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Metadata Record: [https://dspace.lboro.ac.uk/2134/15117](https://dspace.lboro.ac.uk/2134/15117)

Version: Accepted for publication

Publisher: Lippincott, Williams and Wilkins / © American College of Sports Medicine

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Acute effect of Fatmax exercise on the metabolism in overweight and non-overweight girls

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Running title: Effect of exercise on metabolism in girls

Conflicts of Interest and Source of Funding: We thank Tanita Grant-in-Aid for funding the research. No conflicts of interests are reported.
Abstract

Introduction: Acute exercise can reduce postprandial insulin concentrations and increase fat oxidation in adults, which may have important implications for insulin resistance and weight control. However, similar studies with young people or comparing overweight and non-overweight individuals are sparse. Therefore, the acute effect of Fatmax exercise on glucose, insulin and fat oxidation was examined in 12 overweight (OW) and 15 non-overweight (NO) girls. Methods: Participants completed two 2 d conditions in a counter-balanced order. On day one, participants either expended 2.09 MJ (500 kcal) during treadmill exercise at individual Fatmax (EX) or 0.47 MJ (112 kcal) during rest (CON). On day two, capillary blood and expired air samples were taken in the fasting state and at regular intervals for 2 h after high glycaemic index (HGI) breakfast consumption. Subsequently, blood glucose and plasma insulin concentrations were determined and fat oxidation was estimated. Results: Blood glucose was similar between conditions in both groups (P>0.05). Fasting plasma insulin (P=0.047) and total area under the 2 h curve (TAUC, P=0.049) were reduced for EX compared with CON in the NO, but not OW girls (P>0.05). Fasting fat oxidation was higher for EX than CON in the NO girls (P=0.036) and fat oxidation TAUC was higher for EX in both groups of girls (P≤0.05). Conclusion: A bout of Fatmax exercise performed ~16 h before HGI breakfast consumption reduced fasting and postprandial insulin concentrations in NO girls and increased fat oxidation in both OW and NO girls. The higher post-intervention energy and CHO intake in the OW compared with NO girls or differences in metabolism between the two groups may have compromised potential exercise-induced reductions in insulin the OW girls.

Key words: fat oxidation, glucose, insulin, children, adolescents
Introduction

**Paragraph 1** Mounting evidence has shown that high rates of fat oxidation may protect against insulin resistance and weight gain; this is currently a topic of great interest (8, 12, 15). Elevated insulin concentrations and insulin resistance have emerged as serious health concerns in children and adolescents (young people), particularly those with high levels of adiposity (34). Such metabolic complications are exacerbated by the consumption of breakfasts containing high glycaemic index (HGI) carbohydrates, which induce exaggerated glucose and insulin responses (41). Ultimately, this is concerning since the postprandial state contributes to the development of chronic disease (11). Therefore, interventions to improve insulin sensitivity, reduce insulin concentrations and increase fat oxidation in young people are required.

**Paragraph 2** Exercise training can increase insulin sensitivity and fat oxidation in young people (4, 33). In adults, however, it is also well established that such improvements in metabolism are largely a consequence of the acute effects of exercise rather than long-term training adaptations (6, 19). Furthermore, studying the acute effect of exercise reflects the metabolic responses of individuals who do not participate in regular exercise training. Studies in adults have reported that a single bout of aerobic exercise can increase insulin sensitivity (29), reduce postprandial insulin concentrations (6, 20) and increase fat oxidation the next day (6, 13, 32), although the effect on glucose is less clear (6, 20). Fat oxidation and insulin sensitivity may be related; high rates of fat oxidation may reduce the accumulation of fatty acid metabolites (e.g., diacylglycerol, ceramides) within the muscle that interfere with insulin signalling and, consequently, improve insulin sensitivity (12). Importantly, a single bout of exercise can increase the partitioning of fatty acids toward intramuscular triglyceride (IMTG) synthesis, reduce the accumulation of fatty acid metabolites and improve insulin sensitivity in
adults (32). This is relevant for obese young people, as markers of fatty-acid induced insulin resistance are present even in this population (34). In this respect, it is plausible that exercise at Fatmax (the individual exercise intensity corresponding to peak fat oxidation) is particularly beneficial.

Paragraph 3 Despite concerns of overweight and insulin resistance in young people and the well recognised improvements in metabolism after a single exercise bout in adults, studies examining the acute effect of exercise on glucose, insulin and whole body fat metabolism in young people are not available. There are clear differences in metabolism between young people and adults (30) and also between overweight (OW) and non-overweight (NO) young people (3). Thus, the interaction between exercise-induced changes in metabolism and weight status in young people requires examination. Research of this nature may also be best focused on girls, as physical activity levels are lower in girls compared with boys (31) and girls are less likely to eat breakfast daily (39); accordingly, strategies to increase these healthy lifestyle behaviours should specifically target this population. Therefore, the present study examined the acute effect of Fatmax exercise on blood glucose, plasma insulin and fat oxidation in the fasting state and after HGI breakfast consumption in OW and NO girls.
Methods

Participants

**Paragraph 4** After gaining approval from the University Ethical Advisory Committee, 12 OW and 17 NO girls aged 9 to 14 y participated in the study. Two of the NO girls did not complete the 2.09 MJ (500 kcal) exercise bout and were, therefore, removed from statistical analyses. Overweight status was defined using age and sex specific body mass index (BMI) reference points (7). Written informed consent was obtained from the parent and written assent from the participant. The girls were screened using a health history questionnaire; exclusion criteria included: known congenital heart disease, musculoskeletal problems, uncontrolled exercise-induced asthma, diabetes and epilepsy. Stature was measured using a stadiometer (Holtain, Holtain Limited, Dyfed, UK) to the nearest 0.01 m and body mass (BM) to the nearest 0.1 kg using a beam balance scale (Seca Model 888, Hamburg, Germany). Skinfold thickness was measured to the nearest 0.2 mm using a Harpenden skinfold calliper (Baty International, West Sussex, UK) and measurements were taken from three different sites (triceps, subscapular and medial calf) on the right hand side of the body. Each site was measured three times by the same investigator and the median value for each site was used to estimate percentage body fat according to Slaughter et al. (35); fat free mass (FFM) in kg was estimated subsequently. Waist circumference was measured (27) using a Guillick tape measure (Creative Health Products, Plymouth, MI). With the assistance of a parent, the girls provided a self-assessment of their physical maturation using secondary sexual characteristics (37).

Expired air and indirect calorimetry

**Paragraph 5** Expired air was sampled continuously during preliminary exercise tests, for 10 min periods during the 2.09 MJ (500 kcal) exercise bout and for 5 min periods during rest
after the participant lay supine on a bed for 20 min. The flowmeter was attached to a facemask of an appropriate size and breath-by-breath data were displayed online using a gas analysis system (Metalyzer 3B, Cortex, Leipzig, Germany). Calibration procedures were carried out prior to each experimental test, as described previously (42). Breath-by-breath data were interpolated into 1 s intervals for all tests and individual \( \dot{V}O_2 \) and \( \dot{V}CO_2 \) values that were >3 standard deviations (SD) from the mean were removed (22), as were respiratory exchange ratio (RER) values >1. Subsequently, substrate oxidation rates were estimated using stoichiometric equations, with the assumption that the urinary nitrogen excretion rate was negligible and participants were in steady state (9).

**Preliminary measurements**

**Paragraph 6** Participants were familiarised with treadmill (RunRace, TechnoGym, Gambettola, Italy) walking and running before completing two preliminary tests. First, an uphill incremental treadmill test was used to measure peak oxygen uptake (\( \dot{V}O_{2\text{peak}} \)). Treadmill speed (OW 7.1(0.8), NO 8.5(0.4) km·h\(^{-1}\)) was determined for each individual following habituation, in order to attain an exercise duration of 8 to 12 min for all participants. After a 2 min warm-up at 0% gradient, the gradient was increased by 1% each min until volitional exhaustion. The girls were asked to refrain from strenuous exercise and caffeine on the day and food intake 2 h prior to testing. In the absence of a plateau in \( \dot{V}O_2 \), maximal effort was verified using secondary criteria: heart rate (HR) levelling off ≥200 beats·min\(^{-1}\) and RER ≥1.05, in addition to clear subjective symptoms of fatigue. The highest 30 s moving average during the exercise test was recorded as \( \dot{V}O_{2\text{peak}} \).

**Paragraph 7** On a separate occasion, the girls completed a 4 min incremental exercise test to estimate Fatmax. Treadmill speed (OW 4.1(0.3), NO 4.6(0.3) km·h\(^{-1}\)) was determined
individually and based on data from the peak exercise test, in order to ensure each participant was exercising below 80% $\dot{V}O_2$peak throughout. The speed increased by 0.5 km·h⁻¹ every 4 min (1% gradient). Tests were terminated when the RER exceeded 0.95 or the participant was exercising above 80% $\dot{V}O_2$peak. Average $\dot{V}O_2$ and $\dot{V}CO_2$ values from the final min of each stage were used to estimate fat oxidation. Subsequently, Fatmax (% $\dot{V}O_2$peak) was estimated using individual best-fit polynomial curves of fat oxidation rate against % $\dot{V}O_2$peak (42) and the time taken to oxidise 2.09 MJ (500 kcal) at Fatmax was estimated.

Experimental conditions

**Paragraph 8** All participants completed two 2 d conditions in a counter-balanced order (Figure 1). On day one, the girls either expended 2.09 MJ (500 kcal) during treadmill exercise at individual Fatmax (EX) or rested for an equivalent (matched) time period (CON). The exercise was divided into three equal bouts for each participant to aid exercise adherence. During exercise and rest, expired air samples were collected every 20 min and energy expenditure was estimated using stoichiometric equations (9). If required, treadmill speed could then be adjusted accordingly to ensure the girls were exercising at the % $\dot{V}O_2$peak corresponding to individual Fatmax. Treadmill speed was adjusted for six OW (average change ±0.2 km·h⁻¹) and seven NO (average change ±0.4 km·h⁻¹) girls; consequently, exercise duration was re-calculated to ensure an exercise energy expenditure of 2.09 MJ. On day two, the girls reported to the laboratory at 08:00 after a 12 h overnight fast. After fasting measures, they consumed a mixed breakfast meal consisting of foods containing HGI carbohydrates and providing 1 g CHO·kg BM⁻¹ within 15 min. The nutritional content of the breakfast was calculated from information provided by the manufacturer. For a 50 kg participant, the breakfast contained 31.4 g cornflakes, 100.0 g skimmed milk, 34.3 g white bread, 4.3 g margarine, 6.4 g jam and 114.3 g water (calculated GI=73). The GI values for
individual foods were taken from the International Table of GI and Glycemic Load Values (2) and the GI was calculated from the weighted means of the GI values for the component foods (40). On average, the breakfast contained 1385 kJ (331 kcal) for the OW and 955 kJ (228 kcal) for the NO girls. The macronutrient content was 75% CHO, 12% fat and 13% protein. The 2 h postprandial period commenced immediately after breakfast consumption, during which the girls lay supine and capillary blood and expired air samples were collected at regular intervals.

Control of diet and physical activity

**Paragraph 9** With the assistance of a parent, the girls were asked to record their weighted food and drink intake in the 48 h period prior to the Fatmax exercise test and replicate this diet prior to the main experimental trials (EX and CON). As Fatmax is affected by pre-exercise CHO consumption (1), this ensured Fatmax exercise was prescribed under the same conditions as it was measured. As the Fatmax exercise test did not involve a ‘day two’, the girls were required to add their ‘post-intervention diet’ to their food diary on the evening of day one of their first main trial, so that this could also be replicated prior to their second main trial.

Menstrual cycle phase

**Paragraph 10** Ten of the participants (four OW and six NO) had commenced menstruation and completed their experimental conditions 48 h apart to minimise the potential influence of menstrual cycle phase on within-subject comparisons. We attempted to conduct experimental conditions during the early follicular phase (days one to seven) to reduce between-subject variability. However, five (two OW and three NO) of the girls completed their experimental conditions during the early follicular phase, two (two NO) during the late luteal phase and it
was not possible to determine menstrual cycle phase in the remaining three (two OW and one NO) girls due to irregularities in menstrual cycle.

**Blood sampling and analysis**

*Paragraph 11* Capillary blood samples were taken in the fasting state and postprandially at 15, 30, 45, 60, 90 and 120 min from a pre-warmed hand by finger prick using the Unistik 2 single-use lancing device (Owen Mumford, Oxford, UK) and Microvette CB300 EDTA coated capillary blood collection tubes (Sarstedt Ltd, Leicester, UK). Duplicate 25 µl aliquots of whole blood were deproteinised in 250 µl of ice cooled perchloric acid (PCA; 2.5%), centrifuged for 4 min at 2415 × g and stored at -20°C for blood glucose analysis. The remaining whole blood was centrifuged for 4 min at 2415 × g. Plasma was then extracted and stored at -20°C for insulin analysis. Blood glucose concentration was determined spectrophotometrically using the glucose oxidase method (GOD-PAP, Randox, Crumlin, Ireland). Plasma insulin was measured in duplicate using an enzyme-linked immunosorbent assay (ELISA, Mercodia, Uppsala, Sweden). The total 2-h area under the curve (TAUC) for blood glucose and plasma insulin were calculated using the trapezium rule (40). The homeostasis model assessment for insulin resistance (HOMA-IR) (26) was calculated from fasting glucose and insulin. The intra-assay coefficient of variation (CV) for the duplicate samples was 2.0% for blood glucose and 5.8% for plasma insulin.

**Statistical analyses**

*Paragraph 12* Statistical analyses were completed using SPSS software version 18.0 for Windows (SPSS Inc, Chicago, IL, USA). Shapiro-Wilk and Levene’s tests were used to verify normal distribution and homogeneity of variance, respectively. Greenhouse-Geisser correction was used when sphericity could not be assumed. Student’s independent t-tests
were used to compare participant characteristics between the OW and NO groups. Condition by group (2 x 2) mixed measures ANOVA with condition as the repeated factor were used to examine the fasting, peak and TAUC values for glucose, insulin and fat oxidation. Values are expressed as mean(SD), unless stated otherwise, and effect sizes (ES) were calculated for differences that were significant or approached significance for the main outcome variables (i.e., blood glucose, plasma insulin and substrate oxidation). Statistical significance was accepted at P≤0.05.
Results

Participant characteristics

*Paragraph 13* Participant characteristics are displayed in Table 1. Body fat, BMI, body mass, waist circumference and hip circumference were higher in OW compared with NO girls (P<0.003), whereas \( \dot{V}O_{2\text{peak}} \) (mL·kg\(^{-1}\)·min\(^{-1}\)) (P<0.0005) and Fatmax (% \( \dot{V}O_{2\text{peak}} \)) (P=0.024) were higher in NO girls. Two of the OW girls were insulin resistant (HOMA-IR > 3.16) (16).

Energy expenditure and energy intake

*Paragraph 14* During the 2.09 MJ (500 kcal) Fatmax exercise bout, the OW girls exercised at 54(8)% and the NO girls exercised at 63(12)% \( \dot{V}O_{2\text{peak}} \) (P=0.039). Exercise duration was 73(20) min for the OW and for 67(18) min for the NO girls (P=0.422). During the time-matched resting condition, the OW girls tended to expend more energy than the NO girls (0.51(0.11) vs. 0.43(0.12) MJ; P=0.079).

*Paragraph 15* Average daily energy and macronutrient intake in the 48 h prior to EX and CON was similar between the OW and NO girls (P>0.05). However, day one post-intervention (17:30 – 20:00) energy intake was higher in the OW compared with NO girls (4.11(1.87) vs. 2.96(0.84) MJ; P=0.042), whilst CHO (146(85) vs. 101(35) g; P=0.076) and fat (31(12) vs. 23(10) g; P=0.079) intake tended to be higher. The counter-balanced assignment to EX and CON did not affect post-intervention energy or macronutrient intakes between OW and NO nor within OW and NO.

Blood glucose concentration

*Paragraph 16* Blood glucose responses are shown in Figure 2. Postprandial blood glucose concentration increased and peaked at a median (interquartile range) time of 15(15) min for
all conditions, except in the OW CON condition where it peaked at 30 min in four girls and 60 min in one girl (median 22.5(15) min). Fasting, peak, average postprandial and TAUC for blood glucose were similar between EX and CON in both groups of girls (P>0.05) and there were no differences in blood glucose concentrations between the OW and NO girls (P>0.05) (Table 2).

**Plasma insulin concentration**

*Paragraph 17* Plasma insulin responses are shown in Figure 3. Postprandial plasma insulin concentration increased and peaked at a median (interquartile range) time of 15(15) min for both conditions and groups. Fasting (P=0.047, ES: 0.50), average postprandial (P=0.047, ES: 0.50) and TAUC (P=0.049, ES: 0.50) for plasma insulin were lower for EX compared with CON in the NO girls, but there was no difference in peak postprandial plasma insulin (P=0.263). All measures of plasma insulin were similar between conditions in the OW girls (P>0.05). Although not significantly different between conditions, HOMA-IR was 15% lower for EX in the NO girls (P=0.125, ES: 0.40), but 9% higher in the OW girls (P=0.663). Fasting, peak, average postprandial and TAUC for plasma insulin concentration (P≤0.0005) and HOMA-IR (P=0.022, ES: 0.44) were higher in the OW compared with NO girls (Table 2).

**Substrate oxidation**

*Paragraph 18* Fat oxidation (mg·kgFFM\(^{-1}\)·min\(^{-1}\)) for both conditions and groups is displayed in Figure 4. Fasting fat oxidation was higher (P=0.036, ES: 0.53) for EX compared with CON in the NO girls, but similar between conditions in the OW girls (P=0.790). The TAUC for fat oxidation was higher for EX in the NO (P=0.005, ES: 0.66) and OW girls (P=0.040, ES: 0.57). Similarly, the average postprandial rate of fat oxidation was higher for EX in the NO (P=0.003, ES: 0.69) and OW girls (P=0.044, ES: 0.57). When expressed relative to total
energy expenditure (% EE), fasting (P=0.024, ES: 0.56), average postprandial (P=0.016, ES: 0.59) and TAUC (P=0.021, ES: 0.57) for fat oxidation were higher in EX compared with CON in NO, but not OW girls (P>0.05) (Table 2).

Similarly, in the NO girls, CHO oxidation (mg·kgFFM⁻¹·min⁻¹) was lower in EX compared with CON in the fasting state (P=0.021, ES: 0.57) and the TAUC tended to be lower (P=0.093, ES: 0.43), although there was no difference in the average postprandial CHO rate (P=0.167, ES: 0.36). No difference in fasting, postprandial or TAUC for CHO oxidation was found between conditions in the OW girls (P>0.05). Likewise, fasting (P=0.024, ES: 0.56), average postprandial (P=0.017, ES: 0.59) and TAUC (P=0.016, ES: 0.59) for CHO oxidation as % EE were lower in EX compared with CON in the NO girls, but similar between conditions in the OW girls (P>0.05). Substrate oxidation (mg·kgFFM⁻¹·min⁻¹ and % EE) was similar between groups (P>0.05) (Table 2).
Discussion

Paragraph 19 The main findings from the present study were that 2.09 MJ (500 kcal) of Fatmax exercise performed ~16 h before HGI breakfast consumption reduced fasting and postprandial insulin concentrations in NO girls, with no change in blood glucose concentrations. However, exercise did not affect glucose or insulin concentrations in the OW girls. Furthermore, an increase in fat oxidation the day after Fatmax exercise was observed in both the OW and NO girls. To our knowledge, this is the first study to demonstrate these acute exercise-induced metabolic effects in young people.

Paragraph 20 Reductions in insulin ~16 h after a single exercise bout in the NO girls are in agreement with studies in adults (5, 20). However, similar studies in young people do not appear to be available for comparison and few studies have directly examined the potential effect of weight status on this relationship. We were able to identify only one study that has reported glucose and insulin concentrations 12 to 14 h after exercise in young people, although this study was not specifically designed to examine these health markers (24). In support of our findings, 2.51 MJ (600 kcal) of exercise did not affect fasting and postprandial glucose or insulin in overweight adolescent boys; although, in contrast to our study, insulin concentrations were also not reduced in the non-overweight boys (24). However, this previous study included a high-fat breakfast meal and only three blood samples were taken during the immediate 2 h postprandial period (24); thus, a direct comparison between our study and the available literature in young people is limited. In contrast to our findings, exercise performed ~16 h before breakfast reduced postprandial insulin in both overweight and non-overweight women (28) and in obese, but not non-obese, men (10). All participants exercised at the same intensity and duration in these adult studies (10, 28), whereas the girls in the present study expended a set amount of energy; the higher exercise energy expenditure
in the obese men might explain why only they experienced an exercise-induced reduction in postprandial insulin concentration (10). Differences in participant characteristics, breakfast size and composition, and post-exercise energy and macronutrient intake may have also contributed to these disparate findings. In addition, differences in metabolism between young people and adults may have resulted in discrepancies between our study and some of the adult-based literature (30).

**Paragraph 21** Exercise-induced reductions in insulin concentrations in the NO girls may be attributed to enhanced insulin sensitivity. Studies in adults have shown that insulin sensitivity increases up to 72 h after exercise to facilitate muscle glycogen replenishment. The major cellular mechanism controlling this increased insulin sensitivity appears to be increased GLUT-4 translocation to the plasma membrane (38), although other mechanisms have emerged more recently (23). The exercise-induced reduction in insulin with no change in glucose indicates improved insulin sensitivity the morning after exercise in the NO girls; a lower insulin concentration was needed to control the rise in glucose. Although the reduction in HOMA-IR after exercise compared with rest was not statistically significant, it may have been physiologically important and meaningful enough to alter postprandial insulin. Exercise-induced reductions in postprandial insulin with no change in HOMA-IR have also been shown in adults (6, 13), whereas others have reported reduced HOMA-IR (5). It is possible that more sensitive measures of insulin sensitivity (e.g., glucose clamp methods) were required to detect potential differences in insulin sensitivity in our study.

**Paragraph 22** A bout of Fatmax exercise also increased fat oxidation the next day in the OW and NO girls, which appears to be another novel finding in young people and is consistent with studies in overweight (6) and non-overweight (32) adults. Several cellular mechanisms
may contribute to this increased fat oxidation, including increased muscle lipoprotein lipase activity (17), reduced pyruvate dehydrogenase activity (18) and possibly, similar to GLUT-4, the translocation of specific fatty acid transporters to the plasma membrane (21). In adults, acute exercise increased the partitioning of fatty acids toward oxidation and IMTG synthesis, reduced the accumulation of fatty acid metabolites within skeletal muscle and suppressed activation of proinflammatory pathways known to impair insulin action (32). This suggests that elevations in fat oxidation after exercise may have contributed to the reduced insulin concentrations in the NO girls. Furthermore, reducing exercise fat oxidation through the ingestion of a lipolysis inhibitor abolished the exercise-induced reduction in postprandial insulin in men (25). It is, therefore, conceivable that the high rates of fat oxidation during Fatmax exercise can augment potential exercise-induced enhancements in insulin sensitivity. However, a study comparing the acute effect of exercise at Fatmax and exercise eliciting low rates of fat oxidation in young people is required for a more complete understanding of this relationship. The lower insulin concentrations after exercise may have also enhanced lipolysis and contributed to the elevated fat oxidation in the NO girls (14). Interestingly, unlike the NO girls, the OW girls did not experience an increase in fat oxidation as a proportion of total energy expenditure (% EE), suggesting that increased total energy expenditure at least partly explained the increased fat oxidation in the OW girls; thus different mechanisms may control the increased fat oxidation observed the day after exercise in the two groups.

**Paragraph 23** Importantly, exercise-induced reductions in insulin in the NO girls and elevations in fat oxidation in both groups occurred following just a single exercise bout and despite the maintenance of habitual diet, suggesting that these extend to a ‘real world’ setting where diet is not prescribed. Reduced postprandial insulin concentration indicates improved glucose control and may have implications for the prevention of insulin resistance and related
chronic disease. Although insulin was not affected in the OW girls, elevations in fat oxidation may have long term implications for facilitating insulin sensitivity (15) and weight control (8). Future research should consider the time-course of these metabolic improvements in young people, which may persist up to 72 h post-exercise (19). Similarly, the proximity of exercise to breakfast consumption or metabolic assessment may affect exercise-induced changes in metabolism in adults (19, 23), a finding that also requires investigation in young people.

**Paragraph 24** Since insulin concentrations were markedly higher in the OW compared with NO girls, interventions to reduce insulin may be particularly relevant for this population. A number of plausible reasons could explain why exercise did not affect insulin in this group. Dietary analysis indicates that post-exercise energy and/or CHO replacement may have facilitated muscle glycogen restoration and attenuated potential exercise-induced reductions in insulin in the OW girls, as previously reported in adults (6, 29). Indeed, post-intervention energy intake was 72% higher and CHO intake was 69% higher in the OW compared with NO girls and may have resulted in positive energy and CHO balance, although we did not measure energy and CHO balance directly. Interestingly, the OW girls consumed more energy and CHO regardless of whether they completed EX or CON first, which suggests that this dietary ‘compensation’ was not specifically exercise-induced, but may be related to body size or composition. It is also possible that our study design did not permit changes in insulin in the OW girls. We asked all participants to expend a set amount of energy during exercise, expressed in absolute terms rather than relative to body mass to reduce between-participant variability in exercise duration. Consequently, the OW girls expended less energy relative to body mass, which may not have been sufficient to affect insulin in the heavier participants. We also purposefully chose a HGI breakfast known to induce exaggerated postprandial glucose and insulin responses, particularly in OW compared with NO girls (41). Therefore, it
is possible that the exercise was not sufficient to influence the response to this breakfast specifically. Finally, between-group differences in metabolism, such as HOMA-IR, could also underpin the contrasting findings between the OW and NO girls. Although the inclusion of two girls classified as insulin resistant in the OW group did not meaningfully affect our findings, it has been suggested that a single exercise bout may not be a large enough stimulus to increase insulin action in those with reduced fitness and limited cellular mechanisms that might be required to enhance insulin action after exercise (13) and there is evidence that obese young people have a reduced ability to increase insulin sensitivity during a high-CHO diet (36). Future research should address these issues in OW and NO young people by manipulating exercise energy expenditure and post-exercise energy and CHO intake.

Paragraph 25 In conclusion, a bout of Fatmax exercise performed 16 h prior to HGI breakfast consumption reduced fasting and postprandial insulin in NO girls and elevated fat oxidation in both OW and NO girls. Importantly, these metabolic improvements were observed after just a single bout of exercise (2.09 MJ) and despite the maintenance of habitual diet. The higher post-intervention energy and CHO intake in the OW girls and/or metabolic differences between the two groups may have compromised potential exercise-induced reductions in insulin concentrations in the OW girls. Further examination of these issues in young people is warranted.
Acknowledgements
We thank Tanita Grant-in-Aid for funding the research and all of the participants and their parents for their commitment to our study.

Conflict of interest
No conflicts of interests are reported. No companies or manufacturers will benefit from the results of the present study. The results of the present study do not constitute endorsement by the American College of Sports Medicine.
References


Legends

FIGURE 1 – Schematic of 2-d protocol for experimental conditions.

FIGURE 2 – Blood glucose concentration response in EX and CON for the overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min.

FIGURE 3 – Plasma insulin concentration response in EX and CON for the overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min.

FIGURE 4 – Fat oxidation in EX and CON for the overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min.
TABLE 1. Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>OW (n=12)</th>
<th>NO (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>12(1)</td>
<td>12(1)</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>61.2(20.3)</td>
<td>42.2(7.9)</td>
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<tr>
<td>Stature (m)</td>
<td>1.53(0.10)</td>
<td>1.54(0.09)</td>
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<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>25.8(5.2)</td>
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</tr>
<tr>
<td>Estimated body fat (%)</td>
<td>35.2(6.2)</td>
<td>19.1(5.1)</td>
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<tr>
<td>Estimated fat free mass (kg)</td>
<td>39.0(10.3)</td>
<td>34.0(5.5)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>77.5(13.6)</td>
<td>61.7(4.9)</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>90.4(15.7)</td>
<td>74.9(8.5)</td>
</tr>
<tr>
<td>Pubic hair</td>
<td>3(1)</td>
<td>3(2)</td>
</tr>
<tr>
<td>Treadmill ( \dot{V}O_2 \text{peak} ) (mL·kg⁻¹·min⁻¹)</td>
<td>41(6)</td>
<td>51(4)</td>
</tr>
<tr>
<td>Fatmax (% ( \dot{V}O_2 \text{peak} ))</td>
<td>52(10)</td>
<td>63(12)</td>
</tr>
</tbody>
</table>

OW – overweight; NO – non-overweight; pubic hair – self-assessment (37); \( \dot{V}O_2 \text{peak} \) – peak oxygen uptake

\(^a\) significant difference between OW and NO (P≤0.05)

\(^b\) median (interquartile range)
## TABLE 2. Summary of fasting and postprandial responses

<table>
<thead>
<tr>
<th></th>
<th>OW (n=12)</th>
<th>NO (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EX</td>
<td>CON</td>
</tr>
<tr>
<td>Glucose (mmol·L⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>3.79(0.42)</td>
<td>3.81(0.32)</td>
</tr>
<tr>
<td>Peak</td>
<td>5.80(0.46)</td>
<td>5.83(0.61)</td>
</tr>
<tr>
<td>Average postprandial</td>
<td>4.88(0.53)</td>
<td>4.92(0.52)</td>
</tr>
<tr>
<td>2h TAUC</td>
<td>9.52(0.63)</td>
<td>9.60(0.62)</td>
</tr>
<tr>
<td>Insulin (pmol·L⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting a</td>
<td>96(112)</td>
<td>92(79)</td>
</tr>
<tr>
<td>Peak a</td>
<td>745(377)</td>
<td>712(325)</td>
</tr>
<tr>
<td>Average postprandial a</td>
<td>419(253)</td>
<td>439(256)</td>
</tr>
<tr>
<td>2h TAUC a</td>
<td>769(411)</td>
<td>809(451)</td>
</tr>
<tr>
<td>HOMA-IR a</td>
<td>2.90(3.91)</td>
<td>2.65(2.34)</td>
</tr>
<tr>
<td>Fat oxidation (mg·kgFFM⁻¹·min⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>2.01(0.65)</td>
<td>1.96(0.78)</td>
</tr>
<tr>
<td>Average postprandial</td>
<td>1.63(0.42)</td>
<td>1.41(0.39)c</td>
</tr>
<tr>
<td>2h TAUC</td>
<td>6.19(2.86)</td>
<td>4.80(2.55)c</td>
</tr>
<tr>
<td>CHO oxidation (mg·kgFFM⁻¹·min⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>4.87(0.98)</td>
<td>5.18(1.32)</td>
</tr>
<tr>
<td>Average postprandial</td>
<td>6.47(1.27)</td>
<td>6.89(1.72)</td>
</tr>
<tr>
<td>2h TAUC</td>
<td>78.4(25.3)</td>
<td>90.2(37.8)</td>
</tr>
<tr>
<td>Fat oxidation (% total EE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>42(12)</td>
<td>41(17)</td>
</tr>
<tr>
<td>Average postprandial</td>
<td>31(10)</td>
<td>28(10)</td>
</tr>
<tr>
<td>CHO oxidation (% total EE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>58(12)</td>
<td>59(17)</td>
</tr>
<tr>
<td>Average postprandial</td>
<td>69(10)</td>
<td>72(10)</td>
</tr>
</tbody>
</table>

OW – overweight; NO – non-overweight; EX – exercise condition; CON – control condition; TAUC – total area under the curve; HOMA-IR - homeostasis model assessment-insulin resistance; EE – energy expenditure.

a significant difference between OW and NO (P≤0.05)

b significant difference between EX and CON within group for NO (P≤0.05)

c significant difference between EX and CON within group for OW (P≤0.05)
Figure 1  Schematic of 2-d protocol for experimental conditions.
Figure 2  Blood glucose concentration response in EX and CON for the overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min.
Figure 3 Plasma insulin concentration response in EX and CON for the overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min.
Figure 4  Fat oxidation in EX and CON for the overweight (OW) and non-overweight (NO) girls. Breakfast was consumed between 0 and 15 min.