Exercise training improves cutaneous microvascular function in nonalcoholic fatty liver disease

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Pugh CJ, Cuthbertson DJ, Sprung VS, Kemp GJ, Richardson P, Umpleby AM, Green DJ, Cable NT, Jones H. Exercise training improves cutaneous microvascular function in nonalcoholic fatty liver disease. Am J Physiol Endocrinol Metab 305: E50–E58, 2013. First published May 7, 2013; doi:10.1152/ajpendo.00055.2013.—The leading causes of mortality in nonalcoholic fatty liver disease (NAFLD) relate to cardiovascular disease (CVD). The contribution of nitric oxide (NO) to endothelial function, a surrogate of CVD risk, is currently unknown in NAFLD. We hypothesize that NO-mediated cutaneous microvessel function would be impaired in NAFLD compared with controls and that exercise would enhance microvessel function compared with conventional care. Thirteen NAFLD patients (aged 50 ± 3 yr, BMI 31 ± 1 kg/m²) and seven controls (48 ± 4 yr, 30 ± 2 kg/m²) were studied. NAFLD patients were randomized to either 16 wk of exercise or conventional care. Cutaneous microvessel function was examined using laser Doppler flowmetry combined with intradermal microdialysis of G- monomethyl-L-arginine to assay the NO dilator response to local forearm heating. Magnetic resonance imaging and spectroscopy quantified abdominal and liver fat, respectively, and cardiorespiratory fitness was assessed. Differences in NO contribution to cutaneous blood flow between NAFLD and control individuals and between interventions were analyzed using general linear modeling. NO contribution to cutaneous blood flow was similar between NAFLD and controls (P = 0.47). Cardiorespiratory fitness was greater following exercise training compared with conventional care. NO contribution to cutaneous blood flow in response to heating at 42°C was 20.4% CVCmax (95% CI = 4.4, 36.4) greater following exercise training compared with conventional care (P = 0.02). Exercise training improves cutaneous microvascular NO function in NAFLD patients. The benefit of exercise training compared with conventional care strongly supports a role for exercise in the prevention of CVD in NAFLD.

nonalcoholic fatty liver disease; nitric oxide; microvascular function; exercise; exercise training; cutaneous microvessel function

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) is characterized by excess triglyceride accumulation in the liver and is associated with increased liver-related morbidity and mortality. Nevertheless, numerous epidemiological studies have revealed that cardiovascular disease (CVD) accounts for a greater number of deaths than that of liver disease in NAFLD patients (1, 30, 36), and some report CVD to be the leading cause of mortality (15, 30). These findings imply that NAFLD patients are at high risk of cardiovascular events. Endothelial dysfunction, characterized by diminished bioavailability of the antiatherogenic molecule nitric oxide (NO), is the earliest detectable manifestation in the development of atherosclerosis and a strong predictor of future CVD risk in both symptomatic and asymptomatic individuals (17). Although previous cross-sectional studies have shown that NAFLD patients exhibit impaired endothelial function in conduit arteries (34, 42), recent evidence suggests that the microcirculation may be the initial site of endothelial damage in those at risk of CVD (6). Therefore, it is plausible that microvascular dysfunction precedes any impairment found in the larger conduit arteries, suggesting that the microcirculation could be a useful early marker to investigate endothelial dysfunction in high-risk prediabetic patients.

Microvascular endothelial dysfunction can impact upon both peripheral vascular resistance and glucose disposal (21, 43), therefore potentially contributing to pathogenic complications observed in NAFLD such as insulin resistance, hypertension, and hypercholesterolemia (15). Cutaneous vasodilator function reflects generalized microvascular function since it can be used to interrogate endothelial vasodilator pathways (19). Cutaneous microvessel dysfunction correlates with coronary artery endothelial dysfunction (4, 28, 35) and is associated with several CVD risk factors, including obesity (13), hypertension (10, 33), hypercholesterolemia (27), and type 2 diabetes (9, 37, 44), all of which frequently manifest in NAFLD patients (15). Nevertheless, the aforementioned studies utilized iontophoresis of acetylcholine to assay cutaneous vasodilator function, a technique that has accepted limitations since the delivery method is inconsistent and the vasodilator response is not exclusively NO mediated (20).

Alternatively, intradermal microdialysis interrogates the cutaneous circulatory system with greater validity and specificity, allowing the infusion of potent NO blockers that enable the exclusive evaluation of the NO contribution to cutaneous microvessel vasodilatation (11). Given that NO-mediated macro- and microvessel function reflect aggregate atherogenic risk (17), intradermal microdialysis can be used as a surrogate measure of microvascular health and provides a translational model to investigate early CVD risk (19).

No previous study has investigated the cutaneous microcirculation as a model of microvascular disease in NAFLD patients.
Therefore, the aims of the present study were to 1) describe NO-mediated microvascular function in NAFLD patients and 2) utilize a randomized controlled intervention to investigate the effect of supervised exercise training on NO-mediated cutaneous microvascular function. We hypothesized that NO-mediated function would be impaired in NAFLD patients compared with healthy, matched control subjects and that exercise training would induce a greater improvement in NO-mediated function than conventional care in NAFLD subjects.

MATERIALS AND METHODS

Participants

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.

Thirteen NAFLD patients (7 males and 6 females, age 50 ± 3 yr, BMI 31 ± 1 kg/m²) were recruited from hepatology clinics at two tertiary referral centers, and seven control individuals matched for age and BMI (3 males and 4 females, age 48 ± 4 yr, BMI 30 ± 2 kg/m²) were recruited via local advertisement. The diagnosis of NAFLD was based on chronically elevated alanine aminotransferase (ALT) levels (>41 U/l for ≥6 mo) in the presence of an echobright liver on abdominal ultrasonography. Other causes of liver disease were excluded by complete laboratory investigation for viral hepatitis (hepatitis B and C), autoimmune hepatitis, primary biliary cirrhosis, and other metabolic liver diseases (hemochromatosis, α1-antitrypsin deficiency, Wilson’s disease). Hepatic steatosis was confirmed by the presence of ≥5.56% intrahepatocellular triglyceride content, determined by proton magnetic resonance spectroscopy. All participants were sedentary nonsmokers with no history of type 2 diabetes mellitus or excessive alcohol intake (i.e., average weekly consumption of >14 units for females and >21 units for males). None of the participants had any history of ischemic heart disease or any contraindications to exercise. Three NAFLD patients were taking antihypertensive medication (β-blockers, n = 1; calcium channel antagonists, n = 2). Medications were not altered during the course of the study. None of the controls were taking any prescribed medication. Premenopausal women (n = 2) were tested during the early follicular phase of the menstrual cycle, defined as days 1–7 following menstruation. The study conformed to the Declaration of Helsinki and was approved by the National (North West) Research Ethics Committee (United Kingdom). Participants were informed of the methods and study design verbally and in writing before providing written informed consent.

Research Design

Participants attended on two separate occasions to undertake baseline measurements. Visit 1 included anthropometric measurement, a fasting blood sample, assessment of cutaneous NO-mediated vasodilator function, and a cardiorespiratory fitness test. Visit 2 involved MRI and proton magnetic resonance spectroscopy (1H-MRS) to determine abdominal fat volume and liver fat content, respectively. Both visits were completed within 1 wk and were studied at the same time of day (0900) to control for the impact of circadian variation. Measurements were performed following an overnight fast, a 12-h abstinence from caffeine, and a 24-h abstinence from alcohol and strenuous exercise. NAFLD patients were then randomly assigned to either 16 wk of supervised and structured moderate-intensity exercise training or 16 wk of conventional clinical care. Eleven of the 13 NAFLD patients completed the 16-wk intervention period (exercise: n = 6, 45 ± 5 yr, BMI 31 ± 1 kg/m²; conventional care: n = 5, 51 ± 3 yr, BMI 30 ± 21 kg/m²), following which baseline measurements were repeated during visit 3 (Fig. 1).

Anthropometric and Biochemical Assessment

After a full history, a single person recorded all anthropometric measurements (weight, height, and waist and hip circumference).
Following this, a fasting blood sample was taken. All laboratory assays were performed in the clinical biochemistry laboratory at University Hospital Aintree. Plasma samples were analyzed using an Olympus AU2700 analyzer (Beckman Coulter) with standard proprietary reagents and methods as follows: glucose with hexokinase, total cholesterol and HDL with cholesterol esterase/oxidase, triglyceride with glycerol kinase, and liver enzymes, including alanine ALT, aspartate aminotransferase, and γ-glutamyltransferase with International Federation of Clinical Chemistry kinetic UV (without pyridoxal phosphate activation). The intra- and interassay coefficients of variation were ≤10%. LDL was calculated according to the Friedwald formula.

Maximal Oxygen Consumption Test

The fitness test (\(\dot{V}O_2\)peak) was performed on a treadmill ergometer and followed the Bruce protocol (7). Following a 2 min warmup, workload increased with stepwise increments in speed and gradient. Heart rate (Polar Electro Oy) and rate of perceived exertion were monitored throughout (5, 8). \(\dot{V}O_2\)peak was calculated from expired gas fractions (Oxycon Pro, Jaeger, Germany) as the highest consecutive 15-s period of gas exchange (\(\dot{V}O_2\)) data occurring in the last minute before volitional exhaustion.

MRI and Spectroscopy

MRI and \(^1\)H-MRS were performed in a 1.5-T Siemens Symphony scanner (Siemens Medical Solutions, Erlangen, Germany). Abdominal visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (SAT) were calculated from whole body axial T1-weighted fast-spin echo scans, as described previously (23). Liver fat pose tissue (SAT) were calculated from whole body axial T1-microdialysis probe insertion (15 min), as described previously. Upon arrival, participants were instrumented and cannulated for assessments were performed in a quiet, temperature-controlled laboratory. Therefore, data are presented as the NO contribution to cutaneous blood flow. Statistical Analysis

Exercise training intervention. Following a familiarization and induction session, participants were required to attend the university gymnasium on a weekly basis and were provided with full supervision and guidance from a trained exercise physiologist. Based on individual basal fitness level, participants underwent 30 min of moderate-intensity aerobic exercise three times a week at 30% of heart rate reserve, which progressed weekly based on HR responses. At week 12, participants were exercising five times a week for 45 min at 60% of their individually determined heart rate reserve. No dietary modifications occurred throughout the course of the exercise intervention, which was confirmed by the use of a standard food diary. Conventional care intervention. Conventional care consisted of typical lifestyle advice provided at clinical consultation. Participants were simply advised by their hepatologist or clinic nurse to modify their lifestyle by losing weight and increasing their physical activity. There was no supervision or guidance.

Statistical Analysis

Based on previously reported data on our primary outcome of NO contribution (2, 38), we estimated that a two-sample t-test with a 0.05 two-sided significance level would have 80% power to detect a difference in means of 19.6% \(\%\text{CVC}_{\text{max}}\), assuming that the common standard deviation of change score is 14.5% \(\%\text{CVC}_{\text{max}}\), when the sample size in the exercise group is 6. Analyses were performed using the Statistics Package for Social Sciences for Windows, version 17.0 (SPSS, Chicago, IL). Statistical significance was delimited at \(P < 0.05\), and exact \(P\) values are cited (\(P\) values of “0.000” provided by the statistics package are reported as “<0.001”). Data in the text are presented as means (95% confidence interval), unless otherwise stated. First, all data were explored for underlying distribution and transformed if appropriate. Logarithmically transformed data were back transformed to the original units. Differences in clinical characteristics between groups (NAFLD and controls) were compared using independent t-tests. Cutaneous blood flow data is presented as \(\%\text{CVC}_{\text{max}}\) and analysis was performed on this normalized data (11).

To ensure successful increase in NO production with the local heat stimulus and successful blockade of NO production, saline and \(l\)-NMA data (cross-sectional and interventional comparisons) were compared individually using a two-way repeated-measures analysis of variance (ANOVA) (site vs. temperature). A two-factor (group vs. temperature) repeated-measures ANOVA was also employed to compare the contribution of NO (saline \(\%\text{CVC}_{\text{max}}\) minus \(l\)-NMA \(\%\text{CVC}_{\text{max}}\) at equivalent time points) with \(\%\text{CVC}_{\text{max}}\) response between NAFLD patients and controls. Correlation coefficients (2-tailed) were used to describe the strength of relationships between the contribution of NO to \(\%\text{CVC}_{\text{max}}\) vasodilator response (at baseline: 33°C, peak; 42°C and prolonged: 42°C for 30 min) and clinical characteristics in NAFLD patients and controls. For the comparison of exercise vs. conventional care, delta (\(\Delta\)) change from preintervention was calculated and analyzed using analysis of covariance, with pre-exercise data as a covariate (41). Statistically significant interactions were assessed using the least significant difference approach to multiple comparisons (31).

RESULTS

NAFLD vs. Controls: Clinical Characteristics

NAFLD patients and controls were similar in age, BMI, and fitness; however, NAFLD patients exhibited an increased waist circumference \([106\% \text{ [95% confidence interval (CI) = 101}, 111]\) vs. 99 cm \([95\% \text{ CI} = 94}, 103]\), \(P = 0.05\); Table 1). NAFLD patients demonstrated a greater deposition of liver fat \([23.7\% \text{ (95\% CI = 15.8}, 35.4]\) vs. 2.8% \([95\% \text{ CI} = 1.7}, 4.6]\), \(P < 0.001\),
NAFLD vs. Controls: Cutaneous Blood Flow Response During Incremental Heating

In response to local heating, %CVC\textsubscript{max} increased steadily and significantly at the microdialysis site perfused with saline and the site perfused with L-NMMA in all participants ($P < 0.0005$; Fig. 2). However, %CVC\textsubscript{max} was significantly lower at the L-NMMA site in both NAFLD patients and controls ($P < 0.0005$; Fig. 2), suggesting that the response to local heating is partially mediated by the NO dilator system in both groups. A significant microdialysis site $\times$ temperature interaction was evident ($P < 0.001$; Fig. 2), and pairwise comparisons revealed significant differences between the saline and L-NMMA sites from 39.5°C to peak in NAFLD patients and from 40°C to peak in controls ($P < 0.05$).

NAFLD vs. Controls: NO Contribution to Cutaneous Blood Flow During Incremental Heating

There was no significant group $\times$ temperature interaction between NAFLD and controls in NO contribution (saline %CVC\textsubscript{max} - L-NMMA %CVC\textsubscript{max}) to cutaneous blood flow during incremental heating ($P = 0.47$; Fig. 2).

The NO contribution to cutaneous blood flow did not correlate with cardiorespiratory fitness at peak vasodilatation ($r = 0.11, P = 0.73$) or following prolonged heating ($r = 0.14, P = 0.47$).

Table 1. Characteristics of NAFLD ($n = 13$; 7 males and 6 females) and control participants ($n = 7$; 3 males and 4 females)

<table>
<thead>
<tr>
<th></th>
<th>NAFLD</th>
<th>Controls</th>
<th>$P$ Value</th>
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<tbody>
<tr>
<td><strong>Anthropometrics</strong></td>
<td></td>
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<tr>
<td>Age, yr</td>
<td>50 (44, 56)</td>
<td>48 (38, 57)</td>
<td>0.60</td>
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<tr>
<td>Weight, kg</td>
<td>88.6 (81.0, 96.3)</td>
<td>84.4 (74.6, 94.1)</td>
<td>0.46</td>
</tr>
<tr>
<td>BMI, kg/m\textsuperscript{2}</td>
<td>31 (29, 33)</td>
<td>30 (26, 34)</td>
<td>0.64</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>106 (101, 111)</td>
<td>99 (94, 103)</td>
<td>0.05*</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>128 (121, 134)</td>
<td>128 (118, 138)</td>
<td>0.98</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>78 (74, 82)</td>
<td>80 (72, 88)</td>
<td>0.54</td>
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<tr>
<td><strong>Body fat deposition</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver fat, %†</td>
<td>23.7 (15.8, 35.4)</td>
<td>2.8 (1.7, 4.6)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>VAT, liters†</td>
<td>4.6 (3.1, 4.4)</td>
<td>3.7 (3.1, 4.4)</td>
<td>0.08</td>
</tr>
<tr>
<td>SAT, liters</td>
<td>8.7 (7.1, 10.6)</td>
<td>8.5 (5.1, 11.9)</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Liver enzymes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT, U/l†</td>
<td>63 (46, 86)</td>
<td>25 (15, 44)</td>
<td>0.002*</td>
</tr>
<tr>
<td>AST, U/l†</td>
<td>40 (31, 52)</td>
<td>24 (17, 33)</td>
<td>0.02*</td>
</tr>
<tr>
<td>GGT, U/l†</td>
<td>75 (52, 98)</td>
<td>32 (16, 47)</td>
<td>0.01*</td>
</tr>
<tr>
<td><strong>Glucose and lipid profile</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Glucose, mmol/l†</td>
<td>5.1 (4.7, 5.6)</td>
<td>5.0 (4.5, 5.4)</td>
<td>0.52</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>5.6 (5.2, 6.1)</td>
<td>5.3 (4.5, 6.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>Triglyceride, mmol/l</td>
<td>2.5 (1.6, 3.4)</td>
<td>1.7 (0.9, 2.6)</td>
<td>0.23</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.3 (1.1, 1.4)</td>
<td>1.5 (1.3, 1.7)</td>
<td>0.05*</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>3.3 (2.8, 3.9)</td>
<td>2.9 (2.1, 3.6)</td>
<td>0.53</td>
</tr>
<tr>
<td>Cholesterol/HDL ratio†</td>
<td>4.5 (4.1, 4.9)</td>
<td>3.9 (3.2, 4.9)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Cardiorespiratory fitness</strong> (V\textsubscript{O2peak}, ml·kg\textsuperscript{-1}·min\textsuperscript{-1}†)</td>
<td>24.7 (21.5, 28.3)</td>
<td>29.3 (22.1, 38.9)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Data are presented as means (95% confidence intervals). NAFLD, nonalcoholic fatty liver disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; ALT, alanine aminotransferase; GGT, $\gamma$-glutamyltransferase; V\textsubscript{O2peak}, peak oxygen uptake. †Variables analyzed following logarithmic transformation.

*Significant difference between NAFLD and controls ($P < 0.05$).

elevated ALT [63 (95% CI = 46, 86) vs. 25 U/l (95% CI = 15, 44), $P = 0.002$], and reduced HDL [1.3 (95% CI = 1.1, 1.4) vs. 1.5 mmol/l (95% CI = 1.3, 1.7), $P = 0.05$] compared with controls (Table 1). VAT was elevated in NAFLD patients [4.6 (95% CI = 3.1, 4.4) vs. 3.7 liters (95% CI = 3.1, 4.4)], but this did not reach statistical significance ($P = 0.08$; Table 1). There was no difference in SAT between groups ($P > 0.05$; Table 1).

Fig. 2. The effect of incremental and prolonged heating on percent maximum cutaneous vascular conductance (%CVC\textsubscript{max}) in the saline and N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) microdialysis site in nonalcoholic fatty acid liver disease (NAFLD) patients (A) and controls (B) and the contribution of nitric oxide (NO); saline %CVC\textsubscript{max} - L-NMMA %CVC\textsubscript{max} in NAFLD patients and controls (C). Data are presented as means $\pm$ SE. *Significant difference between L-NMMA and saline sites ($P < 0.05$).
0.64) in NAFLD patients. Similarly, no correlation was evident in controls at peak vasodilatation \((r = -0.21, P = 0.65)\) or following prolonged heating \((r = -0.32, P = 0.48)\). NO contribution was not correlated with liver fat or VAT in NAFLD patients or controls \((P > 0.05)\).

**Intervention: Clinical Characteristics**

NAFLD patients who were randomly allocated to supervised exercise training demonstrated 94% compliance to exercise sessions. Following the intervention, cardiorespiratory fitness improved \([\Delta 0.1 (95\% CI = 5.0, 15.3) vs. \Delta 0.9 ml·kg\(^{-1}\)·min\(^{-1}\) (95\% CI = 6.5, 4.8); \(P = 0.01)\], and ALT was reduced \([\Delta -34 (95\% CI = -48, -20) vs. -12 U/l (95\% CI = -27, 3), \(P = 0.04)\] in the exercise-trained group compared with the conventional care group. However, there was no difference between the effect of exercise training and conventional care on BMI, waist circumference, liver fat, adipose tissue, plasma lipid profile, or plasma glucose concentration \((P > 0.05\); Table 2).

**Intervention: Cutaneous Blood Flow Response During Incremental Heating**

Prior to and following both interventions, \%CVC\(_{\text{max}}\) increased significantly in response to local heating at both the saline and L-NMMA sites \((P > 0.0005\); Fig. 3 and 4). Significant interactions \((\text{site} \times \text{temperature})\) between the saline and L-NMMA microdialysis sites both pre- and postintervention were evident \((P > 0.0005\); Fig. 3 and 4). Pairwise comparisons revealed that \%CVC\(_{\text{max}}\) was significantly lower in the L-NMMA site compared with saline between 39 and 42°C preexercise and from 36.5°C to peak postexercise \((P < 0.05\); Fig. 3). Similarly, \%CVC\(_{\text{max}}\) was significantly lower in the L-NMMA site between 39°C and peak preconventional care and 40°C to peak postconventional care \((P < 0.05\); Fig. 4).

**Intervention: NO contribution to Cutaneous Blood Flow During Incremental Heating**

A significant interaction \((\text{intervention} \times \text{temperature})\) in NO contribution to \%CVC\(_{\text{max}}\) was evident in the exercise-trained group \((P = 0.01\); Fig. 3). Subsequent pairwise comparisons revealed differences in NO contribution to \%CVC\(_{\text{max}}\) from 37.5 \([6.7\% \text{CVC}_{\text{max}} (95\% \text{ CI} = 0.6, 12.9), P = 0.04]\) to 42°C \([20.4\% \text{CVC}_{\text{max}} (95\% \text{ CI} = 4.4, 36.4), P = 0.02]\). No interaction in the conventional care group was observed \((P = 0.99\); Fig. 4).

**Comparison of Exercise Training vs. Conventional Care**

There were no significant differences between exercise and conventional care in the individual saline or L-NMMA microdialysis sites \((P > 0.13)\). Nevertheless, a significant interaction was evident for NO contribution to \%CVC\(_{\text{max}}\) \((P = 0.01)\). Pairwise comparisons revealed that the exercise group demonstrated a significantly greater NO response to incremental heating from 37.5 \([11.0\% \text{CVC}_{\text{max}} (95\% \text{ CI} = 0.8, 21.1), P = 0.04]\) to 42°C \([33.6\% \text{CVC}_{\text{max}} (95\% \text{ CI} = 14.0, 53.2), P = 0.004\]; Fig. 5).

**Maximal Cutaneous Vascular Responses During Infusion of 56 mM SNP at 42°C**

Cutaneous blood flow during the 30-min infusion of 56 mM SNP combined with local heating at 42°C was similar between the L-NMMA and saline microdialysis sites in NAFLD patients \([-0.02 \text{CVC} (95\% \text{ CI} = -0.5, 0.5), P = 0.94]\) and controls \([-0.16 \text{CVC} (95\% \text{ CI} = -0.6, 0.3), P = 0.46]\) at baseline. Neither intervention altered maximal cutaneous conductance at either the L-NMMA or saline sites \((P > 0.05)\).

**DISCUSSION**

This is the first study to explore cutaneous microvascular NO-mediated vasodilator function in NAFLD patients.
Systemic microvascular endothelial dysfunction is a key component in the inherent pathogenic complications associated with diseases such as type 2 diabetes, hypertension, and hypercholesterolemia (12, 21). Therefore, an independent assessment of the health of the microvasculature in clinical prediabetic populations such as NAFLD is valuable. The cutaneous principal finding was that supervised exercise training induced an increase in cutaneous NO-mediated vasodilator function in response to local heating compared with the conventional care intervention. These findings suggest that supervised exercise training is an effective management strategy capable of improving NO-mediated cutaneous microvascular function, thus reducing the intrinsic risk of CVD, in NAFLD patients.

**Fig. 3.** The effect of incremental and prolonged heating on %CVC\textsubscript{max} in the saline and \textit{l}-NMMA microdialysis site in NAFLD patients pre- (A) and postexercise training (B) and the contribution of NO (saline minus \textit{l}-NMMA) pre- and postexercise training (C). Data are presented as means ± SE. *Significant difference between \textit{l}-NMMA and saline sites (P < 0.05).

**Fig. 4.** The effect of incremental and prolonged heating on %CVC\textsubscript{max} in the saline and \textit{l}-NMMA microdialysis site in NAFLD patients pre- (pre-con care; A) and postconventional care (post-con care; B) and the contribution of NO (saline minus \textit{l}-NMMA) pre- and post-con care (C). Data are presented as means ± SE. *Significant difference between \textit{l}-NMMA and saline sites (P < 0.05).
Significant difference between exercise and conventional care (P < 0.05).

Several previous studies have demonstrated these changes were not statistically significant compared with visceral fat. Although we observed reductions in both liver fat and adipose tissue volumes (22), we cannot exclude potential contributing effects from these variables on NO microvessel function. Elevated liver fat is an important index of hepatic insulin resistance (3), and visceral fat is intimately associated with insulin resistance, type 2 diabetes risk, and adverse cardiovascular outcomes (34–37). Furthermore, visceral fat is considered a pivotal feature in the pathogenesis of NAFLD, as it is a key source of circulating adipokines, which are also predictive of CVD (14, 32). We also cannot exclude the impact that potential changes in insulin resistance may have had on enhanced NO microvessel function following exercise training, as insulin was not measured in the current study.

Mechanistically, it is also plausible that exercise had a direct therapeutic impact on the cutaneous microvasculature. Previous studies indicate that the episodic increases in shear stress associated with repeated bouts of exercise may be one potential stimulus that enhances NO-mediated vasodilator function (16). Shear stress upregulates endothelial NO synthase production in conduit arteries (18), and restricting the increase in shear stress during exercise prevents improvements in conduit and cutaneous microvascular function (16). Although the current data suggest that exercise training upregulates the NO contribution to local heating, peak vasodilator capacity remained similar following the different treatment regimes. Indeed, the conventional care group elicited a peak vasodilator response similar to that of the exercise group despite an apparent reduction in the contribution of NO. This suggests that compensatory vasodilator mechanisms, for example, prostacyclins, may preserve peak vasodilator responses in the presence of impaired NO-mediated microvascular function. In contrast, exercise training increased NO bioavailability, which may modulate the balance between NO and alternative vasodilatory pathways, causing compensatory vasodilator mechanisms to become redundant. Indeed, the presence of redundancy in vasomotor control is well recognized in other arterial beds (24).

Surprisingly, we observed negligible differences in NO-mediated cutaneous microvessel function between NAFLD patients (prior to randomized intervention) and control individuals of similar age, BMI, and fitness. A previous study using similar microdialysis methodology suggested that NO-mediated cutaneous microvessel dysfunction in healthy individuals was apparent in older subjects and/or those with decreased fitness (2). Given that both the NAFLD and control participants in this study could be classified in the “older” age category, the impact of age on NO microvessel function cannot be discounted. In support of this, Sokolnicki et al. (37) reported no difference in the contribution of NO to cutaneous blood flow during local heating in type 2 diabetic patients and controls of similar age (older individuals) despite differences in BMI being evident between the groups. Clearly, advancing age is an important contributor to microvessel dysfunction and associated CVD risk factors such as hypertension (1, 12). Alternatively, the lack of difference in NO microvessel function could be explained by similar lifestyle practices (i.e., sedentary behavior and obesity). Although we did not observe a causal relationship between cardiorespiratory fitness and NO microvessel function in either group, VO2 peak was similar.
between NAFLD patients and controls. Indeed, compared with normative population-based VO2peak data of healthy individuals of a similar age, both NAFLD and control individuals represent the 20th percentile (25), inferring that both groups adopted a similar excessive sedentary lifestyle. Similarly, BMI did not differ between groups, and both NAFLD and control individuals are classified as obese (BMI $\geq 30$ kg/m$^2$). These data suggest that the intricacies between age and sedentary behavior in diseased (e.g., insulin-resistant) populations require further exploration.

There are a number of noteworthy methodological issues that warrant consideration. Intradermal microdialysis is recognized as the optimal technique (11) to elicit NO blockade, allowing specific evaluation of NO-mediated cutaneous microvessel function during incremental heating. Previous training studies have demonstrated that exercise improves acetylcholine-mediated vasodilatation, which infers an increase in NO production. Although acetylcholine is a precursor of vasodilatation, it is not exclusive to NO, as it accounts for only $\sim$30–60% of the NO-mediated vasodilator response (26). Furthermore, these studies utilized the iontophoresis approach, which has well-accepted limitations (11). Although our patients had unequivocal clinical, biochemical, and radiological evidence of NAFLD, they did not undergo a liver biopsy to provide histopathological staging on the NAFLD disease spectrum. Consequently, it was not possible to differentiate between simple steatosis, characterized by fat infiltration, and nonalcoholic steatohepatitis, characterized by necroinflammatory changes. Finally, a measurement of peripheral insulin resistance was not incorporated within the study, which is clearly an area for future research given the close relationship between insulin resistance and NAFLD and the risk of CVD.

In summary, the findings from this study suggest that exercise training can enhance microvascular function in NAFLD patients via an increase in the bioavailability of the antiatherogenic molecule NO. The therapeutic effects of the supervised exercise training we adopted in the present study were superior to the impact of contemporary conventional care and infer a reduction in CVD risk.

GRANTS

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DISCLOSURES

The authors have no conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

C.J.P., V.S.S., G.J.K., and H.J. performed the experiments; C.J.P., V.S.S., G.J.K., and H.J. performed the experiments; C.J.P., V.S.S., G.J.K., and H.J. analyzed the data; C.J.P., D.J.C., V.S.S., A.M.U., D.J.G., N.T.C., and H.J. interpreted the results of the experiments; C.J.P., V.S.S., and H.J. prepared the figures; C.J.P. and H.J. drafted the manuscript; C.J.P., D.J.C., V.S.S., G.J.K., P.R., A.M.U., D.J.G., N.T.C., and H.J. edited and revised the manuscript; C.J.P., D.J.C., V.S.S., G.J.K., P.R., A.M.U., D.J.G., N.T.C., and H.J. approved the final version of the manuscript; D.J.C., A.M.U., D.J.G., N.T.C., and H.J. contributed to the conception and design of the research.

REFERENCES


23. Jones H, Sprung VS, Pugh CJ, Daousi C, Irwin A, Aziz N, Adams VL, Thomas EI, Bell JD, Kemp GJ, Cuthbertson DJ. Polycystic ovary syndrome with hyperandrogenism is characterized by an increased risk of hepatic steatosis compared with nonhyperandrogenic PCOS phenotypes.


