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

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38 **Abstract**

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

42 **Objective.** To determine the effects of exercise on affinity of chylomicrons, large very low density
43 lipoproteins ($VLDL_1$) and smaller VLDL ($VLDL_2$) for lipoprotein lipase (LPL) mediated TG
44 hydrolysis.

45 **Design.** Within-participant cross-over study

46 **Setting.** A University metabolic investigation unit.

47 **Participants.** Ten overweight/obese men.

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

52 **Main outcome measures.** Circulating TG-rich lipoprotein concentrations; affinity of chylomicrons,
53 $VLDL_1$, $VLDL_2$ for LPL-mediated TG hydrolysis.

54 **Results.** Exercise significantly reduced fasting $VLDL_1$-TG concentration (CON: 0.49(0.33-0.72)
55 $mmol.l^{-1}$, EX: 0.36(0.22-0.59) $mmol.l^{-1}$, [geometric means (95% confidence interval)]; p=0.04). Time-
56 averaged postprandial chylomicron-TG (CON: 0.55±0.10 $mmol.l^{-1}$, EX: 0.39±0.08 $mmol.l^{-1}$,
57 [mean±SEM], p=0.03) and $VLDL_1$-TG (CON: 0.85±0.13 $mmol.l^{-1}$, EX: 0.66±0.10 $mmol.l^{-1}$, p=0.01)
58 concentrations were both lower in EX than CON. Affinity of $VLDL_1$ for LPL-mediated TG
59 hydrolysis increased by 2.2(1.3-3.7) fold (geometric mean (95% confidence interval)) (p=0.02) in the
60 fasted state and 2.6(1.8-2.6) fold (p=0.001) postprandially. Affinity of chylomicrons and $VLDL_2$ was
61 not significantly different between trials.

62 **Conclusions.** Exercise increases affinity of $VLDL_1$ for LPL-mediated TG hydrolysis both fasting and
63 postprandially. This mechanism is likely to contribute to exercise's TG-lowering effect.

64

65

66 **Introduction**

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

74

75 The exercise-induced reduction in postprandial TG concentration is quantitatively greater in very low
76 density lipoproteins (VLDL) than in chylomicrons (13,14), with large VLDL particles ($VLDL_1$, Sf 60-
77 400) being the lipoprotein subclass most affected (13). Kinetic studies have shown that exercise-
78 induced VLDL-TG reductions are due to increased clearance from the circulation, rather than reduced
79 hepatic production (15-17). While exercise has also been shown to increase clearance of
80 chylomicron-like particles (15,18,19), the magnitude of this change is smaller than the increase in
81 clearance of $VLDL_1$-TG (15), and the effect has not been consistently observed (20). Furthermore,
82 although exercise-induced reductions in postprandial TG concentrations are sometimes accompanied
83 by an increase in post-heparin plasma or skeletal muscle lipoprotein lipase (LPL) activity, post-
84 exercise TG reductions are also commonly observed in the absence of increased LPL activity (21,22).
85 The affinity of chylomicrons/chylomicron-like particles for LPL clearance is many fold greater than
86 that of VLDL particles (23); thus the observation that exercise increases $VLDL_1$-TG clearance to a
87 greater extent than chylomicron-like particles (15), taken together with the inconsistent changes to
88 LPL activity in response to exercise (21), suggest that mechanisms other than increased LPL activity
89 are likely to contribute to the exercise-induced increase in $VLDL_1$-TG removal. There is evidence
90 that circulating $VLDL_1$ particles are larger and more TG enriched following exercise (13,15) –
91 changes that might be expected to increase the affinity of these particles for LPL-mediated hydrolysis
92 (24) – and we have recently demonstrated that these exercise-induced changes to the size and TG
93 enrichment of circulating $VLDL_1$ particles explain about half of the variance in the exercise-induced

94 increase in $VLDL_1$ particle clearance in correlational analyses (15). This observation is consistent
95 with exercise-induced changes to $VLDL_1$ particles increasing their affinity for LPL-mediated
96 clearance (15,25): this data interpretation would also explain how exercise could increase clearance of
97 $VLDL_1$-TG without necessarily increasing LPL activity, and why the exercise-induced increase in
98 $VLDL_1$-TG clearance is quantitatively larger than that observed in chylomicron-like particles.
99 However, the hypothesis that exercise increases the affinity of $VLDL_1$ as a substrate for LPL has not
100 been directly tested. The purpose of this study was therefore to determine the effects of exercise on
101 the affinity of TG-rich lipoprotein species (chylomicrons, $VLDL_1$, and $VLDL_2$ (Sf 20-60)) for LPL-
102 mediated TG hydrolysis.
103
104 **Materials and Methods**
105 ***Participants.*** Ten overweight/obese men, aged 35.9±11.8 y (mean±SD), body mass 92.5±19.9 kg,
106 body mass index (BMI) 30.4±5.4 $kg.m^{-2}$, waist circumference 102.2±11.4 cm, and maximal oxygen
107 uptake (VO_{2max}) 36.5±11.8 $ml.kg^{-1}.min^{-1}$ participated in this study. All participants were apparently
108 healthy, normotensive, normoglycemic, non-smokers. None was taking any drugs known to affect
109 lipid or carbohydrate metabolism. The study was conducted in accord with the Declaration of
110 Helsinki and approved by the University of Glasgow Ethics Committee. All participants gave written
111 informed consent.
112
113 ***Study design.*** Participants attended the laboratory for two oral fat tolerance tests (OFTT) in random
114 order with an interval of 7-14 days. On the afternoon prior to one OFTT, participants walked on a
115 treadmill at 50% VO_{2max} for 90 minutes (Exercise trial: EX). They performed no exercise (and did not
116 attend the lab) on the day preceding the other OFTT (Control trial: CON). Participants recorded their
117 dietary intake and refrained from alcohol for the two days prior to the first OFTT, and replicated this
118 prior to the second test. They also performed no exercise, other than the treadmill walk in the
119 exercise trial, during the three days preceding each OFTT.
120

121 ***Preliminary exercise test.*** At least one week prior the first OFTT, a preliminary sub-maximal
122 incremental treadmill test was undertaken to estimate VO_{2max} and determine the walking speed and
123 gradient required to elicit 50% VO_{2max} (26).
124
125 ***Treadmill walk.*** In EX, the 90-minute treadmill walk was completed ~16-18 h before the OFTT: this
126 time interval was chosen to reflect the time scale at which the maximal TG-lowering effects of
127 exercise are evident (21). Expired air samples were collected using Douglas bags to determine
128 oxygen uptake (VO_2) and carbon dioxide production, heart rate was measured (Polar Electroky,
129 Kempele, Finland) and ratings of perceived exertion (27) were obtained at 15-minute intervals during
130 the walk.
131
132 ***Oral fat tolerance tests.*** Participants reported to the metabolic suite in the morning after an overnight
133 12-hour fast. A cannula was placed in an antecubital vein and, after a 10-minute interval, a blood
134 sample was taken. A test breakfast comprising a croissant with butter, and a meal-replacement drink
135 (Complan, Complan Foods Ltd) made with whole milk and double cream, providing 92g fat, 68g
136 carbohydrate, 25g protein and 1277kcal energy, was consumed and further blood samples were
137 obtained 30, 60, 90, 120 and 240 minutes postprandially. Participants rested and consumed only
138 water during this time.
139
140 ***Blood processing and storage.*** Blood samples were collected into potassium EDTA tubes and placed
141 on ice. Plasma was separated within 15 minutes of collection. Plasma for lipoprotein separation was
142 stored at 4°C until processing within 24 hours. The remaining plasma was divided into aliquots and
143 frozen at -80°C until analysis.
144
145 ***Lipoprotein separation.*** Plasma samples (2ml) from 0, 120 and 240 minute time-points were
146 ultracentrifuged to isolate chylomicron (Sf >400), $VLDL_1$ and $VLDL_2$ lipoprotein subfractions as
147 previously described (28). $VLDL_1$ and $VLDL_2$ fractions were assayed for cholesterol, free cholesterol
148 (FC), TG, phospholipid (PL), protein and apolipoprotein B (apoB); $VLDL_1$ and $VLDL_2$ concentration

149 was calculated by summing the concentration of their components; and TG/apoB and CE/TG ratios
150 were calculated as previously described (15). Chylomicron fractions were assayed for TG.
151 Coefficients of variation (CV) were: 2.9% for total cholesterol, 3.8% for TG, 2.2% for FC, 3.8% for
152 PL, 13.2% for apoB and 3.0% for protein.
153
154 Chylomicrons, $VLDL_1$ and $VLDL_2$ were concentrated in preparation for the LPL affinity assays. At
155 the 240-minute time-point, 30ml plasma was ultracentrifuged to separate chylomicrons, which were
156 then concentrated 8-10 fold by centrifugation for 60 minutes at 1,500 x g through a Centriprep®
157 filtering tube. Chylomicron-free plasma at the 240-minute time-point, and 30ml of plasma collected
158 at 0-minute time-point, was then concentrated ~10-fold by ultracentrifugation before lipoprotein
159 separation to obtain concentrated $VLDL_1$ and $VLDL_2$ fractions. The lipoprotein fractions were
160 adjusted to a TG concentration of $0.6mmol.l^{-1}$ by the addition of d $1.006g.ml^{-1}$ density solution.
161
162 ***LPL affinity assays.*** Affinity of chylomicrons, $VLDL_1$ and $VLDL_2$ for LPL was determined using a
163 modified version of a method described previously (29). In brief, LPL from Pseudomonas sp. was
164 prepared in a solution containing: $200mmol.l^{-1}$ Tris-HCl buffer, $130mmol.l^{-1}$ NaCl, $66.7mg.l^{-1}$ sodium-
165 heparin, and $3.3mmol.l^{-1}$ $CaCl_2$ (all Sigma: Poole, UK), at pH 8.2, to give a final LPL activity of
166 $0.1units.ml^{-1}$. For each assay, 70µl of lipoprotein fraction (at $0.6mmol.l^{-1}$ TG concentration) was
167 incubated with 35µl of Tris-HCl buffer at 37°C for 10 minutes, before adding 35µl LPL solution, brief
168 mixing by vortex and further incubation at 37Cº. Separate tubes containing the lipoprotein fractions
169 were incubated in the LPL solution for 0, 5, 10, 15, 20 and 30 minutes, before quenching the reaction
170 by adding 93µl of ice-cold $5mol.l^{-1}$ NaCl, mixing, and placing the tube on ice for >5 minutes.
171 Reactions were performed in triplicate, and two controls [No LPL and no lipoprotein (105µl
172 $1.006g.ml^{-1}$ density solution added), and LPL but no lipoprotein (70µl 1.006 $g.ml^{-1}$ density solution
173 added)] (incubated for 10 minutes) were included for each experiment. Non-esterified fatty acid
174 (NEFA) concentration was determined in each tube using commercially available kits (Wako
175 Chemicals, USA, Inc.). Affinity of lipoproteins for LPL was determined by the rate of NEFA release

176 over the linear portion of 30-minute incubation period before a plateau was achieved. The CV for
177 NEFA release from the LPL-lipoprotein reactions was 9.9% for chylomicrons and 15.1% for $VLDL_1$.
178
179 ***Plasma assays.*** Plasma glucose, TG (Randox Laboratories, Crumlin, UK), NEFA (Wako Chemicals)
180 and small dense LDL (sdLDL) cholesterol (Denka Seiken, Tokyo, Japan) concentrations were
181 analysed, using commercially available enzymatic and turbidimetric kits. Insulin was measured in
182 EDTA plasma using commercially available ELISA kits (Mercodia, Uppsala, Sweden). In the fasted
183 state, total and HDL cholesterol (Roche Diagnostics, Mannheim, Germany) were measured, and LDL
184 cholesterol was calculated using the Friedewald equation (30). CVs for assays not already mentioned
185 above were: 2.0% for glucose, 5.2% for NEFA, 2.8% for HDL cholesterol, 6.3% for sdLDL
186 cholesterol and <4% for insulin.
187
188 ***Data analysis.*** Statistical analyses were performed using Statistica (version 10, StatSoft Inc.) and
189 Minitab (version 17, Minitab Ltd). Data were tested for normality using the Anderson-Darling test
190 and, where they did not approximate a normal distribution, were log-transformed prior to analysis and
191 expressed as geometric means with 95% confidence intervals (95% CI). Time-averaged postprandial
192 concentrations were used as summary measures of postprandial responses (11). Paired *t*-tests were
193 used to compare trials. Differences in lipoprotein affinity for LPL were calculated for absolute (i.e.
194 EX minus CON) and relative (fold) (i.e. EX divided by CON) changes. An *a priori* power
195 calculation, on the basis of our data for intra-subject reproducibility of postprandial TG responses in
196 men, indicated that 10 participants would enable detection of a ~10% exercise-induced change in the
197 TG response with 80% power (31). Significance was accepted at $p<0.05$. Data are presented as
198 means±SEM unless otherwise stated.
199
200 **Results**
201 ***Treadmill walk.*** Participants walked at 5.0±0.1 $km.h^{-1}$ up a gradient of 5.6±0.9%. All participants
202 completed the 90-minute walk without difficulty, with perceived exertion of 10.8±0.4 (‘fairly light’)

203 on the Borg scale of 6–20 (27). Mean VO_2 was 19.9±1.7 ml·kg^{-1}·min^{-1} (51.9±1.5% VO_{2max}), and

204 mean heart rate was 125±3 beats.min^{-1}. Gross energy expenditure of the walk was 3.32±0.29 MJ.

205

206 ***Plasma concentrations in the fasted and postprandial states.*** Table 1 shows fasting and time-

207 averaged postprandial plasma concentrations. Fasting TG was 18% lower (p=0.04) and time-

208 averaged postprandial TG was 13% lower (p=0.03) in EX compared with CON. sdLDL-cholesterol

209 concentrations were lower in EX than CON both fasting and postprandially (p<0.001 for both).

210 There were no significant differences between CON and EX for fasting or postprandial insulin,

211 glucose or NEFA concentrations, or in fasting total, HDL or LDL cholesterol concentrations.

212

213 ***Lipoprotein concentrations and composition in the fasted and postprandial states.*** Figure 1 shows

214 fasting and postprandial chylomicron-TG, $VLDL_1$-TG and $VLDL_2$-TG concentrations. Fasting

215 chylomicron-TG concentrations were negligible in both trials (CON: 0.03±0.014 mmol.l^{-1}, EX:

216 0.02±0.005 mmol.l^{-1}, p=0.25). Exercise significantly reduced fasting $VLDL_1$-TG (CON: 0.49(0.33-

217 0.72) mmol.l^{-1}, EX: 0.36(0.22-0.59) mmol.l^{-1}, geometric means (95% CI); p=0.04), but fasting

218 concentration of $VLDL_2$-TG did not differ between trials (CON: 0.21(0.12-0.36) mmol.l^{-1}, EX:

219 0.18(0.12-0.27) mmol.l^{-1}, geometric means (95% CI); p=0.60). Time-averaged postprandial

220 chylomicron-TG concentrations (CON: 0.55±0.10 mmol.l^{-1}, EX: 0.39±0.08 mmol.l^{-1}, p=0.03) and

221 time-averaged postprandial $VLDL_1$-TG concentrations (CON: 0.85±0.13 mmol.l^{-1}, EX: 0.66±0.10

222 mmol.l^{-1}, p=0.01) were both lower in the exercise compared with the control trial, but the time-

223 averaged postprandial $VLDL_2$-TG concentration did not differ significantly between trials (CON:

224 0.31±0.15 mmol.l^{-1}, EX: 0.27±0.09 mmol.l^{-1}, p=0.60).

225

226 Table 2 shows total $VLDL_1$ and $VLDL_2$ concentration and the constituent lipoprotein molecules

227 fasting and 240 minutes postprandially. Total $VLDL_1$ lipoprotein mass for was 24% lower fasting

228 (p=0.04) and 17% lower postprandially (p=0.049) in EX compared to CON. $VLDL_1$ apo B

229 concentrations were significantly lower fasting, but not postprandially, in EX than CON. Both fasting

230 and postprandial concentrations of $VLDL_1$ TG, cholesteryl ester and free cholesterol were lower in

231 EX than CON. Fasting $VLDL_1$ TG/apo B ratio was 39% higher in EX compared to CON, but this
232 was not statistically significant (p=0.09). At 240 minutes, $VLDL_1$ TG/apoB ratio was similar in the
233 two trials. Similarly, the fasting $VLDL_1$ CE/TG ratio was 26% lower in EX than CON, but this
234 difference was not statistically significant (p=0.16). At 240 minutes $VLDL_1$ CE/TG ratio was similar
235 in the two trials. No differences in $VLDL_2$ concentration or composition were observed between
236 trials.
237
238 ***Lipoprotein affinity for LPL.*** Figure 2 shows NEFA release over the 30-minute incubation period in
239 the LPL-affinity assay for fasting and postprandial $VLDL_1$ and $VLDL_2$, and for postprandial
240 chylomicrons in the two trials. There was no significant change in affinity of chylomicrons for LPL
241 expressed either as fold-change (1.2(0.6- 2.3) fold, p=0.59) or in absolute units (see Table 3).
242 However, exercise increased $VLDL_1$ affinity for LPL by 2.2(1.3-3.7) fold (geometric mean (95% CI))
243 in the fasted state (p=0.02 for fold increase; p-value for absolute increase shown in Table 3) and by
244 2.6(1.8-3.8) fold in the postprandial state (p=0.001 for fold increase; p-value for absolute increase
245 shown in Table 3). Affinity of $VLDL_2$ for LPL was negligible and did not change in response to
246 exercise. In CON, affinity of chylomicrons for LPL was 11.3(6.0-21.6) fold greater (p=0.0001) than
247 that of $VLDL_1$ in the postprandial state, whereas in EX it was 6.0(3.0-12.0) greater (p=0.0007).
248 There was no significant difference in affinity of $VLDL_1$ for LPL between the fasted and postprandial
249 states in either CON (p=0.18) or EX (p=0.28).
250

251 **Discussion**

252 The main novel finding of the present study is that prior exercise significantly increased the affinity of
253 large $VLDL_1$ – but not of chylomicrons or $VLDL_2$ – for clearance by LPL. These findings provide an
254 important advance in our understanding of the mechanism by which exercise lowers TG
255 concentrations and put previous observations in this field into context. Earlier work demonstrated that
256 exercise often lowered TG concentrations without substantially increasing post-heparin plasma or
257 skeletal muscle LPL activity (21,22,32); that the TG reductions induced by exercise were typically
258 larger in VLDL than chylomicrons (13,14); and that exercise increased clearance of VLDL from the

259 circulation, but did not reduce hepatic VLDL production (16,17). However, these observations could
260 not explain how this increased VLDL clearance could occur without a concomitant increase in LPL
261 activity. More recently, it was hypothesised that exercise may increase the affinity of $VLDL_1$ for
262 TG-hydrolysis by LPL (15,25), which received indirect support from observations that exercise
263 upregulated clearance of $VLDL_1$ to a greater extent than chylomicrons (15). This hypothesis is
264 confirmed by the present findings. Exercise increased the affinity of $VLDL_1$ for LPL clearance 2.2-
265 fold in the fasted state and 2.6-fold in the postprandial state, but did not significantly alter the affinity
266 of chylomicrons. Accordingly the affinity of $VLDL_1$ relative to chylomicrons for LPL-mediated
267 clearance in the postprandial state was ~2-fold greater in the exercise compared with the control trial.
268 This is consistent with earlier observations that the exercise-induced increase in $VLDL_1$-TG clearance
269 was twice as great as the exercise-induced increase in chylomicron-like particles (15).
270
271 In contrast to previous observations (13,15), we did not observe a statistically significant increase in
272 size or TG-enrichment of $VLDL_1$ particles after exercise in the present study. However, this does not
273 necessarily imply that exercise-induced changes to $VLDL_1$ size and composition were unimportant in
274 mediating the increase in affinity for LPL-mediated clearance. Circulating $VLDL_1$ particles are all
275 essentially remnant particles, in that they will have had some LPL-mediated hydrolysis of their TG-
276 core by the time any blood sample is taken. Post-exercise $VLDL_1$ particles with greater affinity for
277 LPL would have been exposed proportionately greater TG hydrolysis by the time blood was sampled.
278 Thus, relative to newly secreted $VLDL_1$ particles, circulating post-exercise $VLDL_1$ particles may have
279 had their size and TG-enrichment reduced to a greater extent than $VLDL_1$ particles in the control trial.
280 Thus, it is possible that following exercise, the liver produced larger, more TG-enriched $VLDL_1$
281 particles which contributed to an increased affinity for $VLDL_1$, without this effect being reflected in
282 the composition of the measured circulating $VLDL_1$ particle (25). This suggestion is supported by
283 data from Magkos and colleagues who reported a reduction in hepatic VLDL-apoB100, but not
284 VLDL-TG, secretion following exercise (17), implying secretion of a larger more TG-rich VLDL
285 particle. However, Al-Shayji and coworkers, who considered hepatic production of larger $VLDL_1$
286 particles, rather than total VLDL, found the ratio of $VLDL_1$-TG production to $VLDL_1$-apoB

287 production was similar in control and exercise trials, suggesting that the average size of the secreted
288 $VLDL_1$ particle may not have been influenced by exercise (15). However, it is also important to
289 recognise that $VLDL_1$ particles are heterogeneous, occupying a greater than three-fold range in size
290 and density, and thus considering only 'average' lipoprotein size and composition in the $VLDL_1$ range
291 may not reveal the whole story. Interestingly, Al-Shayji *et al* reported a larger exercise-induced
292 increase in the $VLDL_1$-apoB fractional catabolic rate than the $VLDL_1$-TG fractional catabolic rate
293 (146% *vs* 82% increase) (15), suggesting that exercise was having a proportionately larger effect on
294 clearance of smaller, less TG-rich $VLDL_1$ particles (which have a lower TG/apoB ratio), either via
295 direct particle removal or by TG-removal taking them out of the $VLDL_1$ and into the $VLDL_2$ density
296 range. This interpretation suggests that the observation that circulating $VLDL_1$ particles post-exercise
297 are larger and more TG-enriched reflects the fact that exercise disproportionately increased clearance
298 of smaller, less TG-enriched particles at the lower end of the Sf 60-400 range, leaving proportionally
299 more larger $VLDL_1$ in the circulation. Accordingly, it is possible that the strong correlations between
300 change in $VLDL_1$ apo B fractional catabolic rate and change in $VLDL_1$ TG/apoB and CE/TG ratio
301 previously observed (15) reflects greater clearance of smaller $VLDL_1$ particles following exercise,
302 which would have the net effect of increasing the average TG/apoB and CE/TG ratio of the remaining
303 circulating lipoprotein particles in the $VLDL_1$ density range. This suggestion is supported by recent
304 work from Harrison and colleagues who used nuclear magnetic resonance spectroscopy to quantify 24
305 different VLDL subfractions in the fasted state following exercise (22). They found that in response
306 to exercise, there was a proportionally larger TG reduction in 'medium VLDL' (size range 43-55nm,
307 approximately corresponding to Sf 100-200 (33), i.e. smaller $VLDL_1$) than in 'large VLDL' (size (55-
308 260nm, approximately corresponding to Sf >200 (33), i.e. larger $VLDL_1$), with VLDL particles over
309 120nm in size being virtually unaffected by exercise (22). The authors proposed that their findings
310 were suggestive of independent metabolic regulation of different VLDL pools within the $VLDL_1$
311 range (22). This is an attractive proposition which is consistent with our present and earlier (15)
312 observations on the effects of exercise on $VLDL_1$ metabolism. Further study is needed to both
313 examine the effects of exercise on the affinity of smaller and larger $VLDL_1$ particles for LPL-

314 mediated TG hydrolysis, and to understand the effects of exercise on TG and apoB kinetics of smaller
315 and larger $VLDL_1$ particles.
316
317 This study evaluated the effects of exercise on the affinity of TG-rich lipoproteins for LPL-mediated
318 clearance both fasting and postprandially, finding similar exercise-induced increases in $VLDL_1$
319 affinity under both conditions. There are, however, two factors to consider when interpreting the
320 postprandial data. First, action of LPL on chylomicrons produces chylomicron remnant particles
321 which fall into the Sf 60-400 range. Thus, the isolated postprandial '$VLDL_1$' fraction contains both
322 hepatically-produced $VLDL_1$ particles and chylomicron remnants. However, >95% of postprandial
323 lipoprotein particles in this density range are apoB100 containing (i.e. hepatically-produced) (13),
324 thus it seems unlikely that this relatively small 'contamination' from chylomicron remnants would
325 have substantially influenced the results. A further consideration is that in our *in vitro* assay, we
326 assessed the affinity of isolated postprandial $VLDL_1$ for LPL-mediated clearance without direct
327 competition for chylomicrons, which does not fully reflect the *in vivo* system. Thus, further study
328 which assesses the effects of exercise on affinity of postprandial $VLDL_1$ for LPL-mediated clearance
329 in the presence of chylomicrons is warranted to extend the present findings.
330
331 While this study clearly demonstrated that exercise increased the affinity of $VLDL_1$ particles for
332 clearance by LPL, this effect is unlikely to be solely responsible for the observed TG lowering effect.
333 There was a significant reduction in chylomicron-TG concentration following exercise, which
334 occurred without a statistically significant increase in chylomicron affinity for LPL-mediated
335 clearance. However, as the effect of exercise on chylomicron affinity for LPL-mediated clearance
336 was quite variable, with some participants experiencing a >2-fold increase, it is possible that this
337 contributed to the lowering of chylomicron-TG concentrations in a subset of individuals. We did not
338 measure post-heparin plasma or skeletal muscle LPL activity in the present study, so it is unclear
339 whether this might have contributed to the lower chylomicron TG-concentrations. However, previous
340 studies have suggested that there is also considerable inter-individual variability in exercise-induced
341 changes in LPL activity (34). There is also evidence that exercise-induced changes in blood perfusion

342 to LPL-rich tissues – leading to increased interactions between chylomicron particles and LPL – is
343 likely to contribute to the chylomicron-TG lowering effect (35,36). Thus, it is possible that
344 individually variable combinations of increased affinity of chylomicrons for LPL-mediated clearance,
345 increased LPL activity, and increased blood flow all contributed to the reduction in chylomicron-TG
346 concentration observed after exercise. This is a testable hypothesis which warrants further
347 investigation.

348

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

357

358 In conclusion, the main finding of this study was that exercise substantially increased the affinity of
359 $VLDL_1$ for LPL-mediated TG-hydrolysis both fasting and postprandially. This provides an important
360 piece in the jigsaw of understanding the effects of exercise on the metabolism of TG-rich lipoproteins.

361

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466

Table 1. Fasting and postprandial plasma concentrations

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

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 $VLDL_1$ and $VLDL_2$ in the fasted and postprandial states

		Fasting (0 minutes)			Postprandial (240 minutes)		
		Control	Exercise	p-value	Control	Exercise	p-value
$VLDL_1$	Total lipoprotein concentration (mg.dl^{-1})	84.1 ± 17.4	63.7 ± 13.0	0.04	137.9 ± 22.1	114.6 ± 18.0	0.049
	Apo B (mg.dl^{-1})	1.98 ± 0.36	1.05 ± 0.21	0.01	2.22 ± 0.34	2.01 ± 0.31	0.33
	Triglyceride (mg.dl^{-1})	51.7 ± 11.1	41.0 ± 8.9	0.04*	88.5 ± 14.0	73.9 ± 12.3	0.03
	Cholesteryl ester (mg.dl^{-1})	7.7 ± 1.2	4.8 ± 3.0	0.03	11.6 ± 2.0	8.3 ± 1.2	0.048
	Free cholesterol (mg.dl^{-1})	4.0 ± 0.9	2.6 ± 0.6	0.02	5.9 ± 1.1	4.6 ± 0.8	0.045
	Phospholipid (mg.dl^{-1})	12.8 ± 3.0	9.2 ± 1.9	0.08	21.1 ± 3.7	17.6 ± 8.7	0.13
	Protein (mg.dl^{-1})	8.0 ± 1.6	6.1 ± 1.1	0.12	10.8 ± 2.2	10.2 ± 1.2	0.73
	TG/apoB ratio (mol:mol)	17751 ± 2507	24693 ± 4238	0.09	25103 ± 2304	23072 ± 2561	0.47
	CE/TG ratio (mol:mol)	0.23 ± 0.03	0.17 ± 0.02	0.16	0.18 ± 0.02	0.16 ± 0.01	0.40

VLDL₂	Total lipoprotein concentration (mg.dl^{-1})	49.5 ± 3.6	39.4 ± 2.2	0.15	38.2 ± 1.6	40.0 ± 2.8	0.59
	Apo B (mg.dl^{-1})	4.00 ± 0.57	3.39 ± 0.17	0.50	2.43 ± 0.17	2.53 ± 0.15	0.60
	Triglyceride (mg.dl^{-1})	18.2 ± 3.2	15.0 ± 2.5	0.29	15.8 ± 2.2	16.2 ± 2.3	0.83
	Cholesteryl ester (mg.dl^{-1})	11.3 ± 3.0	9.1 ± 1.5	0.39	7.2 ± 1.5	6.7 ± 1.2	0.44
	Free cholesterol (mg.dl^{-1})	4.0 ± 0.8	3.3± 0.5	0.22	3.0 ± 0.5	2.9 ± 0.4	0.56
	Phospholipid (mg.dl^{-1})	9.8 ± 0.9	8.3 ± 0.5	0.36	7.3 ± 0.5	7.6 ± 0.6	0.70
	Protein (mg.dl^{-1})	6.1 ± 0.4	3.7 ± 0.3	0.08	5.0 ± 0.2	6.6 ± 0.7	0.22
	TG/apoB ratio (mol:mol)	3679 ± 567	3051 ± 400	0.19	5416 ± 993	4169 ± 540	0.20
	CE/TG ratio (mol:mol)	0.59 ± 0.10	0.62 ± 0.06	0.79	0.45 ± 0.08	0.43 ± 0.04	0.78

Values are mean ± SEM, n = 10. *Statistical analysis performed on log-transformed data

Table 3. Lipoprotein Affinity for LPL

	NEFA release (mmol.min^{-1} per mmol lipoprotein per unit LPL activity)					
	Fasting (0 minutes)			Postprandial (240 minutes)		
	Control	Exercise	p-value	Control	Exercise	p-value
Chylomicrons				1.25 (0.94 to 1.66)	1.52 (0.98 to 2.34)	0.53*
$VLDL_1$	0.16 (0.09 to 0.29)	0.35 (0.24 to 0.52)	0.018*	0.08 (0.03 to 0.19)	0.25 (0.15 to 0.42)	0.002*
$VLDL_2$	0.013 (0.004 to 0.044)	0.018 (0.007 to 0.049)	0.60	0.021 (0.006 to 0.070)	0.013 (0.004 to 0.048)	0.34

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), $VLDL_1$-TG (middle panel) and $VLDL_2$-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), $VLDL_1$ in the fasted (middle left) and postprandial (middle right) states and $VLDL_2$ 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

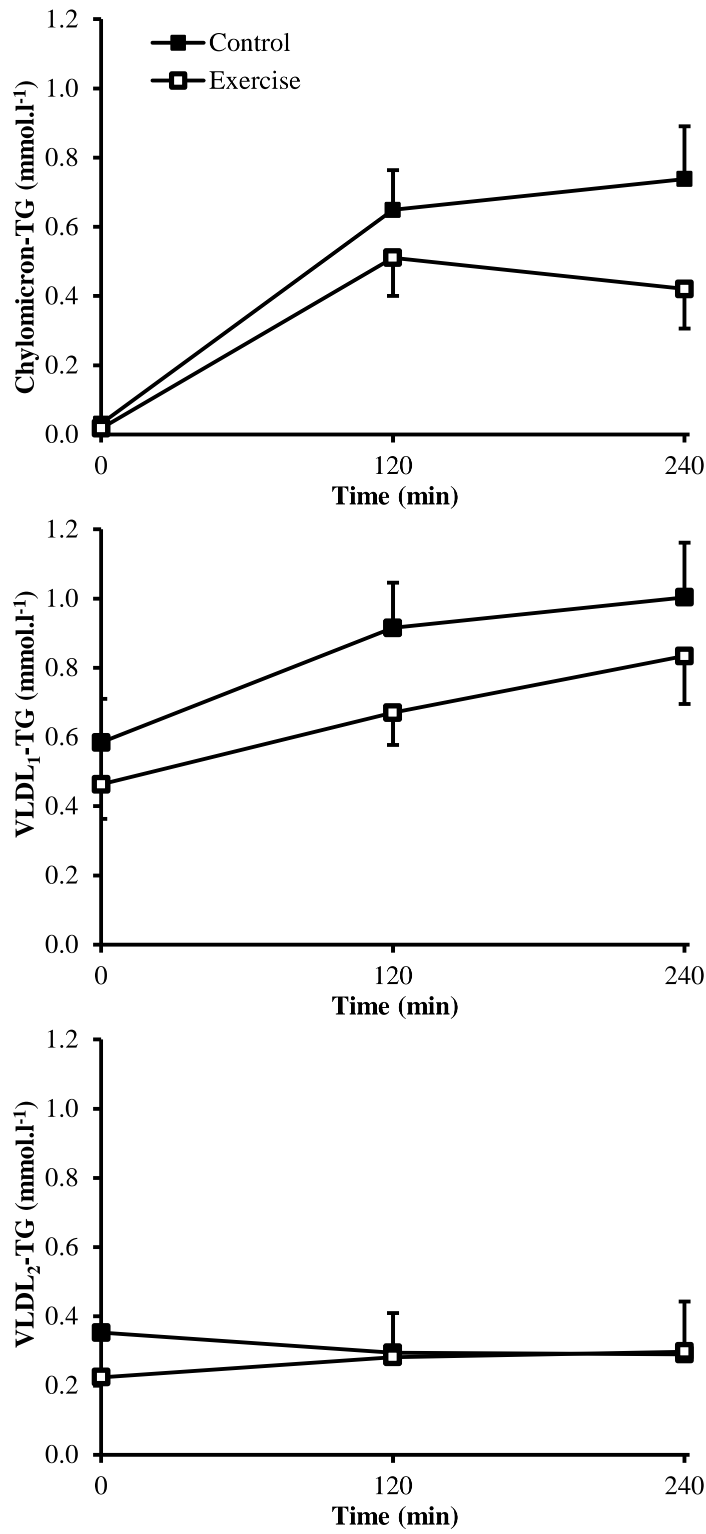

Figure 2