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Moderate exercise increases affinity of large very low density lipoproteins for hydrolysis by lipoprotein lipase

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Abstract

Context. Postprandial triglyceride (TG) concentration is independently associated with cardiovascular disease risk. Exercise reduces postprandial TG concentrations but the mechanisms responsible are unclear.

Objective. To determine the effects of exercise on affinity of chylomicrons, large very low density lipoproteins (VLDL₁) and smaller VLDL (VLDL₂) for lipoprotein lipase (LPL) mediated TG hydrolysis.

Design. Within-participant cross-over study

Setting. A University metabolic investigation unit.

Participants. Ten overweight/obese men.

Interventions. Participants undertook two oral fat tolerance tests, separated by 7-14 days, in which they had blood taken fasting and for 4 hours after a high-fat mixed meal. On the afternoon before one test, they performed a 90-minute treadmill walk at 50% maximal oxygen uptake (EX); no exercise was performed before the control test (CON).

Main outcome measures. Circulating TG-rich lipoprotein concentrations; affinity of chylomicrons, VLDL₁, VLDL₂ for LPL-mediated TG hydrolysis.

Results. Exercise significantly reduced fasting VLDL₁-TG concentration (CON: 0.49(0.33-0.72) mmol.l⁻¹, EX: 0.36(0.22-0.59) mmol.l⁻¹, [geometric means (95% confidence interval)]; p=0.04). Time-averaged postprandial chylomicron-TG (CON: 0.55±0.10 mmol.l⁻¹, EX: 0.39±0.08 mmol.l⁻¹, [mean±SEM], p=0.03) and VLDL₁-TG (CON: 0.85±0.13 mmol.l⁻¹, EX: 0.66±0.10 mmol.l⁻¹, p=0.01) concentrations were both lower in EX than CON. Affinity of VLDL₁ for LPL-mediated TG hydrolysis increased by 2.2(1.3-3.7) fold (geometric mean (95% confidence interval)) (p=0.02) in the fasted state and 2.6(1.8-2.6) fold (p=0.001) postprandially. Affinity of chylomicrons and VLDL₂ was not significantly different between trials.

Conclusions. Exercise increases affinity of VLDL₁ for LPL-mediated TG hydrolysis both fasting and postprandially. This mechanism is likely to contribute to exercise’s TG-lowering effect.
Introduction

Postprandial triglyceride (TG) concentrations are independently associated with risk of cardiovascular events (1-3) and implicated in the atherosclerotic disease process (4,5). Recent Mendelian randomisation studies reasserted the likely causal role of TG-mediated pathways in cardiovascular disease (CVD) (6-9). Moderate intensity exercise lowers postprandial TG concentrations by ~15-25% in a range of population groups at increased risk of cardiovascular disease (10-12) and is recommended as a TG-lowering intervention for patients at high CVD risk (5). However, the mechanism(s) by which exercise lowers TG have not been fully elucidated.

The exercise-induced reduction in postprandial TG concentration is quantitatively greater in very low density lipoproteins (VLDL) than in chylomicrons (13,14), with large VLDL particles (VLDL₁, Sf 60-400) being the lipoprotein subclass most affected (13). Kinetic studies have shown that exercise-induced VLDL-TG reductions are due to increased clearance from the circulation, rather than reduced hepatic production (15-17). While exercise has also been shown to increase clearance of chylomicron-like particles (15,18,19), the magnitude of this change is smaller than the increase in clearance of VLDL₁-TG (15), and the effect has not been consistently observed (20). Furthermore, although exercise-induced reductions in postprandial TG concentrations are sometimes accompanied by an increase in post-heparin plasma or skeletal muscle lipoprotein lipase (LPL) activity, post-exercise TG reductions are also commonly observed in the absence of increased LPL activity (21,22). The affinity of chylomicrons/chylomicron-like particles for LPL clearance is many fold greater than that of VLDL particles (23); thus the observation that exercise increases VLDL₁-TG clearance to a greater extent than chylomicron-like particles (15), taken together with the inconsistent changes to LPL activity in response to exercise (21), suggest that mechanisms other than increased LPL activity are likely to contribute to the exercise-induced increase in VLDL₁-TG removal. There is evidence that circulating VLDL₁ particles are larger and more TG enriched following exercise (13,15) – changes that might be expected to increase the affinity of these particles for LPL-mediated hydrolysis (24) – and we have recently demonstrated that these exercise-induced changes to the size and TG enrichment of circulating VLDL₁ particles explain about half of the variance in the exercise-induced...
increase in VLDL$_1$ particle clearance in correlational analyses (15). This observation is consistent with exercise-induced changes to VLDL$_1$ particles increasing their affinity for LPL-mediated clearance (15,25): this data interpretation would also explain how exercise could increase clearance of VLDL$_1$-TG without necessarily increasing LPL activity, and why the exercise-induced increase in VLDL$_1$-TG clearance is quantitatively larger than that observed in chylomicron-like particles. However, the hypothesis that exercise increases the affinity of VLDL$_1$ as a substrate for LPL has not been directly tested. The purpose of this study was therefore to determine the effects of exercise on the affinity of TG-rich lipoprotein species (chylomicrons, VLDL$_1$, and VLDL$_2$ (Sf 20-60)) for LPL-mediated TG hydrolysis.

Materials and Methods

Participants. Ten overweight/obese men, aged 35.9±11.8 y (mean±SD), body mass 92.5±19.9 kg, body mass index (BMI) 30.4±5.4 kg.m$^{-2}$, waist circumference 102.2±11.4 cm, and maximal oxygen uptake (VO$_{2\text{max}}$) 36.5±11.8 ml.kg$^{-1}$.min$^{-1}$ participated in this study. All participants were apparently healthy, normotensive, normoglycemic, non-smokers. None was taking any drugs known to affect lipid or carbohydrate metabolism. The study was conducted in accord with the Declaration of Helsinki and approved by the University of Glasgow Ethics Committee. All participants gave written informed consent.

Study design. Participants attended the laboratory for two oral fat tolerance tests (OFTT) in random order with an interval of 7-14 days. On the afternoon prior to one OFTT, participants walked on a treadmill at 50% VO$_{2\text{max}}$ for 90 minutes (Exercise trial: EX). They performed no exercise (and did not attend the lab) on the day preceding the other OFTT (Control trial: CON). Participants recorded their dietary intake and refrained from alcohol for the two days prior to the first OFTT, and replicated this prior to the second test. They also performed no exercise, other than the treadmill walk in the exercise trial, during the three days preceding each OFTT.
Preliminary exercise test. At least one week prior the first OFTT, a preliminary sub-maximal incremental treadmill test was undertaken to estimate VO$_{2\text{max}}$ and determine the walking speed and gradient required to elicit 50% VO$_{2\text{max}}$ (26).

Treadmill walk. In EX, the 90-minute treadmill walk was completed ~16-18 h before the OFTT: this time interval was chosen to reflect the time scale at which the maximal TG-lowering effects of exercise are evident (21). Expired air samples were collected using Douglas bags to determine oxygen uptake (VO$_2$) and carbon dioxide production, heart rate was measured (Polar Electroky, Kempele, Finland) and ratings of perceived exertion (27) were obtained at 15-minute intervals during the walk.

Oral fat tolerance tests. Participants reported to the metabolic suite in the morning after an overnight 12-hour fast. A cannula was placed in an antecubital vein and, after a 10-minute interval, a blood sample was taken. A test breakfast comprising a croissant with butter, and a meal-replacement drink (Complan, Complan Foods Ltd) made with whole milk and double cream, providing 92g fat, 68g carbohydrate, 25g protein and 1277kcal energy, was consumed and further blood samples were obtained 30, 60, 90, 120 and 240 minutes postprandially. Participants rested and consumed only water during this time.

Blood processing and storage. Blood samples were collected into potassium EDTA tubes and placed on ice. Plasma was separated within 15 minutes of collection. Plasma for lipoprotein separation was stored at 4°C until processing within 24 hours. The remaining plasma was divided into aliquots and frozen at -80°C until analysis.

Lipoprotein separation. Plasma samples (2ml) from 0, 120 and 240 minute time-points were ultracentrifuged to isolate chylomicron (Sf>400), VLDL$_1$ and VLDL$_2$ lipoprotein subfractions as previously described (28). VLDL$_1$ and VLDL$_2$ fractions were assayed for cholesterol, free cholesterol (FC), TG, phospholipid (PL), protein and apolipoprotein B (apoB); VLDL$_1$ and VLDL$_2$ concentration
was calculated by summing the concentration of their components; and TG/apoB and CE/TG ratios were calculated as previously described (15). Chylomicron fractions were assayed for TG.

Coefficients of variation (CV) were: 2.9% for total cholesterol, 3.8% for TG, 2.2% for FC, 3.8% for PL, 13.2% for apoB and 3.0% for protein.

Chylomicrons, VLDL₁ and VLDL₂ were concentrated in preparation for the LPL affinity assays. At the 240-minute time-point, 30ml plasma was ultracentrifuged to separate chylomicrons, which were then concentrated 8-10 fold by centrifugation for 60 minutes at 1,500 x g through a Centriprep® filtering tube. Chylomicron-free plasma at the 240-minute time-point, and 30ml of plasma collected at 0-minute time-point, was then concentrated ~10-fold by ultracentrifugation before lipoprotein separation to obtain concentrated VLDL₁ and VLDL₂ fractions. The lipoprotein fractions were adjusted to a TG concentration of 0.6mmol.l⁻¹ by the addition of d 1.006g.ml⁻¹ density solution.

**LPL affinity assays.** Affinity of chylomicrons, VLDL₁ and VLDL₂ for LPL was determined using a modified version of a method described previously (29). In brief, LPL from Pseudomonas sp. was prepared in a solution containing: 200mmol.l⁻¹ Tris-HCl buffer, 130mmol.l⁻¹ NaCl, 66.7mg.l⁻¹ sodium-heparin, and 3.3mmol.l⁻¹ CaCl₂ (all Sigma: Poole, UK), at pH 8.2, to give a final LPL activity of 0.1units.ml⁻¹. For each assay, 70μl of lipoprotein fraction (at 0.6mmol.l⁻¹ TG concentration) was incubated with 35μl of Tris-HCl buffer at 37°C for 10 minutes, before adding 35μl LPL solution, brief mixing by vortex and further incubation at 37°C. Separate tubes containing the lipoprotein fractions were incubated in the LPL solution for 0, 5, 10, 15, 20 and 30 minutes, before quenching the reaction by adding 93μl of ice-cold 5mol.l⁻¹ NaCl, mixing, and placing the tube on ice for >5 minutes.

Reactions were performed in triplicate, and two controls [No LPL and no lipoprotein (105μl 1.006g.ml⁻¹ density solution added), and LPL but no lipoprotein (70μl 1.006 g.ml⁻¹ density solution added)] (incubated for 10 minutes) were included for each experiment. Non-esterified fatty acid (NEFA) concentration was determined in each tube using commercially available kits (Wako Chemicals, USA, Inc.). Affinity of lipoproteins for LPL was determined by the rate of NEFA release.
over the linear portion of 30-minute incubation period before a plateau was achieved. The CV for
NEFA release from the LPL-lipoprotein reactions was 9.9% for chylomicrons and 15.1% for VLDL.

Plasma assays. Plasma glucose, TG (Randox Laboratories, Crumlin, UK), NEFA (Wako Chemicals)
and small dense LDL (sdLDL) cholesterol (Denka Seiken, Tokyo, Japan) concentrations were
analysed, using commercially available enzymatic and turbidimetric kits. Insulin was measured in
EDTA plasma using commercially available ELISA kits (Mercodia, Uppsala, Sweden). In the fasted
state, total and HDL cholesterol (Roche Diagnostics, Mannheim, Germany) were measured, and LDL
cholesterol was calculated using the Friedewald equation (30). CVs for assays not already mentioned
above were: 2.0% for glucose, 5.2% for NEFA, 2.8% for HDL cholesterol, 6.3% for sdLDL
cholesterol and <4% for insulin.

Data analysis. Statistical analyses were performed using Statistica (version 10, StatSoft Inc.) and
Minitab (version 17, Minitab Ltd). Data were tested for normality using the Anderson-Darling test
and, where they did not approximate a normal distribution, were log-transformed prior to analysis and
expressed as geometric means with 95% confidence intervals (95% CI). Time-averaged postprandial
concentrations were used as summary measures of postprandial responses (11). Paired t-tests were
used to compare trials. Differences in lipoprotein affinity for LPL were calculated for absolute (i.e.
EX minus CON) and relative (fold) (i.e. EX divided by CON) changes. An a priori power
calculation, on the basis of our data for intra-subject reproducibility of postprandial TG responses in
men, indicated that 10 participants would enable detection of a ~10% exercise-induced change in the
TG response with 80% power (31). Significance was accepted at p<0.05. Data are presented as
means±SEM unless otherwise stated.

Results
Treadmill walk. Participants walked at 5.0±0.1 km.h\(^{-1}\) up a gradient of 5.6±0.9%. All participants
completed the 90-minute walk without difficulty, with perceived exertion of 10.8±0.4 (‘fairly light’)

on the Borg scale of 6–20 (27). Mean VO2 was 19.9±1.7 ml·kg⁻¹·min⁻¹ (51.9±1.5% VO₂max), and mean heart rate was 125±3 beats.min⁻¹. Gross energy expenditure of the walk was 3.32±0.29 MJ.

**Plasma concentrations in the fasted and postprandial states.** Table 1 shows fasting and time-averaged postprandial plasma concentrations. Fasting TG was 18% lower (p=0.04) and time-averaged postprandial TG was 13% lower (p=0.03) in EX compared with CON. sdLDL-cholesterol concentrations were lower in EX than CON both fasting and postprandially (p<0.001 for both). There were no significant differences between CON and EX for fasting or postprandial insulin, glucose or NEFA concentrations, or in fasting total, HDL or LDL cholesterol concentrations.

**Lipoprotein concentrations and composition in the fasted and postprandial states.** Figure 1 shows fasting and postprandial chylomicron-TG, VLDL₁-TG and VLDL₂-TG concentrations. Fasting chylomicron-TG concentrations were negligible in both trials (CON: 0.03±0.014 mmol.l⁻¹, EX: 0.02±0.005 mmol.l⁻¹, p=0.25). Exercise significantly reduced fasting VLDL₁-TG (CON: 0.49(0.33-0.72) mmol.l⁻¹, EX: 0.36(0.22-0.59) mmol.l⁻¹, geometric means (95% CI); p=0.04), but fasting concentration of VLDL₂-TG did not differ between trials (CON: 0.21(0.12-0.36) mmol.l⁻¹, EX: 0.18(0.12-0.27) mmol.l⁻¹, geometric means (95% CI); p=0.60). Time-averaged postprandial chylomicron-TG concentrations (CON: 0.55±0.10 mmol.l⁻¹, EX: 0.39±0.08 mmol.l⁻¹, p=0.03) and time-averaged postprandial VLDL₁-TG concentrations (CON: 0.85±0.13 mmol.l⁻¹, EX: 0.66±0.10 mmol.l⁻¹, p=0.01) were both lower in the exercise compared with the control trial, but the time-averaged postprandial VLDL₂-TG concentration did not differ significantly between trials (CON: 0.31±0.15 mmol.l⁻¹, EX: 0.27±0.09 mmol.l⁻¹, p=0.60).

Table 2 shows total VLDL₁ and VLDL₂ concentration and the constituent lipoprotein molecules fasting and 240 minutes postprandially. Total VLDL₁ lipoprotein mass for was 24% lower fasting (p=0.04) and 17% lower postprandially (p=0.049) in EX compared to CON. VLDL₁ apo B concentrations were significantly lower fasting, but not postprandially, in EX than CON. Both fasting and postprandial concentrations of VLDL₁ TG, cholesteryl ester and free cholesterol were lower in
EX than CON. Fasting VLDL₁ TG/apo B ratio was 39% higher in EX compared to CON, but this
was not statistically significant (p=0.09). At 240 minutes, VLDL₁ TG/apoB ratio was similar in the
two trials. Similarly, the fasting VLDL₁ CE/TG ratio was 26% lower in EX than CON, but this
difference was not statistically significant (p=0.16). At 240 minutes VLDL₁ CE/TG ratio was similar
in the two trials. No differences in VLDL₂ concentration or composition were observed between
trials.

Lipoprotein affinity for LPL. Figure 2 shows NEFA release over the 30-minute incubation period in
the LPL-affinity assay for fasting and postprandial VLDL₁ and VLDL₂, and for postprandial
chylomicrons in the two trials. There was no significant change in affinity of chylomicrons for LPL
expressed either as fold-change (1.2(0.6- 2.3) fold, p=0.59) or in absolute units (see Table 3).
However, exercise increased VLDL₁ affinity for LPL by 2.2(1.3-3.7) fold (geometric mean (95% CI))
in the fasted state (p=0.02 for fold increase; p-value for absolute increase shown in Table 3) and by
2.6(1.8-3.8) fold in the postprandial state (p=0.001 for fold increase; p-value for absolute increase
shown in Table 3). Affinity of VLDL₂ for LPL was negligible and did not change in response to
exercise. In CON, affinity of chylomicrons for LPL was 11.3(6.0-21.6) fold greater (p=0.0001) than
that of VLDL₁ in the postprandial state, whereas in EX it was 6.0(3.0-12.0) greater (p=0.0007).
There was no significant difference in affinity of VLDL₁ for LPL between the fasted and postprandial
states in either CON (p=0.18) or EX (p=0.28).

Discussion
The main novel finding of the present study is that prior exercise significantly increased the affinity of
large VLDL₁ – but not of chylomicrons or VLDL₂ – for clearance by LPL. These findings provide an
important advance in our understanding of the mechanism by which exercise lowers TG
concentrations and put previous observations in this field into context. Earlier work demonstrated that
exercise often lowered TG concentrations without substantially increasing post-heparin plasma or
skeletal muscle LPL activity (21,22,32); that the TG reductions induced by exercise were typically
larger in VLDL than chylomicrons (13,14); and that exercise increased clearance of VLDL from the
circulation, but did not reduce hepatic VLDL production (16,17). However, these observations could not explain how this increased VLDL clearance could occur without a concomitant increase in LPL activity. More recently, it was hypothesised that exercise may increase the affinity of VLDL for TG-hydrolysis by LPL (15,25), which received indirect support from observations that exercise upregulated clearance of VLDL to a greater extent than chylomicrons (15). This hypothesis is confirmed by the present findings. Exercise increased the affinity of VLDL for LPL clearance 2.2-fold in the fasted state and 2.6-fold in the postprandial state, but did not significantly alter the affinity of chylomicrons. Accordingly the affinity of VLDL relative to chylomicrons for LPL-mediated clearance in the postprandial state was ~2-fold greater in the exercise compared with the control trial. This is consistent with earlier observations that the exercise-induced increase in VLDL-TG clearance was twice as great as the exercise-induced increase in chylomicron-like particles (15).

In contrast to previous observations (13,15), we did not observe a statistically significant increase in size or TG-enrichment of VLDL particles after exercise in the present study. However, this does not necessarily imply that exercise-induced changes to VLDL size and composition were unimportant in mediating the increase in affinity for LPL-mediated clearance. Circulating VLDL particles are all essentially remnant particles, in that they will have had some LPL-mediated hydrolysis of their TG-core by the time any blood sample is taken. Post-exercise VLDL particles with greater affinity for LPL would have been exposed proportionately greater TG hydrolysis by the time blood was sampled. Thus, relative to newly secreted VLDL particles, circulating post-exercise VLDL particles may have had their size and TG-enrichment reduced to a greater extent than VLDL particles in the control trial. Thus, it is possible that following exercise, the liver produced larger, more TG-enriched VLDL particles which contributed to an increased affinity for VLDL, without this effect being reflected in the composition of the measured circulating VLDL particle (25). This suggestion is supported by data from Magkos and colleagues who reported a reduction in hepatic VLDL-apoB100, but not VLDL-TG, secretion following exercise (17), implying secretion of a larger more TG-rich VLDL particle. However, Al-Shayji and coworkers, who considered hepatic production of larger VLDL particles, rather than total VLDL, found the ratio of VLDL-TG production to VLDL-apoB
production was similar in control and exercise trials, suggesting that the average size of the secreted VLDL₁ particle may not have been influenced by exercise (15). However, it is also important to recognize that VLDL₁ particles are heterogeneous, occupying a greater than three-fold range in size and density, and thus considering only ‘average’ lipoprotein size and composition in the VLDL₁ range may not reveal the whole story. Interestingly, Al-Shayji et al reported a larger exercise-induced increase in the VLDL₁-apoB fractional catabolic rate than the VLDL₁-TG fractional catabolic rate (146% vs 82% increase) (15), suggesting that exercise was having a proportionately larger effect on clearance of smaller, less TG-rich VLDL₁ particles (which have a lower TG/apoB ratio), either via direct particle removal or by TG-removal taking them out of the VLDL₁ and into the VLDL₂ density range. This interpretation suggests that the observation that circulating VLDL₁ particles post-exercise are larger and more TG-enriched reflects the fact that exercise disproportionately increased clearance of smaller, less TG-enriched particles at the lower end of the Sf 60-400 range, leaving proportionally more larger VLDL₁ in the circulation. Accordingly, it is possible that the strong correlations between change in VLDL₁ apo B fractional catabolic rate and change in VLDL₁ TG/apoB and CE/TG ratio previously observed (15) reflects greater clearance of smaller VLDL₁ particles following exercise, which would have the net effect of increasing the average TG/apoB and CE/TG ratio of the remaining circulating lipoprotein particles in the VLDL₁ density range. This suggestion is supported by recent work from Harrison and colleagues who used nuclear magnetic resonance spectroscopy to quantify 24 different VLDL subfractions in the fasted state following exercise (22). They found that in response to exercise, there was a proportionally larger TG reduction in ‘medium VLDL’ (size range 43-55nm, approximately corresponding to Sf 100-200 (33), i.e. smaller VLDL₁) than in ‘large VLDL’ (size 55-260nm, approximately corresponding to Sf >200 (33), i.e. larger VLDL₁), with VLDL particles over 120nm in size being virtually unaffected by exercise (22). The authors proposed that their findings were suggestive of independent metabolic regulation of different VLDL pools within the VLDL₁ range (22). This is an attractive proposition which is consistent with our present and earlier (15) observations on the effects of exercise on VLDL₁ metabolism. Further study is needed to both examine the effects of exercise on the affinity of smaller and larger VLDL₁ particles for LPL-
mediated TG hydrolysis, and to understand the effects of exercise on TG and apoB kinetics of smaller
and larger VLDL₁ particles.

This study evaluated the effects of exercise on the affinity of TG-rich lipoproteins for LPL-mediated
clearance both fasting and postprandially, finding similar exercise-induced increases in VLDL₁
affinity under both conditions. There are, however, two factors to consider when interpreting the
postprandial data. First, action of LPL on chylomicrons produces chylomicron remnant particles
which fall into the Sf 60-400 range. Thus, the isolated postprandial ‘VLDL₁’ fraction contains both
hepatically-produced VLDL₁ particles and chylomicron remnants. However, >95% of postprandial
lipoprotein particles in this density range are apoB100 containing (i.e. hepatically-produced) (13),
thus it seems unlikely that this relatively small ‘contamination’ from chylomicron remnants would
have substantially influenced the results. A further consideration is that in our in vitro assay, we
assessed the affinity of isolated postprandial VLDL₁ for LPL-mediated clearance without direct
competition for chylomicrons, which does not fully reflect the in vivo system. Thus, further study
which assesses the effects of exercise on affinity of postprandial VLDL₁ for LPL-mediated clearance
in the presence of chylomicrons is warranted to extend the present findings.

While this study clearly demonstrated that exercise increased the affinity of VLDL₁ particles for
clearance by LPL, this effect is unlikely to be solely responsible for the observed TG lowering effect.
There was a significant reduction in chylomicron-TG concentration following exercise, which
occurred without a statistically significant increase in chylomicron affinity for LPL-mediated
clearance. However, as the effect of exercise on chylomicron affinity for LPL-mediated clearance
was quite variable, with some participants experiencing a >2-fold increase, it is possible that this
contributed to the lowering of chylomicron-TG concentrations in a subset of individuals. We did not
measure post-heparin plasma or skeletal muscle LPL activity in the present study, so it is unclear
whether this might have contributed to the lower chylomicron TG-concentrations. However, previous
studies have suggested that there is also considerable inter-individual variability in exercise-induced
changes in LPL activity (34). There is also evidence that exercise-induced changes in blood perfusion
to LPL-rich tissues – leading to increased interactions between chylomicron particles and LPL – is likely to contribute to the chylomicron-TG lowering effect (35,36). Thus, it is possible that individually variable combinations of increased affinity of chylomicrons for LPL-mediated clearance, increased LPL activity, and increased blood flow all contributed to the reduction in chylomicron-TG concentration observed after exercise. This is a testable hypothesis which warrants further investigation.

A further observation was that exercise substantially reduced fasting and postprandial sdLDL cholesterol concentrations. There is accumulating evidence that sdLDL have particularly high atherogenicity (37,38), thus, exercise-induced reductions are likely to have clinically relevant implications for CVD risk. Elevated concentrations of TG-rich lipoproteins facilitate the development of sdLDL by accelerating CETP-mediated neutral lipid exchange, between TG-rich lipoproteins and LDL, leading to TG-enriched LDL particles, which are then acted on by hepatic lipase to produce sdLDL particles (37,38). Thus, by lowering TG-rich lipoprotein concentrations, exercise is likely to have inhibited the neutral lipid exchange process leading to sdLDL formation.

In conclusion, the main finding of this study was that exercise substantially increased the affinity of VLDL₁ for LPL-mediated TG-hydrolysis both fasting and postprandially. This provides an important piece in the jigsaw of understanding the effects of exercise on the metabolism of TG-rich lipoproteins.

References


### Table 1. Fasting and postprandial plasma concentrations

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exercise</th>
<th>p-value</th>
<th>Control</th>
<th>Exercise</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma TG (mmol.l(^{-1}))</strong></td>
<td>1.57 (1.18 to 2.08)</td>
<td>1.31 (0.98 to 1.74)</td>
<td>0.04*</td>
<td>2.72 ± 0.41</td>
<td>2.36 ± 0.31</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Glucose (mmol.l(^{-1}))</strong></td>
<td>5.50 ± 0.22</td>
<td>5.47 ± 0.23</td>
<td>0.81</td>
<td>6.39 ± 0.46</td>
<td>6.35 ± 0.36</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Insulin (mU.l(^{-1}))</strong></td>
<td>12.2 (6.1 to 24.2)</td>
<td>9.1 (6.1 to 13.6)</td>
<td>0.33*</td>
<td>93.5 ± 21.7</td>
<td>77.5 ± 19.9</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>NEFA (mmol.l(^{-1}))</strong></td>
<td>0.66 ± 0.06</td>
<td>0.73 ± 0.05</td>
<td>0.11</td>
<td>0.65 ± 0.06</td>
<td>0.64 ± 0.06</td>
<td>0.88</td>
</tr>
<tr>
<td><strong>3-hydroxybutyrate (mmol.l(^{-1}))</strong></td>
<td>0.07 ± 0.02</td>
<td>0.12 ± 0.05</td>
<td>0.19</td>
<td>0.10 ± 0.03</td>
<td>0.08 ± 0.01</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol.l(^{-1}))</strong></td>
<td>5.44 ± 0.33</td>
<td>5.49 ± 0.36</td>
<td>0.69</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>HDL cholesterol (mmol.l(^{-1}))</strong></td>
<td>1.06 ± 0.09</td>
<td>1.09 ± 0.10</td>
<td>0.38</td>
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<tr>
<td><strong>LDL cholesterol (mmol.l(^{-1}))</strong></td>
<td>3.68 ± 0.28</td>
<td>3.83 ± 0.33</td>
<td>0.19</td>
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<tr>
<td><strong>Plasma small dense LDL (mmol.l(^{-1}))</strong></td>
<td>1.40 ± 0.20</td>
<td>1.17 ± 0.17</td>
<td>0.0002</td>
<td>1.30 ± 0.16</td>
<td>1.15 ± 0.14</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 10. *Statistical analysis performed on log-transformed data and values are geometric mean (95% confidence interval),
Table 2. Concentration and composition of VLDL₁ and VLDL₂ in the fasted and postprandial states

<table>
<thead>
<tr>
<th></th>
<th>Fasting (0 minutes)</th>
<th>Postprandial (240 minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exercise</td>
</tr>
<tr>
<td><strong>VLDL₁</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lipoprotein concentration (mg.ml⁻¹)</td>
<td>84.1 ± 17.4</td>
<td>63.7 ± 13.0</td>
</tr>
<tr>
<td>Apo B (mg.ml⁻¹)</td>
<td>1.98 ± 0.36</td>
<td>1.05 ± 0.21</td>
</tr>
<tr>
<td>Triglyceride (mg.ml⁻¹)</td>
<td>51.7 ± 11.1</td>
<td>41.0 ± 8.9</td>
</tr>
<tr>
<td>Cholesteryl ester (mg.ml⁻¹)</td>
<td>7.7 ± 1.2</td>
<td>4.8 ± 3.0</td>
</tr>
<tr>
<td>Free cholesterol (mg.ml⁻¹)</td>
<td>4.0 ± 0.9</td>
<td>2.6 ± 0.6</td>
</tr>
<tr>
<td>Phospholipid (mg.ml⁻¹)</td>
<td>12.8 ± 3.0</td>
<td>9.2 ± 1.9</td>
</tr>
<tr>
<td>Protein (mg.ml⁻¹)</td>
<td>8.0 ± 1.6</td>
<td>6.1 ± 1.1</td>
</tr>
<tr>
<td>TG/apoB ratio (mol:mol)</td>
<td>17751 ± 2507</td>
<td>24693 ± 4238</td>
</tr>
<tr>
<td>CE/TG ratio (mol:mol)</td>
<td>0.23 ± 0.03</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td><strong>VLDL</strong></td>
<td>Total lipoprotein concentration (mg.dl⁻¹)</td>
<td>49.5 ± 3.6</td>
</tr>
<tr>
<td>----------</td>
<td>------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td></td>
<td>Apo B (mg.dl⁻¹)</td>
<td>4.00 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>Triglyceride (mg.dl⁻¹)</td>
<td>18.2 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>Cholesteryl ester (mg.dl⁻¹)</td>
<td>11.3 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>Free cholesterol (mg.dl⁻¹)</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Phospholipid (mg.dl⁻¹)</td>
<td>9.8 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Protein (mg.dl⁻¹)</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>TG/apoB ratio (mol:mol)</td>
<td>3679 ± 567</td>
</tr>
<tr>
<td></td>
<td>CE/TG ratio (mol:mol)</td>
<td>0.59 ± 0.10</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 10. *Statistical analysis performed on log-transformed data
<table>
<thead>
<tr>
<th></th>
<th>Fasting (0 minutes)</th>
<th>Postprandial (240 minutes)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exercise</td>
<td>p-value</td>
</tr>
<tr>
<td>Chylomicrons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.25 (0.94 to 1.66)</td>
<td>1.52 (0.98 to 2.34)</td>
<td>0.53*</td>
</tr>
<tr>
<td>VLDL₁</td>
<td>0.16 (0.09 to 0.29)</td>
<td>0.35 (0.24 to 0.52)</td>
<td>0.018*</td>
</tr>
<tr>
<td>VLDL₂</td>
<td>0.013 (0.004 to 0.044)</td>
<td>0.018 (0.007 to 0.049)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 10. *Statistical analysis performed on log-transformed data, and values are geometric mean (95% confidence interval)
**Figure Legends**

**Figure 1.** Chylomicron-TG (top panel), VLDL₁-TG (middle panel) and VLDL₂-TG (bottom panel) concentrations in the fasted state (0 mins), and 120 and 240 minutes postprandially in the control and exercise trials. Statistical analyses of these data is described in the results text. N = 10, Values are mean ± SEM.

**Figure 2.** NEFA release over 30-minutes in LPL-affinity assay for chylomicrons in the postprandial state (top right), VLDL₁ in the fasted (middle left) and postprandial (middle right) states and VLDL₂ in the fasted (middle left) and postprandial (middle right) states, in control and exercise trials. N = 10, Values are mean ± SEM. Affinity of lipoproteins for LPL was determined by the rate of NEFA release over the linear portion of the 30-minute incubation period before a plateau was achieved for each individual participant. Values for lipoprotein affinity for LPL and statistical analyses are shown in Table 3.
Figure 1

- **Chylomicron-TG (mmol$l^{-1}$)**
- **VLDL-TG (mmol$l^{-1}$)** (upper graph)
- **VLDL$_2$-TG (mmol$l^{-1}$)** (lower graph)

- **Control**
- **Exercise**