The Effect of Combined Aerobic and Resistance Exercise Training on Vascular Function in Type 2 Diabetes

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OBJECTIVES
The purpose of this study was to examine whether exercise training stimulates a generalized improvement in vascular function in patients with type 2 diabetes mellitus.

BACKGROUND
Exercise is often recommended for patients with type 2 diabetes to improve physical conditioning and glycemic control. This study examined the effect of eight weeks of exercise training on conduit and resistance vessel function in patients with type 2 diabetes, using a randomized crossover design.

METHODS
Both resistance vessel endothelium-dependent and -independent functions were determined by forearm plethysmography and intrabrachial infusions of acetylcholine (ACh) and sodium nitroprusside (SNP), respectively, in 16 patients with type 2 diabetes. Conduit vessel endothelial function was assessed in 15 of these patients using high-resolution ultrasound and flow-mediated dilation of the brachial artery; glyceryl trinitrate (GTN) was used as an endothelium-independent dilator.

RESULTS
Flow-mediated dilation increased from 1.7 $\pm$ 0.5% to 5.0 $\pm$ 0.4% following training ($p < 0.001$). The forearm blood flow ratio to ACh was significantly improved (analysis of variance, $p < 0.05$). Responses to SNP and GTN were unchanged. Endothelium-dependent vasodilation was enhanced in both conduit and resistance vessels.

CONCLUSIONS
If endothelial dysfunction is an integral component of the pathogenesis of vascular disease, as currently believed, this study supports the value of an exercise program in the management of type 2 diabetes. (J Am Coll Cardiol 2001;38:860–6) © 2001 by the American College of Cardiology

Mortality from type 2 diabetes is largely attributable to atherosclerotic macrovascular complications, whereas microvascular dysfunction and consequent retinopathy, neuropathy and nephropathy contribute significantly to morbidity. The importance of the endothelium in maintaining normal vascular function has been increasingly recognized. Endothelium-dependent vasodilation, largely dependent upon nitric oxide (NO), is not only impaired with overt vascular disease but is also associated with conventional vascular risk factors (1) and may improve with appropriate interventions (2–4). Many investigators consider endothelial dysfunction to be an early and integral manifestation of atherosclerotic disease and that improvement in endothelial function reflects antiatherogenic benefit (5). In noninsulin-dependent type 2 diabetic patients, NO-related dilator endothelial function has usually (6,7), although not invariably (8), been found to be depressed.

Exercise training in animals improves NO-dependent vasodilation (9) and upregulates expression of the constitutive NO-synthase (10,11). In addition, recent studies indicate that exercise may improve endothelial function in normal subjects (12) and patients with chronic heart failure (13–16). Although exercise training is recommended for subjects with type 2 diabetes to modify body composition, to maintain cardiorespiratory fitness and to improve glucose tolerance (17), no studies have investigated its effect on vascular function. The purpose of the present study was to determine whether an eight-week exercise training program improved endothelium-dependent or -independent resistance or conduit vessel function.

METHODS

Patients and screening measures. Sixteen patients (14 men, 2 women), aged 52 $\pm$ 2 (SE) years, were recruited. The following were excluded: smokers; those with renal impairment or proteinuria, hepatic impairment, gout or hyperuricemia; hypercholesterolemia (total cholesterol $>6.0$ mmol/l$^{-1}$) or hypertension (systolic blood pressure $>160$ mm Hg). Five patients were taking angiotensin-converting enzyme (ACE) inhibitors; two were taking lipid-lowering therapy; two were taking aspirin, and all but one were taking an oral hypoglycemic (metformin, 3;
Abbreviations and Acronyms

- **ACE** = angiotensin-converting enzyme
- **ACh** = acetylcholine
- **ANOVA** = analysis of variance
- **ECG** = electrocardiogram
- **FBF** = forearm blood flow
- **FMD** = flow-mediated dilation
- **GTN** = glyceryl trinitrate
- **HR** = heart rate
- **L-NMMA** = Nω-monomethyl-L-arginine
- **MAP** = mean arterial pressure
- **NO** = nitric oxide
- **SNP** = sodium nitroprusside

The study protocol was approved by the Royal Perth Hospital Ethics Committee, and patients gave written, informed consent.

**Study design.** Patients were randomized to an 8-week exercise or nontraining period, and the experimental measures in the following text were assessed at entry, after 8 weeks and, following crossover, 16 weeks after entry.

**Vascular function assessment protocols.** Vascular function was assessed 4 h after medication use, fasting and abstaining from alcohol or caffeinated beverages for 12 h and, for individual patients, at the same time of the day. Both endothelium-dependent and -independent vascular functions in resistance vessels were assessed in all 16 patients, whereas high-resolution vascular ultrasonography of conduit vessels was performed in 15 patients (one patient was unavailable for postexercise training assessment).

**Assessment of resistance vessel function.** Investigations were conducted in a quiet, temperature-controlled laboratory. Patients lay supine while pneumatic pressure cuffs (SC10 and SC5, D.E. Hokanson, Bellevue, Washington) and strain-gauges (SG24, Medasonics, Mountain View, California) were positioned for the measurement of forearm blood flow (FBF) by the technique of strain-gauge plethysmography. The cuffs on both wrists were connected to a flow-regulated source of compressed air, and arm cuffs were connected to a rapid inflation device (E20, D.E. Hokanson). The strain-gauges were placed 8 to 10 cm from the olecranon process of each forearm, and care was taken to ensure that they were at the same level on each arm. Output from the gauges passed through an amplifier (SPG16, Medasonics) and was sampled by an online microcomputer at a rate of 75 Hz before being displayed on a monitor in real time. A software program coordinated the acquisition, storage and display of data as well as inflation and deflation of the arm cuffs, ensuring that blood flow measures were synchronized with cuff inflation during recording periods.

Following cuff placement, a 20-gauge arterial cannula (Arrow, Reading, Pennsylvania) was introduced into the brachial artery of the nondominant arm, under local anesthesia with <2 ml of 1% lidocaine (Astra Pharmaceuticals, Sydney, Australia), to transduce pressure, for the infusion of drugs or physiological saline and for sampling of arterial blood. Intra-arterial pressure was measured continuously (Transpac, Abbot Laboratories, Sligo, Ireland) throughout the study. Drug infusions were administered using a constant-rate infusion pump (IVAC 770, IVAC, California). Acetylcholine (ACH) (Miochol; Ciba Vision, Australia) was infused at 10, 20 and 40 μg min⁻¹, each for 3 min, followed by sodium nitroprusside (SNP) (David Bull Laboratories, Melbourne, Australia) at 2, 4 and 8 μg min⁻¹, each for 3 min. All solutions were prepared aseptically from sterile stock solutions or ampules immediately before infusion into the brachial artery.

The study protocol and time frame were identical for every patient. Baseline measurements started 25 min after cannulation of the brachial artery. Blood flow measurements were taken by inflating the wrist cuffs to 220 mm Hg, to exclude the hands from the circulation, and by rapidly inflating the upper arm cuffs to 45 mm Hg for 10 out of every 15 s throughout the baseline and drug infusion periods. Output from the strain-gauges was stored, and the average of the last five flow measurements from each period was used for analysis. Between infusions, the cuffs were deflated, allowing at least 15 min for the FBF to recover from the preceding infusion before further baseline measures were recorded.

**Assessment of conduit vessel function.** Patients lay supine for 20 min before the first ultrasound scan was recorded. Brachial artery diameter, mean arterial pressure (MAP) and heart rate (HR) were determined at rest, after flow-mediated dilation (FMD) induced by reactive hyperemia and after administration of glyceryl trinitrate (GTN).

The monitored, nondominant arm was positioned at an arm-to-torso angle of 80° with the distal forearm supinated and immobilized by foam supports encompassing the limb. A three-lead electrocardiogram (ECG) was used to continuously monitor HR, and MAP was measured using an automated sphygmomanometer on the contralateral arm (Dinamap, 8100, Critikon, Tampa, Florida). A 12-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Aspen, Acuson, Mountain View, California) was used to visualize the artery in the distal third of the upper arm. The probe was held in a constant stable position for the duration of the trial by a stereotactic clamp, and its precise location was recorded and standardized for the repeat session by measurement of the proximal and distal distance of the probe from the radial. Finally, a rapid inflation/deflation pneumatic cuff, placed around the forearm immediately distal to the humeral epicondyles, was used to provide a stimulus for FMD.

Ultrasound parameters were set to optimize longitudinal, B-mode images of the lumen/arterial wall interface, with the focus zone positioned on the near wall to assist visualization of the lumen/arterial wall interface. Once set, operating parameters remained constant throughout each session.
After the initial 20-min stabilization period, baseline scans assessing vessel diameter were recorded over 2 min. The proximal forearm cuff was then inflated to 250 mm Hg for 5 min. Dilation of a forearm conduit artery stimulated by reactive hyperemia, resulting from this ischemic stimulus, provides a measure of endothelium-dependent, largely NO-mediated vasodilation (18). The B-mode measures commenced 30 s before cuff deflation and continued for a period of 3 min after cuff deflation.

When vascular diameter had returned to baseline levels after a period of 15 min, a second baseline scan was recorded. This was followed by sublingual administration of GTN (400 μg), which provides an index of endothelium-independent NO dilation.

Post-test analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software that is independent of investigator bias. Briefly, videotaped images were captured to computer memory using a digital frame grabber. The ECG-gated B-mode frames were then assessed by automated edge detection software, which employs a pixel density and frequency distribution algorithm. Images that coincided with the peak of R waves were analyzed, with approximately 600 individual diameter measures along the artery being assessed per image. The average of these individual diameter measures was calculated and a polynomial regression was then fitted to diameters obtained from successive R waves. Both FMD and GTN values were calculated as percent change from preceding baseline data. **Exercise training regimen.** The eight-week regimen consisted of three 1-h sessions of whole body exercise each week, concentrating on the large muscle groups of the lower limbs. Selected torso and upper body exercises, as long as they did not involve hand gripping or forearm exercise, were also included. Training involved a combination of cycle ergometry, treadmill walking and resistance training, the latter performed on weight-stack machines (Pulsestar, Cheshire, United Kingdom). An exercise “circuit” consisted of seven resistance exercises alternated with eight aerobic exercise (cycling) stations, each performed for 45 s. To conclude the circuit, patients spent 5 min walking on a treadmill. The seven resistance exercises consisted of dual seated leg press, left and right hip extension, pectoral exercises, shoulder extension, seated abdominal flexion and dual leg flexion. Both the intensity and the duration of the exercise program were progressively increased during the first two to three weeks of the program, as individually tolerated. Thereafter, cycle ergometry and treadmill walking were maintained at 70% to 85% of peak HR, determined during a graded incremental exercise test performed to peak endurance capacity (\( V_{O2} \text{peak} \)) before entry to the study. Resistance training intensity was maintained at 55% to 65% of pretraining maximum voluntary contraction, which was determined for all seven exercises in the circuit. **Analysis of data.** All blood flow measures were analyzed by an investigator who was blinded with respect to patient identification. Although the low doses of drugs infused in the study produce negligible systemic effects and showed no effect on blood pressure or HR, it is still desirable to exclude an alteration in overall hemodynamics as a cause of the flow changes seen in the infused forearm. Thus, FBF was measured simultaneously in both arms, although only one arm was infused, and the noninfused arm served as a control. As in earlier studies (2,3,19,20), FBF in the infused arm is described as a ratio to that in the noninfused arm. Changes in the ratio during ACh and SNP infusions are expressed as percentage changes from the baseline immediately preceding each drug administration (21). In addition, FBF is expressed in absolute units (ml/100 ml-1-min -1).

The responses to intrabrachial infusions after exercise training were compared to nonresponding responses using two-way analysis of variance (ANOVA) with repeated measures performed on the three dose levels of ACh and SNP. All other comparisons of training and nontraining periods were undertaken using the Student paired two-sided \( t \) test. Both FMD and GTN responses before and after exercise training were also compared using the Student paired \( t \) test. Pearson product moment correlation coefficients were used to compare changes in the highest dose responses to ACh and changes in the FMD responses, before and after training, to changes in glycated hemoglobin and fasting blood glucose. Results are expressed as means ± SE. A p value <0.05 was considered significant.

**RESULTS**

Six of the 16 patients were randomized to receive exercise training first. All patients completed 24 exercise sessions, and no significant adverse events occurred. There was no effect of the order of randomization on the variables measured, so results after exercise training were compared with those after the nonexercise period irrespective of order of administration. **General effects of exercise training.** There were no significant differences in plasma total, high-density lipoprotein or low-density lipoprotein cholesterol, triglycerides or resting MAP following training. Resting HR, glycated hemoglobin and fasting blood glucose were significantly lower (all \( p < 0.05 \); Table 1). Peak \( V_{O2} \) increased as a result of training, from 23.1 ± 1.2 to 24.8 ± 1.4 ml·kg⁻¹·min⁻¹ (\( p < 0.05 \)). **Forearm conduit vessel function.** Baseline brachial artery diameter was not significantly different before and after the training period (4.5 ± 0.2 vs. 4.3 ± 0.2 mm, \( p = 0.08 \)). The FMD of the brachial artery following forearm ischemia, which is largely endothelium and NO dependent, significantly increased after exercise training, from 1.7 ± 0.5% to 5.0 ± 0.4% (\( p < 0.001 \)). In contrast, endothelium-independent vasodilation to GTN was not significantly different (13.1 ± 1.5% vs. 13.7 ± 2.0%, \( p = 0.7 \); Fig. 1). No significant differences occurred in the change in FMD, before and after training, between patients who were taking
ACE inhibitors (n = 5), compared to those who were not (n = 10; 2.4 ± 0.5 vs. 3.7 ± 0.7%, p = 0.3). Due to the small number of patients taking lipid-lowering medication (n = 2), a t test was not performed to assess the influence of this medication, but the change in FMD before and after training in those taking medication was 2.9 ± 1.9%, compared to 3.3 ± 0.6% in patients who were not medicated.

**Forearm resistance vessel function.** Baseline FBF responses in the infused limb did not significantly differ before and after training (2.88 ± 0.32 vs. 2.13 ± 0.22 ml/100 ml forearm \(-1\) min\(^{-1}\), p = 0.07). The FBF responses to the three dose levels of ACh, related to the preinfusion baseline levels, were on the average higher, but not significantly so, after training (p = 0.06, ANOVA; Table 2). The interaction between treatment and dose was also nonsignificant (p = 0.2). However, the preferred method of analysis relates the flow in the infused arm to that in the noninfused arm \((2.419,21,22)\), and the ratios at the three dose levels of ACh were significantly greater following training (p < 0.05, ANOVA; Fig. 2). The interaction between treatment and dose was not significant (p = 0.2). The effect of exercise training on responses to SNP was not significant (p = 0.6, ANOVA; Fig. 2), nor was the interaction between treatment and dose (p = 0.2). Responses were not dependent on the order of training and nontraining periods, and there was no difference between the nontrained data of those trained first or second (Fig. 3).

**Relationship between changes in vascular function and glycemic control.** Following training, the change in FBF in response to the highest dose of ACh was not significantly correlated with either the change in glycated hemoglobin (r = 0.09, p > 0.1) or in fasting blood glucose concentration (r = 0.14, p > 0.1). Similarly, change in the FMD response, before and after training, was not significantly correlated with either the change in glycated hemoglobin (r = −0.32, p > 0.1) or that in fasting blood glucose concentration (r = −0.10, p > 0.1).

**DISCUSSION**

The principal new finding of this study was that exercise training improved indices of endothelial function in patients with type 2 diabetes. The FBF response to ACh and FMD of the brachial artery, both of which are endothelium and
largely NO dependent, significantly increased. In contrast, no significant changes in resistance vessel response to SNP or conduit artery response to GTN were apparent, although there was a trend toward improved resistance vessel response to SNP. These findings suggest that resistance and conduit vessel vasodilator function are enhanced by exercise training in type 2 diabetic patients. This adaptation appears to be predominantly endothelium dependent, although other mechanisms, such as increased smooth muscle sensitivity to NO or structural changes, cannot be ruled out.

Exercise training and vascular function. Data from animal studies strongly suggest that exercise training or chronic increases in flow induce improvement in NO-dependent vascular function (9,23), including upregulation of constitutive endothelial NO-synthase expression (10,11). In addition, recent studies have investigated the effect of exercise training in humans. We demonstrated that an exercise training program similar to that used in the present study improved endothelium-dependent and -independent vascular function in patients with cardiac failure (16). This accords with other studies in such patients; four weeks of local forearm training improved forearm conduit artery flow-dependent vasodilation, an effect attenuated by infusion of the inhibitor of NO synthesis, Nω-monomethyl-L-arginine (L-NMMA) (13). Also, an eight-week forearm training program improved local flow responses to ACh, whereas endothelium-independent responses were unaltered (15), and six months of cycle training improved basal and stimulated endothelium-dependent, NO-related responses in the lower limb (14). In addition, four weeks of cycle ergometer training improved basal NO function in forearm resistance vessels of healthy volunteers (12). However, the present findings are the first to demonstrate an effect of exercise training in type 2 diabetic patients, and in addition, no previous investigation has combined assessment of both conduit and resistance vessel function. The potential clinical relevance of these findings is highlighted by the critical role of NO as an antiatherogenic molecule (5), the high levels of cardiovascular mortality and morbidity in type 2 diabetes and previous findings that interventions which improve endothelial function (2–4,14,24) are also associated with improved mortality and morbidity.

The beneficial effects of an exercise program on vascular function probably relate to increasing flow and shear stress on the endothelium, although general metabolic effects may also contribute. There is evidence that FMD in conduit vessels is largely dependent on endothelial release of NO (13,18,25–28), as is resistance vessel dilation and hence exercise-induced hyperemia (29–31). Experimentally, repeated exercise induces a sustained increase in endothelial NO-synthase (10,11), implying chronic adaptation of the NO-vasodilator system. It might be expected that an increase in shear stress would not only result from increased blood flow but also from other hemodynamic variables such as increased HR and blood pressure, as well as metabolic effects that would be imposed throughout the vasculature.
This hypothesis would explain why the improvement in vascular function in this study was evident in the upper limb, whereas our exercise program was designed to be one that excluded exercise of the upper limb. That is, the beneficial effect of exercise training appears to be generalized and not restricted to the vascular bed of the specifically trained skeletal muscle. This phenomenon has been recently reported and described (16,32).

Alternative mechanistic explanations may relate to the effect of exercise training on advanced glycation products. Such products are associated with depressed NO function (33,34), and glycated hemoglobin concentration decreased as a result of exercise training in the present study. Fasting blood glucose also decreased, consistent with decreased insulin resistance (17,35–41). This constitutes another possible mechanism for improved vascular function as insulin stimulates the production of NO through the insulin receptor, the effector pathway having some commonality with that for glucose transport (42). Because muscle glucose uptake is dependent, in part, upon skeletal muscle blood flow, the training-induced increase in vasodilator function may contribute to the improved glycemic control, and vice versa, as just outlined. However, in the present study, neither the changes in glycated hemoglobin nor in fasting blood glucose concentrations were correlated with changes in ACh or in FMD responses. A further potential explanation could have been training-induced changes in blood lipid concentrations, as suggested by a recent cross-sectional study (43) demonstrating enhanced ACh responses in the “untrained” forearms of runners who had low plasma cholesterol. This explanation appears unlikely, because vascular function improved in the present study in the absence of change in blood lipid concentrations, although training-induced changes in lipid metabolism cannot be excluded (38). Finally, it is possible that exercise training modulates the effect of reactive oxygen species on NO bioavailability or stimulates other relevant hormonal responses. The precise mechanisms responsible for exercise training–induced changes in vascular function remain to be determined.

Effect of detraining. Because the nontrained data did not differ between the groups training first or second, it does not appear that the effect of exercise training on the vasculature persisted for eight weeks. This is consistent with the time-course of deconditioning associated with other physiologic adaptations to exercise training, such as skeletal muscle metabolic changes (44), which reverse within four to six weeks of exercise cessation. In addition, it accords with the reversal of improvement in FMD of the brachial artery observed six weeks after the cessation of forearm hand-grip exercise in patients with heart failure (13). Therefore, it is likely that an exercise program, at some level as yet undetermined, would be necessary to maintain the vascular benefits of exercise.

Summary. Finally, this study indicates that an exercise training program improves indices of glycemic control, aerobic exercise tolerance and conduit, and resistance vessel endothelial vasodilator function in patients with type 2 diabetes. The latter findings have particularly important clinical significance in diabetes; improvement in endothelial function often parallels antiatherogenic benefits, and vascular complications account for most deaths in type 2 diabetic patients. An exercise program should be considered an integral component of diabetic management for all patients capable of participation.

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