Exercise Training Increases Basal Nitric Oxide Production From the Forearm in Hypercholesterolemic Patients

Tamara V. Lewis, Anthony M. Dart, Jaye P.F. Chin-Dusting, Bronwyn A. Kingwell

Abstract—The objective of this study was to investigate the effects of cycle training on basal nitric oxide (NO) production and endothelium-dependent dilator capacity in hypercholesterolemic patients in whom acetylcholine responsiveness is impaired. Nine sedentary hypercholesterolemic volunteers (total plasma cholesterol >6.0 mmol/L; 2 female) aged 44±3 years (mean±SEM) participated in the study. Subjects remained sedentary for 4 weeks and performed 4 weeks of home-based cycle training (3×30 minutes/week at 65% maximum oxygen consumption [VO₂ max]) in a randomized order. Arteriovenous nitrate/nitrite (NOₓ) gradient was assessed and plethysmography was used to measure the forearm blood flow responses to arterial infusions of acetylcholine, sodium nitroprusside, and N⁶-mono methyl L-arginine. Training increased VO₂ max from 30.4±1.9 to 34.3±1.4 mL kg⁻¹ min⁻¹ (P=0.01). Intrabrachial diastolic blood pressure was reduced from 70±3 to 68±3 mm Hg (P=0.02) with training, whereas systolic pressure did not change. Plasma triglycerides and total, LDL, and HDL cholesterol were not different between interventions. In the sedentary state, there was a positive forearm arteriovenous difference in plasma NOₓ indicating net extraction (6.8±4.0 nmol·100 mL⁻¹·min⁻¹), whereas in the trained state this difference was negative, indicating net production (~5.8±5.8 nmol·100 mL⁻¹·min⁻¹; P=0.03). N⁶-mono methyl L-arginine, at a dose of 4 μmol/min, caused a greater vasoconstriction after training (79.6±3.4% versus 69.9±6.8%; P=0.05). Acetylcholine and sodium nitroprusside induced dose-dependent elevations in forearm blood flow that were unaffected by training. These data suggest that basal release of endothelium-derived NO is increased with 4 weeks of home based training in hypercholesterolemic patients, independently of lipid profile modification. This may contribute to the cardiovascular protective effects of exercise training, including reduced blood pressure. (Arterioscler Thromb Vasc Biol. 1999;19:2782-2787.)

Key Words: exercise ■ endothelium-dependent vasodilation ■ acetylcholine ■ lipids ■ hyperlipidemia

Nitric oxide (NO) has received much recent attention as one potential mediator of some of the vascular benefits derived from regular exercise.¹ A number of studies in both animals and humans have recognized that endothelially derived NO plays a role in blood flow regulation during acute, dynamic exercise.² In particular, it has been postulated that vasodilatation in active muscle promotes a pressure gradient and thus increased blood flow that stimulates NO production from upstream arteries.³ NO mediated dilation of “feed” arteries can therefore permit increased microvascular flow without reduction in muscle perfusion pressure. With regular exercise it appears that there are adaptations in this system that may be partly responsible for the reduction in cardiovascular risk associated with the trained state.

Studies in rats and rabbits have provided evidence for enhanced aortic endothelial dependent vasodilatation and basal release of NO with exercise training.⁴³ Importantly, similar improvements in response to training have been observed in the coronary vasculature of pigs and dogs.⁹⁻¹⁴ All training related studies of endothelial function in humans have been carried out in peripheral vessels. Our laboratory has shown that 4 weeks of cycle training increases basal production of NO from the forearm.¹⁵ In this study, forearm blood flow and blood viscosity were elevated by 230% and 16%, respectively, immediately after a single 30-minute bout of exercise, and 60 minutes after cessation of exercise, forearm blood flow remained elevated by 75%. We postulate that these effects combined with heart rate and pulse pressure elevation would increase shear stress and thus provide a potent stimulus for nitric oxide production.¹⁶,¹⁷ Forearm acetylcholine responsiveness was unaffected by training in this previous study. Increased basal production of NO may therefore contribute to the reduction in blood pressure we and others have previously observed after only 4 weeks of regular exercise.¹⁸⁻²¹

In the current study, we aimed to determine whether similar exercise induced adaptations occur in hypercholesterolemic patients who show impaired forearm responsiveness to acetylcholine.²²⁻²⁴ It is clear from previous studies that total and LDL cholesterol levels are negatively correlated with reactivity to acetylcholine.²⁵ To determine whether mechanisms independent of plasma cholesterol reduction...
could convey beneficial endothelial adaptations in hypercholesterolemic patients, we used a moderate exercise intervention of only 4 weeks duration that we have previously shown not to alter cholesterol levels in normcholesterolemic individuals. In a randomized crossover design, we compared this with 4 weeks of sedentary activity. At the end of each intervention, basal nitric oxide release was examined via both measurement of arteriovenous differences in the nitric oxide metabolites nitrate and nitrite (NO\textsubscript{x}) and the use of forearm venous occlusion plethysmography to measure blood flow responses to intrabrachial infusion of the NO synthase inhibitor, N\textsubscript{5}-monomethyl-L-arginine. Intrabrachial acetylcholine was used to study endothelium-dependent vasodilator reserve and sodium nitroprusside to study smooth muscle sensitivity to NO.

**Methods**

All subjects were recruited from our lipid management clinic on the basis of their elevated total cholesterol and gave their written informed consent for participation in the study, which was performed with the approval of The Ethics Committee from The Alfred Healthcare Group and carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association. Participants were healthy, unmedicated, nonsmokers, with a body mass index of 29 kg/m\textsuperscript{2}, blood pressure <140/90 mm Hg, cholesterol >6.0 mmol/L, triglycerides <4 mmol/L, and maximum oxygen consumption (VO\textsubscript{2} max) <35 mL·min\textsuperscript{-1}·kg\textsuperscript{-1}. No patient had evidence of significant coronary disease on history, examination, or stress testing. Forearm vascular reactivity to NO synthase inhibitors and sodium nitroprusside was used to study endothelium-dependent vasodilator reserve and forearm blood flow was measured using venous occlusion plethysmography with a sealed alloy-filled (gallium and indium), double-strand strain gauge (Medasonic) and recorded for 10 out of every 20 seconds. Hand blood flow was excluded via a wrist cuff inflated to 200 mm Hg, and venous occlusion pressure on the upper arm was between 40 and 50 mm Hg. Before each drug, basal blood flow was obtained from an average of at least 3 measurements. Responses to local sequential infusions (2 mL/min) given in the following order were obtained: the endothelium-dependent vasodilator acetylcholine (24, 48 μg/min), the endothelium-independent vasodilator sodium nitroprusside (0.4, 0.8, 1.6 μmol/min), and the NO synthase inhibitor L-NMMA (2.4 μmol/min). For acetylcholine and sodium nitroprusside, the peak response was ascertained when 3 consecutive measurements showed no further increase in flow (approximately 1 to 2 minutes). The response to L-NMMA was measured after a 10-minute infusion. For all drugs, the response was recorded as the average of 3 steady-state measurements. Rest periods of 5 minutes between drug doses and 15 minutes between drug types was sufficient for flows to return to resting levels for acetylcholine and sodium nitroprusside. For L-NMMA, the rest period between doses was 15 minutes. Intraarterial brachial mean blood pressure was recorded both during measurement of basal flows and immediately after each intervention to ensure that there were no systemic drug effects. Forearm blood flow measurements were made by a research assistant unaware of the training status of the study participants.

**Biochemical Analyses**

Blood for NO\textsubscript{x} analysis was collected into ethylenediaminetetraacetic acid tubes, deproteinized, and plasma concentrations were determined using the Griess reaction with the Cayman chemical kit. This assay reduces all nitrate to nitrite and measures total nitrite and nitrate. Blood for lipid analysis was collected into ethylenediaminetetraacetic acid tubes and placed immediately on ice and then centrifuged at 3000 rpm within 10 minutes of collection. Plasma was frozen at −20°C and analyzed within 5 days of collection. Plasma (5-ml) was loaded into an ultracentrifuge tube, overlay with normal saline (density 1.006), sealed, and spun at 40 000 rpm for 16 hours at 20°C. The tube was sliced and the “bottom” fraction, containing LDL and HDL, was collected volumetrically. Apo B–containing lipoproteins (LDL and IDL) were precipitated by the addition of heparin and manganous chloride leaving only HDL in the supernatant. Cholesterol and triglyceride levels were determined enzymatically in the plasma, and cholesterol was measured in the HDL fraction using a Cobas-BIO Centrifugal Analyser (Roche Diagnostic Systems).
TABLE 1. Blood Pressure and VO2max

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sedentary</th>
<th>Trained</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27±1.0</td>
<td>27±1.0</td>
<td>0.33</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>133±4.0</td>
<td>130±4.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>70±3.0</td>
<td>68±3.0</td>
<td>0.02*</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>92±3.0</td>
<td>90±3.0</td>
<td>0.77</td>
</tr>
<tr>
<td>Resting heart rate (bts/min)</td>
<td>83±3.0</td>
<td>84±2.0</td>
<td>0.73</td>
</tr>
<tr>
<td>VO2max (ml · kg⁻¹ · min⁻¹)</td>
<td>30.4±1.9</td>
<td>34.3±1.4</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. All pressures are intrabrachial. VO2max indicates maximum oxygen consumption.
*Denotes significant difference between sedentary and trained states, P<0.05.

TABLE 2. Plasma Lipids

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Sedentary</th>
<th>Trained</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>6.82±0.39</td>
<td>6.32±0.36</td>
<td>0.49</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>4.60±0.44</td>
<td>4.93±0.34</td>
<td>0.24</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.01±0.14</td>
<td>1.03±0.15</td>
<td>0.51</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.65±0.37</td>
<td>2.75±0.40</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *Denotes significant difference between sedentary and trained states, P<0.05.

Basal Forearm Blood Flow and Vascular Resistance

There was a trend for lower basal forearm blood flow measured before each drug infusion in the trained state, but this failed to reach statistical significance (P=0.14; Figure 2, upper panel). Basal blood flow did, however, fall slightly over the course of the experiment (P=0.05). Forearm vascular resistance was not different after the training and sedentary interventions (P=0.15) nor altered during the course of the protocol (P=0.53; Figure 2, lower panel). There were no order-related influences on basal forearm blood flow (P=0.44) or vascular resistance (P=0.43).

Basal Release of NO

In the sedentary state, there was, on average, a positive forearm NOx arteriovenous difference (2.1±0.9 mmol/L) indicating net consumption of NOx (6.8±4.0 nmol · 100 mL⁻¹ · min⁻¹; Figure 3). After training, however, there was, on average, a negative forearm NOx arteriovenous difference (−1.55±1.75 mmol/L; P=0.02) indicating net production of NOx (−5.8±5.8 nmol · 100 mL⁻¹ · min⁻¹; P=0.03). These data are consistent with increased basal production of nitric oxide in the trained state compared with the sedentary state.

L-NMMA was used to inhibit NO synthase as a functional measure of basal NO release. When absolute blood flows were analyzed using repeated measures ANOVA, a significant dose-dependent vasoconstriction was evident (P=0.002). Blood flow in response to L-NMMA infusion was also lower after the training intervention (P=0.009). Because there was a trend for lower basal forearm blood flow before infusion of L-NMMA in the trained state, we also expressed the responses to L-NMMA infusion as a percentage of basal blood flow. This analysis showed a significant...
interaction between dose and training status \((P=0.05)\), indicating a greater vasoconstriction after training at the higher but not the lower dose of L-NMMA \((79.6\pm 3.4\% \text{ versus } 69.9\pm 6.8\%; P=0.05; \text{ Figure } 4, \text{ upper panel})\).

**Endothelium-Dependent and Independent Vasodilatation**

Acetylcholine caused a dose-dependent increase in forearm blood flow \((P=0.03)\) in both the sedentary and the trained states, which was not different between interventions \((P=0.19; \text{ Figure } 4, \text{ center panel})\). Infusion of the endothelium-independent agonist sodium nitroprusside also caused a dose-dependent increase in forearm blood flow \((P=0.005)\), which was not altered by training \((P=0.46; \text{ Figure } 4, \text{ lower panel})\).

The order of intervention (sedentary versus trained) did not significantly influence any of the blood flow responses to drug infusions.

**Discussion**

This is the first study to provide evidence of enhanced basal NO production after 4 weeks of moderate home-based aerobic training in hypercholesterolemic subjects. Because basal forearm blood flow and vascular resistance were not different between interventions, our findings suggest that training alters the regulation of basal NO production such that more NO is produced at any given flow. At higher flows, this regulatory shift may contribute to increased dilatory capacity. These changes occurred in the absence of any changes in lipid profile. Training did not alter stimulated NO release in response to acetylcholine infusion. Similarly, training did not induce vascular smooth muscle adaptations in reactivity to NO because responses to the NO donor, sodium nitroprusside, were not different between interventions.

The conclusion that basal NO release is increased with training is inferred from both greater vasoconstriction to L-NMMA and the more negative NO arteriovenous difference observed after the training intervention. Evidence of augmented basal NO production has previously been documented in coronary arteries excised from canines after 10 days of treadmill running and in forearm resistance arteries of healthy, previously sedentary, normcholesterolemic humans after 4 weeks of cycle training. Data from the current study indicate that despite impaired vasodilation to acetylcholine, hypercholesterolemic individuals show similar adaptive patterns to normcholesterolemic subjects. We postulate that elevation in shear stress during exercise via increased blood viscosity and elevated heart rate and increased pulse pressure increases production of NO from the forearm during leg exercise. Previous work from our laboratory has documented elevation in both forearm blood flow and blood viscosity immediately after a 30-minute bout of acute exercise. Our findings imply that NO production may remain elevated between training sessions perhaps through upregulation of NO synthase (isoform III) and may subsequently contribute to the blood pressure reduction and elevated heart rate and increased pulse pressure increases production of NO from the forearm during leg exercise. Previous work from our laboratory has documented elevation in both forearm blood flow and blood viscosity immediately after a 30-minute bout of acute exercise.

Responsiveness to acetylcholine has been shown previously to be impaired in the presence of lipid elevation, whereas basal release of NO, as assessed by vasoconstrictor responses to L-NMMA, is similar in hypercholesterolemic individuals and normal healthy subjects. We have also previously shown that training increases basal nitric oxide production but not responsiveness to acetylcholine in normcholesterolemic individuals. L-NMMA and acetylcholine influence NO production through distinct mechanisms. L-NMMA blocks basal nitric oxide production, which is released in response to endothelial shear stress. Acetylcholine, however, acts via the M1 muscarinic receptor on endothelial cells. It is possible that these mechanisms are differentially affected by training. In particular, it is likely that shear stress induced NO release would be modulated by training because exercise acutely increases factors influencing shear stress, including blood flow, blood viscosity, vessel calibre, and heart rate. In addition, the signal transduction mechanisms for shear stress and agonist stimulated NO release appear to be distinct. Although the shear stress...
signaling mechanism is not well understood, it appears to involve a pertussis toxin sensitive G protein, which is distinct from the G protein, predominantly involved in coupling of the endothelial M, muscarinic receptor and in which acetylcholine elicits NO release.

It is possible that the intensity of the training program or the short-term nature of the study contributed to the lack of change in acetylcholine responses because enhanced responsiveness to acetylcholine has been demonstrated using more intense or longer duration programs and in highly-trained athletes. Although most previous studies have not documented whether or not blood lipid changes occurred with training, our previous experience with highly-trained athletes has suggested that enhancement of acetylcholine responsiveness may be related, at least partly, to reduction in total cholesterol. Such effects may involve a pertussis toxin sensitive G protein, which is distinct from the G protein, predominantly involved in coupling of the endothelial M, muscarinic receptor and in which acetylcholine elicits NO release.

In conclusion, the current study provides evidence that a home-based exercise program increases the basal production of NO in hypercholesterolemic patients. Such effects may contribute to the lower blood pressure observed in this and in the previous studies. These changes occurred in the absence of both lipid profile modification or improvement in acetylcholine mediated vasodilation. Thus moderate training for hypercholesterolemic patients has beneficial effects additional to lipid profile modification and may be considered a useful adjunct to conventional lipid lowering therapy.

Acknowledgments

We are grateful for the assistance of Leonie Johnston, Karen Murchie, Luke Robinson, and Tanya Medley. Dr Kingwell is a National Health and Medical Research Council Research Fellow. This work was also supported by the National Heart Foundation of Australia.

References

Exercise Training Increases Basal Nitric Oxide Production From the Forearm in Hypercholesterolemic Patients
Tamara V. Lewis, Anthony M. Dart, Jaye P. F. Chin-Dusting and Bronwyn A. Kingwell

*Arterioscler Thromb Vasc Biol.* 1999;19:2782-2787

doi: 10.1161/01.ATV.19.11.2782

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/19/11/2782

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org/subscriptions/