Exercise training reverses endothelial dysfunction in non-alcoholic fatty liver disease

Christopher J.A. Pugh[1,2], Victoria S. Sprung[1,3], Graham J. Kemp[4,5], Paul Richardson[6], Fariba Shojaee-Moradie[7], A. Margot Umpleby[7], Daniel J. Green[1,2], N. Timothy Cable[1], Helen Jones[1] & Daniel J. Cuthbertson[3,8]

[1]Research Institute for Sport & Exercise Science, Liverpool John Moores University, UK, [2]School of Sport Science, Exercise & Health, The University of Western Australia, [3]Department of Obesity and Endocrinology, University Hospital Aintree, UK, [4]Magnetic Resonance and Image Analysis Research Centre (MARIARC), University of Liverpool, UK, [5]Department of Musculoskeletal Biology, University of Liverpool, UK, [6]Department of Hepatology, Royal Liverpool University Hospital, UK, [7]Diabetes and Metabolic Medicine, Faculty of Health and Medical Sciences, University of Surrey, UK [8]Institute of Ageing and Chronic Disease, University of Liverpool, UK.

Running title: Exercise and endothelial function in NAFLD

Correspondence:
Dr Daniel Cuthbertson
Department of Obesity and Endocrinology, Institute of Ageing and Chronic Disease,
Clinical Sciences, Aintree University Hospital,
Lower Lane, Liverpool L9 7AL
Tel: +44 (0) 151 529 5911
Fax: +44 (0) 151 529 5888
E-mail: daniel.cuthbertson@liverpool.ac.uk

Word Count (abstract): 250

Word count (excluding abstract, figures, tables and references): 4267

This study was funded by the European Federation for the Study of Diabetes (EFSD).

Clinicaltrials.gov identifier: NCT01834300

The authors have nothing to declare.

Abstract

Non-alcoholic fatty liver disease (NAFLD) is an independent risk factor for cardiovascular disease (CVD). Endothelial dysfunction is an early manifestation of atherosclerosis and an important prognostic marker for future cardiovascular events. The aim of this study was two-fold: to examine i) the association between liver fat, visceral adipose tissue (VAT) and endothelial dysfunction in obese NAFLD patients and, ii) the impact of supervised exercise training on this vascular defect. Brachial artery endothelial function was assessed by flow-mediated dilatation (FMD) in 34 obese NAFLD patients and 20 obese controls of similar age and cardiorespiratory fitness (VO_{2peak}) (48±2 vs. 47±2y; 27±1 vs. 26±2ml.kg^{-1}.min^{-1}). Magnetic resonance imaging and spectroscopy quantified abdominal and liver fat, respectively. Twenty-one NAFLD patients completed either 16-weeks of supervised moderate-intensity exercise training (*n*=13) or conventional care (*n*=8). Differences between NAFLD and controls were compared using independent *t*-tests and effects of interventions by analysis of covariance. NAFLD patients had higher liver fat [11.6% (95%CI=7.4, 18.1), *P*<0.0005] and VAT [1.6L (95%CI=1.2, 2.0), *P*<0.0001] than controls and exhibited impaired FMD compared with controls [-3.6% (95%CI=-4.9,-2.2), *P*<0.0001]. FMD was inversely correlated with VAT (*r*= -0.54, *P*=0.001) in NAFLD, although the impairment in FMD remained following covariate adjustment for VAT [3.1% (95%CI= 1.8, 4.5), *P*<0.001]. Exercise training, but not conventional care, significantly improved VO_{2peak} [9.1ml/kg^{-1}/min^{-1} (95%CI=4.1, 14.1); *P*=0.001] and FMD [3.6% (95%CI=1.6, 5.7), *P*=0.002]. Endothelial dysfunction in NAFLD cannot be fully explained by excess VAT, but can be reversed with exercise training; this has potential implications for the primary prevention of CVD in NAFLD.

Word Count: 250

Key words: Non-alcoholic fatty liver disease (NAFLD), flow mediated dilation (FMD), cardiovascular risk, exercise training.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a disease spectrum ranging from simple steatosis, progressing to necro-inflammatory changes (non-alcoholic steatohepatitis) and in a subset, to cirrhosis, fibrosis and end-stage liver disease (20). NAFLD is the most common form of chronic liver disease in western society, affecting 20-30% of the general population (6) and up to ~60% of individuals with type 2 diabetes mellitus (43). NAFLD is regarded as the hepatic manifestation of the metabolic syndrome, co-existing with multiple cardio-metabolic risk factors, including obesity, insulin resistance, hypertension and dyslipidaemia (34).

NAFLD increases the risk of chronic liver disease, yet epidemiological studies suggest that cardiovascular disease (CVD) accounts for more deaths in NAFLD than liver disease, some reporting CVD to be the leading cause of mortality (10, 23, 34). Indeed, there is strong evidence that NAFLD patients are at greater risk of CVD than controls and that NAFLD is an independent predictor of cardiovascular morbidity and mortality (10, 28, 33). Endothelial dysfunction of conduit arteries, measured using the flow-mediated dilatation (FMD) technique (36), is an early manifestation of atherosclerosis and a predictor of future CVD events in both symptomatic and asymptomatic individuals (11). Several studies have reported attenuated FMD in NAFLD patients compared with controls (27, 35, 41). Obesity (42), insulin resistance (3) and elevated visceral fat (26) are characteristics of NAFLD and have all been shown to independently impair FMD. Nevertheless, the relationships between endothelial dysfunction and the various co-morbidities of NAFLD are incompletely understood.

Villanova *et al*. (41) reported a causal association between impaired FMD and insulin resistance, whilst Thakur *et al*. (35) observed endothelial dysfunction in NAFLD independent of obesity, metabolic syndrome and insulin resistance. No study, to date, has quantified liver fat or visceral fat volume, to identify possible associations or mechanisms to explain the impaired FMD observed in NAFLD. Therefore, the first aim of this study was to investigate the relationship between liver fat, visceral adipose tissue (VAT) and endothelial dysfunction in obese NAFLD patients compared with obese controls of similar age and cardiorespiratory fitness.

In the absence of an effective pharmacological treatment to reduce liver fat, lifestyle interventions, incorporating structured exercise and/or dietary modification, are recommended as first-line treatment in NAFLD (7). Several studies have demonstrated the efficacy of exercise training in reducing liver fat (4, 15, 39). Moreover, exercise training has been shown to improve endothelial function in healthy individuals and in populations with high CVD risk (12). We have recently demonstrated that exercise training improves cutaneous microvessel endothelial function in NAFLD patients, compared with conventional clinical care (25), however, the impact of supervised exercise training on conduit arteries, which are of similar size and function as coronary arteries (32), remains unknown.

The second aim of the present study was therefore to undertake a randomised controlled trial design to investigate the effect of supervised exercise training on endothelial function. We hypothesised exercise training would induce greater improvement in FMD than conventional care in NAFLD patients.

Materials & Methods

Participants

All participants were obese (waist circumference ≥94cm for males, ≥80cm for females) and sedentary (<2h low-intensity physical activity per week, with none performing any structured or vigorous physical activity) Caucasians, with no history of excessive alcohol intake (average weekly consumption of <21 units for males and <14 units for females). No participant had a history of type 2 diabetes mellitus or ischaemic heart disease, nor any contraindications to exercise (37). Only non-smokers were recruited. Pre-menopausal women (n=4) were tested during the early follicular phase of the menstrual cycle (days 1-7 of the menstrual cycle, immediately following the onset of menstruation). The same inclusion and exclusion criteria applied to both NAFLD patients and controls. Allocation to the control or NAFLD group was performed following determination of the liver triglyceride content (control < 5.5 % or NAFLD ≥ 5.5% liver fat) by proton magnetic resonance spectroscopy (^{1}H MRS) (31).

NAFLD patients: Thirty-four obese NAFLD patients (age: 48±2y, waist circumference: 107±6cm) were recruited to the study. A single, experienced hepatologist at each of two tertiary referral specialist liver clinics recruited all of the patients. Patients were identified if they had raised transaminases, following careful exclusion of drug causes, viral hepatitis (negative hepatitis B and C serology), autoimmune hepatitis and primary biliary cirrhosis (negative auto-antibody screen) or metabolic disorders such as α_1-antitrypin deficiency or Wilson's disease (normal α1-antitripsin and caeruloplasmin concentrations). Nine NAFLD patients were taking anti-hypertensive medication (β-blocker *n*=3, calcium channel blocker *n*=3, angiotensin converting enzyme inhibitor *n*=3), which were not altered during the course of the study.

Control subjects: Twenty obese controls (age: 47±2y, waist circumference: 101±7cm) were recruited via local advertisement. None were taking any prescribed medication and all had normal liver transaminases.

Ethical considerations

The study conformed to the *Declaration of Helsinki* and was approved by the local research ethics committee. Participants were informed of the methods verbally and in writing before providing written informed consent.

Research Design

Participants reported to the laboratory on two occasions. Measurements were performed following an overnight fast, 12 h abstinence from caffeine and 24 h abstinence from alcohol and strenuous exercise (36). All participants were studied at 09h00am to control for the impact of circadian variation. The first visit included anthropometric measurement, a fasting blood sample, assessment of brachial artery endothelial function and a cardio-respiratory fitness test. The second visit involved whole body magnetic resonance imaging (MRI) with proton magnetic resonance spectroscopy (^{1}H MRS) which was performed within 7 days of the first visit. Thirty-one NAFLD patients were then randomly assigned via a single-blinded computer-generated sequence to 16-weeks of either supervised, moderate-intensity exercise training or conventional care, with measurements repeated after the 16–week intervention (Figure 1).

Experimental Measurements

Anthropometric: After a full medical history and physical examination, a single observer (CJAP) performed all the anthropometric assessments (weight, height, waist and hip circumference).

Biochemical: Blood samples were collected and analysed using the Olympus AU2700 analyser (Beckman Coulter, High Wycombe, UK) with standard proprietary reagents as follows: glucose with hexokinase, total cholesterol and high-density lipoprotein (HDL) with cholesterol esterase/oxidase, triglyceride with glycerol kinase and liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) with International Federation of Clinical Chemistry kinetic UV (without pyridoxal phosphate activation). The intra- and inter-assay coefficients of variation were ≤10%. Low-density lipoprotein (LDL) was calculated according to the Friedwald formula. Insulin, leptin and adiponectin were measured using commercially available radio-immunoassay (Millipore Corp, Billerica, MA); the intra- and inter-assay coefficients of variation were ≤4%, 8% and 6% respectively. Using fasting glucose and insulin concentrations, we calculated steady state beta cell function (%B), insulin sensitivity (%S) and insulin resistance was calculated by the homeostasis model assessment (HOMA-IR) (21) and (HOMA2-IR) (19).

Metabolic syndrome: The diagnosis of metabolic syndrome was according to the American Heart Association Joint Scientific Statement criteria based on the presence of ≥3 of the following: (i) central obesity: waist circumference ≥102 cm (male), ≥88 cm (female), (ii) dyslipidemia: TG ≥1.7 mmol.l^{-1} (150 mg/dl), (iii) dyslipidemia: HDL-C <1.0 mmol.l^{-1} (40 mg/dl) (male), <1.3 mmol.l^{-1} (50 mg/dl) (female), (iv) blood pressure ≥130/85 mmHg, and (v) fasting plasma glucose ≥5.6 mmol.l^{-1} (100 mg/dl) (1). A Framingham risk score, for general cardiovascular risk (10 year) was also calculated for all participants (8).

Vascular Function: Upon arrival, participants rested supine for ~20 min, after which blood pressure was determined from an average of three measurements on the left arm. Participants were then positioned with their right arm extended and immobilised with foam supports at an angle of ~80° from the torso.

For measurement of FMD, a 10 MHz multi-frequency linear array probe attached to a high-resolution ultrasound machine (Terason, Teratech, USA) was used to image the brachial artery in the distal third of the upper right arm. When an optimal image was acquired, the probe was held stable and the ultrasound parameters set to optimise longitudinal B-mode images of the lumen-arterial wall interface. Continuous Doppler peak velocity assessment was also performed, using a 60° isonation angle. Endothelial function was assessed by measuring the change in artery diameter in response to a 5 min ischaemic stimulus, induced by forearm cuff inflation (36) using a rapid-inflation pneumatic device (D.E. Hokanson, Bellevue, WA) with the cuff placed distal to the olecranon process (36). A 1 min baseline recording was acquired before the cuff was inflated (~220 mm Hg) for 5 min. Artery diameter and blood flow velocity recordings resumed 30 s before cuff deflation and continued for 3 min thereafter (36). Peak brachial artery diameter and blood flow velocity, and the time taken to reach these peaks following cuff release, were recorded.

Measurement of endothelium-independent vasodilation then occurred after ~15 min rest. A 1 min baseline recording of the brachial artery was again acquired, before endothelium-independent vasodilation was examined following administration of sublingual glyceryl trinitrate (GTN, 400 µg), a nitric oxide donor. The brachial artery was imaged for 10 min following administration of GTN.

Post-test analysis of brachial artery diameter was undertaken using custom-designed automated edge-detection and wall-tracking software, the validity and reproducibility of which has been demonstrated (44). This software utilises operator independent algorithms to assess images and also to calculate vascular outcomes from the FMD and GTN procedures.

Cardiorespiratory Fitness: A fitness test (VO_{2peak}) on a treadmill ergometer was performed. Following a 2-min warm-up at 2.2km.h^{-1} on a flat gradient, the initial

workload was set at 2.7km.h^{-1} at 5° grade. Thereafter, step-wise increments in speed and gradient were made every minute. Heart rate (Polar Electro Oy, Finland) and rating of perceived exertion were monitored (5). VO_{2peak} was calculated from expired gas fractions (Oxycon Pro, Jaegar, Germany) as the highest consecutive 15s periods of oxygen uptake occurring in the last minute before volitional exhaustion. Criteria for attainment of VO_{2peak} included two of the following: RER $\geq$ 1.15, maximal heart rate within 10bpm of the calculated value, or a VO_2 plateau with an increase in power output.

Magnetic Resonance Imaging: Participants underwent MRI scanning in a 1.5T Siemens Symphony scanner (Siemens Medical Solutions, Erlangen, Germany) at the University of Liverpool Magnetic Resonance and Image Analysis Research Centre. Abdominal subcutaneous adipose tissue (SAT) and abdominal visceral adipose tissue (VAT) were calculated from whole body axial T1-weighted fast spin echo scans (axial scans, 10 mm slice thickness followed by a 10 mm gap using the integral body coil). The abdominal region was defined as the image slices from the slice containing the femoral heads, to the slice containing the top of the liver/base of the lungs. All scans were analysed centrally, and anonymised prior to analysis as previously described (16).

Proton magnetic resonance spectroscopy (^{1}H MRS): In *liver*, NAFLD was defined as intrahepatocellular lipid >5·5% measured by ^{1}H MRS (31). Three voxels of interest were identified in the liver standard sites avoiding ducts and vasculature. In *skeletal muscle,* ^{1}H MRS was used to measure intramyocellular lipid, using a single voxel in each of the tibialis anterior (TA) and soleus muscles, avoiding bone, fascia and the neurovascular bundle. Single voxel spectroscopy was conducted as previously described (16).

Exercise Training: Following a familiarisation session, participants attended the university gymnasium on a weekly basis and were provided with full supervision and

guidance from a trained exercise physiologist. Exercise training comprised a combination of treadmill- and cycle ergometer-based exercise which progressively increased in both intensity and duration throughout the course of the intervention. Based on individual basal fitness level, participants began the intervention with 30 min moderate intensity aerobic exercise 3 times a week at 30% of heart rate reserve (HRR) for the initial 4 weeks. Intensity increased to 45% HRR for the following 4 weeks, until week 8, where HRR remained at 45% but the duration of each session increased to 45 minutes. From week 12, participants were exercising 5 times per week for 45 min at 60% of their individual HRR. There were no dietary modifications throughout the course of the exercise intervention, confirmed by the use of a standard food diary. Three-day food diaries were collected immediately prior to and following the exercise intervention and subsequently analysed for macronutrient intake (total energy, carbohydrate, fat, protein and sugars).

Conventional Care: Conventional care consisted of lifestyle advice provided at clinical consultation. Participants were simply advised by their hepatologist or specialist nurse to modify their lifestyle by healthy eating and increasing their physical activity. There was no supervision or guidance beyond the initial advice.

Statistical Analysis

The primary outcome variable for this study was FMD. Based on previously reported data (14, 30), an absolute mean difference of ≥3.4% with a common standard deviation of 2.6% represents clinically relevant differences between groups. For the trial intervention, previously reported data (14, 29) indicate that an absolute mean difference of ≥3.6% with a common standard deviation of 3.4% represents a clinically relevant improvement.

All data were analysed for distribution and logarithmically transformed where appropriate. Clinical characteristics of NAFLD patients and control individuals were compared using independent *t*-tests. Pearson's and Spearman's correlation coefficients (two-tailed) were used to assess relationships between FMD and the potential covariates. FMD data were then analysed whilst statistically controlling for valid covariates. For the comparison of exercise versus conventional care, delta (Δ) change from pre-intervention was calculated and analysed using analysis of covariance with pre-exercise data as a covariate. Hedge's (g) effect sizes were calculated and statistically significant interactions were assessed using the least significant difference approach to multiple comparisons (24).

All FMD data were analysed and are presented as covariate-controlled for baseline artery diameter measured prior to the induction of hyperaemia in each test; this approach may be more accurate for scaling changes in artery diameter than simple percentage change (2). Analyses were performed using the Statistics Package for Social Sciences for Windows, version 17.0 (SPSS Inc. Chicago, IL, USA). Data are presented as means (95% confidence intervals), unless stated otherwise. Logarithmically-transformed data were back-transformed to the original units for presentation in the text, and statistical significance was taken as $P<0.05$ (values of P of "0.000" provided by the statistics package are reported as "<0.001").

Results

NAFLD vs. controls

The characteristics of all participants are listed in Table 1.

Clinical Characteristics: NAFLD patients and controls were similar in age [0.8y (95% CI=-5.2, 6.9), P=0.79, g=0.09], BMI [1.3kg.m^{-2} (95% CI=-0.9, 3.5), P=0.13, g=0.26] and cardiorespiratory fitness [1.0ml.kg^{-1}.min^{-1} (95% CI=-1.1, 1.2), P=0.58, g=0.16].

Percentage body fat, measured by bio-impedance analysis, was also similar [1.1% (95% CI=-3.4, 5.5), P=0.63, g=-0.14]. However, NAFLD patients demonstrated significantly higher waist circumference [6.2cm (95% CI=0.4, 12.2), P=0.04, g=0.58]. Systolic and diastolic blood pressure was not different between the two groups (P>0.05).

Dietary intake: In the exercise group neither total energy intake (mean±SEM; 0.2±0.3MJ, P=0.44) nor macronutrient composition, specifically protein (-0.6±5.3g, P=0.88), carbohydrates (5.2±12.7g, P=0.51), sugar (-6.2±9.0g, P=0.43) and fat (-4.0±5.9g, P=0.31), of the diet were significantly different, following completion of the exercise intervention, compared with baseline.

Biochemical characteristics: Serum ALT, AST and GGT were significantly higher in the NAFLD patients (P<0.01; Table 1). There was clear evidence of dyslipidaemia in the NAFLD group: serum triglycerides were increased [0.7mmol.L^{-1} (95% CI=0.1, 1.3), P=0.0003, g=0.74] and HDL was reduced [-0.2mmol.L^{-1} (95% CI=-0.3, -0.002), P=0.05, g=-0.74] compared with controls. Fasting glucose [0.2mmol.L^{-1} (95% CI=-0.08, 0.5), P=0.15, g=0.37], fasting insulin [1.1pmol.L^{-1} (95% CI=0.8, 1.7), P=0.50, g=0.36] and HOMA2-IR [1.2 (95% CI=0.8, 1.7), P=0.42, g=0.34] were not different between the two groups.

MRI-derived measures of body composition: Liver fat [11.6% (95% CI=7.4, 18.1), P<0.0005, g=2.23; Figure 2] and VAT [1.6L (95% CI=1.2, 2.0), P<0.0001, g=1.14; Figure 2] were increased in NAFLD patients compared with controls. Total abdominal adipose tissue was greater in NAFLD patients than controls [2.0L (95% CI=0.1, 3.9), P=0.04, g=0.57], but there was no difference in SAT or muscle fat between groups (P=0.91).

Vascular function: Brachial artery FMD was significantly impaired in NAFLD patients when compared with controls [-3.6% (95% CI=-4.9, -2.2), P<0.0001, g=-1.47; Figure

2]. No differences were observed in baseline brachial artery diameter, peak diameter or shear rate between NAFLD patients and controls (P>0.41; Table 2). Nevertheless, it took NAFLD patients significantly longer to reach peak diameter [16.2s (95% CI=0.8, 31.6), P=0.04, g=0.69]. No differences were evident in either endothelium-independent vasodilatation in response to sub-lingual GTN (P=0.72; Table 3) or in endothelium-independent time to peak (P=0.23; Table 3) between groups.

Correlations of FMD: A moderate inverse correlation was observed between FMD and VAT (r= -0.54, P=0.001) in NAFLD patients, although not in controls (r= -0.08, P=0.75). There were no significant correlations between FMD and liver fat in NAFLD patients (r= -0.16, P=0.36) or controls (r= 0.05, P=0.84). FMD did not correlate with any other variable in either NAFLD or controls (P>0.05).

Analysis of Covariance: Impairment in FMD remained in NAFLD patients following covariate adjustment for VAT [3.1% (95% CI= 1.8, 4.5), P<0.001; Figure 2]

Effects of intervention in NAFLD patients

Twenty-one patients completed the trial, n=13 exercise (7 males, 6 females; age 50±3yrs, BMI 30±1kg/m^2) and n=8 conventional care (4 males, 4 females; age 47±5yrs, BMI 30±2kg/m^2; Figure 1).

Clinical Characteristics: NAFLD patients allocated to exercise training demonstrated 92% compliance to exercise sessions. Cardiorespiratory fitness improved [9.1ml.kg^{-1}.min^{-1} (95% CI=4.1, 14.1); P=0.001, g=1.72, Figure 3] and waist circumference decreased [3.5cm (95% CI=7.2, 0.3); P=0.05, g=-0.89] with exercise training compared with conventional care. However, there was no difference in BMI, weight, or blood pressure between interventions (P>0.05; Table 3).

Biochemical characteristics: Fasting glucose decreased with exercise training compared with conventional care [5.0mmol.L^{-1} (95% CI=1.0, 0.05); P=0.03, g=-1.04], but there

was no difference in insulin [1.1pmol.L^{-1} (95% CI=0.8, 1.5); *P*=0.74, *g*=0.04] or HOMA2-IR [0.12 (95% CI=-0.4, 0.6); *P*=0.63, *g*=0.07] following the interventions (Table 3). There was no difference in liver enzymes (*P*>0.05; Table 3), lipid profile (*P*>0.05; Table 3), adiponectin [-0.7ng/ml (95% CI=-3.3, 1.8); *P*=0.54, *g*=0.20] or leptin [-2.2ng/ml (95% CI=-6.5, 2.2); *P*=0.31, *g*=-0.23] between the interventions.

MRI-derived measures of body composition: There was no statistically significant difference in liver fat between exercise training and conventional care [-3.3% (95% CI=-10.0, 3.4), *P*=0.18, *g*=-0.48; Figure 3]. SAT decreased with exercise training when compared with conventional care [-0.5L (95% CI=-0.9, -0.04); *P*=0.04, *g*=-1.0], but there was no significant difference in VAT, total abdominal fat or muscle fat between interventions (*P*>0.05; Figure 3).

Vascular function: FMD improved with exercise training compared with conventional care [3.6% (95% CI=1.6, 5.7), *P*=0.002, *g*=1.68; Figure 3]. There was no difference in baseline or peak arterial diameter, shear rate or time to peak between interventions (*P*>0.05; Table 3). There were no differences in either endothelium-independent vasodilatation in response to sub-lingual GTN or in endothelium-independent time to peak between interventions (*P*>0.05; Table 3).

Discussion

The major findings of this study were, first, that obese NAFLD patients exhibit conduit artery endothelial dysfunction compared with obese controls of similar age and cardiorespiratory fitness, which is not completely explained by excess VAT accumulation. Second, supervised exercise training, but not conventional clinical care, can improve endothelial dysfunction in the absence of changes in liver and visceral fat content. Given that conduit artery endothelial dysfunction reflects CVD risk, our data

suggest that moderate intensity exercise training can reduce intrinsic CVD risk in NAFLD.

This is the first study to investigate the association between liver fat, VAT and endothelial dysfunction in obese NAFLD patients compared with obese controls of similar age and cardiorespiratory fitness. As expected, the NAFLD group had greater abdominal obesity, as evidenced by a larger waist circumference and elevated VAT. Our findings, along with previous studies (27, 41) support the association of NAFLD and impaired FMD. We observed a moderate correlation between FMD and VAT in the NAFLD patients, but no relationship between FMD and liver fat content. Nevertheless, the difference in FMD between NAFLD patients and controls was not fully explained by excess VAT accumulation. This magnitude of FMD impairment in NAFLD compared to controls (3.6%) potentially increases CVD risk by 21% (14), independent of traditional CVD risk factors and ectopic fat accumulation.

Elevated liver fat is regarded as the hepatic manifestation of the metabolic syndrome and is strongly associated with insulin resistance (3). A number of studies have reported that visceral fat is also associated with insulin resistance, as well as adverse cardiovascular outcomes and NAFLD severity (9, 22, 40). In this study, obese NAFLD patients exhibited a marked increase in both liver fat and VAT compared to obese controls, yet surprisingly, we observed no significant difference in insulin resistance between the groups. This finding supports some (35), but not all (41) previous reports, that endothelial dysfunction in NAFLD is independent of insulin resistance. As fundamental features of NAFLD such as excess liver fat, elevated VAT and insulin resistance do not totally explain the decrement in FMD in this study, other less overt pathological features may contribute to endothelial dysfunction, such as the excess

secretion of inflammatory cytokines and adipokines from adipose tissue depots (which need not be proportional to their volume). Our findings therefore cannot exclude an indirect impact of VAT on endothelial dysfunction in NAFLD.

Another novel and clinically relevant aspect of this study was the examination of the potential reversibility of conduit artery endothelial dysfunction with exercise training in NAFLD. Previous studies in these patients have shown that exercise training can modify traditional CVD risk factors, including waist circumference (4) and insulin resistance (39). However, given that endothelial dysfunction is an early marker of atherosclerotic disease, evident prior to overt CVD, and can independently predict future CVD events (11), this study highlights the potential cardio-protective role that exercise may play in NAFLD patients, and the inadequacy of conventional clinical care. Indeed, supervised exercise training resulted in an improvement in FMD of 3.6% compared to conventional care, which could reportedly reduce the risk of a CVD event by ~21% (14). Further, these data suggest that the relative impairment in FMD observed in obese NAFLD patients compared to obese controls at baseline is abolished by 16-weeks of supervised exercise training. This exercise-mediated reduction in CVD risk is of particular clinical importance given that CVD is the leading cause of mortality in NAFLD patients (23). Moreover, supervised exercise training resulted in an improvement in VO_{2Peak} of 9.1ml.kg^{-1}.min^{-1} compared to conventional care, which could reportedly reduce the risk of all-cause mortality and cardiovascular events by 34% and 39% respectively(18).

The observation that exercise training enhances FMD in the present study, although novel in NAFLD patients, is consistent with previous reports that exercise training improves endothelial function in other insulin-resistant states (12), and adds to our own

observations that exercise training improves cutaneous microvessel function via the nitric oxide (NO) pathway in NAFLD patients (25). Although exercise training is associated with improvements in traditional cardiovascular risk factors, these are typically quite modest in magnitude and are unlikely to fully explain the benefits of exercise in terms of cardiovascular risk reduction (12). Regular exercise training has been shown to promote increased NO bioavailability by reducing oxygen free radicals and up-regulating endothelial NO synthase protein (12), independently of improvement in CVD risk factors (13). Increased NO bioavailability is thought to be mediated by recurrent shear stress as a result of repeated exercise bouts (38). Consequently, the chronic benefits in these NAFLD patients imply a direct therapeutic impact of exercise training on the endothelium, likely via an increase in conduit artery NO production.

Surprisingly, the exercise-mediated improvements in endothelial function were not accompanied by a statistically significant reduction in VAT or liver fat. Whilst exercise training induced a clinically important absolute reduction in liver fat of 8.4%, this was not statistically different to the reduction observed following conventional care. A recent meta-analysis has demonstrated that exercise training significantly reduces liver fat in NAFLD patients (17), nevertheless, as endothelial dysfunction in NAFLD was not fully explained by excess fat deposition, the exercise-mediated improvement in FMD, without significant concomitant changes in body composition, is perhaps not surprising. Furthermore, it is important to note that neither liver fat nor VAT were primary outcome measures for this study, as it was designed to investigate exercise-mediated changes in endothelial function.

A strength of this study was that we employed the latest FMD guidelines (36) including measurement of eliciting shear rate and state-of-the-art continuous edge-detection and wall-tracking of high resolution B-mode ultrasound images with simultaneous assessment of blood flow velocity. We also employed a covariate-control for baseline artery diameter in our analysis to scale for artery size in line with recent recommendations (36). Furthermore, we utilised non-invasive gold standard ^{1}H- MRS to precisely quantify liver fat. The limitations of the study generally relate to measurement techniques, although we also acknowledge relatively modest cohort sizes which were not fully matched for gender. Firstly, histological classification of NAFLD and distinction between simple steatosis and steatohepatitis would have provided more detail of the underlying disease. Second, the use of a two-stage hyperinsulinaemic-euglycaemic clamp, with infusion of deuterated glucose, would have provided a more sensitive assessment of insulin sensitivity. Finally, a more comprehensive assessment of adipokine profiles, specifically examining the differences between NAFLD and controls would have strengthened our findings.

In summary, obese NAFLD patients exhibit endothelial dysfunction compared with obese controls of similar age and cardiorespiratory fitness. This impairment is associated with excess VAT accumulation, but cannot be fully explained by this differential in fat deposition. Supervised exercise training improves endothelial dysfunction, possibly via a direct effect on the endothelium mediated by repeated episodic increases in shear stress (38). Exercise prescription should be an integral component of management in this high risk population.

Clinical Perspectives

Moderate intensity exercise training improves established surrogates for CVD risk in NAFLD without significant changes in body composition, and should be considered as a leading management strategy in the prevention of heart disease and stroke in this high risk population. Nevertheless, in order to elicit concomitant improvements in body composition, exercise training interventions of longer duration and/or higher intensity may be required.

Clinical trials number: NCT01834300

Acknowledgements

We would like to thank the *European Foundation for the Study of Diabetes* for funding this study [09/H1008/1].

Grants

European Foundation for the Study of Diabetes (EFSD).

Disclosures:

None of the authors have declared any conflict of interest.

Author Contribution

NAFLD patients were recruited from a hepatology clinic at the Royal Liverpool University Hospital. All data collection, analysis and exercise training sessions were performed at the Research Institute for Sport and Exercise Science at Liverpool John Moores University. CJAP, HJ and DJC were involved in all aspects of the study. VSS assisted with all data collection, exercise sessions and analysis. DJC, HJ, GK and MU contributed to the research design, obtained funding and regulatory approval and had overall intellectual ownership. GJK conducted the data collection and analysis of MRI

and MRS data. FSM conducted all analysis of blood samples. PR was involved in the recruitment and screening of NAFLD patients. DJG and NTC provided expertise regarding the duplex ultrasound technique. All authors were involved in the writing and revision of the manuscript.

References

1. **Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr., International Diabetes Federation Task Force on E, Prevention, Hational Heart L, Blood I, American Heart A, World Heart F, International Atherosclerosis S, and International Association for the Study of O**. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 120: 1640-1645, 2009.

2. **Atkinson G, Batterham AM, Thijssen DH, and Green DJ**. A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. *J Hypertens* 31: 287-291, 2013.

3. **Balletshofer BM, Rittig K, Enderle MD, Volk A, Maerker E, Jacob S, Matthaei S, Rett K, and Haring HU**. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* 101: 1780-1784, 2000.

4. **Bonekamp S, Barone B, Clark J, and Stewart K**. The effects of an exercise training intervention on hepatic steatosis. *Hepatology* 48: 806A, 2008.

5. **Borg G**. *Borg's perceived exertion and pain scales*. Human Kinetics Publishers, 1998.

6. **Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, and Hobbs HH**. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 40: 1387-1395, 2004.

7. **Caldwell S, and Lazo M**. Is exercise an effective treatment for NASH? Knowns and unknowns. *Ann Hepatol* 8 Suppl 1: S60-66, 2009.

8. **D'Agostino RB, Sr., Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, and Kannel WB**. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation* 117: 743-753, 2008.

9. **Despres JP, Lemieux I, Bergeron J, Pibarot P, Mathieu P, Larose E, Rodes-Cabau J, Bertrand OF, and Poirier P**. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. *Arterioscler Thromb Vasc Biol* 28: 1039-1049, 2008.

10. **Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, and Kechagias S**. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 44: 865-873, 2006.

11. **Green DJ, Jones H, Thijssen D, Cable NT, and Atkinson G**. Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? *Hypertension* 57: 363-369, 2011.

12. **Green DJ, Maiorana A, O'Driscoll G, and Taylor R**. Effect of exercise training on endothelium-derived nitric oxide function in humans. *J Physiol* 561: 1-25, 2004.

13. **Green DJ, Walsh JH, Maiorana A, Best MJ, Taylor RR, and O'Driscoll JG**. Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations. *Am J Physiol Heart Circ Physiol* 285: H2679-2687, 2003.

14. **Inaba Y, Chen JA, and Bergmann SR**. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *The international journal of cardiovascular imaging* 26: 631-640, 2010.

15. **Johnson NA, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, and George J**. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology* 50: 1105-1112, 2009.

16. **Jones H, Sprung VS, Pugh CJ, Daousi C, Irwin A, Aziz N, Adams VL, Thomas EL, Bell JD, Kemp GJ, and Cuthbertson DJ**. Polycystic ovary syndrome with hyperandrogenism is characterized by an increased risk of hepatic steatosis compared to nonhyperandrogenic PCOS phenotypes and healthy controls, independent of obesity and insulin resistance. *J Clin Endocrinol Metab* 97: 3709-3716, 2012.

17. **Keating SE, Hackett DA, George J, and Johnson NA**. Exercise and non-alcoholic fatty liver disease: a systematic review and meta-analysis. *J Hepatol* 57: 157-166, 2012.

18. **Kodama S, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, Sugawara A, Totsuka K, Shimano H, Ohashi Y, Yamada N, and Sone H**. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *Jama* 301: 2024-2035, 2009.

19. **Levy JC, Matthews DR, and Hermans MP**. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 21: 2191-2192, 1998.

20. **Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, and Rizzetto M**. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 37: 917-923, 2003.

21. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, and Turner RC**. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 412-419, 1985.

22. **Neeland IJ, Turer AT, Ayers CR, Powell-Wiley TM, Vega GL, Farzaneh-Far R, Grundy SM, Khera A, McGuire DK, and de Lemos JA**. Dysfunctional adiposity and the risk of prediabetes and type 2 diabetes in obese adults. *Jama* 308: 1150-1159, 2012.

23. **Ong JP, Pitts A, and Younossi ZM**. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol* 49: 608-612, 2008.

24. **Perneger TV**. What's wrong with Bonferroni adjustments. *Bmj* 316: 1236-1238, 1998.

25. **Pugh CJ, Cuthbertson DJ, Sprung VS, Kemp GJ, Richardson P, Umpleby AM, Green DJ, Cable NT, and Jones H**. Exercise training improves cutaneous microvascular function in nonalcoholic fatty liver disease. *Am J Physiol Endocrinol Metab* 305: E50-58, 2013.

26. **Romero-Corral A, Sert-Kuniyoshi FH, Sierra-Johnson J, Orban M, Gami A, Davison D, Singh P, Pusalavidyasagar S, Huyber C, Votruba S, Lopez-Jimenez F, Jensen MD, and Somers VK**. Modest visceral fat gain causes endothelial dysfunction in healthy humans. *J Am Coll Cardiol* 56: 662-666, 2010.

27. **Senturk O, Kocaman O, Hulagu S, Sahin T, Aygun C, Konduk T, and Celebi A**. Endothelial dysfunction in Turkish patients with non-alcoholic fatty liver disease. *Intern Med J* 38: 183-189, 2008.

28. **Sookoian S, and Pirola CJ**. Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. *J Hepatol* 49: 600-607, 2008.

29. **Sprung VS, Cuthbertson DJ, Pugh CJ, Aziz N, Kemp GJ, Daousi C, Green DJ, Tim Cable N, and Jones H**. Exercise Training in PCOS Enhances FMD in the Absence of Changes in Fatness. *Med Sci Sports Exerc* 2013.

30. **Sprung VS, Jones H, Pugh CJ, Aziz NF, Daousi C, Kemp GJ, Green DJ, Cable NT, and Cuthbertson DJ**. Endothelial dysfunction in hyperandrogenic polycystic ovary syndrome is not explained by either obesity or ectopic fat deposition. *Clin Sci (Lond)* 126: 67-74, 2014.

31. **Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, and Dobbins RL**. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 288: E462-468, 2005.

32. **Takase B, Uehata A, Akima T, Nagai T, Nishioka T, Hamabe A, Satomura K, Ohsuzu F, and Kurita A**. Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease. *Am J Cardiol* 82: 1535-1539, A1537-1538, 1998.

33. **Targher G, and Arcaro G**. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. *Atherosclerosis* 191: 235-240, 2007.

34. **Targher G, and Byrne CD**. Clinical Review: Nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. *J Clin Endocrinol Metab* 98: 483-495, 2013.

35. **Thakur ML, Sharma S, Kumar A, Bhatt SP, Luthra K, Guleria R, Pandey RM, and Vikram NK**. Nonalcoholic fatty liver disease is associated with subclinical atherosclerosis independent of obesity and metabolic syndrome in Asian Indians. *Atherosclerosis* 223: 507-511, 2012.

36. **Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, and Green DJ**. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol* 300: H2-12, 2011.

37. **Thomson W, Gordon N, and Pescatello L**. ACSMs Guidelines for Exercise Testing and Prescription. Philadelphia, PA: Lippincott, Williams and Wilkins, 2009.

38. **Tinken TM, Thijssen DH, Hopkins N, Dawson EA, Cable NT, and Green DJ**. Shear stress mediates endothelial adaptations to exercise training in humans. *Hypertension* 55: 312-318, 2010.

39. **van der Heijden GJ, Wang ZJ, Chu ZD, Sauer PJ, Haymond MW, Rodriguez LM, and Sunehag AL**. A 12-week aerobic exercise program reduces hepatic fat accumulation and insulin resistance in obese, Hispanic adolescents. *Obesity (Silver Spring)* 18: 384-390, 2010.

40. **van der Poorten D, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, London R, Peduto T, Chisholm DJ, and George J**. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 48: 449-457, 2008.

41. **Villanova N, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, Zoli M, and Marchesini G**. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. *Hepatology* 42: 473-480, 2005.

42. **Williams IL, Chowienczyk PJ, Wheatcroft SB, Patel AG, Sherwood RA, Momin A, Shah AM, and Kearney MT**. Endothelial function and weight loss in obese humans. *Obes Surg* 15: 1055-1060, 2005.

43. **Williamson RM, Price JF, Glancy S, Perry E, Nee LD, Hayes PC, Frier BM, Van Look LA, Johnston GI, Reynolds RM, and Strachan MW**. Prevalence of and risk factors for hepatic steatosis and nonalcoholic Fatty liver disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes Care* 34: 1139-1144, 2011.

44. **Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA, and Green D**. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol* 91: 929-937, 2001.

List of Figures

Figure. 1. Schematic representation of participant recruitment and group allocation.

Figure. 2. Differences in FMD between NAFLD and control groups unadjusted and statistically adjusted for VAT. Data are presented as mean±SD. *Indicates significance between NAFLD and controls (P<0.05).

Figure. 3. Delta (Δ) change in FMD, cardiorespiratory fitness, liver fat and VAT following exercise training and conventional care. Data are presented as mean±SD. *Indicates significant difference between exercise and conventional care (P<0.05).

665 **Table 1**. Baseline characteristics of NAFLD and control participants
666

	NAFLD (*n*=34)	Controls (*n*=20)	*g*	*P*
Clinical Characteristics				
Age (y)	48(44, 51)	47(43, 51)	0.09	0.79
Gender	22M, 12F	8M, 12F		
Weight (kg)	90.6(85.8, 95.5)	84.5(77.6, 91.3)	0.38	0.13
BMI ($kg.m^{-2}$)	31(30, 32)	30(28, 31)	0.26	0.23
Waist circumference (cm)	107(103, 110)	101 (95, 106)	0.58	0.04*
Percentage body fat (%)	35(32, 38)	36(35, 40)	-0.14	0.63
Systolic BP (mm Hg)	128(124, 132)	126(122, 129)	0.21	0.42
Diastolic BP (mm Hg)	79(76, 82)	77(73, 80)	0.26	0.37
Framingham risk (10y CVD)	8.1(6.0, 10.1)	5.4(3.8, 7.0)	0.52	0.07
VO_2 ($l.min^{-1}$) †	2.4(2.09, 2.82)	2.16(1.70, 2.74)	0.21	0.24
VO_{2peak} ($ml.kg^{-1}.min^{-1}$) †	26.9(24.4, 29.5)	25.6(21.9, 30.0)	0.16	0.58
Liver Enzymes				
ALT ($U.l^{-1}$) †	69(54, 84)	28(22, 35)	1.41	<0.001*
AST ($U.l^{-1}$) †	41(34, 48)	25(21, 30)	0.57	0.01*
GGT ($U.l^{-1}$) †	67(45, 89)	37(20,53)	0.63	0.009*
Lipid Profile				
Cholesterol ($mmol.l^{-1}$)	5.4(5.1, 5.8)	5.2(4.8, 5.5)	0.24	0.25
Triglyceride ($mmol.l^{-1}$) †	2.2(1.8, 2.6)	1.5(1.1, 1.8)	0.74	0.003*
HDL ($mmol.l^{-1}$)	1.2(1.1, 1.3)	1.4(1.3, 1.5)	-0.74	0.05*
LDL ($mmol.l^{-1}$)	3.3(3.0,3.6)	3.3(2.8, 3.8)	0.01	0.95
Chol:HDL ratio	4.4(4.0, 4.7)	3.8(3.4, 4.3)	0.54	0.07
Metabolic variables				
Glucose ($mmol.l^{-1}$)	5.1(4.9, 5.3)	4.9(4.7, 5.1)	0.37	0.15
Insulin ($pmol.l^{-1}$) †	88(70, 111) #	77(60, 100) #	0.36	0.50
HOMA-IR †	3.3(2.5, 4.2) #	2.8(2.2, 3.8) #	0.31	0.49
HOMA2-IR †	1.7(1.3, 2.1) #	1.4(1.1, 1.9) #	0.34	0.42
Metabolic syndrome	14/34	2/20		0.009*
Adipose tissue deposition				
Liver fat (%) †	22.5(18.2, 27.9)	1.9(1.2, 3.1)	2.23	<0.001*
VAT (l) †	5.3(4.7, 5.9)	3.3(2.7, 4.1)	1.14	<0.001*
SAT (l)	8.5(7.4, 9.6)	8.4(6.6, 10.2)	0.03	0.91
Total abdominal AT (l)	14.1(13.1, 15.1)	12.1(10.2, 14.0)	0.57	0.04*
VAT:SAT ratio	0.77(0.61, 0.93)	0.51(0.34, 0.66)	0.68	0.03*
Soleus IMCL (CH_2/creatine) †	11.1(8.9, 13.6)	10.2(7.5, 14.0)	0.12	0.66
Tibialis anterior IMCL (CH_2/creatine) †	9.0 (7.5, 10.9)	9.8(7.3, 13.0)	-0.16	0.62
Brachial Artery Function				
Flow-mediated dilation (%)	4.78(4.13, 5.43)	8.37(6.95, 9.78)	-1.47	<0.001*
Baseline diameter (mm)	4.22(3.89, 4.55)	4.01(3.62, 4.41)	0.23	0.41
Peak diameter (mm)	4.42(4.08, 4.77)	4.34(3.92, 4.76)	0.08	0.77
Shear $rate_{AUC}$ ($s^{-1}\times10^3$)	14.6(11.0, 18.3)	15.7(12.0, 19.4)	-0.12	0.69
FMD-mediated time to peak (s)	62(51, 73)	45(37, 54)	0.69	0.04*
GTN-mediated dilation (%)	16(14, 19)	17(15, 19)	-0.18	0.72
GTN-mediated time to peak (s)	412(376, 448)	378(334, 422)	0.33	0.23

667 Data are presented as mean (95% CI). †Data analysed after logarithmic transformation.
668 # indicates analysis of data on *n*=27 NAFLD and *n*=12 controls. *Significant difference
669 between NAFLD and controls.
670
671
672

673

674

Table 2. Changes in the biochemical, metabolic and body composition characteristics of NAFLD patients following supervised exercise training (*n*=13; 7 males, 6 females) and conventional care (*n*=8; 4 males, 4 females)

	Pre Ex	Post Ex	Ex Δ Change	Pre CC	Post CC	CC Δ Change	*g*	*P*
Clinical characteristics								
Weight (kg) †	86.6(79.4, 94.0)	84.5(76.9, 92.2)	-2.1(-3.2, -1.0)	90.8(74.5,107.1)	89.8(73.2,106.3)	-1.1(-2.5, 0.2)	-0.56	0.25
Body mass index ($kg.m^{-2}$) †	30(29.32)	29(28, 31)	-1.0(-1.1, -0.4)	30(26, 34)	30(26, 34)	-0.4(-0.8, 0.4)	-0.86	0.17
Waist (cm)	109(99, 108)	99(94, 104)	-4.6(-6.9, -2.2)	105(94, 116)	104(93, 116)	-1.1(-4.0, 1.9)	-0.89	0.05*
Systolic BP (mm Hg)	127(121, 132)	126(121, 130)	-0.5(-4.2, 4.4)	124(112, 135)	123(116, 131)	-2(-7.1, 4.3)	0.16	0.72
Diastolic BP (mm Hg)	79(75, 82)	77(75, 79)	-0.3(-2.9, 2.6)	74(67, 80)	73(69, 77)	-3.1(-5.6, -0.1)	0.67	0.16
VO_2 ($l.min^{-1}$)	2.29(1.73,2.91)	2.82(2.13,3.61)	0.56(0.26,0.86)	2.45(1.44,3.71)	2.23(1.42,3.21)	-0.23(-0.61,0.14)	1.57	0.003*
VO_{2peak} ($ml.kg^{-1}.min^{-1}$)	26.4(21.8, 30.9)	33.4(27.7, 39.2)	7.0(3.9, 10.1)	27.0(19.3, 34.6)	24.8(19.4, 30.2)	-2.1(-6.0, 1.8)	1.72	0.001*
Liver Enzymes								
ALT ($U.l^{-1}$) †	57.5(38.9, 76.0)	39.8(28.7, 51.0)	-21(-30, -12)	77.1(38.5,115.7)	59.3(33.4, 85.1)	-12(-23,-0.2)	-.062	0.20
AST ($U.l^{-1}$) †	37.0(26.9, 47.1)	29.0(25.1, 32.9)	-10(-14, -6)	48.2(21.3, 75.1)	41.8(24.0, 59.7)	-2(-8, 3)	-1.25	0.10
GGT ($U.l^{-1}$) †	74.1(23.4, 124.8)	54.8(22.7, 86.8)	-20(-30, -11)	81.1(42.9,119.3)	60.6(28.5, 92.7)	-19(-31, -7)	-0.09	0.78
Lipid Profile								
Cholesterol ($mmol.l^{-1}$)	5.4(4.9, 6.0)	5.3(4.8, 5.8)	-0.1(-0.4, 0.1)	5.4(4.8, 6.0)	5.3(4.5, 6.1)	-0.1(-0.4, 0.2)	-2.35	0.93
Triglycerides ($mmol.l^{-1}$)	2.0(1.6, 2.4)	1.9(1.6, 2.2)	-0.2(-.05, 0.02)	2.7(1.1, 4.3)	2.0(1.2, 2.8)	-0.4(-0.8, -0.2)	-1.60	0.18
HDL ($mmol.l^{-1}$)	1.3(1.1, 1.5)	1.4(1.2, 1.5)	0.03(-0.04, 0.1)	1.2(1.1, 1.4)	1.2(1.1, 1.3)	-0.02(-0.1, 0.1)	0.05	0.31
LDL ($mmol.l^{-1}$) †	3.2(2.8, 3.6)	3.1(2.6, 3.5)	-0.1(-0.6, 0.3)	3.3(2.4, 4.2)	3.3(2.6, 3.9)	0.04(-0.5, 0.6)	-0.23	0.55
Chol:HDL ratio †	4.1(3.5, 4.7)	4.0(3.4, 4.6)	-0.1(-0.5, 0.3)	4.6(4.0, 5.2)	4.6(3.9, 5.4)	0.07(-0.5, 0.6)	-0.27	0.59
Metabolic Variables								
Glucose (mmol/l)	4.9(4.5, 5.3)	4.7(4.4, 5.1)	-0.3(-0.5, 0.04)	5.3(4.6, 5.9)	5.5(4.9, 6.1)	0.3(-0.09, 0.6)	-1.04	0.03*
Insulin ($pmol.l^{-1}$) †	78(56, 102)	77(59, 102)	1.0(0.8, 1.2)#	85(45, 1560)	78.1(49.0,124.5)	0.9(0.7, 1.2)#	0.04	0.74

	Pre Ex	Post Ex	Ex Δ Change	Pre CC	Post CC	CC Δ Change	*g*	*P*
HOMA-IR †	2.8(1.8, 3.8)	2.8(2.0, 3.7)	-0.2(-0.9, 0.5)#	3.9(0.9, 6.9)	3.4(1.7, 5.0)	-0.2(-1.0, 0.5)#	0.001	0.98
HOMA2-IR	1.7(1.2, 2.2)	1.5(1.1, 1.9)	-0.4(-0.7,0.004)#	2.3(0.8, 3.8)	1.6(0.8, 2.4)	-0.5(-0.9, -0.1)#	0.07	0.63
Adiponectin ($\mu g.l^{-1}$)	15.6 (9.2, 22.0)	13.4(7.4, 19.4)	-0.7(-3.3, 1.8)#	15.3 (9.7, 20.9)	13.9(8.2, 19.7)	-1.4(-3.4, 0.5)#	0.20	0.54
Leptin ($\mu g.l^{-1}$)	10.6(5.1, 16.2)	9.7(4.8, 14.5)	-1.2(-3.9, 1.6)#	14.2(8.9, 19.5)	14.9(7.7, 21.9)	0.9(-2.3, 4.3)#	-0.23	0.31
Adipose Tissue Deposition								
Liver fat (% CH_2/H_2O) †	27.0(14.7, 39.3)	18.0(11.0, 25.0)	-8.4(-12.5, -4.2)	23.8(16.0, 31.5)	19.8(12.8, 26.8)	-5.0(-10.3, 0.2)	-0.48	0.18
VAT (l)	6.0(4.6, 7.3)	5.9(4.7, 7.0)	0.02(-0.5, 0.5)	4.3(3.5, 5.2)	4.4(3.5, 5.3)	-0.2(-0.9, 0.6)	0.12	0.68
SAT (l)	8.1(6.5, 9.7)	7.6(6.0, 9.3)	-0.4(-0.7, -0.2)	8.4(6.7, 10.1)	8.4(6.4, 10.5)	0.1(-0.3, 0.4)	-1.0	0.04*
Total abdominal AT (l)	14.1(12.5, 16.0)	13.5(12.2, 14.8)	-0.5(-1.1, 0.1)	12.7(11.2, 14.2)	12.8(10.5, 15.1)	0.02(-0.8, 0.9)	-0.47	0.33
VAT:SAT ratio	0.86(0.53, 1.19)	0.90, 0.58, 1.22	0.06(-0.04, 0.1)	0.55(0.36, 0.74)	0.54(0.38, 0.71)	-0.02(-0.2, 0.1)	1.72	0.33
Soleus IMCL (CH_2/creatine)	10.8(7.5, 14.0)	12.2(8.1, 16.4)	1.4(-1.4, 4.2)	11.3(6.2, 16.4)	13.0(6.7, 19.2)	1.7(-2.0, 5.4)	-0.07	0.90
TA IMCL (CH_2/creatine)	11.3(7.7, 14.9)	11.2(8.5, 13.9)	1.1(-2.8, 4.9)	7.5(3.0, 12.1)	13.5(6.2, 20.9)	4.2(-0.8, 9.1)	-0.51	0.32

Ex- Exercise group, CC- Conventional care group. Data are presented as mean (95% CI). Delta (Δ) change from pre-intervention following adjustment for pre-intervention values. †Variables analysed after logarithmic transformation. # Indicates analysis of data on n=10 Ex and n=7 CC. *Significant difference between ΔEx and ΔCC (P<0.05).

Table 3. Changes in the vascular characteristics of NAFLD patients following supervised exercise training (*n*=13; 7 males, 6 females) and conventional care (*n*=8; 4 males, 4 females).

	Pre Ex	Post Ex	Ex Δ Change	Pre CC	Post CC	CC Δ Change	*g*	*P*
Brachial Artery Function								
Flow-Mediated Dilation (%)	4.79(3.45, 6.14)	8.57(7.05, 10.09)	3.47(2.24, 4.71)	5.94(4.33, 7.55)	5.32(4.28, 6.36)	-0.13(-1.72,1.46)	1.68	0.002*
Baseline Diameter (mm)	4.01(3.38, 4.63)	3.95(3.44, 4.46)	0.01(-0.36, 0.36)	3.74(3.22, 4.26)	3.92(3.58, 4.26)	0.08(-0.38, 0.55)	-0.17	0.77
Peak Diameter (mm)	4.20(3.52, 4.88)	4.29(3.73, 4.84)	0.14(-0.24, 0.51)	3.96(3.43, 4.48)	4.13(3.79, 4.46)	0.09(-0.39, 0.57)	0.08	0.87
Shear rate$_{AUC}$ ($s^{-1}\times10^3$)	19.3(9.9, 28.6)	15.0(9.6, 20.4)	-3.1(-7.5, 1.3)	14.4(10.8, 17.9)	14.3(7.2, 21.3)	-2.1(-7.9, 3.8)	-0.13	0.76
Time to peak (s)	68(44, 92)	53(37, 39)	-11(-26, 2.7)	54(32, 77)	41(24, 59)	-19.5(-37.5, -1.4)	0.33	0.47
GTN-mediated dilation (%) †	17.1(11.7, 22.4)	15.9(12.6, 19.2)	-0.8(-4.1, 2.5)	15.8(11.1, 20.4)	15.8(10.6, 21.1)	-0.5(-4.7, 3.7)	-0.05	0.99
GTN-mediated time to peak(s)	432(377, 486)	404(345, 463)	-33(-89, 22)	446(359, 534)	417(342, 492)	-18(-94, 57)	-0.16	0.74

Ex- Exercise group, CC- Conventional care group. Data are presented as mean (95% CI). Delta (Δ) change from pre-intervention following adjustment for pre-intervention values. †Variables analysed after logarithmic transformation. # Indicates analysis of data on *n*=10 Ex and *n*=7 CC. *Significant difference between ΔEx and ΔCC (*P*<0.05).

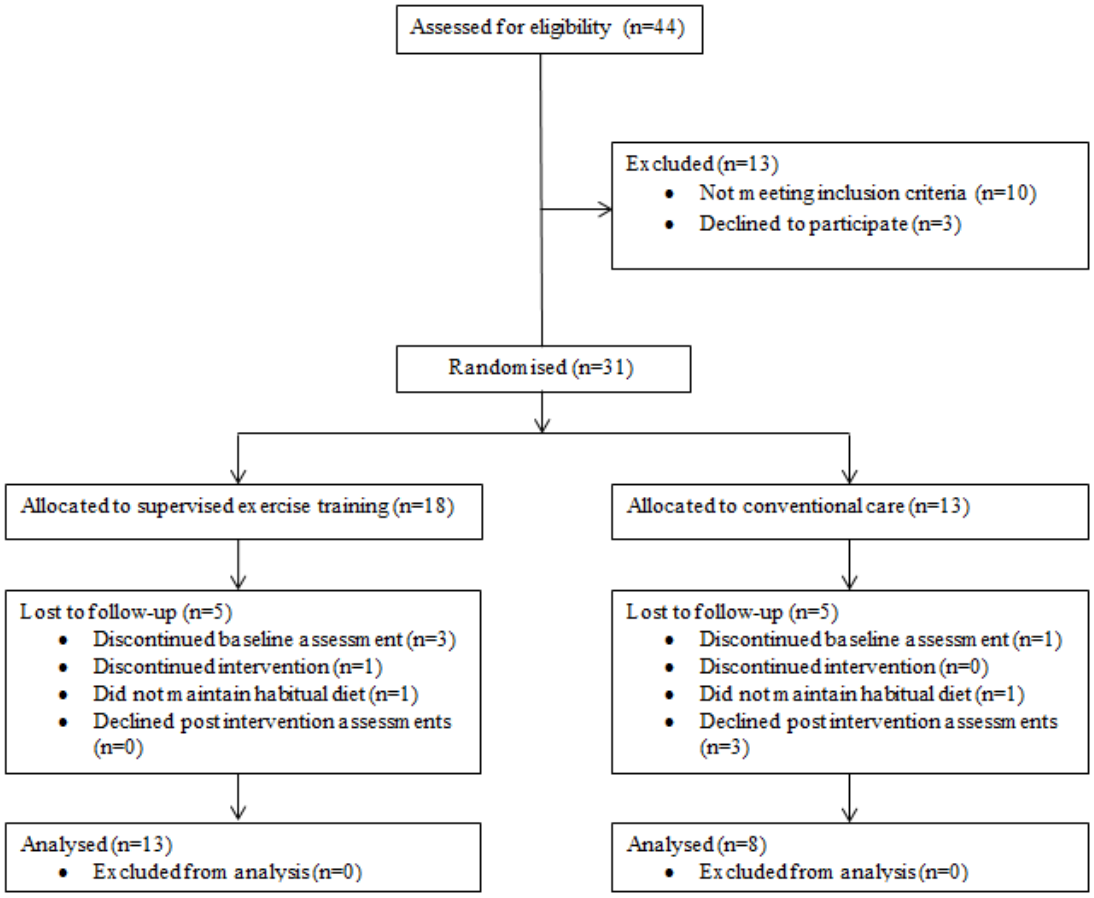

Figure. 1. Schematic representation of participant recruitment and group allocation.

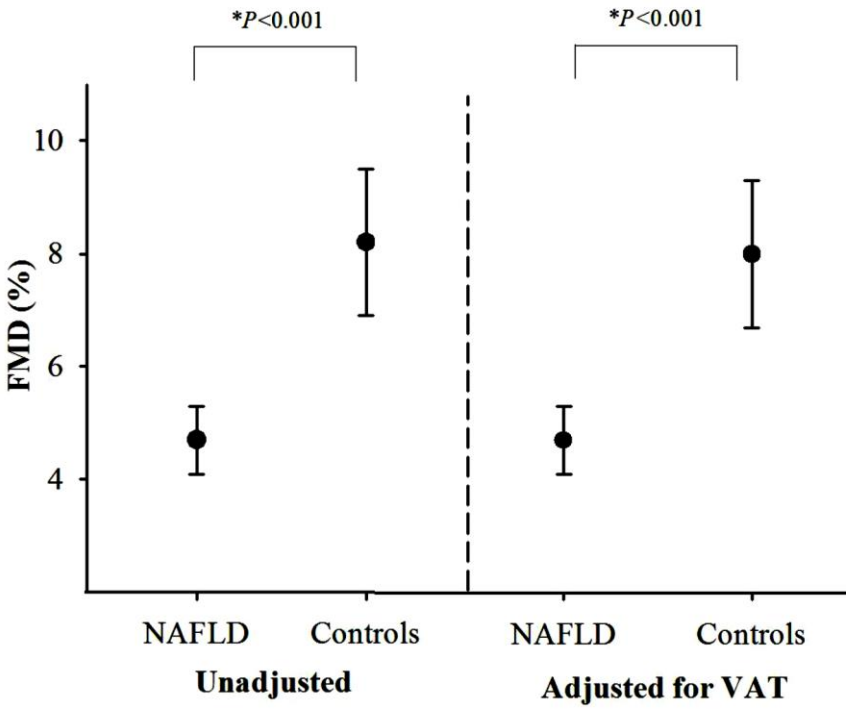

Figure. 2. Differences in FMD between NAFLD and control groups unadjusted and statistically adjusted for VAT. Data are presented as mean±SD. *Indicates significance between NAFLD and controls (P<0.05).

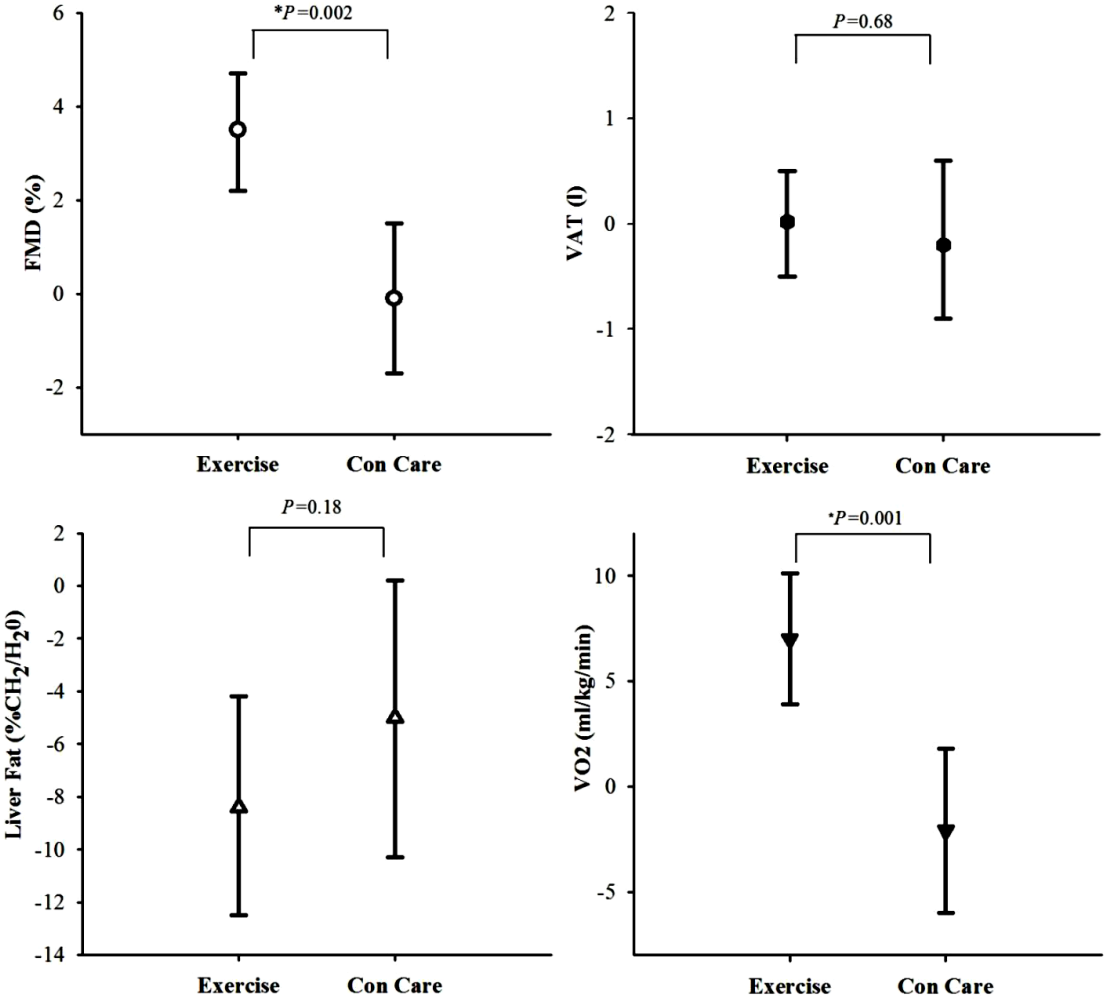

Figure. 3. Delta (Δ) change in FMD, cardiorespiratory fitness, liver fat and VAT following exercise training and conventional care. Data are presented as mean±SD. *Indicates significant difference between exercise and conventional care (P<0.05).