEF and EX-REP, participants exercised on the treadmill at 60% peak $\dot{V}O_2$ in 20 min intervals separated by a standardised period of 5 min seated rest, which was designed to induce a net EE of 32 kJ·kg⁻¹ body mass. Expired air samples were collected and analysed as described previously (Section 2.3) during the third, tenth and seventeenth minute of each 20 min block to calculate the relative exercise intensity. The treadmill speed was adjusted occasionally to ensure the target exercise intensity was met. The exercise EE and oxidation of carbohydrate and fat were estimated via indirect calorimetry [22], assuming 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 [23]. Heart rate was monitored throughout and RPE was recorded during the last 10 s of each expired air sampling period (Section 2.3). Immediately following the cessation of exercise in EX-REP, the exercise-induced net EE was replaced using a milkshake drink composed of strawberry or chocolate milkshake powder and whole milk. The milkshake was prescribed individually and provided 12.9(2.7) g fat (36(2)% of drink total energy), 39.0(8.3) g carbohydrate (49(3)%), 11.7(2.5) g protein (15(1)%) and 1333(271) kJ energy.

2.4.2. Standardisation of dietary intake and physical activity

Participants weighed, recorded and replicated their habitual dietary intake during the 48 h (pre-intervention and intervention day) before day 2 of all experimental conditions. Two-day food records were analysed using dietary analysis software (CompEat Pro Version 5.8.0, Nutrition Systems, Banbury, UK).

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 composition of the cereal snack bar was 1.4 g fat, 12.3 g carbohydrate and 1.0 g protein, which provided 313 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.

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). Raw ActiGraph data files were analysed using custom made data reduction software (KineSoft Software, version 3.3.76, Loughborough University, UK; <http://www.kinesoft.org>). 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 [24].

2.4.3. Day 2: Postprandial day

Participants arrived at the laboratory at ~07:45 after a 12-h overnight fast and provided a fasting capillary blood sample after 10 min seated rest. They then consumed a standardised breakfast 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, within 20 min, at 4 h (Figure 1). Resting expired air samples were collected after each blood sample for 5 min using Douglas bags (Section 2.3). The expired air data for one boy were spurious so results are presented for

17 boys. 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 and the ingested volume was replicated in the subsequent conditions.

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 (61.1% of meal total energy), 1.8 g carbohydrate (32.5%), 0.4 g protein (6.3%) and 95 kJ energy per kilogram body mass. Lunch consisted of white bread, mild cheddar cheese, butter, potato crisps, whole milk and milkshake powder, and provided 1.0 g fat (47.4%), 2.0 g carbohydrate (40.4%), 0.6 g protein (12.2%) and 81 kJ energy per kilogram body mass.

2.5. *Analytical methods*

For each blood sample, the hand was pre-warmed and up to 600 μL of whole blood was collected into potassium-EDTA coated Microvette CB300 tubes (Sarstedt Ltd, Leicester, UK) after the fingertip was pierced (Unistick 3 Extra, Owen Mumford, UK). The whole blood samples were centrifuged immediately at 12,800 g for 15 min (Eppendorf 5415c, Hamburg, Germany) and 200 μL of plasma was 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). The within-batch coefficients of variation for [TAG] and [glucose] were 2.6 and 0.5% 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 [25].

2.6. *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), glucose (TAUC-glucose) and postprandial whole-body EE and substrate oxidation. The TAUC for substrate oxidation were divided by the total duration of the postprandial period (6.5 h). The incremental area under the concentration versus time curve for TAG (iAUC-TAG) and glucose (iAUC-glucose) was calculated using the same method after adjusting for fasting concentrations.

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 and glucose were natural log transformed prior to analysis. These data are presented as geometric mean (95% confidence interval) and analysis is based on ratios of geometric means and 95% confidence intervals (CI) for ratios.

Linear mixed models repeated for condition were used to examine differences in exercise responses, dietary intake, free living physical activity and sedentary time, resting whole-body EE and substrate oxidation, fasting concentrations and TAUC and iAUC responses.

Differences in postprandial [TAG] 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 [26].

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 [27].

3. Results

3.1. Dietary intake

Energy and macronutrient intakes were not different between the conditions on the pre-intervention day ($P \geq 0.25$). Average daily energy intake was 7.9(1.8) MJ, and dietary intake of protein, carbohydrate and fat was 69.2(21.7) g, 254(62) g and 66.6(24.5) g respectively. Energy and absolute protein, carbohydrate and fat intake on the intervention day were higher in EX-REP compared with CON and EX-DEF (effect size (ES) = 0.60 to 1.22, $P < 0.001$); CON and EX-DEF were similar ($P \geq 0.35$) (Table 2). However, no differences were observed across the conditions once the additional energy and macronutrients consumed in the post-exercise milkshake drink were accounted for (ES = 0.01 to 0.12, $P \geq 0.52$). The contribution of protein, carbohydrate and fat to total energy intake was not different across the conditions ($P \geq 0.42$) (Table 2).

3.2. Free-living physical activity and sedentary time

On the pre-intervention day, differences in CPM across the conditions were identified ($P = 0.09$), with simple planned contrasts revealing EX-REP CPM was 71 counts·min⁻¹ lower compared with CON (-26 to -2%, ES = 0.40, $P = 0.03$). No other differences were seen in physical activity levels or sedentary time across the conditions on the pre-intervention day ($P \geq 0.10$). Physical activity levels and sedentary time on the intervention day are displayed in Table 3. No significant differences were seen across the conditions for daily wear time ($P = 0.45$), sedentary time ($P = 0.52$) or time spent in moderate-intensity activities ($P = 0.76$).

Average CPM were higher than CON by 363 counts·min⁻¹ in EX-DEF (ES = 2.35, $P < 0.001$) and by 343 counts·min⁻¹ in EX-REP (ES = 2.26, $P < 0.001$); EX-REP and EX-DEF were similar (20 counts·min⁻¹; $P = 0.73$). Time spent in light-intensity activities was lower than CON by 40 min in EX-DEF (ES = 0.87, $P = 0.005$) and by 24 min in EX-REP (ES = 0.50, $P = 0.09$); EX-REP and EX-DEF were similar (-16 min; $P = 0.19$). Time spent in vigorous-intensity activities was higher than CON by 43 min in EX-DEF (ES = 2.09, $P < 0.001$) and by 47 min in EX-REP (ES = 2.16, $P < 0.001$); EX-REP and EX-DEF were similar (-4 min; $P = 0.78$). No differences were seen in physical activity levels or sedentary time across the conditions when accounting for the time resting or exercising in the laboratory ($P \geq 0.16$). Therefore, any between condition differences were due to the experimental manipulations and not free-living differences.

3.3. Treadmill exercise responses

Participants exercised at 7.1(0.6) and 7.2(0.6) km·h⁻¹, with total durations of 56.4(5.6) and 56.0(5.5) min in EX-DEF and EX-REP, respectively. This elicited a mean oxygen consumption equivalent to 60(1)% peak $\dot{V}O_2$ in both exercise conditions and average respiratory exchange ratios of 0.85(0.03) and 0.86(0.04) in EX-DEF and EX-REP respectively. Mean heart rates were 160(12) and 160(14) beats·min⁻¹ in EX-DEF and EX-REP respectively, which represented 79(6)% of peak heart rate in both exercise conditions. Mean RPE was 11(3) in EX-DEF and 11(2) in EX-REP, corresponding to 'fairly light' on the scale. The estimated net EEs were 1327(260) kJ (32.0(0.2) kJ·kg⁻¹ body mass) and 1329(272) kJ (31.9(0.3) kJ·kg⁻¹ body mass) in EX-DEF and EX-REP respectively. There were no significant differences between EX-DEF and EX-REP in any of these variables ($P \geq 0.25$).

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

Total resting EE over the 6.5 h postprandial period was not different across the conditions (CON 2.2(0.3) MJ, EX-DEF 2.1(0.3) MJ, EX-REP 2.2(0.3) MJ; $P = 0.35$). The relative contribution of fat oxidation to total resting EE tended to be greater in EX-DEF than CON (63(11) vs. 56(13)%; 95% CI -1 to 16%, ES = 0.60, $P = 0.08$), but EX-REP (57(15)%) was similar to EX-DEF (95% CI -14 to 3%, ES = 0.46, $P = 0.17$) and CON (95% CI -7 to 10%, ES = 0.14, $P = 0.68$) (Figure 2). Reciprocally, the relative contribution of carbohydrate oxidation to total resting EE tended to be lower in EX-DEF than CON (37(11) vs. 44(13)%; 95% CI -16 to 1%, ES = 0.60, $P = 0.08$), but EX-REP (43(15)%) was similar to EX-DEF (95% CI -3 to 14%, ES = 0.46, $P = 0.17$) and CON (95% CI -10 to 7%, ES = 0.14, $P = 0.68$) (Figure 2).

3.5. Fasting plasma [TAG] and [glucose]

Average changes in plasma volume between the fasting and 6.5 h postprandial samples were not different across the conditions (CON 0.9%, EX-DEF 1.4%, EX-REP 0.5%; $P = 0.90$). Therefore, the raw plasma [TAG] and [glucose] were not adjusted prior to statistical analyses. Fasting [TAG] and [glucose] are displayed in Table 4. Linear mixed models identified differences in fasting [TAG] across the conditions ($P = 0.04$). Specifically, EX-DEF fasting [TAG] was 19% and 15% lower than CON (ES = 1.15, $P = 0.02$) and EX-REP (ES = 0.91, $P = 0.05$) respectively; CON and EX-REP were similar (-4%; $P = 0.59$). The significant, but small 2% lower fasting [glucose] in EX-DEF compared with EX-REP (ES = 0.38, $P = 0.05$) was the only difference.

3.6. Postprandial plasma [TAG] and [glucose]

Plasma TAG responses over time and across the three conditions are shown in Figure 3.

Linear mixed models revealed differences in postprandial [TAG] over time across conditions (main effect condition $P < 0.001$; main effect time $P < 0.001$; condition by time interaction $P = 0.97$). Mean EX-DEF postprandial [TAG] was 14% and 16% lower than CON (-19 to -9%, $ES = 0.40$, $P < 0.001$) and EX-REP (-21 to -11%, $ES = 0.45$, $P < 0.001$) respectively; CON and EX-REP were similar (2%; -4 to 8%, $P = 0.51$). The EX-DEF TAUC-TAG was 15% and 16% lower than CON ($ES = 0.55$, $P = 0.05$) and EX-REP ($ES = 0.62$, $P = 0.03$) respectively; CON and EX-REP were not different (2%; $P = 0.80$) (Table 4). Specifically, EX-DEF was lower than CON between 0 to 1 h by 13% (-25 to 1%, $ES = 0.56$, $P = 0.07$), 1 to 4.5 h by 17% (-30 to -2%, $ES = 0.63$, $P = 0.03$) and 4.5 to 6.5 h by 12% (-25 to 5%, $ES = 0.40$, $P = 0.14$); EX-DEF was lower than EX-REP between 0 to 1 h by 13% (-25 to 1%, $ES = 0.54$, $P = 0.07$), 1 to 4.5 h by 16% (-29 to -1%, $ES = 0.60$, $P = 0.03$) and 4.5 to 6.5 h by 18% (-30 to -3%, $ES = 0.62$, $P = 0.02$). No differences in TAUC-TAG subsections were observed between CON and EX-REP ($P \geq 0.39$). The iAUC-TAG was 18% lower in EX-DEF compared with EX-REP ($ES = 0.40$, $P = 0.05$), but CON was similar to EX-DEF ($ES = 0.16$, $P = 0.41$) and EX-REP ($ES = 0.24$, $P = 0.23$) (Table 4).

Individual changes (delta) in TAUC-TAG for EX-DEF and EX-REP relative to CON are shown in Figure 4. The reductions in TAUC-TAG were greater than changes in CON for fourteen (78%) boys in EX-DEF and ten (56%) boys in EX-REP. Positive correlations of changes in fasting [TAG] and TAUC-TAG between CON and the two experimental conditions were strong (both $r^2 = 0.77$, $P < 0.001$). The measured physical and physiological characteristics (Table 1), dietary intake (Table 2), free-living physical activity and sedentary time (Table 3), exercise responses and resting whole-body EE and substrate oxidation (Figure 2) did not account meaningfully for the inter-individual variability in delta TAUC-TAG for

EX-DEF or EX-REP. The Pearson's product moment correlation for the individual changes in TAUC-TAG between EX-DEF and EX-REP was small ($r = 0.38$, $P = 0.12$).

No differences in postprandial [glucose] were seen across the conditions (main effect condition $P = 0.91$; main effect time $P < 0.001$; condition by time interaction $P = 0.97$). No significant or meaningful differences were observed in TAUC-glucose ($P = 0.80$; $ES \leq 0.13$) or iAUC-glucose ($P = 0.70$; $ES \leq 0.27$) across the conditions (Table 4).

4. Discussion

The primary finding from the present study was that immediate replacement of the acute exercise-induced energy deficit negates the reduction in fasting and postprandial [TAG] in boys. Furthermore, an exercise-induced energy deficit was required to promote an increase in postprandial whole-body fat oxidation. Therefore, judicious use of energy replacement practices immediately post-exercise may be required in boys to maximise the metabolic health benefits of exercise. To our knowledge, this is the first study to investigate the effect of energy replacement immediately after exercise on postprandial responses in boys.

The reduction in fasting [TAG] in EX-DEF compared with CON and EX-REP supports the majority of previous findings in young people where the exercise-induced energy deficit was maintained in the post-exercise period [28–31]. Although elevated fasting [TAG] are associated with impaired postprandial metabolism in young people [32], fasting [TAG] are highly variable in children [33], and are less predictive of cardiovascular disease risk than nonfasting concentrations in adults [2]. Therefore, studying postprandial [TAG] may be more informative of cardiovascular health in young people.

The reduction in postprandial [TAG] after EX-DEF supports previous studies employing acute moderate- to vigorous-intensity exercise interventions in young people (1.0 to 2.5 MJ)

[7], and has been linked to the total EE of the exercise session in adults [6]. An exercise-induced energy deficit elicits a greater reduction in postprandial [TAG] than an isoenergetic diet-induced energy deficit in girls [11] and pre- and post-menopausal women [12,13]. Recent research demonstrates that replacing the exercise-induced energy deficit diminishes or eliminates the reduction in postprandial [TAG] following acute moderate- and high-intensity exercise interventions in adults [8,14–16]. In comparison, Chiu and colleagues [34] reported that replacing the exercise-induced energy deficit with a glucose solution did not attenuate the reduction in postprandial [TAG] seen after moderate-intensity exercise. The contrasting results in these studies may reflect differences in the exercise intensity, exercise EE and energy replacement timing after exercise. Furthermore, the macro-nutrient composition of the post-exercise replacement meal may contribute to the subsequent postprandial TAG response the following day [16,34]. In this regard, the milkshake provided in the present study (36% fat, 49% carbohydrate, 15% protein) replaced ~75% of the fat and carbohydrate oxidised during the exercise session, whereas the glucose solution provided in the study by Chiu and colleagues [34] replaced the carbohydrate, but not the fat oxidised during exercise. Although this led the authors to argue that the exercise-induced fat deficit may determine the reduction in postprandial [TAG] [34], it has also been suggested that the carbohydrate deficit plays an important role in determining the TAG lowering effect of exercise [16]. Further exercise postprandial studies are clearly warranted to distinguish the effects of an energy and/or substrate deficit [10]. Nevertheless, the current study is the first to extend these findings to young people by showing that immediate replacement of the exercise net EE counter-acts the exercise-evoked reduction in postprandial [TAG] in boys (Table 4, Figure 3).

The trend for an increase in resting whole-body fat oxidation the day after EX-DEF is in agreement with adult studies reporting accompanying reductions in postprandial [TAG] [8,9,16]. Previous exercise postprandial studies in adults have shown that replacing the

exercise-induced energy deficit attenuates the increase in postprandial whole-body fat oxidation [8,16], but this effect may be dependent on the macro-nutrient composition of the post-exercise meal [16]. Resting whole-body fat oxidation in EX-REP was not statistically different to EX-DEF or CON; however, a thorough appraisal of the mean differences and absolute standardised ES revealed that EX-REP was 2% higher than CON (ES = 0.14, $P = 0.68$) but 6% lower than EX-DEF (ES = 0.46, $P = 0.17$). Therefore, immediate replacement of the exercise-induced EE appears to diminish the elevation in whole-body fat oxidation the following day, suggesting an energy deficit may be required to elicit a meaningful increase in whole-body fat oxidation.

The mechanisms underlying the exercise-induced changes in postprandial lipid metabolism and the interaction with energy deficit in young people are unclear. In adults, elevated whole-body fat oxidation following exercise may facilitate the resynthesis of depleted skeletal muscle and/or hepatic glycogen stores through a number of regulatory mechanisms [35], including enhanced lipoprotein lipase activity promoting increased clearance of circulating TAG [36]. Furthermore, hepatic fatty acid flux may be shifted towards oxidation and away from re-esterification resulting in the secretion of fewer, possibly TAG-rich very low-density lipoproteins with a higher affinity for lipoprotein lipase [37]. Immediate replacement of the exercise-induced energy deficit presumably accelerates hepatic and muscle glycogen replenishment [38,39], and attenuates the reduction in postprandial [TAG] and increase in whole-body fat oxidation that normally follows exercise with energy deficit.

The exercise protocol adopted in EX-DEF and EX-REP meets the current physical activity guidelines for health promotion in children and adolescents [40]. While the clinical significance of our findings cannot be determined, high childhood fasting [TAG] is an independent predictor of young adult cardiovascular disease [4], and physical inactivity is associated independently with the clustering of cardiovascular disease risk factors in

childhood and adolescence [41]. Furthermore, low rates of fat oxidation in adults have been implicated in the pathology of obesity [42] and Type 2 diabetes mellitus [43]. Considering childhood cardiovascular disease risk factors track into adulthood [44], interventions that improve the cardiovascular disease risk factor profile should be paediatric orientated [3]. The majority of the postprandial TAG samples (98%) in the current study were below the 2.3 mmol·L⁻¹ threshold proposed as a desirable concentration in young people [45]. Although it is encouraging that EX-DEF resulted in lower postprandial [TAG] and increased resting whole-body fat oxidation in boys with a predominantly healthy postprandial TAG profile, the impact of post-exercise energy intake should be considered carefully to optimise the metabolic health benefits of exercise.

Similar to previous studies in young people [29,31], considerable inter-individual variation was present in the postprandial TAG response following EX-DEF and EX-REP (Figure 4). This suggests that some boys may still experience reductions in postprandial [TAG] when the exercise-induced energy deficit is replaced; however, an energy deficit may be required to maximise the health benefits of exercise in the majority of boys. Previous studies in young people have been unable to elucidate the underlying factors explaining the substantial individual heterogeneity in postprandial [TAG] [29,31]. In adults, the exercise-induced increase in postprandial whole-body fat oxidation is associated negatively with the postprandial lipemic response [8,16]; however, intervention-induced changes in whole-body fat oxidation were not associated with any index of lipemia in the current study. This suggests that exercise-induced changes in postprandial [TAG] and whole-body fat oxidation may occur independently in boys.

Postprandial [glucose] were not different across the conditions in the current study, supporting the majority of previous studies in young people [e.g., 28,30,31]. The boys in the

present study demonstrated a healthy postprandial glucose profile in all three experimental conditions, suggesting the boys exhibited excellent glycemic control.

The present study is limited by the absence of EE quantification during the 5 min rest periods between exercise intervals in EX-DEF and EX-REP, and excess post-exercise oxygen consumption was not measured following the exercise sessions. Although the contribution to the net EE is likely to be relatively small [46], an underestimation of the net EE and incomplete replacement of the energy deficit in EX-REP cannot be ruled out.

5. Conclusion

In conclusion, the findings of the present study demonstrate that immediate replacement of the acute exercise-induced energy deficit eliminates the reduction in fasting and postprandial [TAG] the following day in healthy boys. In addition, meaningful increases in postprandial whole-body fat oxidation appear dependent on the presence of an energy deficit.

Consequently, maintaining a negative energy balance immediately after exercise may be required to maximise the beneficial effect of exercise on postprandial metabolism early in life. Further research is required to determine whether the macronutrient composition of the post-exercise meal is an important determinant of next day postprandial responses to exercise.

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Disclosure Statement

The authors report no conflict of interest.

Author Contributions

AET, LAB, and KT designed the study; AET conducted the research; AET and KT analysed and interpreted the data; AET wrote the manuscript; LAB and KT provided critical revisions to the manuscript; AET, LAB and KT read and approved the final manuscript.

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Table 1 Physical and physiological characteristics

Age (years)	12.3 (0.5)
Body mass (kg)	41.3 (8.4)
Stature (m)	1.50 (0.07)
Body mass index ($\text{kg}\cdot\text{m}^{-2}$)	18.1 (2.4)
Percent body fat (%)	16.5 (5.6)
Lean body mass (kg)	34.1 (4.7)
Genital development*	3 (1)
Pubic hair development*	2 (1)
Peak oxygen uptake ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)	55 (5)

Values are mean (SD) for $n = 18$

*Self-assessment – median (interquartile range)

Table 2 Energy and macronutrient intakes during the intervention day in the control (CON), exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions.

	CON	EX-DEF	EX-REP	CON vs. EX-DEF 95% CI*	CON vs. EX-REP 95% CI*	EX-REP vs. EX-DEF 95% CI*
Energy (MJ·day ⁻¹)	7.0 (1.4)	7.0 (1.6)	8.4 (1.4)	-0.2 to 0.3	1.1 to 1.7 ^b	-1.6 to -1.1 ^c
Protein (g·day ⁻¹)	55.6 (14.0)	56.5 (14.5)	67.5 (13.4)	-0.9 to 2.5	10.0 to 13.5 ^b	-12.7 to -9.2 ^c
CHO (g·day ⁻¹)	224 (36)	225 (45)	268 (41)	-9 to 10	34 to 52 ^b	-52 to -33 ^c
Fat (g·day ⁻¹)	60.4 (19.6)	61.0 (21.0)	72.8 (18.4)	-2.7 to 4.2	8.9 to 15.8 ^b	-15.1 to -8.1 ^c
% energy intake from protein	13 (3)	14 (3)	14 (2)	-0.2 to 0.5	-0.2 to 0.5	-0.4 to 0.3
% energy intake from CHO	55 (5)	54 (6)	54 (4)	-1.3 to 0.6	-1.5 to 0.3	-0.7 to 1.1
% energy intake from fat	32 (6)	32 (6)	32 (5)	-0.6 to 1.0	-0.4 to 1.2	-1.0 to 0.6

Values are mean (SD) for $n = 18$. Values for EX-REP include the post-exercise milkshake drink. *95% confidence interval of the mean absolute difference between the experimental conditions.

CHO, carbohydrate.

^b Significant difference between EX-REP and CON ($P < 0.001$)

^c Significant difference between EX-DEF and EX-REP ($P < 0.001$)

Table 3 Physical activity levels and sedentary time during the intervention day in the control (CON), exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions.

	CON	EX-DEF	EX-REP	CON vs. EX-DEF 95% CI*	CON vs. EX-REP 95% CI*	EX-REP vs. EX-DEF 95% CI*
Daily wear time (min)	823 (778 to 871)	798 (755 to 844)	828 (783 to 876)	-9 to 3%	-6 to 7%	-10 to 3%
Counts per minute	451 (407 to 499)	814 (736 to 900)	794 (718 to 879)	57 to 108% ^a	53 to 103% ^b	-11 to 18%
Sedentary activity (min)	407 (362 to 457)	372 (331 to 418)	390 (347 to 439)	-22 to 7%	-18 to 12%	-19 to 12%
Light activity (min)	303 (275 to 334)	263 (238 to 290)	279 (253 to 308)	-21 to -5% ^a	-16 to 1%	-14 to 3%
Moderate activity (min)	92 (73 to 117)	82 (65 to 104)	84 (66 to 107)	-37 to 26%	-35 to 29%	-31 to 37%
Vigorous activity (min)	6 (4 to 9)	49 (32 to 77)	53 (34 to 82)	436 to 1255% ^a	471 to 1343% ^b	-41 to 49%

Values are geometric mean (95% confidence interval) for $n = 18$. Statistical analyses are based on natural log transformed data. *95% confidence interval for the ratio of geometric means.

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

^b Significant difference between EX-REP and CON ($P < 0.05$)

Table 4 Fasting and postprandial plasma triacylglycerol and glucose concentrations in the control (CON), exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions.

	CON	EX-DEF	EX-REP	CON vs. EX-DEF 95% CI*	CON vs. EX-REP 95% CI*	EX-REP vs. EX-DEF 95% CI*
Triacylglycerol						
Fasting (mmol·L ⁻¹)	0.61 (0.54 to 0.70)	0.49 (0.43 to 0.56)	0.58 (0.51 to 0.67)	-32 to -4% ^a	-19 to 13%	-29 to 0% ^c
TAUC (mmol·L ⁻¹ 6.5 h)	6.61 (5.59 to 7.81)	5.63 (4.76 to 6.66)	6.74 (5.70 to 7.97)	-27 to 0% ^a	-13 to 20%	-29 to -2% ^c
iAUC (mmol·L ⁻¹ 6.5 h)	2.79 (2.19 to 3.56)	2.58 (2.02 to 3.28)	3.14 (2.46 to 4.00)	-24 to 13%	-8 to 37%	-33 to 0% ^c
Glucose						
Fasting (mmol·L ⁻¹)	5.90 (5.71 to 6.10)	5.83 (5.64 to 6.02)	5.96 (5.77 to 6.15)	-3 to 1%	-1 to 3%	-4 to 0% ^c
TAUC (mmol·L ⁻¹ 6.5 h)	45.0 (43.2 to 46.8)	44.5 (42.8 to 46.3)	44.5 (42.8 to 46.3)	-5 to 3%	-4 to 3%	-4 to 4%
iAUC (mmol·L ⁻¹ 6.5 h)	9.12 (7.31 to 11.37)	8.21 (6.58 to 10.24)	8.37 (6.71 to 10.44)	-31 to 18%	-30 to 20%	-25 to 29%

Values are geometric mean (95% confidence interval) for $n = 18$. 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 EX-DEF and CON ($P \leq 0.05$)

^c Significant difference between EX-DEF and EX-REP ($P \leq 0.05$)

Figure legends

- Figure 1** Diagram of the 2-day study protocol. TM, treadmill; $\dot{V}O_2$, oxygen uptake; TAG, triacylglycerol. [†]Evening meal replicated from the first condition.
- Figure 2** Postprandial whole-body fat and carbohydrate oxidation expressed as a percentage of the total energy expenditure (EE) in the control (CON), exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions ($n = 17$). Fat and carbohydrate oxidation were estimated using stoichiometric equations, assuming that the contribution from protein was negligible [22]. Values represent the total area under the substrate oxidation versus time curve divided by the duration of the postprandial period (6.5 h).
- Figure 3** Fasting (F) and postprandial plasma triacylglycerol concentrations ([TAG]) in the exercise with energy-replacement (EX-REP), control (CON) and exercise with energy deficit (EX-DEF) conditions ($n = 18$). Values are mean (SD). Black rectangles denote consumption of breakfast and lunch meals at 08:00 and 12:00, respectively. Main effect condition $P < 0.001$; main effect time $P < 0.001$; condition by time interaction $P = 0.97$.
- Figure 4** Individual changes (delta) in the total area under the plasma triacylglycerol (TAG) concentration versus time curve (TAUC) between the exercise with energy deficit (EX-DEF) and exercise with energy replacement (EX-REP) conditions compared with the control condition (CON): A) EX-DEF minus CON; B) EX-REP minus CON. Participant data are organised 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.

Figure 1

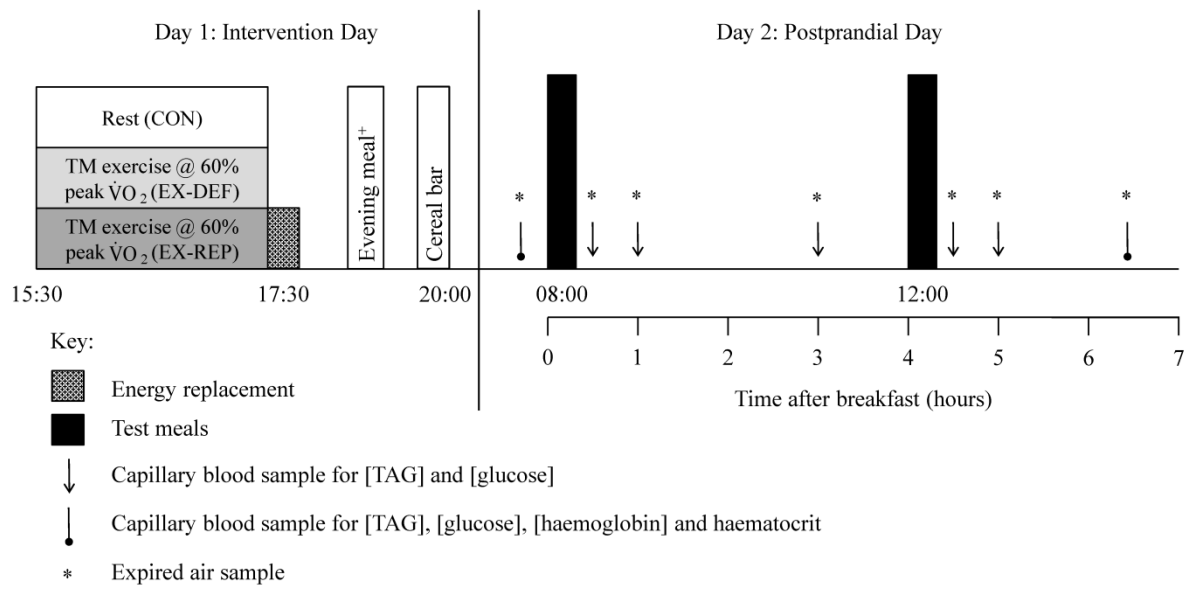


Figure 2

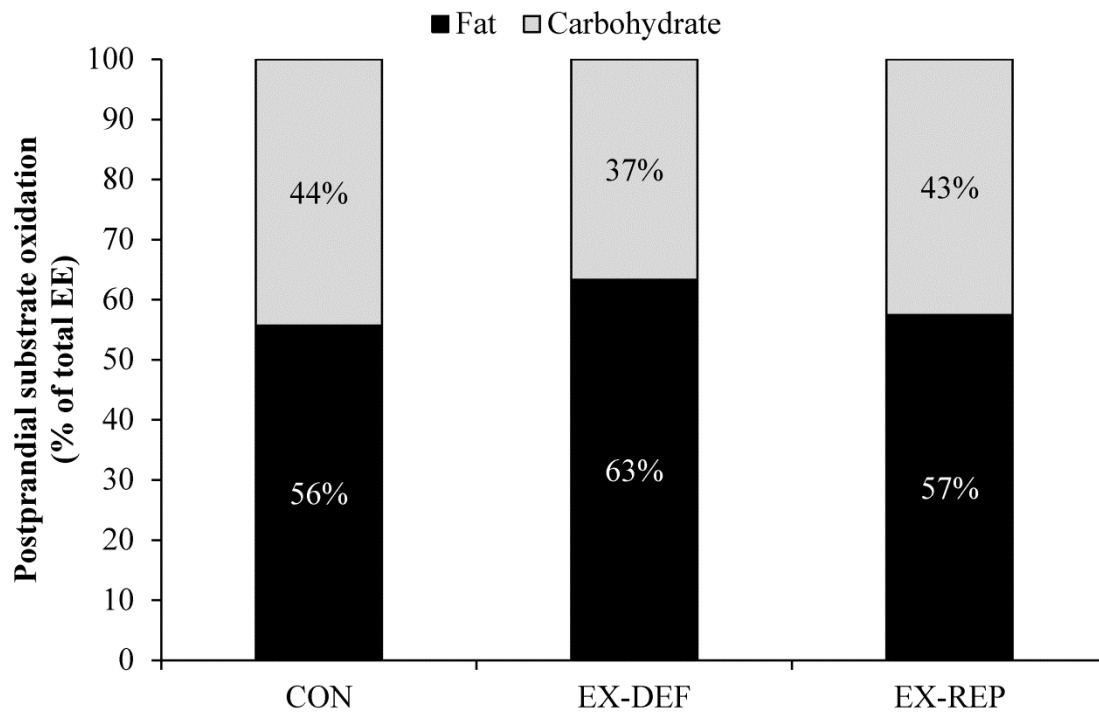


Figure 3

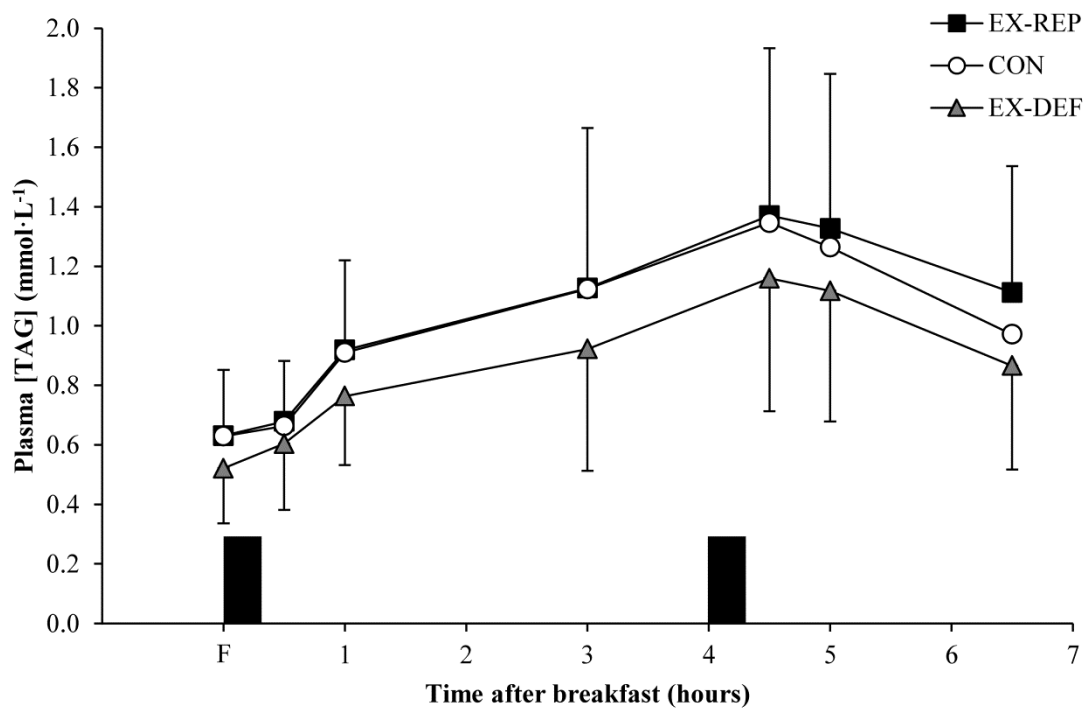


Figure 4

