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**TIME COURSE OF CHANGES IN ENDOTHELIAL FUNCTION FOLLOWING EXERCISE IN
HABITUALLY SEDENTARY MEN**

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ABSTRACT

TIME COURSE OF CHANGES IN ENDOTHELIAL FUNCTION FOLLOWING EXERCISE IN HABITUALLY SEDENTARY MEN. **Catherine H Pullin, Michael F Bellamy, Damien Bailey, Moira Ashton, Bruce Davies Simon Williams, Jonathan Goodfellow, John F Wilson, Malcolm J Lewis.** **JEPonline** 2004;7(4):14-22. Regular exercise reduces mortality from cardiovascular dysfunction or disease. The mechanism(s) underlying this protective effect is unknown but may reflect enhanced nitric oxide activity. Two studies were conducted to investigate the effect of acute and chronic exercise on nitric oxide-mediated endothelium-dependent flow-mediated dilation (FMD) in habitually sedentary healthy male adults. Study A subjects (n=9, 30 ± 4 yrs) conducted daily standardised treadmill exercise for 5 days. Brachial artery FMD was assessed daily and on the following 6 days. During the exercise period, FMD increased steadily, was elevated by Day 3 (P = 0.012) and reached maximal response on Day 6 (192 ± 71 µm at day 6 versus 51 ± 68 µm at baseline, P = 0.002). FMD returned to baseline on Day 9. Study B subjects (n=17, 38 ± 2 yrs) conducted a 4-week bike exercise regime. FMD was assessed pre-training, post-training and following 2-weeks detraining. Post-training FMD was improved (137 ± 64 µm post-training versus 80 ± 59 µm pre-training, P = 0.028) but returned to baseline following detraining (P = 0.226). In both studies, endothelium-independent responses remained similar throughout. Physical exercise progressively, rapidly and reversibly improves FMD. The effects are both rapid and short-lived.

Key Words: Flow-Mediated Dilation, Nitric Oxide, Cardiovascular Risk Factor

INTRODUCTION

Regular sustained exercise reduces morbidity and mortality from cardiovascular dysfunction or disease within the general population (1). Additionally, epidemiological studies have associated lack of physical exercise with increased cardiovascular risk (2). Programmed exercise has become an integral component in the management of hypertension and ischemic heart disease, and increasing evidence supports the beneficial effects of exercise therapy in rehabilitation programs offered to patients following cardiac surgery, chronic heart failure, and hypertension. Physical activity has an important role in both primary and secondary prevention, though the exact mechanism(s) by which it mediates its protective effect is uncertain.

Assessment of endothelial function provides a surrogate marker for the integrity and health of the vasculature. Coronary artery endothelial dysfunction has been shown to be a long-term predictor of atherosclerosis and cardiovascular events (3). Evidence is accumulating to suggest that there is a close association between physical fitness and endothelial function (4). Endothelial function can be determined by measuring vascular responses to increased blood flow. In a healthy artery, increased blood flow along a vessel stimulates endothelial production and release of nitric oxide (5). The released nitric oxide diffuses rapidly into the surrounding vascular smooth muscle cells to cause relaxation via activation of soluble guanylate cyclase. This phenomenon is termed endothelium-dependent flow-mediated dilatation (FMD). Individuals at increased cardiovascular risk typically exhibit impaired vascular responses to increased blood flow, implying reduced nitric oxide bio-availability (6-8).

It is possible that the beneficial effects of exercise on the cardiovascular system are exerted, at least in part, via modulation of the vascular endothelium. Previous studies suggest that long-term physical training improves endothelial function (4). Here we report the endothelial effects of short-term exercise training. The aims of the investigation were to establish whether endothelial function in healthy but habitually sedentary adults could be improved by physical exercise and to determine the time course of any such effect. The study was divided into 2 sections, the first (acute study) designed to investigate the vascular effects of a 5-day training program, and the second (chronic study) designed to establish the effect of a 4-week training program.

METHODS

Subjects

A total of 31 habitually sedentary male subjects were recruited and divided into two study groups (acute study $n = 11$; chronic study $n = 20$). All were selected and screened to eliminate conditions known to be associated with endothelial dysfunction - hypertension (blood pressure $>150/90$ mmHg), hypercholesterolemia (cholesterol >6.5 mmol/L), and diabetes mellitus (fasting blood glucose ≥ 5.2 mmol/L). All exhibited levels of plasma total homocysteine within the normal range (5-16 μ mol/L). None were smokers and none were exposed to heavy passive smoking. All refrained from caffeine intake for >6 hours prior to vascular assessment. Ethical approval was obtained from the Local Research Ethics Committee (Bro Taf Health Authority) and all subjects provided informed written consent.

Study design

Two studies were conducted to investigate the vascular effects of (i) short-term (acute) exercise, and (ii) long-term (chronic) exercise.

Acute Study

Exercise Intervention

The acute study took place over an 11-day period comprising a 5-day exercise regime followed by a 6-day "exercise-free" period. On each of days 1 to 5, subjects performed treadmill exercise following the Bruce protocol (see Table 1) to voluntary exhaustion or for a maximum of 15 min (5 stages), whichever

Table 1. The Bruce Protocol

Stage	Speed (km/hour)	Grade(%)	Time (min)
1	2.7	10	3
2	4.0	12	3
3	5.4	14	3
4	6.7	16	3
5	8.0	18	3

occurred first. Heart rate, both pre- and immediately post-exercise, was recorded using ECG telemetry (Polar Vantage NV™, Polar Electro Oy, Finland), and the total duration of exercise was recorded. On each of days 6 to 11 no exercise was undertaken.

Assessment

Endothelial function was determined on each of days 1 to 11 with the exception of day 7, which was a Sunday. Measurements were made at the same time each day to control for circadian variation, and on days 1 to 5 before exercise. Subjects provided a fasting venous blood sample on day 1.

Subject Compliance

Due to work commitments, two subjects failed to complete the protocol in full. Data collected from these persons are excluded from overall statistical analyses. Nine subjects (mean age 30 ± 4 years) completed the study.

Chronic Study

Exercise Intervention

The chronic study took place over a 6-week period comprising a 4-week physical training regime followed by a 2-week “exercise-free” period. Prior to commencing the study, subjects familiarized themselves with both the equipment and the testing environment. During the 4-week exercise period, subjects exercised 3 times/week on non-consecutive days. At each session, subjects were required to exercise for 30 min on a stationary ergometer (MONARK) at 70% of their pre-training maximum heart rate during weeks 1 and 2, and at 80% during weeks 3 and 4. All exercise sessions were supervised.

Assessment

Subjects underwent assessment on three occasions (i) pre-training (week 0) before commencement of the exercise regime, (ii) post-training (week 4) after completion of the exercise program, and (iii) detraining (week 6) following a two week “exercise-free” period. On each occasion resting heart rate and blood pressure were measured, a fasting venous blood sample was taken, artery function was assessed, and maximal oxygen uptake (VO_{2max}) determined breath-by-breath automated expired gas analysis (MedGraphics®, CPX/D). At week 4, assessment of artery function took place 3 days after the last exercise session. On each occasion, VO_{2max} was determined after assessment of artery function.

Subject Compliance

Twenty subjects entered the study. One subject failed to complete the protocol due to other commitments, and 2 individuals withdrew due to ill health (unrelated to the study). Data collected from these persons are excluded from overall statistical analysis. Seventeen subjects (mean age = 38 ± 2 yrs) completed the study.

Assessment Of Artery Function

Endothelium-dependent and endothelium-independent responses were assessed in the brachial artery (9). Endothelium-dependent flow-mediated vasodilatation (FMD) was determined first. Following 15 min supine rest, baseline measurements of internal brachial artery end-diastolic diameter, blood flow and blood pressure were determined. Brachial artery end-diastolic diameter was measured using high-resolution ($\pm 3\mu m$) ultrasonic vessel ‘wall tracking’ (Vadirec™, Medical Systems Arnhem, Netherlands), blood flow by continuous wave Doppler as the product of internal brachial artery diameter and the mean velocity corrected for Doppler angle (SciMed Dopstation™, SciMed, Bristol, UK), and blood pressure by finger photoplethysmography (Finapres™, Ohmeda, Louisville, CO, USA).

Increased blood flow along the brachial artery was induced secondary to reactive hyperaemia in the hand, resulting from release of a wrist cuff inflated to supra-systolic pressure (systolic pressure + 50mmHg) for 5 min. Brachial artery diameter, blood pressure and blood velocity were recorded at 1, 2, 3, and 5 min after cuff release. Further measurements were made between 10 and 15 min after cuff release to demonstrate vessel recovery. FMD was calculated as the maximum absolute change in artery diameter from baseline at either 1, 2 or 3 min after cuff release.

Brachial artery response to the endothelium-independent vasodilatory agent glyceryl trinitrate (GTN) was then determined. End-diastolic diameter, blood flow and blood pressure were measured 3 min after GTN

administration (400 µg, sublingual), and the biological response was calculated as the absolute change in artery diameter compared to the brachial artery diameter (µm) measured at 10 to 15 min after cuff release.

Blood Sampling and Analyses

For measurement of plasma lipids, blood was collected into SST vacutainers containing clot activators and analysed using a Hitachi 747 Clinical Chemistry analyser. For measurement of plasma total homocysteine, blood was collected into vacutainers containing EDTA, placed immediately on ice and centrifuged (3000 revolutions/min for 10 min at 4°C) within 10 min of collection. The plasma was then removed and stored at -70°C until analysis by HPLC using fluorometric detection. Plasma glucose was measured using a Hitachi 747 Clinical Chemistry analyser.

Statistical Analyses

Statistical analyses were performed using SPSS Version 10 (SPSS Inc., 444N Michigan Avenue, Chicago, Illinois 60611, USA). Changes in selected dependent variables as a function of time were analyzed using a one-factor repeated measures analysis of variance. A *P* value of <0.05 was considered significant. Data reported in the tables and figures are presented as mean ± standard error of the mean.

RESULTS

Acute effect of exercise

Vascular measurements

Significant changes in endothelium-dependent FMD were observed (*P* = 0.006) with FMD being significantly augmented on Days 3 to 8 inclusive as compared to Day 1 (*P* < 0.03) (see Table 2 and Figure 1). FMD progressively increased during the 5-day exercise intervention, reaching a maximal response at Day 6, before declining and returning to a response similar to that at baseline on Day 9 (*P* = 0.45). The corresponding endothelium-independent responses to GTN were unaffected by the exercise intervention (*P* = 0.91).

Table 2. The chronic effects of exercise training on hemodynamic parameters. Data given as mean ± SD.

	<i>Training</i>				
<i>Hemodynamic Data</i>	Day 1	Day 2	Day 3	Day 4	Day 5
<i>Baseline end-diastolic diameter (mm)</i>	3.8 ± 0.4	3.7 ± 0.3	3.7 ± 0.5	3.7 ± 0.3	3.7 ± 0.4
<i>Baseline blood flow (mL/min)</i>	55 ± 35	38 ± 26	41 ± 20	51 ± 38	40 ± 16
<i>FMD peak blood flow (mL/min)</i>	116 ± 180	129 ± 105	134 ± 96	118 ± 162	145 ± 126
<i>GTN peak blood flow (mL/min)</i>	28 ± 23	30 ± 18	27 ± 15	38 ± 20	28 ± 17
<i>Systolic BP (mmHg)</i>	113 ± 16	117 ± 16	111 ± 10	119 ± 10	100 ± 17
<i>Diastolic BP (mmHg)</i>	65 ± 7	65 ± 7	63 ± 7	72 ± 11	64 ± 14
<i>Resting HR (beats/min)</i>	70 ± 9	70 ± 11	65 ± 7	66 ± 7	68 ± 9
<i>Post-ex HR (beats/min)</i>	176 ± 20	180 ± 13	182 ± 10	180 ± 10	178 ± 11
	<i>De-training</i>				
<i>Hemodynamic Data</i>	Day 1	Day 2	Day 3	Day 4	Day 5
<i>Baseline end-diastolic diameter (mm)</i>	3.6 ± 0.5	3.5 ± 0.4	3.6 ± 0.5	3.4 ± 0.4	3.5 ± 0.5
<i>Baseline blood flow (mL/min)</i>	30 ± 25	56 ± 35	57 ± 36	43 ± 55	50 ± 42
<i>FMD peak blood flow (mL/min)</i>	118 ± 174	102 ± 78	128 ± 162	119 ± 111	130 ± 108
<i>GTN peak blood flow (mL/min)</i>	22 ± 20	31 ± 18	32 ± 26	22 ± 17	27 ± 21
<i>Systolic BP (mmHg)</i>	97 ± 13*	99 ± 16*	104 ± 10	107 ± 13	102 ± 11
<i>Diastolic BP (mmHg)</i>	63 ± 15	60 ± 14	62 ± 9	67 ± 7	65 ± 12
<i>Resting HR (beats/min)</i>	61 ± 7*	63 ± 7*	62 ± 8*	63 ± 7*	61 ± 11*
<i>Post-ex HR (beats/min)</i>	no data	no data	no data	no data	no data

*=*P*<0.05 compared to day 1. BP = blood pressure. HR = heart rate.

Throughout the study, both baseline brachial artery end-diastolic diameter and baseline brachial artery blood flow were unchanged (*P* = 0.08 and *P* = 0.71 respectively). The increase in brachial artery blood flow observed during reactive hyperemia (the stimulus for FMD) was similar on each study day (*P* = 0.94), as were the changes in flow observed following GTN administration (*P* = 0.32).

Hemodynamic measurements

A significant change in resting heart rate was observed ($P = 0.003$) (Table 2). As compared to Day 1, heart rate was significantly reduced on Days 6 to 11 inclusive ($P < 0.05$). Similarly, significant changes in resting supine systolic blood pressure were reported ($P = 0.02$). Compared to Day 1, systolic blood pressure was reduced on Days 6 and 8 ($P < 0.03$). Resting supine diastolic blood pressure was unaffected throughout ($P = 0.10$).

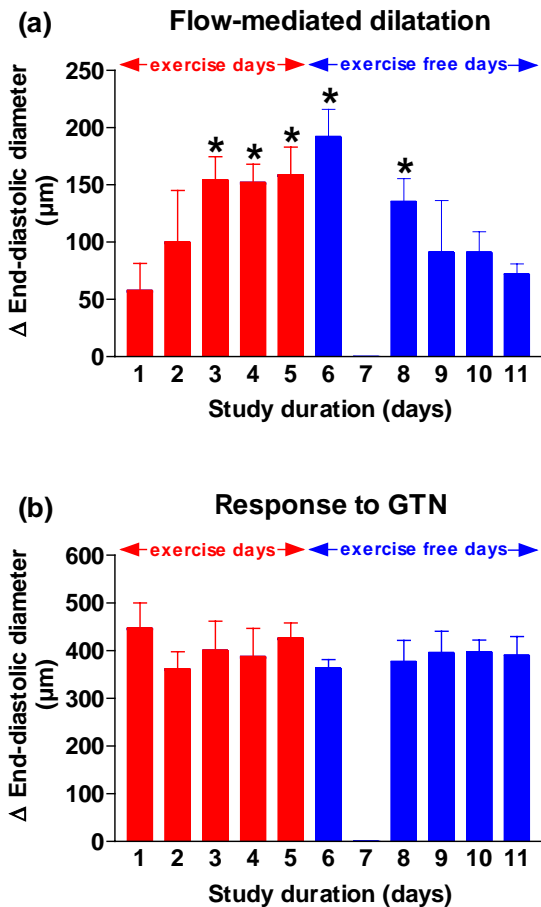


Figure 1. The acute effects of exercise training on brachial artery responses to (a) increased blood flow (FMD), and (b) glyceryl trinitrate administration, as determined by measurement of the absolute change in brachial artery diameter. Data expressed as mean \pm 1 standard error of the mean. Red bars represent training days. Blue bars represent detraining days. $*=P < 0.05$ compared to day 1.

Chronic effect of exercise Vascular measurements

Brachial artery endothelium-dependent FMD was elevated at Week 4 ($P = 0.03$) compared to Week 0 (pre-training) (see Table 3 and Figure 2). FMD was similar at Weeks 0 and 6 ($P = 0.22$). The corresponding endothelium-independent responses to GTN remained similar at Weeks 0, 4 and 6 ($P = 0.93$).

Throughout the study both baseline brachial artery end-diastolic diameter and baseline brachial artery blood flow were unchanged ($P = 0.98$ and $P = 0.89$ respectively). The increase in brachial artery blood flow observed during reactive hyperemia was similar on each study day ($P = 0.31$), as were the changes in flow observed following GTN administration ($P = 0.09$).

Hemodynamic measurements

Compared to Week 0, systolic blood pressure was reduced at Week 4 ($P = 0.006$) though similar at Week 6 ($P = 0.63$) (see Table 3). Diastolic blood pressure and resting heart rate were similar throughout ($P = 0.38$ and $P = 0.10$ respectively).

Biochemical measurements

There were no significant changes in fasting plasma total cholesterol ($P = 0.16$), triglycerides ($P = 0.13$), high-density lipoproteins ($P = 0.59$) or glucose ($P = 0.41$) (see Table 3). However, compared to Week 0, plasma total homocysteine was reduced at Week 4 ($P = 0.04$) though similar at Week 6 ($P = 0.054$).

Maximal effort measurements

Maximal oxygen uptake (VO_{2max}), respiratory exchange ratio and maximal heart rate remained similar throughout ($P > 0.05$) (see Table 3).

Table 3. The chronic effects of exercise training on hemodynamic, biochemical and maximal effort parameters.

	<i>Pre-training</i>	<i>Post-training</i>	<i>De-training</i>
<i>Haemodynamic Data</i>			
<i>Baseline end-diastolic diameter (mm)</i>	3.9 ± 0.4	3.9 ± 0.4	3.9 ± 0.5
<i>Baseline blood flow (mL/min)</i>	31 ± 22	28 ± 23	29 ± 26
<i>FMD peak blood flow (mL/min)</i>	167 ± 94	131 ± 54	149 ± 83
<i>GTN peak blood flow (mL/min)</i>	24 ± 12	24 ± 22	16 ± 12
<i>Systolic blood pressure (mmHg)</i>	118 ± 14	106 ± 20*	121 ± 18
<i>Diastolic blood pressure (mmHg)</i>	76 ± 11	77 ± 16	80 ± 11
<i>Resting heart rate (beats/min)</i>	62 ± 8	58 ± 10	61 ± 10
<i>Biochemical Data</i>			
<i>Total cholesterol (mmol/L)</i>	5.35 ± 0.98	5.33 ± 1.07	5.58 ± 1.12
<i>High-density lipoproteins (mmol/L)</i>	1.33 ± 0.26	1.28 ± 0.21	
<i>Glucose (mmol/L)</i>	4.78 ± 0.37	4.62 ± 0.31	4.74 ± 0.43
<i>Total homocysteine (µmol/L)</i>	10.3 ± 3.8	9.4 ± 3.5*	9.4 ± 3.2
<i>Maximal Effort Data</i>			
<i>VO₂max (L/min)</i>	2.75 ± 0.58	2.87 ± 0.59	2.90 ± 0.50
<i>Respiratory exchange ratio</i>	1.23 ± 0.08	1.26 ± 0.05	1.25 ± 0.08
<i>Heart rate (beats/min)</i>	182 ± 10	178 ± 14	180 ± 14

Data given as mean ± SD. *=*P*<0.05 compared to pre-training week.

DISCUSSION

Previous studies suggest a potential beneficial role for exercise in a wide and diverse selection of disorders. Physical training has been shown to have advantageous effects on cardiovascular (2), hormonal, metabolic (10) and neurological (11) function. While the beneficial effects of long-term physical activity on cardiovascular health are widely accepted, the exact mechanism by which exercise mediates this benefit remains unclear. The principle finding of this open-design investigation was that regular exercise improved brachial artery endothelial function in habitually sedentary but apparently healthy male adults. Throughout the study, vascular responses to GTN were unchanged implying that exercise improved FMD by enhancing endothelium-derived NO bio-availability, rather than by increasing the sensitivity of vascular smooth muscle cells to NO. Importantly, this study demonstrated that the beneficial endothelial effects of exercise are both rapid and short-lived. Endothelial function was improved within days of commencing an exercise regime. However, as soon as regular physical activity ceased, endothelial function declined rapidly. These findings may indicate that in order to achieve long-term improvement of endothelial function, exercise sessions need to be frequent, regular and maintained.

A study conducted to explore the cardiovascular health benefits of exercise (12) concluded that for the full benefits to be achieved (i.e. to maximally reduce the chance of experiencing a cardiovascular event), exercise needs to be moderate, regular and sustained. A separate study that examined cardiovascular risk reported that people who exercised at least 3 days/week had the most favourable coronary risk profile (13). These reports are in keeping with the observation reported here, which showed that exercising 3 times/week significantly improves FMD. The same study also reported that even those who exercised only once per week had an improved cardiovascular risk profile compared to sedentary individuals. This supports observations from a prior study from our laboratory indicating that exercise exerts a graded effect upon endothelial function (14).

Evidence to support there being an association between activity levels and endothelial function continues to accumulate. Exercise has now been reported to improve endothelium-mediated responses in individuals both with and without established vascular disease (15,16) in both the central and peripheral vascular beds. Together, these

observations support exercise as mediating its beneficial effects on cardiovascular health by generalized modification of endothelial function.

The studies reported here do not investigate the specific mechanisms behind the endothelial response to exercise, but do support there being a role for modification of the functional properties of the endothelium in the cardio-protective effects of exercise. It is physiologically plausible that exercise could exert its vascular health effects by increasing NO bio-availability. The anti-atherogenic properties of NO are well established, and enhanced NO bio-availability would be expected to have important physiological consequences.

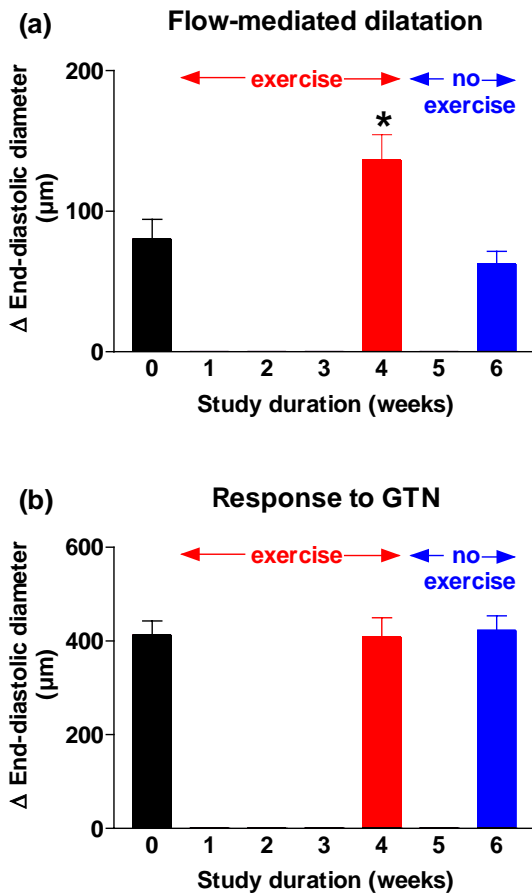


Figure 2. The effects of chronic exercise training on brachial artery responses to (a) increased blood flow (FMD), and (b) glyceryl trinitrate administration, as determined by measurement of absolute change in brachial artery diameter. Data expressed as mean \pm 1 standard error of the mean. Black bars represent the pre-training 'exercise-free' week, red bars the end of the training period, and blue bars the end of the 'exercise-free' detraining weeks. $*=P<0.05$ compared to week 0.

Hemodynamic forces including pulsatile stretch and shear stress play a crucial role in the regulation of eNOS, the enzyme responsible for endothelial NO production. Endothelial eNOS mRNA expression is upregulated in cultured endothelial cells exposed to laminar shear stress (17). *In vivo*, physical activity, which intermittently increases blood flow, increases coronary artery nitric oxide activity in dogs (18) and also appears to regulate nitric oxide formation in humans (19). Exercise may limit the progression of vascular disease by upregulating eNOS and increasing the availability of anti-atherosclerotic NO. It is possible that upregulation of eNOS may be responsible for the improved endothelial function observed in the present study.

Alternatively, exercise could increase NO bioavailability by reducing NO deactivation by free radicals. Exercise up-regulates the free radical scavenging enzyme superoxide dismutase (20) that may limit NO degradation by enhancing anti-oxidant capacity. Indeed, evidence is accumulating to suggest that atherosclerosis is, at least in part, governed by the effect of shear stress on endothelial NOS and superoxide dismutase (21).

Physical activity could mediate its health effects by favorably modifying other ‘conventional’ risk factors, though beneficial effects of exercise have been reported previously in the absence of an effect upon either blood pressure or lipid profile (22). In the studies reported here, the beneficial effects of exercise were also manifested as a reduction in resting heart rate and systolic blood pressure, an effect that could be attributed to an increase in nitric oxide activity.

The observation that there was no change in lipid profile following chronic exercise was not unexpected. Previous studies, although somewhat conflicting in their findings, suggest that exercise sessions need to be sustained for long periods before changes in lipid profile occur (23). A small but significant reduction in plasma total homocysteine seen following the four week exercise program is apparent, but the physiological implications of this remain uncertain and further investigation is required before firm conclusions can be reached concerning its physiological relevance.

The unaltered VO₂max observed in the chronic study suggests physical fitness was unaffected by the exercise training. The activity level therefore appears to have been sufficient to improve vascular function but not VO₂max. This finding supports previous work, which concludes that the quality and quantity of exercise needed to obtain health-related benefits may differ from that required to improve cardio-respiratory and muscular endurance related fitness (24).

Although a precise mechanism cannot be established, the endothelial effects of exercise reported in this study appears to be clear-cut. However, several limitations need to be borne in mind. It is difficult to perform a study of this design in a blinded fashion due to the nature of the intervention. Both sections of the investigation were conducted in an open manner with subjects acting as their own control. Additionally, these studies were conducted in male subjects only, and although there is no reason to suspect that these findings cannot be directly extrapolated to include females, it cannot be assumed.

This study would suggest that in the absence of regular exercise, healthy individuals exhibit “sub-optimal” vascular endothelial responses to increased blood flow, consistent with the view that physical inactivity is a risk factor for cardiovascular disease in the general population. In conclusion, this study demonstrates that the endothelial effects of exercise are both rapid and short-lived. These observations indicate that for the full cardiovascular benefits of physical activity to be achieved, exercise sessions need to be regular, frequent and maintained. Whatever the mechanism by which regular exercise mediates its beneficial cardiovascular effects, it seems likely that the endothelium has a pivotal role.

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