European Journal of Clinical Nutrition (2004) 58, 1327–1335
 0954-3007/04 $30.00
www.nature.com/ejcn

ORIGINAL COMMUNICATION

Effect of prior exercise on lipemia after a meal of moderate fat content

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Objective: Prior exercise has been repeatedly shown to reduce lipemia after meals of exaggerated fat content (over 60% of total energy). The aim of the present study was to investigate whether the same applies to meals closer to the composition of the typical Western diet and explore whether exercise affects the release of dietary fat into the bloodstream.
Design: Randomized counterbalanced.
Setting: Laboratory.
Subjects: Nine healthy young male volunteers.
Intervention: Subjects consumed a meal of moderate fat content (35% of total energy, 0.66 g/kg body mass) 14 h after having either cycled for 1 h at 70–75% of maximal heart rate or rested. Macadamia nuts were used as the main source of dietary fat to trace its entry into the circulation because of their unusual fatty acid composition. Blood samples were drawn before the meal and for 8 h postprandially.
Results: Plasma triacylglycerol concentrations and total area under the triacylglycerol concentration *vs* time curve (AUC) were significantly lower after exercise ($P = 0.001$ and 0.003, respectively; effect size for the latter, 0.84). However, incremental (above baseline) AUC was not affected by exercise significantly. When controlling for differences in baseline plasma concentrations, only the fatty acids that were more abundant in the meal than in plasma triacylglycerols were decreased in the early postprandial period following exercise, implying either a suppressive effect of exercise on the rate of triacylglycerol release from the intestine or a more rapid chylomicron clearance after meal consumption.
Conclusions: Exercise performed between 15 and 14 h before a meal of moderate fat content reduced postprandial lipemia, mainly by lowering fasting triacylglycerols. The effect of exercise on postprandial triacylglycerol metabolism may be mediated, at least in part, by attenuated release of dietary fat from the intestine.
European Journal of Clinical Nutrition (2004) **58,** 1327–1335. doi:10.1038/sj.ejcn.1601968
Published online 31 March 2004

Keywords: fatty acids; intestine; macadamia nuts; postprandial lipemia; triacylglycerols

Introduction

A number of clinical studies (reviewed by Karpe, 1999) have associated the presence or development of coronary artery disease with postprandial lipemia, rendering its mitigation a justifiable target for intervention strategies to improve public health. Physical exercise has been repeatedly shown to be effective to this end, especially when performed on the day before a fat-rich meal (Aldred *et al*, 1994; Tsetsonis & Hardman, 1996a, b; Tsetsonis *et al*, 1997; Gill *et al*, 1998, 2001a, b, 2002, 2003; Zhang *et al*, 1998; Malkova *et al*, 1999, 2000; Gill & Hardman, 2000; Herd *et al*, 2001; Koutsari & Hardman 2001; Koutsari *et al*, 2001; Thomas *et al*, 2001; Petitt *et al*, 2003). However, there does not seem to be a chronic effect extending beyond 2 days after the interruption of training (Hardman *et al*, 1998; Herd *et al*, 1998, 2000).

A common feature of the studies cited above is the high fat content of the test meal (at least 1.0 g/kg body mass; over 60% of total energy). This greatly exceeds usual fat intakes and may exaggerate the observed differences between exercise and rest. For the sake of applicability, it would be interesting to examine the magnitude of the effect, if any, of prior exercise on lipemia after a meal of a composition closer to that of a typical Western diet. Therefore, the main purpose

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***Contributors*: MK performed the major part of data collection, data analysis, and writing of the manuscript, as this work was her master's thesis. AP was the main contributor in designing the study, trained MK and collaborated in data collection, data analysis and writing of the manuscript. VM also contributed to the writing of the manuscript.**
Received 5 December 2003; revised 22 January 2004; accepted 3 February 2004; published online 31 March 2004

of the present study was to determine whether exercise reduces lipemia after a meal of moderate fat content (35% of total energy).

The mechanism of the exercise-induced decrease in postprandial lipemia favored by most researchers (eg, Tsetsonis & Hardman, 1996a; Hardman, 1998; Zhang *et al*, 1998; Petitt *et al*, 2003) is an increase in lipoprotein lipase (LPL) activity in the capillaries of the exercised muscles, which should accelerate the clearance of circulating triacylglycerols (TG) in the postprandial state. This hypothesis has been based on the fact that the attenuation of postprandial lipemia is observed within the time frame of enhanced LPL expression and activity after acute exercise (Seip & Semenkovich, 1998), but experimental evidence is either weak (Herd *et al*, 2001; Gill *et al*, 2003) or not supportive (Malkova *et al*, 2000; Gill *et al*, 2001b; Thomas *et al*, 2001). An alternative (or complementary) mechanism, decreased secretion of TG by the liver, has gained indirect experimental support (Malkova *et al*, 2000; Gill *et al*, 2001a). A third possibility, reduced output of dietary TG from the intestine, has been usually dismissed by the argument that too many hours have elapsed since the exercise stimulus (Tsetsonis & Hardman, 1996b; Gill & Hardman, 2000; Gill *et al*, 2001a). However, this hypothesis has not been tested. Therefore, the second purpose of this study was to examine whether exercise affects the entry of dietary fat into the bloodstream. Thus, the hypotheses tested were that (i) exercise decreases lipemia after a moderate-fat meal and (ii) exercise decreases the rate of fat release by the intestine.

Subjects and methods

Subjects

Nine men, aged 20–25 years, participated in the study. Their body mass, height, and body mass index (mean $\pm$SEM) were 84.9 ± 4.0 kg, 1.80 ± 0.02 m, and 24.7 ± 0.8 kg/m^2. Subjects were nonsmokers, were not suffering from any apparent acute or chronic illness, and were not taking any medication or dietary supplements. Additionally, they were normolipidemic and euglycemic. 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 placed 1 week apart in a random counterbalanced design. Each trial was conducted over 2 days. In the afternoon of day 1, subjects either exercised for 1 h (from 5 to 6 pm) or abstained from exercise. Exercise was performed in a microprocessor-controlled cycle ergometer (Kettler KX1, Ense-Parsit, Germany) at a constant power output corresponding to 70–75% of each subject's predicted maximal heart rate (HRmax, 220-age). This power output had been determined by an incremental maximal test in the same ergometer during the preliminary visit at which they had been recruited in the study. Heart rate was monitored by a Polar Accurex monitor (Kempele, Finland). Subjects had dinner no later than 8 pm of day 1.

In the morning of day 2, subjects arrived at the laboratory after an overnight fast. A cannula was inserted into a forearm vein and, after 5 min of rest, 5-ml baseline blood samples were obtained with the subjects seated. Blood was transferred into precooled test tubes containing EDTA to prevent clotting and was immediately centrifuged at $1500 \times g$ at 4°C for 5 min. Plasma was promptly removed, divided into aliquots for the determination of lipids, glucose, and insulin, and stored at −20°C.

Immediately after providing the fasting blood sample, at 8 am, subjects consumed a test meal within 15 min. The meal consisted of skimmed milk, cornflakes, bread, orange juice, low fat ham, and macadamia nuts. The latter have been used to trace the entry of dietary fat into the circulation due to their unusual fatty acid profile, that is, a high proportion of monounsaturated fatty acids (Fielding *et al*, 2000). The composition of the meal for an 85-kg individual (the mean body mass of the participants) is shown in Table 1. The meal contained, per kg body mass, 0.66 g of fat, 2.14 g of carbohydrate, and 0.62 g of protein. Its energy content was 71 kJ/kg (corresponding to that of a main daily meal), derived from fat by 35%, carbohydrate by 50%, and protein by 15%.

Blood samples were drawn at 0.5, 1, 2, 3, 4, 5, 6, and 8 h postprandially as described for the baseline sample. The cannula was kept patent by flushing with normal saline. Subjects remained in the lab during this period, sitting or sleeping. They were allowed no food or drink except for water *ad libitum*. Water intake was recorded during the first trial and was repeated during the second trial.

To control for the effect of prior diet on the outcome measures of the study, subjects recorded their food intake and the time of food consumption during the 2 days preceding the first test meal and repeated this diet before the second test meal. They were asked to abstain from alcohol during those days and from caffeine during the day preceding each test meal. Finally, they were asked to perform

Table 1 Composition of the test meal for an 85-kg subject

Food	*Quantity*	*Fat (g)*	*Carbohydrate (g)*	*Protein (g)*	*Energy (kJ)*
Milk	328 ml	0.0	15.7	12.1	466
Cornflakes	61 g	0.5	50.4	4.9	943
Bread	121 g	2.3	59.9	10.2	1259
Orange juice	425 ml	0.0	49.7	0.9	846
Ham	115 g	2.5	2.9	19.6	472
Macadamia nuts	66 g	50.9	3.1	5.2	2055
Sum		56.2	181.8	52.8	6043

Data were derived from Holland *et al* (1991) and Nutrition Facts food labels.

no exercise other than the experimental bout during the 2 days before each test meal.

Assays

For the determination of plasma TG and nonesterified fatty acids (NEFA), thin layer chromatography (TLC) and gas chromatography (GC) were performed. All the samples of each participant were analyzed on the same day. In all, 0.5 ml of plasma was mixed with 2.5 ml 2-propanol – heptane – 0.5 mol/l H_2SO_4, 40:10:1 (v/v/v), after the addition of heptadecanoic acid and triheptadecanoylglycerol (both from Sigma, St. Louis, MO) as internal standards. After 10 min, 1 ml of heptane and 1.5 ml of 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 nitrogen, and applied onto silica gel TLC plates (Sigma). The plates were developed with petroleum ether - diethyl ether - acetic acid, 80:20:1 (v/v/v), and lipid spots were located under ultraviolet 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 of methanolic sodium methoxide (Sigma) at 50°C for 10 min. Then 0.5 ml of boron trifluoride (Fluka, Buchs, Switzerland) was added and incubation was repeated as before (Kramer *et al*, 1997). The fatty acid methyl esters thus produced were extracted with 1.5 ml of 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 ionization detector. The column temperature was programmed from 140°C to 220°C at 5°C/min. The carrier gas was helium 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 to those of pure methyl esters purchased from Sigma and were quantified by comparing the area under their peaks to 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 3. The intra- and inter-assay coefficient of variation for the whole analysis was 8%.

The fatty acid composition of the test meal was determined by analyzing a portion of it corresponding to approximately one-sixth of the meal shown in Table 1. After homogenizing the ingredients in a food blender, a few milligrams of the slurry were incubated with sodium methoxide and boron trifluoride as described above. Fatty acid methyl esters were then extracted and separated by GC as described above.

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). All samples were analyzed on the same day for each parameter. The intra-assay coefficients of variation were 1.8% and 3.8%, respectively.

Dietary analysis

Dietary records were analyzed in Microsoft Access by the use of a food database 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 *vs* time were calculated using the trapezoidal rule. Additionally, incremental AUC was calculated for TG by subtracting the area attributable to the baseline TG concentration from total AUC.

Values are expressed as means ± SEM. 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 plasma concentrations were detected by two-way (treatment × time) ANOVA with repeated measures on both factors. Pairwise comparisons were performed through simple main effect analysis. When we wanted to compare postprandial values between exercise and rest at individual time points while compensating for differences in baseline values, we performed simple contrast analysis.

Differences between exercise and rest with respect to AUC, as well as between baseline plasma TG and the test meal with respect to percentage fatty acid distribution were examined by Student's *t*-test. Linear correlation analysis was carried out by Pearson's product–moment correlation. The level of statistical significance was set at $\alpha = 0.05$. SPSS (version 10.0) was used for all analyses (SPSS Inc., Chicago, IL).

Results

Heart rate during the cycling exercise bout was 146 ± 4 beats/min, corresponding to 74 ± 2% of HRmax. Power output was 163 ± 10 W and the estimated net energy expenditure of exercise was 2.3 ± 0.1 MJ or 28 ± 2 kJ/kg body mass, as calculated by the ergometer microprocessor based on a mechanical efficiency of 25%. The energy content of the dinner on day 1 was 3.8 ± 0.4 MJ and was derived from 113 ± 38 g carbohydrate, 40 ± 11 g fat, and 27 ± 2 g protein (47%, 41%, and 12% of energy, respectively). The fatty acid composition of the dinner was 30% saturated, 57% monounsaturated, and 13% polyunsaturated.

In all, 13 fatty acids were detected in considerable amounts in the test meal, namely, laurate (12:0), myristate (14:0), palmitate (16:0), palmitoleate (16:1ω7), stearate (18:0), oleate (18:1ω9), *cis*-vaccenate (18:1ω7), linoleate (18:2ω6), γ-linolenate (18:3ω6), α-linolenate (18:3ω3), gondoate (20:1ω9), arachidonate (20:4ω6), and lignocerate (24:0).

Table 2 Percentage molar fatty acid composition of the test meal and baseline plasma TG

Fatty acid	*Meal*	*Plasma*	*Meal/plasma*
12:0	0.31	—	—
14:0	1.11	1.87	0.59
16:0	10.33	30.43	0.34
16:1ω7	16.74	1.95	8.58
18:0	2.94	4.87	0.60
18:1ω9	57.31	36.83	1.56
18:1ω7	3.48	2.51	1.39
18:2ω6	4.35	19.29	0.23
18:3ω6	0.02	0.23	0.09
18:3ω3	0.35	0.57	0.61
20:1ω9	2.56	0.36	7.11
20:3ω6	—	0.24	—
20:4ω6	0.12	0.85	0.14
24:0	0.38	—	—
Sum	100.00	100.00	

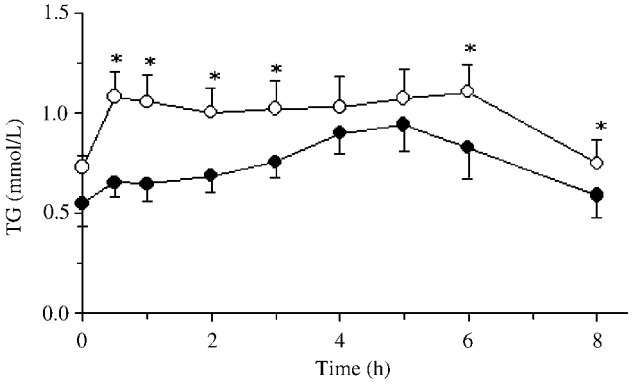

Figure 1 Fasting (0 h) and postprandial plasma triacylglycerol (TG) concentrations attributable to a meal of moderate fat content consumed 14 h after exercise (●) or rest (○). Values are means ± SEM for nine subjects. *Significantly different between exercise and rest ($P \leqslant 0.05$).

Their molar distribution is presented in Table 2. On the other hand, 12 fatty acids were detected in considerable amounts in plasma, namely, 14:0, 16:0, 16:1ω7, 18:0, 18:1ω9, 18:1ω7, 18:2ω6, 18:3ω6, 18:3ω3, 20:1ω9, dihomo-γ-linolenate (20:3ω6), and 20:4ω6. Their mean molar distribution in the TG of the baseline samples (pooled data from both trials) is also presented in Table 2. 16:1ω7, 18:1ω9, 18:1ω7, and 20:1ω9 (all monounsaturated fatty acids) were significantly more abundant in the test meal compared to plasma TG ($P<0.001$), reflecting the unique fatty acid profile of macadamia nuts, which accounted for 91% of the meal's fat (see Table 1).

Plasma total TG concentrations at baseline and during the postprandial period with and without prior exercise are shown in Figure 1. TG were significantly lower in the exercise trial. Simple main effect analysis showed a borderline significant difference between the two trials at baseline ($P=0.055$) and significant differences at 0.5, 1, 2, 3, 6, and 8 h ($P \leqslant 0.05$). In the control trial, TG peaked at 0.5 h and remained relatively constant up to 6 h, whereas, in the exercise trial, TG peaked at 5 h. The greatest differences between the two trials were observed from 0.5 to 2 h postprandially. TG concentration almost returned to baseline 8 h after the meal in both trials.

The AUC of TG concentration *vs* time was significantly reduced after exercise (5.98 ± 0.80 *vs* 8.05 ± 0.82 mmol/lh, $P=0.003$). The effect size (calculated as the difference between means divided by the SD of the control trial) was −0.84, which is considered large (Cohen, 1998). The observed power (0.96, computed by the statistical software) was also high. In contrast, incremental AUC was not significantly different between exercise and control (1.89 ± 0.41 *vs* 2.32 ± 0.50 mmol/lh, respectively). Finally, a positive correlation was found between baseline TG concentration and total AUC in both trials ($r=0.816$, $P=0.007$ for control; $r=0.731$, $P=0.025$ for exercise).

The postprandial responses of the individual fatty acids of plasma TG resembled the one of total TG described above: all were significantly lower in the exercise trial ($P<0.05$), with most of the significant differences located at 0.5–2 h. However, there were marked differences in the kinetics of the fatty acids, depending on their abundance in the test meal *vs* plasma TG and independent of chain length, degree of unsaturation or concentration. Three patterns emerged. Those fatty acids that were less abundant in the test meal compared to baseline plasma TG (ie, 14:0, 16:0, 18:0, 18:2ω6, 18:3ω6, 18:3ω3, 20:3ω6, and 20:4ω6, see Table 1) peaked at 0.5 h and gradually declined afterwards in the control trial, whereas, in the exercise trial, they exhibited a near plateau between 0.5 and 5 h. This pattern is typified by 16:0 and 18:2ω6 at the left panel of Figure 2. The two fatty acids being strikingly more abundant (over 7-fold) in the test meal (i.e., 16:1ω7 and 20:1ω9) increased almost linearly up to 6 h and declined at 8 h in the control trial, whereas, in the exercise trial, they increased up to 4 h, plateaued up to 6 h, and declined at 8 h (Figure 2, right). Finally, the two fatty acids having a meal-to-plasma ratio of around 1.5 (ie, 18:1ω9 and 18:1ω7) exhibited the pattern of total TG described above (not shown).

To compare the postprandial responses of the individual fatty acids of plasma TG while controlling for differences at baseline, we performed simple contrast analysis at each postprandial time point. This post-hoc test showed significantly lower concentrations in the exercise trial for the fatty acids that were more abundant in the test meal than in baseline plasma TG (ie, 16:1ω7, 18:1ω9, 18:1ω7, and 20:1ω9). These differences were located at 1 h for all four fatty acids and, additionally, at 6 h for 16:1ω7 as well as 0.5 h for 20:1ω9.

Data on plasma NEFA concentrations are presented in Figure 3. Total NEFA did not differ significantly between the two trials, decreasing after the meal and up to 3 h in the

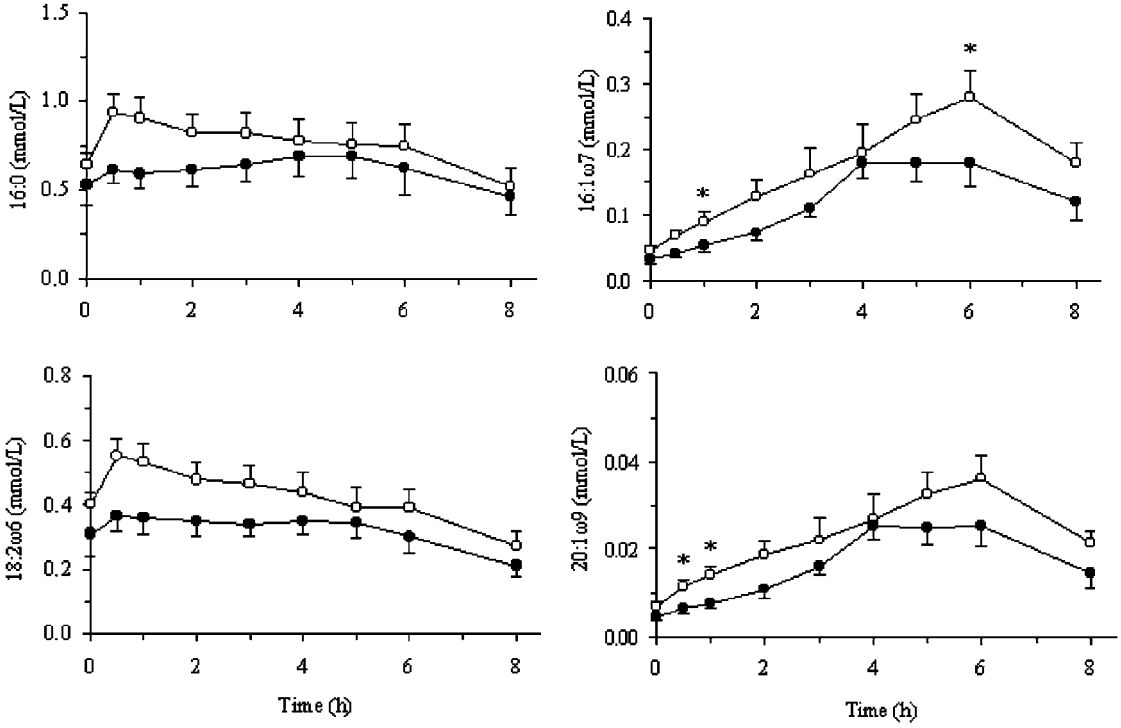

Figure 2 Postprandial plasma kinetics of four TG acyl groups exhibiting two distinct patterns, that is, either an early peak (left) or a late peak (right). Open circles correspond to the control trial and full circles to the exercise trial. Values are means ± SEM for nine subjects. *Significantly different between exercise and control while compensating for differences at baseline (simple contrast analysis, $P \leqslant 0.05$).

control trial or 2 h in the exercise trial. This decrease was followed by an increase until the end of the observation period. The concentrations at 8 h did not reach baseline in either trial. The corresponding curves of the individual NEFA were qualitatively the same as those of total NEFA, the only remarkable difference being that 16:1ω7 and 20:1ω9 exceeded their baselines at 8 h in both trials (Figure 3). Additionally, these were the only NEFA that differed significantly between exercise and control when simple contrast analysis was performed. The differences were located at 1 and 6 h for 16:1ω7, as well as 1 and 2 h for 20:1ω9. In all cases, the exercise values were significantly lower than the corresponding control values when corrected for baseline differences.

No differences were observed in the plasma glucose and insulin concentrations between the two trials (Figure 4). Glucose peaked at 0.5 h and then decreased gradually, stabilizing at baseline by 3 h. Insulin peaked at 0.5 h and then decreased gradually, reaching baseline at 6 h.

In accordance with the lack of significant differences in plasma total NEFA, glucose, and insulin concentrations, there were no significant differences in the AUC of these parameters between exercise and control. The respective values were 1.15 ± 0.18 *vs* 1.04 ± 0.13 mmol/lh for NEFA, 40.7 ± 0.8 *vs* 42.4 ± 1.4 mmol/lh for glucose, and 263 ± 33 *vs* 278 ± 45 mU/lh for insulin.

Discussion

We have examined the influence of acute exercise on postprandial lipemia caused by a meal of moderate fat content (35% of total energy, 0. 66 g/kg body mass). This is the first attempt to determine whether exercise can suppress the lipemic response to a meal approximating the macronutrient composition of the typical Western diet, that is (in terms of energy) 35–40% fat, about 47% carbohydrate, and about 16% protein (Williams, 2002). In fact, with the exception of Murphy *et al*. (2000), who employed a diet providing 47% of energy from fat over the course of 1 day, all studies on the effect of exercise on postprandial lipemia have used test meals containing 60–93% of energy as fat, or 1.0–1.5 g/kg body mass (Maruhama *et al*, 1977; Schlierf *et al*, 1987; Klein *et al*, 1992; Aldred *et al*, 1994; Hardman & Aldred, 1995; Tsetsonis & Hardman, 1996a, b; Tsetsonis *et al*, 1997; Gill *et al*, 1998, 2001a, b, 2002, 2003; Hardman *et al*, 1998; Herd *et al*, 1998, 2000, 2001; Zhang *et al*, 1998; Malkova *et al*, 1999; Gill & Hardman, 2000; Koutsari & Hardman, 2001; Koutsari *et al*, 2001; Thomas *et al*, 2001; Petitt *et al*, 2003). Since the lipemic response to a fat meal 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 not have a significant effect on lipemia after a meal of moderate fat content.

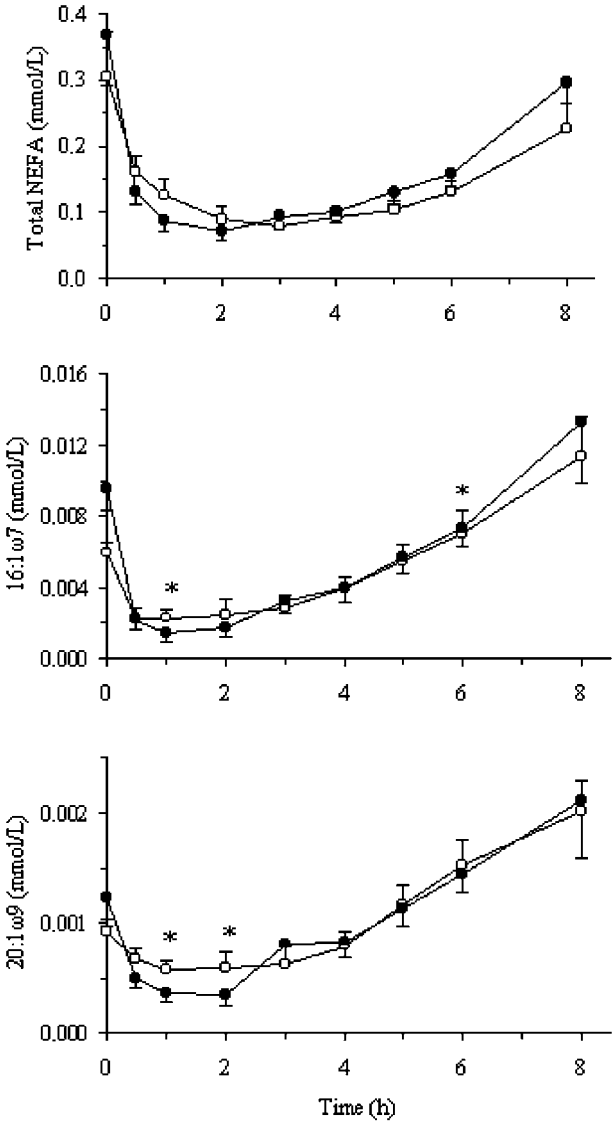

Figure 3 Fasting and postprandial plasma concentrations of total NEFA and two individual NEFA being the most abundant in the test meal relative to plasma TG. Open circles correspond to the control trial and full circles to the exercise trial. Values are means ± SEM for nine subjects. *Significantly different between exercise and control while compensating for differences at baseline (simple contrast analysis, $P \leqslant 0.05$).

We chose the exercise regimen with applicability and efficiency in mind. Duration did not exceed the maximum recommended for developing and maintaining cardiorespiratory fitness and weight control (60 min per session) (ACSM, 1998), while intensity was in the middle of the recommended range (55–90% HRmax), in order to produce an energy expenditure slightly above the minimum shown to decrease lipemia after high-fat meals (Gill & Hardman, 2000; Thomas *et al*, 2001).

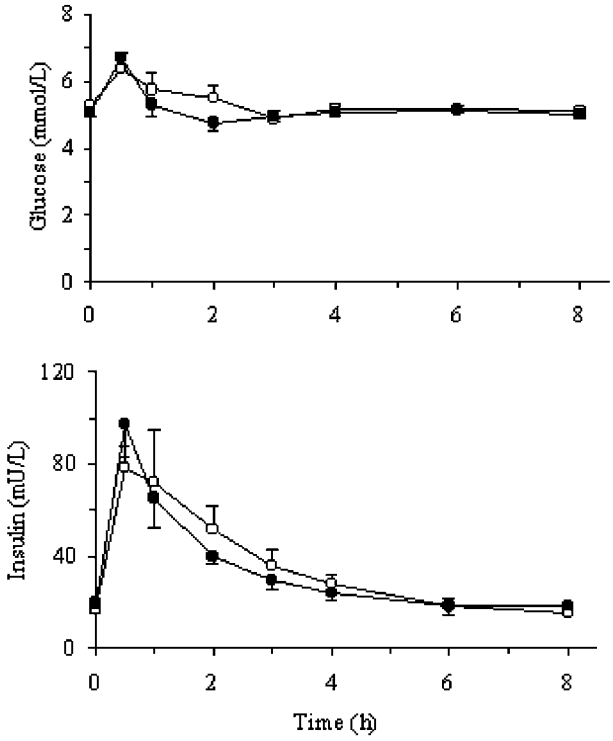

Figure 4 Fasting and postprandial plasma concentrations of glucose and insulin. Open circles correspond to the control trial and full circles to the exercise trial. Values are means ± SEM for nine subjects.

Our findings indicate that 1 h of exercise classified as hard in terms of relative intensity (ACSM, 1998), performed between 15 and 14 h before the consumption of a moderate-fat meal, caused a significant reduction in postprandial lipemia expressed as the total AUC of TG concentration *vs* time. This is in agreement with the majority of the studies that have compared this outcome measure or an equivalent one, time-averaged concentration, after a high-fat meal consumed 12–18 h following aerobic exercise *vs* rest (Tsetsonis & Hardman, 1996a (moderate intensity trial), 1996b; Tsetsonis *et al*, 1997; Hardman *et al*, 1998; Gill *et al*, 1998, 2001a, b, 2003; Malkova *et al*, 1999, 2000; Gill & Hardman, 2000; Herd *et al*, 2001; Koutsari & Hardman, 2001; Koutsari *et al*, 2001). Three studies have found no significant difference between exercise and rest (Tsetsonis & Hardman, 1996a (low-intensity trial); Herd *et al*, 1998; Petitt *et al*, 2003). This is apparently due to the low energy expenditure of the exercise performed, since the energy expenditure of prior exercise correlates positively with the suppression of postprandial lipemia (Gill & Hardman, 2003; Pettit & Cureton, 2003). Total AUC decreased by 26% with exercise in the present work, well within the respective values in the studies using high-fat meals. In terms of SD units (ie, effect size), the effect of exercise was one of the largest in the relevant literature.

Contrary to total AUC, incremental AUC, which reflects the net lipemic response to fat intake, was not affected significantly by prior exercise. This can be explained by the fact that most (79%) of the difference between exercise and control in total AUC was due to the decrease in baseline TG concentration (by 25%). Most of the relevant studies have found significant decreases in incremental AUC with exercise (Aldred *et al*, 1994; Tsetsonis & Hardman, 1996a (moderate intensity trial), 1996b; Tsetsonis *et al*, 1997; Zhang *et al*, 1998; Hardman *et al*, 1998; Malkova *et al*, 1999; Gill & Hardman, 2000; Gill *et al*, 2001a, b, 2003; Herd *et al*, 2001; Koutsari & Hardman, 2001; Thomas *et al*, 2001). None of the studies that found a significant decrease in total AUC has reported a nonsignificant change in incremental AUC, although a few of them have not compared incremental AUC (Gill *et al*, 1998; Malkova *et al*, 2000; Koutsari *et al*, 2001). The most probable reason for the different finding in the present study is the moderate fat content of the test meal, suggesting that a moderate fat intake does not produce a net lipemic response large enough to be affected by exercise significantly.

Previous reports have suggested that increased LPL-mediated TG clearance (Tsetsonis & Hardman, 1996a; Hardman, 1998; Zhang *et al*, 1998; Petitt *et al*, 2003) and/or decreased hepatic VLDL secretion (Malkova *et al*, 2000; Gill *et al*, 2001a; Gill & Hardman, 2003) are likely to mediate the reductions in postprandial lipemia seen following exercise. These mechanisms are likely to have contributed to the TG-lowering effect of exercise observed in the present study, but our data suggest that exercise may also have diminished the entry of intestinally derived TG into the circulation. To test this hypothesis, we have taken advantage of the unusual fatty acid profile of macadamia nuts. To enhance their utility, we minimized other sources of fat (by using skimmed milk and low-fat ham) in the test meal. This resulted in a considerable enrichment of the test meal, relative to plasma TG, in four fatty acids (16:1ω7, 18:1ω9, 18:1ω7, and 20:1ω9). These were the only fatty acids of plasma TG that were significantly lower in the exercise trial postprandially after correcting for differences at baseline, thus representing net decreases in the postprandial lipemic response. We consider this an indication of attenuated release of dietary fat from the intestine after exercise. Consistent with this possibility is the delayed peak in the plasma TG concentration following exercise (contrary to most relevant reports with high-fat meals). This effect may be due to changes in the availability of substrates for chylomicron formation from the previous meal(s) inside the enterocytes, reduced entry of fatty acids and monoacylglycerols as a result of changes in the biological activity or expression of proteins involved in the pathways of their uptake from the intestinal lumen, increased oxidation of dietary fatty acids in the enterocytes, decreased TG synthesis, and/or decreased chylomicron secretion. None of these possibilities has been tested experimentally. It is tempting to speculate that if such an effect is manifest 14 h after exercise, a similar effect may have been exerted on the lipemia after the meal that was consumed within 2 h of the end of exercise (on the evening before the test meal) and that this effect may have contributed to the reduced fasting plasma TG concentration of the following morning. Evidently, further research is needed to examine the possible involvement of the intestine in the response of postprandial lipemia to exercise.

An alternative explanation for the reduction in the fatty acids that were more abundant in the test meal than in plasma TG with exercise may be the upregulation of muscle LPL in conjuction with its apparent preference to hydrolyze TG incorporated into chylomicrons rather than VLDL (Malkova *et al*, 2000), since the fatty acids in question will be primarily present in chylomicrons. However, this mechanism alone cannot explain the NEFA data, as will be discussed shortly.

In addition to plasma TG, we have measured three parameters related to their metabolism, namely, NEFA, glucose, and insulin. In accordance with most of the relevant studies (Tsetsonis & Hardman, 1996a; Gill *et al*, 1998, 2001b; Herd *et al*, 1998, 2001; Malkova *et al*, 1999, 2000; Koutsari & Hardman, 2001; Koutsari *et al*, 2001), the postprandial response of total NEFA was not significantly different between exercise and control. This was also the case for the individual NEFA except for the ones most abundant in the test meal relative to plasma TG (ie, 16:1ω7 and 20:1ω9).

A comparison of the postprandial responses of the latter two NEFA between the two trials lends further support to an involvement of the intestine in the reduction of postprandial lipemia by exercise. Skeletal muscle has been shown to entrap all NEFA produced by the hydrolysis of lipoprotein-borne TG in its capillaries (Evans *et al*, 2002). Therefore, if only the increased activity of muscle LPL was responsible for the reduction in postprandial lipemia, there would have been no significant difference in the concentration of any plasma NEFA between the two trials despite the preference of the enzyme for chylomicron-borne TG. On the contrary, however, 16:1ω7 and 20:1ω9, as NEFA, were significantly lower after exercise at almost the same time points at which they were significantly lower as TG acyl groups. This suggests a decreased release of these fatty acids from the intestine into the bloodstream. An alternative explanation for this observation is increased LPL activity and NEFA entrapment in adipose tissue after exercise, possibly as a result of increased sensitivity to insulin, which increases LPL activity and decreases hornone-sensitive lipase activity (Frayn, 1998).

In unison with all the relevant studies (Tsetsonis & Hardman, 1996a, b; Tsetsonis *et al*, 1997; Gill *et al*, 1998, 2001a, b; Hardman *et al*, 1998; Herd *et al*, 1998, 2001; Malkova *et al*, 1999; Gill & Hardman, 2000; Malkova *et al*, 2000; Koutsari & Hardman, 2001; Koutsari *et al*, 2001; Petitt *et al*, 2003), the postprandial glycemic response did not differ between exercise and control, confirming the strong homeostasis of plasma glucose. No difference was found in the insulinemic response either. The relevant literature is split in this respect, with some studies being in agreement with ours

(Tsetsonis & Hardman, 1996a; Herd *et al*, 1998, 2001; Malkova *et al*, 2000; Gill *et al*, 2001b; Koutsari & Hardman, 2001), some reporting significantly lower posprandial insulin AUC after exercise (Hardman *et al*, 1998; Malkova *et al*, 1999; Gill & Hardman, 2000; Gill *et al*, 2001a; Koutsari *et al*, 2001), and some producing different outcomes for different exercise conditions or subject characteristics (Tsetsonis & Hardman, 1996b; Tsetsonis *et al*, 1997; Gill *et al*, 1998; Petitt *et al*, 2003). The insulinemic response in the present study was markedly higher than in the other studies due to the higher carbohydrate content of the test meal (2.14 *vs* 1.44 g/kg at the most).

In conclusion, hard exercise performed between 15 and 14 h before the consumption of a meal of moderate fat content had a large reducing effect on postprandial lipemia. This effect was mainly due to the decrease in baseline plasma TG concentration and was more pronounced in the early postprandial hours. During this period, only the fatty acids that were more abundant in the test meal than in plasma TG were significantly lower following exercise when controlling for differences at baseline. This suggests a possible effect on the rate of TG release from the intestine, although other mechanisms are likely to have accounted for a large part of the TG lowering effect of exercise. Finally, further research with meal compositions and exercise regimens applicable to everyday conditions should enable us to define the limits of exercise in terms of its ability to mitigate postprandial lipemia.

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