Effect of exercise performed immediately before a meal of moderate fat content on postprandial lipaemia

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The majority of the studies that have found a lowering effect of exercise on postprandial lipaemia have employed exercise 12–18 h before a test meal of exaggerated fat content (over 60% total energy). The aim of the present study was to investigate whether this effect is manifest when exercise is performed immediately before a test meal of moderate fat content. Eleven healthy young men cycled for 45 min at 62% maximal heart rate or rested, and, immediately afterwards, consumed a meal of moderate fat content (35% total energy, 0.65 g/kg body mass) in a random counterbalanced design. Blood samples were drawn before exercise, before the meal, and for 8 h postprandially. No significant differences were observed in plasma triacylglycerol concentrations and areas under the triacylglycerol time curves between exercise and rest, although exercise reduced the postprandial lipaemic response by 17%. Non-esterified fatty acids, glucose, and insulin did not differ significantly between the trials. In conclusion, moderate exercise performed immediately before a meal of a fat content typical to the Western diet had only a modest effect on postprandial lipaemia.

Exercise: Fatty acids: Postprandial lipaemia: Triacylglycerols

Postprandial lipaemia is considered to be associated with the presence or development of coronary artery disease (Karpe, 1999). Many studies (for reviews, see Gill & Hardman, 2003; Pettit & Cureton, 2003) have shown that acute exercise mitigates the elevation of plasma triacylglycerol (TG) concentration after a fatty meal, adding to the potential health benefits of physical activity. However, most of these studies have employed test meals containing exceedingly high amounts of fat (at least 1.0 g/kg body mass or over 60% total energy), which may magnify the effect of exercise. We have recently examined the effect of exercise on lipaemia after a meal of a fat content closer to that of the typical Western diet (35% total energy) and have, nevertheless, found a significant reduction in postprandial lipaemia (Kolifa et al, 2004).

In most of the studies addressing the effect of acute exercise on postprandial lipaemia, the exercise bout was performed on the day (12–18 h) before the test meal. This choice of timing has been probably dictated by the prevailing hypothesis that the effect of exercise is due to a delayed and sustained activation of lipoprotein lipase (LPL) in the capillaries of the exercised muscles, which accelerates the clearance of circulating TG in the postprandial state (Hardman, 1998). There are only two studies in which exercise ended 1 h (Zhang et al, 1998) or just before the test meal (Murphy et al, 2000) and six studies that employed exercise during the postprandial period (Cohen & Goldberg, 1960; Chinnici & Zauner, 1971; Schlierf et al, 1987; Klein et al, 1992; Hardman & Aldred, 1995; Zhang et al, 1998). As with the papers in which exercise was performed on the day before the test meal, most of the studies that employed exercise close to the meal have found significant reductions in postprandial lipaemia (Cohen & Goldberg, 1960; Schlierf et al, 1987; Klein et al, 1992; Hardman & Aldred, 1995; Zhang et al, 1998; Murphy et al, 2000). However, Zhang et al, (1998) observed this effect when exercise was performed before but not after the meal. However, again, these studies have used high-fat test meals. Encouraged by our finding that exercise performed on the day before a moderate-fat meal reduced postprandial lipaemia (Kolifa et al, 2004), we chose to examine whether this is also the case when exercise is performed immediately before such a meal. In addition, in order to make our findings more applicable to the general population, we employed exercise of moderate intensity as opposed to high intensity in our previous study (Kolifa et al, 2004).

Experimental methods

Subjects

Eleven sedentary young men who responded to a public invitation participated in the study. Their mean age, body...
mass, height, and BMI were 21.7 (SEM 0.6) years, 73.2 (SEM 2.1) kg, 1.80 (SEM 0.02) m, and 22.5 (SEM 0.5) kg/m². Subjects were non-smokers, were not suffering from any apparent acute or chronic illness, and were not taking any medication or dietary supplement. They were informed orally and in writing of the design and possible risks of the study and consented to participate. The study was designed and carried out according to the guidelines of the University of Thessaloniki ethics committee.

**Design**

Each subject took part in two trials (exercise and control) separated by 7 d in a random counterbalanced design. For each trial, the participant arrived at the laboratory at approximately 07.45 hours after an overnight fast. A cannula was inserted into a forearm vein and, after sitting for 5 min, 5 ml blood were drawn. Blood was immediately transferred into a pre-cooled test tube containing EDTA to prevent clotting. A small portion was removed, divided into samples for the determination of lipids, glucose, and insulin, and stored at −20°C.

After the first blood sampling, participants either cycled for 45 min on a microprocessor-controlled cycle ergometer (Kettler KX1; Kettler, Ense-Parsit, Germany) or rested. Cycling was performed at a constant power output corresponding to 60–65 % of each participant’s predicted maximal heart rate (HR max; 220 – age). This power output had been determined by an incremental test during a preliminary visit 1 week before the first trial. Heart rate was monitored by a Polar Accurex monitor (Polar Electro Oy, Kempele, Finland).

At the end of the cycling or resting period, a second blood sample (used as baseline in determining postprandial responses) was drawn by the aforementioned procedure. Immediately afterwards, participants consumed a test meal within 5 min. The meal was a standard liquid product, Resource Energy (Novartis Nutrition, Revel, France). Each subject consumed an amount providing 70kJ/kg body mass (typical energy content of a main daily meal). Of total energy, 35 % was derived from fat (which was of plant origin), 50 % from carbohydrate, and 15 % from protein. Thus, each subject received 0.65 g fat, 2.09 g carbohydrate, and 0.63 g protein/kg body mass.

Additional blood samples were obtained at 0.5, 1, 2, 3, 4, 5, 6, and 8 h postprandially as described earlier. The cannula was kept patent by flushing with normal saline. Subjects remained in the laboratory during this period, sitting or sleeping. They were allowed no food or drink except for water ad libitum.

To control for the effect of previous diet on the outcome measures of the study, subjects recorded their food intake and the time of food consumption during the 2 d preceding the first trial and repeated this diet before the second trial. They were asked to refrain from alcohol during those days and from caffeine during the day preceding each trial as well as on the day of the trial.

**Assays**

Plasma volume relative to that at the first blood sampling was determined according to Dill & Costill (1974) after measuring haemoglobin by a kit from Spinreact (Santa Coloma, Spain) and packed cell volume by microcentrifugation.

For the determination of plasma TG and non-esterified fatty acids (NEFA), TLC and GC were performed. Plasma (0.5 ml) was mixed with 2.5 ml 2-propanol–heptane–0.5 m H₂SO₄ (40:10:1, by vol.), after the addition of heptadecanoic acid and triheptadecanoylglycerol (both from Sigma, St Louis, MO, USA) as internal standards to control for incomplete recovery in the ensuing analytical steps. After 10 min, 1 ml heptane and 1.5 ml water were added and the mixture was stirred vigorously in order to afford extraction of the lipids (Dole, 1956). The upper layer was removed, condensed under a stream of N₂, and applied onto silica gel TLC plates (Sigma). The plates were developed with petroleum ether–diethyl ether–acetic acid (80:20:1, by vol.), and lipid spots were located under UV light after spraying with a solution of dichlorofluorescein in ethanol. The spots corresponding to TG and NEFA were excised separately and incubated in 0.5 ml methanolic sodium methoxide (Sigma) at 50°C for 10 min. Then 0.5 ml boron trifluoride (Fluka, Buchs, Switzerland) were added and incubation was repeated as before (Kramer et al. 1997). The fatty acid methyl esters thus produced were extracted with 1.5 ml hexane and separated in a Hewlett Packard 5890 Series II chromatograph (Waldbronn, Germany) equipped with a 30 m long BPX70 capillary column from SGE (Ringwood, Victoria, Australia) and a flame ionisation detector. The column temperature was programmed from 140 to 220°C at 5°C/min. The carrier gas was He at a flow rate of 0.67 ml/min (at 140°C).

Methyl esters of individual fatty acids were identified in the chromatograms by comparing their retention times with those of pure methyl esters purchased from Sigma and were quantified by comparing the area under their peaks with that of methyl heptadecanoate (derived from the internal standards) with the aid of the HP 3365 ChemStation software from Hewlett Packard. Total NEFA were calculated as the sum of individual NEFA and total TG as the sum of individual TG acyl groups divided by three. The intra-assay CV for the whole analysis was 8 %. Additionally, plasma TG were assayed photometrically with a reagent kit from BEST (Athens, Greece).

The fatty acid composition of the test meal was determined in the same way except that lipids were not separated by TLC, as we were interested in the total fatty acid intake regardless of source (i.e. TG, NEFA, etc.). Glucose was assayed by a photometric method using a kit from BEST (Athens, Greece). Insulin was assayed by enzyme immunoassay using a kit from DRG (Marburg, Germany).

**Dietary analysis**

Dietary records of the 2 d preceding each trial were analysed in Microsoft Access by the use of a food database.
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created in our laboratory on the basis of published data (Holland et al. 1991).

Calculations and statistical analysis
As summary measures of the responses of plasma TG, NEFA, glucose, and insulin to the test meal, areas under the curves (AUC) of their concentrations v. time were calculated starting from the value just before meal consumption and using the trapezoidal rule.

Data are presented as means with their standard errors. The distribution of all dependent variables was examined by the Kolmogorov–Smirnov test and was found not to differ significantly from normal. Significant differences between exercise and rest with respect to relative plasma volume and plasma concentrations were detected by two-way (treatment X time) ANOVA with repeated measures on both factors. Pair-wise comparisons were performed through simple main effect analysis. Differences between exercise and rest with respect to AUC were examined by Student’s t test. The level of statistical significance was set at α=0.05 and the P values quoted are two-sided. The SPSS (version 10.0) was used for all analyses (SPSS Inc., Chicago, IL, USA).

Results
Daily energy intake during the 2 d preceding each trial was 137 (SEM 6) kJ/kg body mass and was derived from 48 (SEM 2) % carbohydrate, 38 (SEM 2) % fat, and 14 (SEM 1) % protein. Heart rate during the cycling exercise bout was 124 (SEM 1) beats/min, corresponding to 62 (SEM 1) % predicted HRmax. Power output was 102 (SEM 6) W and the estimated net energy expenditure of exercise was 1-1 (SEM 0-1) MJ or 15 (SEM 1) kJ/kg body mass, as calculated by the ergometer microprocessor based on a mechanical efficiency of 25 %. Relative plasma volume did not change significantly between the trials at each time point or across time within each trial, therefore no adjustment was made to measured concentrations.

The test meal contained seven fatty acids in considerable amounts, namely, palmitate (16 : 0), stearate (18 : 0), oleate (18 : 1n-9), cis-vaccenate (18 : 1n-7), linoleate (18 : 2n-6), γ-linolenate (18 : 3n-6), and α-linolenate (18 : 3n-3). Five additional fatty acids were detected in considerable amounts in plasma, namely, myristate (14 : 0), palmitoleate (16 : 1n-7), gondoate (20 : 1n-9), dihomo-γ-linolenate (20 : 3n-6), and arachidonate (20 : 4n-6).

Plasma total TG concentrations during the exercise and control trials are shown in Fig. 1. The two curves were monophasic and qualitatively similar, with peak TG concentrations occurring 2–3 h postprandially. Although the exercise curve was below the control curve at all postprandial sampling points (with the greatest difference at 3 h), no significant difference was found between the two trials. TG concentrations returned to pre-meal values 8 h postprandially in both trials.

The AUC of postprandial TG concentration v. time was not significantly different between the exercise and control trials (6.83 (SEM 0.63) v. 7.46 (SEM 0.67) mmol/l X h, respectively). Likewise, the incremental AUC, calculated from the total AUC by subtracting the area attributable to the baseline TG concentration, was not significantly different between exercise and control (2.91 (SEM 0.37) v. 3.51 (SEM 0.60) mmol/l X h, respectively). The effect size (calculated as the difference between means divided by the standard deviation of the control trial) of exercise on both total and incremental AUC was −0.30, while the observed power (computed by the statistical software) was 0.15 and 0.18, respectively. Results were essentially the same when the data from the photometric assay of plasma TG were used.

As with total TG, the concentrations and AUC of the individual fatty acids of plasma TG did not differ significantly between the two trials. However, when we examined the curves of the percentage of each fatty acid v. time, we noticed that those fatty acids that had higher percentages in the meal than in plasma TG had lower percentages in the exercise trial compared with rest, whereas those fatty acids that had lower percentages in the meal than in plasma TG had higher percentages in the exercise trial compared with rest. It thus seemed as if exercise hindered the approach of the fatty acid profile of plasma TG towards that of the meal.

There were no significant differences between the two trials in plasma NEFA, glucose, and insulin concentrations (Fig. 2), except for NEFA at the end of exercise (P=0.027). The corresponding curves of the individual NEFA were similar to those of total NEFA. Likewise, there were no significant differences in the AUC of these parameters between exercise and control. These AUC were (for exercise and control, respectively): 1.76 (SEM 0.18) v. 1.71 (SEM 0.11) mmol/l X h for total NEFA; 44.4 (SEM 1.18) v. 43.5 (SEM 1.1) mmol/l X h for glucose; 256 (SEM 25) v. 243 (SEM 22) μU/l X h for insulin.

Discussion
In the present study we examined the effect of exercise on postprandial lipaemia, when exercise is performed immediately before a meal containing 0.65 g fat/kg body mass (35 % total energy). This is the second study from our laboratory utilising a meal of moderate fat content. In contrast, the vast majority of the other studies in this field have employed meals containing 60–93 (average 67) % energy

![Fig. 1. Fasting and postprandial plasma triacylglycerol (TG) concentrations after a meal of moderate fat content consumed immediately after exercise (●) or rest (○). Values are means for eleven subjects, with standard errors of the mean represented by vertical bars. There was no significant difference between the trials.](https://www.cambridge.org/core/core?ip-address=54.191.40.80&timestamp=1497322136319&message=subject-to-the-Cambridge-Core-terms-of-use-available-at-https://www.cambridge.org/core/terms)
as fat or 1·0–1·5 (average 1·3) g/kg body mass. Using such high amounts of fat limits the applicability of the results to everyday conditions, as the fat content of the typical Western diet is 35–40 % as energy (Ministry of Agriculture, Fisheries & Food, 1997). Since postprandial lipaemia is expected and has been shown to be positively related to the amount of fat ingested (Dubois et al. 1998), one could claim that exercise might have a significant effect on lipaemia after a high-fat but not after a regular-fat meal. However, in our previous study we showed that high-fat meals. We employed an exercise protocol performed immediately before the consumption of a moderate-fat meal reduced the net lipaemic response to the meal (i.e. incremental TG AUC) by 17 %. Although this effect is notable, it was not significant statistically. This contrasts with most of the studies in which exercise was performed close to the test meal and which found significant decreases in indices of postprandial lipaemia (Cohen & Goldberg, 1960; Schlierf et al. 1987; Klein et al. 1992; Hardman & Aldred, 1995; Murphy et al. 2000). On the other hand, Chinnici & Zauner (1971) found no significant effect of exercise (performed postprandially) on postprandial lipaemia, while Zhang et al. (1998) found a significant decrease in postprandial lipaemia when the meal was consumed 1 h after the end of exercise but not when it was consumed 1 h before the onset of exercise.

The absence of a significant lowering effect of exercise on postprandial lipaemia in the present study may be attributed to the low energy expenditure of exercise along with the moderate fat content of the test meal. Energy expenditure has been positively correlated with the suppression of postprandial lipaemia (Gill & Hardman, 2003; Pettit & Cureton, 2003) and, compared with the present study, was higher in most of the aforementioned studies that found a significant decrease in postprandial lipaemia (Cohen & Goldberg, 1960; Schlierf et al. 1987; Hardman & Aldred, 1995; Zhang et al. 1998). Specifically, the energy expenditure of exercise was 36 kJ/kg in Zhang et al. 1998 (compared with 15 kJ/kg in the present study). The other three studies did not specify this parameter but, judging from exercise intensity and duration, it was apparently higher than in the present study. Moreover, energy expenditure was lower in the study that found no significant change in postprandial lipaemia (Chinnici & Zauner, 1971). There are, however, two studies that found significant decreases in postprandial lipaemia despite a low energy expenditure (Klein et al. 1992; Murphy et al. 2000). This discrepancy with the present study may be due to the high fat content of their test meals. Finally, the low statistical power of the present study may be a reason for the absence of significant differences; it would require approximately eighty-five subjects to increase power to 0·8. However, the sample size of eleven in the present study is the median of all studies on the effect of exercise on postprandial lipaemia.

We have observed an interesting dependence of the reduction in the lipaemic response of each fatty acid in plasma TG by exercise on its abundance in the test meal. Energy expenditure was lower in the study that found significant decreases in indices of postprandial lipaemia (Cohen & Goldberg, 1960; Schlierf et al. 1987; Klein et al. 1992; Hardman & Aldred, 1995; Murphy et al. 2000). This contrasts with most of the studies in which exercise was performed close to the test meal and which found significant decreases in indices of postprandial lipaemia (Cohen & Goldberg, 1960; Schlierf et al. 1987; Klein et al. 1992; Hardman & Aldred, 1995; Murphy et al. 2000). On the other hand, Chinnici & Zauner (1971) found no significant effect of exercise (performed postprandially) on postprandial lipaemia, while Zhang et al. (1998) found a significant decrease in postprandial lipaemia when the meal was consumed 1 h after the end of exercise but not when it was consumed 1 h before the onset of exercise.

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We have observed an interesting dependence of the reduction in the lipaemic response of each fatty acid in plasma TG by exercise on its abundance in the test meal compared with plasma TG. Specifically, the higher the difference in the percentage of a fatty acid between the meal and plasma TG, the higher the attenuating effect of exercise on its postprandial response. This may be attributed to a reduced release of dietary fat from the intestine into the bloodstream or to an increased activity of muscle LPL, as the latter has been shown to hydrolyse...
TG preferentially in chylomicrons rather than in VLDL (Evans et al. 2002). However, experimental evidence for an LPL-mediated mechanism is either weak (Herd et al. 2001; Gill et al. 2003) or not supportive (Malkova et al. 2000; Gill et al. 2001; Thomas et al. 2001). Additionally, the delayed nature of LPL up regulation does not explain the findings of a significant reduction in postprandial lipaemia when exercise is performed close to the test meal (Cohen & Goldberg, 1960; Schlief et al. 1987; Klein et al. 1992; Hardman & Aldred, 1995; Zhang et al. 1998; Murphy et al. 2000). An alternative explanation for the present observation could be an effect of exercise on the postprandial hepatic clearance of chylomicron-TG and/or secretion of VLDL-TG, since the liver plays an important role in the uptake and release of dietary fatty acids (Heath et al. 2003). Direct testing of the possible intestinal and hepatic involvement in the effect of exercise on postprandial lipaemia is warranted.

We found no significant effect of exercise on postprandial plasma NEFA, glucose, and insulin, except for a rise in NEFA concentration at the end of exercise, which is apparently due to the stimulation of lipolysis in adipose tissue. These findings are in accordance with those of Hardman & Aldred (1995), as well as Murphy et al. (2000), with the exception that the latter found no change in NEFA concentration. None of the other studies that employed exercise close to the test meal has examined these parameters.

In conclusion, moderate exercise performed immediately before a meal of a fat content typical to the Western diet had a modest but non-significant effect on postprandial lipaemia. Exercise caused a greater attenuation in the postprandial response of the fatty acids that were more abundant in the test meal than in plasma TG, implying effects on dietary lipid absorption or effects on the clearance of circulating TG. Future research should clarify the interplay of the amount of dietary fat, exercise dosage, and exercise timing, as well as reveal the mechanisms mediating the effect of exercise on postprandial lipaemia.

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References


