Effects of prior moderate exercise on exogenous and endogenous lipid metabolism and plasma factor VII activity

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ABSTRACT

Moderate exercise reduces postprandial triacylglycerol concentrations, which are a risk marker for coronary heart disease. The present study sought to determine the qualitative nature of exercise-induced changes in lipid metabolism and their association (if any) with changes in factor VII activation. Eleven normotriglyceridaemic men, aged 51.7 ± 6.1 years (mean ± S.D.), participated in two oral fat tolerance tests after different pre-conditions: control (no exercise), and exercise (90 min of brisk walking the day before). Venous blood samples were obtained in the fasted state and for 8 h after ingestion of a high-fat meal (1.32 g of fat, 1.36 g of carbohydrate, 0.30 g of protein and 10 mg of [1,1,1-13C] tripalmitin [kg −1 body mass]). Prior exercise reduced postprandial plasma triacylglycerol concentrations by 25 ± 3% (mean ± S.E.M.), with lower concentrations in the Svedberg flotation rate (Sf) 20–400 (very-low-density lipoprotein) fraction accounting for 79 ± 10% of this reduction. There was no effect on plasma factor VII coagulant activity or on the concentration of the active form of factor VIIa. Prior exercise increased postprandial serum 3-hydroxybutyrate and plasma fatty acid concentrations, decreased serum postprandial insulin concentrations and increased exogenous (8 h13C breath excretion of 15.1 ± 0.9% of ingested dose compared with 11.9 ± 0.8%; P = 0.00001) and endogenous postprandial fat oxidation. These data raise the possibility that reduced hepatic secretion of very-low-density lipoprotein plays a role in the attenuation of plasma triacylglycerol concentrations seen after exercise, although it is possible that increased triacylglycerol clearance also contributes to this effect.

INTRODUCTION

Disturbances in triacylglycerol metabolism occurring during the postprandial period have been implicated in the progression of atherosclerosis by a number of possible mechanisms. An early suggestion was that remnants of postprandial lipoproteins may be directly atherogenic [1], but it has since been proposed that repeated episodes of exaggerated postprandial lipaemia are atherogenic because they lead to low concentrations

Key words: chylomicron, exercise, factor VII, fat ingestion, fat oxidation, fatty acids, insulin, ketone bodies, triacylglycerol, very-low-density lipoprotein.

Abbreviations: FVIIa, factor VII in the activated form; FVIIc, factor VII coagulant activity; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LPL, lipoprotein lipase; Sf, Svedberg flotation rate; VLDL, very-low-density lipoprotein; VCO2, carbon dioxide production; VO2, oxygen uptake.

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of high-density lipoprotein (HDL) cholesterol and a preponderance of small, dense low-density lipoprotein (LDL) and small HDL\textsubscript{\textit{x}} particles [2]. Factor VII coagulant activity (FVIIc) is increased in the postprandial state [3–5], largely due to increased circulating concentrations of factor VII in the activated form (FVIIa) [3]. Plasma FVIIc is positively associated with concentrations of plasma triacylglycerol [6], and has been reported to be associated with the risk of fatal ischaemic heart disease in two studies [7,8], although not in a third [9]. Thus interventions that can attenuate postprandial lipaemia may delay both the chronic and acute phases of coronary heart disease.

Current exercise guidelines recommend that adults should engage in moderate-intensity exercise [10], and a number of studies have shown that this type of exercise, performed some hours before a meal, can attenuate postprandial plasma triacylglycerol concentrations [11–17], with an associated increase in postprandial fat oxidation [14,18]. However, the qualitative nature of these changes and the mechanisms responsible are poorly understood. In an attempt to provide some insight into these mechanisms and their association (if any) with changes in FVIIc activity and FVIIa concentration, we examined the effects of prior moderate-intensity exercise on chylomicron-triacylglycerol and very-low-density lipoprotein (VLDL)-triacylglycerol concentrations in the postprandial state, and on the postprandial oxidation of fatty acids derived from the meal (exogenous) and of fatty acids already stored within the body (endogenous). This was achieved by tracing the metabolic fate of the ingested dietary lipid using \textsuperscript{13}C-labelled fatty acids incorporated within the test meal. Middle-aged men were chosen for study, as the prescription of moderate-intensity exercise is particularly applicable for this age group; in addition, these subjects, by virtue of their age and/or gender, are at greater risk of coronary heart disease than the populations in which the influence of exercise on postprandial lipoprotein metabolism has been investigated previously [19].

**METHODS**

**Subjects**

A total of 11 men (age 51.7 ± 6.1 years; body mass 74.7 ± 13.5 kg; body mass index 24.2 ± 3.7 kg·m\textsuperscript{-2}; sum of four skin-folds 58.0 ± 19.2 mm; body fat 27.2 ± 5.6 % [20]; waist circumference 87.8 ± 10.1 cm; maximum heart rate 178 ± 7 beats·min\textsuperscript{-1}; maximal oxygen uptake (V\textsubscript{O\textsubscript{2}} max) 38.9 ± 5.6 ml·min\textsuperscript{-1}·kg\textsuperscript{-1} (means ± S.D.)) participated in the study, which was conducted in accordance with the Declaration of Helsinki (1989) of the World Medical Association and with the approval of the Loughborough University Ethical Advisory Committee.

Most of the men engaged in some physical activity, for example walking or gardening, at least once per week, and three performed activities such as jogging, cycling or fitness classes on three to five occasions per week. All were informed of the procedures and risks involved, and gave their written consent to take part. They were apparently healthy non-smokers who displayed no symptoms of coronary artery disease during a clinical exercise stress test. None was taking any drugs thought to affect lipid or carbohydrate metabolism. Six subjects exhibited the E3/E3 apolipoprotein E phenotype, three subjects possessed the E4/E3 phenotype and two possessed the E3/E2 phenotype.

**Preliminary exercise testing**

After a familiarization session to determine each subject’s comfortable walking speed, two preliminary exercise tests were conducted. In the first, the steady-state relationship between submaximal V\textsubscript{O\textsubscript{2}} and treadmill gradient was established. In the second, V\textsubscript{O\textsubscript{2}} max was determined during incremental uphill treadmill walking. The gradient required to elicit 60 % V\textsubscript{O\textsubscript{2}} max was interpolated.

**Study design**

Subjects participated in two oral fat tolerance tests in a randomized, balanced design with an interval of 7 days and with different pre-conditions. In one trial, subjects walked on a treadmill for 90 min at an intensity of approx. 60 % V\textsubscript{O\textsubscript{2}} max on the day prior to the oral fat tolerance test (exercise trial). In the other trial, subjects performed no exercise on the day preceding the oral fat tolerance test (control trial).

Subjects weighed and recorded their dietary intake for the 2 days before the first oral fat tolerance test, and replicated this before the second fat tolerance test. In addition, subjects agreed to avoid foods naturally enriched in \textsuperscript{13}C (they were given a list describing such foods) and performed no exercise, other than the treadmill walk in the exercise trial, during the 3 days preceding each oral fat tolerance test. Subjects abstained from alcohol consumption on the day preceding each fat tolerance test.

**Treadmill walk**

The walk began between 14.30 hours and 15.30 hours. Expired air samples were collected into Douglas bags at 15-min intervals during the walk, and V\textsubscript{O\textsubscript{2}} and carbon dioxide production (VCO\textsubscript{2}) were determined using standard techniques. Energy expenditure was calculated using indirect calorimetry (see Calculations and statistics section). Heart rate was monitored by short-range telemetry (Polar PE 3000 Sport Tester; Polar Electroxy, Kempele, Finland), and ratings of perceived exertion [21] were obtained at 15-min intervals during the walk.
Oral fat tolerance tests
Subjects reported to the laboratory after an overnight fast of at least 12 h, having travelled by car. A cannula was introduced into an antecubital or forearm vein and, after a 10-min interval, a 6-min expired air sample was taken into a Douglas bag. $V_O_2$ and $V_CO_2$ were determined by standard techniques. In addition, a specimen of expired air was collected into a breath collection bag (Quinton, Milwaukee, WI, U.S.A.) to determine baseline $^{13}C$ excretion. A baseline blood sample was then obtained.

Subjects then consumed the test meal, which comprised two parts: a heated lipid/casein/glucose/sucrose emulsion containing 10 mg·kg$^{-1}$ body mass [1,1,1-$^{13}C$] tripalmitin (99% atom % excess; Masstrace Inc., Woburn, MA, U.S.A.), and a high-fat, cereal-based meal consisting of whipping cream, oats, fruit, chocolate and nuts. The emulsion was prepared as described in a previous publication [22]; this form of labelled lipid delivery has been shown to result in nearly complete absorption (~99%) of the tracer [22]. Together, the test meal provided 99 ± 18 g of fat, 102 ± 18 g of carbohydrate, 23 ± 4 g of protein and 5.7 ± 1.0 MJ of energy (means ± S.D.). Subjects ingested 1.5 g of paracetamol with the test meal as a marker of gastric emptying [23,24]. Blood samples were obtained 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 480 min after finishing the test meal (total volume of blood sampled was ~220 ml per trial). Prior to each hourly blood sample, expired air samples were collected into Douglas bags and breath collection bags. During the 8 h postprandial period, subjects rested and only water was consumed; this was provided ad libitum in the first trial and consumption was replicated in the second trial. After 8 h subjects were given a standard meal with a low natural abundance of $^{13}C$. Further breath samples for the determination of $^{13}CO_2$ were obtained ~15 h and ~24 h after ingestion of the test meal; subjects were instructed to refrain from exercise and alcohol consumption until after these collections were made and to consume an evening meal (to be replicated in the second trial) containing a low natural abundance of $^{13}C$.

Plasma separation
Blood samples were collected into 9 ml and 5 ml pre-cooled EDTA Monovettes (Sarstedt, Leicester, U.K.), 4.5 ml non-heparinized serum Monovettes and 5 ml citrated Monovettes. Blood collected into the EDTA Monovettes was separated in a refrigerated centrifuge within 15 min of collection. Plasma was pooled and 3.6 ml was dispensed into a tube containing 90 μl of a preservative cocktail [44 g of EDTA, 2.93 g of chomaphenicol, 3.67 g of sodium azide, 2.93 g of gentamicin sulphate and 366670 units of Trasylol (Bayer plc, Newbury, U.K.) per litre] and refrigerated overnight for subsequent lipoprotein fractionation. The remaining EDTA plasma was divided into aliquots and stored at ~20 °C. Blood collected into the serum Monovettes was allowed to clot for 60 min before separation and storage at ~70 °C. Blood collected into citrated Monovettes was centrifuged at room temperature before storage at ~70 °C.

Lipoprotein fractionation
Chylomicron [Svedberg flotation rate (Sf) > 400] and VLDL (Sf 20–400) fractions were obtained by sequential flotation in an ultracentrifuge (Beckman Optima TLX; Beckman Instruments Inc., London, U.K.) on the day after sample collection, using a procedure based on the method described by Havel et al. [25]. Duplicate 0.75 ml plasma samples were layered under a NaCl solution of density 1.006 g/ml in polyallomer open-top ultracentrifuge tubes (product no. 347357; Beckman Instruments Inc.). These were ultracentrifuged for 20 min at 4 °C in a swinging-bucket rotor (Beckman TLS 55) at 59000 g (average), and chylomicrons were isolated by removing the top layer in a tube slicer (Beckman CentriTube slicer). Each pair of chylomicron infranates was transferred into a bell-topped ultracentrifuge tube (product no. 362248; Beckman Instruments Inc.), and these were ultracentrifuged in an angle-head rotor (Beckman 100.4) for 2.5 h at 4 °C at 417000 g (average). VLDLs were isolated by removing the top layer in a tube slicer (Beckman CentriTube slicer). The infranate contained the Sf < 20 fraction.

Analytical methods
Triacylglycerol concentrations were determined on the day after collection, in the chylomicron, VLDL and Sf < 20 fractions as well as in total plasma, by an enzymic colorimetric method (Boehringer Mannheim G.m.b.H., Lewes, E. Sussex, U.K.) using a centrifugal analyser (Cobas-Bio; Roche, Basle, Switzerland). Recovery of triacylglycerol in the lipoprotein fractions was 91.1 ± 0.8% in the control trial and 89.4 ± 1.4% in the exercise trial. The coefficient of variation for samples run on the same day was 6.3% for chylomicron triacylglycerol, 4.1% for VLDL triacylglycerol and 1.1% for total plasma triacylglycerol. The between-day coefficient of variation was 1.7% for total plasma triacylglycerol. Quality control sera (Roche) were used to ensure accuracy and precision.

Previously frozen EDTA plasma was analysed for HDL cholesterol by a MnCl$_2$/phosphotungstic acid precipitation method (only at baseline), and for total cholesterol (only at baseline), glucose (all from Boehringer Mannheim), fatty acids (Wako Chemicals) and paracetamol (Cambridge Life Sciences, Cambridge, U.K.) by enzymic colorimetric methods using a centrifugal analyser (Cobas-Mira; Roche). Serum was analysed for insulin by RIA (COAT-A-COUNT Insulin;
Figure 1 Plasma triacylglycerol, VLDL triacylglycerol and chylomicron triacylglycerol concentrations in the fasted state and for 8 h after consumption of a mixed high-fat meal in control and prior moderate exercise trials

The upper panel shows plasma triacylglycerol concentrations following the control (■) and exercise (▲) protocols. The lower panel shows VLDL triacylglycerol (▲, △) and chylomicron triacylglycerol (●, ○) concentrations following the control (▲, ●) and exercise (△, ○) protocols. The black rectangle denotes ingestion of the meal. Values are means ± S.E.M. (n = 11). Summary results of postprandial responses are shown in Table 2.

Diagnostic Products Corp., Los Angeles, CA, U.S.A.), and for 3-hydroxybutyrate by an enzymic colorimetric method (Sigma Diagnostics, Poole, Dorset, U.K.). LDL subclass distribution was determined at baseline using the method described by Griffin et al. [26]. All samples for one subject were analysed in the same run. Quality-control sera (from Roche, Boehringer Mannheim, Sigma and Sero AS, Billingstad, Norway) were used to ensure accuracy. Within-batch coefficients of variation were 0.8% for glucose, 0.9% for total cholesterol, 2.8% for HDL cholesterol, 0.9% for fatty acids, 0.7% for paracetamol, 2.3% for 3-hydroxybutyrate and 3.9% for insulin. Apolipoprotein E phenotypes were determined by isoelectric focusing using Western blot techniques [27].

Determination of breath enrichment of 13C

Specimen breath samples were transferred into evacuated gas sample containers (Exetainers; Labco, High Wycombe, U.K.) for analysis in duplicate by continuous-flow isotope-ratio MS (20–20 stable isotope analyser; Europa Scientific Ltd) as detailed previously. Excretion of 13C on the breath was expressed as the percentage of the administered 13C label excreted per h, and as the cumulative percentage dose excreted over 8 h (the controlled study period) and 24 h [22].

Determination of FVIIa and FVIIc in plasma

All assays for factor VII were performed on citrated plasma in duplicate and the results averaged. Plasma FVIIc was measured by a one-stage bioassay using a rabbit brain thromboplastin (Diagen, Thame, Oxon, U.K.) and factor VII-deficient substrate plasma prepared as described previously [28]. The results of the assay were expressed as a percentage of the performance of an ‘in house’ standard plasma calibrated in terms of an international primary standard plasma [National Institute of Biological Standards and Control (NIBSC), Potters Bar, Herts., U.K.; code labelled 84/665]. Plasma FVIIa was measured by a one-stage clotting assay employing a soluble mutant recombinant tissue factor which possesses cofactor activity for FVIIa, but fails to support activation of factor VII (a gift from Dr James H. Morrissey, Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, U.S.A.) [29]. Coagulation times were converted into FVIIa concentration (ng ml⁻¹) by comparison with a standard curve produced using a freeze-dried preparation of purified recombinant FVIIa (NIBSC; code labelled 89/688), serially diluted with commercial factor VII-deficient human plasma (George King Biomedical, Overland Park, KS, U.S.A.).

Calculations and statistics

Summary measures of the postprandial responses were calculated (using the trapezium rule) as the time-averaged areas under the plasma or serum concentration against time curves. After subtraction of the baseline concentrations, these values represented the time-averaged postprandial rises in concentration. The peak plasma paracetamol concentration and time to peak concentration were used to assess gastric emptying. These indices have been shown to correlate well with the rate of gastric emptying [23,24]. Energy expenditure and total
Table 1  Plasma and serum concentrations, substrate utilization and energy expenditure in the fasted state in the control and prior exercise trials

*P* values denote level of significance (paired *t*-test) between control and exercise trials.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Exercise</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasma triacylglycerol (mmol·l⁻¹)</td>
<td>1.03 ± 0.11</td>
<td>0.82 ± 0.12</td>
<td>0.0003</td>
</tr>
<tr>
<td>VLDL triacylglycerol (mmol·l⁻¹)</td>
<td>0.54 ± 0.09</td>
<td>0.37 ± 0.09</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chylomicron triacylglycerol (mmol·l⁻¹)</td>
<td>0.02 ± 0.004</td>
<td>0.01 ± 0.002</td>
<td>0.14</td>
</tr>
<tr>
<td>Plasma total cholesterol (mmol·l⁻¹)</td>
<td>4.84 ± 0.25</td>
<td>4.80 ± 0.25</td>
<td>0.65</td>
</tr>
<tr>
<td>Plasma HDL cholesterol (mmol·l⁻¹)</td>
<td>1.02 ± 0.10</td>
<td>1.06 ± 0.10</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum insulin (pmol·l⁻¹)</td>
<td>99.0 ± 9.8</td>
<td>85.7 ± 7.9</td>
<td>0.007</td>
</tr>
<tr>
<td>Plasma glucose (mmol·l⁻¹)</td>
<td>4.84 ± 0.25</td>
<td>4.80 ± 0.25</td>
<td>0.65</td>
</tr>
<tr>
<td>Plasma fatty acids (mmol·l⁻¹)</td>
<td>0.02 ± 0.004</td>
<td>0.01 ± 0.002</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum 3-hydroxybutyrate (mmol·l⁻¹)</td>
<td>0.06 ± 0.02</td>
<td>0.23 ± 0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>Fat oxidation rate (g·h⁻¹)</td>
<td>5.5 ± 0.6</td>
<td>6.4 ± 0.3</td>
<td>0.11</td>
</tr>
<tr>
<td>Carbohydrate oxidation rate (g·h⁻¹)</td>
<td>5.4 ± 1.6</td>
<td>3.2 ± 0.6</td>
<td>0.14</td>
</tr>
<tr>
<td>Energy expenditure (kJ·h⁻¹)</td>
<td>302 ± 12</td>
<td>303 ± 13</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Table 2  Time-averaged postprandial concentrations/activities and time-averaged postprandial increases in concentration/activity above that observed in the fasted state in the control and prior exercise trials

*P* values denote the level of significance of difference (paired *t*-test) between control and exercise trials.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Exercise</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasma triacylglycerol (mmol·l⁻¹)</td>
<td>1.80 ± 0.20</td>
<td>1.38 ± 0.19</td>
<td>0.0002</td>
</tr>
<tr>
<td>Postprandial concentration</td>
<td>0.77 ± 0.14</td>
<td>0.56 ± 0.10</td>
<td>0.002</td>
</tr>
<tr>
<td>VLDL triacylglycerol (mmol·l⁻¹)</td>
<td>0.95 ± 0.14</td>
<td>0.66 ± 0.14</td>
<td>0.00002</td>
</tr>
<tr>
<td>Postprandial increase in concentration</td>
<td>0.41 ± 0.08</td>
<td>0.28 ± 0.06</td>
<td>0.0008</td>
</tr>
<tr>
<td>Chylomicron triacylglycerol (mmol·l⁻¹)</td>
<td>0.30 ± 0.05</td>
<td>0.22 ± 0.03</td>
<td>0.006</td>
</tr>
<tr>
<td>Postprandial concentration</td>
<td>241.4 ± 28.2</td>
<td>204.9 ± 18.9</td>
<td>0.013</td>
</tr>
<tr>
<td>Plasma glucose (mmol·l⁻¹)</td>
<td>142.4 ± 19.0</td>
<td>119.2 ± 11.9</td>
<td>0.052</td>
</tr>
<tr>
<td>Postprandial concentration</td>
<td>5.8 ± 0.15</td>
<td>5.88 ± 0.10</td>
<td>0.46</td>
</tr>
<tr>
<td>Plasma fatty acids (mmol·l⁻¹)</td>
<td>0.15 ± 0.11</td>
<td>0.40 ± 0.08</td>
<td>0.011</td>
</tr>
<tr>
<td>Postprandial concentration</td>
<td>0.41 ± 0.02</td>
<td>0.48 ± 0.03</td>
<td>0.003</td>
</tr>
<tr>
<td>Serum 3-hydroxybutyrate (mmol·l⁻¹)</td>
<td>0.10 ± 0.02</td>
<td>0.16 ± 0.03</td>
<td>0.009</td>
</tr>
<tr>
<td>Plasma FVIIc (% of standard)</td>
<td>98.2 ± 10.5</td>
<td>99.6 ± 11.3</td>
<td>0.71</td>
</tr>
<tr>
<td>Postprandial activity</td>
<td>6.2 ± 2.6</td>
<td>3.4 ± 3.1</td>
<td>0.60</td>
</tr>
<tr>
<td>Plasma FVIIa (ng·ml⁻¹)</td>
<td>2.56 ± 0.48</td>
<td>2.00 ± 0.56</td>
<td>0.27</td>
</tr>
<tr>
<td>Postprandial concentration</td>
<td>0.96 ± 0.24</td>
<td>1.17 ± 0.30</td>
<td>0.31</td>
</tr>
<tr>
<td>Plasma paracetamol</td>
<td>0.13 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.64</td>
</tr>
<tr>
<td>Peak concentration (mmol·l⁻¹)</td>
<td>59 ± 13</td>
<td>49 ± 6</td>
<td>0.47</td>
</tr>
</tbody>
</table>
substrate utilization were calculated using indirect calorimetry, assuming no protein oxidation [30]. Cumulative $^{13}$CO$_2$ excretion was used to estimate oxidation of fatty acids contained in the test meal (i.e. exogenous lipid oxidation), after application of a correction factor to account for [1,1,1-$^{13}$C] tripalmitin, which was oxidized but not excreted on the breath (see Results). Means were compared using Student’s $t$-tests for correlated means, adopting a 5% level of significance. Data are presented as means ± S.E.M. unless otherwise stated.

RESULTS

Responses during treadmill walking
The mean $V_o_2$ during the 90 min walk was $25.1 ± 0.8 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, which represented $64.9 ± 1.2$% of $V_o_2$ max. Gross energy expenditure was $3.37 ± 0.22 \text{ MJ}$ and the mean heart rate was $140 ± 4 \text{ beats} \cdot \text{min}^{-1}$. The subjects rated the walk as $12.6 ± 0.5$ on the Borg scale, which corresponds to a perception of exertion between ‘fairly light’ and ‘somewhat hard’ [21].

Triacylglycerol concentrations in the fasted and postprandial states
The total plasma, chylomicron and VLDL triacylglycerol responses to the test meal are shown in Figure 1. In the fasted state, the total plasma triacylglycerol concentration was lower in the exercise trial than in the control trial, with this difference being largely accounted for by a lower triacylglycerol concentration in the VLDL fraction (see Table 1 for $P$ values and concentrations). Time-averaged postprandial total plasma, chylomicron and VLDL triacylglycerol concentrations and the time-averaged postprandial increases above baseline in total plasma and VLDL triacylglycerol concentrations were all smaller in the exercise trial than in the control trial (Table 2). There was no obvious effect of apolipoprotein E phenotype on these responses.

Concentrations of plasma FVIIc and FVIIa
Neither the time-averaged postprandial plasma activity nor the time-averaged postprandial rise in plasma activity of FVIIc differed between trials (Table 2). Similarly, the time-averaged plasma concentration and the time-
Table 3  Postprandial substrate utilization and energy expenditure in the control and prior exercise trials

P values denote the level of significance of difference (t-test) between control and exercise trials.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Exercise</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 h total fat oxidation (g)</td>
<td>47.3 ± 2.7</td>
<td>56.9 ± 2.8</td>
<td>0.002</td>
</tr>
<tr>
<td>8 h total carbohydrate oxidation (g)</td>
<td>54.4 ± 8.0</td>
<td>30.6 ± 6.4</td>
<td>0.002</td>
</tr>
<tr>
<td>8 h energy expenditure (kJ)</td>
<td>2734 ± 140</td>
<td>2741 ± 122</td>
<td>0.81</td>
</tr>
<tr>
<td>13C excretion as 13CO2 (% of ingested dose)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 h</td>
<td>11.9 ± 0.8</td>
<td>15.1 ± 0.9</td>
<td>0.00001</td>
</tr>
<tr>
<td>24 h</td>
<td>22.0 ± 1.6</td>
<td>27.6 ± 1.5</td>
<td>0.002</td>
</tr>
<tr>
<td>8 h exogenous fat oxidation* (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assuming 50% recovery on breath of oxidized 13C</td>
<td>23.3 ± 1.8</td>
<td>29.2 ± 1.8</td>
<td>0.000002</td>
</tr>
<tr>
<td>Assuming 100% recovery on breath of oxidized 13C</td>
<td>11.7 ± 0.9</td>
<td>14.6 ± 0.9</td>
<td>0.000002</td>
</tr>
</tbody>
</table>

*Assuming that oxidation of 13C-tripalmitin is representative of exogenous fat oxidation.

Averaged postprandial rise in the plasma concentration of FVIIa did not differ between trials (Table 2).

Concentrations of other plasma and serum constituents in the fasted and postprandial states

In the fasted state, plasma fatty acid, serum 3-hydroxybutyrate and HDL cholesterol concentrations were higher, and the serum insulin concentration was lower, in the exercise trial than the control trial (Table 1). Fasting plasma glucose concentrations tended to be lower in the exercise trial than in the control trial (Table 1). The fasting plasma total cholesterol concentration did not differ between trials (Table 1). The LDL subclass distribution ($n = 10$) did not differ between trials (control: $18 \pm 3\%$ LDL-I, $43 \pm 6\%$ LDL-II, $39 \pm 7\%$ LDL-III; exercise: $17 \pm 3\%$ LDL-I, $40 \pm 5\%$ LDL-II, $43 \pm 8\%$ LDL-III).

Time-averaged postprandial plasma fatty acid and serum 3-hydroxybutyrate concentrations were higher in the exercise trial than in the control trial, as was the time-averaged postprandial increase in plasma glucose concentration. Both the time-averaged postprandial insulin concentration in the exercise trial than in the control trial (see Table 2 for P values and concentrations, and Figure 2 for the postprandial responses).

Neither peak plasma paracetamol concentration nor time to peak concentration differed between trials (Table 2).

Substrate utilization and energy expenditure

In the fasted state, fat oxidation, carbohydrate oxidation and energy expenditure did not differ between trials (Table 1). Total postprandial fat oxidation was higher and carbohydrate oxidation was lower in the exercise trial than in the control trial (Table 3).

Cumulative excretion of $^{13}$C on the breath was higher in the exercise trial than in the control trial, both over the 8 h period during which directly measured VCO$_2$ measurements were obtained and over the full 24 h observation period (see Table 3 for P values and data, and Figure 3 for the responses). The 8 h values for exogenous fat oxidation calculated from $^{13}$CO$_2$ excretion, assuming recoveries of oxidized $^{13}$C on the breath of 50% and 100%, are shown in Table 3. These recovery percentages reflect the low [31] and high [32] extremes reported in the literature for recovery of $^{13/18}$CO$_2$ on the breath after administration of NaH$^{13/18}$CO$_3$.
DISCUSSION

The present study has shown that a prior session of moderate-intensity exercise significantly attenuated post-absorptive and postprandial plasma triacylglycerol concentrations in normotriglyceridaemic middle-aged men. Plasma triacylglycerol concentrations were decreased after exercise in every subject. Thus moderate exercise can improve triacylglycerol metabolic capacity in a population group who, by nature of their age [19,33,34] and/or gender [19,34], are at increased risk of coronary heart disease compared with the young adults [11–16], middle-aged women [18] and postmenopausal women [17] studied previously.

Exercise reduced triacylglycerol concentrations in both the chylomicron (Sf > 400) and VLDL (Sf 20–400) fractions, with the decrease in the latter accounting for almost four-fifths of the reduction in plasma triacylglycerol observed in the postprandial state. The Sf 20–400 fraction does not solely contain hepatically derived VLDL particles in the postprandial state, as small chylomicrons and chylomicron remnants are also present. However, it seems likely that the decrease in triacylglycerol in this lipoprotein fraction largely reflected differences in hepatically derived VLDL particles, as (1) there were large differences between trials in the fasted state (when there are no chylomicrons present), and (2) in the postprandial state, concentrations of apolipoprotein B48 in the Sf 20–400 fraction are at least an order of magnitude lower than the concentrations of apolipoprotein B-100 in this fraction [35,36].

Lower concentrations of triacylglycerol in the fasted state accounted for half of the decrease in the postprandial triacylglycerol concentration seen after exercise. The lower fasting concentration may also have contributed to the smaller postprandial rise in triacylglycerol seen after exercise, by reducing competition for clearance of triacylglycerol-rich lipoproteins by lipoprotein lipase (LPL). However, the mechanism(s) via which moderate-intensity exercise induces the observed triacylglycerol attenuation (in both the fasting and postprandial states) are not obvious. Exercise may have increased the rate of triacylglycerol clearance, and/or reduced the rate of hepatic VLDL secretion. Although it is also theoretically possible that the rate of appearance of chylomicrons from the gut was reduced after exercise, it seems unlikely that this was a large determinant of the lower plasma triacylglycerol concentrations, for a number of reasons. Firstly, exercise was performed on the afternoon before the oral fat tolerance test, and it is unlikely that effects on gastrointestinal blood flow would have persisted into the following day. This view is supported by the plasma paracetamol data, which showed no significant differences in peak concentration nor time to peak between the control and exercise trials. In fact, the paracetamol concentration peaked numerically slightly earlier in the exercise trial, implying that gastric emptying was not impaired, and if anything was slightly faster, in the exercise trial. Moreover, the rate of rise of chylomicron triacylglycerol concentrations was similar in the control and exercise trials for the first 2 h postprandially, and the peak concentration did not occur later in the exercise trial, which also argues against a delayed appearance of chylomicrons into the circulation following exercise.

It is often assumed that exercise-induced decreases in plasma triacylglycerol concentrations are due to increased triacylglycerol clearance. This is based on a body of evidence showing that prolonged, vigorous exercise can increase triacylglycerol clearance [37,38] and LPL activity [38–42]. However, when moderate-intensity exercise of shorter duration has been evaluated, the findings have been less clear. It has been shown that 90 min of moderate-intensity cycling [43] did not increase triacylglycerol clearance, and recent reports have shown that, despite significantly attenuating postprandial lipaemia, 90 min of moderate-intensity exercise did not increase LPL activity in skeletal muscle [44] or increase the clearance rate of an intravenously administered bolus of an artificial triacylglycerol emulsion [45]. These data suggest that the attenuation in plasma triacylglycerol observed following moderate exercise is not necessarily due to increased (LPL-mediated) triacylglycerol clearance. Chylomicrons and VLDL are both cleared by the same pathway (hydrolysis of lipoprotein triacylglycerol by the action of LPL) [46], but the presence of chylomicrons [47] or chylomicron-like particles [48] in the circulation almost completely inhibits the clearance of VLDL-sized lipoproteins, resulting in their accumulation in the plasma; accordingly, clearance of triacylglycerol in the postprandial state largely reflects clearance of large chylomicron-sized particles. Thus, while the decrease of 0.08 ± 0.03 mmol/l in the chylomicron triacylglycerol concentration seen after exercise in the present study is likely to reflect increased triacylglycerol clearance (although it is not clear whether this reflects increased LPL activity or reduced competition for LPL due to the smaller VLDL pool size), it seems unlikely that this was the sole mediator of the observed 0.30 ± 0.05 mmol/l decrease in the postprandial VLDL triacylglycerol concentration seen after exercise.

This raises the possibility that hepatic VLDL secretion was reduced by exercise. The potential role of this effect as a mediator of exercise-induced reductions in plasma triacylglycerol concentrations has been largely overlooked, and we are not aware of any data evaluating this in humans. However, in rats, hepatic VLDL secretion rates are reduced by training [49–51], although data evaluating the acute effects of a single exercise session are lacking. The mechanism(s) responsible for such a decrease are not immediately apparent. Substrate delivery to the liver is an important determinant of VLDL apolipoprotein B-100 secretion [52], and thus the elevated
fasting and postprandial plasma fatty acid concentrations observed in the exercise trial would normally be expected to induce a higher, not lower, rate of VLDL secretion. However, the situation is not clear cut, as, in the liver, fatty acids are either re-esterified into triacylglycerol or enter the mitochondrial matrix for β-oxidation or ketone body formation, according to the concentration of malonyl-CoA [53]. Prior exercise increased serum 3-hydroxybutyrate concentrations (a surrogate measure of hepatic β-oxidation) by over 50%, demonstrating a shift in partitioning of the fatty acid flux to the liver towards ketogenesis and oxidation rather than re-esterification and VLDL synthesis. It is not unreasonable to suggest that this shift may have induced a decrease in hepatic VLDL output, an interpretation supported by studies in rats by Fukuda and co-workers [51]. These authors reported that exercise-training-induced reductions in plasma triacylglycerol concentrations were matched by similar decreases in the hepatic triacylglycerol secretion rate, with a concomitant increase in hepatic ketone body production, and concluded that altered partitioning of fatty acids between esterification and oxidation was one of the causative factors in the triacylglycerol-lowering effect of exercise. However, as direct evidence is not available in humans, this warrants further study.

Prior exercise had a profound effect on substrate utilization. This is in agreement with previous data reporting that in both the fasted [54,55] and postprandial [14,18] states prior exercise increased whole-body fat oxidation. However, the present study is the first (to our knowledge) to evaluate the qualitative effects of prior moderate exercise on exogenous and endogenous fat oxidation. Whole-body fat oxidation was 9.6 ± 2.2 g greater over the 8 h postprandial observation period, with increased exogenous fat oxidation accounting for between 2.9 ± 0.3 g (assuming 100% recovery) and 5.9 ± 0.6 g (assuming 50% recovery) of this elevation. It is possible that a proportion of the labelled tripalmitin used to calculate exogenous fat oxidation was ‘recycled’ into the VLDL triacylglycerol pool before oxidation. However, as this still reflects oxidation of newly ingested lipid (whatever the intermediate pathway), breath 13CO2 excretion still provides a valid measure of ‘exogenous’, as opposed to stored or ‘endogenous’, fat oxidation. Thus increases in both exogenous and endogenous fat oxidation contributed to the augmentation in fat oxidation seen after exercise, and this is likely to reflect increased hepatic and skeletal muscle fat oxidation. Further investigation is required to ascertain the relative importance of these two tissues in eliciting these changes; however, in light of the 3-hydroxybutyrate data and the liver’s high metabolic requirements (at rest, the liver accounts for over one-quarter of whole-body oxygen uptake, which is equivalent to the oxygen requirement of skeletal muscle [56]), the potential contribution of increased hepatic fat oxidation should not be underestimated.

The LDL subclass distribution was not changed on the morning after exercise. This is perhaps not surprising, as the residence time for LDL particles in the circulation is of the order of days [57], so changes in LDL profile are not likely to have been evident so soon after a triacylglycerol-lowering intervention. Further study is necessary to ascertain whether a short-term moderate exercise intervention can influence LDL subclass distribution.

Fasting and postprandial insulin concentrations were lower after exercise, coupled with somewhat lower (fasting; \( P = 0.08 \)) or similar (postprandial) glucose concentrations, suggesting that exercise may have influenced insulin sensitivity. However, it is interesting to note that the postprandial increase in glucose concentration was higher in the exercise trial than in the control trial. This might be a consequence of the somewhat lower fasting glucose concentration after exercise, but we can offer no obvious explanation for this finding.

This study confirmed the transient activation of factor VII following a fat-rich meal, and provided new information by showing that this response is uninfluenced by the concentration of VLDL triacylglycerol in the postprandial phase. This observation suggests that triacylglycerol-rich lipoproteins of intestinal origin are likely to be more important for factor VII activity than hepatic VLDL, in accordance with an earlier observation. Thus FVIIc generally declines when fat is reduced in the diet [58,59], even when replacement of fat induces an increase in the fasting (i.e. endogenous) triacylglycerol concentration [60]. It also appears that lipolysis of lipoproteins is required for factor VII activation, as factor VII levels are normal in the massive hypertriglyceridaemia in patients with familial complete LPL deficiency [61]. Thus the finding that moderate exercise did not influence factor VII activation in the present study is consistent with our interpretation that mechanisms other than increased triacylglycerol clearance were largely responsible for the lower plasma triacylglycerol concentrations seen after exercise.

In conclusion, prior moderate exercise attenuated both fasting and postprandial triacylglycerol concentrations, and increased both postprandial exogenous and endogenous fat oxidation. However, exercise did not alter postprandial FVIIc activity or FVIIa concentration, suggesting that the plasma concentration of hepatic VLDL is of less importance for factor VII activation than the level of triacylglycerol-rich lipoproteins of intestinal origin. A large proportion of the exercise-induced attenuation in plasma triacylglycerol was attributable to a decrease in VLDL triacylglycerol, which is consistent with reduced hepatic VLDL secretion being the major mediator of this change, although the role of increased lipoprotein triacylglycerol clearance cannot be ruled out. This, together with other data [44,45], raises the possibility that, in contrast with prolonged and intense exercise, moderate-intensity exercise may attenuate
plasma triacylglycerol concentrations through mechanisms other than increased triacylglycerol clearance. This is a testable hypothesis which warrants further investigation.

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