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Prior exercise and postprandial substrate extraction across the human leg

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Malkova, D., R. D. Evans, K. N. Frayn, S. M. Humphreys, P. R. M. Jones, and A. E. Hardman. Prior exercise and postprandial substrate extraction across the human leg. *Am J Physiol Endocrinol Metab* 279: E1020–E1028, 2000.—Prior exercise decreases postprandial plasma triacylglycerol (TG) concentrations, possibly through changes to skeletal muscle TG extraction. We measured postprandial substrate extraction across the leg in eight normolipidemic men aged 21–46 yr. On the afternoon preceding one trial, subjects ran for 2 h at $64 \pm 1\%$ of maximal oxygen uptake (exercise); before the control trial, subjects had refrained from exercise. Samples of femoral arterial and venous blood were obtained, and leg blood flow was measured in the fasting state and for 6 h after a meal (1.2 g fat, 1.2 g carbohydrate/kg body mass). Prior exercise increased time averaged postprandial TG clearance across the leg (total TG: control, 0.079 ± 0.014 ml·100 ml tissue⁻¹·min⁻¹; exercise, 0.158 ± 0.023 ml·100 ml tissue⁻¹·min⁻¹, $P < 0.01$), particularly in the chylomicron fraction, so that absolute TG uptake was maintained despite lower plasma TG concentrations (control, 1.53 ± 0.13 mmol/l; exercise, 1.01 ± 0.16 mmol/l, $P < 0.001$). Prior exercise increased postprandial leg blood flow and glucose uptake (both $P < 0.05$). Mechanisms other than increased leg TG uptake must account for the effect of prior exercise on postprandial lipemia.

triacylglycerol; glucose; arteriovenous differences; leg blood flow

IMPAIRED CLEARANCE of triacylglycerol (TG)-rich lipoproteins constitutes an atherogenic influence on other lipoprotein species (16): when their residence time in the circulation is prolonged, these particles exchange their TG for cholesteryl ester from the cholesterol-rich lipoproteins. The result is a depletion of high-density lipoprotein (HDL) cholesterol and a preponderance of small, dense low-density lipoprotein particles, a combination known as the atherogenic lipoprotein phenotype (1). Case-control studies support the clinical relevance of these interactions, showing that indexes of postprandial lipemia are highly discriminatory in predicting the presence or ab-

sence of coronary artery disease (21, 32). Humans spend most of their lives in the postprandial state, so impaired processing of TG-rich lipoproteins, reflecting a poor metabolic capacity for TG, is likely to be one determinant of atherogenesis. Nevertheless, little is known about potential modulators of postprandial lipemia or their mechanisms of action.

Exercise may be such a modulator; a single session has been shown to reduce the lipemic response to a fatty meal consumed some hours afterwards by $>20\%$ (30, 35). One plausible mechanism is enhanced removal after exercise of TG from TG-rich lipoproteins into skeletal muscle. This speculation is supported by evidence for exercise-induced changes to the activity of skeletal muscle lipoprotein lipase (LPL), the rate-limiting enzyme of TG hydrolysis and, hence, removal. Exercise induces an increase in LPL gene expression in human skeletal muscle. This increase is transient and delayed, the peak LPL protein response occurring >8 h after exercise (25), which fits well with the findings of whole body studies where reduced lipemia was evident when the test meal was consumed 12–15 h after an exercise session (19, 30, 35).

Nevertheless, there is little direct evidence of the consequences of exercise-induced changes to muscle LPL activity for the postprandial partitioning of TG and their relation to reduced postprandial plasma TG concentrations. Thus the purpose of the present study was to examine the influence of a prolonged session of prior exercise on postprandial extraction of TG across the leg. We interpreted measurements of arteriovenous differences across the leg to reflect mainly skeletal muscle metabolism. There is evidence that chylomicron-TG is hydrolyzed by LPL in preference to very low density lipoprotein (VLDL)-TG (22); therefore, we determined TG extraction in individual lipoprotein fractions.

SUBJECTS AND METHODS

Subjects. Eight healthy men aged 21–46 yr with body mass index of 24.6 ± 1.8 kg/m², body fat level of $17.6 \pm 4.6\%$, and

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maximal oxygen uptake ($\dot{V}O_{2\max}$) of 56.8 ± 5.3 ml·kg⁻¹·min⁻¹ (all means \pm SD) volunteered to participate. Lean tissue (muscle plus bone) comprised 72.5% (range 67.1–77.1%) of their leg volumes, assessed as described below. The Ethical Advisory Committee of Loughborough University and the Central Oxford Research Ethics Committee approved the study protocol. The purpose of the study and the risks involved in taking part were explained to the subjects before their written informed consent was obtained. All subjects were normolipidemic, with plasma concentrations in the fasted state of 3.54 ± 0.32 mmol/l for total cholesterol, 1.09 ± 0.07 mmol/l for HDL cholesterol, and 0.91 ± 0.09 mmol/l (all means \pm SD) for TG. They were all nonsmokers, and none used any medication. Five took part in physically active recreations and three in structured endurance training programs.

Preliminary exercise tests. Two preliminary exercise tests were conducted. In the first, $\dot{V}O_{2\max}$ was determined during uphill running at a constant speed (range 2.9 to 3.8 m/s). In the second, the steady-state relationship between submaximal $\dot{V}O_2$ and treadmill speed was established. The treadmill running speed, which elicited 60% of $\dot{V}O_{2\max}$ was interpolated on an individual basis.

Study design. Each subject underwent two oral fat tolerance tests in a randomized fully balanced design, with an interval of 2 or 3 wk and with different preconditions. On one occasion (exercise trial), subjects ran on a motorized treadmill for 2 h at 60% $\dot{V}O_{2\max}$. Approximately 3 h after the run (and at the corresponding time on the control trial), they ate a standard low-fat evening meal [5.35 ± 0.31 MJ (means \pm SD), with 10% of energy from fat and 71% from carbohydrate]. The next morning, they were taken to the laboratory for the oral fat tolerance test. On the other occasion (control trial), no exercise was performed, but other procedures were identical.

Other than the treadmill runs, only activities of daily living and slow walking for personal transportation were permitted during the 3 days preceding each fat tolerance test. Subjects weighed and recorded all the food and drink they consumed during the 2 days leading up to the first oral fat tolerance test, replicating this before the second test. Subjects refrained from drinking alcohol for 2 days before each test.

Treadmill runs. Subjects began running at 1400, >1 h after a light lunch. Expired air samples were collected every 15 min using Douglas bags. These were analyzed for oxygen with a paramagnetic analyzer (570A; Taylor-Servomex, Crowborough, UK) and for carbon dioxide with an infrared analyzer (Lira MSA model 303; Mines Safety Appliances Britain, Coatbridge, Scotland). Gas volumes were measured by means of a dry-gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and corrected to STPD. $\dot{V}O_2$ and carbon dioxide production were calculated using the Haldane transformation. Heart rate was monitored using short-range telemetry (Sport-Tester; Polar Electro, Tampere, Finland).

Fat tolerance tests. Subjects reported to the laboratory at 0800 after a 12-h fast, 16 h after the end of exercise. They were placed in the supine position, and the right groin was exposed. Under aseptic conditions, the skin overlying the right femoral vessels was anesthetized with 1% lidocaine, and the femoral artery was cannulated via a guide-wire in a cephalad direction (20 Fr, 8 cm, Leadercath, Vygon, Ecouen, France). The ipsilateral femoral vein was then cannulated in a caudal direction with the same technique. Sterile physiological saline (0.9% wt/vol) without heparin was continuously infused through each cannula (femoral artery, 50 ml/h; femoral vein, 30 ml/h) to maintain patency. For sampling proce-

dures, the vascular line dead space was aspirated before blood samples were removed and then flushed with saline.

Subjects then rested for 30 min before two basal blood samples were taken, simultaneously, from the femoral artery and the femoral vein, with an interval of 20 min. The test meal was then consumed over a maximum of 10 min. This consisted of whipping cream, cereal, coconut, nuts, chocolate, and fruit and was given according to body mass (per kg body mass: 1.2 g fat, 1.2 g carbohydrate, 0.2 g protein, and 61 kJ energy). For our subjects, this meant 98 ± 8 g fat, 98 ± 8 g carbohydrate, and 16 ± 1 g protein, with a total energy value of 4.95 ± 0.42 MJ (all means \pm SD), 69% of which came from fat. Additional blood samples were obtained 20, 40, 60, 90, and 120 min after completion of the meal and then hourly for 6 h. Arterial blood pressure and leg and subcutaneous abdominal adipose tissue blood flows were measured immediately after each blood sample. Blood pressure was measured with an automated sphygmomanometer. Calf blood flow was determined by venous occlusion plethysmography below the right knee (10) and calculated according to the principle outlined by Sumner (29). This approach to the measurement of leg blood flow has been validated previously (28). Blood flow was measured in adipose tissue, a major site of postprandial TG uptake, by the ¹³³Xe washout technique (18), using a CsI-scintillation detector as described previously (24). Subjects remained immobile for periods of 10 min during measurements of blood flows.

Analytical methods. Blood samples were dispensed into precooled EDTA, heparinized, and plain Monovettes (Sarstedt, Leicester, UK). Heparinized blood was immediately used to measure hematocrit and oxygen saturation (Co-oximeter, Instrumentation Laboratory, Warrington, UK). Blood samples in plain tubes were allowed to clot for 1 h at room temperature before serum was separated. A portion of each EDTA blood sample was rapidly deproteinized with perchloric acid (70 g/l) for measurement of 3-hydroxybutyrate concentration, and the remainder was used to prepare plasma within 15 min. Plasma was recovered after low-speed centrifugation at 4°C. From samples obtained at the second baseline and at 2, 3, 4, 5, and 6 h, one aliquot was kept overnight on ice at 4°C until chylomicron- and VLDL-rich fractions were prepared by density-gradient ultracentrifugation. Further aliquots were stored at -20°C until analysis.

Blood 3-hydroxybutyrate concentrations were measured with an enzymatic method adapted to a Monarch centrifugal analyzer (Instrumentation Laboratory). Plasma concentrations of total cholesterol and HDL cholesterol (fasting state only; both Roche, Lewes, UK), glucose (Roche, Basel, Switzerland), and nonesterified fatty acids (NEFA; Wako, Neuss, Germany) were determined with enzymatic colorimetric methods using a centrifugal analyzer (Cobas-Mira, Roche). By use of the same analyzer, the concentrations of total TG (all time points, in frozen samples) and TG in VLDL- and chylomicron-rich fractions (0, 2, 3, 4, 5, and 6 h, in fresh samples) were measured enzymatically, with correction for free glycerol (12).

Serum insulin was quantified by radioimmunoassay (Coat-a-Count; Diagnostic Products, Los Angeles, CA). Apart from TG analysis in chylomicron- and VLDL-rich fractions, all samples from each subject were analyzed in the same batch. Accuracy and precision were maintained using quality-control sera (Roche).

Lipoprotein fractionation by density-gradient ultracentrifugation. The chylomicron-rich fraction was prepared by layering 0.75 ml of plasma underneath NaCl solution [density 1.006 g/ml, with 0.01% (wt/vol) EDTA] in thin-walled open-topped centrifuge tubes (11 × 34 mm). The tubes were then

centrifuged in an ultracentrifuge (Optima TLX, Beckman Instruments, High Wycombe, Bucks, UK) for 20 min in a swinging-bucket rotor (Beckman, TLS 55) at 30,000 rpm. The chylomicron-rich fraction was then separated by slicing. The VLDL-rich fraction was prepared using the infranatant from 0.75 ml of plasma, prepared as described above, which was transferred into bell-topped centrifuge tubes (13 × 51 mm). These tubes were filled with NaCl solution, sealed, and centrifuged for 2 h 30 min in a fixed angle rotor (Beckman, TLA 100.4) at 100,000 rpm. The VLDL-rich fraction was then separated by slicing. Both separated fractions were aspirated into preweighed glass tubes. Within-batch coefficients of variation for concentrations of TG in chylomicron- and VLDL-rich fractions were 4.8 and 5.2%, respectively.

Anthropometry. Height and body mass were determined by standard methods. Body density was predicted from the logarithm of the sum of skinfold thicknesses at four sites and used to estimate body fatness (6). Leg, muscle, and bone volumes were determined by anthropometry (14, 15) with ultrasound to measure subcutaneous fat thickness.

Calculations. Variables for substrate extraction across the leg were calculated as described previously (22). Concentrations of TG (total or in lipoprotein fractions) and glucose in plasma (P) were converted to those in whole blood (B) using the hematocrit (H): $B = P \times (1 - H)$. The Dillon factor (5) was used to correct for distribution of glucose between plasma and erythrocytes. Arteriovenous (a-v) differences were calculated for whole blood concentrations. Two measures of substrate extraction were calculated: absolute extraction (uptake) as $a-v_{\text{substrate}} \times \text{leg blood flow}$, and substrate clearance as uptake/arterial concentration. Clearance, therefore, measures the "efficiency of removal."

Adipose tissue blood flow was calculated from the product of the decay slope and the partition coefficient (18). The partition coefficient for human adipose tissue was assumed to have a value of 10 g/ml. This value has recently been confirmed as applicable with reasonable accuracy over a wide range of body mass index (13). The washout curves were treated as monoexponential.

Leg vascular resistance was calculated as mean blood pressure divided by leg blood flow.

Energy expenditure and substrate oxidation during running were estimated from $\dot{V}O_2$ and carbon dioxide production using indirect calorimetry and neglecting protein oxidation (8).

Statistical analysis. Results are shown as means \pm SE unless otherwise stated. Variables were compared between trials by repeated-measures ANOVA to examine the effects of trial, time, and (where appropriate) sampling site. In addition, basal values and summary measures of postprandial responses (time averaged areas under response vs. time curves) were compared by *t*-test for correlated data. Where data were not normally distributed (3-hydroxybutyrate), comparisons were performed after logarithmic transformation. Statistical procedures were performed using Statistica for Windows 95, version 5.0 (Stat Soft, Tulsa, OK).

RESULTS

Cardiorespiratory and metabolic responses during running. The average $\dot{V}O_2$ during running, $37.0 \pm 2.7 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, represented $64 \pm 3\%$ of $\dot{V}O_{2\text{max}}$. An estimated $53 \pm 9 \text{ g}$ of fat and $333 \pm 49 \text{ g}$ of carbohydrate were oxidized, with a gross energy expenditure of $7.21 \pm 0.87 \text{ MJ}$, $28 \pm 4\%$ from fat and $72 \pm 4\%$ from carbohydrate. Average values for heart rate and respi-

ratory exchange ratio were $166 \pm 14 \text{ beats/min}$ and 0.91 ± 0.01 (all means \pm SD), respectively.

Plasma TG concentrations in basal and postprandial states. Arterial and femoral venous plasma concentrations of total, VLDL- and chylomicron-TG are shown in Fig. 1, with summary statistics in Table 1. Basal values of total and VLDL-TG were significantly lower in

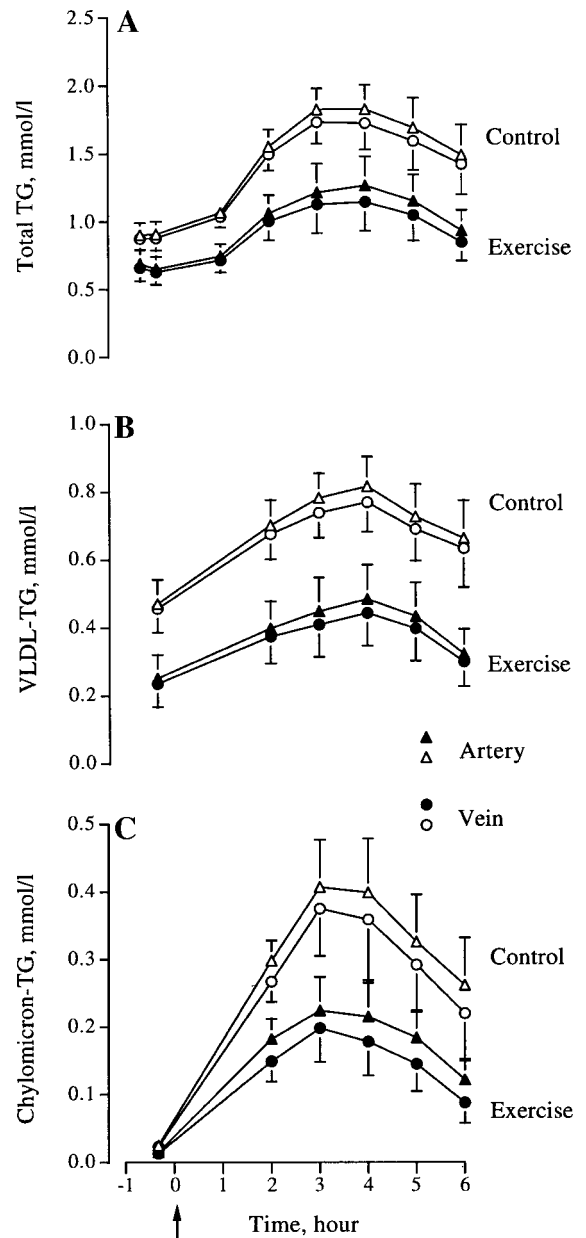


Fig. 1. Arterial and femoral venous concentrations of triacylglycerol (TG) in control and exercise trials in the fasting state and after a high-fat mixed meal completed at time 0, indicated by the arrow. A: total TG; B: very low density lipoprotein (VLDL)-TG; C: chylomicron-TG. Values are means \pm SE for 8 men. Analysis by repeated-measures ANOVA shows that the total TG concentration and the TG concentration in each fraction were all significantly lower ($P < 0.01$) in the exercise trial than in the control trial. All TG concentrations were significantly lower in vein than in artery ($P < 0.001$). Statistical analyses based on areas under concentration vs. time curves are given in Table 1.

Table 1. Arterial concentrations of total, VLDL- and chylomicron-TG in the basal and postprandial states

	Total TG		VLDL-TG		Chylomicron-TG	
	Control	Exercise	Control	Exercise	Control	Exercise
Basal	0.91 ± 0.09	0.67 ± 0.1*	0.47 ± 0.07	0.25 ± 0.07*		
Postprandial	1.53 ± 0.13§	1.01 ± 0.16†‡	0.70 ± 0.08§	0.40 ± 0.09†‡	0.26 ± 0.04	0.15 ± 0.03†

Values are means ± SE. Postprandial values are time averaged over the 6-h observation period, based on areas under arterial concentration curve vs. time curves. VLDL, very low density lipoprotein; TG, triacylglycerol. Units are mmol/l. Significance of difference between control and exercise trials: * $P < 0.05$, † $P < 0.01$; significance of difference between basal and postprandial values: ‡ $P < 0.01$, § $P < 0.001$.

the exercise trial than in the control trial. Basal values for the concentration of TG measured in the fraction separated as chylomicrons were not distinguishable from zero. Postprandial concentrations of total, VLDL-TG, and chylomicron-TG were all significantly lower in the exercise trial, as were the postprandial increases in these variables above the basal level.

Cardiovascular responses. Neither leg blood flow nor leg vascular resistance was significantly different in the basal state between trials (Fig. 2). Postprandially, leg blood flow was significantly higher in the exercise trial, and leg vascular resistance was significantly lower [time averaged areas under the response vs. time

curves: leg blood flow, control 1.6 ± 0.2 ml·100 ml tissue⁻¹·min⁻¹, exercise 2.2 ± 0.3 ml·100 ml tissue⁻¹·min⁻¹ ($P < 0.01$); leg vascular resistance, control 62.3 ± 4.9 mmHg·ml⁻¹·100 ml tissue⁻¹·min; exercise 46.8 ± 7.3 mmHg·ml⁻¹·100 ml tissue⁻¹·min ($P = 0.03$)]. Changes in mean arterial blood pressure were small and did not differ between trials (average values: control, 84 ± 1 mmHg; exercise, 81 ± 2 mmHg). Average adipose tissue blood flow in the postprandial state, calculated over the whole period between basal and 6-h time points, was not significantly different between trials (control, 4.0 ± 0.5 mmHg ml⁻¹·100 ml tissue⁻¹·min⁻¹; exercise 4.3 ± 1.0 mmHg ml⁻¹·100 ml tissue⁻¹·min⁻¹).

Substrate extraction across the leg. Data comparing TG extraction across the leg are presented as uptake (Table 2 and Fig. 3) and clearance (Table 2 and Fig. 4). In the basal state, none of these measures differed significantly between trials. Postprandially, uptake of total TG increased significantly from basal in both exercise and control trials, but there was no such increase in the uptake of VLDL-TG. There were no significant differences between trials in postprandial uptake of TG (an absolute measure of extraction) (main effect of trial: total TG, $P = 0.21$; VLDL-TG, $P = 0.32$; chylomicron-TG, $P = 0.20$), but postprandial clearance (a measure of fractional removal) was higher in the exercise trial for total, VLDL-TG, and chylomicron-TG. TG clearance was higher in the exercise trial for a given arterial TG concentration (Fig. 5).

Uptake of glucose in the fasted state did not differ significantly between trials but was greater postprandially in the exercise trial (Fig. 3; ANOVA, $P < 0.01$, t -test for area under the uptake curve vs. time curve, $P = 0.04$). Glucose clearance was not significantly different in the fasted state ($P = 0.06$) or when evaluated as the time averaged area under the postprandial response curve vs. time curve (control, 0.007 ± 0.001 ml·100 ml tissue⁻¹·min; exercise, 0.010 ± 0.002 ml·100 ml tissue⁻¹·min, $P = 0.07$). However, analysis by ANOVA showed that postprandial glucose clearance was higher during the exercise trial ($P < 0.01$).

Responses of glucose, insulin, NEFA, lactate, and 3-hydroxybutyrate. Arterial concentrations of plasma glucose, plasma NEFA, plasma lactate, blood 3-hydroxybutyrate, and serum insulin are shown in Fig. 6. There were no significant differences in plasma NEFA concentrations, either in the basal state ($P = 0.07$) or postprandially ($P = 0.08$), but the change over time

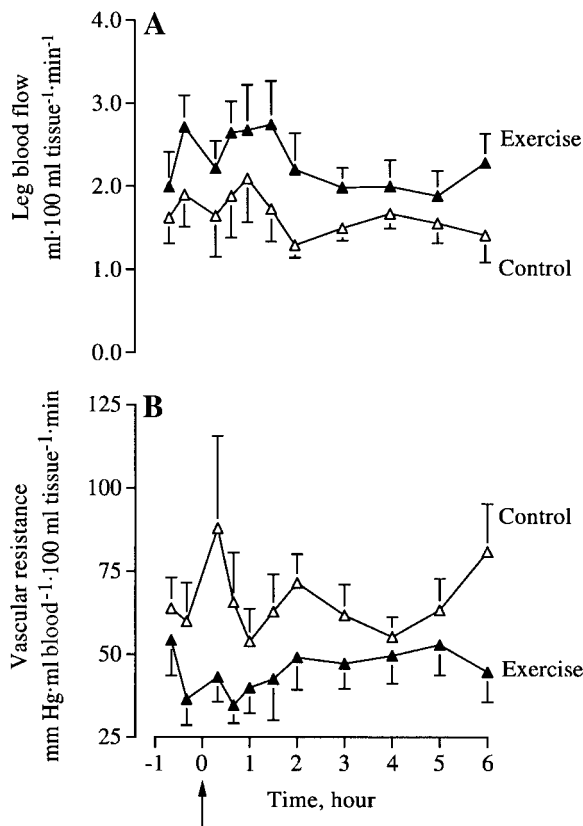


Fig. 2. Leg blood flow (A) and vascular resistance (B) for control and exercise trials in the fasting state and after a high-fat mixed meal completed at time 0 h, indicated by the arrow. Values are means ± SE for 8 men. Analysis by repeated-measures ANOVA showed that blood flow was significantly higher in the exercise trial than in the control trial ($P = 0.04$) and that vascular resistance was significantly lower ($P = 0.02$). Statistical analyses based on areas under response vs. time curves are given in RESULTS.

Table 2. Extraction of total, VLDL- and chylomicron-TG across the leg in the basal and postprandial states

	Total TG		VLDL-TG		Chylomicron-TG	
	Control	Exercise	Control	Exercise	Control	Exercise
Basal						
Uptake	0.028 ± 0.014	0.039 ± 0.013	0.019 ± 0.011	0.021 ± 0.007		
Clearance	0.059 ± 0.021	0.123 ± 0.036	0.070 ± 0.037	0.242 ± 0.089		
Postprandial						
Uptake	0.066 ± 0.010‡	0.091 ± 0.014‡	0.030 ± 0.005	0.032 ± 0.007	0.025 ± 0.004‡	0.031 ± 0.007‡
Clearance	0.079 ± 0.014	0.158 ± 0.023*	0.077 ± 0.015	0.190 ± 0.043*	0.195 ± 0.039	0.446 ± 0.072†

Values are means ± SE. Postprandial values are time averaged over the 6-h observation period, based on areas under variable curve vs. time curves. Units for uptake are $\mu\text{mol} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$. Units for clearance are $\text{ml} \cdot 100 \text{ ml tissue}^{-1} \cdot \text{min}^{-1}$. Significance of difference between control and exercise trials: * $P < 0.05$, † $P < 0.01$; significance of difference between basal and postprandial values: ‡ $P < 0.01$.

differed significantly between trials (interaction, $P < 0.01$). 3-Hydroxybutyrate concentration was higher in the exercise trial, both in the basal state ($P < 0.01$) and postprandially (ANOVA, $P < 0.01$; area under the concentration vs. time curve, $P < 0.01$) with a different pattern of change over time (interaction, $P = 0.02$). Plasma lactate concentration was lower in the exercise trial in the basal state ($P = 0.02$) and in the postprandial state (ANOVA, $P = 0.02$).

DISCUSSION

Prior exercise decreased plasma TG concentrations during the hours after consumption of a high-fat mixed meal by more than one-third. By contrast with earlier reports suggesting that exercise largely influences chylomicron-TG (2, 33), >70% of this decrease was attributable to the lower concentration of TG in VLDL. Our functional measures of substrate extraction across the leg during the postprandial period help elucidate the metabolic basis of these effects.

In the fasted state, plasma TG concentration was 0.25 mmol/l lower after exercise, as observed previously. This effect has often (26) been attributed to an increase in muscle LPL activity (17, 25), the assumption being that increased enzyme activity leads to greater muscle TG uptake. A new finding of our study was that, although prior exercise may have enhanced the efficiency of LPL action as measured by leg TG clearance (Table 2 and Fig. 4), the absolute uptake of TG across the leg in the fasted state was relatively unchanged in the fasted state.

Mechanisms other than increased leg TG uptake that might account for the lower basal TG concentration in the exercise trial include increased adipose tissue uptake or/and a suppressive effect on hepatic VLDL secretion. The former seems unlikely for two reasons. First, we were able to measure TG uptake across subcutaneous abdominal adipose tissue in three subjects with the use of methods described previously (9). For each individual, prior exercise reduced, rather than increased, TG uptake [control 0.165 (range, 0.109–0.243) $\mu\text{mol} \cdot 100 \text{ g tissue}^{-1} \cdot \text{min}^{-1}$; exercise 0.092 (range, 0.027–0.164) $\mu\text{mol} \cdot 100 \text{ g tissue}^{-1} \cdot \text{min}^{-1}$]. Second, adipose tissue LPL activity has been reported to be unchanged by exercise in fasted subjects (25).

By contrast, prior exercise may well have decreased VLDL-TG secretion rate in the fasting state. Blood 3-hydroxybutyrate concentration was higher after exercise, suggesting enhanced hepatic fatty acid oxidation. This would decrease the availability of fatty acids for re-esterification and thus decrease VLDL-TG secretion, in line with published data showing (in rats) that training decreases VLDL secretion (20, 27). To the authors' knowledge, no comparable data are available for humans.

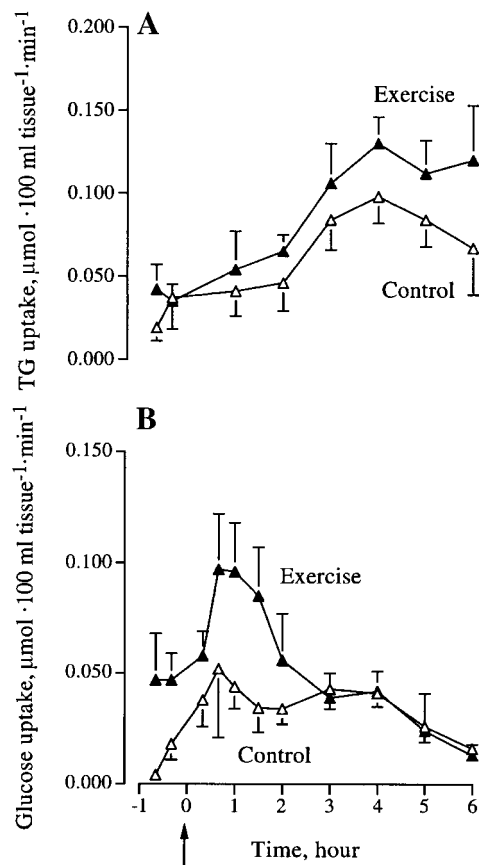


Fig. 3. Uptake of TG (A) and glucose (B) in control and exercise trials in the fasting state and after a high-fat mixed meal completed at time 0 h, indicated by the arrow. Values are means ± SE for 8 men. Analysis by repeated-measures ANOVA shows that uptake of glucose was significantly higher in the exercise trial ($P < 0.01$). Area under the glucose uptake vs. time curve significantly higher in exercise trial ($P = 0.04$).

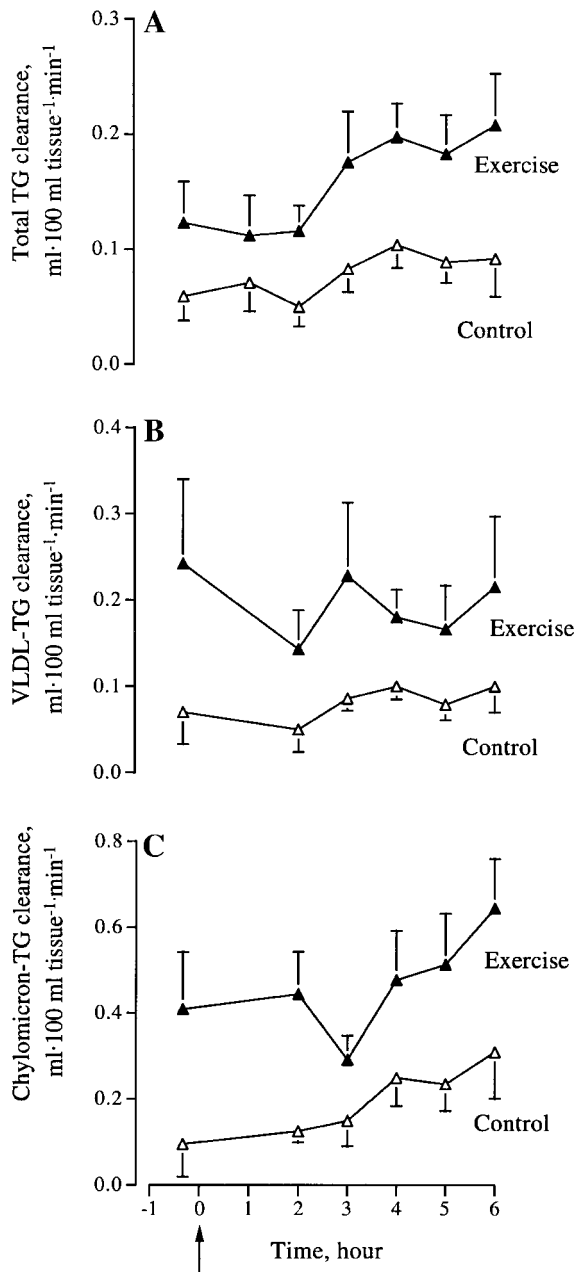


Fig. 4. Clearance of TG across the leg in control and exercise trials in the fasting state and after a high-fat mixed meal completed at time 0 h, indicated by the arrow. A: total TG. B: VLDL-TG. C: chylomicron-TG. Values are means \pm SE for 8 men. Analysis by repeated-measures ANOVA shows that clearance was higher for the exercise trial than for control trial (total, $P = 0.03$; VLDL, $P = 0.04$; chylomicron, $P < 0.01$). Statistical analyses based on areas under clearance vs. time curves are given in Table 2.

These are the first data to describe the effects of prior exercise on leg TG uptake and clearance during the postprandial period. Although the decrease in TG concentrations was particularly clear during the postprandial period, there was no convincing evidence that prior exercise increased the rate of leg TG uptake. The main effect, as shown so clearly in Fig. 5, was to increase the efficiency of leg TG clearance, so that leg TG uptake

was maintained despite a much lower plasma TG concentration.

The higher leg TG clearance can be assumed to reflect an effect of prior exercise on muscle LPL. Exercise increases mRNA for skeletal muscle LPL (25) and, hence, enzyme activity (17) over a time scale that fits well with our observations, i.e., maximal increase >8 h after exercise (26). Our data show that this is functionally important in postprandial leg TG clearance and is sufficient to override the inhibition of muscle LPL activity by insulin (7). The improvement of leg TG clearance by prior exercise was greater in the chylomicron fraction than in the VLDL fraction, probably because LPL appears to hydrolyze larger, more TG-rich lipoproteins better than smaller particles (22).

Assuming that the appearance rate of chylomicron-TG was not delayed by prior exercise (and we found no evidence for a later peak in concentration that might suggest this), then whole body chylomicron-TG uptake must have been increased. We can exclude a significant increase in muscle uptake, so (provided that myocardial uptake of chylomicron-TG was similar in both trials) uptake into adipose tissue may have been enhanced. Postprandial subcutaneous abdominal adipose tissue blood flow was not higher after exercise, however, so greater uptake would require an increase in adipose tissue LPL activity, but there is no evidence on this for the postprandial state.

Whole body chylomicron uptake may have been greater after exercise simply because VLDL-TG concentration was lower, reducing competition for hydrolysis by LPL. The major part of the difference between trials in postprandial TG concentration was attributable to VLDL-TG. As suggested for the fasted state, this might reflect a lower rate of VLDL-TG synthesis and secretion, secondary to greater oxidation in the liver of fatty acids. The higher postprandial 3-hydroxy-

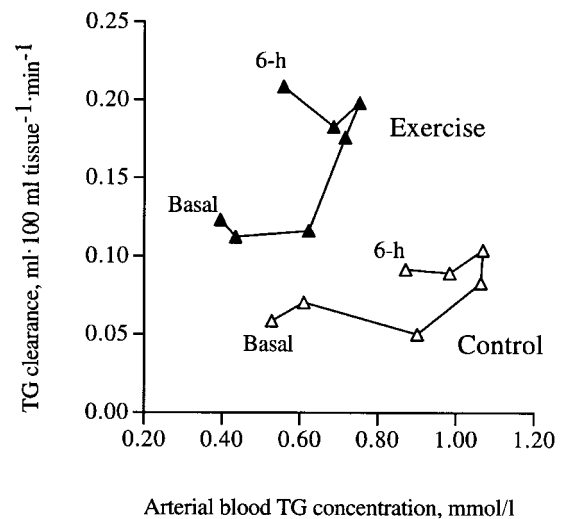


Fig. 5. Relationship between arterial plasma TG concentration and leg TG clearance in the basal state and for 6 h after consumption of a high-fat mixed meal for control and exercise trials. Each data point represents the mean value for 8 men.

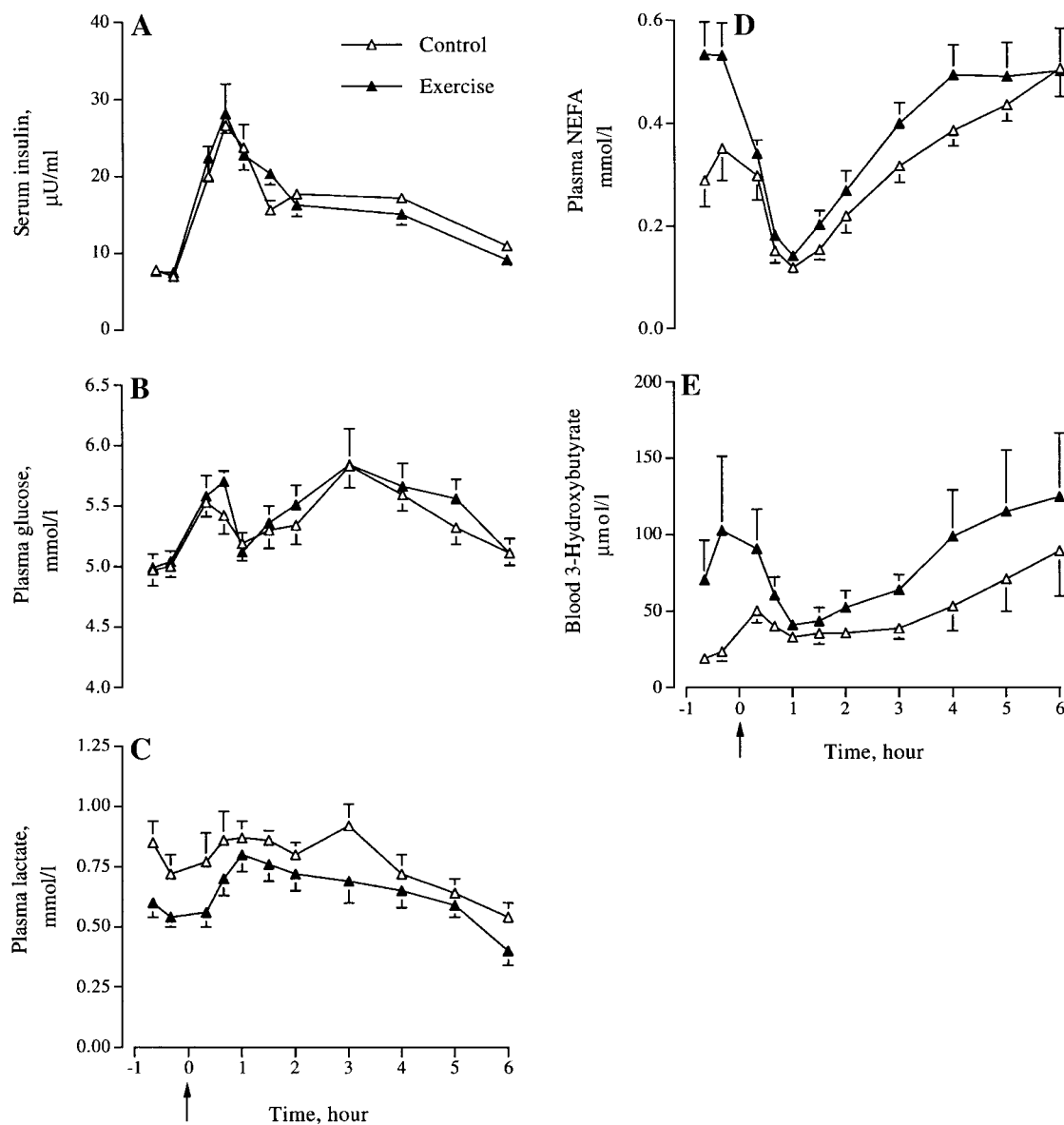


Fig. 6. Arterial concentrations of insulin (A), glucose (B), lactate (C), nonesterified fatty acids (NEFA, D), and 3-hydroxybutyrate (E) in control and exercise trials in the fasting state and after a high-fat mixed meal completed at time 0 h, indicated by the arrow. Values are means \pm SE for 8 men. Plasma lactate concentrations were significantly lower in the exercise trial than in the control trial in the fasted state ($P = 0.02$) and postprandially (repeated-measures ANOVA, $P = 0.02$; area under concentration vs. time curve, $P = 0.09$). Blood 3-hydroxybutyrate concentrations were higher in the exercise trial in the basal state ($P < 0.01$) and postprandially (repeated-measures ANOVA, $P < 0.01$; area under concentration vs. time curve, $P < 0.01$). Changes over time in plasma NEFA and blood 3-hydroxybutyrate concentrations were significantly different between trials (interaction $P < 0.01$, $P = 0.02$, respectively).

butyrate response in the exercise trial provides indirect evidence for this.

The finding that prior exercise enhanced postprandial muscle blood flow measured the next day is intriguing and, to the authors' knowledge, novel. It is consistent with reports that leg blood flow is higher in endurance-trained men than in normally active controls (11). One mechanism may be that enhanced sensitivity to insulin in trained people reinforces its hemodynamic effects, which include decreasing vascular resistance (Fig. 2). A single session of exercise im-

proves insulin sensitivity in physically active individuals (4), so this might explain our findings. A limitation to this reasoning is that concentrations of serum insulin and plasma glucose after the test meal were not influenced by exercise, but this does not preclude an effect on insulin action.

The higher leg blood flow in the exercise trial increased glucose delivery, facilitating enhanced glucose uptake during hyperinsulinemia. It takes ~ 20 h for a 70-kg person having ingested 500–600 g of carbohydrate to replenish muscle glycogen depleted by exercise

(3). In our study, where subjects exercised at 64% of $\dot{V}O_{2\max}$ for 2 h, utilizing a total of ~330 g of carbohydrate, both muscle and liver glycogen would have been considerably reduced (23). The evening meal consumed after exercise contained 250 g of carbohydrate, clearly insufficient fully to restore these glycogen stores. Consequently, prior exercise led to enhanced leg glucose uptake for utilization in muscle glycogen synthesis. Low muscle glycogen might also explain the higher plasma NEFA concentrations after exercise (34). Low liver glycogen might explain the lower basal plasma lactate in the exercise trial if hepatic uptake of lactate, an important gluconeogenic precursor, was enhanced.

We have interpreted our measurements of a-v differences across the leg to reflect mainly changes to skeletal muscle metabolism. Some contamination of venous samples with drainage from adipose tissue may occur with these techniques, but if the venous cannula is inserted in a caudal direction, as in our study, this is minimal (31). Furthermore, the legs we studied were lean (15), muscle plus bone typically comprising 73% of leg volume. Consequently, we are confident that the measurements we present reflect mainly the metabolism of skeletal muscle.

In summary, prior exercise enhanced TG clearance across the leg, allowing postprandial tissue uptake of TG-derived fatty acids to be maintained despite a markedly lower arterial plasma TG concentration. Postprandial leg blood flow was higher after exercise, and this served to increase leg glucose uptake during the early part of the postprandial period, presumably to facilitate muscle glycogen replenishment.

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