

Effect of exercise training on endothelium-derived nitric oxide function in humans

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Vascular endothelial function is essential for maintenance of health of the vessel wall and for vasomotor control in both conduit and resistance vessels. These functions are due to the production of numerous autacoids, of which nitric oxide (NO) has been the most widely studied. Exercise training has been shown, in many animal and human studies, to augment endothelial, NO-dependent vasodilatation in both large and small vessels. The extent of the improvement in humans depends upon the muscle mass subjected to training; with forearm exercise, changes are restricted to the forearm vessels while lower body training can induce generalized benefit. Increased NO bioactivity with exercise training has been readily and consistently demonstrated in subjects with cardiovascular disease and risk factors, in whom antecedent endothelial dysfunction exists. These conditions may all be associated with increased oxygen free radicals which impact on NO synthase activity and with which NO reacts; repeated exercise and shear stress stimulation of NO bioactivity redresses this radical imbalance, hence leading to greater potential for autacoid bioavailability. Recent human studies also indicate that exercise training may improve endothelial function by up-regulating eNOS protein expression and phosphorylation. While improvement in NO vasodilator function has been less frequently found in healthy subjects, a higher level of training may lead to improvement. Regarding time course, studies indicate that short-term training increases NO bioactivity, which acts to homeostatically regulate the shear stress associated with exercise. Whilst the increase in NO bioactivity dissipates within weeks of training cessation, studies also indicate that if exercise is maintained, the short-term functional adaptation is succeeded by NO-dependent structural changes, leading to arterial remodelling and structural normalization of shear. Given the strong prognostic links between vascular structure, function and cardiovascular events, the implications of these findings are obvious, yet many unanswered questions remain, not only concerning the mechanisms responsible for NO bioactivity, the nature of the cellular effect and relevance of other autacoids, but also such practical questions as the optimal intensity, modality and volume of exercise training required in different populations.

(Resubmitted 17 May 2004; accepted after revision 14 September 2004; first published online 16 September 2004)

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Introduction

The endothelium produces numerous paracrine substances, including nitric oxide (NO), which help maintain the health of the vascular wall and regulate vasomotor function. Nitric oxide (NO) is a labile, lipid soluble gas synthesized in endothelial cells from the amino-acid L-arginine through the action of endothelial nitric oxide synthase (eNOS) (Palmer *et al.* 1988). It is

released both basally and in response to pharmacological stimulation (Vallance *et al.* 1989). The likely physiological stimulus to endothelial NO production has been identified as increased flow through the vessel lumen (Pohl *et al.* 1986; Rubanyi *et al.* 1986), with acute NO-mediated vasodilatation tending to normalize shear stress (Hutcheson & Griffith, 1991; Koller & Kaley, 1991; Neibauer & Cooke, 1996; Dimmeler & Zeiher, 2003). These findings raised the possibility that NO may contribute to exercise

hyperaemia, as exercise is associated with increased pulse pressure and pulsatility, and that repeated exposure to increased shear stress, as a result of exercise training, may improve the bioavailability of NO.

Exercise training is associated with reduction in primary (Hakin *et al.* 1999; Sesso *et al.* 2000; Myers *et al.* 2002) and secondary vascular events (Jolliffe *et al.* 2001), but the effect of exercise on conventional risk factors (Blair *et al.* 1984; Tran & Weltman, 1985; Holloszy *et al.* 1986; Blair, 1993; Williams, 1996; Malfattoo *et al.* 1998; Kelley, 1999; Smith *et al.* 1999; El-Sayed *et al.* 2000) cannot solely account for the magnitude of risk reduction, since the association with reduced mortality is independent of these risk factors (Dimmeler & Zeiher, 2003). Moreover, improvements in conduit and resistance vessel endothelial function can occur independent of changes in cardiovascular risk factors (Green *et al.* 2003). Direct shear-stress-mediated effects on the vascular endothelium may therefore provide a plausible explanation for the reduction in coronary events associated with exercise training. The purpose of this review is to summarize the extant and evolving evidence from human studies regarding the contribution of endothelium-derived NO to exercise hyperaemia and the impact of exercise training on NO vasodilator function.

How is the contribution of NO to exercise and training studied in humans?

In humans, the rapid half-life of NO and its various non-vascular sources have rendered direct assessment of endothelial production difficult (Vallance *et al.* 1995) but NO bioassay, dependent upon the vasodilator effect of NO, has become a practical surrogate for endothelial function assessment *in vivo*. After the elegant method of Vallance *et al.* (1989), intrabrachial or coronary infusion of locally active doses of inhibitors of eNOS, such as N^G -monomethyl-L-arginine (L-NMMA), has been used to examine basal NO bioactivity in resting muscle or the contribution of NO to exercise hyperaemia. Using a similar approach, dose-response curves to intra-arterial infusion of endothelium-dependent NO vasodilators such as acetylcholine (ACh), the response to which is at least partly NO mediated (Newby *et al.* 1997), have been used to assay changes in the stimulated release of NO induced by exercise training. Similarly, sodium nitroprusside (SNP) infusion is commonly undertaken to assess endothelium-independent NO vasodilator function. Evaluating the responses to endothelium-dependent and -independent NO vasodilators provides information about the contribution to blood flow control of different components of the NO-dilator system (Benjamin *et al.* 1995). This methodological approach has typically been employed to provide an assessment of resistance vessel function in studies of the skeletal muscle

vasculature (Shepherd, 1983), utilizing strain-gauge plethysmographic measurement of limb blood flow responses.

A less invasive method, that of flow-mediated dilatation (FMD), has been devised to assess conduit vessel NO-mediated endothelial function (Celermajer *et al.* 1992), particularly that of the brachial artery (Corretti *et al.* 2002). This method involves direct imaging of large artery dilator responses to shear-stress-induced FMD consequent to a brief period of limb ischaemia. Assuming the occluding cuff is placed distal to the scanned artery (Doshi *et al.* 2001) and that the period of ischaemia does not exceed 5 min (Mullen *et al.* 2001a), the increase in arterial diameter in response to this stimulus is almost exclusively mediated by NO (Joannides *et al.* 1995; Doshi *et al.* 2001), and FMD therefore provides an index of conduit artery endothelium-dependent NO function (Ganz & Vita, 2003). Sublingual nitroglycerine (GTN) administration is often undertaken to assess endothelium-independent NO vasodilator function in the same manner that SNP is used in invasive studies.

It is important to consider some of the limitations of each of the above approaches in the context of measurement of exercise hyperaemia and exercise training effects. Strain-gauge plethysmography reliably measures relative changes induced by pharmacological agents to a resting muscle bed (Joyner *et al.* 2001), but has several compelling limitations for measurement of exercise hyperaemia (Rowell, 1993); crucially, plethysmography provides data with poor temporal resolution and is highly sensitive to movement, necessitating measurement of 'exercise' flows from brief periods of rest following exercise bouts. Perhaps as a consequence, plethysmographic measures of exercise flows significantly underestimate those derived from thermodilution (Andersen & Saltin, 1985). Nonetheless, most of what we know about mechanisms responsible for exercise hyperaemia is derived from fundamental observations utilizing plethysmography (Joyner *et al.* 2001), and, importantly, studies of the effect of training on the vasculature, which typically involve pharmacological assay in resting vessel beds before and after the intervention, are not subject to the motion artefact limitations.

High resolution Doppler ultrasonography is becoming an increasingly common method to directly visualize and measure arterial diameter and blood flow through conduit arteries in humans, including during FMD studies (Radegran, 1997; Radegran & Saltin, 1999; Maiorana *et al.* 2001a; Green *et al.* 2002b). This method possesses the advantages of being non-invasive, provides absolute blood flow measures, greatly improves temporal resolution for measurement during exercise hyperaemia (Green *et al.* 2002c) and is potentially less affected by

motion artefact than plethysmography. However, both arterial diameter and Doppler velocity measures are critically dependent upon image quality, which is operator dependent, and velocity is also importantly modulated by the insonation angle (Logason *et al.* 2001), which should be less than 60 deg. Furthermore, many groups fail to utilize edge-detection and wall-tracking software, which markedly improves the sensitivity of the technique and renders analysis less subjective (Woodman *et al.* 2001; Green *et al.* 2002c). In the context of the use of FMD in exercise training studies, Hijmering *et al.* (2002) recently demonstrated that acute changes in sympathetic outflow, similar in magnitude to those observed in ageing and disease and in response to training, can acutely blunt FMD by 50%. Whether this can be attributed to a decreased flow stimulus as a result of the downstream sympathetic vasoconstriction remains to be determined, but it warrants caution in the use of FMD in repeated measures studies in which the intervention may also modulate sympathetic tone. Finally, dose–response curves to FMD cannot be constructed at present, as the relationship between shear stress and the NO dependence of FMD has not been comprehensively assessed and appears complex (Doshi *et al.* 2001; Mullen *et al.* 2001b; Pyke *et al.* 2004).

In summary, all methods used to assess regional blood flow *in vivo* possess limitations and yet the oldest and arguably most compromised method, strain-gauge plethysmography, has yielded the greatest body of extant knowledge regarding the effects of acute and chronic exercise on the vasculature. Newer technologies will provide greater insight, but the approaches to which they are applied must in the first instance be physiologically sound. Several studies, including one which examined the effects of exercise training (Lind *et al.* 2000; Eskurza *et al.* 2001; Green *et al.* 2004), have demonstrated poor correlations between ACh and FMD measures derived in the same subjects and, although this may be indicative of differential NO contributions in resistance and conduit vessels, the possibility also exists that distinct physiological pathways are being interrogated.

Does endothelium-derived NO contribute to exercise hyperaemia in humans?

Skeletal muscle. Indirect evidence for a role of NO in skeletal muscle exercise hyperaemia is provided by the increased levels of plasma nitrite (Ambring *et al.* 1994) and urinary nitrate (Bode-Böger *et al.* 1994) in response to prolonged aerobic exercise, but these measures do not reliably reflect endothelium-derived NO. Therefore, despite technical difficulties associated with measuring blood flow during exercise, a number of investigators have attempted to determine NO involvement in the hyperaemic response to exercise in the peripheral circulation. In an early study, flow was measured

using plethysmography during periods of inactivity immediately following two intensities of wrist flexor exercise and concurrent infusion of L-NMMA had no significant effect on blood flow (Wilson & Kapoor, 1993). Furthermore, a small reduction in exercise hyperaemia during static hand gripping following NO inhibition could be explained by changes in resting flow (Endo *et al.* 1994). However, both of these studies measured blood flow after the cessation of muscular contraction and blood flow during recovery may be under different control from that during exercise (Joyner & Dietz, 1997).

The role of NO in skeletal muscle vasodilatation may increase with the duration of exercise. While it is unlikely that NO plays a major role in the dilator response to a single muscle contraction (Brock *et al.* 1998), it may be important during prolonged exercise. L-NMMA infusion during intermittent forearm hand gripping at 15%, 30% and 45% of maximum grip strength induced a small, but significant, reduction in exercise-induced vasodilatation (Gilligan *et al.* 1994). Importantly, the greatest effect of L-NMMA was evident at the highest infusion rate of the drug, indicating that the dose of an antagonist may be critical during an exercise study, when increased blood flow effectively dilutes a constant dose delivery. Indeed, Katz *et al.* (1996) indicated that the effect of L-NMMA decreased with increasing intensity of exercise. In another study, L-NMMA infusion attenuated the blood flow response to prolonged forearm hand gripping by 20–30% (Dyke *et al.* 1995). Furthermore, Duffy *et al.* (1999a,b) recently confirmed a role for both NO and vasodilator prostanoids in mediating hyperaemia in response to forearm handgrip exercise. However, sequential inhibition of these two vasodilators had no additional effect on reducing blood flow over and above that induced by inhibition of one, highlighting the possibility that redundant mechanisms may contribute to blood flow control during continuous exercise. This was recently confirmed in a comprehensive study (Schrage *et al.* 2004) which used Doppler ultrasound of the brachial artery during prolonged forearm handgrip exercise at low intensity to demonstrate that N^G -nitro-L-arginine methyl ester (L-NAME), a more potent NOS inhibitor than L-NMMA, reduced exercise blood flow by 20%, and that prostaglandin inhibition transiently decreased exercise hyperaemia, but that the combination of approaches was not additive. The authors concluded that both NO and prostaglandins contribute significantly to exercise hyperaemia, that their effects appear independent and that factors other than NO restore blood flow responses following PG inhibition (Schrage *et al.* 2004). This study also emphasized the importance of localized administration of blockers during, rather than prior to, contractions and reinforced the importance of redundancy in the control of exercise hyperaemia (Boushel & Kjær, 2004).

In the lower limbs, Hickner *et al.* (1997), using microdialysis probes, measured a reduction in blood flow of 50% following the infusion of L-NMMA during continuous dynamic exercise of the vastus lateralis. However this technique requires further validation and these results have not been supported by other studies of lower limb exercise. L-NMMA had no effect on blood flow measured using Doppler ultrasound during steady-state submaximal or peak voluntary knee extension exercise (Radegran & Saltin, 1999). Similarly, when blood flow to the lower limbs was determined using femoral thermol-dilution, L-NMMA did not reduce blood flow during supine cycling, despite decreasing glucose flux across the limb (Bradley *et al.* 1999). Leg exercise is associated with a high perfusion-to-muscle mass ratio, and flow may be increased over 10-fold. Possibly, this hyperaemia dilutes L-NMMA concentration to an inadequate blocking level. In a recent report, systemic intravenous infusion of L-NAME, a potent inhibitor of NO production, failed to reduce blood flow during submaximal leg extensor exercise, leading the authors to conclude that NO is not essential to exercise hyperaemia (Fransden *et al.* 2001). However, this interpretation may be considered controversial, since mean arterial pressure increased and vascular conductance was lower during L-NAME infusion. Furthermore, an increase in arterial pressure is likely to reduce sympathetic outflow during exercise (Scherrer *et al.* 1990), and this may mask a potential blood flow reduction due to eNOS inhibition (Sheriff *et al.* 2000). This possibility is supported by the recent findings of Schrage *et al.* (2004), who used localized exercise and infusions to avoid the confounding influences of reflex responses and observed a contribution of NO to exercise hyperaemia of similar magnitude to Sheriff *et al.* (2000).

In summary of the above results in the skeletal muscle circulation, the evidence for a role of NO in the hyperaemic response to prolonged exercise in the forearms, which have a relatively small muscle mass, is reasonably convincing. Because the increased blood flow in these studies was modest (3–6 times resting levels), the dilution of constant dose infusion of NO antagonists would be less than with leg exercise. Evidence is less compelling from studies involving the lower limbs. While this disparity may reflect regional differences between vascular beds (Green *et al.* 2004), it may depend upon greater dilution of NO antagonists during leg exercise. Further carefully designed studies will be required to clarify this issue. There is also evidence that redundancy of mechanisms of vasodilatation exist so that inhibition of one pathway may result in up-regulation of another (Boushel & Kjær, 2004; Schrage *et al.* 2004). A final problem concerns blood flow measurement techniques. For example, as summarized by Radegran & Saltin (1998), studies indicating an effect of L-NMMA have mostly been undertaken using plethysmography, necessitating

measurement of flow during brief rest periods between contractions or exercise bouts. Flows measured using ultrasound/Doppler actually during exercise have, to date, not supported much role for NO during muscular contraction but have indicated an effect at rest and during recovery, at least in the lower limbs. Most of the existing data can therefore be rationalized if it is accepted that NO contributes to resting and postexercise hyperaemia, whilst the picture regarding NO contribution during exercise is possibly confounded by reflex effects of systemic doses of NO antagonists (Sheriff *et al.* 2000; Schrage *et al.* 2004). In the one recent study which used appropriately delivered potent NOS inhibition and measured flows during exercise using ultrasound Doppler, the magnitude of NO contribution was approximately 20%. The contribution of NO to exercise hyperaemia in humans therefore appears to be of around 20–30%, but redundancy exists and NO may not be obligatory for the hyperaemia observed during exercise.

We believe one final point is germane, that the shear-stress-mediated effects of exercise, and consequent production and bioactivity of NO, differ qualitatively and quantitatively according to the exercise involved. In a recent study we examined brachial artery blood flow during lower limb exercise (Green *et al.* 2002b) and found that brachial artery infusion of L-NMMA decreased forearm blood flow during cycling at 60, 100 and 160 W, compared to the resting state. This finding suggests that systemic production of NO increases during exercise, even in vascular beds feeding metabolically inactive tissue. A subsequent study found that changes in HR alone in the absence of exercise were not associated with this phenomenon and, hence, it may be due to pulse pressure dynamics stimulating the endothelium throughout the circulation during exercise (Green *et al.* 2002a). When the contributions of NO to incremental handgrip and cycle exercise (see Fig. 1) were directly compared, mean flows were higher during handgrip, but the pattern of flow differed; handgrip exercise was associated with incremental increase in antegrade flow, whilst cycling induced a pattern of oscillatory antegrade systolic, retrograde diastolic flow in the resting upper limb which was amplified by exercise intensity (Fig. 1). Hence, whilst average forearm blood flow may be higher during handgrip exercise, the physiological stimulus for NO production may be paradoxically greater during cycling due to the pattern of oscillatory stretch on the endothelial cell membrane.

Cardiac muscle. In contrast to the disparate findings in skeletal muscle, studies performed in the coronary circulation have generally indicated a role for NO in epicardial coronary vasodilatation during exercise. Experimental studies of coronary blood flows in humans

rely upon increases in cardiac metabolism achieved by increasing ventricular rate using cardiac pacing. Indirect evidence for a role of NO in metabolic vasodilatation comes from a study showing an increase in by-products of NO in coronary sinus blood following pacing in subjects without coronary artery disease (CAD) risk factors (Minamino *et al.* 1998). No increase was evident in

subjects with CAD risk factors but coronary sinus adenosine was elevated (Minamino *et al.* 1998), raising the possibility that adenosine production may be a compensatory mechanism maintaining myocardial blood flow in the presence of endothelial dysfunction. In other studies, L-NMMA abolished pacing-induced epicardial vessel dilatation, indicating that NO

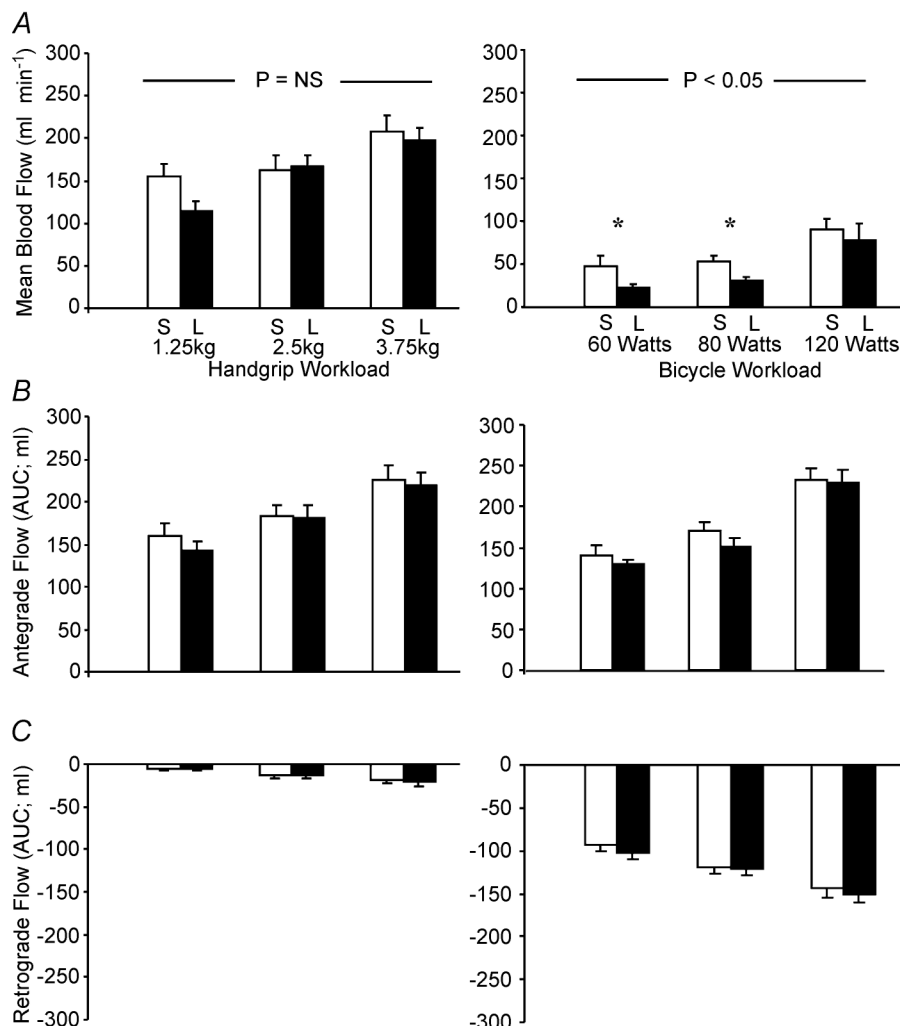


Figure 1. Effect of localized handgrip (left panels) and cycle ergometer (right panels) exercise on brachial artery haemodynamics

Mean blood flow (A), antegrade (B) and retrograde (C) flows during exercise under saline (S; light bars) and L-NMMA (L; dark bars) infusion are displayed. L-NMMA significantly decreased flows under the cycle condition only (2-way ANOVA). Note that, although mean flows are lower during cycle exercise, the magnitude of antegrade flows are similar to handgrip exercise and there is substantial retrograde flow during cycling which is not evident during handgrip. This systolic antegrade, diastolic retrograde flow pattern and its accompanying oscillatory shear stress on the endothelium may explain the paradoxical greater contribution of NO during cycling than handgrip exercise, despite the greater mean flow during handgrip exercise. This difference in flow patterns may also resolve another conundrum in the literature, that exercise training studies involving localized handgrip training have not always produced significant improvement in NO bioactivity (Green *et al.* 1994, 1996a; Franke *et al.* 1998), whilst studies which have utilized typical 'whole body' exercise training regimes, predominantly involving lower limb exercise (cycling, running, etc.), have observed improvements in NO-mediated vasodilator capacity, even in the untrained upper limbs (Kingwell *et al.* 1997b; Maiorana *et al.* 2000, 2001a; Linke *et al.* 2001; Walsh *et al.* 2003a,b). An acute bout of cycle exercise may be a more potent stimulus to endothelial NO production in the forearm vasculature than a bout of localized handgrip exercise. (Green *et al.* in press).

contributes to vasodilatation in these conduit vessels in patients free of CAD risk factors (Quyyumi *et al.* 1995a) and in those with angiographically normal coronary arteries (Tousoulis *et al.* 1997). L-NMMA also reduced pacing-induced dilatation of large epicardial arteries, but not of microvessels (Egashira *et al.* 1996). However, there have been negative studies in those with angiographically normal arteries, perhaps related to the shortcomings of angiography (Shiode *et al.* 1996; Nishikawa & Ogawa, 1997). In yet another study, NO was involved in pacing-induced hyperaemia in patients with risk factors for CAD, including some with mildly irregular arteries (Duffy *et al.* 1999a) despite risk factors leading to a reduction in vasodilatation (Quyyumi *et al.* 1995a), and an impaired response to stimulated NO release (Quyyumi *et al.* 1995b). In patients with CAD, NO production increased by pacing was abolished at the sites of stenoses (Tousoulis *et al.* 1997). In summary, there is strong evidence for a role of endothelium-derived NO in the metabolic vasodilatation of healthy coronary epicardial vessels, but NO-dependent vasodilatation decreases in the presence of risk factors and is absent or much reduced in grossly atherosclerotic vessels. Furthermore, in a recent review, Feigl and colleagues make the important point that, although the majority of studies indicate a decrease in epicardial coronary diameter during NO inhibition, coronary flows are not usually affected, indicating that downstream arteriolar dilatation adequately compensates for the decreased NO bioactivity (Tune *et al.* 2004). Hence, whilst NO inhibition doesn't steepen the relationship between coronary venous oxygen tension and myocardial oxygen consumption, indicating that it is not a metabolic vasodilator (Tune *et al.* 2002), it does play an important physiological role in epicardial dilatation, possibly as a result of 'ascending vasodilatation' via increased shear stress following downstream metabolic vasodilatation (Segal, 1992; Green *et al.* 1996b).

In summary, studies performed in humans indicate general agreement that NO contributes to resting and postexercise blood flows, although its contribution during exercise remains controversial. NO blockade reduces epicardial coronary diameters during pacing in the majority of studies, whereas the reduction in skeletal muscle blood flow during exercise is usually no greater than that at baseline (Radegran & Saltin, 1998; Bradley *et al.* 1999), suggesting that NO may not be an obligatory component of skeletal muscle exercise hyperaemia in humans. Despite the continuing controversy regarding the precise contribution of NO to exercise hyperaemia, regular exercise training is associated with increased vasodilator capacity (Sinoway *et al.* 1986, 1987; Green *et al.* 1996b) and longitudinal studies provide convincing evidence for the antiatherogenic effect of regular physical activity (Lee *et al.* 2000; Sesso *et al.* 2000; Myers *et al.* 2002). These

phenomena may be due, in part, to up-regulation of endothelial function as a result of exercise training.

Does exercise training improve vascular NO function in healthy animals?

Insights into the time course of the effect of exercise training. Animal studies provide insight into the time course of effect of exercise training on NO vasodilator function. Short-term exercise-training in rats, of 2–4 weeks, increased endothelial NO synthesis in skeletal muscle arterioles and increased the vasodilator responses to ACh and L-arginine, but not to sodium nitroprusside (SNP), suggesting enhanced endothelium and NO-dependent function but unchanged smooth muscle cell sensitivity to NO (Sun *et al.* 1994). A subsequent study of similar duration also demonstrated augmented dilator response in rat muscle arterioles following training, which was partially abolished by L-NMMA infusion (Koller *et al.* 1995). Vascular structure was not obviously altered. These findings suggest that increased production of endothelial NO constitutes an initial phase in the adaptive response to exercise training. In large conduit vessels, improved endothelium-dependent vasodilatation has been observed after as few as 7 days of endurance training in pigs (McAllister & Laughlin, 1997). In another study, 4 weeks of training not only enhanced ACh-induced vasodilatation of the rat aorta but also increased eNOS protein levels in aortic tissue (Delp *et al.* 1993; Delp & Laughlin, 1997). In rabbits, 8 weeks of treadmill running increased ACh reactivity in the aorta and pulmonary arteries but not in the carotid artery, a vessel which is partly protected from dramatic changes in flow and shear stress (Chen & Li, 1993). Similarly, 4 weeks of daily exercise in rats improved flow-induced dilatation in skeletal muscle arteries, but not in mesenteric vessels, which are perhaps not subjected to the shear stress stimulus of exercise hyperaemia (Sun *et al.* 1998).

In contrast to short to moderate periods of exercise training, studies over a longer duration have not consistently shown augmented NO-related endothelial function. NO-dependent vasodilatation was unaltered after 16–20 weeks of training in pigs (McAllister *et al.* 1996) and 16 weeks in rats despite improved arterial compliance (Kingwell *et al.* 1997a). This seems to indicate that improvement in endothelium-dependent vasodilator responses in the periphery is a transient phenomenon that is lost with long-term training. Further, there is evidence that the expression of eNOS, initially increased by training, also exhibits this time dependence; daily exercise for 1 week resulted in an increased expression of eNOS protein in porcine pulmonary arteries and enhanced ACh-mediated relaxation (Johnson *et al.* 2001), whereas changes were not present after 16 weeks of exercise training

(Johnson & Laughlin, 2000). Paralleling the time course of these changes, in various animal models, prolonged exercise training enlarges the diameter of arteries (Leon & Bloor, 1968; Wyatt & Mitchell, 1978; Kramsch *et al.* 1981; Lash & Bohlen, 1992); yet this vascular remodelling, in contrast to increased capillarization, also appears to be an endothelium- and NO-dependent phenomenon (Kamiya & Togawa, 1980; Langille & O'Donnell, 1986; Zarins *et al.* 1987; Gibbons & Dzau, 1994; Rudic *et al.* 1998; Prior *et al.* 2003), but one which partly supplants the acutely responsive vasodilator mechanisms.

In animal studies of the coronary circulation, early reports indicated that exercise training increased sensitivity to vasodilator agents in dogs (DiCarlo *et al.* 1989) and increased transport capacity in miniature swine (Laughlin *et al.* 1989). Evidence that exercise training enhances NO-mediated dilatation was found in a study of short-term, 7–10 days, treadmill exercise training in dogs (Wang *et al.* 1993). This intervention increased dilatation of the circumflex artery following both ACh infusion and reactive hyperaemia and infusion of nitro-L-arginine, an NO-synthase inhibitor, eliminated these responses. Training did not change the response to SNP. Evidence that eNOS gene expression is enhanced in the coronary vasculature by exercise training was first published by Sessa *et al.* (1994), who found that nitrite and NO production, and eNOS gene expression, were increased in coronary arterioles following 10 days of training in dogs. In a porcine model, 16 weeks of training increased eNOS mRNA (Woodman *et al.* 1997) and increased bradykinin-induced vasodilatation (Muller *et al.* 1994) in coronary resistance vessels, suggesting that enhanced NO and endothelium-dependent dilatation was persisting in these vessels. Clear evidence of withdrawal of this mechanism in the coronary circulation with longer term exercise training is not currently available. Additionally, however, 16–22 weeks of training augmented vasodilator responses to adenosine in the large epicardial arteries of pigs, even after removal of the endothelium. This one piece of evidence suggests that changes in vasomotor response in coronary arteries with longer-term training might be due, at least in part, to adaptations within smooth muscle (Oltman *et al.* 1992).

In summary, animal studies investigating both the peripheral and coronary vasculature suggest that short-term exercise training enhances eNOS, and NO production and bioactivity, producing a short-term buffer to the increased shear associated with exercise. After extended training, at least in the peripheral circulation, the increased production of NO and possibly other mediators induces structural changes in the vessels resulting in an increase in lumen diameter (Brown, 2003; Prior *et al.* 2003). Shear stress is hence 'structurally' normalized and endothelial NO activity returns towards initial levels (see Does exercise training modify vascular

structure by a NO-dependent mechanism? and Figs 2 and 3).

Can normal endothelial function be enhanced by exercise training?

Does localized exercise training improve vascular NO function in healthy humans? The first investigation to elucidate mechanisms associated with blood flow changes following exercise training was conducted in young males (Green *et al.* 1994). Four weeks of handgrip training reduced minimum vascular resistance following an ischaemic stimulus in the trained limb but not the untrained contralateral arm, nor in sedentary controls. However, neither the forearm blood flow response to the endothelium-dependent vasodilator methacholine chloride nor to SNP was altered following training. It was concluded that either NO did not contribute to enhanced vasodilatation in the forearm, or that the relatively brief period of conditioning and low stimulus intensity were insufficient to generate a detectable bioassay response in young fit healthy subjects. To examine the latter hypothesis, a subsequent study examined the effect of long-term repetitive conditioning, using local ACh and SNP infusion into the preferred and non-preferred limbs of elite tennis players (Green *et al.* 1996a). We observed enhanced forearm and wrist girth, grip strength and forearm volume in the preferred limbs and significantly higher peak vasodilator capacity, indicating a unilateral structural vascular enlargement (Fig. 3). However, neither L-NMMA, ACh nor SNP dose–response curves were significantly different between limbs, indicating no difference in either basal or stimulated NO dilator function. Similar findings were evident in studies comparing involved and control arms following recovery from forearm immobilization after casting (Green *et al.* 1997).

These studies suggest that forearm muscle training, that is, localized exercise of a small muscle group, does not influence endothelial function in apparently healthy subjects, despite demonstrable increased capacity for peak blood flow. The lack of benefit may have several explanations, one being that the muscle mass recruited or haemodynamics associated with handgrip studies may not induce shear-stress-mediated improvement in NO function (see Fig. 1). Alternatively, the vasculature remodelling evident in these studies may structurally normalize shear stress and negate the requirement for NO functional adaptations (see Insights into the time course of the effect of exercise training). Finally, it may be that exercise training of healthy subjects with normal endothelial function does not induce benefits in terms of the NO-vasodilator system, whereas benefit may be possible in those with antecedent endothelial dysfunction (see Can endothelial dysfunction be improved by exercise training?).

Does whole body exercise exert a generalized effect on NO vasodilator function? Exercise involving large muscle groups of the legs is likely to produce systemic changes in vascular haemodynamics not apparent with localized exercise of the upper limb (see Fig. 1). In normal subjects, Kingwell *et al.* (1997b) examined the effect of 4 weeks of cycling on NO bioactivity in the forearm. Following training, L-NMMA-induced vasoconstriction was increased, suggesting enhanced basal NO function. Contrarily, there was no significant effect of training on the response to ACh or SNP, suggesting that stimulated endothelium-dependent and -independent function were unaltered. However, following a 10 week programme of daily aerobic and anaerobic exercise training in young military recruits, FMD of the brachial artery was significantly improved while GTN-mediated vasodilatation was not (Clarkson *et al.* 1999). This study also involved predominantly lower limb exercise, indicating that the improvement in endothelial function was likely

to be systemic in nature although, despite an intensive training regime, the change in FMD was relatively small, increasing from 2.2 to 3.9%. Even the post-training FMD was lower than commonly reported in sedentary healthy subjects, the explanation for which remains unclear as the baseline vessel diameters were not substantially different from those in other studies (Neunteufl *et al.* 1997; Maiorana *et al.* 2001a; Walsh *et al.* 2003a; Walsh *et al.* 2003b).

Recently, in a randomised cross-over designed study of combined aerobic and resistance exercise in healthy middle-aged men, training did not significantly affect endothelium-dependent or -independent function (Maiorana *et al.* 2001b). This finding is in contrast to similar training studies in chronic heart failure (CHF) (Maiorana *et al.* 2000; Linke *et al.* 2001) and type 2 diabetic subjects (Maiorana *et al.* 2001a). NO-related endothelial function is impaired in these conditions and it is therefore probable that depressed endothelial function

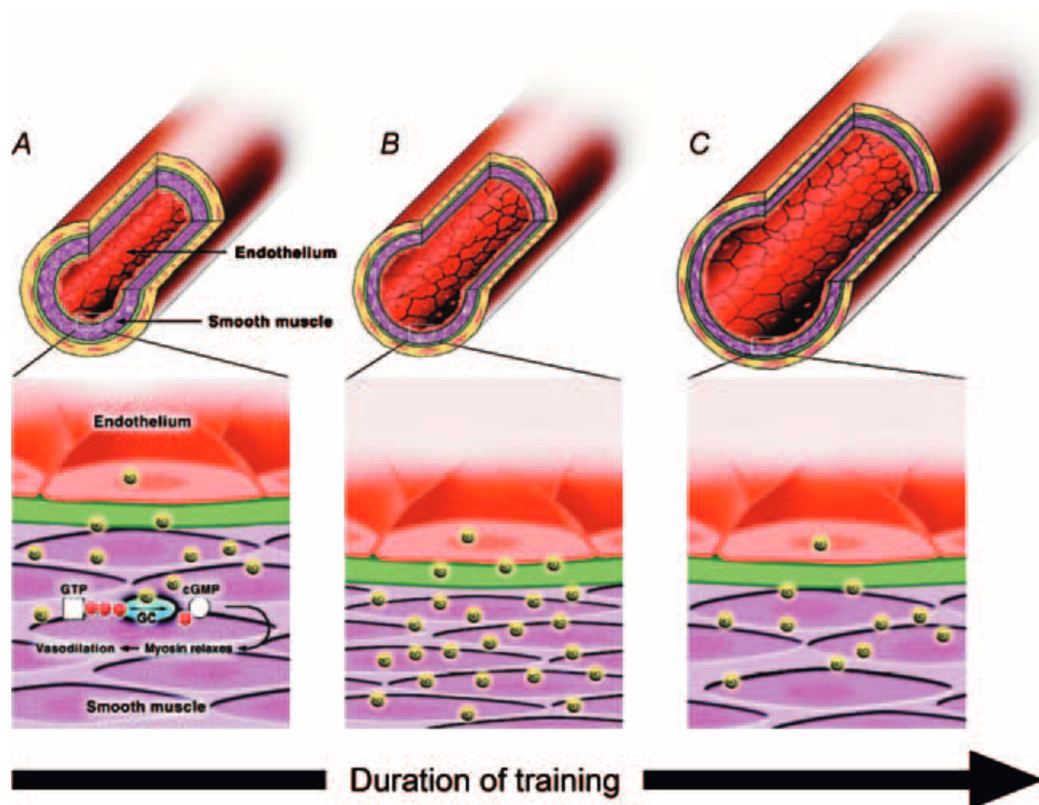


Figure 2. Hypothesized response of arteries to increased flow and shear stress following varying durations of exercise training

In the untrained vessel (left panel), basal release of NO causes subjacent smooth muscle cell vasodilatation which acts to homeostatically regulate wall shear. In response to medium-term exercise training (middle panel), acute increase in shear stress, associated with repetitive exposure to increased flow during bouts of exercise, stimulates increased endothelial NO production and consequent vasodilatation. Up-regulation of the NO-dilator system, including eNOS expression, occurs to buffer increased shear stress. Following long-term exercise training (right panel), structural adaptation occurs, possibly in part due to NO-mediated remodelling, resulting in chronic increase in vessel calibre which 'structurally normalises' shear stress. NO function returns towards baseline levels. Figure based on Maiorana *et al.* (2003). Permission granted by Adis International Ltd.

is more capable of augmentation by moderate exercise training than is the well preserved function of healthy subjects (see Can endothelial dysfunction be improved by exercise training?), which may, however, be improved by more intense training (Clarkson *et al.* 1999). This theory is supported by findings that regular physical activity may partially reduce the age-related decline in NO-related endothelial function (Yasue *et al.* 1990; Gerhard *et al.* 1996), possibly by reducing the impact of oxidative stress (DeSouza *et al.* 2000; Taddei *et al.* 2000).

In summary, localized exercise training, at least of the forearm, does not improve NO vasodilator function in healthy subjects and the evidence for benefit from larger muscle group exercise is relatively modest. It appears that a beneficial effect of exercise training in those with *a priori* normal vascular function may require a high volume of the exercise, if indeed it is possible at all.

Can endothelial dysfunction be improved by exercise training?

Numerous conditions relevant to cardiovascular health are associated with depressed endothelial function, including impaired NO dilator system activity. The potential for beneficial impact of exercise on the endothelium has assumed particular relevance because endothelial dysfunction, characterized by decreased NO-vasodilator activity, appears to be an important early and integral manifestation of vascular disease. Evidence for this includes the following: (i) endothelial dysfunction occurs initially at coronary branch points as do atherosclerotic plaques (McLenachan *et al.* 1990), (ii) endothelial dysfunction precedes and predicts the development of atherosclerotic disease in normal coronary arteries post-cardiac transplant (Fish *et al.* 1988), (iii) endothelial dysfunction is evident in children and adolescents with risk factors for future cardiovascular disease (Celermajer *et al.* 1992, 1993a,b, 1994, 1996; Watts *et al.* 2004a,b), (iv) interventions of known benefit in terms of cardiovascular risk also improve NO-mediated endothelial function (O'Driscoll *et al.* 1997a,b, 1999), (v) coronary (Al Suwaidi *et al.* 2000; Schachinger *et al.* 2000; Heitzer *et al.* 2001; Hollenberg *et al.* 2001) and peripheral (Neunteufl *et al.* 2000; Kuvin *et al.* 2001; Perticone *et al.* 2001b; Gokce *et al.* 2002a) vascular endothelial dysfunction predict cardiovascular events, and (vi) improvement in endothelial function improves prognosis (Modena *et al.* 2002).

The mechanisms proposed to explain depressed NO activity in subjects with cardiovascular disease and risk factors include a loss of endothelial synthesis of NO due to inadequate substrate or cofactors or abnormalities in enzymatic activity. Alternatively, a reduction in NO bioavailability may occur due to quenching by superoxide

anions associated with an abnormal redox state (Ohara *et al.* 1993; Berliner *et al.* 1995; Kojda & Harrison, 1999). However, the mechanisms are not well defined and probably depend upon the specific aetiology. In the majority of subject groups the abnormality appears localized to the endothelium; responses to endothelium-independent NO donors are typically not impaired. The exception may be those subjects with

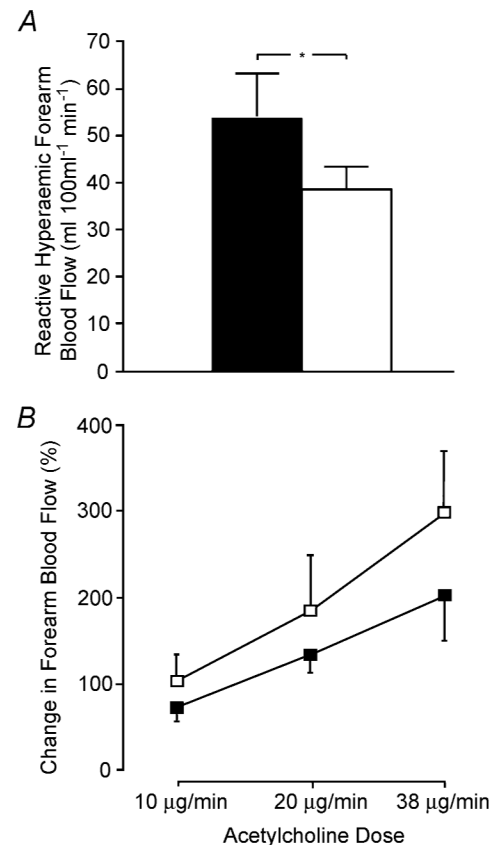


Figure 3. Reactive hyperaemic forearm blood flow (A) and change in forearm blood flow response to three doses of acetylcholine (ACh; B) in the preferred (■) and non-preferred (□) forearms of elite single-handed tennis players (* $P < 0.05$) (Green *et al.* 1996a)

Peak reactive hyperaemia provides an index of resistance vessel structure and the higher preferred limb data indicates that chronic or recurrent episodic changes in blood flow induce arterial remodelling in humans which enhances vasodilator capacity. Responses to ACh, which provide an index of stimulated endothelium-dependent activity, did not differ between the limbs, indicating that chronic training is not associated with altered endothelium-dependent vascular responses. This study supports the schema presented in Fig. 2, that NO production and bioactivity initially produce a short-term buffer to the increased shear associated with exercise. Following extended training, arterial remodelling results in an increase in lumen diameter (Brown, 2003; Prior *et al.* 2003) which 'structurally' normalizes shear and endothelial NO activity returns towards initial levels. Figure based on Green *et al.* (1996a). Permission granted by the American Physiological Society.

Table 1. Summary of exercise training studies of conduit and/or resistance vessel function in healthy humans and subjects with cardiovascular disease and risk factors

Study	Methods	Exercise training	Effect of NO-mediated vasodilator function	NO improvement?
Normal subjects:				
Localized exercise				
Green <i>et al.</i> 1994	Healthy young controls. VOP	Handgrip, 4 weeks, 3 × weekly	No difference in ACh, SNP or L-NMMA responses after training. Peak reactive hyperaemia improved	↔
Franke <i>et al.</i> 1998	Young healthy subjects. VOP	Handgrip, 4 weeks, 3 × weekly	Response to exercise increased. No change in ACh + exercise responses	↔
Bank <i>et al.</i> 1998	Controls for study of CHF patients. VOP	Handgrip, 30 min, 4–6 times/week	ACh improved after training. No change SNP	↑
Whole body exercise				
Kingwell <i>et al.</i> 1997b	Male sedentary 4 week cycle training. VOP	Cycling, 4 weeks, 3 × weekly, 30 min	Training ↑ L-NMMA response at 2 μmol only. No difference ACh or SNP First suggestion that leg training improves forearm responses	↔ ^a ↑ ^b
Bergholm <i>et al.</i> 1999	Fit young males. VOP	Running. High intensity 4 × 1 h running session, weekly	ACh and L-NMMA ↓ after training. No change SNP. Effect may be due to ↓ antioxidant concentrations	↓
Clarkson <i>et al.</i> 1999	Healthy army recruits Brachial D/U	Running, 10 weeks Daily 3 mile run + strength sessions High volume exercise	FMD increased from 2.2 to 3.9%. No change GTN	↑
Higashi <i>et al.</i> 1999a	Middle-aged controls for study of hypertensives. VOP	Walking, 30 mins, 5–7/week, 12 weeks	ACh response increased. No change in isosorbide dinitrate	↑
DeSouza <i>et al.</i> 2000	Normal middle aged sedentary. VOP	Walking, 3 months home based No control group or period	Training improved ACh responses. No changes in SNP responses	↑
Disease states:				
Maiorana <i>et al.</i> 2001b	Normals. Middle-aged. VOP	Circuit training, 8 weeks, 3 × week	No changes ACh or SNP or L-NMMA	↔
Goto <i>et al.</i> 2003	Normal young males. VOP	Cycle exercise, 30 min, 5–7 × weekly, 12 weeks 25%, 50% and 75% $\dot{V}O_{2,max}$ groups	Moderate intensity improved ACh responses. L-NMMA abolished this improvement No improvement in low or high intensity groups Oxidant stress increased, high intensity group	↔ ^c ↔ ↑ ^d ↔ ^e

CHF	Localized exercise Hornig <i>et al.</i> 1996	Radial artery D/U	Handgrip, 4 weeks training, daily for 30 min	Training improved FMD, especially the L-NMMA sensitive component	↑
	Katz <i>et al.</i> 1997	VOP	Handgrip, 30 min daily, 8 weeks	Yes ACh ↑. GTN no change	↑
	Bank <i>et al.</i> 1998	VOP	Handgrip, 30 min, 4–6 times/week	No improvement in ACh responses after training. No change SNP	↔
	Hambrecht <i>et al.</i> 2000b	Radial D/U	Handgrip, 6 × daily, 4 weeks	ACh dilatation increased, L-arginine potentiated the effect. No change in GTN	↑
Whole body exercise	Hambrecht <i>et al.</i> 1998	Doppler flow velocity + angiography of femoral artery	Cycle training, 6 months, 6 × day, 10 min, 80% HR _{max}	ACh mediated blood flow response increased. L-NMMA response also increased. No change GTN.	↑
	Maiorana <i>et al.</i> 2000	VOP	Circuit training, 8 weeks, 3 × week	ACh increased. SNP response also significantly improved.	↑
	Linke <i>et al.</i> 2001	Radial artery D/U.	4 weeks cycle training, 6 × day, 10 min, 80% HR _{max}	Leg training improved ACh and FMD responses in the forearm. No change in GTN.	↑
	CAD	Whole body exercise Hambrecht <i>et al.</i> 2000c	Coronary bed. Flow wire/Doppler	Cycle training, 6 × day, 10 min, 4 weeks 80% HR _{max}	ACh responses and peak flow velocity improved.
Gielen <i>et al.</i> 2003		Coronary bed Intravascular ultrasound/angiography	Home based follow-up to above study Bicycle exercise, 20 min daily, 5 months	ACh responses improved, improved flow and velocity to ACh. No changes in response to GTN.	↑
Gokce <i>et al.</i> 2002b		Brachial and tibial D/U	Leg exercise, 40 min, 3x weekly, 10 weeks	FMD increased significantly in the tibial artery, with increase (NS) also in the brachial. No changes in GTN responses.	↑
Walsh <i>et al.</i> 2003a		Brachial D/U	Circuit training, 8 weeks, 3 × week	FMD improved. No change in GTN	↑
Hambrecht <i>et al.</i> 2003	Coronary bed Intravascular ultrasound/angiography	Cycle or rowing, 3 × day, 10 min, 4 weeks	Peak flow velocity response to ACh increased eNOS expression and phosphorylation increased in coronary artery samples.	↑	

(continued)

Table 1. Continued

Study	Methods	Exercise training	Effect of NO-mediated vasodilator function	NO improvement?
Hypertensives Whole body exercise Higashi <i>et al.</i> 1999a	VOP	Walking, 30 mins, 5–7/week, 12 weeks	ACh increase sig in hypertensives. No difference isosorbide dinitrate LNNMA abolished training enhancement in ACh response.	↑
Higashi <i>et al.</i> 1999b	VOP	Walking, 30 mins, 5–7/week, 12 weeks	Max RHBF response increased only in hypertensives. L-NMMA abolished this enhancement	↑
Hypercholesterolaemia Whole body exercise Lewis <i>et al.</i> 1999	Hyperchols not medicated. VOP	Cycle training, 4 weeks, 3 × 30 min/week	L-NMMA responses greater after training at 4 μmol. No effect of training on ACh or SNP responses	↑ ^f ↔ ^g
Walsh <i>et al.</i> 2003b	Hyperchols on and off medication. VOP and Brachial D/U	Circuit training, 8 weeks, 3 × week	ACh and FMD responses improved in overall and in medicated subjects. No change SNP or GTN. L-NMMA improved in unmedicated subjects.	↑ ^h ↔ ⁱ ↑ ^j
Obesity Whole body exercise Sciacqua <i>et al.</i> 2003	Adults. VOP	Walking, 30 min daily, 3 × weekly, 10–16 weeks	In those who lost weight: improved response to upper dose of ACh. No change in SNP responses	↑
Watts <i>et al.</i> 2004a	Adolescents. Brachial D/U	Circuit training, 8 weeks, 3 × week	FMD increased. Improvement not related to change in adiposity	↑
Watts <i>et al.</i> 2004b	Children. Brachial D/U	Structured play/games, 1 h, 3 × week	FMD increased	↑
Woo <i>et al.</i> 2004	Overweight children. Brachial D/U	Diet + exercise versus diet alone design 6 weeks with 12 month follow-up	At 6 weeks, both group improved FMD Only the exercise group maintained the FMD benefit at 12 months. No changes in GTN measures.	↑
Diabetes Whole body exercise Lavrencic <i>et al.</i> 2000	Polymetabolic syndrome. Brachial D/U	Bicycle exercise, 3 × weekly, 12 weeks	FMD increased. No change in GTN responses	↑
Maiorana <i>et al.</i> 2001a	Type 2 diabetics. VOP and Brachial D/U	Circuit training, 8 weeks, 3 × week	ACh and FMD increased. No change in SNP or GTN	↑
Fuchsjaeger-Mayrl <i>et al.</i> 2002	Type 1 diabetes. Brachial D/U	Cycle exercise, 1 h, 2–3 × weekly, 4 months	FMD increased significantly. No significant change in GTN, despite trend	↑

VOP, venous occlusion plethysmography; D/U, Doppler ultrasound arterial assessment; L-NMMA, N^G-monomethyl-L-arginine; ACh, acetylcholine, an endothelium-dependent NO vasodilator; SNP, sodium nitroprusside, an endothelium-independent NO vasodilator; FMD, flow-mediated vasodilatation, an endothelium- and NO-dependent vasodilator stimulus; GTN, glyceryl trinitrate, an endothelium-independent vasodilator; CHF, chronic heart failure; CAD, coronary artery disease; ↑, improvement in NO vasodilator function; ↓, deterioration in NO vasodilator function; ↔, no change in NO vasodilator function. ^aStimulated function; ^bbasal function; ^clow intensity; ^dmoderate intensity; ^ehigh intensity; ^fbasal function; ^gstimulated function; ^hbasal function in unmedicated subjects; ⁱstimulated function in unmedicated subjects; ^jmedicated subjects.

advanced vascular disease, where the abnormality may spread to involve the vascular smooth muscle component of the NO dilator system (Nakamura *et al.* 1996).

The detailed effects of exercise training in various populations with endothelial dysfunction are summarized in Table 1 and fully reviewed elsewhere (Maiorana *et al.* 2003). Briefly, exercise training of both localized muscle groups (Hornig *et al.* 1996; Katz *et al.* 1997; Hambrecht *et al.* 2000b) and whole body exercise, predominantly of the lower limbs (Hambrecht *et al.* 1998, 2000c, 2003; Higashi *et al.* 1999a; Maiorana *et al.* 2000, 2001a; Linke *et al.* 2001; Gokce *et al.* 2002b; Schmidt *et al.* 2002; Gielen *et al.* 2003; Walsh *et al.* 2003a,b) are associated with improvement in measures of NO vasodilator function in subjects with cardiovascular risk factors and disease. Indeed, the consistency of the published data indicating that exercise training improves endothelial function in heterogeneous groups in whom it is initially depressed is remarkable, and contrasts with training studies of subjects with normal endothelial function. This strongly suggests that subjects with impaired endothelial function may be more amenable to improvement in NO function as a result of training than healthy subjects. Also, as mentioned above, the beneficial effects of exercise are generalized if the exercise involves large muscle groups; lower limb exercise improves upper limb conduit and resistance vessel function (Linke *et al.* 2001; Maiorana *et al.* 2001a; Schmidt *et al.* 2002; Walsh *et al.* 2003a,b). Several other findings, which provide specific physiological insight, can be gleaned from studies of patients with endothelial dysfunction.

Does improved vascular function relate to improved functional capacity?

The majority of exercise training studies in humans have been undertaken in patients with chronic heart failure, probably due to the well established endothelial dysfunction evident in this group (Treasure *et al.* 1990; Kubo *et al.* 1991; Drexler *et al.* 1992; Katz *et al.* 1992, 1993) and the clinical significance of peripheral abnormalities which limit $\dot{V}_{O_{2peak}}$ (Wilson *et al.* 1984; Kraemer *et al.* 1993). These studies indicate that exercise training, predominantly of the lower limbs, improves NO-mediated vasodilator function in the vasculature of the exercised muscle (Hambrecht *et al.* 1998) and also in the untrained upper limb (Maiorana *et al.* 2000; Linke *et al.* 2001). Increases in endothelium-dependent vasodilatation in vessels supplying the trained musculature were significantly correlated with changes in functional capacity (Hambrecht *et al.* 1998) and a small increase in stroke volume (Hambrecht *et al.* 2000a). These studies in CHF subjects therefore suggest that improved NO-mediated vascular function as a result of training

may improve cardiac function and $\dot{V}_{O_{2peak}}$, possibly by improving vascular compliance and decreasing afterload.

Hypercholesterolaemia is associated with impaired endothelium-dependent vascular relaxation (Creager *et al.* 1990; Vita *et al.* 1990; Drexler *et al.* 1991; Chowienczyk *et al.* 1992; Casino *et al.* 1993; Sorensen *et al.* 1994), which, in mice, correlates with reduced aerobic capacity (Maxwell *et al.* 1998). Exercise training in hypercholesterolaemic mice partially reverses endothelial dysfunction, and this is correlated with improved aerobic capacity (Niebauer *et al.* 1999). These findings raise the question of whether exercise training alone, or in combination with lipid lowering, may improve NO vasodilator function and functional capacity in humans. Cycle training in unmedicated subjects enhanced basal NO bioactivity but did not alter ACh-stimulated vasodilatation nor smooth muscle sensitivity to SNP in the forearm (Lewis *et al.* 1999) and, in a randomised, cross-over design study, 8 weeks of training also failed to alter endothelial function in unmedicated subjects (Walsh *et al.* 2003b). Conversely, FMD and ACh responses improved in subjects taking 3-hydroxy-3-methylglutaryl CoA (HMGCoA) reductase inhibitor lipid-lowering therapy (Walsh *et al.* 2003b). This disparity may be due to the longer duration and initially greater severity of hypercholesterolaemia in the treated subjects and their depression of NO-related vasodilatation compared to the untreated and normal subjects, again consistent with the greater potential for improving depressed compared to normal function. Alternatively, as HMGCoA reductase inhibitors and exercise training have both been reported to increase eNOS activity (Griffin *et al.* 2001; Bates *et al.* 2002), and perhaps both affect the oxidant state (Powers *et al.* 1999; Carneado *et al.* 2002), there is a hypothetical possibility of synergistic benefit from the two interventions (Dimmeler & Zeiher, 2003).

In summary, it appears that improvement in NO vasodilator function is associated with improved functional capacity in patients with CHF, due perhaps to enhanced coronary perfusion or decreased afterload. This is important clinically, as $\dot{V}_{O_{2peak}}$ strongly correlates with prognosis in CHF (Mancini *et al.* 1991; Myers *et al.* 1998). Whether exercise training contributes to improved $\dot{V}_{O_{2peak}}$ in other groups such as subjects with hypercholesterolaemia, hypertension or diabetes remains to be seen, but positive evidence has emerged in animals (Maxwell *et al.* 1998; Niebauer *et al.* 1999) and a possible synergism may exist between pharmacological approaches known to improve NO function, such as statins and ACE inhibitors, and shear-stress-mediated exercise training effects. Future studies will need to be specifically designed to address this possibility.

Is improvement in NO function with training related to training-induced improvement in risk factors?

Cardiovascular risk factors such as hypercholesterolaemia (Creager *et al.* 1990; Vita *et al.* 1990; Drexler *et al.* 1991; Chowienczyk *et al.* 1992; Casino *et al.* 1993; Sorensen *et al.* 1994), hypertension (Panza *et al.* 1990; Brush *et al.* 1992; Treasure *et al.* 1992; Egashira *et al.* 1995; Higashi *et al.* 1997), diabetes (McVeigh *et al.* 1992; Karusa *et al.* 1995; Ting *et al.* 1996; Watts *et al.* 1996; Williams *et al.* 1996; O'Driscoll *et al.* 1999) and obesity (Arcaro *et al.* 1999; Perticone *et al.* 2001a) are associated with impaired NO-vasodilator function and treatment with pharmacological agents can reverse this (O'Driscoll *et al.* 1997a,b, 1999; Widlansky *et al.* 2003). These findings raise the possibility that exercise training may improve NO-mediated vasodilator function via its effects on cardiovascular risk factors (Blair *et al.* 1984; Tran & Weltman, 1985; Holloszy *et al.* 1986; Blair, 1993; Williams, 1996; Malfattoo *et al.* 1998; Kelley, 1999; Smith *et al.* 1999; El-Sayed *et al.* 2000).

Improvement in basal (Lewis *et al.* 1999) and stimulated (Walsh *et al.* 2003b) endothelial function with exercise training have been reported in the absence of a decrease in serum lipids, and reduction in lipid levels are not necessarily associated with improved endothelial function (Jodoin *et al.* 1999). Exercise-induced improvements in endothelial function in hypercholesterolaemia therefore appear to result from mechanisms independent of the effect of exercise training on serum cholesterol. In hypertensive subjects, 12 weeks of daily walking increased forearm reactive hyperaemia (Higashi *et al.* 1999b) and the blood flow response to ACh (Higashi *et al.* 1999a), the latter effect abolished by L-NMMA, indicating that the improvement following training was due to augmented bioactivity of NO. There was a reduction in blood pressure as a result of exercise training in this study, but it did not correlate with improved endothelial function indicating that the vascular function benefit is likely to be independent of the pressure lowering effect of training. The effect of exercise training on endothelial function in type 2 diabetes also appears to be independent of changes in glucose tolerance. Diabetic rats with impaired aortic endothelial function were randomised to sedentary, exercise trained and food restricted groups (Sakamoto *et al.* 1998). Both exercise training and food restriction reduced plasma glucose and insulin, and increased insulin sensitivity, but only exercise training improved endothelium-dependent relaxation to histamine and increased urinary excretion of nitrite. Similarly, in humans, exercise training enhanced endothelium-dependent reactivity to ACh and FMD in forearm resistance and conduit vessels but these changes were not significantly related to changes in fasting blood glucose or glycated haemoglobin, suggesting that

improved endothelial function may occur independently of glycaemic control (Maiorana *et al.* 2001a).

Similar results were evident in obese children and adolescents; improvement in FMD with training was not associated with changes in lipid fractions, haemodynamic variables or glycaemic control (Watts *et al.* 2004a; Watts *et al.* 2004b). Indeed, improvements in endothelial function occurred independent of changes in body weight and BMI, lending some support to studies in overweight and obese adults which have demonstrated that low cardiovascular fitness may be a more important predictor of cardiovascular mortality than BMI (Barlow *et al.* 1995; Blair *et al.* 2001; Farrell *et al.* 2002).

In summary, improvement in cardiovascular risk factors does not appear to be essential for improvement in NO vasodilator function. Indeed, in a pooled analysis of 75 subjects with diverse risk factors who undertook a similar training regimen, training did not significantly alter plasma lipids, blood pressure, blood glucose, waist:hip or BMI, despite significant improvement in both conduit and resistance vessel NO vasodilator function, and there were no correlations between changes in any risk factor variables and the improvements in vascular function. At a minimum, these data indicate that the beneficial effects of relatively short-term exercise training on vascular function are not solely mediated by the effects of exercise on cardiovascular risk factors (Green *et al.* 2003).

What mechanisms are responsible for the beneficial effect of exercise training on NO vasodilator function?

It seems likely that a direct shear-stress-related mechanism is largely responsible for the beneficial effects of repeated exercise, but it is also known that NO reacts with oxygen free radicals and that the latter can quench NO bioactivity. It is possible that the repeated induction of NOS activity with exercise training prolongs the half-life of NO by reducing its degradation by free radicals in these conditions (Fukai *et al.* 2000) or by directly decreasing free radical production (Adams *et al.* 2002). In this context, Ennezat *et al.* (2001) recently observed markedly increased expression of genes encoding antioxidant enzymes in skeletal muscle, in the absence of changes in eNOS levels, in patients with CHF following training. This is obviously an area requiring much further study.

In the most recent of their impressive series of studies on exercise training on vascular function, Hambrecht *et al.* (2003) examined the effects of 4 weeks of cycle ergometer exercise on ACh responses in the left internal mammary artery of patients with stable CAD and in matched controls. Training increased peak flow velocity responses to ACh by 56% and flow-mediated dilatation responses to adenosine by 150% while no changes were evident in the

untrained control group. A unique aspect of this study was that within 36 h of the final training session and 24 h of the repeat vascular function assessments, subjects underwent coronary bypass surgery from which a section of the internal mammary artery was harvested and used for *in vitro* ACh and SNP assessment, immunohistochemistry, eNOS mRNA isolation and protein quantification. These cross-sectional analyses revealed significantly higher eNOS mRNA and protein expression in the trained subjects compared to controls and substantially higher eNOS phosphorylation which correlated with *in vivo* ACh-mediated vasodilator capacity. These unique human data reveal that exercise training improves endothelial function *in vivo* by up-regulating eNOS protein expression and by increased phosphorylation of this enzyme. These effects are consistent with a shear-stress mechanism for enhanced NO bioactivity with training, although reduced degradation of NO by oxidant species cannot be ruled out as a contributing mechanism (Fukai *et al.* 2000).

Is there an optimal volume of exercise training for vascular effects?

Two studies raise the possibility that there may be an optimal threshold of training, beyond which exercise may not improve endothelial function. In the only published study which has demonstrated a deleterious effect of exercise training on vascular function in humans, Bergholm *et al.* (1999) reported that 3 months of high intensity running reduced endothelium-dependent function but not endothelium-independent function. The degree of endothelial dysfunction following training was significantly correlated with a decrease in serum uric acid and was most severe in subjects with the greatest improvement in $\dot{V}_{O_{2max}}$. The authors postulated that the training-induced decrease in circulating antioxidant levels may adversely affect endothelial function in the highly trained or overtrained state. Goto *et al.* (2003) recently studied the effects of 12 weeks of exercise undertaken at low (25% $\dot{V}_{O_{2max}}$), moderate (50% $\dot{V}_{O_{2max}}$) and high (75% $\dot{V}_{O_{2max}}$) intensity in young healthy men. ACh-mediated forearm vasodilatation improved in the moderate intensity group only, and this occurred in the absence of changes in blood-borne measures of oxidative stress. In contrast, ACh responses did not improve, nor did they deteriorate, in the high intensity group in whom increased oxidative stress was evident. Taken together, these intriguing studies suggest that low intensity exercise may fall below the threshold for improvement in NO-related vascular function, that moderate intensity exercise enhances endothelial NO bioavailability, whilst any improvement in vascular function as a result of enhanced NO production following high-intensity exercise may be abolished through scavenging by free

radicals generated at high intensities. These findings parallel recent data on the effects of exercise intensity on cardiovascular disease prevention (Manson *et al.* 2002; Tanasescu *et al.* 2002).

Does exercise training modify vascular structure by a NO-dependent mechanism?

It has been known for many years that exercise training is associated with structural vascular enlargement, for example autopsy and angiographic studies demonstrate enlarged coronary arteries in athletes (Currens & White, 1961; Pellicia *et al.* 1990) and physically fit individuals (Rose *et al.* 1967; Mann *et al.* 1972; Hildick-Smith *et al.* 2000), indicating that physical conditioning may induce a change in arterial calibre (i.e. 'arterial remodelling'). Similarly, cross-sectional studies have consistently reported enlargement of both skeletal muscle conduit (Zeppilli *et al.* 1995; Schmidt-Trucksass *et al.* 2000; Dinunno *et al.* 2001; Huonker *et al.* 2003) and resistance (Sinoway *et al.* 1986; Green *et al.* 1996a) vessels in athletes relative to matched controls. Exercise training studies support these findings, with increases in resistance vessel structure (Sinoway *et al.* 1987; Green *et al.* 1994) and resting conduit vessel diameter (Miyachi & Iemitsu Okutsu, 1998; Dinunno *et al.* 2001) reported following exercise training in healthy men. Findings from animal studies regarding epicardial and resistance coronary vasculature concur (Brown, 2003).

A link between chronic changes in flow and modification of vascular structure is supported by a classic study which examined rabbit carotid arteries after unilateral ligation-mediated chronic decreases in flow (Langille & O'Donnell, 1986). The diameter of the ligated vessel exposed to a 70% reduction in flow for a period of 2 weeks was significantly smaller than the contralateral control vessel. In addition, this change in vascular structure was dependent upon the presence of the endothelium, indicating that changes in vessel structure secondary to chronic changes in flow may be dependent upon the release of a labile factor from endothelial cells. A similar conclusion was derived from an earlier study which found that shear stress was autoregulated after initial perturbation by an arteriovenous fistula (Kamiya & Togawa, 1980). More recent studies confirm that the stimulus to arterial remodelling is shear stress (Tuttle *et al.* 2001) and that vessels enlarge to homeostatically regulate wall shear in a NO-dependent manner (Tronc *et al.* 1996).

The above data are consistent with the evolving hypothesis that exercise training induces structural enlargement of conduit vessels which is dependent upon shear-stress-mediated NO release and may be an adaptive response which acts to mitigate the increases in transmural pressure and wall stress brought about by repeated exercise bouts (Rodbard, 1975; Zamir, 1977;

Guyton & Hartley, 1985; Langille *et al.* 1989; Tronc *et al.* 1996; Lloyd *et al.* 2001; Prior *et al.* 2003). This structural remodelling and consequent normalization of shear obviates the need for ongoing functional dilatation, including enhanced NO dilator system function (Brown, 2003). In contrast, microvascular angiogenesis, whilst endothelium (and VEGF) dependent, appears to occur primarily in responses to hypoxia rather than shear stress and NO is not obligatory (Lloyd *et al.* 2001; Prior *et al.* 2003). Indeed, evidence is emerging that the contribution of substances such as NO to exercise training-mediated adaptations in vascular structure and function may be vessel calibre dependent with larger vessels, which are exposed to higher shear stress forces, possessing greater capacity for NO production (Laughlin *et al.* 2003a,b; Green *et al.* 2004). Given that acute bouts of exercise induce changes in local haemodynamic conditions and shear stress which differ according to branch order of the vascular tree, it is likely that the NO-mediated contribution to vascular adaptations differs at distinct loci.

Collectively, the above data from both animal and human studies of exercise training suggest that functional and structural adaptations of the vasculature to exercise training alter with training duration and intensity and the vessel beds involved. Brown, in an excellent review of coronary remodelling in response to exercise, concluded that vascular remodelling differs depending on the size and location of vessels along the vascular tree; capillaries and small arterioles appear to undergo longitudinal network extension whilst resistance and conduit arteries circumferentially expand (Brown, 2003). In humans, this might be considered a hypothesis to fit the current data; more evidence is required regarding the time course of changes in resistance, conduit and microvessels, particularly in the coronary circulation, and concerning functional mechanisms aside from those which are NO dependent.

What is the effect of exercise cessation and detraining on NO function?

Localized and systemic exercise-induced changes in endothelial function are quickly lost following the cessation of relatively short duration training. Six weeks after handgrip training was ceased in patients with CHF, radial artery diameters had returned to pre-training size (Hornig *et al.* 1996). Similarly, endothelial function returned to baseline 8 weeks following cessation of training in patients with CHF (Maiorana *et al.* 2000), CAD (Walsh *et al.* 2003a), hypercholesterolaemia (Walsh *et al.* 2003b) and type 2 diabetes (Maiorana *et al.* 2001a) and in obese adolescents (Watts *et al.* 2004a,b), all of whom had undergone an initial 8 weeks of training. This is consistent with the time course of deconditioning associated with other physiological adaptations to exercise training. These

data suggest therefore that an exercise programme, at some level as yet undetermined, would be necessary to maintain vascular benefits in these extremely common and important conditions for which an exercise programme should be prescribed. Gielen *et al.* (2003) recently found that a 5-month period of moderate volume home-based exercise maintained a proportion, but not the entire benefit, of a preceding intensive hospital based programme. To date, no data exist on the effect of detraining on endothelial function in healthy subjects.

Summary and conclusions

In an excellent recent review for this journal, Booth *et al.* (2002) suggested that humans possess a genome which was selected during an era of obligatory physical activity, whereas contemporary Western lifestyle is characteristically sedentary. In this context, habitual exercise restores perturbed homeostatic mechanisms towards the normal physiological range. Analysis of the studies presented in this review reveal data consistent with this hypothesis. The majority of those studies undertaken in subjects with *a priori* impaired NO-related vasodilator function produced improvement in conduit or resistance vessel function, whereas studies in healthy normal subjects have been less consistent. That is, depressed endothelial function is more amenable to improvement by exercise training than is 'normal' endothelial function in the young and healthy, who might need a higher intensity or volume of exercise training for benefit to be apparent. This raises the intriguing possibility of a threshold effect for training on endothelial function which may be determined by age, fitness or pathological state. Studies are now beginning to emerge regarding the optimal modality and intensity of exercise for improvement in vascular structure and function and intriguing relationships between these factors, endothelial function and oxidative stress are becoming apparent.

Endothelial function may improve after as few as 7 days in trained animals (Wang *et al.* 1993; McAllister & Laughlin, 1997). In healthy humans, 4 weeks of training improved basal NO-related endothelial function in healthy subjects (Kingwell *et al.* 1997b). However, studies that have identified improved stimulated endothelium-dependent vasodilatation have prescribed at least 10 weeks of training in normal subjects (Clarkson *et al.* 1999; DeSouza *et al.* 2000). Pathological states associated with depressed NO bioactivity may respond more rapidly to training, with endothelial function improving after as little as 4 weeks (Hornig *et al.* 1996; Hambrecht *et al.* 2000c), but returning rapidly to baseline following the cessation of training (Hornig *et al.* 1996; Maiorana *et al.* 2000, 2001a). Conditions in which exercise training can improve NO-dependent

endothelial vasodilator function include CAD, type 2 diabetes, obesity, hypercholesterolaemia, hypertension and CHF. In the latter, peripheral abnormalities typically limit $\dot{V}_{O_{2peak}}$ and improvement in vasodilator function correlates with changes in functional capacity and may improve cardiac function by decreasing afterload. Few studies have investigated the relationship between improvements in vasodilator function and functional capacity in other conditions.

It is likely that exercise training improves NO vasodilator function by both direct and indirect means. Conventional risk factors impair NO-mediated endothelial function and beneficial training effects on risk factor profiles are widely accepted, although the evidence is less convincing in the secondary prevention setting in which pharmacotherapy has a greater impact. Nonetheless, exercise training consistently improves NO function in patients with cardiovascular risk factors and disease, including those on optimal medical regimes and in the absence of risk factor modulation. These findings strongly implicate a direct effect of exercise on the vasculature, mediated through intermittent increases in endothelial shear stress. This effect may, in turn, be mediated through up-regulation of eNOS or decreased free radical degradation of NO, with evidence beginning to emerge for both possibilities (Fukai *et al.* 2000; Dimmeler & Zeiher, 2003; Hambrecht *et al.* 2003). The possibility that synergistic benefits accrue from combined pharmacological and exercise-mediated shear stress strategies remains to be investigated in humans.

Vascular remodelling with increase in vessel diameter occurs with prolonged exercise (Leon & Bloor, 1968; Kramsch *et al.* 1981; Miyachi *et al.* 2001; Prior *et al.* 2003). This process teleologically constitutes a longer term mechanism for reducing shear stress on a more sustained basis, allowing NO bioactivity to return towards pre-training levels. This hypothetical schema, which promotes shear stress as a homeostatically regulated variable, explains the observation that improvement in NO-related vasodilatation is observed in short to medium term studies, whereas longer term training studies, associated with arterial remodelling, have not usually reported improved endothelial function. Whilst the mechanisms responsible for mediating vascular structural change remain largely undefined, there is strong evidence that NO plays an important role in arterial remodelling (Kamiya & Togawa, 1980; Langille & O'Donnell, 1986; Zarins *et al.* 1987; Gibbons & Dzau, 1994; Tronc *et al.* 1996; Rudic *et al.* 1998; Prior *et al.* 2003). These findings reinforce the conclusion that future questions regarding the physiological effects of exercise training on the endothelium will be best resolved if studies consider the direct effects of various interventions on the vessel wall.

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