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 [VO2max]) 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 NG mono methyl L-arginine. Training increased VO2max 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). NG 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.4–9 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 normocholesterolemic 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 intervention, basal nitric oxide release was examined via both this with 4 weeks of sedentary activity. At the end of each

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 $<29 \text{ kg/m}^2$, blood pressure $<140/90 \text{ mm Hg}$, cholesterol $>6.0 \text{ mmol/L}$, triglycerides $<4 \text{ mmol/L}$, and maximum oxygen consumption ($VO_2 max$) $<30 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. No patient had evidence of significant coronary disease on history, examination, or biochemical blood tests, assessment of blood pressure, and $VO_2 max$. 

Study Design

Nine sedentary volunteers (2 female, aged 44±3 years; mean±SEM) remained sedentary for 4 weeks and performed 4 weeks of cycle training in random order. After each intervention subjects were required to attend the laboratory on 2 separate days. The first of these was for $VO_2 max$ assessment. On the second occasion, which occurred at least 48 hours after the last exercise bout, forearm arteriovenous NO$_x$ gradient and forearm vascular reactivity to acetylcholine, sodium nitroprusside, and N$^\omega$-monomethyl-L-arginine (L-NMMA) was assessed as described below.

Throughout the study, subjects were advised to make no changes to their lifestyle, especially diet, including alcohol intake and recreational physical activity.

Exercise Training

Exercise bikes were provided for home use and training consisted of 30-minute cycling sessions 3 times per week performed at 65% of predetermined maximum heart rate. This was calculated as resting heart rate plus 65% of the difference between resting and maximum heart rate. Each session was preceded by a 5-minute warm-up during which workload was incremented to achieve the target workload at the completion of the warm-up. A 5-minute cool-down concluded each session. Compliance was assessed by diary and validated with maximum oxygen consumption assessment.

$VO_2 max$ was assessed during a graded exercise test on an electrically braked cycle ergometer as described previously. This test consisted of 1-minute periods of bicycle exercise commencing at zero workload and increasing by 20 watts each minute until any further increase in workload was prevented by fatigue. The criteria for establishment of $VO_2 max$ included a plateau in the oxygen consumption with increasing work rate, a respiratory exchange ratio $>1.1$, and failure to maintain the required workrate despite encouragement. We defined $VO_2 max$ as the average $VO_2$ during the final 30 seconds of exercise. Oxygen and carbon dioxide measurements were made using a Medical Graphics Corporation 2001 CAD/Net Cardiopulmo-

Biochemical Analyses

Blood for NO$_x$ analysis was collected into ethylenediaminetetraacetic acid tubes, deproteinized, and plasma concentrations were determined using the Griess reaction with the Cayman chemical kit 780001. This assay reduces all nitrate to nitrite and measures total nitrate, which indicates nitric oxide formation, as the Griess conversion product. We calculated arteriovenous differences in NO$_x$ and multiplied this value by forearm blood flow to derive net forearm consumption or production. This method provides a more accurate assessment of nitric oxide production or consumption or production. This method provides a more accurate assessment of nitric oxide production or consumption from the forearm than single measures of venous or arterial NO$_x$, which are susceptible to the effects of changes in volume of distribution and nitrate accumulation.

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 then frozen at $-20°C$ and analyzed within 5 days of collection. Plasma (5-ml) was loaded into an ultracentrifuge tube, overlaid 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 manganese 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).
Results

We verified that acetylcholine responses were indeed impaired \( (P<0.05) \) in the hypercholesterolemic individuals by comparison with an age-matched \( (42 \pm 3 \text{ years}; n=8) \) control group with a total plasma cholesterol of \( 4.42 \pm 0.24 \text{ mmol/L} \) and triglycerides of \( 1.39 \pm 0.14 \text{ mmol/L} \). \( P<0.001 \) compared with the protocol \( (P=0.14; \text{Figure 1}) \). After training, however, there was, on average, a negative forearm NO\(_x\) arteriovenous difference \( (-1.55 \pm 1.75 \text{ mmol/L}; P=0.02) \) indicating net production of NO\(_x\) \( (-5.8 \pm 5.8 \text{ mmol} \cdot 100 \text{ mL}^{-1} \cdot \text{min}^{-1}; P=0.03) \). 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

### 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 \( (P=0.14; \text{Figure 2, upper panel}) \). 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 seden-

### 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, \( P<0.05 \).
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 \(79.6\pm3.4\% \text{ versus } 69.9\pm6.8\%; \quad P=0.05; \quad \text{Figure 4, upper panel}\).

**Endothelium-Dependent and Endothelium-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 \((P=0.19; \quad \text{Figure 4, center panel})\). 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 \((P=0.46; \quad \text{Figure 4, lower panel})\).

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 the 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\(^9\) and in forearm resistance arteries of healthy, previously sedentary, normocholesterolemic humans after 4 weeks of cycle training.\(^{15}\) Data from the current study indicate that despite impaired vasodilation to acetylcholine, hypercholesterolemic individuals show similar adaptive patterns to normocholesterolemic subjects. We postulate that elevation in shear stress during exercise via increased blood viscosity\(^{17}\) and elevated heart rate and increased pulse pressure\(^{16}\) 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.\(^{15}\) Our findings imply that NO production may remain elevated between training sessions perhaps through upregulation of NO synthase (isoform III)\(^9\) and may subsequently contribute to the blood pressure reduction, elevation in 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.\(^{15-17}\) 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,33 which is distinct from the G protein, predominantly involved in coupling of the endothelial M, muscarinic receptor32,34–36 via 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 programs37 and in highly-trained athletes.25 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.25 Like improvement in acetylcholine responsiveness, such modifications in lipid profile usually only occur after intense, long-duration training programs.

As expected, 4 weeks of training did not significantly alter any of the lipoprotein parameters measured. In studies that have reported an increase in HDL cholesterol18 and a decrease in LDL cholesterol39 and triglycerides,40,41 subjects have usually trained at between 70% to 90% of their predetermined maximum heart rate, 3 to 5 times per week for at least 14 weeks.41–43 Furthermore, Superko reported jogging for at least 6 km/wk for 7 months was required to induce a change in lipid profile.44 Prescription of high-intensity exercise to alter lipid levels may not be appropriate for hypercholesterolemic patients who are at elevated risk of myocardial ischemia. However, it is evident from the current study that exercise may convey benefit beyond lipid profile modification via elevation in basal NO production.

In conclusion, the current study provides evidence that a home-based exercise program increases the basal production of nitric oxide from the forearm.45 Increases basal production of nitric oxide from the forearm.45

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


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*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
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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