Exercise training reduces liver fat and increases rates of VLDL clearance, but not VLDL production in NAFLD

Shojaee-Moradie F1, Cuthbertson DJ2, Barrett M1, Jackson NC1, Herring R4, Thomas EL3, Bell J3, Kemp GI2, Wright J4, Umpleby AM1

1Diabetes and Metabolic Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK; 2Metabolism and Nutrition Research Group, Institute of Ageing and Chronic Disease, University of Liverpool, UK; 3Section of Investigative Medicine, Endocrinology and Metabolism, Imperial College London, UK; Centre for Diabetes, Endocrinology and Research, Royal Surrey County Hospital, Guildford, UK

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

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

Design A 16 week randomised controlled trial.

Patients 27 sedentary patients with NAFLD.

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

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

Results: In the exercise group VO2max increased by 31 ± 6% (mean ± SEM) and IHCL decreased from 19.6% (14.8, 30.0) to 8.9% (5.4, 17.3) (median (IQR)) with no significant change in VO2max or IHCL in the control group (change between groups p = 0.001 and p = 0.02, respectively). Exercise training increased VLDL1-TG and apoB fractional catabolic rates, a measure of clearance, (change between groups p = 0.02 and p = 0.01, respectively), and VLDL1-apoB production rate (change between groups p = 0.006), with no change in VLDL1-TG production rate. Plasma TG did not change in either group.

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

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

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

VLDL secreted by the liver can be separated into large TG-rich VLDL1 and smaller denser VLDL2 which has a lower TG content. There is evidence that these two VLDL species are independently regulated (5). VLDL is initially assembled as a primordial particle (pre-VLDL) when apolipoprotein B100 (apoB) is cotranslationally lipidated in the endoplasmic reticulum by microsomal transfer protein (MTP). Pre-VLDL can either be retained and degraded, or further lipidated to form VLDL2. This particle can then either be secreted or converted to VLDL1 following the addition of more TG in the liver. The hydrolysis of VLDL1-TG by lipoprotein lipase (LPL) also generates VLDL2 in the circulation. Thus V LDL2 has two sources. VLDL1-TG by lipoprotein lipase (LPL) also generates TG-rich VLDL1 and smaller denser VLDL2 which has a lower TG content. There is evidence that these two VLDL sources.

Table 1. Subject characteristics and biochemistry

| Age yr | 52.4 ± 2.2 | 97.3 ± 12.2 | <0.001 | 52.8 ± 3.0 | NS | 21 U | <0.001 |
| Body weight kg | 101.3 ± 2.64 | 36.5 ± 1.0 | <0.001 | 31.7 ± 1.0 | NS | 10.0 ± 2.5 | <0.001 |
| BMI kg/m² | 31.6 ± 0.8 | 30.5 ± 1.0 | <0.001 | 31.6 ± 1.0 | NS | 10.0 ± 2.5 | <0.001 |
| Waist circumference cm | 109.3 ± 19 | 105.0 ± 2.5 | 0.005 | 110.0 ± 3.9 | NS | 109.6 ± 4.3 | NS |
| VO₂max ml kg⁻¹ min⁻¹ | 25.5 ± 1.1 | 33.0 ± 1.5 | <0.001 | 23.3 ± 1.0 | <0.001 | 23.8 ± 1.3 | NS |
| Fasting glucose mmol/l | 6.0 ± 0.2 | 5.9 ± 0.2 | <0.001 | 6.4 ± 1.7 | 0.02 | 170.0 ± 17 | NS |
| Fasting insulin pmol/l | 32.5 ± 2.9 | 361.0 ± 17 | 0.007 | 164.0 ± 17 | <0.001 | 170.0 ± 17 | NS |
| HOMA2% | 46.5 ± 4.9 | 361.0 ± 17 | 0.007 | 164.0 ± 17 | <0.001 | 170.0 ± 17 | NS |
| Adipose tissue-R | 79.8 ± 8.0 | 58.2 ± 8.8 | 0.03 | 75.9 ± 9.4 | 0.02 | 86.6 ± 15.7 | NS |
| NEFA mmol/l | 0.45 ± 0.03 | 0.41 ± 0.04 | NS | 0.48 ± 0.05 | NS | 0.50 ± 0.05 | NS |
| Total cholesterol mmol/l | 5.0 ± 0.2 | 4.7 ± 0.2 | NS | 5.1 ± 0.2 | NS | 5.1 ± 0.2 | NS |
| TG mmol/l | 2.0 ± 0.2 | 1.8 ± 0.2 | NS | 1.6 ± 0.2 | NS | 1.9 ± 0.2 | NS |
| LDL-Cholesterol mmol/l | 3.8 ± 0.1 | 3.3 ± 0.2 | 0.03 | 3.6 ± 0.2 | 0.07 | 3.2 ± 0.2 | NS |
| HDL-Cholesterol mmol/l | 1.01 ± 0.06 | 1.03 ± 0.06 | NS | 1.09 ± 0.09 | NS | 1.09 ± 0.09 | NS |
| Alanine transaminase U/l | 51.3 ± 5.3 | 36.8 ± 5.2 | 0.01 | 40.9 ± 6.2 | 0.04 | 31.1 ± 4.7 | NS |
| Aspartate transaminase U/l | 36.9 ± 3.2 | 29.4 ± 3.5 | 0.02 | 29.0 ± 2.5 | NS | 26.3 ± 1.84 | NS |
| γ-glutamyl transaminase U/l | 53.5 ± 10.2 | 36.3 ± 7.5 | 0.02 | 37.0 ± 4.5 | NS | 33.8 ± 4.9 | NS |

Materials and Methods

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

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

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

**Study measurements.** Prior to and following the 16 week intervention period measurements of physical fitness (VO₂max) were made and a 7 day diet diary was completed. On a separate visit, measurements of fasting VLDL₁ and VLDL₂-apoB and TG kinetics and arterial stiffness (by pulse wave velocity) were made. Body composition (total, subcutaneous and visceral fat volumes) was measured by magnetic resonance imaging (MRI) and IHCL, intramyocellular (IMCL) and pancreatic lipid content was measured by magnetic resonance spectroscopy (¹³H-MRS).

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

**Measurement of VO₂max.** VO₂max was performed within four days of the metabolic study using an electronically braked bicycle ergometer (Lode; Excalibur Sport, Groningen, the Netherlands) equipped with a computerised breath (oxygen [O₂]/carbon dioxide [CO₂]) analyzer system (Medical Graphics, St Paul, MN, USA). An electrocardiogram (ECG) was undertaken during the exercise test to monitor participants’ heart rate and exclude latent ischemic heart disease.

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

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

**Measurement of body composition and intracellular fat.** Subjects fasted for 6 hours before the scans. Whole body MR imaging for body fat content and ¹³H MRS measurements of pancreatic fat, IHCL and IMCL (tibialis anterior and soleus muscle) was measured on an Intera 1.5T Achieva multinuclear system (Philips Medical Systems, Best, Holland) as previously reported (17, 18). NAFLD was defined as mean IHCL > 5.5%. For more details see the Supplementary Material.

**Metabolic Study Protocol.** Participants attended the CEDAR center, Royal Surrey County Hospital, Guildford on two occasions before (0 week) and after the intervention (16 weeks). The participants were asked to refrain from vigorous exercise for 72h before the study, abstain from drinking alcoholic beverages for 24h, and attend after an overnight fast. A primed (1 mg/kg) intravenous (IV) infusion of ¹³C-leucine (1 mg/kg/h) and a bolus of ²H₅ glycerol (75 µmol/kg) were administered. Blood samples were taken at regular time intervals for 9 hours.

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

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

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

Ten-year cardiovascular risk was calculated using the 10 year Framingham Risk Score (FRS) (22). Homeostatic Model Assessment (HOMA2) was used to assess whole body insulin sensitivity (HOMA2-%S) (23). Adipose tissue insulin resistance (Adipo-IR) was calculated by multiplying fasting plasma NEFA concentration with fasting serum insulin concentration.

Percent Change in IHCL was calculated as Pre-Post intervention/Pre x100. Changes in other measurements were calculated as Pre-Post intervention.

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

**Baseline characteristics**

Body weight, BMI and baseline biochemical characteristics (plasma lipid profile and liver enzyme concentrations) were not significantly different at 0 weeks between groups (Table 1). Similarly, there were no significant baseline differences in cardiorespiratory fitness (VO_{2max}), IHCL, pancreatic fat or fat distribution (Table 2). IHCL in all participants (n = 27) at 0 week correlated positively with fasting plasma TG concentration (r = 0.439, P = .02) and abdominal visceral fat (r = 0.411, P = .03).

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

**Body weight, BMI and fitness**

Body weight and BMI decreased by 3.6 ± 0.8% and 3.8 ± 0.9% respectively after 16 weeks exercise training with no change in controls (change exercise vs. change control, P < .001 and, P = .02) (Table 1). In both groups, total energy intake and macronutrient composition remained unchanged after 16 weeks compared with baseline (Supplemental Table 1).

In the exercise group VO_{2max} increased significantly by 31 ± 6% after 16 weeks with no change in controls (change exercise vs. group control, P < .001) (Figure 1, Table 1).

**Liver enzymes (Table 1)**

After 16 weeks intervention there were within group decreases in the exercise group in ALT, AST and GGT concentrations (both P < .01) (between groups for insulin, P = .02). HOMA2-%S (a measure of insulin sensitivity) increased by 42.5 ± 11.6% in the exercise group (P = .002) with no change in controls (between group, P = .003).

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

After 16 weeks, there was a significant decrease in IHCL content (% decrease 52.2% (29.0, 61.8); median (IQR)) in the exercise group, with no change in controls (change exercise vs. change control P = .02). There was no significant change in pancreatic fat. All measured adipose tissue depots also significantly decreased with no change in controls (Table 2). The percentage change in IHCL between 0 and 16 weeks in all patients (exercise and control group) correlated negatively with the change in VO_{2max} (r = -0.45, P < .02) and correlated positively with the change in total body fat and visceral fat (r = 0.54, P = .004; r = 0.41, P = .03).

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

After 16 weeks exercise there was a within-group decrease in fasting plasma glucose and serum insulin concentrations in the exercise group (both P < .01) (between groups for insulin, P = .02). HOMA2-%S (a measure of insulin sensitivity) increased by 42.5 ± 11.6% in the exercise group (P = .002) with no change in controls (between group, P = .003).

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

Both systolic and diastolic blood pressure (BP) measurements decreased by 5.4 ± 1.8% and 6.2 ± 2.7% in the exercise group after 16 weeks exercise (P = .01, P = .04) (between groups P = .04, P = .02 respectively). After 16 weeks, pulse wave velocity, a measure of atrial elasticity, improved in the exercise group (P = .05) although between groups this was not significant. The Framingham risk score decreased 14 ± 4% (P = .001) after exercise with no change in controls (between groups, P < .05). The percentage change in IHCL between 0 and 16 weeks in all patients correlated positively with the change in the FRS (r = 0.62, P = .001).

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

At baseline, there were no differences in plasma or lipoprotein fraction lipids between groups. Total chole-
terol, TG and HDL cholesterol concentrations did not change from baseline in either group at 16 weeks. After 16 weeks exercise there was a significant within-group decrease (P = .03) in plasma LDL cholesterol concentration.

NEFA concentration did not change in either group. However adipose tissue IR decreased by 24 ± 10% in the exercise group (P = .03) with no change in controls (change between groups, P = .02). After 16 weeks there was no significant change in VLDL1-TG or VLDL1 apoB concentration in either group. However VLDL2 TG, cholesterol and apoB concentration were reduced (P < .01, P < .02, P = .04) in the exercise group with no change in controls. The particle size of VLDL1 (TG/apoB) was reduced in the exercise group (P = .03), and was different between groups (P = .04).

**Table 3.** VLDL TG kinetics (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Pre Ex n = 15</th>
<th>Post Ex n = 15</th>
<th>Within group P</th>
<th>Pre Control n = 12</th>
<th>Post Control n = 12</th>
<th>Within group P</th>
<th>Between group P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL1-TG mmol/l</td>
<td>1.24 ± 0.15</td>
<td>1.04 ± 0.11</td>
<td>NS</td>
<td>1.00 ± 0.12</td>
<td>1.05 ± 0.15</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL2-TG mmol/l</td>
<td>0.17 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>&lt;0.01</td>
<td>0.13 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>VLDL-TG mmol/l</td>
<td>1.41 ± 0.17</td>
<td>1.15 ± 0.11</td>
<td>&lt;0.01</td>
<td>1.13 ± 0.15</td>
<td>1.19 ± 0.16</td>
<td>NS</td>
<td>0.08</td>
</tr>
<tr>
<td>VLDL1-Chol mmol/l</td>
<td>0.31 ± 0.04</td>
<td>0.29 ± 0.04</td>
<td>NS</td>
<td>0.29 ± 0.04</td>
<td>0.32 ± 0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL-Chol mmol/l</td>
<td>0.10 ± 0.07</td>
<td>0.07 ± 0.01</td>
<td>&lt;0.02</td>
<td>0.09 ± 0.03</td>
<td>0.09 ± 0.02</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>VLDL-Chol mmol/l</td>
<td>0.41 ± 0.04</td>
<td>0.36 ± 0.04</td>
<td>0.02</td>
<td>0.38 ± 0.06</td>
<td>0.40 ± 0.06</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>VLDL1-TG FCR pools/d*</td>
<td>8.25 ± 1.07</td>
<td>9.80 ± 1.51</td>
<td>&lt;0.05</td>
<td>9.09 ± 0.80</td>
<td>8.62 ± 1.02</td>
<td>NS</td>
<td>0.06</td>
</tr>
<tr>
<td>VLDL1-TG catabolism FCR pools/d**</td>
<td>6.82 ± 1.16</td>
<td>8.14 ± 1.31</td>
<td>0.05</td>
<td>7.46 ± 0.78</td>
<td>5.92 ± 0.53</td>
<td>NS</td>
<td>0.02</td>
</tr>
<tr>
<td>VLDL2-TG transfer FCR pools/d**</td>
<td>1.22 ± 0.16</td>
<td>1.44 ± 0.38</td>
<td>NS</td>
<td>1.63 ± 0.48</td>
<td>2.71 ± 1.35</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL1-TG PR mg/kg/d**</td>
<td>230.9 ± 20.3</td>
<td>232.5 ± 12.9</td>
<td>NS</td>
<td>218.4 ± 30.6</td>
<td>213.9 ± 24.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL2-TG PR pools/d**</td>
<td>10.44 ± 0.70</td>
<td>11.62 ± 1.48</td>
<td>NS</td>
<td>12.05 ± 1.52</td>
<td>13.16 ± 3.18</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL1-TG PR mg/kg/d**</td>
<td>40.7 ± 4.6</td>
<td>33.9 ± 5.1</td>
<td>NS</td>
<td>37.6 ± 6.5</td>
<td>49.6 ± 10.7</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL2-TG PR mg/kg/d**</td>
<td>5.03 ± 0.83</td>
<td>4.23 ± 0.93</td>
<td>NS</td>
<td>6.7 ± 1.3</td>
<td>8.3 ± 3.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL1-TG PR mg/kg/d**</td>
<td>235.6 ± 20.2</td>
<td>236.4 ± 13.0</td>
<td>NS</td>
<td>225.1 ± 30.4</td>
<td>222.3 ± 25.5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL1-TG transfer mg/kg/d**</td>
<td>35.7 ± 4.7</td>
<td>29.7 ± 4.6</td>
<td>NS</td>
<td>30.9 ± 4.8</td>
<td>41.3 ± 8.5</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

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

**Table 4.** VLDL apoB kinetics (mean±SEM)

<table>
<thead>
<tr>
<th></th>
<th>Pre Ex n = 15</th>
<th>Post Ex n = 15</th>
<th>Within group p value</th>
<th>Pre Control n = 12</th>
<th>Post Control n = 12</th>
<th>Within group p value</th>
<th>Between-group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL1-apoB mg/l</td>
<td>18.4 ± 2.2</td>
<td>20.2 ± 2.8</td>
<td>NS</td>
<td>16.7 ± 1.5</td>
<td>17.3 ± 2.3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL1-apoB mg/l</td>
<td>12.9 ± 1.4</td>
<td>9.7 ± 1.0</td>
<td>0.04</td>
<td>11.2 ± 1.8</td>
<td>11.1 ± 1.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL1-apoB mg/l</td>
<td>66.2 ± 7.0</td>
<td>49.8 ± 4.0</td>
<td>0.03</td>
<td>56.5 ± 7.2</td>
<td>60.12 ± 8.7</td>
<td>NS</td>
<td>0.04</td>
</tr>
<tr>
<td>VLDL1-apoB mg/l</td>
<td>12.9 ± 1.5</td>
<td>11.6 ± 1.1</td>
<td>NS</td>
<td>12.1 ± 1.4</td>
<td>14.8 ± 3.5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL1-apoB FCR pools/day</td>
<td>7.18 ± 0.57</td>
<td>10.93 ± 1.49</td>
<td>0.02</td>
<td>10.91 ± 1.76</td>
<td>8.88 ± 1.06</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>VLDL1-apoB FCR pools/day</td>
<td>9.58 ± 0.66</td>
<td>10.39 ± 1.49</td>
<td>&lt;0.01</td>
<td>9.87 ± 1.86</td>
<td>7.89 ± 1.23</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>VLDL1-apoB FCR pools/day</td>
<td>1.19 ± 0.16</td>
<td>0.54 ± 0.1</td>
<td>0.005</td>
<td>1.04 ± 0.25</td>
<td>0.99 ± 0.3</td>
<td>NS</td>
<td>0.06</td>
</tr>
<tr>
<td>VLDL1-apoB FCR pools/day</td>
<td>12.3 ± 1.3</td>
<td>11.8 ± 1.3</td>
<td>NS</td>
<td>16.9 ± 3.0</td>
<td>12.9 ± 1.8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL1-apoB mg/kg/d</td>
<td>3.67 ± 0.65</td>
<td>5.54 ± 0.49</td>
<td>0.003</td>
<td>4.92 ± 0.80</td>
<td>3.96 ± 0.60</td>
<td>NS</td>
<td>0.006</td>
</tr>
<tr>
<td>VLDL1-apoB mg/kg/d</td>
<td>4.05 ± 0.42</td>
<td>3.22 ± 0.44</td>
<td>NS</td>
<td>4.93 ± 1.00</td>
<td>3.98 ± 0.60</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL1-apoB mg/kg/d</td>
<td>0.52 ± 0.09</td>
<td>0.50 ± 0.10</td>
<td>NS</td>
<td>0.74 ± 0.23</td>
<td>0.89 ± 0.24</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL1-apoB mg/kg/d</td>
<td>3.52 ± 0.04</td>
<td>2.72 ± 0.01</td>
<td>NS</td>
<td>4.19 ± 1.09</td>
<td>3.09 ± 0.69</td>
<td>NS</td>
<td>0.013</td>
</tr>
<tr>
<td>VLDL1-apoB mg/kg/d</td>
<td>4.19 ± 0.66</td>
<td>6.04 ± 0.50</td>
<td>0.004</td>
<td>5.66 ± 0.95</td>
<td>4.85 ± 0.49</td>
<td>NS</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The figure shows the difference in VO2max at 0 and 16 weeks in a) Exercise group and b) Control group.
VLDL\textsubscript{1} and VLDL\textsubscript{2} ApoB kinetics (Table 4, Figure 2)

VLDL\textsubscript{1} and VLDL\textsubscript{2}-apoB kinetics were not different at baseline between groups. VLDL\textsubscript{1}-apoB FCR was increased at 16 weeks in the exercise group ($P = .02$) with no change in controls (between groups, $P = .01$). This was due to an increase in the catabolism FCR ($P < .01$) while the transfer FCR (to VLDL\textsubscript{2}) was decreased ($P = .005$). There was no change in VLDL\textsubscript{2}-apoB FCR. VLDL\textsubscript{1}-apoB PR and...
Discussion

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

It is well documented that VLDL1-TG and apoB FCR increases with acute exercise (24) but this effect is not sustained 48h after exercise (25). In the current study subjects abstained from exercise for 72h prior to the measurement of VLDL kinetics in order to measure the chronic, rather than the acute, effects of exercise. NAFLD is highly associated with peripheral and hepatic insulin resistance (26, 27), as observed in our participants who had a fasting insulin concentration double that reported in healthy subjects. There was an improvement in insulin sensitivity, as measured by HOMA %S, with exercise training, as has also been demonstrated previously in type 2 diabetes mellitus and overweight subjects (20, 15). We have also shown in a different subset of patients with NAFLD that 4 months of exercise training (with a similar-sized effect on fitness and IHCL to the current study) improved peripheral but not hepatic insulin sensitivity (16). In the current study an improvement in peripheral insulin sensitivity was also demonstrated with the decrease in adipose-IR. LPL activity is regulated by insulin (28) and 20-weeks endurance exercise training in healthy men, which increased VO2max by 13%, has previously been shown to significantly increase postheparin plasma lipoprotein lipase (29). Increased LPL activity would provide a mechanism for the increase in VLDL1-TG and apoB FCR observed in the current study. Notably for VLDL1-TG and apoB FCR it was the catabolic pathway that was increased rather than the transfer of TG to VLDL2. The increased clearance of TG from the systemic circulation, while the production rate of TG was simultaneously maintained, would enable the liver to export some of the stored TG for hydrolysis in skeletal muscle to sustain increased demand for fatty acids during exercise.

The reduction in body weight in the exercise group is unlikely to have mediated the increase in VLDL1-TG and apoB clearance since previous studies have shown weight loss in obese men, following a low calorie diet, reduces VLDL-apoB production rate with no effect on VLDL-apoB FCR (30). Similarly in obese women, a hypocaloric diet has been shown to have no effect on either VLDL-TG or VLDL-apoB FCR (31).

The failure of exercise training to lower VLDL1-apoB and TG production rate and to increase VLDL1-apoB production rate was unexpected. This differs from a study in patients with type 2 diabetes where exercise training for 6 months, resulting in a 16% increase in VO2max, reduced VLDL-apoB production rate (20). Liver fat was not measured in the latter study, patients were on oral hypoglycemic treatment (metformin and sulfonylureas) and some of the participants were African-Caribbean, a group known to have a lower propensity for NAFLD (32). The increase in VLDL1-apoB production rate following exercise training in the current study may be explained by the marked decrease in fasting insulin concentration in response to the improved peripheral insulin sensitivity, while at the same time hepatic insulin resistance was maintained. Insulin regulates VLDL assembly (6, 7, 8), thus a lowering of insulin will increase apoB secretion. It has also been shown in mice that triglycerides can rescue apoB from posttranslational degradation (33). The up-regulation of VLDL1-apoB production rate in response to exercise training could increase TG export and therefore assist in the reduction of liver fat. This could also explain the maintenance of plasma TG levels despite a decrease in liver fat.

The findings of this study differ from a previous study of patients with NAFLD where 16 weeks exercise training at an exercise intensity comparable to the current study had no effect on VLDL-TG and apoB kinetics (14). The discordant findings most likely reflect a greater improvement in both cardiorespiratory fitness and thus a greater reduction in IHCL in our study participants. VO2max increased by 31% in the current study compared to only a 9% increase in the previous study (14). An alternative or additional explanation is that total VLDL-TG and apoB (20–400) were measured in the previous study, rather than VLDL1 and VLDL2 as in the current study. There is evidence that VLDL1 and VLDL2 are independently regulated (5) and that exercise primarily affects VLDL1 kinetics (34), and so the effect of exercise on VLDL1 may not be revealed by measurements on total VLDL. VLDL1 carries more TG compared to VLDL2 per particle and LPL has been shown to have a preference for TG-rich particles (35).

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

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

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

Acknowledgments

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

Address all correspondence and requests for reprints to: Margaret Umpleby, Diabetes and Metabolic Medicine, The Leggett Building, University of Surrey, Daphne Jackson Rd, Manor Park, Guildford GU2 7WG, UK., Phone: 01483 688579 Fax: 01483 688501 E mail: m.umpleby@surrey.ac.uk

This work was supported by the European Foundation for the Study of Diabetes, Rheindorfer Weg 3, 40 591 Dusseldorf, Germany.

Clinical Trial registry number NCT01834300

Disclosure statement: The authors have nothing to disclose.

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

References


17. Thomas EL, Hamilton G, Patel N, et al. Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and