HDL-apoA-I kinetics in response to 16 weeks exercise training in men with non-alcoholic fatty liver disease (NAFLD)

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

HDL-apoA-I kinetics in response to 16 weeks exercise training in men with non-alcoholic fatty liver disease (NAFLD)

Running title: Effect of exercise on HDL kinetics in patients with NAFLD

Martin B. Whyte[1], Fariba Shojaee-Moradie[1], Sharaf E. Sharaf[1], Daniel J. Cuthbertson[2], Graham J. Kemp[2], Mark Barrett[1], Nicola C. Jackson[1], Roselle A. Herring[3], John Wright[3], E. Louise Thomas[4], Jimmy Bell[4], and A. Margot Umpleby[1]

1. Faculty of Health & Medical Sciences, University of Surrey, Guildford, Surrey, UK

2. Institute of Ageing and Chronic Disease, University of Liverpool, Liverpool, UK

3. Centre for Diabetes, Endocrinology, and Research, Royal Surrey County Hospital, Guildford, UK

4. Research Centre for Optimal Health, School of Life Sciences, University of Westminster, UK

MBW data analysis, manuscript writing; FSM data acquisition, laboratory analysis; SES data acquisition and laboratory analysis; NCJ: laboratory and data analysis; RAH, JW and MB data acquisition; ELT and JB imaging data acquisition and analysis; DJC, GJK and AMU: conception and design of the work, data analysis. All authors were involved in manuscript revision and approved the final version.

Author contact:

Dr.Martin Whyte, 21PGM00, Leggett Building, Daphne Jackson Road, University of Surrey

Guildford, UK

Email: m.b.whyte@surrey.ac.uk
Phone: +44 1483 68 8669

Word count: 3853

Number of tables and figures: 6

Abstract

Non-alcoholic fatty liver disease (NAFLD) is characterised by low circulating concentration of high-density lipoprotein cholesterol (HDL-C) and raised triacylglycerol (TAG). Exercise reduces hepatic fat content, improves insulin resistance and increases clearance of very-low density lipoprotein-1 ($VLDL_1$). However, the effect of exercise on TAG and HDL-C metabolism is unknown. We randomised male participants to 16 weeks of supervised, moderate-intensity aerobic exercise (n=15) or conventional lifestyle advice (n=12). Apolipoprotein A-I (apoA-I) and VLDL-TAG and apolipoprotein B (apoB) kinetics were investigated using stable isotopes (1-^{13}C-leucine and 1,1,2,3,3-$^{2}H_5$ glycerol) pre and post intervention. Participants underwent MRI/spectroscopy to assess changes in visceral fat. Results are mean ± standard deviation.

At baseline, there were no differences between exercise and control groups for age (52.4±7.5 *vs* 52.8±10.3 years), BMI (31.6±3.2 *vs* 31.7±3.6 kg/m^2) and waist circumference (109.3±7.5 *vs* 110.0±13.6 cm). Percentage liver fat was 23.8 (interquartile range 9.8–32.5%).

Exercise reduced body weight (101.3±10.2 to 97.9±12.2 kg; P<0.001) and hepatic fat content (from 19.6%, IQR 14.6-36.1% to 8.9% (4.4-17.8%); P=0.001) and increased the fraction HDL-C concentration (measured following ultracentrifugation) and apoA-I pool size with no change in the control group. However, plasma and $VLDL_1$ TAG concentrations and HDL-apoA-I fractional catabolic rate (FCR) and production rate (PR) did not change significantly with exercise. Both at baseline (all participants), and after exercise, there was an inverse correlation between apoA-I pool size and VLDL TAG and apoB pool size. The modest effect of exercise on HDL metabolism may be explained by the lack of effect on plasma and $VLDL_1$ TAG.

Keywords: NALFD, exercise, HDL

Introduction

The presence of non-alcoholic fatty liver disease (NAFLD) is associated with an increased risk of cardiovascular disease (CVD) (29). Whether NAFLD contributes to the development of CVD, or is an epiphenomenon, is unsettled (8). Adverse cardiovascular outcomes may be mediated via the proatherogenic plasma lipid profile that is seen with NAFLD. This includes a low concentration of high-density lipoprotein cholesterol (HDL-C), raised triacylglycerol (TAG) and raised small, dense low-density lipoprotein (LDL) (2). Intravascular exchange of excess VLDL–TAG and HDL cholesteryl ester, mediated by cholesterol ester transfer protein (CETP), results in TAG accrual within the HDL particle. Lipolysis of HDL–TAG will then create smaller HDL particles which are more rapidly removed from the circulation than larger HDL, thereby reducing HDL concentration. Very-low density lipoprotein (VLDL) is secreted by the liver and comprises the large TAG-rich $VLDL_1$ and the smaller, TAG-poor $VLDL_2$. In individuals with abdominal obesity, dysfunctional $VLDL_1$ metabolism is responsible for increased HDL apolipoprotein A-I (apoA-I) catabolism and low plasma HDL-C (11; 12; 19; 46; 48).

In obese men, weight reduction of 5 to 10 kg with a low-fat diet can reduce hepatic VLDL-apoB secretion and decrease both HDL apoA-I catabolism and production (30; 36). Exercise training, with or without dietary intervention, is also an effective treatment for reducing liver fat in patients with NAFLD (4; 22). We have shown that 16 weeks supervised exercise training in men with NAFLD resulted in a decrease in intra-hepatocellular lipid (IHCL) content, and an increase in very-low density lipoprotein-1 triacylglycerol ($VLDL_1$-TAG) and apolipoprotein B (apoB) fractional catabolic rates (FCR) (a measure of clearance) as well as increased $VLDL_1$-apoB production rate (38). This suggested that exercise led to greater production of VLDL by the liver as well as greater clearance of the VLDL particle thereafter. Consequently, it is possible that the effect of exercise on VLDL kinetics that we observed will translate into effects on HDL kinetics. Thus far, only one study has reported on the effect of physical activity on HDL-apoA-I kinetics. (43; 48). Using exogenously labelled, iodinated, HDL it was found that HDL-apoA-I FCR decreased by 6% and HDL-apoA-I production rate (PR) increased by 13%, in response to one-year of exercise training in sedentary overweight participants. Hitherto, no studies have been made of the effect of exercise on HDL kinetics in NAFLD.

We examined HDL kinetics from samples collected from our previous study of supervised exercise training in men with NAFLD. We hypothesised that exercise would increase the clearance of large, TAG-rich $VLDL_1$ ($VLDL_1$-TAG) thereby decreasing the clearance of apoA-I, and increasing the HDL apoA-I pool size.

Methods

Participants

The study design has been reported previously (11; 38). The study received NHS Research Ethics Committee approval and was registered at clinicaltrials.gov (NCT 01834300). All participants gave written informed consent. Males aged 40-65 years and body mass index (BMI) 27-40 kg/m^2, with suspected NAFLD (referred for investigation with raised serum transaminases and/or indication of hepatic steatosis on ultrasound or liver biopsy) were eligible.

Exclusion criteria were: NAFLD secondary to drug treatments, viral hepatitis, autoimmune hepatitis or primary biliary cirrhosis; history of type 2 diabetes mellitus, ischaemic heart disease; any contraindications to exercise; fasting plasma TAG >3.0 mmol/l or total cholesterol levels > 7.0 mmol/l; current smokers; weekly alcohol consumption >21 units; contraindications to magnetic resonance imaging (MRI) such as cardiac pacemakers, metal implants; use of fibrates or beta-blocker medication.

Participants were randomised to either exercise training or lifestyle advice. Participants were randomized to one of the two groups using a list generated by computer randomization, (Statistical Analysis System version 9.1, PROC PLAN software; SAS Institute). Supervised exercise training consisted of 16 weeks of gym-based or other modes of exercise to suit the participants' lifestyle, at moderate intensity (40-60% heart rate reserve) for a minimum of 20 minutes initially (progressing towards 1 hour as the programme developed) 4 to 5 times per week. Participants received weekly supervision from an exercise trainer, usually in person (11; 38).

The control group was advised to exercise and received standard lifestyle advice but with no further communication from the exercise trainer and no supervision. Both groups were asked to continue their usual diet. Participants made no dietary modifications - as confirmed by three-day food diaries collected immediately before and after the intervention and analysed for macronutrient intake.

Metabolic measurements were made at Centre for Diabetes and Endocrine Research (CEDAR) centre, Royal Surrey County Hospital, Guildford, UK. Magnetic Resonance Imaging (MRI) and proton magnetic resonance spectroscopy (^{1}H-MRS) measurements were made at the MRI unit, Hammersmith Hospital, London.

Experimental procedures

Body Composition and intra-hepatocellular fat measurements

Height, weight and waist-to-hip ratio were measured before each metabolic study. All MRI studies were performed on a 1.5T multinuclear scanner (Achieva, Philips Medical Systems, Best, Netherlands) as previously described (42). Briefly, images were acquired using whole body axial T1 - weighted spin echo sequence using a body coil and no respiratory gating (typical parameters: repetition time (TR) 560 ms; echo time (TE) 18 ms; slice thickness 10 mm; interslice gap 10 mm; flip angle 90 degrees; number of excitations 1). Subjects were positioned in the magnet in a prone position with their arms straight above their head and were scanned from their fingertips to their toes. Images were acquired as 9 equal stacks of 12 slices at the isocentre of the magnet. Images were analysed by Vardis (Vardis Group, London, UK) using SliceOmatic, (Tomovision, Montreal, Canada). 1H MRS of liver: Spectra were acquired using a PRESS sequence without water suppression (typical parameters: TR 1500 ms; TE 135 ms; voxel size 20x20x20 mm; flip angle 90 degrees, number of excitations 64). Transverse images of the liver were used to ensure positioning of the voxel, which was placed in an area of the liver avoiding the gall bladder, adipose tissue and main blood vessels. Spectra were analyzed using the AMARES (advanced method for accurate, robust, and efficient spectral fitting) algorithm included in the MRUI software package. Peak areas for all resonances were obtained and lipid resonances quantified with reference to water after correcting for T_1 and T_2.(41)

Cardiorespiratory fitness assessment

VO_{2max} was performed on an electronically-braked bicycle ergometer (Lode; Excaliber Sport) with breath analyser (Medical Graphics). Heart rate was measured throughout. After 2-min warm up at 50 W, resistance increased step-wise at 20 W/min until volitional exhaustion (7).

Metabolic study

The participants were asked to refrain from exercise activity for 48 hours prior to the two metabolic studies (baseline visit and at 16 weeks) and to fast for 13 hours beforehand. Upon arrival, patients were weighed, and an intravenous cannula was placed in a superficial vein for administration of isotopes and another in the contralateral arm for blood sampling. Two basal blood samples were taken for the determination of basal enrichments of leucine and glycerol in $VLDL_1$, $VLDL_2$ and HDL

147 fractions; and for enrichment of plasma glycerol and α ketoisocaproic acid (KIC). A primed (1 mg/kg)
148 infusion of 1-^{13}C-leucine (1 mg/kg/h, for 9 hours) and a bolus of [1,1,2,3,3-$^{2}H_5$ glycerol (75 µmol/kg)
149 were then administered at 0 min. Blood samples were taken from 0-540 min, as we reported
150 previously (38). The plasma samples for ultracentrifugation were stored at 4^{o}C until analysis on the
151 following day. All other plasma samples were kept at -80^{o}C until analysis.

152

153 **Analytical methods**

154 After removal of $VLDL_1$ (sf >60) and $VLDL_2$ (sf 20-60) by sequential centrifugation, a mixture of
155 intermediate-density (IDL) and LDL was removed at an adjusted density of 1.063 kg/L at 147000g for
156 20 hours using sodium bromide. The HDL fraction was isolated at a density of 1.21 kg/L following
157 ultracentrifugation for 24 hours at 218000g, 4^{o}C (Beckman Coulter Optima LE80-K ultracentrifuge
158 using a Type 50.4 Ti rotor (High Wycombe, UK). The HDL fraction thus collected was adjusted for
159 volume (2 mL) using saline and stored at -80^{o}C for further analysis of HDL-C and apoA-I
160 concentration and enrichment of HDL-apoA-I. Fractionated and unfractionated plasma HDL-C
161 concentration was measured with Cobas MIRA (Roche, Welwyn Garden City, UK).

162

163 Isolation of $VLDL_1$ and $VLDL_2$ TAG and apoB as well as measurements of enrichment and
164 concentration of $^{2}H_5$-glycerol in TAG and 1-^{13}C-leucine in apoB have been explained in detail in a
165 previous publication on this study (38).

166 ApoA-I from the HDL fraction (400ul) was precipitated in 8 mL of ice-cold methanol:diethyl ether
167 (V:V), mixed vigorously and centrifuged at 1792 g for 20 min at 4^{o}C. The precipitate was further
168 extracted with ice-cold diethyl ether and centrifuged as before. The precipitate was dried and
169 dissolved in sample buffer, pH 6.8, in preparation for polyacrylamide gel electrophoresis (PAGE).
170 Samples were loaded on polyacrylamide gels (10% resolving &1% stacking) and ran overnight as
171 previously reported (27). Following PAGE, the bands for ApoA-I were visualised by silver stain (Bio-
172 Rad, USA), excised from the gel and hydrolysed in the presence of 6M HCl at 120^{o}C for 24 h. The free
173 amino acids were further purified by cation exchange chromatography using (Dowax AG-50W-X8
174 100-200 mesh).

175 Isotopic enrichment of ^{13}C leucine from apoA-I and apoB were measured in oxazolinone derivative
176 applied on gas chromatography mass spectroscopy GCMS (GCMS; GC system, Agilent 5973C) in
177 negative CI mode with methane as reagent gas (38). Ions monitored were 209 *m/z* ^{12}C and 210 *m/z*
178 ^{13}C leucine, tracer/tracee ratios were calculated for the time course of the study.

Isotopic enrichment of plasma α-ketoisocaproic acid (KIC), a measure of intracellular leucine enrichment for apoB and apoA-I synthesis, was measured by GCMS (38). Plasma glucose, NEFA and TAG, total cholesterol, and lipoprotein fraction cholesterol and TAG were measured with enzymatic reagents with Cobas Mira analyser (38). ApoA-I concentration in the HDL fraction was analysed by immunoturbidimetric method (Horiba ABX, France) with a Cobas MIRA analyser (Horiba ABX, France) inter assay cv 3.17% and intra-assay cv 5.5%. Insulin and plasma adiponectin were measured by radioimmunoassay purchased from Millipore Ltd, MA, USA. The intra-assay cv was 6% and 5% respectively. Fetuin A was measured by ELISA (Epitope Diagnostics), with intra-assay cv 4.8%. Irisin was measured by ELISA (Phoenix Pharmaceuticals), with intra-assay cv 4.1%.

Data analysis

The kinetics of HDL-apoA-I, production rate (PR) and fractional clearance rate (FCR) were calculated using tracer:tracee ratio (TTR) of apoA-I between 2 and 9 hours. This is the period when the enrichment curves of apoA-I are linear, the enrichment of α-KIC is at steady state and apoA-I concentration is unchanged. TTR was calculated as tracer/tracee in samples after the infusion minus tracer/tracee at baseline.

During fasting the apoA-I concentration is at steady state and fractional secretion rate (FSR) is equal to the FCR (27).

FCR (pools/day) = (rate of increase of apoA-I TTR per min/ α-KIC TTR at steady state) x 24 x 60.

The production rate (PR) was calculated from the FCR and the pool size as follows: apoA-I PR (mg/kg/day) = FCR x HDL-apo-I pool size.

Apo-A-I pool size was calculated using concentration (mean of apoA-1 concentration in four samples) and plasma volume (PV) and body weight (BW). ApoA-I pool size (mg/kg) = HDL-apoA-I concentration x PV / BW.

PV was calculated as PV (mL) = 1578 x body surface area (m^2) (32).

Body surface area (BSA) was calculated using BW in kg (DuBois) as follows:

BSA (m^2) = (BW 0.245) x (height x 0.725) x 0.007184

Kinetics of apoB and TAG in $VLDL_1$ and $VLDL_2$ fractions were calculated using SAAM II model as reported in an earlier publication (38). Homeostasis model assessments of insulin resistance (HOMA2- IR) was calculated using the HOMA calculator version 2.2 (10).

209

210 **Statistical analysis**

211 This is a post-hoc analysis of a previously reported randomised controlled trial powered to detect a
212 20% within-group reduction in VLDL-apoB production with 80% power at the 5% level (38).

213 Statistical analysis of the data was performed using SPSS for Windows v25 (IBM Corp. Armonk, NY).
214 Results are means ± standard deviation unless stated otherwise. Data were tested for normality
215 using Shapiro-Wilk. Basal comparisons were performed using Student's independent *t* test
216 (parametric) or Mann-Whitney U (non-parametric). The differences between baseline and 16 weeks
217 were compared within groups using paired *t*-tests or Wilcoxon (nonparametric) and between groups
218 using student's *t* test for parametric data and Mann-Whitney U test for nonparametric data.

219 Correlations between metabolic variables were determined using Spearman's rho correlation
220 coefficient. A two-tailed probability level with P value ≤0.05 was considered statistically significant.

221

Results

Subject characteristics

We have reported on the characteristics of the study population previously (11; 38). At baseline there were no differences between exercise and control groups for age (52.4 ±.7.5 *vs* 52.8 ± 10.3 years; P=0.99), BMI (31.6 ± 3.2 *vs* 31.7 ± 3.6 kg/m^2; P=0.956) and waist circumference (109.3 ± 7.5 *vs* 110.0 ± 13.6 cm; P=0.872). Percentage liver fat was 23.8 (IQR 9.8 – 32.5%).

In the exercise training group there was a significant within-group change in body weight (101.3 ± 10.2 to 97.9 ± 12.2 kg; P<0.001). This equated to loss of 3.6% of their baseline weight; n=13 of the exercise group achieved at least modest (≤3%) weight loss and n=6 achieved >3% weight loss. The exercise group also showed significant change in: BMI (31.6 ± 3.2 to 30.5 ± 3.7 kg/m^2; P=0.001), fasting glucose (6.0 ± 0.8 to 5.8 ± 0.7mmol/L; P=0.005), HOMA2 S% (32.5 ± 11.0 to 45.6 ± 18.9%; P=0.002), VO_{2max} (25.5 ± 4.1 to 33.0 ± 5.8 mL/kg/min; P<0.001), IHCL content (median 19.6%, IQR 14.6-36.1) to 8.9% (4.4-17.8); P=0.001 and alanine aminotransferase (ALT), from 51.1 ± 20.6 to 36.8 ± 20.0 iU/L; P=0.013. However, no effect was seen with exercise on adiponectin (5560 ± 2636 ng/mL to 5901 ± 2806 ng/mL; P=0.226), irisin (138.8 ± 25.6 to 131.1 ± 22.4 ng/mL; P=0.187) or Fetuin A (483.9 ± 82.8 to 471.0 ± 97.2 µg/mL; P=0.402).

By contrast, in the control group, significant within-group changes were only seen in glucose (5.9 ± 0.5 to 5.6 ± 0.3mmol/L; P=0.016) and ALT concentrations (40.9 ± 21.5 to 31.1 ± 16.3 iU/L; P=0.041). Consequently, there were significant between-group changes in weight (P<0.001), BMI (P=0.016), waist circumference (P=0.026), insulin sensitivity (P=0.003) and VO_{2max} (P<0.001).

Lipid profile

As we have reported (38), baseline lipid profiles were similar in the exercise training and control groups. Plasma TAG, $VLDL_1$-TAG (**Table 1**), NEFA and total cholesterol concentrations did not change within, or between, groups. Plasma LDL-C decreased in the exercise training group (from 3.8 ± 0.5 to 3.3 ± 0.6mmol/L; P=0.03). The fraction HDL-C decreased with exercise (**Table 1**) but there was no change in plasma HDL-C, measured without ultracentrifugation, (from 1.01 ± 0.22 to 1.03 ± 0.23 mmol/L; P=0.234). The ratio of total cholesterol to fractional HDL-C was also significantly reduced after the exercise training. There were no significant changes in the control group after the 16 weeks intervention (**Table 1**).

HDL-apoA-I kinetics

HDL-apoA-I pool-size increased significantly after 16 weeks exercise training (P=0.046) (**Table 2**) with no change in the control group. However, between-group changes in HDL-apoA-I pool-size were not different. There were no within- or between-group changes in HDL-apoA-I FCR or HDL-apoA-I PR (**Table 2**).

Relationship between HDL-apoA-I, $VLDL_1$-apoB and $VLDL_2$-apoB at baseline

At baseline, HDL-apoA-I FCR (but not HDL-apo-A-I PR) correlated positively with ALT, aspartate aminotransferase (AST), and Fetuin A and correlated negatively with fraction HDL-C (rho -0.423; P=0.028) and adiponectin (rho -0.547; P=0.003) (**Table 3**).

HDL-apo-A-I PR positively correlated with Fetuin A and negatively with $VLDL_2$ apoB PR (rho -0.417; P=0.03) and negatively with irisin (rho -0.539; P=0.004).

Baseline HDL-apoA-I pool-size (n=27) correlated inversely with total VLDL-TAG pool-size (rho -0.533; P=0.005; **Figure 1**), $VLDL_1$-TAG pool-size (rho -0.542; P=0.004) and $VLDL_2$-TAG pool-size (rho -0.385; P=0.047) and correlated positively with $VLDL_1$-TAG FCR (rho 0.431; P=0.026).

HDL-apoA-I pool-size was also inversely correlated with total VLDL apoB pool-size (rho -0.464; P=0.015) and with $VLDL_2$ apo-B pool-size (rho -0.497; P=0.009). HDL-apoA-I pool-size correlated positively with $VLDL_1$ and $VLDL_2$ apoB FCR (rho 0.416; P=0.032 and rho 0.474; P=0.013 respectively) (**Table 3**).

Correlations with delta changes post intervention from baseline in lipid kinetics.

We have previously reported that exercise increased $VLDL_1$ apoB FCR from 7.18 ± 0.57 to 10.93 ± 1.49 pools/day compared with 10.91 ± 1.76 to 8.88 ± 1.06 pools/day in control (P=0.01 between groups). Furthermore, that $VLDL_1$-TAG FCR changed from 8.25 ± 1.07 to 9.80 ± 1.51 pools/day with exercise *versus* 9.09 ± 0.80 to 8.62 ± 1.02 pools/day in controls (P=0.06 between groups). (38)

Correlation between delta changes post exercise intervention from baseline for HDL-apoA-I and $VLDL_1$- and $VLDL_2$-TAG and apoB and other variables are tabulated in **Table 4**. The Δ HDL-apoA-I pool-

size inversely correlated with Δ VLDL-apoB pool-size (rho -0.729; P=0.002), Δ $VLDL_1$-TAG pool-size (rho -0.650; P=0.009) and Δ total VLDL-TAG pool-size (rho -0.586; P=0.022). The Δ HDL-apoA-I pool-size correlated positively with Δ $VLDL_1$-apoB FCR (rho=0.596, p=0.019) and with $VLDL_1$-TAG FCR (rho=0.555; P=0.049). These relationships were not seen in the control group (**Table 5**).

The Δ body weight significantly correlated with Δ apoB PR (rho -0.560; P = 0.002). All other correlations between Δ baseline to post-intervention, for HDL-apoA-I PR, HDL-apo-A-I FCR, body weight, HDL-C:apoA-I ratio, IHCL and total visceral fat with other variables are tabulated for all participants, n=27 (**Appendix 1**).

Discussion

We report, for the first time, the effect of an exercise intervention on HDL kinetics in patients with NAFLD. Although there was an increase in fraction HDL-C concentration and apoA-I pool size, HDL-apoA-I FCR and PR did not change significantly. Both at baseline, and after exercise, there was an inverse correlation between apoA-I pool size and VLDL TAG and apoB pool size which confirms the well documented inverse relationship between HDL and VLDL metabolism (45). Similarly, at baseline there were also striking positive relationships between apoA-I pool size and the clearance of $VLDL_1$ TAG and apoB.

There is evidence that $VLDL_1$ and $VLDL_2$ are independently regulated (28) and that exercise primarily affects $VLDL_1$ kinetics (16). As we reported previously, 16 weeks of exercise increased $VLDL_1$-TAG and apoB FCR in these subjects (38) and the current study shows that the change in these measurements (with exercise) negatively correlated with the change in apoA-I pool size.

Exercise had no effect on $VLDL_2$ TAG and apoB FCR and thus perhaps, not surprisingly, there was no correlation between the change in these measurements with exercise and apoA-I pool size. The modest effect of exercise on HDL metabolism may be explained by the lack of effect on plasma and $VLDL_1$ TAG concentration. Although IHCL was reduced, it was not normalised and the liver continued to export excessive amounts of TAG as measured by VLDL-TAG production rate. (38) A longer duration of exercise may be required to reduce IHCL to normal and achieve a significant change in HDL metabolism.

To date, the only published study of the effect of exercise training on HDL-apoA-I kinetics was by Zmuda *et al* (48). They showed that in overweight participants, with baseline HDL-C < 40mg/dL (1.03 mmol/L), a one-year exercise intervention reduced body weight by 1.2 kg and increased HDL apoA-I and HDL-C concentrations. Underlying this was a reduction in apoA-I clearance as well as an increase in apoA-I production. Murine models suggest that exercise increases the expression of proteins involved in cholesterol efflux, including liver X receptor-α (LXRα) (21) and ATP-binding cassette A1 (ABCA1) (15). This could have the effect of increasing hepatic clearance of HDL. However, little-to-no effect of exercise on HDL parameters was seen when baseline HDL-C > 44mg/dL (1.14 mmol/L) (48) and so these observations may represent regression to the mean. Furthermore, the methodology used in that paper comprised exogenously radio-labelled HDL which was then re-injected, and plasma kinetics measured over 10 days. This methodology has inherent uncertainty as to whether the tracer has identical metabolic properties to the tracee (35).

From studies of knock-out mice, it has been suggested that HDL formation regulates VLDL-TAG production, resulting in an inverse relationship between plasma HDL-C and TAG concentration (31). However, our data rather suggests that VLDL clearance lowers VLDL TAG, thereby reducing the intravascular exchange of TAG between VLDL and HDL - which in turn may increase HDL apoA-I pool size. This concept is supported by the study of Verges *et al* (46).

There are conflicting data for the effect of exercise training on HDL-C concentration in NAFLD, with either no effect (11; 39), or improvement (33). In T2DM, increased HDL-C concentration has been reported in response to aerobic exercise training after 12-26 weeks (1; 3; 25). However, 12-weeks of resistance training had no effect on HDL-C levels (20). The diverse prescription of duration, frequency and intensity of exercise will all contribute to the heterogeneity of response to the effect of exercise on lipoproteins (18; 24).

Whereas exercise, without weight loss, produces a 20–30% relative reduction in intrahepatic lipid (18), it has been suggested that for an effect of exercise to be seen on HDL-C, at least modest weight loss (≥3%) is required (40). In our study, exercise led to 3.6% weight loss and improvement in HOMA2-IR and fraction HDL-C. However, we did not observe a correlation between the degree of weight loss and change in HDL production or clearance.

In recent years, HDL functionality has been considered a better predictor of cardiovascular disease risk than HDL-C concentration (37). NAFLD is associated with reduced HDL efflux (13) and exercise is associated with increased HDL particle size (17; 40; 44) and cholesterol-efflux capacity (23). We used the fraction HDL-C : apoA-I ratio as a surrogate marker for particle size but found no change with exercise.

HDL-apoA-I FCR correlated with ALT and AST levels at baseline (although not with IHCL). It is unclear whether the magnitude of intra-hepatic fat impacts on the hepatocytes through higher hepatic lipase (HL) activity and hence increased clearance of HDL. In this study we did not measure post-heparin lipase activity. Previous studies have shown the activity of hepatic lipase to be increased in obese men (26; 34) and women (6; 9) with high intra-abdominal fat levels.

The present study has several strengths. This was a randomized controlled trial in which the exercise group was supervised by research staff and had a distinct intensity of exercise comprising an aerobic dose consistent with physical activity recommendations. We allowed at least 48 hours from the final exercise session before metabolic studies thereby removing any acute effect of exercise on HDL metabolism (14). HDL-C concentration was measured following isolation of the HDL-C fraction by

ultracentrifugation. This is more precise and accurate than kit assays (47). In addition, we utilized endogenous stable isotope labelling to assess HDL metabolism *in vivo*.

This study was not an evaluation of the effects of exercise independent of its effect on body weight. For this reason, the results observed might also be achieved by dieting. However, exercise has a particular benefit in reducing hepatic fat (4; 22), which was evident in our study. The exercise programme was free-living and so energy output was not quantified. However, all participants received weekly support from a trainer to maintain commitment to the protocol. As there are pronounced differences in fat metabolism between sexes (5), this study was limited to male participants.

In conclusion, a 16-week exercise programme reduced body weight and hepatic fat content but without significant changes to HDL kinetics. The strong relationship between the change in VLDL-TAG pool size and change in HDL apoA-I pool size, in response to exercise, confirms that VLDL-TAG is a determinant for HDL concentration.

384 **Table 1 -** Lipid profile (mean ± SD)

	Exercise (Pre) n=15	Exercise (Post) n=15	Within group *P* value	Control (Pre) n=12	Control (Post) n=12	Within group *P* value	Between group *P* value
Fraction HDL-C (mmol/L)	0.75 ± 0.19	0.93 ± 0.21	**0.028**	0.93 ± 0.32	0.88 ± 0.25	0.702	0.097
Fraction HDL-apoA-I (g/L)	0.76 ± 0.12	0.80 ± 0.11	0.140	1.24 ± 0.56	1.06 ± 0.12	0.314	0.068
TC : fraction HDL-C ratio	6.6 ± 2.4	5.4 ± 2.0	**0.0035**	7.0 ± 3.0	6.3 ± 2.3	0.320	0.573
Fraction HDL-C : apoA-I ratio	1.06 ± 0.17	1.14 ± 0.19	0.186	1.16 ± 0.57	1.06 ± 0.12	0.546	0.307
Plasma TAG (mmol/L)	1.92 (1.05-2.73)	1.69 (1.30-2.24)	0.155	1.25 (1.07-2.21)	1.57 (1.33-2.56)	0.388	0.683
$VLDL_1$ TAG (mmol/L)	0.99 (0.86-1.45)	0.99 (0.76-1.39)	0.256	0.87 (0.65-1.47)	1.00 (0.67-1.15)	0.347	0.683

385 apoA-I: apolipoprotein A-I, NEFA: non-esterified fatty acids, TC: total cholesterol, TAG:
386 triacylglycerol, $VLDL_1$: very-low density lipoprotein-1

387

388

389 **Table 2** - HDL-apoA-I kinetics (mean ± SD)

	Exercise (Pre) n=15	**Exercise (Post) n=15**	**Within group *P* value**	**Control (Pre) n=12**	**Control (post) n=12**	**Within group *P* value**	**Between group *P* value**
HDL-apoA-I pool size (mg/kg)	17.4 ± 2.9	18.9 ± 2.9	**0.046**	17.9 ± 4.9	19.3 ± 4.4	0.396	0.965
HDL-apoA-I FCR (pools/day)	0.26 ± 0.59	0.24 ± 0.77	0.449	0.18 ± 0.07	0.18 ± 0.06	0.932	0.585
HDL-apoA-I PR (mg/kg/day)	4.4 ± 1.1	4.4 ± 1.2	0.984	3.2 ± 1.3	3.5 ± 1.5	0.573	0.648

390 FCR: fractional catabolic rate, PR: production rate

391

392

393

Table 3- Correlations between HDL-apoA-I and VLDL kinetics at baseline (n=27).

VLDL: Very low density lipoprotein, LDL: low-density lipoprotein, HDL: high density lipoprotein, PS: pool size, FCR: fractional catabolic rate, PR: production rate

	HDL-apoA-I Fractional Clearance Rate (pools/day)	HDL-apoA-I Production Rate (mg/kg/day)	HDL-apoA-I Pool size
$VLDL_1$-apoB pool size (mg)	rho 0.230 P = 0.249	rho -0.006 P = 0.977	rho -0.364 P = 0.062
$VLDL_2$-apoB pool size (mg)	rho -0.074 P = 0.713	rho -0.344 P = 0.079	**rho -0.497** **P = 0.009**
Total VLDL apoB pool size (mg)	rho 0.157 P = 0.435	rho -0.121 P = 0.547	**rho -0.464** **P = 0.015**
$VLDL_1$-apoB FCR (pools/day)	rho -0.198 P = 0.323	rho 0.037 P = 0.855	**rho 0.416** **P = 0.032**
$VLDL_2$-apoB FCR (pools/day)	**rho -0.434** **P = 0.024**	rho -0.177 P = 0.378	**rho 0.474** **P = 0.013**
$VLDL_1$-apoB PR (mg/kg/day)	rho 0.008 P = 0.969	rho -0.125 P = 0.535	rho -0.110 P = 0.584
$VLDL_2$-apoB PR (mg/kg/day)	**rho -0.416** **P = 0.031**	**rho -0.417** **P = 0.030**	rho 0.033 P = 0.871
Total VLDL-apoB PR (mg/kg/day)	rho -0.134 P = 0.506	rho -0.173 P = 0.390	rho 0.004 P = 0.984
$VLDL_1$-TAG pool size (µmol/kg)	rho 0.133 P = 0.508	rho -0.129 P = 0.520	**rho -0.542** **P = 0.004**
$VLDL_2$-TAG pool size (µmol/kg)	rho 0.213 P = 0.285	rho 0.002 P = 0.990	**rho -0.385** **P = 0.047**
Total VLDL-TAG pool (µmol/kg)	rho 0.253 P = 0.204	rho -0.090 P = 0.655	**rho -0.533** **P = 0.005**
$VLDL_1$ TAG PR (mg/kg/day)	rho 0.003 P = 0.987	rho -0.205 P = 0.305	rho -0.373 P = 0.056
$VLDL_2$-TAG PR (mg/kg/day)	rho 0.099 P = 0.624	rho -0.051 P = 0.800	rho -0.212 P = 0.287
$VLDL_1$-TAG FCR (pools/day)	rho -0.310 P = 0.116	rho -0.116 P = 0.564	**rho 0.431** **P = 0.026**
$VLDL_2$-TAG FCR	rho -0.189	rho -0.169	rho 0.056

(pools/day)	P = 0.345	P = 0.399	P = 0.782
Ratio of fraction HDL to ApoA-I	rho 0.162 P = 0.180	rho 0.143 P = 0.47	rho -0.091 P = 0.652
Plasma TAG (mmol/L)	rho 0.309 P = 0.168	rho 0.079 P = 0.696	rho -0.378 P = 0.053
Plasma HDL-C (mmol/L)	rho -0.052 P=0.799	rho 0.164 P = 0.413	rho 0.346 P = 0.077
Fraction HDL-C (mmol/L)	**rho -0.423** **P = 0.028**	rho -0.061 P = 0.763	**rho 0.546** **P = 0.004**
ALT (iU/L)	**rho 0.505** **P = 0.007**	rho 0.325 P = 0.098	rho -0.235 P = 0.238
AST (iU/L)	**rho 0.442** **P = 0.021**	rho 0.375 P = 0.054	rho 0.012 P = 0.953
IHCL (%)	rho 0.357 P = 0.068	rho 0.364 P = 0.062	rho -0.054 P = 0.788
Adiponectin (ng/mL)	**rho -0.547** **P = 0.003**	rho -0.338 p = 0.084	rho 0.308 P = 0.118
Irisin (ng/mL)	rho -0.256 P = 0.197	**rho -0.539** **p = 0.004**	**rho -0.386** **P = 0.047**
Fetuin A (µg/mL)	**0.583** **P = 0.001**	**rho 0.552** **p = 0.003**	rho 0.029 P = 0.886

398

399

400 **Table 4**- Correlations between changes in HDL kinetics and changes in VLDL kinetics at 16
401 weeks (exercise group, n=15).

402

	HDL-apoA-I FCR	**HDL-apoA-I Prod rate**	**HDL-apoA-I Pool size**
$VLDL_1$-apoB pool size (mg)	rho 0.132 P = 0.639	rho -0.025 P = 0.930	rho -0.507 P = 0.054
$VLDL_2$-apoB pool size (mg)	rho -0.207 P = 0.459	rho -0.368 P = 0.177	rho -0.232 P = 0.405
Total VLDL-apoB pool size (mg)	rho -0.011 P = 0.970	rho -0.332 P = 0.226	**rho -0.729** **P = 0.002**
$VLDL_1$-apoB FCR (pools/day)	rho -0.164 P = 0.558	rho -0.054 P = 0.850	**rho 0.596** **P = 0.019**
$VLDL_2$-apoB FCR (pools/day)	rho 0.275 P = 0.321	rho 0.350 P = 0.201	rho 0.104 P = 0.713
$VLDL_1$-apoB PR (mg/kg/day)	rho 0.036 P = 0.889	rho 0.021 P = 0.940	rho 0.382 P = 0.160
$VLDL_2$-apoB PR (mg/kg/day)	rho -0.046 P = 0.869	rho -0.189 P = 0.499	rho -0.196 P= 0.483
Total VLDL-apoB PR (mg/kg/day)	rho -0.089 P = 0.752	rho -0.096 P = 0.732	rho 0.429 P = 0.111
$VLDL_1$-TAG pool size (µmol/kg)	rho 0.050 P = 0.860	rho -0.161 P = 0.567	**rho -0.650** **P = 0.009**
$VLDL_2$-TAG pool size (µmol/kg)	rho -0.186 P = 0.508	rho -0.168 P = 0.550	rho 0.061 P = 0.830
Total VLDL-TAG pool size (µmol/kg)	rho -0.025 P = 0.930	rho -0.218 P = 0.435	**rho -0.586** **P = 0.022**
$VLDL_1$-TAG PR (mg/kg/day)	rho 0.137	rho 0.071	rho 0.007

	P = 0.655	P = 0.817	P = 0.100
$VLDL_2$-TAG PR (mg/kg/day)	rho -0.559 P = 0.059	rho -0.573 P = 0.051	rho 0.217 P = 0.499
$VLDL_1$-TAG FCR (pools/day)	rho 0.027 P = 0.929	rho 0.154 P = 0.616	**rho 0.555** **P = 0.049**
$VLDL_2$-TAG FCR (pools/day)	rho -0.441 P =0.152	**rho -0.622** **P = 0.031**	rho -0.224 P = 0.484

403 **Table 5** - Correlations between changes in HDL kinetics with changes in VLDL kinetics at 16
404 weeks (control group, n=12).

	HDL-apoA-I Pool size	**HDL-apoA-I Fractional clearance rate**	**HDL-apoA-I Production rate**
$VLDL_1$-apoB pool size (mg)	rho -0.497 P = 0.104	rho -0.350 P = 0.265	**rho -0.608** **P = 0.036**
$VLDL_2$-apoB pool size (mg)	rho -0.573 P = 0.051	rho -0.357 P = 0.255	**rho -0.622** **P = 0.031**
VLDL-apoB pool size (mg)	rho -0.536 P = 0.073	rho -0.515 P = 0.087	**rho -0.722** **P = 0.008**
$VLDL_1$-TAG pool size (µmol/kg)	rho 0.091 P = 0.779	rho -0.217 P = 0.499	rho -0.098 P = 0.762
Total VLDL-TAG pool size (µmol/kg)	rho 0.105 P = 0.746	rho 0.056 P = 0.863	rho 0.084 P = 0.795
$VLDL_1$-apoB FCR (pools/day)	rho 0.042 P = 0.897	rho 0.196 P = 0.542	rho 0.217 P = 0.499
$VLDL_1$-apoB PR (mg/kg/day)	rho -0.035 P = 0.914	rho -0.021 P = 0.948	rho -0.007 P = 0.983
Total VLDL-apoB PR (mg/kg/day)	rho 0.063 P = 0.846	rho 0.007 P = 0.983	rho 0.056 P = 0.863
$VLDL_1$-TAG FCR (pools/day)	rho -0.266 P = 0.404	rho 0.126 P = 0.697	rho -0.042 P = 0.897
$VLDL_2$-TAG FCR (pools/day)	rho -0.224 P = 0.484	rho 0.385 P = 0.217	rho 0.028 P = 0.931
$VLDL_1$-TAG PR (mg/kg/day)	rho -0.126 P = 0.697	rho 0.140 P = 0.665	rho 0.021 P = 0.948
$VLDL_2$-TAG PR (mg/kg/day)	rho -0.336 P = 0.286	rho 0.098 P = 0.762	rho -0.168 P = 0.602

405

406

Appendix 1- Correlation between changes in HDL kinetics, weight, HDL-c : apoA-1 ratio, IHCL and total visceral fat with changes in VLDL and TAG kinetics. Delta changes are at 16 weeks (n=27). IHCL: intra-hepatocellular lipid

	Fraction HDL-C to apoA-I ratio	HDL-apoA-I Pool size	HDL-apoA-I Fractional Clearance Rate	HDL-apoA-I Production rate	Weight	IHCL	Total visceral fat
$VLDL_1$-apoB pool size (mg)	rho 0.082 P = 0.683	**rho - 0.429** **P = 0.026**	rho -0.024 P = 0.905	rho -0.208 P = 0.297	rho 0.019 P = 0.925	rho -0.263 P = 0.186	rho 0.209 P = 0.296
$VLDL_2$-apoB pool size (mg)	rho -0.223 P = 0.263	**rho - 0.409** **P = 0.034**	rho -0.194 P = 0.332	rho -0.366 P = 0.061	**rho 0.481** **P = 0.011**	rho 0.355 P = 0.069	rho 0.013 P = 0.947
VLDL-apoB pool size (mg)	rho -0.071 P = 0.724	**rho -0.627** **P < 0.001**	rho -0.159 P = 0.428	**rho -0.428** **P = 0.026**	rho 0.327 P = 0.096	rho -0.104 P = 0.606	rho 0.151 P = 0.454
$VLDL_1$-apoB FCR (pools/day)	rho -0.164 P = 0.415	**rho 0.413** **p = 0.032**	rho -0.055 P= 0.784	rho 0.050 P = 0.804	**rho -0.540** **P = 0.004**	rho -0.133 P = 0.508	**rho -0.491** **P = 0.009**
$VLDL_2$-apoB FCR (pools/day)	rho 0.001 P = 0.995	rho 0.069 P = 0.732	rho 0.342 P = 0.081	rho 0.334 P = 0.089	rho -0.313 P = 0.112	rho -0.143 P = 0.475	rho -0.172 P = 0.392
$VLDL_1$-apoB PR (mg/kg/day)	rho -0.220 P = 0.271	rho 0.251 P = 0.207	rho -0.026 P= 0.897	rho -0.025 P = 0.901	**rho -0.622** **P < 0.001**	**rho -0.423** **P = 0.028**	**rho -0.461** **P = 0.016**
$VLDL_2$-apoB PR (mg/kg/day)	rho -0.369 P = 0.058	rho -0.271 P = 0.171	rho 0.138 P = 0.493	rho -0.072 P = 0.721	rho 0.133 P = 0.507	rho 0.107 P = 0.596	rho -0.188 P = 0.348
VLDL-apoB PR (mg/kg/day)	rho -0.342 P = 0.080	rho 0.277 P = 0.162	rho -0.047 P = 0.815	rho -0.045 P = 0.825	**rho -0.560** **P = 0.002**	**rho -0.409** **P = 0.034**	**rho -0.531** **P = 0.004**
$VLDL_1$ TAG PR (mg/kg/day)	rho -0.125 P = 0.550	rho 0.068 P = 0.745	rho 0.082 P = 0.696	rho 0.053 P = 0.801	rho 0.129 P = 0.540	rho 0.138 P = 0.509	rho -0.065 P = 0.756
$VLDL_2$ TAG PR	rho -0.063	rho -0.201	rho -0.106	rho = -0.248	rho 0.193	rho 0.359	rho 0.117

(mg/kg/day)	P = 0.768	P = 0.347	P = 0.622	P = 0.243	P = 0.367	P = 0.085	P = 0.585
Total VLDL TAG PR (mg/kg/day)	rho -0.172 P = 0.412	rho 0.052 P = 0.804	rho 0.158 P = 0.449	rho 0.133 P = 0.526	rho 0.179 P = 0.391	rho 0.175 P = 0.404	rho -0.045 P = 0.832
Total VLDL TAG pool size (µmol/kg)	rho 0.087 P = 0.667	rho -0.250 P = 0.209	rho 0.017 P = 0.934	rho -0.013 P = 0.947	**rho 0.655** **P < 0.001**	**rho 0.399** **P = 0.039**	**rho 0.393** **P = 0.043**
$VLDL_1$ TAG pool size (µmol/kg)	rho 0.150 P = 0.455	rho -0.225 P = 0.260	rho -0.033 P = 0.869	rho -0.042 P = 0.835	**rho 0.584** **P = 0.001**	rho 0.286 P = 0.148	**rho 0.405** **P = 0.036**
$VLDL_2$ TAG pool size (µmol/kg)	rho -0.217 P = 0.278	rho -0.262 P = 0.187	rho -0.081 P = 0.689	rho -0.180 p = 0.369	**rho 0.529** **P = 0.005**	**rho 0.451** **P = 0.018**	rho 0.221 P = 0.268
$VLDL_1$ TAG FCR (pools/day)	rho -0.112 P = 0.596	rho 0.250 P = 0.228	rho 0.118 P = 0.575	rho 0.118 P = 0.575	**rho -0.430** **P = 0.032**	rho -0.089 P = 0.671	**rho -0.483** **P = 0.014**
$VLDL_2$ TAG FCR (pools/day)	rho 0.03 P = 0.888	rho -0.150 P = 0.483	rho -0.063 P = 0.771	rho -0.259 P = 0.221	**rho -0.431** **P = 0.036**	rho -0.341 P = 0.103	rho -0.137 P = 0.525
Fraction HDL:ApoA-I ratio		rho 0.044 P = 0.828	rho -0.199 P = 0.320	rho -0.033 P = 0.870	rho -0.188 P = 0.347	rho -0.020 P = 0.923	rho 0.321 P = 0.102
HDL-apoA-I Pool size			rho -0.047 P = 0.817	**rho 0.428** **P = 0.026**	rho -0.287 P = 0.146	rho -0.040 P = 0.842	rho -0.188 P = 0.348
HDL-apoA-I FCR				**rho 0.826** **P <0.001**	rho -0.136 P = 0.498	rho 0.070 P = 0.729	rho 0.090 P = 0.655

HDL-apoA-I PR	[redacted]	rho -0.093 P = 0.645	rho 0.129 P = 0.522	rho 0.020 P = 0.923

410

411 **References**

412

413 1. Alam S, Stolinski M, Pentecost C, Boroujerdi MA, Jones RH, Sonksen PH, Umpleby AM: The effect
414 of a six-month exercise program on very low-density lipoprotein apolipoprotein B secretion in type 2
415 diabetes. J Clin Endocrinol Metab 2004;89:688-694
416 2. Amor AJ, Perea V: Dyslipidemia in nonalcoholic fatty liver disease. Curr Opin Endocrinol Diabetes
417 Obes 2019;26:103-108
418 3. Bacchi E, Negri C, Targher G, Faccioli N, Lanza M, Zoppini G, Zanolin E, Schena F, Bonora E,
419 Moghetti P: Both resistance training and aerobic training reduce hepatic fat content in type 2
420 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 Randomized Trial). Hepatology
421 2013;58:1287-1295
422 4. Berzigotti A, Saran U, Dufour JF: Physical activity and liver diseases. Hepatology 2016;63:1026-
423 1040
424 5. Blaak E: Gender differences in fat metabolism. Curr Opin Clin Nutr Metab Care 2001;4:499-502
425 6. Blackburn P, Lemieux I, Lamarche B, Bergeron J, Perron P, Tremblay G, Gaudet D, Despres JP:
426 Hypertriglyceridemic waist: a simple clinical phenotype associated with coronary artery disease in
427 women. Metabolism 2012;61:56-64
428 7. Borg M LH: Perceived Exertion and Pulse Rate during Graded Exercise in Various Age Groups. Acta
429 Medica Scandinavica 1967;181:194-206
430 8. Brouwers M, Simons N, Stehouwer CDA, Isaacs A: Non-alcoholic fatty liver disease and
431 cardiovascular disease: assessing the evidence for causality. Diabetologia 2020;63:253-260
432 9. Carr MC, Knopp RH, Brunzell JD, Wheeler BS, Zhu X, Lakshmanan M, Rosen AS, Anderson PW:
433 Effect of raloxifene on serum triglycerides in women with a history of hypertriglyceridemia while on
434 oral estrogen therapy. Diabetes Care 2005;28:1555-1561
435 10. Cavalot F: Do data in the literature indicate that glycaemic variability is a clinical problem?
436 Glycaemic variability and vascular complications of diabetes. Diabetes Obes Metab 2013;15 Suppl
437 2:3-8
438 11. Cuthbertson DJ, Shojaee-Moradie F, Sprung VS, Jones H, Pugh CJ, Richardson P, Kemp GJ, Barrett
439 M, Jackson NC, Thomas EL, Bell JD, Umpleby AM: Dissociation between exercise-induced reduction
440 in liver fat and changes in hepatic and peripheral glucose homoeostasis in obese patients with non-
441 alcoholic fatty liver disease. Clin Sci (Lond) 2016;130:93-104
442 12. Emerging Risk Factors C, Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L,
443 Thompson A, Sarwar N, Kizer JR, Lawlor DA, Nordestgaard BG, Ridker P, Salomaa V, Stevens J,
444 Woodward M, Sattar N, Collins R, Thompson SG, Whitlock G, Danesh J: Separate and combined
445 associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative
446 analysis of 58 prospective studies. Lancet 2011;377:1085-1095
447 13. Fadaei R, Poustchi H, Meshkani R, Moradi N, Golmohammadi T, Merat S: Impaired HDL
448 cholesterol efflux capacity in patients with non-alcoholic fatty liver disease is associated with
449 subclinical atherosclerosis. Sci Rep 2018;8:11691
450 14. Ferguson MA, Alderson NL, Trost SG, Essig DA, Burke JR, Durstine JL: Effects of four different
451 single exercise sessions on lipids, lipoproteins, and lipoprotein lipase. J Appl Physiol (1985)
452 1998;85:1169-1174
453 15. Ghanbari-Niaki A, Khabazian BM, Hossaini-Kakhak SA, Rahbarizadeh F, Hedayati M: Treadmill
454 exercise enhances ABCA1 expression in rat liver. Biochem Biophys Res Commun 2007;361:841-846
455 16. Gill JM, Al-Mamari A, Ferrell WR, Cleland SJ, Sattar N, Packard CJ, Petrie JR, Caslake MJ: Effects of
456 a moderate exercise session on postprandial lipoproteins, apolipoproteins and lipoprotein remnants
457 in middle-aged men. Atherosclerosis 2006;185:87-96
458 17. Halverstadt A, Phares DA, Wilund KR, Goldberg AP, Hagberg JM: Endurance exercise training
459 raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-

460 density lipoprotein independent of body fat phenotypes in older men and women. Metabolism
461 2007;56:444-450
462 18. Hashida R, Kawaguchi T, Bekki M, Omoto M, Matsuse H, Nago T, Takano Y, Ueno T, Koga H,
463 George J, Shiba N, Torimura T: Aerobic vs. resistance exercise in non-alcoholic fatty liver disease: A
464 systematic review. J Hepatol 2017;66:142-152
465 19. Ji J, Watts GF, Johnson AG, Chan DC, Ooi EM, Rye KA, Serone AP, Barrett PH: High-density
466 lipoprotein (HDL) transport in the metabolic syndrome: application of a new model for HDL particle
467 kinetics. J Clin Endocrinol Metab 2006;91:973-979
468 20. Kadoglou NP, Fotiadis G, Athanasiadou Z, Vitta I, Lampropoulos S, Vrabas IS: The effects of
469 resistance training on ApoB/ApoA-I ratio, Lp(a) and inflammatory markers in patients with type 2
470 diabetes. Endocrine 2012;42:561-569
471 21. Kazeminasab F, Marandi M, Ghaedi K, Esfarjani F, Moshtaghian J: Effects of A 4-Week Aerobic
472 Exercise on Lipid Profile and Expression of LXRalpha in Rat Liver. Cell J 2017;19:45-49
473 22. Keating SE, Hackett DA, George J, Johnson NA: Exercise and non-alcoholic fatty liver disease: a
474 systematic review and meta-analysis. J Hepatol 2012;57:157-166
475 23. Khan AA, Mundra PA, Straznicky NE, Nestel PJ, Wong G, Tan R, Huynh K, Ng TW, Mellett NA, Weir
476 JM, Barlow CK, Alshehry ZH, Lambert GW, Kingwell BA, Meikle PJ: Weight Loss and Exercise Alter the
477 High-Density Lipoprotein Lipidome and Improve High-Density Lipoprotein Functionality in Metabolic
478 Syndrome. Arterioscler Thromb Vasc Biol 2018;38:438-447
479 24. Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS, Bales CW, Henes S,
480 Samsa GP, Otvos JD, Kulkarni KR, Slentz CA: Effects of the amount and intensity of exercise on
481 plasma lipoproteins. N Engl J Med 2002;347:1483-1492
482 25. Lehmann R, Vokac A, Niedermann K, Agosti K, Spinas GA: Loss of abdominal fat and improvement
483 of the cardiovascular risk profile by regular moderate exercise training in patients with NIDDM.
484 Diabetologia 1995;38:1313-1319
485 26. Lemieux I, Pascot A, Couillard C, Lamarche B, Tchernof A, Almeras N, Bergeron J, Gaudet D,
486 Tremblay G, Prud'homme D, Nadeau A, Despres JP: Hypertriglyceridemic waist: A marker of the
487 atherogenic metabolic triad (hyperinsulinemia; hyperapolipoprotein B; small, dense LDL) in men?
488 Circulation 2000;102:179-184
489 27. Li X, Stolinski M, Umpleby AM: Development of a method to measure prebetaHDL and alphaHDL
490 apoA-I enrichment for stable isotopic studies of HDL kinetics. Lipids 2012;47:1011-1018
491 28. Malmstrom R, Packard CJ, Caslake M, Bedford D, Stewart P, Yki-Jarvinen H, Shepherd J, Taskinen
492 MR: Effects of insulin and acipimox on VLDL1 and VLDL2 apolipoprotein B production in normal
493 subjects. Diabetes 1998;47:779-787
494 29. Morrison AE, Zaccardi F, Khunti K, Davies MJ: Causality between non-alcoholic fatty liver disease
495 and risk of cardiovascular disease and type 2 diabetes: A meta-analysis with bias analysis. Liver Int
496 2019;39:557-567
497 30. Ng TW, Watts GF, Barrett PH, Rye KA, Chan DC: Effect of weight loss on LDL and HDL kinetics in
498 the metabolic syndrome: associations with changes in plasma retinol-binding protein-4 and
499 adiponectin levels. Diabetes Care 2007;30:2945-2950
500 31. Parks JS, Chung S, Shelness GS: Hepatic ABC transporters and triglyceride metabolism. Curr Opin
501 Lipidol 2012;23:196-200
502 32. Pearson TC, Guthrie DL, Simpson J, Chinn S, Barosi G, Ferrant A, Lewis SM, Najean Y:
503 Interpretation of measured red cell mass and plasma volume in adults: Expert Panel on
504 Radionuclides of the International Council for Standardization in Haematology. Br J Haematol
505 1995;89:748-756
506 33. Pugh CJ, Spring VS, Kemp GJ, Richardson P, Shojaee-Moradie F, Umpleby AM, Green DJ, Cable
507 NT, Jones H, Cuthbertson DJ: Exercise training reverses endothelial dysfunction in nonalcoholic fatty
508 liver disease. Am J Physiol Heart Circ Physiol 2014;307:H1298-1306

34. Purnell JQ, Kahn SE, Albers JJ, Nevin DN, Brunzell JD, Schwartz RS: Effect of weight loss with reduction of intra-abdominal fat on lipid metabolism in older men. J Clin Endocrinol Metab 2000;85:977-982
35. Ramakrishnan R: Studying apolipoprotein turnover with stable isotope tracers: correct analysis is by modeling enrichments. J Lipid Res 2006;47:2738-2753
36. Riches FM, Watts GF, Hua J, Stewart GR, Naoumova RP, Barrett PH: Reduction in visceral adipose tissue is associated with improvement in apolipoprotein B-100 metabolism in obese men. J Clin Endocrinol Metab 1999;84:2854-2861
37. Ronsein GE, Heinecke JW: Time to ditch HDL-C as a measure of HDL function? Curr Opin Lipidol 2017;28:414-418
38. Shojaee-Moradie F, Cuthbertson DJ, Barrett M, Jackson NC, Herring R, Thomas EL, Bell J, Kemp GJ, Wright J, Umpleby AM: Exercise Training Reduces Liver Fat and Increases Rates of VLDL Clearance But Not VLDL Production in NAFLD. J Clin Endocrinol Metab 2016;101:4219-4228
39. 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;55:1738-1745
40. Swift DL, Houmard JA, Slentz CA, Kraus WE: Effects of aerobic training with and without weight loss on insulin sensitivity and lipids. PLoS One 2018;13:e0196637
41. Thomas EL, Hamilton G, Patel N, O'Dwyer R, Dore CJ, Goldin RD, Bell JD, Taylor-Robinson SD: Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. Gut 2005;54:122-127
42. Thomas EL, Parkinson JR, Frost GS, Goldstone AP, Dore CJ, McCarthy JP, Collins AL, Fitzpatrick JA, Durighel G, Taylor-Robinson SD, Bell JD: The missing risk: MRI and MRS phenotyping of abdominal adiposity and ectopic fat. Obesity (Silver Spring) 2012;20:76-87
43. Thompson PD, Yurgalevitch SM, Flynn MM, Zmuda JM, Spannaus-Martin D, Saritelli A, Bausserman L, Herbert PN: Effect of prolonged exercise training without weight loss on high-density lipoprotein metabolism in overweight men. Metabolism 1997;46:217-223
44. Varady KA, Bhutani S, Klempel MC, Kroeger CM: Comparison of effects of diet versus exercise weight loss regimens on LDL and HDL particle size in obese adults. Lipids Health Dis 2011;10:119
45. Verges B: Abnormalities in lipoprotein kinetics in Type 2 diabetes. Clinical Lipidology 2010;5:277-289
46. Verges B, Adiels M, Boren J, Barrett PH, Watts GF, Chan D, Duvillard L, Soderlund S, Matikainen N, Kahri J, Robin I, Taskinen MR: Interrelationships between the kinetics of VLDL subspecies and HDL catabolism in abdominal obesity: a multicenter tracer kinetic study. J Clin Endocrinol Metab 2014;99:4281-4290
47. Warnick GR, Nauck M, Rifai N: Evolution of methods for measurement of HDL-cholesterol: from ultracentrifugation to homogeneous assays. Clin Chem 2001;47:1579-1596
48. Zmuda JM, Yurgalevitch SM, Flynn MM, Bausserman LL, Saratelli A, Spannaus-Martin DJ, Herbert PN, Thompson PD: Exercise training has little effect on HDL levels and metabolism in men with initially low HDL cholesterol. Atherosclerosis 1998;137:215-221

Figure legend

Figure 1. Correlation of ApoA-I pool size with VLDL-TG pool size at baseline (n=27)