

WestminsterResearch

<http://www.westminster.ac.uk/westminsterresearch>

Exercise Training Reduces Liver Fat and Increases Rates of VLDL Clearance, but not VLDL Production in NAFLD

Shojaee-Moradie, F., Cuthbertson, D.J., Barrett, M., Jackson, N.C., Herring, R, Thomas, E.L., Bell, J.D., Kemp, G.J., Wright, J. and Umpleby, A.M.

This is an author's accepted manuscript of an article to be published in the Journal of Clinical Endocrinology and Metabolism. The final definitive version will be available online at: <http://press.endocrine.org/journal/jcem>

The WestminsterResearch online digital archive at the University of Westminster aims to make the research output of the University available to a wider audience. Copyright and Moral Rights remain with the authors and/or copyright owners.

Whilst further distribution of specific materials from within this archive is forbidden, you may freely distribute the URL of WestminsterResearch: (<http://westminsterresearch.wmin.ac.uk/>).

In case of abuse or copyright appearing without permission e-mail repository@westminster.ac.uk

25 **Abstract**

26

27 **Context** Randomised controlled trials in non-alcoholic fatty liver disease (NAFLD) have shown that
28 regular exercise, even without calorie restriction, reduces liver steatosis. A previous study has shown
29 that 16 weeks supervised exercise training in NAFLD did not affect total VLDL kinetics.

30 **Objective** To determine the effect of exercise training on intrahepatocellular fat (IHCL) and the
31 kinetics of large triglyceride-(TG)-rich VLDL₁ and smaller denser VLDL₂ which has a lower TG
32 content.

33 **Design** A 16 week randomised controlled trial.

34 **Patients** 27 sedentary patients with NAFLD.

35 **Intervention** Supervised exercise with moderate-intensity aerobic exercise or conventional lifestyle
36 advice (control).

37 **Main outcome** Very low density lipoprotein1 (VLDL₁) and VLDL₂-TG and apolipoproteinB (apoB)
38 kinetics investigated using stable isotopes before and after the intervention.

39 **Results** In the exercise group VO_{2max} increased by 31±6% (mean±SEM) and IHCL decreased from
40 19.6% (14.8, 30.0) to 8.9% (5.4, 17.3) (median (IQR)) with no significant change in VO_{2max} or IHCL
41 in the control group (change between groups p<0.001 and p=0.02, respectively). Exercise training
42 increased VLDL₁-TG and apoB fractional catabolic rates, a measure of clearance, (change between
43 groups p=0.02 and p=0.01, respectively), and VLDL₁-apoB production rate (change between groups
44 p=0.006), with no change in VLDL₁ -TG production rate. Plasma TG did not change in either group.

45 **Conclusion** An increased clearance of VLDL₁ may contribute to the significant decrease in liver fat
46 following 16 weeks of exercise in NAFLD. A longer duration or higher intensity exercise
47 interventions may be needed to lower plasma TG and VLDL production rate.

48

49 **Introduction**

50 NAFLD, the most prevalent liver disease in the developed world (1), increases the risk of chronic
51 liver disease, hepatocellular carcinoma and cardiovascular disease, and is associated with increased
52 visceral fat, hypertriglyceridaemia and insulin resistance (2).

53 Hepatic steatosis is the result of an imbalance between triglyceride (TG) synthesis and TG export.
54 TGs stored and secreted by the liver are synthesised from fatty acids generated from three main
55 sources: hepatic de novo lipogenesis; circulating non-esterified fatty acids (NEFA), originating from
56 adipose tissue; and fatty acids derived from the remnants of the TG rich lipoproteins, VLDL and
57 chylomicrons (3) which are generated when these lipoproteins are cleared from the circulation by the
58 lipolytic action of lipoprotein lipase (LPL) and hepatic lipase (4).

59 VLDL secreted by the liver can be separated into large TG-rich VLDL₁ and smaller denser VLDL₂
60 which has a lower TG content. There is evidence that these two VLDL species are independently
61 regulated (5). VLDL is initially assembled as a primordial particle (pre-VLDL) when apolipoprotein
62 B100 (apoB) is co-translationally lipidated in the endoplasmic reticulum by microsomal transfer
63 protein (MTP). Pre-VLDL can either be retained and degraded, or further lipidated to form VLDL₂.
64 This particle can then either be secreted or converted to VLDL₁ following the addition of more TG in
65 the liver. The hydrolysis of VLDL₁-TG by lipoprotein lipase (LPL) also generates VLDL₂ in the
66 circulation. Thus VLDL₂ has two sources. Insulin regulates VLDL assembly by decreasing apoB
67 mRNA translation (6), inhibiting the expression of MTP (7) and promoting apoB degradation via
68 autophagy (8). In NAFLD, with intrahepatic lipids in copious supply, increases in both VLDL-apoB
69 and VLDL-TG production rate (PR) contribute to the atherogenic lipid profile (9).

70 Lifestyle intervention is the first line of treatment for NAFLD. Weight loss (5-10%) through diet, with
71 or without exercise, has been shown to reduce hepatic steatosis (10,11). A number of randomised
72 controlled trials have also shown that regular exercise, even without calorie restriction, reduces liver
73 steatosis (12,13). A previous study has shown that 16 weeks supervised exercise training in men and
74 women with NAFLD did not affect total VLDL kinetics (14). In the current study we examined the
75 effect of 16 weeks supervised exercise training in men with NAFLD on VLDL₁ and VLDL₂ kinetics,
76 using a protocol which we have shown previously to very effective at increasing fitness (15).

77 **Methods**

78 **Study design** The study was approved by the English National Health Service (NHS) Ethics
79 Committee and the University of Surrey Ethics Committee. The study was performed at one centre, in
80 Guildford, Surrey. This study is part of a larger collaborative study investigating the metabolic impact
81 of exercise supervision in patients with NAFLD (16). Informed consent was obtained from the study
82 participants prior to inclusion into the study.

83 **Study participants** Twenty nine sedentary male patients, confirmed to have NAFLD were recruited
84 through the English NHS primary and secondary care providers in the local area. There were two
85 dropouts, one in each group. Twenty seven patients completed the study (Table 1) The diagnosis of
86 NAFLD was made in patients who had been referred for investigation of raised serum transaminases,
87 indication of hepatic steatosis on ultrasound or by liver biopsy (n=4, two in each group; none of these
88 patients had non-alcoholic steatohepatitis). It was not possible to exclude NASH from subjects who
89 were not recruited by biopsy. Patients were excluded if the diagnosis of NAFLD was secondary to
90 drug treatments, if there was evidence of viral hepatitis, autoimmune hepatitis or primary biliary
91 cirrhosis, or metabolic disorders, if they had a history of type 2 diabetes mellitus, ischaemic heart
92 disease or had any contraindications to exercise, clinical hyperlipidaemia (fasting plasma TG >3.0
93 mmol/l or total cholesterol levels > 7.0 mmol/l), if they were current smokers, had a history of
94 excessive alcohol intake (weekly consumption of >21 units), had MRI contraindications (cardiac
95 pacemakers, metal implants), or were taking any fibrates or beta blockers.

96 Participants were asked to complete a Physical Activity Readiness Questionnaire to identify those not
97 suitable for physical activity. Motivation was assessed through questions relating to willingness to
98 increase exercise levels and confidence in complying with exercising four times per week. Suitable
99 participants were randomised to one of two groups using a list generated by computer randomisation,
100 (Statistical Analysis System v 9.1, PROC PLAN software). One group received a structured
101 supervised exercise programme with an exercise physiologist. The other group received standard
102 lifestyle advice (control group) with no further communication from the exercise physiologist. Both
103 groups were asked to continue their usual diet.

104

105 **Study measurements** Prior to and following the 16 week intervention period measurements of
106 physical fitness (VO_{2max}) were made and a 7 day diet diary was completed. On a separate visit,
107 measurements of fasting VLDL₁ and VLDL₂-apoB and TG kinetics and arterial stiffness (by pulse
108 wave velocity) were made. Body composition (total, subcutaneous and visceral fat volumes) was
109 measured by magnetic resonance imaging (MRI) and IHCL, intramyocellular (IMCL) and pancreatic
110 lipid content was measured by magnetic resonance spectroscopy (¹H-MRS).

111 **Physical training protocol** Participants allocated to the supervised group exercised at moderate
112 intensity (40-60% heart rate reserve) for 20 minutes initially (progressing towards 1 hour as the
113 programme developed) 4-5 times per week for 16 weeks. Types of activities were either gym based
114 aerobic plus resistance exercise, or outdoor aerobic activities and resistance exercise as discussed with
115 the exercise physiologist. Participants received weekly exercise supervision by the exercise
116 physiologist usually in person, otherwise by telephone, to assess their progress.

117 **Measurement of VO_{2max}** VO_{2max} was performed within four days of the metabolic study using an
118 electronically braked bicycle ergometer (Lode; Excalibur Sport, Groningen, the Netherlands)
119 equipped with a computerised breath (oxygen [O₂]/carbon dioxide [CO₂]) analyser system (Medical
120 Graphics, St Paul, MN, USA). An electrocardiogram (ECG) was undertaken during the exercise test
121 to monitor participants' heart rate and exclude latent ischaemic heart disease.

122 **Measurement of pulse wave velocity** is described in the Supplementary Material.

123 **Diet diaries** Quantification of dietary intake in all participants was assessed by diet diary, and
124 analysed by Dietplan 6 (Release 6.60b4 with Windows VistaService Pack 1. Forestfield Software Ltd,
125 Horsham, West Sussex, UK).

126 **Measurement of body composition and intracellular fat** Subjects fasted for 6 hours before the scans.
127 Whole body MR imaging for body fat content and ¹H MRS measurements of pancreatic fat, IHCL and
128 IMCL (tibialis anterior and soleus muscle) was measured on an Intera 1.5T Achieva multinuclear
129 system (Philips Medical Systems, Best, Holland) as previously reported (17, 18). NAFLD was defined
130 as mean IHCL > 5.5%. For more details see the Supplementary Material.

131 **Metabolic Study Protocol** Participants attended the CEDAR centre, Royal Surrey County Hospital,
132 Guildford on two occasions before (0 week) and after the intervention (16 weeks). The participants

133 were asked to refrain from vigorous exercise for 72h before the study, abstain from drinking alcoholic
134 beverages for 24h, and attend after an overnight fast. A primed (1mg/kg) intravenous infusion of 1-
135 ¹³C-leucine (1 mg/kg/h) and a bolus of ²H₅ glycerol (75μmol/kg) were administered. Blood samples
136 were taken at regular time intervals for 9 hours.

137 **Laboratory protocols** VLDL₁ (Svedberg flotation rate 60–400) and VLDL₂ (Svedberg flotation rate
138 20–60) fractions were isolated from plasma by sequential ultracentrifugation (19). ApoB and TG were
139 isolated from VLDL₁ and VLDL₂ hydrolysed, derivatised and isotopic enrichment measured by gas
140 chromatography mass spectrometry as described in the Supplementary Material. Concentration
141 measurements are also described in the Supplementary Material.

142 **Power calculation** The primary endpoint for this study was VLDL-apoB production rate. Based on a
143 previous study in type 2 diabetes where a 6 month exercise program reduced VLDL-apoB production
144 rate by 48% (20), the study was powered to detect a 20% within-group reduction in VLDL-apoB
145 production with 80% power at the 5% level.

146 **Data analysis** The measurements of enrichment of free glycerol in plasma and glycerol enrichment of
147 TG in VLDL₁ and VLDL₂ particles were used to determine VLDL₁ and VLDL₂-TG fractional
148 catabolic rate (FCR) using the modelling software SAAM II (SAAM Institute, Seattle, WA) as
149 previously described (23). The model was also used to determine the kinetic parameters of VLDL₁
150 and VLDL₂ apoB using plasma αKIC enrichment and 1-¹³C leucine enrichment of VLDL₁ and
151 VLDL₂-apoB. VLDL₁-TG and apoB FCR had two components, VLDL₁ FCR transfer (to VLDL₂) and
152 VLDL₁ FCR catabolism (direct removal from circulation). Production rate (PR) was calculated as the
153 product of VLDL₁ and VLDL₂-FCR and their respective pool sizes. VLDL₁ and VLDL₂-TG and apoB
154 pool sizes were calculated from VLDL₁ and VLDL₂-TG and apoB concentrations in
155 ultracentrifugation fractions and plasma volume as previously described (21). (For more details of the
156 models see the Supplementary Material). Total VLDL-TG and VLDL-apoB pool sizes were
157 calculated by the addition of VLDL₁ and VLDL₂-TG and apoB pool sizes respectively. Particle sizes
158 of VLDL₁ and VLDL₂ were calculated by dividing TG pool size by apoB pool size. Total VLDL-TG
159 PR was calculated by summation of VLDL₁-TG PR and VLDL₂-TG hepatic PR.

160

161 Ten-year cardiovascular risk was calculated using the 10 year Framingham Risk Score (FRS) (22).
162 Homeostatic Model Assessment (HOMA2) was used to assess whole body insulin sensitivity
163 (HOMA2-%S) (23). Adipose tissue insulin resistance (Adipo-IR) was calculated by multiplying
164 fasting plasma NEFA concentration with fasting serum insulin concentration.
165 Percent Change in IHCL was calculated as Pre-Post intervention/Pre x100. Changes in other
166 measurements were calculated as Pre-Post intervention.

167

168 **Statistical analysis** Statistical analysis of the data was performed using SPSS version 21.0 for
169 Window (Chicago: SPSS Inc.). IHCL is shown as median (interquartile range). All other results are
170 means \pm SEM. Non parametric data was log-transformed. Basal comparisons were performed using
171 Student's *t* test. Within-group changes between baseline and 16 weeks were compared using paired *t*
172 tests. The change between baseline and 16 weeks was compared between groups using student's *t* test
173 for parametric data and Mann-Whitney U test for nonparametric data. Correlations were assessed by
174 Pearson's correlation coefficient and Spearman's rho correlation coefficient when the data were not
175 normally distributed. A p value <0.05 was taken as statistically significant.

176

177 **Results**

178 *Baseline characteristics*

179 Body weight, BMI and baseline biochemical characteristics (plasma lipid profile and liver enzyme
180 concentrations) were not significantly different at 0 weeks between groups (Table 1). Similarly, there
181 were no significant baseline differences in cardiorespiratory fitness (VO_{2max}), IHCL, pancreatic fat or
182 fat distribution (Table 2). IHCL in all participants (n=27) at 0 week correlated positively with fasting
183 plasma TG concentration ($r=0.439$, $p=0.02$) and abdominal visceral fat ($r=0.411$, $p=0.03$).

184

185 *Effects of intervention (exercise training vs. control)*

186 *Body weight, BMI and fitness*

187 Body weight and BMI decreased by $3.6\pm 0.8\%$ and $3.8\pm 0.9\%$ respectively after 16 weeks exercise
188 training with no change in controls (change exercise vs. change control $p<0.001$ and, $p=0.02$) (Table
189 1). In both groups, total energy intake and macronutrient composition remained unchanged after 16
190 weeks compared with baseline (Supplementary Table 1).

191 In the exercise group VO_{2max} increased significantly by $31\pm 6\%$ after 16 weeks with no change in
192 controls (change in exercise group vs. control, $p<0.001$) (Figure 1, Table 1).

193 *Liver enzymes (Table 1)*

194 After 16 weeks intervention there were within group decreases in the exercise group in ALT, AST and
195 GGT concentrations ($p<0.01$, $p<0.02$ and $p<0.03$, respectively). ALT also decreased in controls
196 ($p<0.04$) with no change in either AST or GGT.

197 *Body composition and ectopic fat (Table 2)*

198 After 16 weeks, there was a significant decrease in IHCL content (% decrease 52.2% (29.0, 61.8);
199 median (IQR)) in the exercise group, with no change in controls (change exercise vs. change control
200 $p=0.02$). There was no significant change in pancreatic fat. All measured adipose tissue depots also
201 significantly decreased with no change in controls (Table 2). The percentage change in IHCL between
202 0 and 16 weeks in all patients (exercise and control group) correlated negatively with the change in

203 VO_{2max} ($r=-0.45$, $p<0.02$) and correlated positively with the change in total body fat and visceral fat (;
204 $r=0.54$, $p=0.004$; $r=0.41$, $p=0.03$).

205 *Insulin sensitivity, fasting plasma insulin and glucose concentration (Table 1)*

206 After 16 weeks exercise there was a within-group decrease in fasting plasma glucose and serum
207 insulin concentrations in the exercise group (both $p<0.01$) (between groups for insulin, $p=0.02$).
208 HOMA2-%S (a measure of insulin sensitivity) increased by $42.5\pm 11.6\%$ in the exercise group
209 ($p=0.002$) with no change in controls (between group, $p=0.003$).

210 *Blood pressure, pulse wave velocity and Framingham risk factor scores (Table 2)*

211 Both systolic and diastolic blood pressure measurements decreased by $5.4\pm 1.8\%$ and $6.2\pm 2.7\%$ in the
212 exercise group after 16 weeks exercise ($p=0.01$, $p=0.04$) (between groups $p=0.04$, $p=0.02$
213 respectively). After 16 weeks, pulse wave velocity, a measure of arterial elasticity, improved in the
214 exercise group ($p=0.05$) although between groups this was not significant. The Framingham risk score
215 decreased $14\pm 4\%$ ($p=0.001$) after exercise with no change in controls (between groups, $p<0.05$). The
216 percentage change in IHCL between 0 and 16 weeks in all patients correlated positively with the
217 change in the FRS ($r=0.62$, $p=0.001$).

218 *Plasma and fraction lipids (Tables 1, 3 and 4)*

219 At baseline, there were no differences in plasma or lipoprotein fraction lipids between groups. Total
220 cholesterol, TG and HDL cholesterol concentrations did not change from baseline in either group at
221 16 weeks. After 16 weeks exercise there was a significant within-group decrease ($p=0.03$) in plasma
222 LDL cholesterol concentration. NEFA concentration did not change in either group. However adipose
223 tissue IR decreased by $24\pm 10\%$ in the exercise group ($p=0.03$) with no change in controls (change
224 between groups, $p=0.02$). After 16 weeks there was no significant change in VLDL1-TG or VLDL1
225 apoB concentration in either group. However VLDL₂ TG, cholesterol and apoB concentration were
226 reduced ($p<0.01$, $p<0.02$, $p=0.04$) in the exercise group with no change in controls. The particle size
227 of VLDL₁ (TG/apoB) was reduced in the exercise group ($p=0.03$), and was different between groups
228 ($p=0.04$).

229 *VLDL₁- and VLDL₂-TG kinetics (Table 3, Figure 2)*

230 At baseline VLDL₁ and VLDL₂-TG kinetics did not differ between groups. After 16 weeks VLDL₁-
231 TG FCR was increased in the exercise group ($p<0.05$) due to an increase in the VLDL₁-TG
232 catabolism FCR ($p=0.05$). The percent change in IHCL in all participants was negatively correlated
233 with the change in VLDL₁-TG catabolism FCR ($r=-0.74$, $p<0.001$) (Fig 2) and positively correlated
234 with the change in VLDL₁-TG transfer FCR ($r=0.63$, $p=0.001$). There was no change in VLDL₂-TG
235 FCR, VLDL₁-TG PR, VLDL₂-TG PR and total VLDL-TG PR within or between groups.

236 *VLDL₁- and VLDL₂-apoB kinetics (Table 4, Figure 2)*

237 VLDL₁- and VLDL₂-apoB kinetics were not different at baseline between groups. VLDL₁-apoB FCR
238 was increased at 16 weeks in the exercise group ($p=0.02$) with no change in controls (between groups,
239 $p=0.01$). This was due to a increase in the catabolism FCR ($p<0.01$) while the transfer FCR (to
240 VLDL₂) was decreased ($p=0.005$). There was no change in VLDL₂-apoB FCR. VLDL₁-apoB PR and
241 total VLDL-apoB PR increased in the exercise group ($p= 0.003$, $p=0.004$) (between groups $p=0.006$,
242 $p=0.02$). The percent change in IHCL between 0 and 16 weeks in all participants correlated
243 negatively with the change in VLDL₁-apoB PR ($r=-0.48$, $p=0.01$) (Fig 2).

245 **Discussion**

246 We have demonstrated for the first time that a 16 week supervised exercise intervention which
247 significantly improved cardiorespiratory fitness and reduced liver fat by over 50% in men with
248 NAFLD increased the FCR (a measure of clearance) of both VLDL₁-TG and apoB.

249 It is well documented that VLDL₁-TG and apoB FCR increases with acute exercise (24) but this effect
250 is not sustained 48h after exercise (25). In the current study subjects abstained from exercise for 72h
251 prior to the measurement of VLDL kinetics in order to measure the chronic, rather than the acute,
252 effects of exercise. NAFLD is highly associated with peripheral and hepatic insulin resistance (26,27),
253 as observed in our participants who had a fasting insulin concentration double that reported in healthy
254 subjects. There was an improvement in insulin sensitivity, as measured by HOMA %S, with exercise
255 training, as has also been demonstrated previously in type 2 diabetes mellitus and overweight subjects
256 (20,15). We have also shown in a different subset of patients with NAFLD that 4 months of exercise
257 training (with a similar-sized effect on fitness and IHCL to the current study) improved peripheral but
258 not hepatic insulin sensitivity (16). In the current study an improvement in peripheral insulin
259 sensitivity was also demonstrated with the decrease in adipose-IR. LPL activity is regulated by insulin
260 (28) and 20-weeks endurance exercise training in healthy men, which increased VO_{2max} by 13%, has
261 previously been shown to significantly increase post-heparin plasma lipoprotein lipase (29). Increased
262 LPL activity would provide a mechanism for the increase in VLDL₁-TG and apoB FCR observed in
263 the current study. Notably for VLDL₁-TG and apoB FCR it was the catabolic pathway that was
264 increased rather than the transfer of TG to VLDL₂. The increased clearance of TG from the systemic
265 circulation, while the production rate of TG was simultaneously maintained, would enable the liver to
266 export some of the stored TG for hydrolysis in skeletal muscle to sustain increased demand for fatty
267 acids during exercise.

268 The reduction in body weight in the exercise group is unlikely to have mediated the increase in
269 VLDL₁-TG and apoB clearance since previous studies have shown weight loss in obese men,
270 following a low calorie diet, reduces VLDL-apoB production rate with no effect on VLDL-apoB FCR

271 (30). Similarly in obese women, a hypocaloric diet has been shown to have no effect on either VLDL-
272 TG or VLDL-apoB FCR (31).

273 The failure of exercise training to lower VLDL₁-apoB and TG production rate and to increase
274 VLDL₁-apoB production rate was unexpected. This differs from a study in patients with type 2
275 diabetes where exercise training for 6 months, resulting in a 16% increase in VO_{2max}, reduced VLDL-
276 apoB production rate (20). Liver fat was not measured in the latter study, patients were on oral
277 hypoglycemic treatment (metformin and sulphonylureas) and some of the participants were African-
278 Caribbean, a group known to have a lower propensity for NAFLD (32). The increase in VLDL₁-apoB
279 production rate following exercise training in the current study may be explained by the marked
280 decrease in fasting insulin concentration in response to the improved peripheral insulin sensitivity,
281 while at the same time hepatic insulin resistance was maintained. Insulin regulates VLDL assembly
282 (6,7,8), thus a lowering of insulin will increase apoB secretion. It has also been shown in mice that
283 triglycerides can rescue apoB from posttranslational degradation (33). The up-regulation of VLDL₁-
284 apoB production rate in response to exercise training could increase TG export and therefore assist in
285 the reduction of liver fat. This could also explain the maintenance of plasma TG levels despite a
286 decrease in liver fat.

287 The findings of this study differ from a previous study of patients with NAFLD where 16 weeks
288 exercise training at an exercise intensity comparable to the current study had no effect on VLDL-TG
289 and apoB kinetics (14). The discordant findings most likely reflect a greater improvement in both
290 cardiorespiratory fitness and thus a greater reduction in IHCL in our study participants. VO_{2max}
291 increased by 31% in the current study compared to only a 9% increase in the previous study (14). An
292 alternative or additional explanation is that total VLDL-TG and apoB (sf 20-400) were measured in
293 the previous study, rather than VLDL₁ and VLDL₂ as in the current study. There is evidence that
294 VLDL₁ and VLDL₂ are independently regulated (5) and that exercise primarily affects VLDL₁
295 kinetics (34), and so the effect of exercise on VLDL₁ may not be revealed by measurements on total
296 VLDL. VLDL₁ carries more TG compared to VLDL₂ per particle and LPL has been shown to have a
297 preference for TG-rich particles (35).

298 In NAFLD, CV events are the most common cause of mortality (36). Both the FRS, which has been
299 shown to accurately predict the actual 10-year CV disease risk in patients with NAFLD (37), and
300 arterial stiffness, an indicator of CVD and independent predictor of the corresponding risk and LDL
301 cholesterol were decreased following exercise training. The reduced LDL cholesterol may be related
302 to the small weight loss (38). These measures demonstrate that 16 weeks exercise training can reduce
303 CVD risk in NAFLD.

304 The correlation between liver fat and cardiorespiratory fitness suggests the latter is the main driver for
305 reduced IHCL in the exercise group. However the small weight loss in the exercise group will have
306 contributed to the reduction in IHCL (11). Both endurance and resistance exercise with and without
307 weight loss have been shown to reduce liver fat (39). The decrease in IHCL following exercise was
308 not accompanied by any change in IMCL. This has also been reported in a previous exercise study in
309 obese subjects (13). A recent meta-analysis of 33 studies examining the effect of lifestyle
310 interventions on ectopic fat deposition in overweight and obese adults showed only a non-significant
311 trend toward reductions in IMCL (40). Although the meta-analysis suggested pancreatic fat reduced
312 with exercise, this was not found in the current study. There have been few studies specifically
313 addressing effects of exercise intervention on pancreatic fat.

314 In conclusion, with an exercise intervention in non-diabetic men with NAFLD that significantly
315 improved fitness and cardio-metabolic health, and produced a significant reduction in IHCL, the liver
316 continued to export excessive amounts of TG in VLDL. This may reflect the failure to normalise
317 IHCL and restore hepatic insulin sensitivity. A longer duration or higher intensity exercise
318 intervention, or a combined approach with calorie restriction, may be required to achieve this and to
319 lower plasma TG and VLDL production rate.

320

321

322 **Acknowledgements**

323 We are grateful to Dr Roman Hovorka, University of Cambridge, UK for creating the VLDL TG and
324 apolipoprotein B models.

325

326

327 **Contribution statement**

328 AMU, DJC, FSM and GJK designed the study, FSM. JW and RH performed the clinical studies. MB
329 supervised the exercise intervention. FSM and NCJ performed the laboratory work, supervised by
330 AMU. JB and ELT performed the MRI and MRS measurements, AMU was the lead writer. All
331 authors reviewed the manuscript. AMU is the guarantor of this work and, as such, had full access to
332 all the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

333

334 **Figure legends**

335 Figure 1: VO_{2max} at 0 and 16 weeks in a) Exercise group and b) Control group

336 Figure 2. a) VLDL₁ (V1) apoB FCR, b) VLDL₁-TG FCR c) VLDL₁-apoB production rate and d)
337 VLDL₁-TG production rate, at 0 weeks (solid bar) and 16 weeks (hatched bar) in the exercise and
338 control groups, e) relationship between percent change in IHCL and the change in VLDL₁-apoB
339 production rate and f) relationship between percent change in IHCL and the change in VLDL₁-TG
340 catabolism FCR. Black circles: exercise group; open circles: control group.

341

342 **References**

- 343 1. **Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M.** Global
344 Epidemiology of Non-Alcoholic Fatty Liver Disease-Meta-Analytic Assessment of
345 Prevalence, Incidence and Outcomes. *Hepatology*. 2016;64 (1):73-84.
- 346 2. **Marchesini G, Brizi M, Morselli-Labate AM, et al.** Association of nonalcoholic fatty liver
347 disease with insulin resistance. *Am J Med*. 1999;107(5):450-455.
- 348 3. **Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ.** Sources
349 of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty
350 liver disease. *J Clin Invest*. 2005;115(5):1343-1351.
- 351 4. **Dallinga-Thie GM, Franssen R, Mooij HL, et al.** The metabolism of triglyceride-rich
352 lipoproteins revisited: new players, new insight. *Atherosclerosis*. 2010;211(1):1-8.
- 353 5. **Malmstrom R, Packard CJ, Caslake M, et al.** Effects of insulin and acipimox on VLDL1
354 and VLDL2 apolipoprotein B production in normal subjects. *Diabetes*. 1998;47(5):779-787.
- 355 6. **Sidiropoulos KG, Pontrelli L, Adeli K.** Insulin-mediated suppression of apolipoprotein B
356 mRNA translation requires the 5' UTR and is characterized by decreased binding of an
357 insulin-sensitive 110-kDa 5' UTR RNA-binding protein. *Biochemistry*. 2005;44(37):12572-
358 12581.
- 359 7. **Au WS, Kung HF, Lin MC.** Regulation of microsomal triglyceride transfer protein gene by
360 insulin in HepG2 cells: roles of MAPKerk and MAPKp38. *Diabetes*. 2003;52(5):1073-1080.
- 361 8. **Sparks JD, O'Dell C, Chamberlain JM, Sparks CE.** Insulin-dependent apolipoprotein B
362 degradation is mediated by autophagy and involves class I and class III phosphatidylinositide
363 3-kinases. *Biochem Biophys Res Commun*. 2013;435(4):616-620.
- 364 9. **Adiels M, Taskinen MR, Packard C, et al.** Overproduction of large VLDL particles is
365 driven by increased liver fat content in man. *Diabetologia*. 2006;49(4):755-765.
- 366 10. **Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI.** Reversal of
367 nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate
368 weight reduction in patients with type 2 diabetes. *Diabetes*. 2005;54(3):603-608.
- 369 11. **Lazo M, Solga SF, Horska A, et al.** Effects of a 12-month intensive lifestyle intervention on
370 hepatic steatosis in adults with type 2 diabetes. *Diabetes Care*. 2010;33(10):2156-2163.
- 371 12. **Hallsworth K, Fattakhova G, Hollingsworth KG, et al.** Resistance exercise reduces liver
372 fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut*.
373 2011; 60(9):1278-1283.
- 374 13. **Johnson NA, Sachinwalla T, Walton DW, et al.** Aerobic exercise training reduces hepatic
375 and visceral lipids in obese individuals without weight loss. *Hepatology*. 2009;50(4):1105-
376 1112.

- 377 14. **Sullivan S, Kirk EP, Mittendorfer B, Patterson BW, Klein S.** Randomized trial of
378 exercise effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty
379 liver disease. *Hepatology*. 2012;55(6):1738-1745.
- 380 15. **Shojaee-Moradie F, Baynes KC, Pentecost C, et al.** Exercise training reduces fatty acid
381 availability and improves the insulin sensitivity of glucose metabolism. *Diabetologia*..
382 2007;50(2):404-413.
- 383 16. **Cuthbertson DJ, Shojaee-Moradie F, Sprung VS, et al.** Dissociation between exercise-
384 induced reduction in liver fat and changes in hepatic and peripheral glucose homeostasis in
385 obese patients with non-alcoholic fatty liver disease. *Clin Sci (Lond)*. 2016;130(2):93-104.
- 386 17. **Thomas EL, Hamilton G, Patel N, et al.** Hepatic triglyceride content and its relation to
387 body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy
388 study. *Gut*. 2005;54(1): 122-127.
- 389 18. **Thomas EL, Fitzpatrick JA, Malik SJ, Taylor-Robinson SD, Bell JD.** Whole body fat:
390 content and distribution. *Prog Nucl Magn Reson Spectrosc*. 2013;73:56-80.
- 391 19. **Watts GF, Mandalia S, Brunt JN, Slavin BM, Coltart DJ, Lewis B.** Independent
392 associations between plasma lipoprotein subfraction levels and the course of coronary artery
393 disease in the St. Thomas' Atherosclerosis Regression Study (STARS). *Metabolism*.
394 1993;42(11):1461-1467.
- 395 20. **Alam S, Stolinski M, Pentecost C, et al.** The effect of a six-month exercise program on
396 very low-density lipoprotein apolipoprotein B secretion in type 2 diabetes. *J Clin Endocrinol*
397 *Metab*. 2004;89(2):688-694.
- 398 21. **Sarac I, Backhouse K, Shojaee-Moradie F, et al.** Gender differences in VLDL1 and
399 VLDL2 triglyceride kinetics and fatty acid kinetics in obese postmenopausal women and
400 obese men. *J Clin Endocrinol Metab*. 2012;97(7):2475-2481.
- 401 22. **D'Agostino RB, Vasan RS, Pencina MJ, et al.** General cardiovascular risk profile for use
402 in primary care the Framingham Heart Study. *Circulation*. 2008;117(6):743-753.
- 403 23. **Levy JC, Matthews DR, Hermans MP.** Correct homeostasis model assessment (HOMA)
404 evaluation uses the computer program. *Diabetes Care*. 1998;21(12): 2191-2202.
- 405 24. **Al-Shayji IA, Caslake MJ, Gill JM.** Effects of moderate exercise on VLDL and Intralipid
406 kinetics in overweight/obese middle-aged men. *Am J Physiol Endocrinol Metab*.
407 2012;302(3):E349-355.
- 408 25. **Bellou E, Magkos F, Kouka T, et al.** Effect of high-intensity interval exercise on basal
409 triglyceride metabolism in non-obese men. *Appl Physiol Nutr Metab*. 2013;38(8):823-829.
- 410 26. **Kotronen A, Seppälä-Lindroos A, Bergholm R, Yki-Järvinen H.** Tissue specificity of
411 insulin resistance in humans: fat in the liver rather than muscle is associated with features of
412 the metabolic syndrome. *Diabetologia*. 2008;51(1):130-138.
- 413 27. **Bugianesi E, Gastaldelli A, Vanni E, et al.** Insulin resistance in non-diabetic patients with

- 414 non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia*. 2005;48(4):634-642.
- 415 28. **Pollare T, Vessby B, Lithell H.** Lipoprotein lipase activity in skeletal muscle is related to
416 insulin sensitivity. *Arterioscler Thromb*. 1991;11(5):1192-1203.
- 417 29. **Bergeron J, Couillard C, Després JP, et al.** Race differences in the response of postheparin
418 plasma lipoprotein lipase and hepatic lipase activities to endurance exercise training in men:
419 results from the HERITAGE Family Study. *Atherosclerosis*. 2001;159(2):399-406.
- 420 30. **Chan DC, Gan SK, Wong AT, Barrett PH, Watts GF.** Association between skeletal
421 muscle fat content and very-low-density lipoprotein-apolipoprotein B-100 transport in
422 obesity: effect of weight loss. *Diabetes Obes Metab*. 2014;16(10):994-1000.
- 423 31. **Mittendorfer B, Patterson BW, Klein S.** Effect of weight loss on VLDL-triglyceride and
424 apoB-100 kinetics in women with abdominal obesity. *Am J Physiol Endocrinol Metab*.
425 2003;284(3):E549-556.
- 426 32. **Kallwitz ER, Guzman G, TenCate V, et al.** The histologic spectrum of liver disease in
427 African-American, non-Hispanic white, and Hispanic obesity surgery patients. *Am J*
428 *Gastroenterol*. 2009;104(1):64-69.
- 429 33. **Zhang YL, Hernandez-Ono A, Ko C, Yasunaga K, Huang LS, Ginsberg HN.** Regulation
430 of hepatic apolipoprotein B-lipoprotein assembly and secretion by the availability of fatty
431 acids. I. Differential response to the delivery of fatty acids via albumin or remnant-like
432 emulsion particles. *J Biol Chem*. 2004;279(18):19362-19374.
- 433 34. **Gill JM, Al-Mamari A, Ferrell WR, et al.** Effects of a moderate exercise session on
434 postprandial lipoproteins, apolipoproteins and lipoprotein remnants in middle-aged men.
435 *Atherosclerosis*. 2006;185(1):87-96.
- 436 35. **Bjorkegren J, Packard CJ, Hamsten A, et al.** Accumulation of large very low density
437 lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride
438 emulsion reflects competition for a common lipolytic pathway. *J Lipid Res*. 1996;37(1): 76-
439 86.
- 440 36. **Targher G, Day CP, Bonora E.** Risk of cardiovascular disease in patients with nonalcoholic
441 fatty liver disease. *N Engl J Med*. 2010;363(14): 1341-1350
- 442 37. **Vlachopoulos C, Manesis E, Baou K, et al.** Increased arterial stiffness and impaired
443 endothelial function in nonalcoholic Fatty liver disease: a pilot study. *Am J Hypertens*. 2010;
444 23(11):1183-1189.
- 445 38. **Dattilo AM, Kris-Etherton PM.** Effects of weight reduction on blood lipids and
446 lipoproteins: a meta-analysis. *Am J Clin Nutr*. 1992;56(2):320-328.
- 447 39. **Musso G, Cassader M, Rosina F, Gambino R.** Impact of current treatments on liver
448 disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease
449 (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia*.
450 2012;55(4):885-904

451 40. **Hens W, Taeyman J, Cornelis J, Gielen J, Van Gaal L, Vissers D.** The Effect of Lifestyle
452 Interventions on Excess Ectopic Fat Deposition Measured by Noninvasive Techniques in
453 Overweight and Obese Adults: A Systematic Review and Meta-Analysis. *J Phys Act Health.*
454 2016;13(6):671-694
455
456
457

Table 1 Subject characteristics and biochemistry

	Pre Ex n=15	Post Ex n=15	Within group p value	Pre Control n=12	Post Control n=12	Within group p value	Between group p value
Age yr	52.4 ±2.2			52.8±3.0			NS
Body weight kg	101.3±2.64	97.3±12.2	< 0.001	102.3±6.1	102.9±6.4	NS	< 0.001
BMI kg/m ²	31.6±0.8	30.5±1.0	< 0.001	31.7±1.0	31.6±1.2	NS	0.02
Waist circumference cm	109.3±1.9	105.0±2.5	0.005	110.0±3.9	109.6±4.3	NS	0.03
VO _{2max} ml kg ⁻¹ min ⁻¹	25.5±1.1	33.0±1.5	< 0.001	23.3±1.0	23.8±1.3	NS	< 0.001
Fasting glucose mmol/l	6.0±0.2	5.8±0.2	0.005	5.9±0.2	5.6±0.1	0.02	NS
Fasting insulin pmol/l	183±17	138±16	0.007	164±17	170±17	NS	0.02
HOMA2 %S	32.5±2.9	45.6±4.9	0.002	36.1±3.7	34.5±3.2	NS	0.003
Adipose tissue-IR	79.8±8.0	58.2±8.8	0.03	75.9±9.4	86.6±15.7	NS	0.02
NEFA mmol/l	0.45±0.03	0.41±0.04	NS	0.48±0.05	0.50±0.05	NS	NS
Total Cholesterol mmol/l	5.0±0.2	4.7±0.2	NS	5.1±0.2	5.1±0.2	NS	NS
TG mmol/l	2.0±0.2	1.8±0.2	NS	1.6±0.2	1.9±0.2	NS	NS
LDL-Cholesterol mmol/l	3.8±0.1	3.3±0.2	0.03	3.6±0.2	3.2±0.2	0.07	NS
HDL-Cholesterol (mmol/l)	1.01±0.06	1.03±0.06	NS	1.09±0.09	1.09±0.08	NS	NS
Alanine transaminase U/l	51.1±5.3	36.8±5.2	0.01	40.9±6.2	31.1±4.7	0.04	NS
Aspartate transaminase U/l	36.9±3.2	29.4±3.5	0.02	29.0±2.5	26.3±1.84	NS	NS
Gamma glutamyl transaminase U/l	53.5±10.2	36.3±7.5	0.03	37.0±4.5	33.8±4.9	NS	NS

Table 2 Body composition and vascular measurements

	Pre Ex n=15	Post Ex n=15	Within group p value	Pre Control n=12	Post Control n=12	Withi n group p value	Betwee n group p value
IHCL %	19.6(14.8,30.0)	8.9(5.4,17.3)	<0.001	12.5(6.9,32.9)	12.6(9.2,26.1)	NS	0.02
IMCL (Sol)	21.7 ± 3.8	19.0 ± 3.5	0.09	19.2±1.8	20.5 ± 2.6	NS	0.07
IMCL (Tib)	8.8 ± 1.0	8.8 ± 1.4	NS	13.2±5.4	8.2 ± 1.2	NS	NS
Pancreatic fat	13.7 ± 5.0	8.8 ± 1.6	NS	8.2±3.3	10.9 ± 3.5	NS	NS
Total Internal fat kg	9.5 ± 0.6	7.9 ± 0.6	<0.001	9.9 ± 0.9	9.6 ± 0.9	NS	0.03
Visceral Fat kg	5.7 ± 0.4	4.7 ± 0.4	<0.001	5.7 ± 0.55	5.4 ± 0.6	NS	0.06
Abdominal Subcut Fat kg	7.0 ± 7.2	6.3 ± 0.7	<0.001	7.8 ± 1.0	7.91 ± 1.1	NS	0.003
Total body fat kg	31.6±2.0	27.3±1.9	<0.001	34.8 ± 3.2	34.5 ± 3.5	NS	0.004
Total subcut fat kg	22.1 ± 1.7	19.5 ± 1.6	<0.001	24.9 ± 2.4	24.9 ± 2.6	NS	0.001
Systolic BP mm Hg	133.4 ± 4.2	128.8 ± 4.2	0.01	131.3 ± 4.5	134.4 ± 3.8	NS	0.04
Diastolic BP mm Hg	83.4 ± 2.3	78.2 ± 2.6	0.04	83.8 ± 3.0	86.3 ± 3.0	NS	0.02
PWV (m/s)	7.94 ± 0.26	7.67 ± 0.25	0.05	7.58 ± 0.27	7.77 ± 0.28	NS	NS
Framingham Risk scores	14.4 ± 1.4	12.4 ± 1.4	0.001	14.2 ± 2.5	13.6 ± 1.9	NS	<0.05

Intrahepatocellular fat (IHCL) presented as median (interquartile range)

Sol, soleus; Tib, tibialis; visc, visceral; subcut, subcutaneous; PWV, pulse wave velocity

Table 3. VLDL TG kinetics (mean ± SEM)

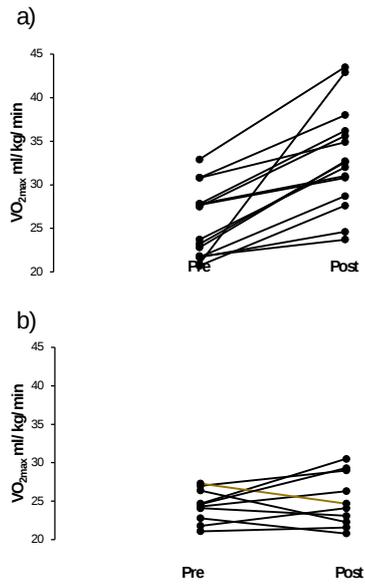
	Pre Ex n=15	Post Ex n=15	Within group P	Pre Control n=12	Post Control n=12	Within group P	Between group P
VLDL ₁ -TG mmol/l	1.24 ±0.15	1.04±0.11	NS	1.00±0.12	1.05±0.15	NS	NS
VLDL ₂ -TG mmol/l	0.17±0.02	0.11±0.01	< 0.01	0.13±0.02	0.14±0.02	NS	0.01
VLDL-TG mmol/l	1.41±0.17	1.15±0.11	< 0.01	1.13±0.15	1.19±0.16	NS	0.08
VLDL ₁ -Chol mmol/l	0.31 ±0.04	0.29±0.04	NS	0.29±0.04	0.32±0.05	NS	NS
VLDL ₂ -Chol mmol/l	0.10 ±0.07	0.07±0.01	< 0.02	0.09±0.03	0.09±0.02	NS	< 0.01
VLDL-Chol mmol/l	0.41 ±0.04	0.36±0.04	0.02	0.38±0.06	0.40±0.06	NS	0.01
VLDL ₁ -TG FCR pools/d*	8.25±1.07	9.80±1.51	< 0.05	9.09±0.80	8.62±1.02	NS	0.06
VLDL ₁ -TG catabolism FCR pools/day**	6.82±1.16	8.14±1.31	0.05	7.46±0.78	5.92±0.53	NS	0.02
VLDL ₁ -TG transfer FCR pools/day**	1.22±0.16	1.44±0.38	NS	1.63±0.48	2.71±1.35	NS	NS
VLDL ₁ -TG PR mg/kg/d*	230.9 ±20.3	232.5±12.9	NS	218.4±30.6	213.9±24.1	NS	NS
VLDL ₂ -TG FCR pools/d**	10.44 ±0.70	11.62±1.48	NS	12.05±1.52	13.16±3.18	NS	NS
VLDL ₂ -TG PR mg/kg/d**	40.7 ±4.6	33.9±5.1	NS	37.6±5.5	49.6±10.7	NS	NS
VLDL ₂ -TG hepatic PR mg/kg/d**	5.03±0.83	4.23±0.93	NS	6.7±1.3	8.3±3.1	NS	NS
VLDL-TG PR mg/kg/d**	235.6±20.2	236.4±13.0	NS	225.1±30.4	222.3±25.5	NS	NS
VLDL ₁ to VLDL ₂ - TG transfer mg/kg/d**	35.7 ± 4.7	29.7±4.6	NS	30.9±4.8	41.3±8.5	NS	NS

460 *n=13 and **n=12 in exercise group due to problems with sample analysis

Table 4. VLDL apoB kinetics (mean±SEM)

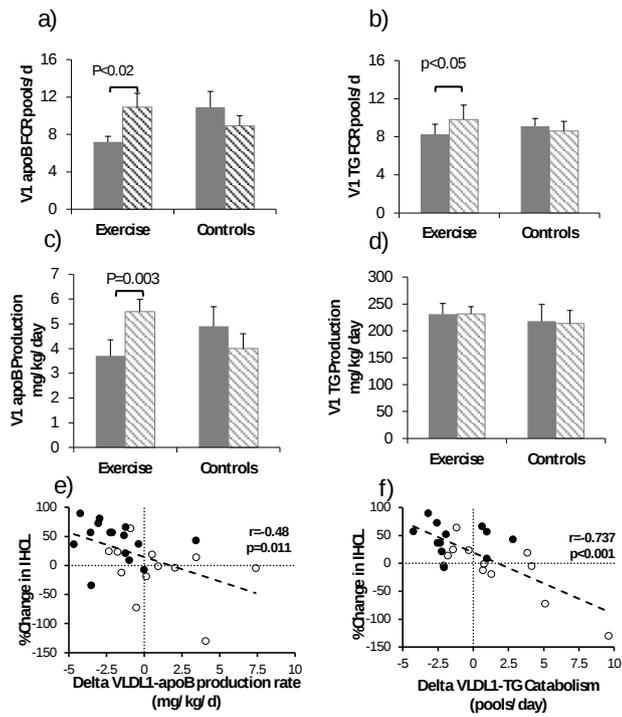
	Pre Ex n=15	Post Ex n=15	Within group p value	Pre Control n=12	Post Control n=12	Within group p value	Between- group p value
VLDL ₁ apoB concentration mg/l	18.4±2.2	20.2±2.8	NS	16.7±1.5	17.3±2.3	NS	NS
VLDL ₂ -apoB concentration mg/l	12.9±1.4	9.7±1.0	0.04	11.2±1.8	11.1±1.1	NS	NS
VLDL ₁ - TG/V ₁ apoB	66.2±7.0	49.8±4.0	0.03	56.5±7.2	60.12±8.7	NS	0.04
VLDL ₂ - TG/V ₂ apoB	12.9±1.5	11.6±1.1	NS	12.1±1.4	14.8±3.5	NS	NS
VLDL ₁ -apoB FCR pools/day	7.18±0.57	10.93±1.49	0.02	10.91±1.76	8.88±1.06	NS	0.01
VLDL ₁ -apoB catabolism FCR pools/day	5.98± 0.66	10.39±1.49	<0.01	9.87±1.86	7.89±1.23	NS	0.01
VLDL ₁ -apoB transfer FCR pools/day	1.19±0.16	0.54±0.1	0.005	1.04±0.25	0.99±0.3	NS	0.06
VLDL ₂ -apoB FCR pools/day	12.3±1.3	11.8±1.3	NS	16.9±3.0	12.9±1.8	NS	NS
VLDL ₁ -apoB PR mg/kg/d	3.67±0.65	5.54±0.49	0.003	4.92±0.80	3.96±0.60	NS	0.006
VLDL ₂ -apoB PR mg/kg/d	4.05±0.42	3.22±0.44	NS	4.93±1.00	3.98±0.60	NS	NS
VLDL ₂ -apoB hepatic PR mg/kg/d	0.52±0.09	0.50±0.10	NS	0.74±0.23	0.89±0.24	NS	NS
VLDL ₁ - to VLDL ₂ transfer mg/kg/d	3.52±0.04	2.72±0.01	NS	4.19±1.09	3.09±0.69	NS	0.013
VLDL apoB PR mg/kg/d	4.19±0.66	6.04±0.50	0.004	5.66±0.95	4.85±0.49	NS	0.02

Figure 1



462

Figure 2



463
464