**Postprandial triacylglycerol in adolescent boys: a case for moderate exercise**

Keith Tolfrey[1], Alex Doggett[1], Craig Boyd[1], Susan Pinner[1], Adam Sharples[1], and Laura Barrett[2]

[1]Research Institute for Health and Social Change (RIHSC), Department of Exercise and Sport Science, Manchester Metropolitan University, Alsager, UK
[2]Exercise and Health Research Group, School of Sport and Exercise Sciences, Loughborough University, Loughborough, UK

Corresponding author:
Dr. Keith Tolfrey
Research Institute for Health and Social Change (RIHSC)
Department of Exercise and Sport Science
MMU Cheshire
Alsager
ST7 2HL
UK
☎ + 44 1509 223283
Fax + 44 1509 226301
⌨ K.Tolfrey@lboro.ac.uk

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ABSTRACT

**Purpose:** To compare the effects of 60 min bouts of intermittent moderate and vigorous exercise on postprandial plasma triacylglycerol (TAG) metabolism in eight healthy, adolescent boys (mean(SD) age 13(0.3) y. **Methods:** Participants completed three conditions in a counter-balanced order. On day one, they either rested for 110 min (CON), completed $6 \times 10$ min blocks of intermittent treadmill exercise at 53% peak $\dot{V}O_2$ (MOD), or $6 \times 10$ min blocks at 75% peak $\dot{V}O_2$ (VIG). On day two following a 12 h fast, a capillary blood sample was taken for [TAG] and [glucose] ($mmol \cdot L^{-1}$) and then a high fat milkshake was consumed ($1.50 \ g \cdot kg^{-1}$ fat, $1.22 \ g \cdot kg^{-1}$ CHO, and $0.22 \ g \cdot kg^{-1}$ protein; $80 \ kJ \cdot kg^{-1}$). Further blood samples were taken every hour over a 6 h postprandial rest period for [TAG] and [glucose]. **Results:** Estimated energy expenditure was 45% higher in VIG than MOD (95% CI 23 to 72%). Fasting [TAG] and [glucose] did not differ between the conditions. Average [TAG] over the postprandial period was lower by 24% in MOD (95% CI -47 to 9%, P = 0.06) and 21% in VIG (95% CI -42 to 8%, P = 0.08) than CON, with no meaningful difference (4%; 95% CI -27 to 48%, P = 0.50) between MOD and VIG. The total area under the [TAG] vs. time curve ($mmol \cdot L^{-1}$ 6 h) was lower by 24% in MOD (95% CI -42 to 0%, P = 0.05) and 20% in VIG (95% CI -37% to 0%, P = 0.07) than CON. MOD and VIG were not different to each other (4%; 95% CI -18 to 32%, P = 0.54). **Conclusion:** Both 60 min of moderate and vigorous intermittent exercise reduced postprandial [TAG]. However, the extra energy expended in the vigorous condition did not produce a dose-related reduction compared with the moderate intensity condition.

INTRODUCTION

**Paragraph Number 1** Even in the absence of clinically overt symptoms, the paediatric origins of atherosclerosis are well established (17). The progression of nascent fatty streaks to occlusive thrombotic fibrous plaques is a complicated multifactorial process that normally takes place over several decades (6). However, it has been suggested that preventive measures should be considered early in life (12,22). An elevated concentration of plasma triacylglycerol ([TAG]) postprandially has been linked to atherogenic progression (26). It has been shown repeatedly in adults that this postprandial lipaemia (PPL) may be attenuated by an acute bout of exercise up to 16 hours prior to the consumption of a fatty meal (19,25). In contrast, it has only been possible to locate a single study that sought to identify whether a similar exercise induced reduction in PPL might be evident during adolescence (4). In this study, both continuous walking (-14%) and intermittent-games activity (-26%) resulted in reductions in the total area under the plasma [TAG] versus time curve in these normolipidaemic 15 year old boys compared with a non-exercise control condition. Two independent groups of boys completed the different exercise bouts in this study so any direct comparisons between them are likely to be confounded by inter-individual differences in metabolism. Furthermore, the intermittent exercise bout differed in both intensity and duration compared with the continuous walking; thus, it is difficult to identify what might have been the primary cause of the disparate effects on PPL in these late adolescent boys.

**Paragraph Number 2** Current recommendations for young people suggest that 60 min of at least moderate intensity physical activity should be accumulated on five or more days a week (7,9). The reduction in PPL seen after an hour of walking at ~59%

peak $\dot{V}O_2$ (4) provides initial evidence that this might have some credence where TAG metabolism is concerned in young people. However, this has yet to be confirmed in any other studies within this population and extrapolation beyond a single acute exercise bout has yet to be demonstrated. Furthermore, a recent study with a random selection of 1732 children and adolescents suggested that just one hour per day of moderate intensity physical activity may not be sufficient to prevent clustering of cardiovascular disease risk factors that are increasingly prevalent in young people (3). Despite the shift in emphasis from exercise for fitness to physical activity for health that occurred in the 1990s, there are still some strong advocates of vigorous intensity work (e.g., 30). Therefore, rather than increasing the amount of moderate activity it might be prudent to increase the intensity whilst maintaining the recommended daily duration of 60 min. Consequently, this study was designed to compare the effects of 60 min of moderate and vigorous intermittent exercise on PPL in adolescent boys. This work was an advance on that published previously (4) as a within measures research design was used to reduce inter-individual variability and total exercise time was fixed at 60 min in both experimental conditions. Although this did not allow us to isolate the independent effect of exercise intensity on PPL, this research design was chosen purposely as it reflects current physical activity recommendations for young people.

METHODS

**Participants**

**Paragraph Number 3** Thirteen adolescents (four girls) volunteered to participate in this study. A parent of each participant gave their written informed consent whilst written and verbal assent was also provided by the adolescents. All study procedures

were approved by the University Research Ethics Committee. Results are presented for eight boys because two of the girls did not adhere to the fasting protocol asked of them whereas the two other girls and one boy did not complete the 60 min of intermittent vigorous exercise. Information from a general health and physical activity questionnaire indicated that half of the boys participated in organised sports and regular physical activity of a general nature. In contrast the remaining boys classified themselves as sedentary. The baseline participant characteristics are shown in Table 1.

INSERT TABLE 1 ABOUT HERE

**Preliminary measurements**

**Paragraph Number 4** A self-reported general health questionnaire indicated that the boys appeared to be in good overall health. They were not taking any substances known to influence lipid or carbohydrate metabolism and no reported contraindications to moderate or vigorous exercise were evident. Body mass was measured to the nearest 0.1 kg (Seca, Hamburg, Germany) and stature to the nearest 0.01 m (Holtain, Crosswell, UK) with the boys clad in running shorts and socks. The measurement of body mass was repeated on the first day of each experimental condition in order to calculate the quantity of milkshake required to induce the lipaemic response on day two (below). Skinfold thickness was measured to the nearest 0.2 mm using Harpenden callipers (John Bull, St. Albans, UK). All measurements were taken from the right hand side of the body, with the median of three measurements calculated as the fold thickness. Triceps and subscapular skinfold thicknesses were used to estimate the boys' percent body fat (%BF) using maturation, race, and sex-specific equations (27). A self-assessment of secondary sexual

characteristics by the boys was used to estimate physical maturity. The boys used drawings of the five stages of genitalia and pubic hair development to provide this information (24). The parents were asked to assist the boys with this assessment by (i) discussing the schematic illustrations with them and (ii) comparing their son's genital and pubic hair development with the schematics and accompanying written descriptions.

**Paragraph Number 5** Before any further data were collected, the boys completed a structured habituation session on the treadmill in a temperature-controlled laboratory (18 to 21$^{o}$C). This session lasted between 20 and 30 min depending on prior experience and subjectively determined participant confidence. After a 30 min rest period each participant completed $6 \times 4$ min steady rate exercise bouts with the treadmill inclined at 1%. The speed for the initial jog was 6.0 km·h$^{-1}$, increments of 0.5 km·h$^{-1}$ were used to increase the intensity progressively for subsequent bouts, and a one min standing rest period interrupted each bout. Heart rate (HR) was monitored continuously via radio telemetry (Polar Accurex Plus, Kempele, Finland) whenever the boys exercised on the treadmill and ratings of perceived exertion (RPE) were measured using the 6-20 scale (8) in the final 15 seconds of exercise bouts. Expired gas samples were collected into 100 L Douglas bags (Cranlea and Company, Birmingham, UK) during the final min of each progressive bout. Oxygen and carbon dioxide concentrations in each Douglas bag were analysed using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Servomex 1400, Sussex, UK) calibrated against gases of known concentration before each series of six bags. The volume of expired gas was determined using a dry gas meter (Harvard, Kent, UK). For each sample, oxygen consumption ($\dot{V}O_2$), expired carbon dioxide ($\dot{V}CO_2$),

minute ventilation ($\dot{V}_E$), and respiratory exchange ratio were calculated. These submaximal $\dot{V}O_2$ data were used subsequently to, (i) choose an appropriate speed for each individual at which their peak $\dot{V}O_2$ could be determined (below), and (ii) to establish the relationship between $\dot{V}O_2$ and treadmill speed to provide an initial estimate for the two experimental exercise conditions described below.

**Paragraph Number 6** On a separate day, after an initial warm-up, the boys ran at a fixed speed while the treadmill belt was raised by 1% each min until volitional exhaustion. Expired gas samples were collected into Douglas bags in each successive min and analysed as described previously. Both HR and RPE were measured as described previously. To verify an exhaustive effort, each participant satisfied all of the following criteria on termination of the treadmill test due to volitional exhaustion: (i) a plateau in $\dot{V}O_2$ ($\leq 3\%$) with an increase in treadmill gradient; (ii) a maximum heart rate ($HR_{max}$) $\geq 95\%$ of age-predicted maximum (220- chronological age); and (iii) respiratory exchange ratio $\geq 1.10$. It is worth noting that an equipment failure invalidated the initial peak $\dot{V}O_2$ data for three boys in the sample. Although this error did not influence any of the other values reported in this study, its influence on the data was not identified until after these boys had completed the moderate intensity exercise condition (below). Consequently all eight of the participants completed repeat peak $\dot{V}O_2$ tests using the methods described above and this peak value was used to determine the relative intensity at which the two experimental exercise conditions were completed. This accounts for the relatively large variance in the moderate experimental condition (% peak $\dot{V}O_2$) (Table 2) compared with the vigorous condition.

**Experimental study design and procedures**

**Paragraph Number 7** A within-measures design was used in which the boys completed three separate conditions each separated by a fixed period of 14 days. A two-day model was used for each of these conditions. On day one the participants either, (a) rested in the laboratory for 110 min (CON); (b) completed 60 min of intermittent exercise designed initially to elicit a moderate intensity of 60% peak $\dot{V}O_2$ (MOD); or (c) completed 60 min of intermittent exercise designed to elicit a vigorous intensity of 75% peak $\dot{V}O_2$ (VIG). An intermittent exercise model had to be used because initial pilot work with the participants suggested that they would not be able to sustain 60 min of continuous exercise at the required intensities. The exercise was on a motorised treadmill (Woodway, Waukesha, USA) and the three conditions were completed at 17:20 on day one in a counter-balanced order across the sample. Each 60 min bout of exercise was completed in $6 \times 10$ min blocks separated by passive rest periods of equal duration. During each 10 min interval of exercise, samples of expired gas were collected in the fourth and tenth min and analysed using the procedures described previously to verify the relative exercise intensity and to estimate exercise energy expenditure (EE; 35). The treadmill speed was adjusted periodically throughout each condition in an effort to match the target exercise intensities of 60% and 75% peak $\dot{V}O_2$ for MOD and VIG respectively (Table 2). The boys recorded their nutritional intake and physical activity in the 48 h period leading up to day two of the first assigned experimental condition. This included any meals and drinks consumed in the period following the experimental manipulation on day one of first assigned condition. This information was used to replicate their diet and activity patterns for the two subsequent conditions. The boys were reminded verbally of this requirement to replicate their nutritional intake just prior to the second and third conditions

respectively. In addition, the boys were asked to minimise their engagement in physical activity, other than the prescribed treadmill exercise, in this 48 hour period; however, no measurements were taken to verify this. Before leaving the laboratory on day one of each experimental condition, the boys were reminded that they could drink plain water but should not consume any food after 19:45 that evening. They were also asked to remain as inactive as possible after leaving the laboratory in an effort to minimise this as an extraneous factor on PPL measured during day two.

INSERT TABLE 2 ABOUT HERE

**Paragraph Number 8** Day two for each condition was identical in that the boys arrived at the laboratory at 07:45 following a 12 hour fast. After providing an initial fasting capillary blood sample, a high fat test milkshake was consumed within 10 min and then six further blood samples were taken at hourly intervals. The timing of the postprandial period commenced when the boys started to consume the milkshake (08:00) and was standardised so that it occurred ~14.7 h after the treadmill exercise or rest period finished the previous day. During this postprandial period the boys were asked to remain seated throughout whilst they read, played on a computer games console, or watched DVD films. One litre of plain water was provided and the boys were asked to drink this in small quantities divided equally over the six hours. A schematic representation of the design is provided in Figure 1.

INSERT FIGURE 1 ABOUT HERE

**Paragraph Number 9** The milkshake was a 2:1 mix of vanilla dairy ice cream and double cream with 10 g of either powdered strawberry or chocolate flavour added. It provided 1.50 g of fat (70% of total energy), 1.22 g of carbohydrate (25%), and 0.22 g of protein (5%) per kilogram of body mass (80 kJ·kg$^{-1}$). None of the boys reported any problems when consuming the milkshake or during the 6 h postprandial period.

**Analytical methods**

**Paragraph Number 10** The fasting and postprandial capillary blood samples were used to quantify [TAG] and glucose concentration ([glucose]). Haematocrit and haemoglobin concentration were determined from the fasting and final samples to estimate change in plasma volume (10). Prior to each capillary blood sample the whole hand was warmed for five min in water heated to 40$^{o}$C whilst the participant remained seated. The hand was dried thoroughly and cleaned with a steret before the tip of the finger was pierced (Softclix Pro, Basel, Switzerland). After the initial drop had been discarded, between 300 and 600 µL of whole blood was collected into potassium-EDTA coated microvette CB 300 tubes (Sarstedt Ltd, Leicester, UK) and then centrifuged immediately at 12,800g for 15 min (Eppendorf 5410, Hamburg, Germany). Plasma was separated immediately after centrifugation and 20 µL were removed and then diluted 50 times by the addition of 980 µL of ice-cold saline (0.9%; 4) to prevent any freeze-drying effect as a result of storage. This procedure was repeated so that two aliquots of diluted plasma were stored at -70$^{o}$C for three months for subsequent analysis by enzymatic, colourimetric methods (Randox Laboratories Ltd) with the use of a centrifugal analyser (Cobas Mira Plus, Roche, Basel, Switzerland). The predilution procedure precluded the dilution step of the assay when [TAG] and [glucose] were measured and three times the sample volume

recommended in the Randox kit assay procedure was used. This resulted in the concentration of the sample for analysis being the same as that in the original assay procedure (4). The within-batch coefficients of variation for [TAG] and [glucose] using the methods described above were 2.6% and 2.7% respectively.

**Statistical analyses**

**Paragraph Number 11** The data were stored and analysed using the Statistical Package for the Social Sciences (SPSS for Windows Version 12.0.1; SPSS Inc., Chicago, USA). Descriptive statistics (means (SD)) representing the physical and physiological characteristics at baseline (Table 1) were calculated for the 8 boys for whom complete data were available. The Shapiro-Wilk test was used to check all data for normality. All data were normally distributed (P>0.05) precluding any transformation prior to inferential analyses. Homogeneity of variance was checked using Mauchly's test of sphericity and if this assumption was violated the degrees of freedom were adjusted accordingly (Greenhouse-Geisser). Differences in relative exercise intensity at which MOD and VIG were completed were confirmed with paired Student's t-tests (Table 2) and 95% confidence intervals (CI) for the mean paired differences were calculated. These procedures were also used to analyse potential changes in haemoglobin concentration and haematocrit. The total six hour area under the plasma concentration versus time curves for TAG (TAUC-TAG) and glucose (TAUC-glucose) were calculated using the trapezium rule for each experimental condition. Incremental versions of these were also calculated after accounting for respective fasting concentrations (IAUC-TAG and IAUC-glucose) across the conditions. The TAUC, IAUC, and fasting concentrations for TAG and glucose were compared across the experimental conditions using separate one-way

within measures ANOVA. Separate 3 x 7 (condition by time) within measures ANOVA were used to identify differences in [TAG] and [glucose] over the postprandial period. *A priori* simple contrasts with CON as the reference category were used to follow up any significant main effects from the omnibus ANOVAs. Bivariate relationships between TAUC, IAUC, and variables shown in Table 2 were quantified using Pearson's product moment correlation. The 95% CI for mean absolute and percentage differences between conditions and for correlations were calculated using the *t* distribution and n – 1 degrees of freedom. Percentage changes over time for paired results were calculated after the data had been transformed using a natural logarithm. The mean percentage difference was then obtained from $100 \cdot (e^{\text{mean difference}} - 1)$. All results are presented as mean (SD).

RESULTS

**Intermittent exercise**

**Paragraph Number 12** The intermittent treadmill exercise data (Table 2) show that VIG was considerably more strenuous than MOD when expressed in both absolute and relative terms. Subsequently, the mean estimated exercise EE was 45% higher in VIG compared with MOD (95% CI 23 to 72%). As mentioned previously, the larger % peak $\dot{V}O_2$ variance for MOD was a function of the enforced peak $\dot{V}O_2$ reassessments due to equipment failure. For six of the eight boys, MOD ranged from 53 to 65% peak $\dot{V}O_2$, but the two remaining boys only exercised at 38 and 39% in this condition. Consequently, the relationship between MOD EE (kJ) and the six hour IAUC-TAG was strong despite the sample size (Figure 2; r = -0.88 (95% CI -0.47 to -0.98), P = 0.004). Changes in both haemoglobin concentration and haematocrit from the fasting to the 6 hour postprandial sample were small (ranging from -2.8% to 1.7%

across the experimental conditions) precluding a correction in [TAG] and [glucose] to account for changes in plasma volume.

INSERT FIGURE 2 ABOUT HERE

**Plasma Triacylglycerol (TAG)**

**Paragraph Number 13** Fasting [TAG] were not different between the three conditions (Table 3; P = 0.33). Changes in plasma [TAG] over time and across the three experimental conditions are shown in Figure 3. The plasma [TAG] was lower in both MOD and VIG compared with CON (main effect condition, P = 0.04; main effect time, P ≤ 0.0005; condition by time interaction, P = 0.60). The percentage differences (95% CI) in [TAG] over the entire study period were: MOD vs. CON -24% (-47 to 9%, P = 0.06); VIG vs. CON -21% (-42 to 8%, P = 0.08); VIG vs. MOD 4% (-27 to 48%, P = 0.50). *A priori* simple contrasts at 3 hours postprandially showed that the [TAG] was 32% lower for MOD (95% CI -52 to -4%; P = 0.03) and 30% lower for VIG (95% CI -49 to -4%; P = 0.04) compared with CON, but not different to each other (mean difference 3%, 95% CI -22 to 37%; P = 0.50). This postprandial sample time was chosen because it has been shown previously that peaks in [TAG] following a high fat test meal are likely to occur after approximately 3 hours. The ANOVA analysis for the TAUC-TAG values between conditions was significant (P = 0.04). The 95% CIs for the relative differences (%, Table 4) suggest that both MOD and VIG were different to CON (Table 4). Although the ANOVA analysis for IAUC-TAG was not significant (P = 0.08), the 95% CIs for the paired comparisons between conditions suggest that this might have been a sample size artefact (Table 4).

**Plasma Glucose**

**Paragraph Number 14** The ANOVA analysis for fasting [glucose] was not significant (P = 0.17). Changes in plasma [glucose] over time and across the three experimental conditions are shown in Figure 4. The plasma [glucose] was similar across the three conditions (main effect condition, P = 0.20; main effect time, P ≤ 0.0005; condition by time interaction, P = 0.06). The alpha value for the interaction reflected the lower glucose concentration at 1 hour in VIG compared with CON and MOD. However, no other between condition differences were evident across the six hour postprandial period. Neither TAUC- nor IAUC-glucose were different between the three conditions (Table 4).

DISCUSSION

**Paragraph Number 15** The main finding from the current study was that single 60 min bouts of intermittent moderate or vigorous intensity exercise completed ~15 hours before consuming a high fat milkshake reduced PPL compared with rest in adolescent boys. However, the changes in TAG metabolism were not amplified by the greater EE or more intense exercise characteristic of the VIG condition compared with MOD. As far as we are aware, this is the first study to compare directly the effect of two different exercise conditions on PPL in adolescent boys. The novel features of this work are that the exercise bouts were based on the most common current internationally recognised population specific guidelines for daily physical activity and the within measures research design allowed direct comparison of the three conditions.

**Paragraph Number 16** The evidence linking exercise EE to reduced PPL in adults is well established (16,19,25). The large differences in exercise intensity and EE between MOD and VIG suggested that a greater attenuation in PPL would have been anticipated in the exercise requiring the greater effort. In contrast, the effects on TAG metabolism were very similar when compared with CON. Interestingly, the wider within-condition EE variation seen in MOD shared a strong relationship with IAUC-TAG (Figure 2). This implies that a dose-response might exist during adolescence, yet it did not surface when comparing MOD with VIG. There is some evidence supporting a dose-response between postprandial metabolism and EE (16). In this study, 1.5 and 3.1 MJ were expended at 50% of $\dot{V}O_2$ max resulting in 9% and 23% reductions in TAUC-TAG respectively in 11 pre-menopausal women. The only other study that has included adolescents as participants suggested that increased exercise EE between two exercise conditions was probably the cause of the 12% greater attenuation in PPL (-14% vs. -26%; 4). In this study, however, the intermittent-games activity precluded a valid estimation of EE and the between participants design meant that any direct comparisons between the different experimental conditions may have been confounded by inter-individual differences. In the current study, the reductions in TAUC-TAG and IAUC-TAG in MOD are larger than those after 60 min of uphill walking at ~59% peak $\dot{V}O_2$ reported by Barrett et al. (4). The relative (kJ·kg$^{-1}$) exercise EE was similar between the studies, but the 50 min of passive rest interspersed with the $6 \times 10$ min exercise bouts far exceeded the 9 min permitted in the Barrett et al. (4) study. Altena et al. (2) suggested that the greater excess post exercise oxygen consumption (EPOC) during these rest periods may influence PPL. Given $\dot{V}O_2$ was not measured during the periods of passive rest, empirical evidence to

support this hypothesis is still not available. Moreover, EPOC should have been higher in VIG than MOD, yet the reductions in PPL were similar.

**Paragraph Number 17** Measures to standardise dietary intake and physical activity in our study and Barrett's (4) were similar, as was the relative contribution of fat to total energy (~70%) in the high fat meal. However, the total energy supplied by the high fat meal and the absolute quantity of fat consumed were both greater in the current study. Despite these differences, the lipaemic response to the meal in the eight 12 year old boys was quite small across the three conditions; of the 168 individual samples [TAG] $\leq$ 1 mmol·L$^{-1}$ in 82%. Some parallels might be drawn with a recent study of nine healthy, young men who consumed a test meal that had only a moderate proportion of fat (35%) (20). A small net lipaemic response was given as the probable cause for the non-significant reduction in the IAUC-TAG after exercise (20). Although the test meal in the current study is consistent with most others in the field, the fasting postprandial [TAG]s and areas under the plasma concentration curves were considerably lower than those reported for the older boys by Barrett et al. (4), yet similar to Kolifa et al. (20). The comparison of MOD and VIG with CON is dependent to a large extend on the CON response. Given this appeared to be blunted, compared with that reported by Barrett et al. (4), this may have limited the extent to which it was possible for the boys to experience a reduction in PPL in the two exercise conditions.

**Paragraph Number 18** Ethical restrictions when working with healthy adolescents do not allow measurements that may clarify the mechanisms underpinning the reductions seen in PPL. Therefore, until suitable non-invasive techniques are available we will have to assume that the mechanisms seen in adults also operate during

adolescence. The ~15 h time delay between exercise and the high fat meal should have allowed any potential exercise-induced increases in muscle lipoprotein lipase activity (m-LPLA) to occur (36). Consequently, enhanced hydrolysis of plasma TAG to non-esterified fatty acids (NEFA) and subsequent uptake by either muscle or adipose tissue may have happened during the postprandial period (11). This mechanism, however, is not supported universally when moderate intensity exercise provides the stimulus (14,15). Absolute fat oxidation is optimised during moderate intensity exercise (1) and about 30 to 50% of this is derived from muscle- and/or lipoprotein-derived TAG (33). With increasing exercise intensity, akin to VIG in our study, intramuscular TAG (IMTAG) use declines (32) because of a down regulation in hormone sensitive lipase (34). It is, therefore, possible that greater IMTAG use during MOD offset the higher exercise EE associated with VIG and partly contributed to the similar reductions seen in PPL between these conditions. Again, unless it becomes possible to measure LPLA and IMTAG in adolescents, this will continue to be mere speculation. Finally, the similarity in the glucose response across the conditions points to good homeostatic control in the healthy boys who volunteered to complete this study. This appears to be a consistent finding in studies employing a similar research design (4,5,13,14,16,18,23,31) although there are others that have reported small changes (15,21).

**Paragraph Number 19** The boys in our study completed 60 min bouts of exercise matching the most common internationally recommended level of daily physical activity (7,9). To confer health benefits, the guidelines suggest that 60 min of physical activity that is at least moderate in intensity, involving large muscle groups, should be accumulated five times or more a week by all young people (7,9). Our findings show

that single bouts of moderate or vigorous exercise bouts lasting 60 min in total provide an adequate stimulus to alter TAG metabolism following a fatty meal. Although extrapolation beyond this acute response is not possible, the results do provide some evidence to support the physical activity recommendations. Despite a recent upsurge in favour of more vigorous exercise in preference to moderate for those capable of enduring it (28,29,30), changes in PPL reported here do not support this where postprandial TAG metabolism is concerned in adolescent boys.

**Paragraph Number 20** It is clear that our study is not without its limitations; some of which preclude a more mechanistic understanding of the responses reported above whilst another may reduce our ability to provide incontrovertible conclusions. A combination of small sample size and heterogeneous responses across the experimental conditions resulted in 95% CI that were large. Consequently, although most of the mean differences between the two exercise conditions and CON show that the exercise exerted a meaningful effect, the statistical analyses were often borderline when using the traditional 0.05 boundary. We believe that a meaningful alteration in TAG metabolism has occurred in the postprandial period following the acute bouts of exercise, but confirmation with a larger sample size would be prudent.

**Paragraph Number 21** In conclusion, 60 min of intermittent moderate and vigorous running exercise reduced postprandial lipaemia in a small group of healthy adolescent boys. An increase in exercise intensity from 53% to 75% peak $\dot{V}O_2$ and the subsequent augmentation in exercise energy expenditure were not sufficient to demonstrate a dose-response in TAG metabolism. These results support the most common current international physical activity recommendations for young people.

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**Figure Legends**

Figure 1  Study protocol.

Figure 2  The relationship between incremental triacylglycerol area under the 6 hour time curve and moderate intermittent exercise absolute energy expenditure.

Figure 3  Fasting (F) and postprandial plasma triacylglycerol concentrations for the control (CON), moderate intensity (MOD), and vigorous intensity (VIG) exercise conditions. Data are mean (95% CI); n = 8. Black rectangle is when the milkshake was consumed. Main effect for condition ($P = 0.04$), main effect for time ($P \leq 0.0005$); condition by time interaction ($P = 0.60$).

Figure 4  Fasting (F) and postprandial plasma glucose concentrations for the control (CON), moderate intensity (MOD), and vigorous intensity (VIG) exercise conditions. Data are mean (95% CI); n = 8. Black rectangle is when the milkshake was consumed. Main effect for condition ($P = 0.20$), main effect for time ($P = 0.01$); condition by time interaction ($P = 0.06$).

**Table Legends**

Table 1        Participant physical and physiological characteristics at baseline (n=8)

| | Mean (SD) | Range |
|---|---|---|
| Age (y) | 12.9 (0.3) | 12.4 to 13.5 |
| Body mass (kg) | 45 (11) | 28 to 61 |
| Stature (m) | 1.58 (0.11) | 1.37 to 1.73 |
| Body mass index (kg·m$^{-2}$) | 17.8 | 15.1 to 21.4 |
| Pubic hair (stage) | 3 | 2 to 5 |
| Genetalia (stage) | 3 | 2 to 5 |
| Body fat (%) | 15 (4) | 10 to 23 |
| Peak HR (beats·min$^{-1}$) | 204 (5) | 200 to 214 |
| Peak RER | 1.13 (0.03) | 1.10 to 1.18 |
| Peak RPE | 19 (1) | 17 to 20 |
| Peak $\dot{V}O_2$ (mL·kg$^{-1}$·min$^{-1}$) | 52 (7) | 38 to 59 |

HR – heart rate, RER – respiratory exchange ratio, RPE – rating of perceived exertion

$\dot{V}O_2$ – oxygen uptake

Table 2      60 minute intermittent treadmill exercise profiles across experimental conditions

| | Moderate intensity | Vigorous intensity | Mean Difference | 95% CI* | alpha[†] |
|---|---|---|---|---|---|
| Heart rate (beats·min$^{-1}$) | 147 (14) | 175 (14) | 28 | 8 to 48 | 0.015 |
| Percent peak HR (%) | 72 (8) | 85 (5) | 14 | 4 to 23 | 0.013 |
| Oxygen uptake (L·min$^{-1}$) | 1.25 (0.38) | 1.76 (0.42) | 0.51 | 0.33 to 0.69 | P<0.0005 |
| Percent peak $\dot{V}O_2$ (%) | 53 (10) | 75 (1) | 22 | 14 to 31 | P<0.0005 |
| Respiratory exchange ratio | 0.87 (0.04) | 0.90 (0.02) | 0.03 | -0.00 to 0.07 | 0.069 |
| Energy expenditure (kJ) | 1538 (463) | 2182 (509) | 644 | 418 to 871 | P<0.0005 |
| Energy expenditure (kJ·kg$^{-1}$) | 33.9 (6.4) | 48.9 (6.5) | 15.0 | 9.0 to 21.0 | 0.001 |

All data are mean (SD); HR – heart rate (n=7), $\dot{V}O_2$ – oxygen uptake;

* 95% confidence interval of the absolute difference between moderate and vigorous exercise; [†] paired Student's t-test

Table 3  Fasting (baseline) plasma triacylglycerol (TAG) and glucose (GLU) concentrations across experimental conditions

| | CON | MOD | VIG | CON vs. MOD 95% CI* | CON vs. VIG 95% CI |
|---|---|---|---|---|---|
| Triacylglycerol (mmol·L$^{-1}$)[a] | 0.63 (0.23) | 0.53 (0.17) | 0.54 (0.28) | -0.25 to 0.06 | -0.29 to 0.12 |
| | | | | -34 to 10% | -46 to 17% |
| Glucose (mmol·L$^{-1}$)[b] | 4.74 (0.31) | 4.40 (0.44) | 4.49 (0.50) | -0.68 to -0.01 | -0.73 to 0.23 |
| | | | | -14 to 0% | -15 to 5% |

Data are mean (SD); *95% CI – confidence interval data are absolute (mmol·L$^{-1}$) and relative differences (%) between conditions;

CON – control condition; MOD – moderate intensity exercise condition; VIG – vigorous intensity exercise condition

a – ANOVA $F_{(2,14)}$ = 1.2, P = 0.33; b – ANOVA $F_{(2,14)}$ = 2.0, P = 0.17

Table 4        Total and incremental areas under the triacylglycerol and glucose concentration versus time curves

| | CON | MOD | VIG | CON vs. MOD 95% CI* | CON vs. VIG 95% CI |
|---|---|---|---|---|---|
| TAUC-TAG (mmol·L$^{-1}$ 6 h)[a] | 5.26 (1.66) | 3.99 (1.27) | 4.22 (1.45) | -2.55 to 0.00<br>-42 to 0% | -2.19 to 0.10<br>-37 to 0% |
| IAUC-TAG (mmol·L$^{-1}$ 6 h)[b] | 1.48 (0.61) | 0.80 (0.32) | 0.96 (0.89) | -1.25 to -0.11<br>-66 to -9% | -1.13 to 0.07<br>-71 to -6% |
| TAUC-glucose (mmol·L$^{-1}$ 6 h)[c] | 29.67 (1.94) | 28.60 (1.71) | 28.42 (2.30) | -2.73 to 0.58<br>-9 to 2% | -2.93 to 0.43<br>-10 to 1% |
| IAUC-glucose (mmol·L$^{-1}$ 6 h)[d] | 1.22 (2.65) | 2.22 (2.59) | 1.48 (2.55) | -0.98 to 2.98<br>negative values preclude calculation | -3.22 to 3.75 |

Data are mean (SD); *95% CI – confidence interval data are absolute (mmol·L$^{-1}$ 6 h) and relative differences (%) between conditions ;

CON – control condition; MOD – moderate intensity exercise condition; VIG – vigorous intensity exercise condition

TAUC – total area under the concentration versus time curve; TAG – triacylglycerol

a – ANOVA $F_{(2,14)}$ = 4.3, P = 0.04; b – ANOVA $F_{(2,14)}$ = 3.0, P = 0.08; c – ANOVA $F_{(2,14)}$ = 1.5, P = 0.26; d – ANOVA $F_{(2,14)}$ = 0.4, P = 0.68

## Day 1
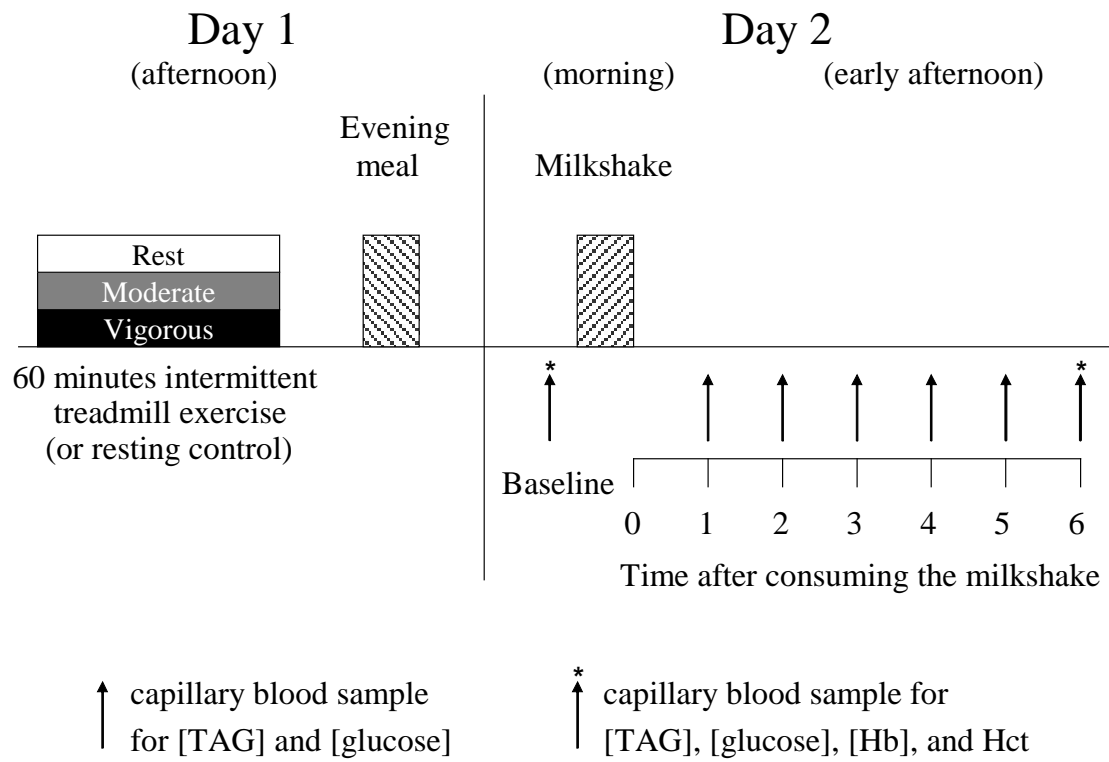
(afternoon)

## Day 2

(morning)          (early afternoon)

Evening
meal

Milkshake

| Rest |
| Moderate |
| Vigorous |

60 minutes intermittent
treadmill exercise
(or resting control)

\*                                                                    \*

Baseline

0    1    2    3    4    5    6

Time after consuming the milkshake

↑ capillary blood sample
  for [TAG] and [glucose]

\*
↑ capillary blood sample for
  [TAG], [glucose], [Hb], and Hct

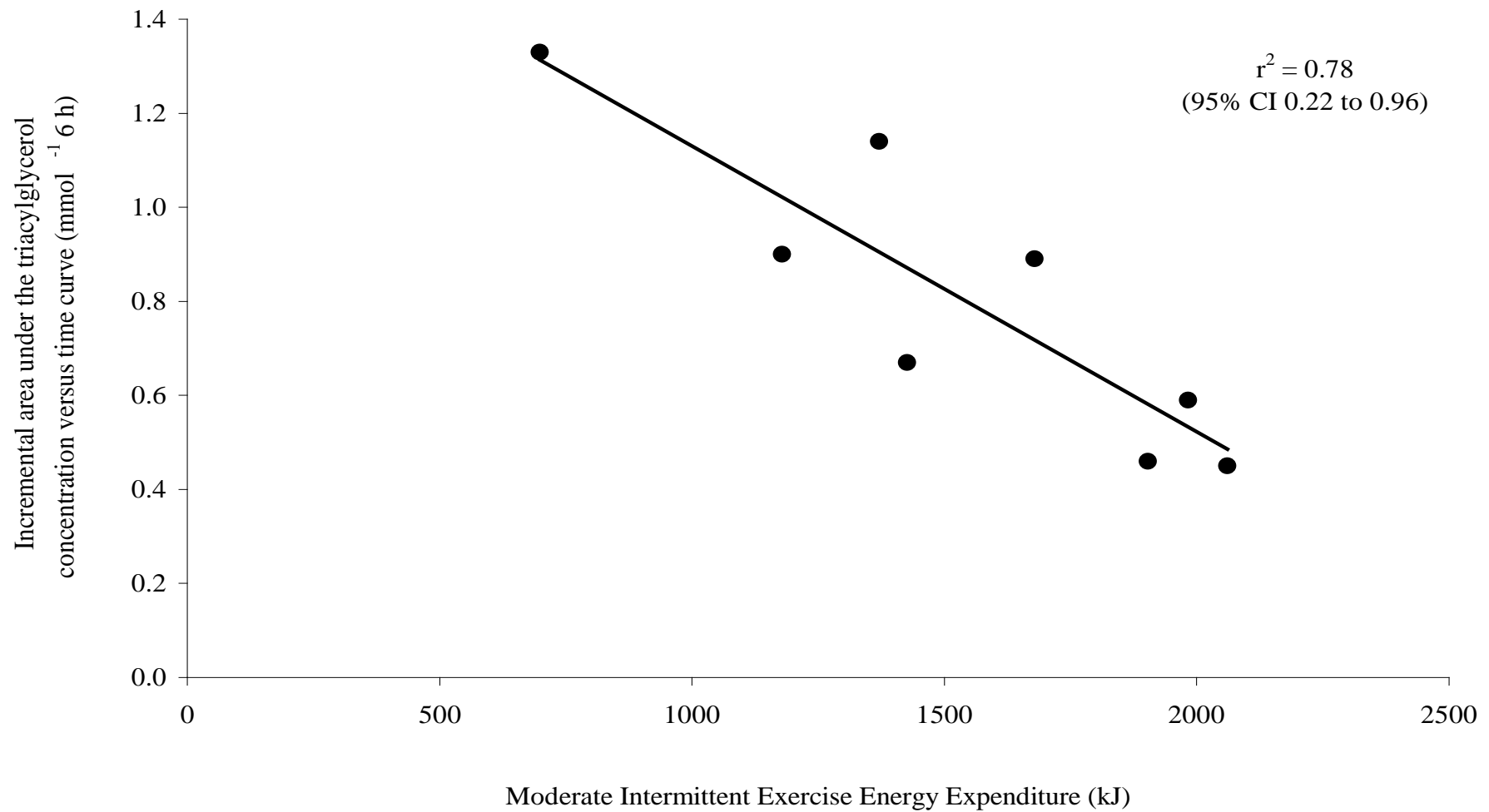Figure 1        Study protocol

**Figure 2** The relationship between incremental triacylglycerol area under the 6 hour time curve and moderate intermittent exercise absolute energy expenditure
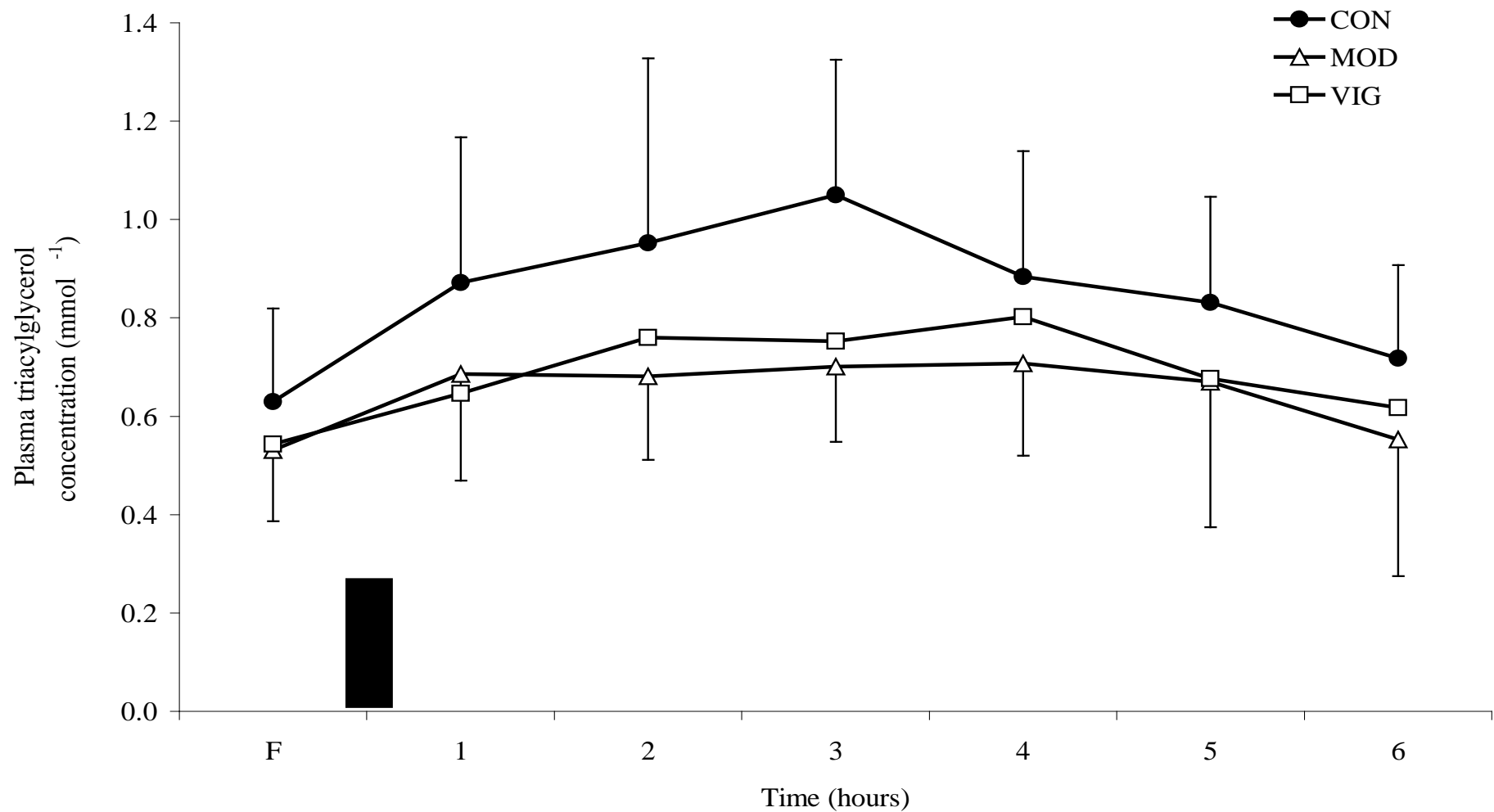
Figure 3      Fasting (F) and postprandial plasma triacylglycerol concentrations for the control (CON), moderate intensity (MOD), and vigorous intensity (VIG) exercise conditions. Data are mean (95% CI); n = 8. Black rectangle is when the milkshake was consumed. Main effect for condition (P = 0.04), main effect for time (P ≤ 0.0005); condition by time interaction (P = 0.60).
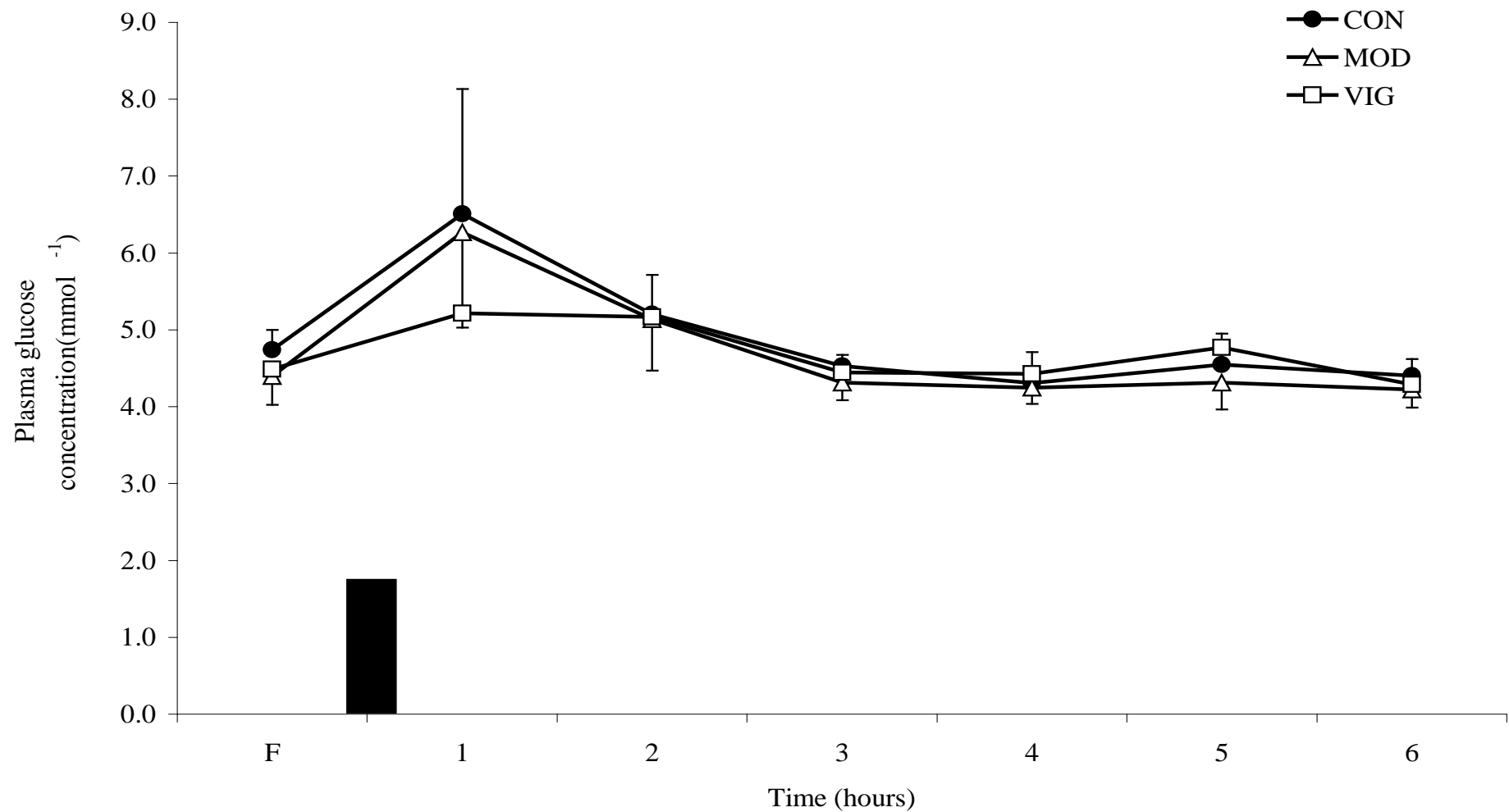
Figure 4    Fasting (F) and postprandial plasma glucose concentrations for the control (CON), moderate intensity (MOD), and vigorous intensity (VIG) exercise conditions. Data are mean (95% CI); n = 8. Black rectangle is when the milkshake was consumed. Main effect for condition (P = 0.20), main effect for time (P = 0.01); condition by time interaction (P = 0.06).