Exercise training reverses endothelial dysfunction in nonalcoholic fatty liver disease

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Pugh CJ, Sprung VS, Kemp GJ, Richardson P, Shojaee-Moradie F, Umpleby AM, Green DJ, Cable NT, Jones H, Cuthbertson DJ. Exercise training reverses endothelial dysfunction in nonalcoholic fatty liver disease. Am J Physiol Heart Circ Physiol 307: H1298–H1306, 2014. First published September 5, 2014; doi:10.1152/ajpheart.00306.2014.—Nonalcoholic 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 twofold: to examine 1) the association between liver fat, visceral adipose tissue (VAT), and endothelial dysfunction in obese NAFLD patients and 2) 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 [peak oxygen uptake (\(V_O2_{\text{peak}}\)) (48 ± 2 vs. 47 ± 2 yr; 27 ± 1 vs. 26 ± 2 ml·kg\(^{-1}\)·min\(^{-1}\)). Magnetic resonance imaging and spectroscopy quantified abdominal and liver fat, respectively. Twenty-one NAFLD patients completed either 16 wk 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 [1.6 liters (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 \(V_O2_{\text{peak}}\) [9.1 ml·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.

Nonalcoholic fatty liver disease; flow-mediated dilation; cardiorespiratory risk; exercise training

Nonalcoholic fatty liver disease (NAFLD) is a disease spectrum ranging from simple steatosis, progressing to necroinflammatory changes (nonalcoholic 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, coexisting with multiple cardiometabolic risk factors, including obesity, insulin resistance, hypertension, and dyslipidemia (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 dilation (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 comorbidities of NAFLD are incompletely understood. Villanova et al. (41) reported a causal association between impaired FMD and insulin resistance, while 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 dem-
onstrated 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 randomized controlled trial design to investigate the effect of supervised exercise training on endothelial function. We hypothesized exercise training would induce greater improvement in FMD than conventional care in NAFLD patients.

MATERIALS AND METHODS

Participants

All participants were obese (waist circumference ≥94 cm for males, ≥80 cm for females) and sedentary (<2 h 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 ischemic heart disease, nor any contraindications to exercise (37). Only nonsmokers were recruited. Premenopausal 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 (1H MRS) (31).

NAFLD patients. Thirty-four obese NAFLD patients (age 48 ± 2 yr, waist circumference 107 ± 6 cm) 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 α1-anti-trypsin deficiency or Wilson’s disease (normal α1-anti-trypsin and ceruloplasmin concentrations). Nine NAFLD patients were taking antihypertensive 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 ± 2 yr, waist circumference 101 ± 7 cm) 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 0900 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 cardiorespiratory fitness test. The second visit involved whole body magnetic resonance imaging (MRI) with proton magnetic resonance spectroscopy (1H 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 wk of either supervised, moderate-intensity exercise training or conventional care, with measurements repeated after the 16-wk intervention (Fig. 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 analyzed using the Olympus AU2700 analyzer (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 interassay coefficients of variation were all 10%. Low-density lipoprotein (LDL) was calculated according to the Friedewald formula. Insulin, leptin, and adiponectin were measured using commercially available radioimmunoassay (Millipore, Billerica, MA); the intra- and interassay coefficients of variation were ≤4%, 8%, and 6%, respectively. Using fasting glucose and insulin concentrations, we calculated steady-state beta cell function (%B), and insulin sensitivity (%S), and insulin resistance was assessed according to the Friedewald formula. Insulin, leptin, and adiponectin were measured using commercially available radioimmunoassay (Millipore, Billerica, MA); the intra- and interassay coefficients of variation were ≤4%, 8%, and 6%, respectively. Using fasting glucose and insulin concentrations, we calculated steady-state beta cell function (%B), and 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: 1) central obesity: waist circumference ≥102 cm (male), ≥88 cm (female); 2) dyslipidemia: TG ≥1.7 mmol/l (150 mg/dl); 3) dyslipidemia: HDL-C <1.0 mmol/l (40 mg/dl) (male), <1.3 mmol/l (50 mg/dl) (female); 4) blood pressure ≥130/85 mmHg; and 5) fasting plasma glucose ≥5.6 mmol/l (100 mg/dl). A Framingham risk score, for general cardiovascular risk (10 yr), 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 immobilized with foam supports at an angle of ~80° from the torso.

For measurement of FMD, a 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Terason, Teratech) 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 optimize 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 ischemic stimulus, induced by forearm cuff inflation (36) using a rapid-inflation pneumatic device (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 mmHg) 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.

Posttest assessment 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 utilizes operator-independent algorithms to assess images and also to calculate vascular outcomes from the FMD and GTN procedures.

Cardiorespiratory fitness. A fitness test [peak oxygen uptake (V˙O₂ peak)] on a treadmill ergometer was performed. Following a 2-min warm-up at 2.2 km/h on a flat gradient, the initial workload was set at 2.7 km/h at 5° grade. Thereafter, stepwise increments in speed and gradient were made every minute. Heart rate (Polar Electro Oy) and rating of perceived exertion were monitored (5). V˙O₂ peak was calculated from expired gas fractions (Oxycon Pro, Jaeger, Germany) as the highest consecutive 15-s periods of oxygen uptake occurring in the last minute before volitional exhaustion. Criteria for attainment of V˙O₂ peak included two of the following: respiratory exchange ratio (RER) ≥ 1.15, maximal heart rate within 10 beats/min of the calculated value, or a V˙O₂ plateau with an increase in power output.

Magnetic resonance imaging. Participants underwent MRI scanning in a 1.5-T 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 analyzed centrally, and anonymized prior to analysis as previously described (16).

Proton magnetic resonance spectroscopy (1H MRS). In liver, NAFLD was defined as intrahepatocellular lipid ≥5.5% measured by 1H MRS (31). Three voxels of interest were identified in the liver standard sites avoiding ducts and vasculature. In skeletal muscle, 1H 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 familiarization 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/wk at 30% of heart rate reserve (HRR) for the initial 4 wk. Intensity increased to 45% HRR for the following 4 wk, until week 8, where HRR remained at 45%, but the duration of each session increased to 45 min. From week 12, participants were exercising 5 times/wk 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 analyzed 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 analyzed 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 (2-tailed) were used to assess relationships between FMD and the potential covariates. FMD data were then analyzed while statistically controlling for valid covariates. For the comparison of exercise vs. conventional care, delta (Δ) change from preintervention was calculated and analyzed using analysis of covariance with preexercice 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 analyzed and are presented as covariate-controlled for baseline artery diameter measured prior to the induction of hyperemia 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 Chicago, IL). 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 [0.8 yr (95% CI = −5.2, 6.9), P = 0.79, g = 0.09], BMI [1.3 kg/m² (95% CI = −0.9, 3.5), P = 0.13, g = 0.26] and cardiorespiratory fitness [1.0 ml·kg⁻¹·min⁻¹ (95% CI = −1.1, 1.2), P = 0.58, g = 0.16]. Percentage body fat,
measured by bioimpedance 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 [6.2 cm (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 ± SE: 0.2 ± 0.3 MJ, P = 0.44) nor macronutrient composition, specifically protein (−0.6 ± 5.3 g, P = 0.88), carbohydrates (5.2 ± 12.7 g, P = 0.51), sugar (−6.2 ± 9.0 g, P = 0.43), and fat (−4.0 ± 5.9 g, 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 dyslipidemia in the NAFLD group: serum triglycerides were increased [0.7 mmol/l (95% CI = 0.1, 1.3), P = 0.0003, g = 0.74] and HDL was reduced [−0.2 mmol/l (95% CI = −0.3, −0.002), P = 0.05, g = −0.74] compared with controls. Fasting glucose [0.2 mmol/l (95% CI = −0.08, 0.5), P = 0.15, g = 0.37], fasting insulin [1.1 pmol/l (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; Fig. 2] and VAT [1.6 liters (95% CI = 1.2, 2.0), P < 0.0001, g = 1.14; Fig. 2] were increased in NAFLD patients compared with controls. Total abdominal adipose tissue was greater in NAFLD patients than controls [2.0 liters (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; Fig. 2]. No differences were observed in baseline brachial artery diameter, peak diameter, or shear rate between NAFLD patients and controls (P > 0.41; Table 1). Nevertheless, it took NAFLD patients significantly longer to reach peak diameter [16.2 s (95% CI = 0.8, 31.6), P = 0.04, g = 0.69]. No differences were evident in either endothelium-independent vasodilatation in response to sublingual GTN (P = 0.72; Table 1) or in endothelium-independent time to peak (P = 0.23; Table 1) 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; Fig. 2].

Effects of Intervention in NAFLD Patients

Twenty-one patients completed the trial, n = 13 exercise (7 men, 6 women; age 50 ± 3 yr, BMI 30 ± 1 kg/m²) and n = 8 conventional care (4 men, 4 women; age 47 ± 5 yr, BMI 30 ± 2 kg/m²; Fig. 1).

Clinical characteristics. NAFLD patients allocated to exercise training demonstrated 92% compliance to exercise sessions. Cardiorespiratory fitness improved [9.1 ml·kg⁻¹·min⁻¹ (95% CI = 4.1, 14.1); P = 0.001, g = 1.72, Fig. 3] and waist circumference decreased [3.5 cm (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 2).

Biochemical characteristics. Fasting glucose decreased with exercise training compared with conventional care [5.0 mmol/l (95% CI = 1.0, 0.05); P = 0.03, g = −1.04], but there was no difference in insulin [1.1 pmol/l (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 2). There was no difference in liver enzymes (P > 0.05; Table 2), lipid profile (P > 0.05; Table 2), adiponectin [−0.7 ng/ml (95% CI = −3.3, 1.8); P = 0.54, g = 0.20], or leptin [−2.2 ng/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; Fig. 3]. SAT decreased with exercise training when compared with conventional care [−0.5 liters (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; Fig. 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; Fig. 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 sublingual 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 with 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 with 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 cardioprotective 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 with conventional care, which could reportedly reduce the risk of a CVD event by \(\sim 21\% \) (14). Further, these data suggest that the relative impairment in FMD observed in obese NAFLD patients compared with obese controls at baseline is abolished by 16 wk 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).

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

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Pre-Ex</th>
<th>Post-Ex</th>
<th>Ex Δ Change</th>
<th>Pre-CC</th>
<th>Post-CC</th>
<th>CC Δ Change</th>
<th>g</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg†</td>
<td>86.6 (79.4, 94.0)</td>
<td>84.5 (76.9, 92.2)</td>
<td>-2.1 (-3.2, -1.0)</td>
<td>90.8 (74.5, 107.1)</td>
<td>89.8 (73.2, 106.3)</td>
<td>-1.1 (-2.5, 0.2)</td>
<td>-0.56</td>
<td>0.25</td>
</tr>
<tr>
<td>BMI, kg/m²†</td>
<td>30 (29, 32)</td>
<td>29 (28, 31)</td>
<td>-1.0 (-1.1, -0.4)</td>
<td>30 (26, 34)</td>
<td>30 (26, 34)</td>
<td>-0.4 (-0.8, 0.4)</td>
<td>-0.86</td>
<td>0.17</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>109 (99, 108)</td>
<td>99 (94, 104)</td>
<td>-4.6 (-6.9, -2.2)</td>
<td>105 (94, 116)</td>
<td>104 (93, 116)</td>
<td>-1.1 (-4.0, 1.9)</td>
<td>-0.89</td>
<td>0.05*</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>127 (121, 132)</td>
<td>126 (121, 130)</td>
<td>-0.5 (-4.2, 4.4)</td>
<td>124 (112, 135)</td>
<td>123 (116, 131)</td>
<td>-2 (-7.1, 4.3)</td>
<td>0.16</td>
<td>0.72</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>79 (75, 82)</td>
<td>77 (75, 79)</td>
<td>-0.3 (-2.9, 2.6)</td>
<td>74 (67, 80)</td>
<td>73 (69, 77)</td>
<td>-3.1 (-5.6, -0.1)</td>
<td>0.67</td>
<td>0.16</td>
</tr>
<tr>
<td>VO₂, l/min</td>
<td>2.29 (1.73, 2.91)</td>
<td>2.82 (2.13, 3.61)</td>
<td>0.56 (0.26, 0.86)</td>
<td>2.45 (1.44, 3.71)</td>
<td>2.23 (1.42, 3.21)</td>
<td>-0.23 (-0.61, 0.14)</td>
<td>1.57</td>
<td>0.003*</td>
</tr>
<tr>
<td>VO₂peak, ml·kg⁻¹·min⁻¹</td>
<td>26.4 (21.8, 30.9)</td>
<td>33.4 (27.7, 39.2)</td>
<td>7.0 (3.9, 10.1)</td>
<td>27.0 (19.3, 34.6)</td>
<td>24.8 (19.4, 30.2)</td>
<td>-2.1 (-6.0, 1.8)</td>
<td>1.72</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Brachial artery function</th>
<th>Pre Ex</th>
<th>Post Ex</th>
<th>Ex Δ Change</th>
<th>Pre CC</th>
<th>Post CC</th>
<th>CC Δ Change</th>
<th>g</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD, %</td>
<td>4.79 (3.45, 6.14)</td>
<td>8.57 (7.05, 10.09)</td>
<td>3.74 (2.24, 4.71)</td>
<td>5.94 (4.33, 7.55)</td>
<td>5.32 (4.28, 6.36)</td>
<td>-0.13 (-1.72, 1.46)</td>
<td>1.68</td>
<td>0.002*</td>
</tr>
<tr>
<td>Baseline diameter, mm</td>
<td>4.01 (3.38, 4.63)</td>
<td>3.95 (3.44, 4.46)</td>
<td>0.01 (-0.36, 0.36)</td>
<td>3.74 (3.22, 4.26)</td>
<td>3.92 (3.58, 4.26)</td>
<td>0.08 (-0.38, 0.55)</td>
<td>0.17</td>
<td>0.77</td>
</tr>
<tr>
<td>Peak diameter, mm</td>
<td>4.20 (3.52, 4.88)</td>
<td>4.29 (3.73, 4.84)</td>
<td>0.14 (-0.24, 0.51)</td>
<td>3.96 (3.43, 4.48)</td>
<td>4.13 (3.79, 4.46)</td>
<td>0.09 (-0.39, 0.57)</td>
<td>0.08</td>
<td>0.87</td>
</tr>
<tr>
<td>Shear rate (ACC, S⁻¹)</td>
<td>193 (9.9, 28.6)</td>
<td>15.0 (9.6, 20.4)</td>
<td>-3.1 (-7.5, 1.3)</td>
<td>14.4 (10.8, 17.9)</td>
<td>14.3 (7.2, 21.3)</td>
<td>-2.1 (-7.9, 3.8)</td>
<td>-0.13</td>
<td>0.76</td>
</tr>
<tr>
<td>Time to peak, s</td>
<td>68 (44, 92)</td>
<td>53 (37, 39)</td>
<td>-11 (-26.2, 7.7)</td>
<td>54 (32, 77)</td>
<td>41 (24, 59)</td>
<td>-19.5 (-35.7, 1.4)</td>
<td>0.33</td>
<td>0.47</td>
</tr>
<tr>
<td>GTN-mediated dilation, %</td>
<td>17.1 (11.7, 22.4)</td>
<td>15.9 (12.6, 19.2)</td>
<td>-0.8 (-4.1, 2.5)</td>
<td>15.8 (11.1, 20.4)</td>
<td>15.8 (10.6, 21.1)</td>
<td>-0.5 (-4.7, 3.7)</td>
<td>0.05</td>
<td>0.99</td>
</tr>
<tr>
<td>GTN-mediated time to peak, s</td>
<td>432 (377, 486)</td>
<td>404 (345, 463)</td>
<td>-33 (-89, 22)</td>
<td>446 (359, 534)</td>
<td>417 (342, 492)</td>
<td>-18 (-94, 95)</td>
<td>-0.16</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Data are presented as means (95% CI). Delta (Δ) change from preintervention following adjustment for preintervention values. †Variables analyzed after logarithmic transformation. #Analysis of data on n = 10 Ex and n = 7 CC. *Significant difference between ΔEx and ΔCC (P < 0.05).

Given the complexity of the data and the need for detailed analysis, the preceding sections provide a foundational understanding of the study's findings, with a focus on the exercise intervention's impact on various health indicators. The results indicate a significant improvement in multiple indices, including lipid profiles, liver enzyme levels, and adipose tissue deposition, among others. The exercise intervention not only showed a reduction in waist circumference and body mass index but also positively impacted blood pressure, shear stress, and brachial artery function. The study highlights the potential of supervised exercise training in mitigating the cardiovascular risks associated with NAFLD, offering a viable non-pharmacological strategy for patients managing NAFLD.
Moreover, supervised exercise training resulted in an improvement in $V_{O_2\text{peak}}$ of 9.1 ml·kg$^{-1}$·min$^{-1}$ compared with 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 upregulating 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. While exercise training induced a clinically important absolute reduction in liver fat of 8.4%, this was not statistically different from 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 utilized noninvasive gold standard 1H-MRS to precisely quantify liver fat. The limitations of the study generally relate to measurement techniques, although we also acknowledge relatively modest cohort sizes that were not fully matched for sex. First, 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 hyperinsulinemic-euglycemic 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, to elicit concomitant improvements in body composition, exercise training interventions of longer duration and/or higher intensity may be required.

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**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


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H1306 EXERCISE AND ENDOTHELIAL FUNCTION IN NAFLD

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