Exercise Training Reduces Liver Fat and Increases Rates of VLDL Clearance But Not VLDL Production in NAFLD

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Context: Randomized controlled trials in nonalcoholic fatty liver disease (NAFLD) have shown that regular exercise, even without calorie restriction, reduces liver steatosis. A previous study has shown that 16 weeks of supervised exercise training in NAFLD did not affect total very low-density lipoprotein (VLDL) kinetics.

Objective: The objective of the study was 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: This was a 16-week randomized controlled trial.

Patients: A total of 27 sedentary patients with NAFLD participated in the trial.

Intervention: The intervention was composed of supervised exercise with moderate-intensity aerobic exercise or conventional lifestyle advice (control).

Main Outcome: $VLDL_1$ and $VLDL_2$-TG and apolipoprotein B (apoB) kinetics were investigated using stable isotopes before and after the intervention.

Results: In the exercise group, maximal oxygen uptake increased by 31% ± 6% (mean ± SEM) and IHCL decreased from 19.6% (14.8%, 30.0%) to 8.9% (5.4%, 17.3%) (median [interquartile range]) with no significant change in maximal oxygen uptake or IHCL in the control group (change between groups, $P < .001$ and $P = .02$, respectively). Exercise training increased $VLDL_1$-TG and apoB fractional catabolic rates, a measure of clearance, (change between groups, $P = .02$ and $P = .01$, respectively), and $VLDL_1$-apoB production rate (change between groups, $P = .006$), 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 after 16 weeks of exercise in NAFLD. A longer duration or higher-intensity exercise interventions may be needed to lower the plasma TG and VLDL production rate. (***J Clin Endocrinol Metab*** **101: 4219–4228, 2016)**

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Abbreviations: Adipo-IR, adipose tissue insulin resistance; apoB, apolipoprotein B; BMI, body mass index; BP, blood pressure; FCR, fractional catabolic rate; FRS, Framingham risk score; HOMA, homeostatic model assessment; IHCL, intrahepatocellular fat; IMCL, intramyocellular; LDL, low-density lipoprotein; LPL, lipoprotein lipase; NAFLD, nonalcoholic fatty liver disease; NEFA, nonesterified fatty acid; PR, production rate; TG, triglyceride; VLDL, very low-density lipoprotein; VO_{2max}, maximal oxygen uptake.

Nonalcoholic fatty liver disease (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, hypertriglyceridemia, 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 (NEFAs), originating from adipose tissue; and fatty acids derived from the remnants of the TG-rich lipoproteins, very low-density lipoprotein (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 cotranslationally lipidated in the endoplasmic reticulum by microsomal transfer protein. 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 $VLDL_1$ after the addition of more TG in the liver. The hydrolysis of $VLDL_1$-TG by 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 microsomal transfer protein (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 randomized 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 of 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 of supervised exercise training in men with NAFLD on $VLDL_1$ and $VLDL_2$ kinetics, using a protocol that we have shown previously to be very effective at increasing fitness (15).

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 National Health Service 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

Table 1. Subject Characteristics and Biochemistry

	Before Exercise (n = 15)	After Exercise (n = 15)	Within-Group *P* Value	Before Control (n = 12)	After Control (n = 12)	Within-Group *P* Value	Between-Group P Value
Age, y	52.4 ±.2.2			52.8 ± 3.0			NS
Body weight, kg	101.3 ± 2.64	97.3 ± 12.2	<.001	102.3 ± 6.1	102.9 ± 6.4	NS	<.001
BMI, kg/m^2	31.6 ± 0.8	30.5 ± 1.0	<.001	31.7 ± 1.0	31.6 ± 1.2	NS	.02
Waist circumference, cm	109.3 ± 1.9	105.0 ± 2.5	.005	110.0 ± 3.9	109.6 ± 4.3	NS	.03
VO_{2max}, mL/kg · min	25.5 ± 1.1	33.0 ± 1.5	<.001	23.3 ± 1.0	23.8 ± 1.3	NS	<.001
Fasting glucose, mmol/L	6.0 ± 0.2	5.8 ± 0.2	.005	5.9 ± 0.2	5.6 ± 0.1	.02	NS
Fasting insulin, pmol/L	183 ± 17	138 ± 16	.007	164 ± 17	170 ± 17	NS	.02
HOMA2%S	32.5 ± 2.9	45.6 ± 4.9	.002	36.1 ± 3.7	34.5 ± 3.2	NS	.003
Adipo-IR	79.8 ± 8.0	58.2 ± 8.8	.03	75.9 ± 9.4	86.6 ± 15.7	NS	.02
NEFAs, 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
TGs, 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	.03	3.6 ± 0.2	3.2 ± 0.2	.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	.01	40.9 ± 6.2	31.1 ± 4.7	.04	NS
Aspartate transaminase, U/L	36.9 ± 3.2	29.4 ± 3.5	.02	29.0 ± 2.5	26.3 ± 1.84	NS	NS
γ-Glutamyl transaminase, U/L	53.5 ± 10.2	36.3 ± 7.5	.03	37.0 ± 4.5	33.8 ± 4.9	NS	NS

Abbreviation: HDL, high-density lipoprotein; HOMA2%S, Homa2 insulin sensitivity; NS, not significant.

was made in patients who had been referred for investigation of raised serum transaminases, an 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 or 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; if they had a history of excessive alcohol intake (weekly consumption of > 21 U); if they had magnetic resonance imaging contraindications (cardiac pacemakers, metal implants); or if they 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 randomization, (Statistical Analysis System version 9.1, PROC PLAN software; SAS Institute). 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 after the 16-week intervention period, measurements of physical fitness (maximal oxygen uptake [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, sc, and visceral fat volumes) was measured by magnetic resonance imaging, and intrahepatocellular fat (IHCL), intramyocellular (IMCL) and pancreatic lipid content was measured by magnetic resonance spectroscopy.

Physical training protocol

Participants allocated to the supervised group exercised at moderate intensity (40%–60% heart rate reserve) for 20 minutes initially (progressing toward 1 hour as the program developed) four to five 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 4 days of the metabolic study using an electronically braked bicycle ergometer (Lode; Excalibur Sport) equipped with a computerized breath (oxygen/carbon dioxide) analyzer system (Medical Graphics). An electrocardiogram 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 Supplemental Material.

Diet diaries

Quantification of dietary intake in all participants was assessed by a diet diary and analyzed by Dietplan 6 (release 6.60b4 with Windows VistaService Pack 1; Forestfield Software Ltd).

Measurement of body composition and intracellular fat

Subjects fasted for 6 hours before the scans. Whole-body magnetic resonance imaging for body fat content and ^{1}H-magnetic resonance spectroscopy 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) as previously reported (17, 18). NAFLD was defined as mean IHCL greater than 5.5%. For more details see the Supplemental Material.

Metabolic study protocol

Participants attended the CEDAR center, Royal Surrey County Hospital (Guildford, United Kingdom) on two occasions before (0 wk) and after the intervention (16 wk). The participants were asked to refrain from vigorous exercise for 72 hours before the study, abstain from drinking alcoholic beverages for 24 hours, and attend the CEDAR center after an overnight fast. A primed (1 mg/kg) iv infusion of 1-^{13}C-leucine (1 mg/kg · h) and a bolus of $^2H_5$ glycerol (75 μmol/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 the plasma by sequential ultracentrifugation (19). apoB and TG were isolated from $VLDL_1$ and $VLDL_2$, hydrolyzed, derivatized, and isotopic enrichment measured by gas chromatography mass spectrometry as described in the Supplemental Material. Concentration measurements are also described in the Supplemental Material.

Power calculation

The primary end point for this study was VLDL-apoB production rate. Based on a previous study in type 2 diabetes in which 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 modeling software SAAM II (SAAM Institute) as previously described (21). The model was also used to determine the kinetic parameters of $VLDL_1$ and $VLDL_2$ apoB using plasma α-ketoisocaproate 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). The 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

Table 2. Body Composition and Vascular Measurements

	Before Exercise (n = 15)	After Exercise (n = 15)	Within-Group *P* Value	Before Control (n = 12)	After Control (n = 12)	Within-Group *P* Value	Between-Group *P* Value
IHCL, %	19.6 (14.8, 30.0)	8.9 (5.4, 17.3)	<.001	12.5 (6.9, 32.9)	12.6 (9.2, 26.1)	NS	.02
IMCL (Sol)	21.7 ± 3.8	19.0 ± 3.5	.09	19.2 ± 1.8	20.5 ± 2.6	NS	.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	<.001	9.9 ± 0.9	9.6 ± 0.9	NS	.03
Visceral fat, kg	5.7 ± 0.4	4.7 ± 0.4	<.001	5.7 ± 0.55	5.4 ± 0.6	NS	.06
Abdominal sc fat, kg	7.0 ± 7.2	6.3 ± 0.7	<.001	7.8 ± 1.0	7.91 ± 1.1	NS	.003
Total body fat, kg	31.6 ± 2.0	27.3 ± 1.9	<.001	34.8 ± 3.2	34.5 ± 3.5	NS	.004
Total sc fat, kg	22.1 ± 1.7	19.5 ± 1.6	<.001	24.9 ± 2.4	24.9 ± 2.6	NS	.001
Systolic BP, mm Hg	133.4 ± 4.2	128.8 ± 4.2	.01	131.3 ± 4.5	134.4 ± 3.8	NS	.04
Diastolic BP, mm Hg	83.4 ± 2.3	78.2 ± 2.6	.04	83.8 ± 3.0	86.3 ± 3.0	NS	.02
PWV, m/s	7.94 ± 0.26	7.67 ± 0.25	.05	7.58 ± 0.27	7.77 ± 0.28	NS	NS
FRS	14.4 ± 1.4	12.4 ± 1.4	.001	14.2 ± 2 .5	13.6 ± 1.9	NS	<.05

Abbreviations: NS, not significant; PWV, pulse wave velocity; Sol, soleus; Tib, tibialis; visc, visceral. IHCL is presented as median (interquartile range).

volume as previously described (21). (For more details of the models, see the Supplemental 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 (HOMA)-2 was used to assess whole-body HOMA2 insulin sensitivity (23). Adipose tissue insulin resistance (Adipo-IR) was calculated by multiplying fasting plasma NEFA concentration with fasting serum insulin concentration.

Percentage change in IHCL was calculated as before and after intervention/before intervention × 100. Changes in other measurements were calculated as before and after intervention.

Statistical analysis

Statistical analysis of the data was performed using SPSS version 21.0 for Window (SPSS Inc). IHCL is shown as median (interquartile range). All other results are means ± SEM. Nonparametric data were log transformed. Basal comparisons were performed using a 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 a Student's *t* test for parametric data and a 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 value of $P < .05$ was taken as statistically significant.

Results

Baseline characteristics

Body weight, body mass index (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). The 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 of exercise training with no change in controls (change in exercise group vs control, $P < .001$ and, $P = .02$, respectively) (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 in exercise group vs control, $P < .001$) (Figure 1 and Table 1).

Liver enzymes (Table 1)

After 16 weeks of intervention, there were within-group decreases in the exercise group in Alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyltransferase concentrations ($P < .01$, $P < .02$, and $P < .03$, respectively). Aminotransferase also decreased in controls ($P < .04$) with no change in either aspartate aminotransferase or gamma-glutamyltransferase.

Body composition and ectopic fat (Table 2)

After 16 weeks, there was a significant decrease in IHCL content (percentage decrease 52.2% (29.0, 61.8); median [interquartile range]) in the exercise group, with

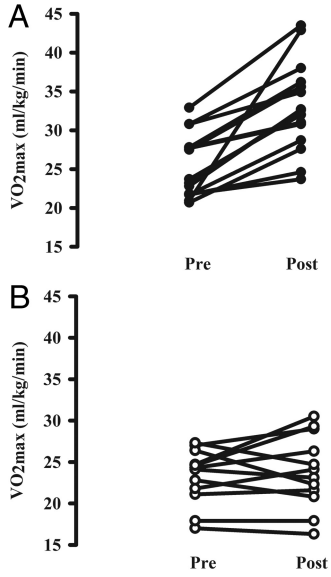

Figure 1. VO_{2max} at 0 and 16 weeks in exercise group (A) and control group (B).

no change in controls (change in exercise group vs 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 insulin, and glucose concentration (Table 1)

After 16 weeks of 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 insulin sensitivity (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 of exercise ($P = .01$ and $P = .04$, respectively) (between groups $P = .04$ and $P = .02$, 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 cholesterol, TG, and high-density lipoprotein cholesterol concentrations did not change from baseline in either group at 16 weeks. After 16 weeks of exercise, there was a significant within-group decrease ($P = .03$) in plasma low-density lipoprotein (LDL) cholesterol concentration. The NEFA concentration did not change in either group. However, Adipo-IR decreased by 24% ± 10% in the exercise group ($P = .03$) with no change in controls (change be-

Table 3. VLDL-TG Kinetics (Mean ± SEM)

	Before Exercise (n = 15)	After Exercise (n = 15)	Within-Group *P* Value	Before Control (n = 12)	After Control (n = 12)	Within-Group *P* Value	Between-Group *P* Value
$VLDL_1$-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	<.01	0.13 ± 0.02	0.14 ± 0.02	NS	.01
VLDL-TG, mmol/L	1.41 ± 0.17	1.15 ± 0.11	<.01	1.13 ± 0.15	1.19 ± 0.16	NS	.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	<.02	0.09 ± 0.03	0.09 ± 0.02	NS	<.01
VLDL-Chol, mmol/L	0.41 ± 0.04	0.36 ± 0.04	.02	0.38 ± 0.06	0.40 ± 0.06	NS	.01
$VLDL_1$-TG FCR, pools/d[a]	8.25 ± 1.07	9.80 ± 1.51	<.05	9.09 ± 0.80	8.62 ± 1.02	NS	.06
$VLDL_1$-TG catabolism FCR, pools/d[b]	6.82 ± 1.16	8.14 ± 1.31	.05	7.46 ± 0.78	5.92 ± 0.53	NS	.02
$VLDL_1$-TG transfer FCR, pools/d[b]	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[a]	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[b]	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[b]	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[b]	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[b]	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[b]	35.7 ± 4.7	29.7 ± 4.6	NS	30.9 ± 4.8	41.3 ± 8.5	NS	NS

Abbreviations: Chol, cholesterol; NS, not significant.

[a] n = 13 in exercise group due to problems with sample analysis.

[b] n = 12 in exercise group due to problems with sample analysis.

Table 4. VLDL apoB Kinetics (Mean ± SEM)

	Before Exercise (n = 15)	After Exercise (n = 15)	Within-Group *P* Value	Before Control (n = 12)	After 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	.04	11.2 ± 1.8	11.1 ± 1.1	NS	NS
$VLDL_1$-TG/$VLDL_1$apoB	66.2 ± 7.0	49.8 ± 4.0	.03	56.5 ± 7.2	60.12 ± 8.7	NS	.04
$VLDL_2$-TG/$VLDL_2$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/d	7.18 ± 0.57	10.93 ± 1.49	.02	10.91 ± 1.76	8.88 ± 1.06	NS	.01
$VLDL_1$-apoB catabolism FCR, pools/d	5.98 ± 0.66	10.39 ± 1.49	<.01	9.87 ± 1.86	7.89 ± 1.23	NS	.01
$VLDL_1$-apoB transfer FCR, pools/d	1.19 ± 0.16	0.54 ± 0.1	.005	1.04 ± 0.25	0.99 ± 0.3	NS	.06
$VLDL_2$-apoB FCR, pools/d	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	.003	4.92 ± 0.80	3.96 ± 0.60	NS	.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	.013
VLDL-apoB PR, mg/kg · d	4.19 ± 0.66	6.04 ± 0.50	.004	5.66 ± 0.95	4.85 ± 0.49	NS	.02

Abbreviation: NS, not significant.

tween groups, $P = .02$). After 16 weeks there was no significant change in $VLDL_1$-TG or $VLDL_1$-apoB concentration in either group. However, $VLDL_2$ TG, cholesterol, and apoB concentration were reduced ($P < .01$, $P < .02$, and $P = .04$, respectively) in the exercise group with no change in controls. The particle size of $VLDL_1$ (TG/apoB) was reduced in the exercise group ($P = .03$) and was different between groups ($P = .04$).

$VLDL_1$- and $VLDL_2$-TG kinetics (Table 3 and 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 ($P < .05$) due to an increase in the $VLDL_1$-TG catabolism FCR ($P = .05$). The percentage change in IHCL in all participants was negatively correlated with the change in $VLDL_1$-TG catabolism FCR ($r = -0.74$, $P < .001$) (Figure 2) and positively correlated with the change in $VLDL_1$-TG transfer FCR ($r = 0.63$, $P = .001$). 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 and 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 ($P = .02$) with no change in controls (between groups, $P = .01$). This was due to an increase in the catabolism FCR ($P < .01$), whereas the transfer FCR (to $VLDL_2$) was decreased ($P = .005$). There was no change in $VLDL_2$-apoB FCR. $VLDL_1$-apoB PR and total VLDL-apoB PR increased in the exercise group ($P = .003$, $P = .004$) (between groups, $P = .006$, $P = .02$). The percentage change in IHCL between 0 and 16 weeks in all participants correlated negatively with the change in $VLDL_1$-apoB PR ($r = -0.48$, $P = .01$) (Figure 2).

Discussion

We have demonstrated for the first time that a 16-week supervised exercise intervention that significantly improved cardiorespiratory fitness and reduced liver fat by greater than 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 increase with acute exercise (24), but this effect is not sustained 48 hours after exercise (25). In the current study, subjects abstained from exercise for 72 hours prior to the measurement of VLDL kinetics 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 HOMA2 insulin sensitivity, with exercise training, as demonstrated previously in type 2 diabetes mellitus and overweight subjects (15, 20,). 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 Adipo-IR. LPL activity is regulated by insulin (28), and 20-week endurance exercise training in healthy men, which increased VO_{2max} 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 $VLDL_1$-TG and apoB FCR observed in

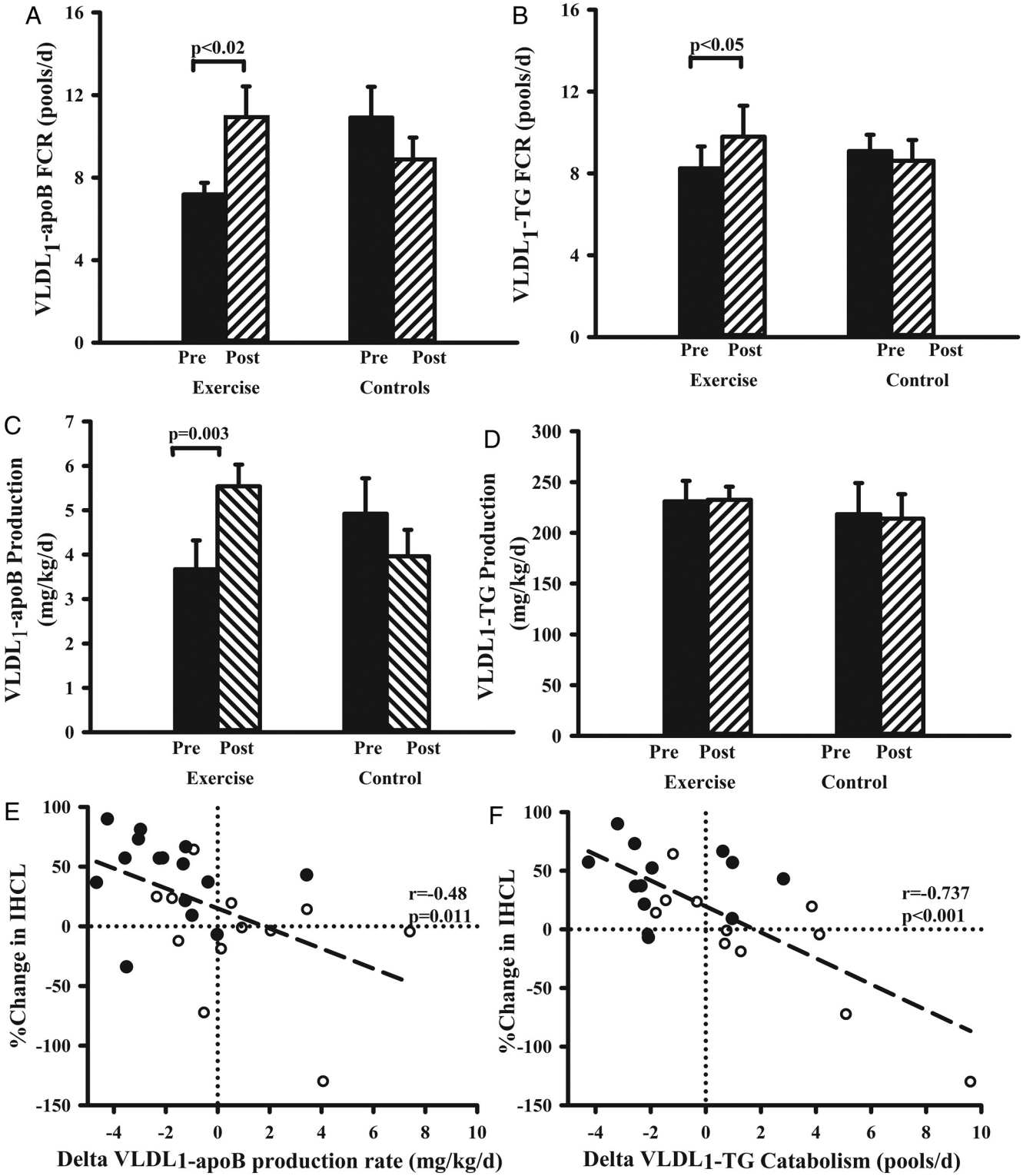

Figure 2. $VLDL_1$ apoB FCR (A), VLDL1-TG FCR (B), $VLDL_1$-apoB production rate (C), and VLDL1-TG production rate (D) at 0 weeks (Pre, solid bar) and 16 weeks (Post, hatched bar) in the exercise and control groups. Relationship between percentage change in IHCL and the change in $VLDL_1$-apoB production rate (E) and the relationship between percentage change in IHCL and the change in $VLDL_1$-TG catabolism FCR (F) are shown. Black circles, exercise group; open circles, control group.

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 $VLDL_2$. An 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 the skeletal muscle to sustain the 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 because 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 in which exercise training for 6 months, resulting in a 16% increase in VO_{2max}, reduced the 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 $VLDL_1$-apoB production rate after 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, whereas at the same time, hepatic insulin resistance was maintained. Insulin regulates VLDL assembly (6–8); thus, a lowering of insulin will increase apoB secretion. It has also been shown in mice that TGs can rescue apoB from posttranslational degradation (33). The up-regulation of the $VLDL_1$-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 in which 16 weeks of exercise training at an exercise intensity comparable with 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 with 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), so the effect of exercise on $VLDL_1$ may not be revealed by measurements on total VLDL. $VLDL_1$ carries more TG compared with $VLDL_2$ per particle, and LPL has been shown to have a preference for TG-rich particles (35).

In NAFLD, cardiovascular events are the most common cause of mortality (36). Both the FRS, which has been shown to accurately predict the actual 10-year cardiovascular disease risk in patients with NAFLD (37), and arterial stiffness, an indicator of cardiovascular disease and independent predictor of the corresponding risk and LDL cholesterol, were decreased after exercise training. The reduced LDL cholesterol may be related to the small weight loss (38). These measures demonstrate that 16 weeks of exercise training can reduce cardiovascular disease 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 after 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 was 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 cardiometabolic health and produced a significant reduction in IHCL, the liver continued to export excessive amounts of TGs 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 the plasma TG and VLDL production rate.

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Contribution statement includes the following: A.M.U., D.J.C., F.S.-M., and G.J.K. designed the study, and F.S.-M., J.W., and R.H. performed the clinical studies. M.B. supervised the exercise intervention. F.S.-M. and N.C.J. performed the laboratory work, which was supervised by A.M.U. J.B. and E.L.T.

performed the magnetic resonance imaging and magnetic resonance spectroscopy measurements, and A.M.U. was the lead writer. All authors reviewed the manuscript. A.M.U. 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.

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References

1. **Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M.** Global epidemiology of non-alcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence and outcomes. *Hepatology*. 2016;64(1):73–84.
2. **Marchesini G, Brizi M, Morselli-Labate AM, et al.** Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med*. 1999;107(5):450–455.
3. **Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ.** Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest*. 2005;115(5):1343–1351.
4. **Dallinga-Thie GM, Franssen R, Mooij HL, et al.** The metabolism of triglyceride-rich lipoproteins revisited: new players, new insight. *Atherosclerosis*. 2010;211(1):1–8.
5. **Malmstrom R, Packard CJ, Caslake M, et al.** Effects of insulin and acipimox on VLDL1 and VLDL2 apolipoprotein B production in normal subjects. *Diabetes*. 1998;47(5):779–787.
6. **Sidiropoulos KG, Pontrelli L, Adeli K.** Insulin-mediated suppression of apolipoprotein B mRNA translation requires the 5′ UTR and is characterized by decreased binding of an insulin-sensitive 110-kDa 5′ UTR RNA-binding protein. *Biochemistry*. 2005;44(37):12572–12581.
7. **Au WS, Kung HF, Lin MC.** Regulation of microsomal triglyceride transfer protein gene by insulin in HepG2 cells: roles of MAPKerk and MAPKp38. *Diabetes*. 2003;52(5):1073–1080.
8. **Sparks JD, O'Dell C, Chamberlain JM, Sparks CE.** Insulin-dependent apolipoprotein B degradation is mediated by autophagy and involves class I and class III phosphatidylinositide 3-kinases. *Biochem Biophys Res Commun*. 2013;435(4):616–620.
9. **Adiels M, Taskinen MR, Packard C, et al.** Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia*. 2006;49(4):755–765.
10. **Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI.** Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes*. 2005;54(3):603–608.
11. **Lazo M, Solga SF, Horska A, et al.** Effects of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. *Diabetes Care*. 2010;33(10):2156–2163.
12. **Hallsworth K, Fattakhova G, Hollingsworth KG, et al.** Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut*. 2011;60(9):1278–1283.
13. **Johnson NA, Sachinwalla T, Walton DW, et al.** Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology*. 2009;50(4):1105–1112.
14. **Sullivan S, Kirk EP, Mittendorfer B, Patterson BW, Klein S.** Randomized trial of exercise effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty liver disease. *Hepatology*. 2012;55(6):1738–1745.
15. **Shojaee-Moradie F, Baynes KC, Pentecost C, et al.** Exercise training reduces fatty acid availability and improves the insulin sensitivity of glucose metabolism. *Diabetologia*. 2007;50(2):404–413.
16. **Cuthbertson DJ, Shojaee-Moradie F, Sprung VS, et al.** Dissociation between exercise-induced reduction in liver fat and changes in hepatic and peripheral glucose homoeostasis in obese patients with non-alcoholic fatty liver disease. *Clin Sci (Lond)*. 2016;130(2):93–104.
17. **Thomas EL, Hamilton G, Patel N, et al.** Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. *Gut*. 2005;54(1):122–127.
18. **Thomas EL, Fitzpatrick JA, Malik SJ, Taylor-Robinson SD, Bell JD.** Whole body fat: content and distribution. *Prog Nucl Magn Reson Spectrosc*. 2013;73:56–80.
19. **Watts GF, Mandalia S, Brunt JN, Slavin BM, Coltart DJ, Lewis B.** Independent associations between plasma lipoprotein subfraction levels and the course of coronary artery disease in the St. Thomas' Atherosclerosis Regression Study (STARS). *Metabolism*. 1993;42(11):1461–1467.
20. **Alam S, Stolinski M, Pentecost C, et al.** The effect of a six-month exercise program on very low-density lipoprotein apolipoprotein B secretion in type 2 diabetes. *J Clin Endocrinol Metab*. 2004;89(2):688–694.
21. **Sarac I, Backhouse K, Shojaee-Moradie F, et al.** Gender differences in VLDL1 and VLDL2 triglyceride kinetics and fatty acid kinetics in obese postmenopausal women and obese men. *J Clin Endocrinol Metab*. 2012;97(7):2475–2481.
22. **D'Agostino RB, Vasan RS, Pencina MJ, et al.** General cardiovascular risk profile for use in primary care the Framingham Heart Study. *Circulation*. 2008;117(6):743–753.
23. **Levy JC, Matthews DR, Hermans MP.** Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care*. 1998;21(12):2191–2202.
24. **Al-Shayji IA, Caslake MJ, Gill JM.** Effects of moderate exercise on VLDL1 and intralipid kinetics in overweight/obese middle-aged men. *Am J Physiol Endocrinol Metab*. 2012;302(3):E349–E355.
25. **Bellou E, Magkos F, Kouka T, et al.** Effect of high-intensity interval exercise on basal triglyceride metabolism in non-obese men. *Appl Physiol Nutr Metab*. 2013;38(8):823–829.
26. **Kotronen A, Seppälä-Lindroos A, Bergholm R, Yki-Järvinen H.** Tissue specificity of insulin resistance in humans: fat in the liver rather than muscle is associated with features of the metabolic syndrome. *Diabetologia*. 2008;51(1):130–138.
27. **Bugianesi E, Gastaldelli A, Vanni E, et al.** Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia*. 2005;48(4):634–642.
28. **Pollare T, Vessby B, Lithell H.** Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. *Arterioscler Thromb*. 1991;11(5):1192–1203.
29. **Bergeron J, Couillard C, Després JP, et al.** Race differences in the response of postheparin plasma lipoprotein lipase and hepatic lipase activities to endurance exercise training in men: results from the HERITAGE Family Study. *Atherosclerosis*. 2001;159(2):399–406.
30. **Chan DC, Gan SK, Wong AT, Barrett PH, Watts GF.** Association between skeletal muscle fat content and very-low-density lipoprotein-apolipoprotein B-100 transport in obesity: effect of weight loss. *Diabetes Obes Metab*. 2014;16(10):994–1000.
31. **Mittendorfer B, Patterson BW, Klein S.** Effect of weight loss on VLDL-triglyceride and apoB-100 kinetics in women with abdominal obesity. *Am J Physiol Endocrinol Metab*. 2003;284(3):E549-E556.
32. **Kallwitz ER, Guzman G, TenCate V, et al.** The histologic spectrum of liver disease in African-American, non-Hispanic white, and Hispanic obesity surgery patients. *Am J Gastroenterol*. 2009;104(1):64–69.
33. **Zhang YL, Hernandez-Ono A, Ko C, Yasunaga K, Huang LS, Ginsberg HN.** Regulation of hepatic apolipoprotein B-lipoprotein assembly and secretion by the availability of fatty acids. I. Differential

response to the delivery of fatty acids via albumin or remnant-like emulsion particles. *J Biol Chem*. 2004;279(18):19362–19374.
34. **Gill JM, Al-Mamari A, Ferrell WR, et al.** Effects of a moderate exercise session on postprandial lipoproteins, apolipoproteins and lipoprotein remnants in middle-aged men. *Atherosclerosis*. 2006; 185(1):87–96.
35. **Bjorkegren J, Packard CJ, Hamsten A, et al.** Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *J Lipid Res*. 1996;37(1):76–86.
36. **Targher G, Day CP, Bonora E.** Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med*. 2010; 363(14):1341–1350.
37. **Vlachopoulos C, Manesis E, Baou K, et al.** Increased arterial stiffness and impaired endothelial function in nonalcoholic fatty liver disease: a pilot study. *Am J Hypertens*. 2010;23(11):1183–1189.
38. **Dattilo AM, Kris-Etherton PM.** Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. *Am J Clin Nutr*. 1992; 56(2):320–328.
39. **Musso G, Cassader M, Rosina F, Gambino R.** Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia*. 2012; 55(4):885–904.
40. **Hens W, Taeyman J, Cornelis J, Gielen J, Van Gaal L, Vissers D.** The effect of lifestyle interventions on excess ectopic fat deposition measured by noninvasive techniques in overweight and obese adults: a systematic review and meta-analysis. *J Phys Act Health*. 2016;13(6): 671–694.