European Heart Journal (2003) **24**, 1681–1689

Effects of exercise training on conduit and resistance vessel function in treated and untreated hypercholesterolaemic subjects

Jennifer H. Walsh[a,b,c,d], Gerald Yong[c,d], Craig Cheetham[a,c,d], Gerald F. Watts[b], Gerard J. O'Driscoll[c,d], Roger R. Taylor[b,c], Daniel J. Green[a,c,d]*

[a] *School of Human Movement and Exercise Science, The University of Western Australia, Perth, Australia*
[b] *University Department of Medicine, The University of Western Australia, Perth, Australia*
[c] *Department of Cardiology, Royal Perth Hospital, Western Australia, Perth, Australia*
[d] *Cardiac Transplant Unit, Royal Perth Hospital, Western Australia, Perth, Australia*

Received 11 February 2003; received in revised form 26 May 2003; accepted 2 July 2003

KEYWORDS
Blood flow;
Cholesterol;
Endothelial function;
Nitric oxide;
Ultrasound;
Exercise

Aims Despite the importance of both lipid metabolism and physical activity to cardiovascular health, few studies have examined the effect of exercise training on vascular function in hypercholesterolaemic humans.
Methods and results A randomized, cross-over design investigated the effect of 8 weeks of combined aerobic and resistance exercise training on conduit and resistance vessel function in 11 untreated subjects with hypercholesterolaemia and 11 subjects taking lipid-lowering medication. High-resolution vascular ultrasonography following forearm ischaemia and glyceryl trinitrate administration determined conduit vessel endothelium-dependent and independent function. Strain-gauge plethysmography, with intra-aerial infusions of acetylcholine, sodium nitroprusside and N^G-monomethyl-L-arginine, determined resistance vessel function. Flow-mediated dilation and the forearm blood flow response to acetylcholine improved significantly following training in the treated subgroup (both $P<0.05$) but not the untreated, although the blood flow response to N^G-monomethyl-L-arginine was augmented following training in the untreated subjects ($P<0.05$), indicating greater basal nitric oxide bioactivity. Training did not alter responsiveness to glyceryl trinitrate or sodium nitroprusside.
Conclusions Combined aerobic and resistance training improves endothelium-dependent conduit and resistance vessel function in hypercholesterolaemic subjects taking lipid-lowering medications and basal nitric oxide bioactivity in untreated hypercholesterolaemic subjects. Exercise training may provide additional cardiovascular benefits for hypercholesterolaemic patients including those taking lipid-lowering medication.

Introduction

Endothelium-derived, nitric oxide (NO) release is an important component of vasomotor regulation and depressed function of the endothelial NO system is an early manifestation of atherosclerosis.[1] Subjects with known cardiovascular disease,[2–4] or even cardiovascular risk factors,[2,3,5,6] exhibit impaired endothelium-dependent vasomotor responses. Pharmacological interventions associated with reduced cardiovascular mortality and morbidity also improve endothelial function.[7–10] In addition, recent studies indicate that endothelial dysfunction may predict cardiovascular outcomes.[11–13]

* Correspondence to: Dr D. J. Green, University of Western Australia, Human Movement and Exercise Science, Crawley, Perth, Western Australia 6009, Australia. Tel.: +61-893802361; fax: +61-893801039
E-mail address: brevis@cyllene.uwa.edu.au (D.J. Green).

0195-668X/03/$ - see front matter © 2003 Published by Elsevier Ltd on behalf of The European Society of Cardiology.
doi:10.1016/S0195-668X(03)00384-1

Table 1 Resting, exercise and biochemical characteristics in trained and untrained hypercholesterolaemic subjects

	All subjects (*n*=22)		Treated (*n*=11)		Untreated (*n*=11)	
	Untrained	Trained	Untrained	Trained	Untrained	Trained
Plasma lipids (mmol l^{-1})						
Total cholesterol	5.4 (0.2)	5.3 (0.2)	4.6 (0.3)	4.4 (0.2)	6.3 (0.1)[a]	6.2 (0.2)[a]
LDL cholesterol	3.4 (0.2)	3.4 (0.2)	2.6 (0.2)	2.6 (0.1)	4.2 (0.1)[a]	4.1 (0.2)[a]
HDL cholesterol	1.3 (0.1)	1.2 (0.1)	1.3 (0.1)	1.2 (0.1)	1.3 (0.1)	1.3 (0.1)
Triglycerides	1.6 (0.2)	1.4 (0.2)	1.5 (0.2)	1.3 (0.2)	1.8 (0.3)	1.6 (0.2)
Fasting blood glucose (mmol-l^{-1})	4.9 (0.1)	4.9 (0.1)	4.9 (0.2)	4.9 (0.1)	4.9 (0.1)	4.9 (0.1)
$\dot{V}O_{2peak}$ (l min^{-1})	2.25 (0.13)	2.41 (0.15)[b]	2.05 (0.19)	2.25 (0.23)	2.45 (0.18)	2.57 (0.20)
Exercise duration (sec)	975 (67)	1084 (76)[b]	881 (94)	987 (119)[b]	1070 (95)	1181 (96)[b]
Weight (kg)	79.1 (2.9)	79.9 (3.0)	77.2 (4.6)	77.8 (4.8)	81.0 (3.8)	81.9 (3.9)
Resting MAP (mmHg)	83 (2)	81 (2)	81 (3)	78 (3)	84 (3)	84 (3)
Resting HR (beats min^{-1})	59 (2)	58 (2)	59 (3)	58 (3)	59 (3)	57 (2)

[a]Significantly different from treated group ($P<0.001$).
[b]Significantly different from untrained period ($P<0.005$).
Values are mean±(SE). LDL, low-density lipoprotein; HDL, high-density lipoprotein; $\dot{V}O_{2\ peak}$, peak oxygen consumption; MAP, mean arterial pressure; HR, heart rate.

A number of animal[14–16] and human[17–24] studies suggest that exercise training can enhance endothelium-mediated vasomotor tone. Specifically, animal studies indicate that the increase in arterial wall shear stress during exercise results in increased constitutive NO-synthase expresssion[15] and enhanced vasodilator capacity.[16] Human studies suggest that exercise training leads to sustained whole-body improvement of vascular function, not only that of the exercising musculature.[22,24–27]

Treatment of hypercholesterolaemia is associated with improved vascular function.[7–9,28] For example, O'Driscoll et al.[7] demonstrated that 4 weeks of simvastatin treatment improved endothelium-dependent dilator function. A further 3 months of therapy induced additional improvement without further benefit to the lipid profile. These and other findings indicate that statin therapy may have effects on the vasculature other than those associated with lipid-lowering alone.

Although both lipid control and physical activity are important to cardiovascular health, few studies have examined the effect of exercise training on vascular function in hypercholesterolaemic humans. In untreated subjects, 4 weeks of three times weekly cycle training has been reported to increase basal NO bioactivity in resistance vessels, determined using the NO-synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA).[29] However, neither the effect of exercise training in subjects taking statin therapy nor that on conduit vessel function have been studied. Hence, our aim was to use a randomized, cross-over design to determine whether exercise training has a beneficial effect on conduit and resistance vessel function in treated and untreated hypercholesterolaemic subjects.

Methods

Subjects

Twenty-two sedentary (16 male, six female),currently or previously hypercholesterolaemic, but otherwise healthy, subjects (11 treated, 11 untreated) were recruited from lipid clinics or via public advertisement. Average age was 52±2 years, BMI, 26±0.7 kg m^{-2} and waist:hip ratio, 0.90±0.02 without difference between the treated and untreated subgroups. Initial screening required total cholesterol >6.5 mmol l^{-1} and/or low-density lipoprotein (LDL) cholesterol >4.0 mmol l^{-1} in untreated subjects and documentation that these levels had been previously attained in the treated, who must have been taking an HMG-CoA reductase inhibitor in stable dose for at least 3 months (nine on atorvastatin, one simvastatin and one cervistatin), the average of their previously highest LDL-cholesterol being 5.6±0.2 mmol l^{-1}. Lipid results at the time of study are reported in Table 1. Four of the treated subgroup were also taking aspirin; one amlodipine (subject was normotensive for study duration) and one constant dose oestradiol. Medications remained unaltered during the study. No untreated subject was taking any medication.

Subjects were excluded if they were current smokers; premenopausal female; post-menopausal female taking cyclical hormone therapy; hypertensive (resting BP>160/90); diabetic; asthmatic; displayed evidence of coronary or valvular heart disease from history, examination and exercise electrocardiography; performed >2 sessions of light-moderate exercise per week; were unable to exercise due to physical limitations; or were taking angiotensin-converting enzyme inhibitor medication. The investigation conforms with the principles outlined in the Declaration of Helsinki. The Royal Perth Hospital Ethics Committee approved the protocol and all subjects gave written informed consent.

Study design

After preliminary screening and baseline assessments, subjects were randomly assigned to remain sedentary or perform exercise training for 8 weeks, followed by cross-over (Fig. 1). Such a cross-over design has proved satisfactory to study the effect of this relatively short training period in several studies.[22,24,30] Assessments of vascular function and exercise capacity were made at baseline, 8 weeks and 16 weeks. A familiarization exercise capacity assessment was made prior to baseline measurements. The exercise training protocol and assessment procedures are summarized below, having previously been extensively reported.[22,24,30,31] Subjects were requested to make

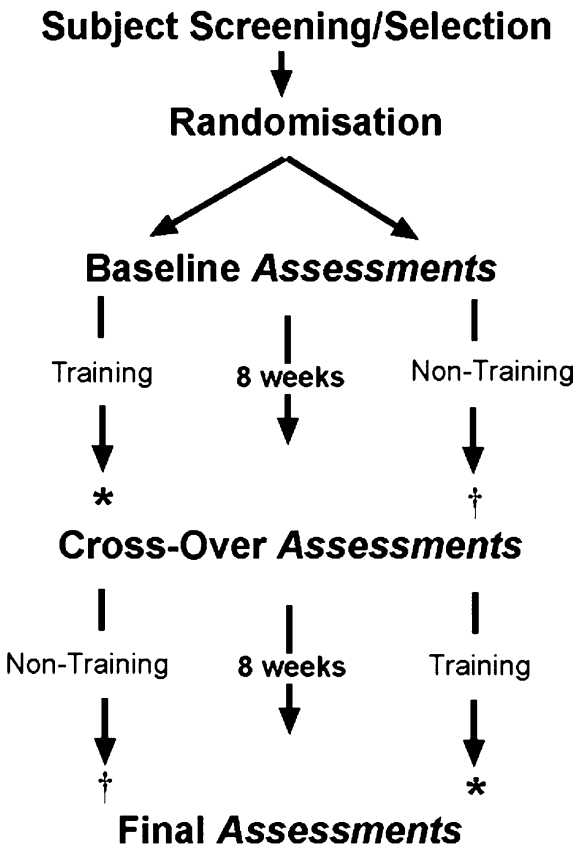

Fig. 1 Study design. Subjects underwent a randomized, controlled crossover study involving 8 weeks of training and non-training periods. All subjects had assessments at baseline, 8 weeks and 16 weeks. Baseline data did not significantly differ from the post-sedentary data and there was no evidence of a carry-over effect in the post-sedentary data of those subjects trained first. We therefore compared the post-training data (*) to that following the untrained control period (†).

no changes to their diet or other routines. Those who exercised first returned to their previous sedentary lifestyle for the control phase and this was confirmed by interview.

Tests of forearm resistance and conduit vessel function were conducted, at separate attendances, in a quiet, temperature-controlled environment and 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 subjects. In 20 subjects (10 treated, 10 untreated), resistance vessel function was examined by plethysmography, as was the peak reactive hyperaemic blood flow response to a 10-min ischaemic stimulus ($RHBF_{10}$), an index of vascular structure.[32]

Assessment of conduit vessel function

The procedure for conduit vessel function assessment using vascular ultrasonography has been described previously.[24] Analysis of brachial artery diameters was performed at the time of the ECG R wave, that is, at end-diastole. The same experienced sonographer performed the analysis using custom-designed edge-detection and wall-tracking software which minimizes investigator bias and has the power to detect an absolute change in FMD of 2.5% in cross-over design study with only five subjects.[33] 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.[33]

Assessment of resistance vessel function

Within 1 week of conduit vessel assessment, subjects returned for resistance vessel and peak reactive hyperaemic assessment ($RHBF_{10}$). The procedures have been fully described previously.[22,24,30]

Following $RHBF_{10}$, a 10-min rest period was observed to allow forearm blood flow (FBF) to return to baseline. A 20-gauge cannula was inserted into the brachial artery of the non-dominant arm to infuse vasoactive agents and sterile saline, and for blood sampling and measurement of intra-aterial pressure. Baseline plethysmography measurements were made 20 min after cannulation and during the following infusions as previously described: acetylcholine (ACh) at 10, 20, and 40 μg min^{-1}, each for 3 min; sodium nitroprusside (SNP) at 2, 4 land 8 μg min^{-1}, each for 3 min, and N^G-monomethyl-L-arginine (L-NMMA) at 2, 4 and 8 μmol min^{-1}, each for 4 min. The latter was infused last because of its more prolonged duration of action.

Assessment of exercise capacity

Maximal oxygen uptake VO_{2peak} and exercise test duration were determined from a graded maximal exercise test which was performed on an electronically braked bicycle ergometer (Orival 400, Lode). In all subjects, at all presentations, initial resistance was set at 50 W and increased in 25 W increments every 3 min until volitional exhaustion. Oxygen uptake was calculated as previously described.[30]

Exercise training protocol

Subjects performed two supervised combined aerobic and resistance circuit training sessions at the Cardiac Gymnasium, Royal Perth Hospital and one home training session per week. The focus was 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 manoeuvre. The 8-week 'circuit' training protocol involved a combination of resistance training, cycle ergometry and treadmill walking fully described in recent publications.[22,24,30,31] Home training sessions were individually prescribed and involved subjects performing continuous aerobic exercise at 70–85% peak heart rate for up to 45–60 min. To ensure compliance, sessions were recorded in a diary and heart rates were recorded using Polar heart rate monitors (Polar Electro Oy; Kempele, Finland).

Analysis of data

Forearm blood flow is presented as the raw data and as a ratio of that in the infused arm to that in the non-infused arm, changes in the ratio being expressed as percentage changes from the baseline immediately preceding the drug infusion period.[34,35] Forearm blood flow responses to the drug infusions following the exercise training period are compared to those following the non-training period using two-way analysis of variance (ANOVA) with repeated measures for the three doses of each drug. To account for period and order effects associated with our cross-over design, we additionally used a mixed model ANOVA which

included assessment of random effects by subject and accounted for period and order effects. To compare responses to training in other discrete variables, the Student paired t-test was used. Responses to training in the treated and untreated subjects were examined separately and also compared between groups using ANOVA. Data are reported as means±SE. Significance was set at $P<0.05$.

Results

Six treated and five untreated subjects were randomized to train during the first 8 weeks. All subjects completed 16 'circuit' training and eight training sessions without adverse event.

Effect of training order on vascular function

No differences were evident between baseline responses, that is the responses preceding the exercise or non-exercise protocol, and those following the non-exercise control period in either the sub-group trained first or those trained second (Fig. 1). As a single group, the effect of exercise training on FMD was not different between those who trained first (Δ=2.7±1.0%) or second (Δ=1.2±1.5%; $P>0.42$) and untrained FMD did not differ between those who trained first and those who trained second ($P>0.034$). Similarly, FBF responses to ACh were not dependent on training order ($P>0.05$). In fact, the difference between trained and untrained data was, on average, greater in those subjects who trained first, suggesting no significant persistence of the training effect. We therefore present results for the post-training data compared to the non-exercise control period data.

General effects of exercise training

Plasma total and LDL-cholesterol levels were significantly higher in the untreated than the treated subjects ($P<0.001$). Lipid and glucose levels did not change throughout the study (Table 1). Resting HR and MAP were not significantly altered while peak oxygen consumption (VO_2) tended to increase following training in each of the two subgroups, significantly in the total group ($P<0.05$). Exercise test duration increased significantly in each subgroup ($P<0.01$; Table 1).

Conduit vessel function

Basal brachial artery diameters were not significantly altered by training in either the treated (3.7±0.2 vs 3.7±0.2 mm; $P=0.84$) or untreated (3.8±0.2 mm vs 3.8±0.2 mm; $P=0.95$) subgroup. Exercise training significantly increased FMD in the treated subgroup (3.7±1.1 to 7.2±1.4%; $P=0.03$; paired t-test) but the trend was not significant in the untreated subgroup (5.5±1.0 vs 5.9±0.8%; $P=0.70$; Fig. 2). Endothelium-independent vasodilation in response to GTN was not significantly changed with training in either the treated (14.1±1.4 vs 14.8±1.7%; $P=0.61$) or untreated subgroups (15.7±1.3 vs 14.7±1.1%; $P=0.42$; Fig. 2).

Peak reactive hyperaemic response

In the treated subgroup, $RHBF_{10}$ was not significantly altered with exercise training in either the infused, non-dominant, (35.0±4.5 vs 29.0±2.3 ml 100 ml^{-1} min^{-1}) or non-infused, dominant, (28.5±4.9 vs 23.6±2.8 ml 100 ml^{-1} min^{-1}) arm. Likewise, $RHBF_{10}$ was not altered with training in the untreated subgroup in either the infused (34.7±3.9 vs 31.3±3.5 ml 100 ml^{-1} min^{-1}) or non-infused (26.4±3.6 vs 25.8±2.5 ml 100 ml^{-1} min^{-1}) arm.

Resistance vessel function

Baseline FBFs in the infused and non-infused arms were not significantly affected by training. In the infused arm, baseline FBF values preceding each drug infusion were not significantly different, indicating adequate washout periods (Table 2). Analysis of variance with repeated measures indicated a significant interaction between training status and ACh dose in the treated subgroup ($P<0.05$). Analysis at individual doses of ACh revealed a significant training effect on raw FBF at the highest dose in the treated subgroup ($P=0.02$; paired t-test). The magnitude of the training effect in the treated and untreated subgroups was not significantly different (ANOVA) even though the response in the untreated group failed to reach significance. Forearm blood flow responses to SNP and L-NMMA were not significantly altered with training (Table 2).

Fig. 3 shows percentage changes in the ratios of flow in the infused arm to the non-infused arm, the preferred method of analysis,[34,35] in response to ACh, SNP and L-NMMA infusions in both subgroups. When responses to the highest dose of ACh (40 μg min^{-1}) were compared, exercise training increased the percentage change in FBF ratio in subjects taking medication ($P<0.01$) but not in untreated subjects although the difference between the responses of the two subgroups was not statistically significant. In the untreated group, ANOVA revealed a significant dose/training interaction for change in the FBF ratio response to L-NMMA ($P=0.045$), consistent with greater vasoconstriction at the higher doses following training (Fig. 3). The FBF ratio response to L-NMMA was not altered in the treated subgroup. Training had no effect on the FBF ratio response to SNP in either group.

Discussion

The present study is the first to measure changes in both conduit and resistance vessel function in treated and untreated hypercholesterolaemic subjects in response to exercise training. Eight weeks of predominantly lower-limb, combined aerobic and resistance exercise training improved both conduit and resistance vessel endothelium and largely NO-dependent dilator function in hypercholesterolaemic subjects on HMG-CoA reductase inhibitor therapy but with persisting depression of endothelial

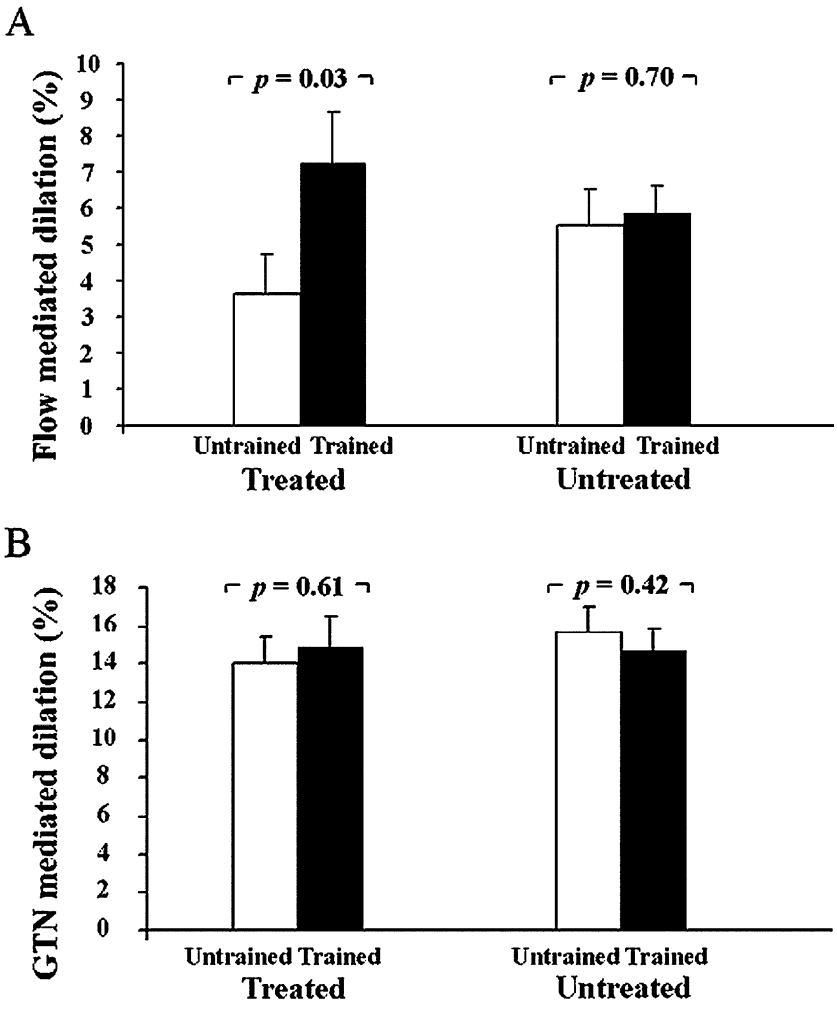

Fig. 2 (A) Endothelium-dependent, flow-mediated dilation (FMD) of the brachial artery and (B) endothelium-independent, glyceryl trinitrate (GTN)-mediated dilation of the brachial artery in treated and untreated hypercholesterolaemic subjects following an 8-week training (solid bars) and non-training period (open bars). Values are means±SE. FMD was significantly greater following training in subjects taking medication (P=0.03).

function. In untreated subjects, training significantly increased basal NO bioactivity, but not forearm conduit or resistance vessel dilator function. That is, in both groups, there was evidence of greater, but differently increased NO bioactivity. Training did not alter responses to the NO donors, SNP and GTN, nor to peak reactive hyperaemia in either subgroup, suggesting substantially unchanged smooth muscle cell sensitivity to exogenous NO and unchanged vascular structure.

FMD and FBF response to ACh examine different vascular beds but are both endothelium, and largely NO-dependent, responses. The possibility that the improvement in endothelium-dependent dilation resulted from underlying changes in vasodilator capacity is unlikely given that $RHBF_{10}$ was not altered with training. Peak reactive hyperaemia in response to a 10-min ischaemic stimulus ($RHBF_{10}$) is a maximal stimulus to flow and is considered an index of vessel structure,[32] not, therefore, dependent upon endothelium-mediated function.[36] Hence, the lack of significant change in $RHBF_{10}$ in response to training suggests that our assessments of FMD and ACh-mediated changes are not compromised by any underlying changes in vascular structure. Improved endothelium-dependent dilator function following exercise training, found in the treated group who had persistently depressed endothelial function, is consistent with studies in those with cardiac failure,[19,22] coronary artery disease,[21] hypertension,[20] and type 2 diabetes[24] in addition to some studies in healthy subjects.[17] These findings are significant in view of the evidence that endothelial dysfunction is an early event in the development of atherosclerosis[2] and coronary and/or peripheral vessel endothelial dysfunction can predict cardiovascular prognosis.[11–13]

The most likely mechanism for the improvement in endothelium-dependent function, although different in the two groups, is related to shear stress-mediated up-regulation of NO-synthase expression resulting from increased blood flow,[15,37] although reduced quenching of NO, as by superoxide radicals, could be relevant.[38] As fully discussed elsewhere,[26,27] it seems that repeated episodes of exercise, in the form of exercise training,

Table 2 Forearm blood flow: raw values for the infused arm in trained and untrained hypercholesterolaemic subjects

	Treated (*n*=10)		Untreated (*n*=10)	
	Untrained	Trained	Untrained	Trained
ACh				
Baseline	2.4(0.4)	2.7(0.3)	2.2(0.4)	2.5(0.3)
10 μg min^{-1}	5.0(1.6)	5.3(0.8)	4.0(1.1)	4.2(1.0)
20 μg min^{-1}	10.1(2.4)	14.1(2.3)	7.4(1.8)	11.1(2.6)
40 μg min^{-1}	12.7(2.7)	23.7(3.1)[a]	11.1(2.1)	16.7(3.0)
SNP				
Baseline	2.8(0.7)	2.6(0.4)	2.1(0.4)	2.6(0.3)
2 μg min^{-1}	8.7(0.8)	9.1(0.8)	5.7(0.9)	8.2(1.0)
4 μg min^{-1}	12.5(1.0)	13.3(1.2)	11.1(1.6)	10.6(1.5)
8 μg min^{-1}	17.8(1.6)	19.8(1.4)	16.6(2.3)	15.2(1.9)
L-NMMA				
Basline	2.6(0.6)	2.6(0.4)	2.3(0.5)	2.7(0.4)
2 μmol min^{-1}	1.9(0.4)	2.0(0.3)	1.9(0.4)	2.1(0.2)
4 μmol min^{-1}	1.5(0.3)	1.9(0.3)	1.6(0.3)	1.8(0.2)
8 μmol min^{-1}	1.7(0.2)	1.9(0.1)	1.7(0.3)	1.7(0.2)

[a]Significantly different from untrained period (P=0.02; paired t-test).
Values are mean±(SE). Exercise training significantly increased the FBF response to the highest dose of ACh in subjects taking medication (P<0.03; ANOVA).

lead to a sustained and generalized increase in the potential for NO bioactivity within the vasculature. In the current study, exercise training, which avoided upper limb exercise, improved NO-dependent vasodilator function in one group, and basal NO bioactivity in the other, in the forearm vessel. Such a general effect on the vasculature has been found previously by us,[22,24] and others.[23,26] Furthermore, during a discrete session of lower limb, cycle exercise, an increase in NO-dilator bioactivity in the upper limb vessels has recently been found which, because of the very short half-life of NO, indicates that the NO has been produced in those vessels.[27] The effect is probably largely due to the generalized impact of haemodynamic variables acting through vessel wall shear stress[39] but hormonal or other effects accompanying exercise could contribute.

The improvement in basal NO bioactivity, shown by the greater response to infused L-NMMA, in the untreated subgroup is consistent with the findings of Lewis et al.[29] who reported 4 weeks of three times weekly cycle training improved basal NO bioactivity without change in ACh-mediated endothelium-dependent dilation of forearm resistance vessels. Both L-NMMA and ACh influence endothelial cell NO production and bioactivity, but through different mechanisms; L-NMMA is a competitive antagonist of NO synthase and NO production, including basal production and bioactivity, while ACh stimulates NO-synthase activity via the muscarinic receptor. It is possible that training influences these mechanisms differently and dependent upon pre-training status; training significantly augmented basal NO bioactivity in the untreated subjects but did not significantly alter responsiveness to either FMD or ACh, although there was a trend for increase in the latter. However, in treated subjects, training did improve the stimulated vasodilator responses. Probably relevant is that the untreated subjects possessed relatively normal vascular function at baseline whereas the treated hypercholesterolaemic subjects had clearly impaired baseline function. For example, FMD in the untreated subjects, although lower on average, was not significantly different from that of recently studied healthy control subjects of similar age (5.5±1.1 vs 7.1±0.4%; P=ns), while that of the treated subjects was significantly depressed (3.7±1.1% vs 7.1±0.4%; P<0.05). Previous studies indicate that subjects with healthy endothelium fail to improve stimulated vasodilator function following short-term exercise training programmes,[25,30,35,40] though longer duration[18,23] or more intense training[17] may prove effective. An alternate explanation for the disparate findings of our two subgroups involves the possibility that HMG-CoA reductase inhibitor therapy might facilitate improvement in endothelium-dependent vasodilator function resulting from exercise training. Statins have been reported to have various beneficial effects on the endothelium, perhaps independent of their lipid-lowering effects[7–9,28] and, in particular, have been reported to upregulate NO-synthase expression.[8,28] Exercise training has also been associated with increases NO-synthase expression, at least in animals,[15,16] and it is reported that both exercise[38] and statins[28] can reduce oxidant stress, which is an important determinant of NO bioactivity.[41] Hence, there may be synergism between the two interventions; at the least, the results indicate that statin therapy, and its associated improvement in plasma lipids, does not necessarily deny the possibility of benefit to vascular function from an exercise programme.

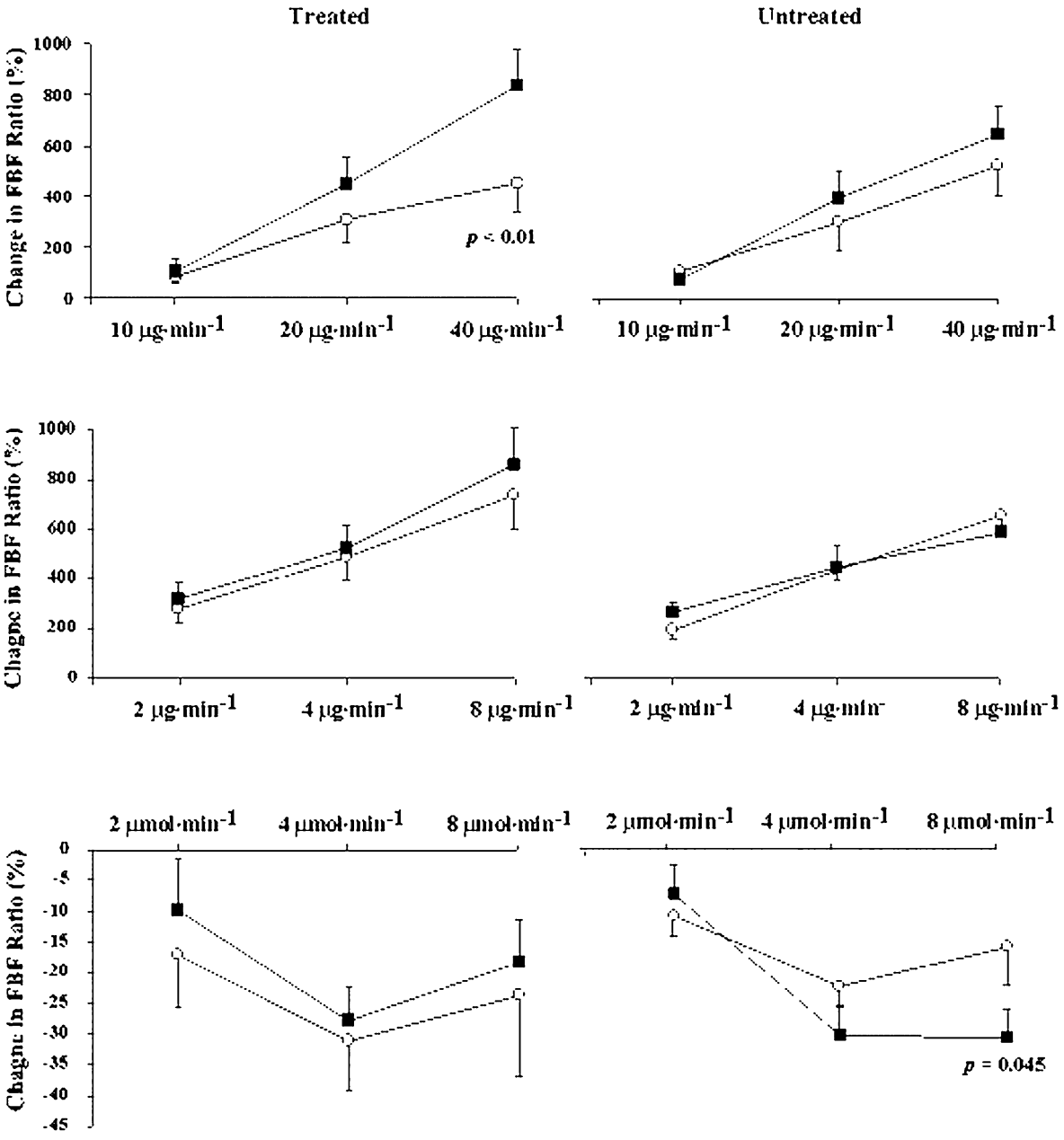

Fig. 3 Forearm blood flow (FBF) responses to acetylcholine (ACh; upper panels), sodium nitroprusside (SNP; middle panels) and N^G-monomethyl-L-arginine (L-NMMA; lower panels) in treated (left) and untreated (right) hypercholesterolaemic subjects before (○) and after training (■). FBF is presented as the percentage change in the ratio of the infused to non-infused arm flows relative to the baseline preceding the drug infusion.[34,35] Values are means±SE. Training significantly increased the FBF response to the highest dose of ACh in the treated subjects ($P<0.01$). In the untreated subjects, vasoconstriction to L-NMMA increased significantly following training ($P=0.045$).

The disparate findings in baseline vascular function between the groups was somewhat surprising in view of the well documented, beneficial effect of lipid-lowering medication on endothelium-dependent vascular function.[7–9,28] However, although the plasma cholesterol of our treated subjects had been substantially reduced and was well within the normal range, as mentioned above, their vascular function remained significantly depressed compared to that of normal subjects. This is consistent with the fact that these treated subjects were known to be hypercholesterolaemic for a longer period (3.2±0.7 vs 1.9±0.6 years) and had significantly higher initial, untreated levels of LDL-cholesterol; 5.6±0.2 vs 4.9±0.2 $mmol^{-1}l^{-1}$ ($P=0.03$) than the untreated subjects. Hence, more extreme or longer duration hypercholesterolaemia, not unexpected in a group submitted to therapy, probably explain differences in baseline FMD between the two subgroups. The finding of near-normal baseline vascular function in the untreated group, with LDL>4 mmol l^{-1} on average, is also surprising but may relate to the relatively shorter duration and less severe hypercholesterolaemia in this subgroup. Of course, not

all groups of untreated hypercholesterolaemic subjects would have such little depressed endothelial function. In summary, the above findings support the concept that our training protocol was not sufficient to improve relatively normal vasodilator function but was sufficient to improve depressed vasodilator function. In addition, the current study does not exclude the possibility of improvement in NO-dependent vasodilator function by exercise in untreated hypercholesterolaemic subjects having depressed endothelial function.

There are several potential limitations of the present study. The treated subjects studied were on several different HMG-CoA reductase inhibitors, rather than being prospectively placed on a single drug. However, subjects had been stable on medication typical of a community group for a minimum of 3 months. As mentioned above, while LDL cholesterol had been effectively lowered, FMD remained abnormal but it should be impressed that the subjects were not selected as those having residual depression of endothelial function. Another possible limitation arises with the use of cross-over design, which allows intra-subject comparisons, but raises the possibility of a carry-over effect in those trained first. However, the results indicate that the order of administering training had little effect on the outcome and, additionally, that the effect of our short-term training programme does not persist for 8 weeks, conclusions consistent with previous findings.[22,24,30] Another possible limitation of the study relates to the nature of the exercise training protocol, which largely excluded upper limb exercise but which emphasizes the general nature of the beneficial vascular responses to exercise as previously described.[22–24,26] While the programme used was predominantly gymnasium based, similar programmes could be adapted for home use, although it is possible that other training modalities, intensities and frequencies of exercise may elicit different outcomes.

In conclusion, this the first study to report that exercise training can improve aerobic capacity and conduit and resistance vessel endothelium-dependent dilator function in subjects currently treated for hypercholesterolaemia. In addition, subjects with untreated hypercholesterolaemia demonstrated enhanced basal NO bioactivity following a novel 8-week combined resistance and aerobic training programme. The improvements in vascular function occurred in the absence of any change in plasma cholesterol, triglycerides or haemodynamic variables and appear to be generalized throughout the circulation. While this study and accumulated evidence suggests that improvement is unlikely to be as substantial when therapy has returned endothelial function to near normal levels, these findings suggest that moderate exercise training may have beneficial cardioprotective effects in hypercholesterolaemic subjects, even in those taking lipid-lowering medication.

Acknowledgements

This study was supported by the National Health and Medical Research Council of Australia (project grant number 211997). We would like to thank Dr Valerie Burke for her invaluable statistical advice.

References

1. Drexler H, Hornig B. Endothelial dysfunction in human disease. *J Mol Cell Cardiol* 1999;**31**:51–60.
2. Zeiher AM, Drexler H, Wollschlager H et al. Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation* 1991; **83**:391–401.
3. Egashira K, Inou T, Hirooka Y et al. Impaired coronary blood flow response to acetylcholine in patients with coronary risk factors and proximal atherosclerotic lesions. *J Clin Invest* 1993;**91**:29–37.
4. Schroeder S, Enderle MD, Ossen R et al. Noninvasive determination of endothelium-mediated vasodilation as a screening test for coronary artery disease: Pilot study to assess the predictive value in comparison with angina pectoris, exercise electrocardiography, and myocardial perfusion imaging. *Am Heart J* 1999;**138**:731–9.
5. Celermajer DS, Sorensen KE, Bull C et al. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects related to coronary risk factors and their interaction. *J Am Coll Cardiol* 1994; **24**:1468–74.
6. Creager MA, Cooke JP, Mendelsohn ME et al. Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest* 1990;**86**:228–34.
7. O'Driscoll G, Green D, Taylor R. Simvastatin, an HMG-Coenzyme. A reductase inhibitor improves endothelial function within 1 month. *Circulation* 1997;**95**:1126–31.
8. Laufs U, La Fata V, Plutzky J et al. Upregulation of endothelial nitric oxide sythase by HMG CoA reductase inhibitors. *Circulation* 1998; **97**:1129–35.
9. Jarvisalo MJ, Toikka JO, Vasankari T et al. HMG CoA reductase inhibitors are related to improved systemic endothelial function in coronary artery disease. *Atherosclerosis* 1999;**147**:237–42.
10. O'Driscoll G, Green D, Maiorana A et al. Improvement in endothelial function by angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 1999; **33**:1506–11.
11. Suwaidi JA, Hamasaki S, Higano ST et al. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 2000;**101**:948–54.
12. Heitzer T, Schlinzig T, Krohn K et al. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001;**104**:2673–8.
13. Halcox JPJ, Schenke WH, Zalos G et al. Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 2002;**106**:653–8.
14. Niebauer J, Maxwell AJ, Lin PS et al. Impaired aerobic capacity in hypercholesterolaemic mice: partial reversal by exercise training. *Am J Physiol* 1999;**276**:1346–54.
15. Sessa WC, Prichard K, Seyedi N et al. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ Res* 1994; **74**:349–53.
16. Sun D, Huang A, Koller A et al. Short-term daily exercise activity enhances endothelial NO synthesis in skeletal muscle arterioles of rats. *J Appl Physiol* 1994;**76**:2241–7.
17. Clarkson P, Montgomery HE, Mullen MJ et al. Exercise training enhances endothelial function in young men. *J Am Coll Cardiol* 1999; **33**:1379–85.
18. Kingwell BA, Tran B, Cameron JD et al. Enhanced vasodilation to acetylcholine in athletes is associated with lower plasma cholesterol. *Am J Physiol* 1996;**270**:H2008–13.
19. Hambrecht R, Fiehn E, Weigl C et al. Regular physical exercise corrects endothelial dysfunction and improves exercise capacity in patients with chronic heart failure. *Circulation* 1998; **98**:2709–15.
20. Higashi Y, Sasaki S, Kurisu S et al. Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide. *Circulation* 1999;**100**:1194–202.

21. Hambrecht R, Wolf A, Gielen S et al. Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med* 2000;**342**:454–60.
22. Maiorana A, O'Driscoll G, Dembo L et al. Effect of aerobic and resistance exercise training on vascular function in heart failure. *Am J Physiol* 2000;**279**:H1999–2005.
23. DeSouza CA, Shairo LF, Clevenger CM et al. Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation* 2000; **102**:1351–7.
24. Maiorana A, O'Driscoll G, Cheetham C et al. The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. *J Am Coll Cardiol* 2001;**38**:860–6.
25. Kingwell BA, Sherrard B, Jennings GL et al. Four weeks of cycle training increases basal production of nitric oxide from the forearm. *Am J Physiol* 1997;**272**:H1070–7.
26. Linke A, Schoene N, Gielen S et al. Endothelial dysfunction in patients with chronic heart failure: systemic effects of lower-limb exercise training. *J Am Coll Cardiol* 2001;**37**:392–7.
27. Green D, Cheetham C, Mavaddat L et al. Effect of lower limb exercise on forearm vascular function: contribution of nitric oxide. *Am J Physiol* 2002;**283**:H899–907.
28. Kalinowski L, Dobrucki LW, Brovkovych V et al. Increased nitric oxide bioavailability in endothelial cells contributes to the pleiotropic effect of cerivastatin. *Circulation* 2002;**105**:933–8.
29. Lewis TV, Dart AM, Chin-Dusting JPF et al. Exercise training increases basal nitric oxide production from the forearm in hypercholesterolaemic patients. *Arterioscler Thromb* 1999;**19**:2782–878.
30. Maiorana A, O'Driscoll G, Dembo L et al. Exercise training, vascular function, and functional capacity in middle-aged subjects. *Med Sci Sports Exerc* 2001;**33**:2022–8.
31. Maiorana A, O'Driscoll G, Cheetham C et al. Combined aerobic and resistance exercise training improved functional capacity and strength in CHF. *J Appl Physiol* 2000;**88**:1565–70.
32. Patterson GC, Whelan RF. Reactive hyperaemia in the human forearm. *Clin Sci (Colch)* 1955;**14**:197–211.
33. Woodman R, Playford D, Watts G et al. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol* 2001;**91**:929–37.
34. Benjamin N, Valver A, Collier J et al. Measuring forearm blood flow and interpreting the responses to drugs and mediators. *Hypertension* 1995;**25**:918–23.
35. Green D, Cable N, Fox C et al. Modification of forearm resistance vessels by exercise training in young men. *J Appl Physiol* 1994; **77**:1829–33.
36. Mullen MJ, Kharbanda RK, Cross J et al. Heterogenous nature of flow-mediated dilation in human conduit arteries in vivo. *Circ Res* 2001;**88**:145–51.
37. Miller VM, VanHoutte PM. Enhanced release of endothelium-derived factor(s) by chronic increases in blood flow. *Am J Physiol* 1988; **255**:H446–51.
38. Miyazaki H, Oh-ishi S, Ookawara T et al. Strenuous endurance training in humans reduces oxidative stress following exhausting exercise. *Eur J Appl Physiol* 2001;**84**:1–6.
39. Niebauer J, Cooke JP. Cardiovascular effects of exercise: role of endothelial shear stress. *J Am Coll Cardiol* 1996;**28**:1652–60.
40. Green DJ, Fowler DT, O'Driscoll JG et al. Endothelium-derived nitric oxide activity in forearm vessels of tennis players. *J Appl Physiol* 1996;**81**:943–8.
41. Cai H, Harrison D. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 2000;**87**:840–4.