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

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Abstract

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 $VLDL_1$ and smaller denser $VLDL_2$ 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 ($VLDL_1$) and $VLDL_2$-TG and apolipoproteinB (apoB) kinetics investigated using stable isotopes before and after the intervention.

Results In the exercise group VO_{2max} 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 VO_{2max} or IHCL in the control group (change between groups $p<0.001$ and $p=0.02$, respectively). Exercise training increased $VLDL_1$-TG and apoB fractional catabolic rates, a measure of clearance, (change between groups $p=0.02$ and $p=0.01$, respectively), and $VLDL_1$-apoB production rate (change between groups $p=0.006$), with no change in $VLDL_1$-TG production rate. Plasma TG did not change in either group.

Conclusion An increased clearance of $VLDL_1$ 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.

Introduction

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 synthesised from fatty acids generated from three main sources: hepatic de novo lipogenesis; circulating non-esterified fatty acids (NEFA), originating from adipose tissue; and fatty acids derived from the remnants of the TG rich lipoproteins, VLDL and chylomicrons (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 $VLDL_1$ and smaller denser $VLDL_2$ 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 co-translationally lipidated in the endoplasmic reticulum by microsomal transfer protein (MTP). Pre-VLDL can either be retained and degraded, or further lipidated to form $VLDL_2$. This particle can then either be secreted or converted to $VLDL_1$ following the addition of more TG in the liver. The hydrolysis of $VLDL_1$-TG by lipoprotein lipase (LPL) also generates $VLDL_2$ in the circulation. Thus $VLDL_2$ has two sources. Insulin regulates VLDL assembly by decreasing apoB mRNA translation (6), inhibiting the expression of MTP (7) and promoting apoB degradation via autophagy (8). In NAFLD, with intrahepatic lipids in copious supply, increases in both VLDL-apoB and VLDL-TG production rate (PR) contribute to the atherogenic lipid profile (9).

Lifestyle intervention is the first line of treatment for NAFLD. Weight loss (5-10%) through diet, with or without exercise, has been shown to reduce hepatic steatosis (10,11). A number of randomised controlled trials have also shown that regular exercise, even without calorie restriction, reduces liver steatosis (12,13). A previous study has shown that 16 weeks supervised exercise training in men and women with NAFLD did not affect total VLDL kinetics (14). In the current study we examined the effect of 16 weeks supervised exercise training in men with NAFLD on $VLDL_1$ and $VLDL_2$ kinetics, using a protocol which we have shown previously to very effective at increasing fitness (15).

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 centre, 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 non-alcoholic 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, ischaemic heart disease or had any contraindications to exercise, clinical hyperlipidaemia (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 units), had MRI 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 randomised to one of two groups using a list generated by computer randomisation, (Statistical Analysis System v 9.1, PROC PLAN software). One group received a structured supervised exercise programme 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 (VO_{2max}) were made and a 7 day diet diary was completed. On a separate visit, measurements of fasting $VLDL_1$ and $VLDL_2$-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 (^{1}H-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 programme 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 VO_{2max} VO_{2max} 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 [O_2]/carbon dioxide [CO_2]) analyser system (Medical Graphics, St Paul, MN, USA). An electrocardiogram (ECG) was undertaken during the exercise test to monitor participants' heart rate and exclude latent ischaemic 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 analysed by Dietplan 6 (Release 6.60b4 with Windows VistaService 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 ^{1}H 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 centre, 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 (1mg/kg) intravenous infusion of 1-^{13}C-leucine (1 mg/kg/h) and a bolus of $^{2}H_5$ glycerol (75μmol/kg) were administered. Blood samples were taken at regular time intervals for 9 hours.

Laboratory protocols $VLDL_1$ (Svedberg flotation rate 60–400) and $VLDL_2$ (Svedberg flotation rate 20–60) fractions were isolated from plasma by sequential ultracentrifugation (19). ApoB and TG were isolated from $VLDL_1$ and $VLDL_2$ hydrolysed, 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 $VLDL_1$ and $VLDL_2$ particles were used to determine $VLDL_1$ and $VLDL_2$-TG fractional catabolic rate (FCR) using the modelling software SAAM II (SAAM Institute, Seattle, WA) as previously described (23). The model was also used to determine the kinetic parameters of $VLDL_1$ and $VLDL_2$ apoB using plasma αKIC enrichment and 1-^{13}C leucine enrichment of $VLDL_1$ and $VLDL_2$-apoB. $VLDL_1$-TG and apoB FCR had two components, $VLDL_1$ FCR transfer (to $VLDL_2$) and $VLDL_1$ FCR catabolism (direct removal from circulation). Production rate (PR) was calculated as the product of $VLDL_1$ and $VLDL_2$-FCR and their respective pool sizes. $VLDL_1$ and $VLDL_2$-TG and apoB pool sizes were calculated from $VLDL_1$ and $VLDL_2$-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 $VLDL_1$ and $VLDL_2$-TG and apoB pool sizes respectively. Particle sizes of $VLDL_1$ and $VLDL_2$ were calculated by dividing TG pool size by apoB pool size. Total VLDL-TG PR was calculated by summation of $VLDL_1$-TG PR and $VLDL_2$-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=0.02) and abdominal visceral fat (r=0.411, p=0.03).

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<0.001 and, p=0.02) (Table 1). In both groups, total energy intake and macronutrient composition remained unchanged after 16 weeks compared with baseline (Supplementary Table 1).

In the exercise group VO_{2max} increased significantly by 31±6% after 16 weeks with no change in controls (change in exercise group *vs.* control, p<0.001) (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 (p<0.01, p<0.02 and p<0.03, respectively). ALT also decreased in controls (p<0.04) with no change in either AST or GGT.

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=0.02). 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

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±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±1.8% and 6.2±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 atrial elasticity, improved in the
214 exercise group (p=0.05) although between groups this was not significant. The Framingham risk score
215 decreased 14±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±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 $VLDL_2$ 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 $VLDL_1$ (TG/apoB) was reduced in the exercise group (p=0.03), and was different between groups
228 (p=0.04).

$VLDL_1$- and $VLDL_{2-}$ TG kinetics (Table 3, Figure 2)

At baseline $VLDL_1$ and $VLDL_2$-TG kinetics did not differ between groups. After 16 weeks $VLDL_1$-TG FCR was increased in the exercise group ($p<0.05$) due to an increase in the $VLDL_1$-TG catabolism FCR ($p=0.05$). The percent change in IHCL in all participants was negatively correlated with the change in $VLDL_1$-TG catabolism FCR ($r=-0.74$, $p<0.001$) (Fig 2) and positively correlated with the change in $VLDL_1$-TG transfer FCR ($r=0.63$, $p=0.001$). There was no change in $VLDL_2$-TG FCR, $VLDL_1$-TG PR, $VLDL_2$-TG PR and total VLDL-TG PR within or between groups.

$VLDL_{1-}$ and $VLDL_2$ ApoB kinetics (Table 4, Figure 2)

$VLDL_{1-}$ and $VLDL_2$-apoB kinetics were not different at baseline between groups. $VLDL_1$-apoB FCR was increased at 16 weeks in the exercise group ($p=0.02$) with no change in controls (between groups, $p=0.01$). This was due to a increase in the catabolism FCR ($p<0.01$) while the transfer FCR (to VLDL2) was decreased ($p=0.005$). There was no change in $VLDL_2$-apoB FCR. $VLDL_1$-apoB PR and total VLDL-apoB PR increased in the exercise group ($p= 0.003$, $p=0.004$) (between groups $p=0.006$, $p=0.02$). The percent change in IHCL between 0 and 16 weeks in all participants correlated negatively with the change in $VLDL_1$-apoB PR ($r=-0.48$, $p=0.01$) (Fig 2).

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 $VLDL_1$-TG and apoB.

It is well documented that $VLDL_1$-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 VO_{2max} by 13%, has previously been shown to significantly increase post-heparin plasma lipoprotein lipase (29). Increased LPL activity would provide a mechanism for the increase in $VLDL_1$-TG and apoB FCR observed in the current study. Notably for $VLDL_1$-TG and apoB FCR it was the catabolic pathway that was increased rather than the transfer of TG to $VLDL_2$. 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 $VLDL_1$-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 $VLDL_1$-apoB and TG production rate and to increase $VLDL_1$-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 VO_{2max}, reduced VLDL-apoB production rate (20). Liver fat was not measured in the latter study, patients were on oral hypoglycemic treatment (metformin and sulphonylureas) and some of the participants were African-Caribbean, a group known to have a lower propensity for NAFLD (32). The increase in $VLDL_1$-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 $VLDL_1$-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. VO_{2max} 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 (sf 20-400) were measured in the previous study, rather than $VLDL_1$ and $VLDL_2$ as in the current study. There is evidence that $VLDL_1$ and $VLDL_2$ are independently regulated (5) and that exercise primarily affects $VLDL_1$ kinetics (34), and so the effect of exercise on $VLDL_1$ may not be revealed by measurements on total VLDL. $VLDL_1$ carries more TG compared to $VLDL_2$ 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 non-significant 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 non-diabetic 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 normalise 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.

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Contribution statement

AMU, DJC, FSM and GJK designed the study, FSM. JW and RH performed the clinical studies. MB supervised the exercise intervention. FSM 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.

Figure legends

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

Figure 2. a) $VLDL_1$ (V1) apoB FCR, b) VLDL1-TG FCR c) $VLDL_1$-apoB production rate and d) VLDL1-TG production rate, at 0 weeks (solid bar) and 16 weeks (hatched bar) in the exercise and control groups, e) relationship between percent change in IHCL and the change in $VLDL_1$-apoB production rate and f) relationship between percent change in IHCL and the change in $VLDL_1$-TG catabolism FCR. Black circles: exercise group; open circles: control group.

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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

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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	Within group p value	Between 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

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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

*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_1apoB	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_2apoB	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**

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Figure 1

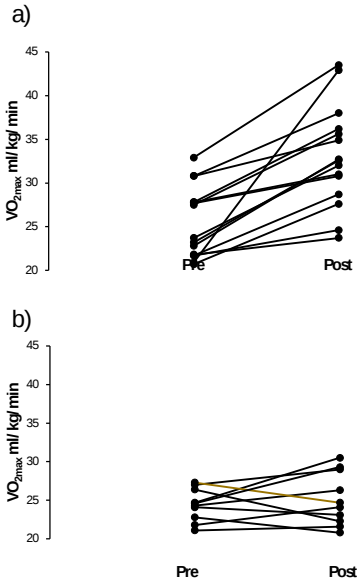

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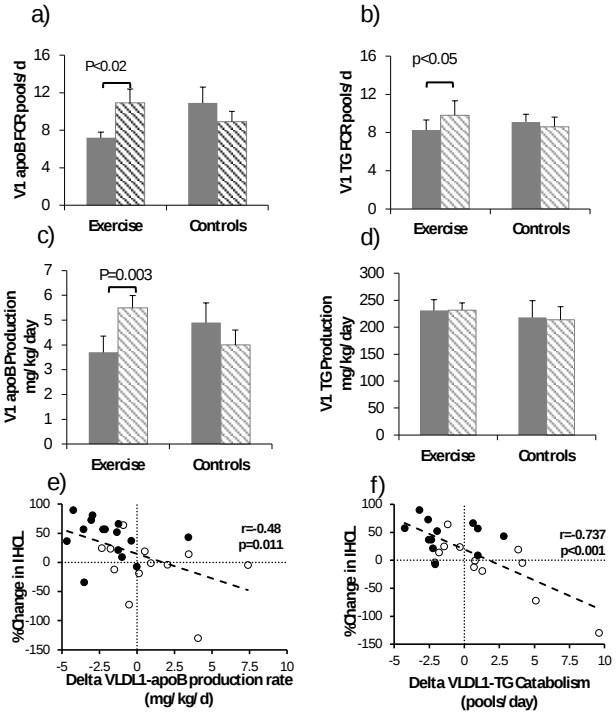
Figure 2



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