Exercise-induced improvement in endothelial dysfunction is not mediated by changes in CV risk factors: pooled analysis of diverse patient populations

Daniel J. Green, Jennifer H. Walsh, Andrew Maiorana, Matthew J. Best, Roger R. Taylor, and J. Gerard O’Driscoll. 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–H2687, 2003. First published August 21, 2003; 10.1152/ajpheart.00519.2003.—We have pooled data from a series of our exercise training studies undertaken in groups with a broad range of vascular (dys)function to the examine the hypothesis that exercise-induced improvements in the conduit and/or resistance vessel function are related to improvements in risk factors for cardiovascular (CV) disease. Endothelium-dependent and -independent conduit vessel function were assessed by using wall tracking of high-resolution ultrasound images of the brachial artery response to flow-mediated dilation (FMD) and glyceryl trinitrate. Resistance vessel function was assessed using intrabrachial administration of acetylcholine (ACh), sodium nitroprusside, and 3-$\text{N}^\text{G}$-monomethyl-L-arginine. Randomized cross-over studies of 8-wk exercise training were undertaken in untreated hypercholesterolemic (n = 11), treated hypercholesterolemic (n = 11), coronary artery disease (n = 10), chronic heart failure (n = 12), Type 2 diabetic (n = 15), and healthy control subjects (n = 16). Exercise training did not significantly alter plasma lipids, blood pressure, blood glucose, waist-to-hip ratio, or body mass index values, despite significant improvement in both FMD and ACh responses. There were no correlations between changes in any risk factor variables and indexes of either resistance or conduit vessel function. We conclude that, in these subjects with antecedent vascular dysfunction, the beneficial effects of relatively short-term exercise training on vascular function are not solely mediated by the effects of exercise on CV risk factors.

NITRIC OXIDE (NO), released from the vascular endothelium, is a potent vasodilator substance that possesses a myriad of antiatherogenic properties. Several lines of evidence suggest that endothelial dysfunction is an early event in the atherosclerotic process; endothelial dysfunction is present in subjects with cardiovascular risk factors (3, 4, 7, 11) and disease (36, 50), and interventions that improve cardiovascular mortality and morbidity are also associated with improved endothelial function (20, 28, 41, 42). In addition, recent studies (1, 15, 44, 51) indicate that endothelial dysfunction in coronary and also peripheral arteries predicts cardiovascular events. Because some evidence suggests that NO contributes to exercise hyperemia (10, 22), recent studies in humans have investigated the possibility that repeated bouts of exercise upregulate the NO dilator system and improve endothelial function (5, 8, 16, 17, 19, 23, 24, 29–31, 52, 53).

The mechanisms responsible for the beneficial effects of exercise training on endothelial function are controversial. Exercise training has been variably reported to improve several risk factors for cardiovascular disease, such as hypercholesterolemia, obesity, glycemic control, and hypertension, factors that are also associated with endothelial dysfunction. Some early studies of exercise training in humans suggested that the improvement in endothelial function observed was secondary to amelioration of these coincident risk factors (25). An alternate explanation is that repeated exposure of the vasculature to increased shear stress, a primary physiological stimulus to NO production, may explain upregulation of the NO-dilator system (39). In the present study, we aimed to examine the hypothesis that exercise-induced improvement in conduit and/or resistance vessel endothelial function is associated with improvement in risk factors for cardiovascular disease.

METHODS

Subjects

A list of subject groups and their baseline characteristics are reported in Table 1. Inclusion criteria required untreated hypercholesterolemic subjects (UTHC; n = 11) to have an initial total cholesterol of >6.5 mmol/l and/or a low-density lipoprotein (LDL) of >4.0 mmol/l, and none were taking any...
medication. Treated hypercholesterolemic subjects (THC; \( n = 11 \)) were taking a HMG-CoA reductase inhibitor in a stable dose for at least 3 mo (9 on atorvastatin, 1 simvastatin, and 1 cerivastatin) and had documentation that the total cholesterol was \( >6.5 \text{ mmol/l} \) and/or LDL \( >4.0 \text{ mmol/l} \) before treatment. Four THC subjects were also taking aspirin; 1 amlodipine (subject was normotensive for study duration) and 1 constant dose of estradiol. Coronary artery disease (CAD) subjects \( (n = 10) \) had CAD requiring surgical (coronary artery bypass grafting) or nonsurgical revascularization (percutaneous transluminal coronary angioplasty). All were taking aspirin, 9 were on HMG-CoA reductase inhibitor (statin) therapy, 7 were on \( \beta \)-blocking therapy, 5 were on an angiotensin-converting enzyme (ACE) inhibitor, 2 were on a proton pump inhibitor, and 1 each was on a diuretic, a calcium channel blocking drug, and cholestyramine. Chronic heart failure (CHF) subjects \( (n = 12) \) were all classified between New York Heart Association class I and class III, possessed left ventricular ejection fraction fraction of 26 ± 3%, and did not have overt evidence of congestive (right heart) failure at the time of study. Eleven CHF patients were taking ACE inhibitors; 8 were on aspirin; 7 were on warfarin; 6 took a diuretic; 4 took digoxin; 5 were on statin therapy; 3 were taking a nitrate; 3 were taking a potassium supplement; 2 were on carvedilol; and 2 were on an antiarrhythmic drug. All but 1 of the Type 2 diabetic subjects \( (T2D; n = 15) \) were taking oral hypoglycemic medication; 5 were taking an ACE inhibitor; 2 were on statin therapy, and 2 were taking aspirin. None had evidence of micro- or macrovascular disease. None of the healthy control subjects \( (CON; n = 16) \) were taking medication. For all subjects taking medication, treatment did not alter throughout the study period. All subjects were recruited from hospital clinics or via public advertisement.

Subjects were excluded if they were current smokers, hypertensive \( \text{[resting blood pressure (BP)]} > 160/90 \text{ mmHg} \); hypercholesterolemic \( \text{(total cholesterol} > 6.0 \text{ mmol/l or LDL} > 4.0 \text{ mmol/l}}; \text{except the UTHC subgroup)} \); diabetic \( \text{(except the T2D group)} \); asthmatic; displayed evidence of coronary or valvular heart disease from history, examination, and exercise electrocardiography \( \text{(except the CAD and CHF subgroups)} \); performed \( >5 \) sessions of light-moderate exercise per week; or were unable to exercise due to physical limitations. No subject had undergone a surgical procedure within the 3 mo preceding the study. The Royal Perth Hos-

### Table 1. Baseline characteristics of untreated and treated HC, CAD, heart failure, and Type 2 diabetic patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Untreated HC</th>
<th>Treated HC</th>
<th>CAD</th>
<th>Type 2 Diabetes</th>
<th>Heart Failure</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>15</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Age, yr</td>
<td>50 ± 3</td>
<td>55 ± 2</td>
<td>55 ± 2</td>
<td>51 ± 2</td>
<td>60 ± 2</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>26.7 ± 1.1</td>
<td>26.9 ± 1.0</td>
<td>28.8 ± 1.2</td>
<td>30.4 ± 1.0</td>
<td>28.7 ± 1.1</td>
<td>27.3 ± 1.1</td>
</tr>
<tr>
<td>Waist/hip, %</td>
<td>0.89 ± 0.02</td>
<td>0.87 ± 0.03</td>
<td>0.85 ± 0.02</td>
<td>1.00 ± 0.02</td>
<td>1.04 ± 0.02</td>
<td>0.91 ± 0.02</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total, mmol/l</td>
<td>6.3 ± 0.1</td>
<td>4.6 ± 0.3</td>
<td>4.3 ± 0.4</td>
<td>4.6 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>4.2 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.3</td>
<td>2.4 ± 0.2</td>
<td>2.8 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>5.1 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.0 ± 0.2</td>
<td>11.2 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycated hemoglobin, %</td>
<td>5.22 ± 0.10</td>
<td>5.23 ± 0.10</td>
<td>5.21 ± 0.10</td>
<td>8.55 ± 0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>86 ± 4</td>
<td>82 ± 2</td>
<td>90 ± 4</td>
<td>103 ± 2</td>
<td>84 ± 3</td>
<td>88 ± 3</td>
</tr>
<tr>
<td>Resting HR, beats/min</td>
<td>56 ± 2</td>
<td>61 ± 3</td>
<td>55 ± 2</td>
<td>70 ± 3</td>
<td>69 ± 4</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>( V_{O2 \text{ peak}}, \text{ml-kg}^{-1} \cdot \text{min}^{-1} )</td>
<td>30.4 ± 2.4</td>
<td>26.1 ± 1.5</td>
<td>27.0 ± 1.7</td>
<td>22.3 ± 1.1</td>
<td>19.5 ± 1.2</td>
<td>28.2 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of subjects. HC, hypercholesterolemia; CAD, coronary artery disease; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; MAP, mean arterial pressure; HR, heart rate; \( V_{O2 \text{ peak}} \), peak \( O_2 \) uptake.

### Study Design

Study designs and assessment techniques for each group are described in individual papers and were almost identical \((30–32, 52, 53)\). After preliminary screening and baseline assessments, subjects were randomly assigned to remain sedentary or perform exercise training for 8-wk periods, followed by crossover. The exercise training protocol and assessment procedures are outlined below. Subjects were requested to make no changes to their diet, therapy, or other routines for the duration of the study. Interventions commenced within 7 days of the completion of baseline assessments, and all repeat assessments, including resistance and conduit vessel function, were performed with 7 days of the cessation of exercise training or control periods.

### Assessment of Vascular Function

Vascular function assessments were conducted in a quiet, temperature-controlled environment at separate attendances if both conduit and resistance vessel function were assessed. Repeat investigations were performed at the same time of day for individual subjects. Subjects fasted for 8 h, abstained from alcohol and caffeine for 12 h, and did not perform any exercise for 24 h before assessments. Conduit vessel function was assessed by flow-mediated dilation (FMD) of the brachial artery in all UTHC, THC, CAD, and T2D subjects. Forearm resistance vessel function was examined by plethysmography in 10 UTHC, 10 THC, 8 CAD, and all T2D, CHF, and CON subjects.

### Assessment of Conduit Vessel Function

As the subject rested in the supine position, the nondominant arm was extended and immobilized with foam supports explained on the imaged arm immediately distal to the olecranon.
process to provide a stimulus to forearm ischemia (6). A 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Aspen; Acuson, CA) was used to image the brachial artery in the distal third of the upper arm. When an optimal image was attained, the probe was held stable in a stereotactic clamp. Ultrasound parameters were set to optimize longitudinal, B-mode images

After a 20-min rest, baseline images were recorded on a S-VHS video cassette recorder (SVO-9500 MDP, Sony; Tokyo, Japan) over 2 min. The forearm cuff was then inflated to 200 mmHg for 5 min. Images were recorded 30 s before cuff deflation and for 2 min after deflation. After a 10-min rest, to allow arterial diameter to return to baseline, another 2-min baseline recording was made before a sublingual 400-µg spray dose of glyceryl trinitrate (GTN) with images recorded for a further 5 min.

Brachial artery diameters were analyzed using customized edge detection and wall-tracking software, which minimizes investigator bias and has the power to detect an absolute change in FMD of 2% in a cross-over design study with only six subjects (54). Briefly, an edge-detection algorithm averages >300 diameter measurements per frame, with 20–30 frames assessed per second. Those average diameter measures that coincide with the ECG R wave (also autodetected), that is, occurring at end diastole, were subsequently analyzed using a third-order polynomial curve (54). FMD and GTN responses were then calculated from the peak value derived from this polynomial curve and related to the average of all R wave-gated diameters collected during the baseline period preceding either the FMD or GTN manipulations. The mean intraobserver coefficient of variation of repeated measures of FMD using this software is 6.7%, which is significantly lower than that for traditional manual methods (54).

Assessment of Resistance Vessel Function

While the subject was in the supine position, a 20-gauge cannula (Arrow) was inserted into the brachial artery of the nondominant arm, under local anesthesia with <2 ml of 1% lignocaine, to infuse vasoactive agents and sterile saline and for blood sampling and measurement of intra-arterial pressure. Subjects were then positioned with elbows at heart level and hands at a comfortable height to allow forearm venous drainage. Pneumatic cuffs (SC10 and SC5, D. E. Hokanson) and strain gauges (SG 24, Medasonics; Fremont, CA) were positioned for forearm blood flow (FFB) measurements. Wrist and upper arm cuffs were connected to rapid inflation devices (E-20 and AG 101, Hokanson); strain gauges were positioned 8–10 cm distal to the olecranon process of each arm. Strain-gauge placement and hand and elbow elevation were the same for repeat tests. An online microcomputer (SPG 16, Medasonics) sampled amplified output from the strain gauges at 75 Hz, which was displayed in real time. A software program controlled cuff inflation/deflation as well as data acquisition, storage, and display to ensure blood flow measurements were synchronized with upper arm cuff inflation.

Arterial pressure was monitored continuously with a Hewlett-Packard 78353A monitoring system. Acetylcholine (ACH, Miochol-Ciba Vision; New South Wales, Australia) was infused at 10, 20, and 40 µg/min, each for 3 min, and sodium nitroprusside (SNP, David Bull Laboratories; Victoria, Australia) at 2, 4, and 8 µg/min, each for 3 min using a constant-rate infusion pump (IVAC 770). Nω-monomethyl-L-arginine (L-NMMA, Cinalfa; Laufelfingen, Switzerland) was infused at 2, 4, and 8 µmol/min, each for 4 min. All solutions were prepared aseptically immediately before infusion.

The study protocol was identical for each subject. Baseline measurements were made 20 min after cannulation. Blood flow measurements were made after inflation of the wrist cuffs to 200 mmHg to exclude the hands from the circulation and by rapidly inflating the upper arm cuffs to 45 mmHg to occlude venous flow for 10 s out of every 15 s during baseline and drug infusion periods. For each data collection period, the last five measurements of FBF were averaged to give a representative flow for that period. There was a minimum of a 10-min rest between ACh and SNP infusions and 15 min between SNP and L-NMMA infusions. The latter was infused last because of its more prolonged duration of action.

Assessment of Maximal Exercise Capacity

VO₂ peak, HR, BP, rate-pressure product, and exercise duration (in s) were determined from a graded maximal exercise test that was performed on an electronically braked bicycle ergometer (Orival 400, Lode). Initial resistance was set between 20 and 60 W and increased in 20- to 25-W increments, depending on subject ability, every 3 min until fatigue or termination, according to standard indications for stopping an exercise test (12). HR and rhythm were continuously recorded by 12-lead electrocardiogram.

Volumetric oxygen consumed (VO₂) and carbon dioxide produced (VCO₂) during exercise were calculated from minute ventilation and measured by using mass flow ventilometry and simultaneous mixing chamber analysis of expired gas fractions. Gas analyzers and flow probes were calibrated before each test. VO₂ and VCO₂ (expressed in l/min and ml·kg⁻¹·min⁻¹) were recorded during the final 40 s of each stage of the test. VO₂ peak was calculated as the average of the two highest consecutive 20-s periods of gas exchange data occurring in the last minute before volitional exhaustion, which was generally due to leg fatigue or breathlessness. Each subject performed a familiarization VO₂ peak test before their definitive VO₂ peak assessment at each phase of testing.

 Anthropometric Assessment

Body weight and height were measured before each exercise test, and body mass index was calculated. Skinfolds were measured using spring-loaded calipers (Harpenden) at eight standard sites using an anthropometric steel tape (Luftkin): relaxed arm, flexed arm, waist, hip, thigh, and the waist-to-hip ratio (waist/hip) was calculated.

Assessment of Muscular Strength

Maximal isotonic voluntary contractile strength (MVC) was assessed for seven distinct muscle groups using the one repetition maximum (1 RM) technique and custom-designed pin-loaded weight stack resistance equipment (Pulsestar; Cheshire, UK), with minimum 2.5-kg increments. These machines were also used during the exercise training program (see Exercise Training Protocol). The seven resistance exercises consisted of the following: dual-seated leg press, left and right hip extension, pectoral exercises, shoulder extension, seated abdominal flexion, and dual leg flexion. Subjects were instructed in correct lifting technique to avoid Valsalva maneuver and hand gripping. MVC was calculated as the sum of strength measures on each apparatus.
Exercise Training Protocol

Subjects performed three sessions of exercise per week composed of either three supervised combined aerobic and resistance circuit training sessions or two supervised circuit training sessions in addition to one home exercise training session per week, monitored for compliance (CAD, UTHC, and THC). Circuit training sessions were performed at the Cardiac Gymnasium, Royal Perth Hospital, with the focus on the large muscles of the lower limbs. Upper body exercises did not involve the forearm, and subjects were instructed to avoid hand gripping. They were also instructed on correct lifting techniques to avoid the Valsalva maneuver.

The 8-wk “circuit” training protocol involved a combination of resistance training, cycle ergometry, and treadmill walking. The resistance exercises (listed above) were alternated with cycle stations at a work-to-rest ratio of 45:15 s. Subjects performed one lift every 3 s, completing 15 lifts in the 45-s work period. At completion of the circuit, subjects performed an additional 5 min of treadmill walking. Training intensity and duration were progressively increased during the first 2 to 3 wk, as tolerated. Resistance intensity commenced at 55% of pretraining 1 RM and increased to 65% at week 4. Cycling and treadmill walking intensities were initially 70% of peak HR, determined from a pretest graded maximal exercise test, and were increased up to 85% of peak HR at week 6.

Home training sessions, where performed, were individually prescribed and involved subjects performing continuous aerobic exercise at 70–85% maximal HR for up to 45–60 min. To ensure compliance, sessions were recorded in a diary, and HR were recorded using Polar heart rate monitors (Polar Electro Oy, Kempele, Finland).

Analysis of Data

In plethysmographic, resistance vessel function studies, FBF responses were initially calculated as a ratio of that in the infused arm to that in the noninfused arm, and changes in the ratio being expressed as percentage changes from the baseline immediately preceding the drug infusion period (2). FBF responses to each drug infusion were then expressed as the area under the curve (AUC) of percent changes in FBF ratio responses to the three doses of the drug. To compare trained and untrained data for all variables, including conduit and resistance vessel responses, Student’s paired t-test was used. To examine relationships between variables at baseline (i.e., pretraining), we performed univariate analysis between all variables and baseline FMD, GTN, ACh-AUC, SNP-AUC, and L-NMMA-AUC, thereby providing correlation coefficients and associated significance levels. This approach was repeated for change in all variables (i.e., trained-untrained) and change in FMD, GTN, AUC ACh, AUC SNP, and AUC L-NMMA. Results of these univariate analyses were then used to select variables for stepwise multivariate linear regression analysis. Finally, to avoid the possibility of “regression to the mean” in the comparison of relationships between changes in variables with training, we created a multivariate model, as indicated above, which included both baseline values and posttraining data. Data are reported as means ± SE. Significance was set at P < 0.05. In the majority of cases for correlation between variables, a minimum of 45 matched pairs were available. Power analysis indicates that, assuming a two-tailed 5% test, this number is sufficient to detect a significant correlation of 0.40 with 80% power (26).

RESULTS

The results of exercise training within each group are comprehensively described in individual papers (30–32, 52, 53). The purpose of pooling the data in the present analysis was to provide adequate power to examine relationships between variables both before training and as a result of training; previous papers do not include correlation analyses.

Relationship Between Variables Before Training

Correlation coefficients for the relationships between variables before training are presented in Table 2. Before training, FMD significantly correlated with \( \dot{V}O_2_{peak} \) (n = 45; r = 0.368; P = 0.013) as did the FBF response to ACh, expressed as the area under the dose-response curve (AUC-ACh) (n = 69; r = 0.266; P = 0.027). The presence of a significant relationship be-

<table>
<thead>
<tr>
<th>Table 2. Correlations between baseline cardiovascular risk factor data and measures of conduit and resistance vessel endothelium-dependent and -independent vasodilation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conduit Vessel Function, %</strong></td>
</tr>
<tr>
<td>Endothelium-dependent</td>
</tr>
<tr>
<td>FMD</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Resting MAP, mmHg</td>
</tr>
<tr>
<td>Resting HR, beats/min</td>
</tr>
<tr>
<td>( \dot{V}O_2_{peak} ), ml·kg(^{-1})·min(^{-1})</td>
</tr>
<tr>
<td>Exercise time, s</td>
</tr>
<tr>
<td>Body mass, kg</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
</tr>
<tr>
<td>Waist/hip, %</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
</tr>
<tr>
<td>Glycated hemoglobin, %</td>
</tr>
</tbody>
</table>

Data are correlation coefficients (n, number of matched pairs). AUC, area under the dose-response curve; FMD, flow-mediated dilation; GTN, glyceryl trinitrate; ACh, acetylcholine; SNP, sodium nitroprusside; L-NMMA; N\(^\text{G}\)-monomethyl-L-arginine. * P < 0.05; † P < 0.01; ‡ P < 0.001.
between FMD and exercise capacity was further confirmed by correlation between exercise test duration and FMD ($n = 47; r = 0.466; P < 0.001$). No significant correlations were evident among the responses to SNP, GTN, or l-NMMA and either VO$_2$peak or exercise test duration.

There was an inverse relationship between age and FMD ($n = 47; r = -0.326; P = 0.025$) and also age and AUC-ACh ($n = 71; r = -0.276; P = 0.020$). In addition, baseline FMD was inversely correlated with glycated hemoglobin concentration ($n = 42; r = -0.376; P = 0.014$), whereas ACh-AUC correlated directly with high-density lipoprotein (HDL) concentration ($n = 65; r = 0.248; P = 0.046$) and inversely with the waist/hip ($n = 65; r = -0.422; P < 0.001$). SNP-AUC also correlated directly with HDL concentration ($n = 62; r = 0.295; P < 0.05$) and inversely with measures of body composition (body mass: $n = 67; r = -0.462; P < 0.001$; body mass index: $n = 67; r = -0.413; P < 0.001$; waist/hip: $n = 65; r = -0.301; P < 0.001$).

No significance was found between baseline measures of conduit and resistance vessel endothelium-dependent (FMD vs. ACh: $n = 43; r = 0.191; P = 0.220$) or -independent (SNP vs. GTN: $n = 41; r = -0.015; P = 0.925$) function. In addition, no correlations were evident at baseline between basal and stimulated endothelium-dependent NO dilator function in either conduit (l-NMMA vs. FMD: $n = 42; r = -0.243; P = 0.121$) or resistance vessels (l-NMMA vs. ACh: $n = 68; r = 0.148; P = 0.229$).

From the above univariate results, age, VO$_2$peak, exercise test duration, and glycated hemoglobin were entered as independent variables in a stepwise multiple regression analysis in an effort to predict pretraining FMD. This revealed peak duration was the single best predictor ($R = 0.49$, adjusted $R^2 = 0.217$) and that no other variables contributed significantly to the prediction of FMD beyond this. Stepwise regression was also performed for the effect of age, VO$_2$peak, waist/hip, and HDL cholesterol on ACh responses. This analysis revealed the waist/hip as the best predictor ($R = 0.423$, adjusted $R^2 = 0.165$), with an additional significant contribution of age ($R = 0.485$, adjusted $R^2 = 0.209$). Stepwise regression for the effect of body weight, body mass index, waist/hip ratio, and HDL cholesterol on SNP responses indicated weight as the single best predictor ($R = 0.508$, adjusted $R^2 = 0.249$). Multivariate regression analyses were not performed on GTN and l-NMMA responses due to lack of significant univariate relationships.

### Effects of Exercise Training

The effects of exercise training on physiological and vascular function responses are shown in Table 3. Training significantly increased FMD from $3.3 \pm 0.4$ to $5.9 \pm 0.5\% (P < 0.001)$, whereas the response to GTN was not altered (Table 3). The FBF-ACh AUC response significantly increased from $405 \pm 43$ to $637 \pm 67\% (P = 0.001)$ after training as did the FBF ratio to the highest dose of ACh (ACh-dose 3 $359 \pm 33$ to $537 \pm 58\% (P = 0.002$). The responses to SNP-AUC, SNP-dose 3, l-NMMA-AUC, and l-NMMA-dose 3 did not change with training.

Training significantly improved VO$_2$peak from $2.15 \pm 0.06$ to $2.37 \pm 0.06$ l/min ($P < 0.001$) and also VO$_2$peak when expressed relative to body mass ($P < 0.001$). This evidence for improvement in exercise capacity was reinforced by a significant increase in exercise test duration ($392 \pm 33$ to $1,096 \pm 40\ s; P < 0.0001$) and decrease in resting HR (Table 4). Exercise training was also associated with a significant reduction in peripheral adiposity ($161 \pm 5$ vs. $153 \pm 7\ mm; P = 0.001$, Table 4), without a change in girths measured at corresponding landmarks, whereas muscular strength increased ($435.9 \pm 13.8\ vs. 481.1 \pm 13.7\ mm; P = 0.0001$, Table 3). These data infer a change in body composition with exercise training favoring an increase in lean body mass.

Plasma lipids, blood pressure, and resting blood glucose did not change with training, although there was a significant decrease in glycated hemoglobin concentration ($6.41 \pm 0.22$ vs. $6.26 \pm 0.20\% (P < 0.05$, Table 4). This improvement in glycated hemoglobin was largely due to an effect in the T2D subjects (33).

### Table 3. Effect of exercise training on vascular function and exercise variables in all subjects

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trained</th>
<th>n</th>
<th>P</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>VO$_2$peak, l/min</td>
<td>2.15 ± 0.06</td>
<td>2.37 ± 0.06</td>
<td>73</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>VO$_2$peak, ml/kg·min$^{-1}$</td>
<td>25.6 ± 0.7</td>
<td>28.0 ± 0.7</td>
<td>73</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Exercise time to exhaustion, s</td>
<td>932 ± 33</td>
<td>1096 ± 40</td>
<td>73</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Muscular strength, kg</td>
<td>435.9 ± 13.8</td>
<td>483 ± 13.7</td>
<td>74</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>FMD, %</td>
<td>3.3 ± 0.4</td>
<td>5.9 ± 0.5</td>
<td>47</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>GTN, %</td>
<td>14.2 ± 0.7</td>
<td>13.9 ± 0.9</td>
<td>46</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>ACh-AUC, %</td>
<td>405 ± 43</td>
<td>637 ± 67</td>
<td>71</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>SNP-AUC, %</td>
<td>772 ± 46</td>
<td>849 ± 46</td>
<td>67</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>l-NMMA-AUC, %</td>
<td>-45 ± 5</td>
<td>-48 ± 5</td>
<td>68</td>
<td>0.92</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of matched pairs.

### Table 4. Effect of exercise training on cardiovascular risk factors in all subjects

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trained</th>
<th>n</th>
<th>P</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, kg</td>
<td>85.7 ± 1.9</td>
<td>85.7 ± 1.8</td>
<td>74</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>28.2 ± 0.5</td>
<td>28.2 ± 0.4</td>
<td>74</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Waist/hip, %</td>
<td>0.95 ± 0.01</td>
<td>0.94 ± 0.01</td>
<td>72</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Sum of skinfolds, mm</td>
<td>161 ± 5</td>
<td>153 ± 7</td>
<td>73</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Segment girths, mm</td>
<td>322.3 ± 3.1</td>
<td>321.4 ± 3.1</td>
<td>73</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Resting HR, beats/min</td>
<td>64 ± 1</td>
<td>61 ± 1</td>
<td>72</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Resting MAP, mmHg</td>
<td>89 ± 1</td>
<td>88 ± 2</td>
<td>72</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Peak HR, beats/min</td>
<td>158 ± 3</td>
<td>160 ± 2</td>
<td>71</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Plasma lipids, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.03 ± 0.12</td>
<td>4.9 ± 0.12</td>
<td>73</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>3.04 ± 0.12</td>
<td>3.02 ± 0.10</td>
<td>67</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.11 ± 0.03</td>
<td>1.12 ± 0.03</td>
<td>67</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.91 ± 0.13</td>
<td>1.78 ± 0.10</td>
<td>73</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>7.04 ± 0.45</td>
<td>6.88 ± 0.38</td>
<td>45</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Glycated hemoglobin, %</td>
<td>6.41 ± 0.22</td>
<td>6.26 ± 0.20</td>
<td>42</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects.
Relationship Between Variables After Exercise Training

Relationship between baseline data and changes with training. The change in FMD after exercise training was significantly negatively correlated with baseline FMD (n = 47; r = −0.533; P < 0.001) and, similarly, the change in ACh-AUC after exercise training was significantly negatively correlated with the baseline ACh-AUC (n = 71; r = −0.363; P < 0.01) and directly correlated with age (n = 71; r = 0.318; P = 0.007).

No correlation was observed among baseline lipid fractions, resting MAP, blood glucose, glycated hemoglobin, weight, body mass index, or sum of skinfolds and change in FMD or change in ACh-AUC.

Relationship between changes in variables with training. Correlations between training-induced changes in endothelium-dependent and -independent, conduit and resistance vessel function, and other variables are presented in Table 5. The change in FMD following training was not correlated with the change in $VO_2$peak (n = 45; r = −0.084; $P = 0.585$) or change in exercise test duration (n = 47; r = −0.090; $P = 0.549$). Likewise, the change in ACh-AUC was not correlated with the change in $VO_2$peak (n = 69; r = 0.113; $P = 0.356$) or change in exercise test duration (n = 71; r = 0.043; P = 0.723). No correlations were observed among changes in FMD or in ACh AUC and changes in lipid fractions, resting MAP, blood glucose, glycated hemoglobin, weight, waist/hip, body mass index, sum of skinfolds, or muscular strength (Table 5).

No significance was found between changes in conduit and resistance vessel endothelium-dependent (FMD vs. ACh: n = 43; r = −0.037; P = 0.816) or -independent (SNP vs. GTN: n = 39; r = −0.222; P = 0.175) function with training, or between changes in basal and stimulated endothelium-dependent NO dilator function in either conduit (L-NMMA vs. FMD: n = 40; r = −0.046; P = 0.777) or resistance vessels (L-NMMA vs. ACh: n = 66; r = −0.053; P = 0.660).

Because of the absence of relationships between changes in cardiovascular risk factor variables and changes in conduit and resistance vessel function on univariate analysis, multivariate regression analysis was not performed.

DISCUSSION

The primary purpose of the present study was to determine whether improvements in vascular function with training are associated with the potentially beneficial effects of exercise training on risk factors for endothelial dysfunction and cardiovascular disease, such as serum lipid concentrations, BP, obesity, or glycemic control. It is generally accepted that exercise training decreases mortality from cardiovascular disease, both in the primary and secondary prevention settings (21, 38, 43, 46, 49). Whereas the mechanism(s) responsible for this cardioprotective effect of exercise training has often been ascribed to the beneficial effects of training on risk factors for cardiovascular disease, it has also been suggested that the beneficial effects of regular exercise cannot solely be accounted for by the reduction of risk factors, because the association with reduced mortality is independent of other coronary risk factors (9). Vascular function, and in particular NO-mediated endothelial function, is now considered a useful independent measure of atherosclerotic disease risk (27), because 1) NO possesses a number of antiatherogenic properties (18); 2) endothelial dysfunction is evident in subjects who possess risk factors for, and overt evidence of, cardiovascular disease (3, 4, 7, 11, 36, 50); 2) many interventions that ameliorate risk factors and decrease cardiovascular mortality and morbidity are also associated with improvement in endothelial function (20, 28, 41, 42); and 4) endothelial dysfunction in coronary and also peripheral artery diseases predict cardiovascular events (1, 15, 44, 51).

We and others have previously demonstrated that exercise training improves endothelial function and,

Table 5. Correlations between changes (trained-untrained) in cardiovascular risk factor data and changes in conduit and resistance vessel endothelium-dependent and -independent vasodilation as a result of training

<table>
<thead>
<tr>
<th></th>
<th>Conduit Vessel Function (Δ)</th>
<th>Resistance Vessel Function (Δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endothelium-dependent</td>
<td>Endothelium-independent</td>
</tr>
<tr>
<td></td>
<td>FMD, %</td>
<td>ACh</td>
</tr>
<tr>
<td>Resting MAP, mmHg</td>
<td>−0.055 (n = 44)</td>
<td>−0.058 (n = 68)</td>
</tr>
<tr>
<td>Resting HR, beats/min</td>
<td>−0.123 (n = 44)</td>
<td>0.088 (n = 68)</td>
</tr>
<tr>
<td>$VO_2$peak, ml·kg⁻¹·min⁻¹</td>
<td>−0.084 (n = 45)</td>
<td>0.113 (n = 69)</td>
</tr>
<tr>
<td>Exercise time, s</td>
<td>−0.090 (n = 47)</td>
<td>0.043 (n = 71)</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>0.000 (n = 47)</td>
<td>−0.042 (n = 70)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>−0.014 (n = 47)</td>
<td>0.085 (n = 68)</td>
</tr>
<tr>
<td>Waist/hip, %</td>
<td>0.024 (n = 44)</td>
<td>0.027 (n = 69)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>−0.103 (n = 45)</td>
<td>0.064 (n = 63)</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/l</td>
<td>0.238 (n = 43)</td>
<td>0.278 (n = 41)</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>0.058 (n = 45)</td>
<td>0.098 (n = 39)</td>
</tr>
<tr>
<td>Glycated hemoglobin, %</td>
<td>−0.208 (n = 42)</td>
<td>−0.083 (n = 37)</td>
</tr>
</tbody>
</table>

Data are correlation coefficients (n, number of matched pairs).
furthermore, that this improvement may be general- 
ized to vascular beds not directly associated with the 
exercise stimulus (24, 29–31, 52, 53). It is not clear, 
however, whether the beneficial effects of training on 
endothelial function are a consequence of training-
induced improvements in risk factor profiles or to some 
other mechanism(s). The data analyzed in the present 
study reveal that exercise training did not significantly 
alter plasma lipids, blood pressure, blood glucose, gly-
cated hemoglobin, waist/hip, or body mass index, de-
spite significant improvement in both FMD and ACh 
response. We conclude that, in these subjects with 
anteecedent vascular dysfunction, the beneficial effects 
of short-term exercise training on conduit and resis-
tance vessel function are not solely mediated by the 
effects of exercise on cardiovascular risk factors.

The above conclusion is at odds with some previous 
findings. For example, it has been suggested that ex-
ercise training-mediated improvements in vascular 
function may occur as a result of improvements in 
plasma lipid concentrations (25). Before exercise train-
ing in the present study, the response to ACh positively 
correlated with HDL, whereas glycated hemoglobin 
and waist/hip were inversely correlated with FMD. 
Furthermore, glycated hemoglobin and subcutaneous 
body fat decreased after training. However, there was 
no correlation between the changes in any of these 
variables and those in either FMD or ACh responses, 
indicating that improvements evident in vascular func-
tion were not associated with modulation of these con-
ventional risk factors.

An alternate explanation for the effect of exercise 
training on vascular function is that exercise exerts a 
direct effect on the vasculature by generating a recur-
rent intermittent increase in shear stress, a known 
physiological stimulus to NO bioactivity (37). Animal 
studies have found that exercise training is associated 
with an increase in vascular NO production (45, 48) 
and upregulation of NO synthase expression (45), at-
tributable to repeated episodes of vascular wall shear 
stress. Furthermore, we recently demonstrated that, 
during a discrete session of lower-limb exercise, an 
increase in NO-dilator bioactivity occurs in the resting 
upper-limb vessels, which, because of the very short 
life of NO, indicates that the NO has been pro-
duced in those vessels (14). The effect is probably 
largely due to the generalized impact of hemodynamic 
variables acting through vessel wall shear stress (39).

Hence, recurrent augmentation of vessel wall shear 
stress, as a consequence of repeated exercise-induced 
hemodynamic changes, likely results in a generalized 
increase in NO bioactivity throughout the vasculature 
(24, 29–31, 52, 53), which would explain the improve-
ments in resistance and conduit vessel function we 
observed. Interestingly though, we did not observe 
evidence of improved basal NO function in the present 
study, because no changes were evident in 1-NMMA 
responses. Future studies will be required to determine 
the precise relationship between vascular shear stress 
and changes in endothelial function, and measures of 
oxidative stress will also be essential to determination 
of changes in bioavailability of NO (13).

An interesting secondary outcome of this study re-
lates to the relationship between improvements in vas-
cular function and those in measures of exercise capac-
ity. Some recent animal and human studies have ex-
amined the hypothesis that decreased NO-mediated 
dilator capacity and consequent decreased blood flow 
and oxygen transport to active skeletal muscle during 
exercise, attenuates aerobic capacity (16, 35, 40). Limb 
blood flow and $V_{O2\text{peak}}$ were impaired in hypercholes-
terolemic mice with endothelial dysfunction and in 
wild-type mice administered a NO synthase inhibitor 
(35). The latter suggests that depressed vasodilator 
function can limit exercise capacity under some condi-
tions. In a subsequent study, 4 wk of exercise training 
improved both NO bioactivity and $V_{O2\text{peak}}$ in normal 
mice with diet-induced and genetically induced hyper-
cholesterolemia (40). Taken together, these studies are 
consistent with the proposal that impaired endothelial 
function inhibits exercise-induced redistribution of 
blood flow to skeletal muscle during exercise, that loss 
of endothelium-dependent vasodilator function can be 
rate limiting to oxygen delivery and exercise perfor-
ance (35), and that training-induced correction of 
resistance vessel endothelial dysfunction and corre-
spondingly preferential redistribution of blood flow to 
the working muscles during exercise might be related 
to enhanced $V_{O2\text{peak}}$. In humans, a number of exercise 
training studies have reported significant correlations 
between improvements in reactive hyperemic res-
sponses in trained muscle vascular beds and $V_{O2\text{peak}}$ in 
healthy subjects (34) or $V_{O2\text{peak}}$ in CHF patients (8, 
47). In a well-conducted recent trial involving a small 
number of CHF patients, Hambrecht et al. (16) re-
ported a significant correlation between improvement 
in $V_{O2\text{peak}}$ following 6 mo of cycle ergometer training 
and an increase in ACh-mediated femoral artery blood 
flow. In the present study, we observed significant 
correlations between FMD and both $V_{O2\text{peak}}$ and exer-
cise test duration across the groups before exercise 
training. In addition, the area under the ACh dose-
response curve at baseline, dependent on resistance 
vessel vasodilation, also correlated with baseline 
$V_{O2\text{peak}}$. At first glance it would be tempting to con-
clude from these data that exercise capacity is depend-
tent to some extent on vasodilator function, yet if this 
was the case, then changes in vascular function with 
training should correlate with changes observed in 
$V_{O2\text{peak}}$ and exercise test duration. No such correla-
tions were evident, indicating to us that improvement 
in functional capacity as a result of exercise training is 
not dependent on generalized improvement in conduit 
or resistance vessel vasodilator function. The disparity 
between the above studies and our results may relate 
to the subject groups studied, the magnitude of vascu-
lar dysfunction evident at entry, different training 
protocols used, or, quite likely, because we studied the 
 systemic (forearm) vascular response to training 
rather than vascular function specifically in the 
trained (lower limb) muscles.

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There are several potential limitations of the present study. A broad range of subjects were examined, with differing etiologies, medications, and disease severity. However, medications were not altered in any subject across the period of study, and the purpose of pooling the data from the various groups studied (30–32, 52, 53) was to provide adequate power to examine relationships between variables both before training and as a result of training. Furthermore, it might be argued that detection of relationships between variables is facilitated by a study of groups with a wide variation in baseline characteristics. Another possible statistical limitation relates to the possibility that we failed to observe relationships between changes in vascular function and changes in cardiovascular risk factors with training due to regression to the mean. We think this unlikely, however, because we repeated our univariate analysis for the prediction of change in vascular function measures with the inclusion of both pre- and posttraining data in the model as independent variables rather than delta scores for training baseline. Finally, we cannot exclude the possibility that a larger cohort of subjects or a more prolonged period of exercise training may have uncovered an effect of exercise training on some cardiovascular risk factors. However, we have demonstrated that in the relatively short time frame of the present study, there were minimal changes in cardiovascular risk factors despite significant improvements in vascular function, indicating that cardiovascular risk factors may not be the only factors involved in improvement in vascular function.

In summary, we did not observe a relationship between the changes in risk factors for cardiovascular disease and the improvements we observed in conduit factors involved in improvement in vascular function. Cant improvements in vascular function, indicating the frame of the present study, there were minimal drug and posttraining data in the model as independent variables for the prediction of change in vascular function and changes in cardiovascular risk factors with training due to regression to the mean. We think this unlikely, however, because we repeated our univariate analysis for the prediction of change in vascular function measures with the inclusion of both pre- and posttraining data in the model as independent variables rather than delta scores for training baseline. Finally, we cannot exclude the possibility that a larger cohort of subjects or a more prolonged period of exercise training may have uncovered an effect of exercise training on some cardiovascular risk factors. However, we have demonstrated that in the relatively short time frame of the present study, there were minimal changes in cardiovascular risk factors despite significant improvements in vascular function, indicating that cardiovascular risk factors may not be the only factors involved in improvement in vascular function.

In summary, we did not observe a relationship between the changes in risk factors for cardiovascular disease and the improvements we observed in conduit factors involved in improvement in vascular function.


