

Subacute effects of a maximal exercise bout on endothelium-mediated vasodilation in healthy subjects

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Abstract

We evaluated vascular reactivity after a maximal exercise test in order to determine whether the effect of exercise on the circulation persists even after interruption of the exercise. Eleven healthy sedentary volunteers (six women, age 28 ± 5 years) were evaluated before and after (10, 60, and 120 min) a maximal exercise test on a treadmill. Forearm blood flow (FBF) was measured by venous occlusion plethysmography before and during reactive hyperemia (RH). Baseline FBF, analyzed by the area under the curve, increased only at 10 min after exercise ($P = 0.01$). FBF in response to RH increased both at 10 and 60 min vs baseline ($P = 0.004$). Total excess flow for RH above baseline showed that vascular reactivity was increased up to 60 min after exercise (mean \pm SEM, before: 526.4 ± 48.8 ; 10 min: 1053.0 ± 168.2 ; 60 min: 659.4 ± 44.1 ml 100 ml⁻¹ min⁻¹ · s; $P = 0.01$ and 0.02 , respectively, vs before exercise). The changes in FBF were due to increased vascular conductance since mean arterial blood pressure did not change. In a time control group ($N = 5$, 34 ± 3 years, three women) that did not exercise, FBF and RH did not change significantly ($P = 0.07$ and 0.7 , respectively). These results suggest that the increased vascular reactivity caused by chronic exercise may result, at least in part, from a summation of the subacute effects of successive exercise bouts.

Key words

- Vascular reactivity
- Exercise test
- Blood flow
- Plethysmography

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Introduction

Exercise training reduces overall morbidity and mortality in the general population (1) and in subjects with established cardiovascular disease (2). Although the mechanisms involved in this protective effect are complex and not completely understood, improved vascular function, i.e., increased vasodilatory reactivity, has been ob-

served after exercise training in patients with chronic heart failure (3), in patients with type 2 diabetes (4) and in healthy subjects (5). Vascular tone and reactivity are under the direct influence of endothelium function via the release of chemical mediators such as nitric oxide, which also modulates platelet aggregation, thrombosis, inflammation, leukocyte adherence, and cell proliferation (6). Thus, disruption of the functional integrity

of the vascular endothelium plays a key role in all stages of atherogenesis, from lesion initiation to plaque rupture (6).

Regular aerobic exercise can both prevent the age-associated loss in endothelium-dependent vasodilation and restore the function lost as a result of sedentary aging (5). Twelve weeks of moderate exercise, but not mild- or high-intensity exercise, increased endothelium-dependent vasodilation through increased nitric oxide production and release in healthy subjects (7). The chronic effects of exercise develop from the interaction of physiological responses that occur during (acute effects) and after each session of physical effort (subacute effects). Therefore, it is important to evaluate the subacute effects of exercise to understand the whole process of adaptation. However, little is known about the physiological phenomenon that occurs after a single session of physical exertion. Baynard et al. (8) demonstrated that endurance-trained men appear to have a greater peak vasodilatory capacity compared to resistance-trained men, and acute maximal exercise on a treadmill increased the vasodilatory capacity, measured only once after physical effort, in both groups. Copeland et al. (9) observed increases in forearm blood flow (FBF) after acute handgrip exercise, and these increases were greater in the aerobically trained than in the resistance-trained individuals. It is not known whether the subacute effects of exercise on vascular reactivity also occur in healthy sedentary subjects and, if they actually occur, for how long after exercise they are sustained.

On the basis of these considerations, the purpose of the present study was to determine peripheral blood flow and vascular reactivity for 2 h after a single bout of maximal aerobic exercise in healthy subjects.

Subjects and Methods

Eleven healthy subjects (six women; mean age 28 ± 5 years) volunteered to participate

in the study, which was approved by the Institutional Ethics Committee (CEP CCM/HUAP 030/04). All subjects gave written informed consent to participate in the study. Exclusion criteria were the presence of diabetes, hypertension, known cardiovascular disease, smoking habit, and the use of medications. The subjects fasted for at least 2 h and did not perform vigorous exercise in the preceding 24 h or ingested caffeine on the day of the evaluation. All tests were performed in the morning, after a 20-min period of supine rest in a quiet, air-conditioned room (constant temperature of 23° to 25°C).

FBF was measured with a mercury-filled silastic strain-gauge plethysmograph (EC6, D.E., Hokanson, Inc., Bellevue, WA, USA). A cuff was placed around the upper arm, a strain gauge around the widest part of the right forearm and an additional cuff around the wrist to occlude hand circulation. The arm was supported by a device at the level of the heart. One minute before each measurement and throughout FBF measurement, the wrist cuff was inflated to 200 mmHg. FBF was determined by rapidly inflating the upper cuff to 40 mmHg for 10 s to occlude venous circulation, followed by a 10-s deflation, during each 20-s cycle. An average of nine 20-s plethysmographic cycles were used for resting FBF measured as the rate of volume change during venous occlusion and expressed as ml/100 ml of forearm tissue volume/min. Reactive hyperemia (RH) was induced by inflating the upper arm cuff to 200 mmHg for 5 min, after which the cuff was deflated for 10 s and FBF measured for 3 min thereafter as described above. The area under the curve (AUC) both at baseline and during RH was calculated as a flow-time index to provide values of total FBF in each situation. Vascular reactivity was considered to be the total excess flow above baseline, calculated as the difference between the AUC during RH and at baseline. The plethysmographic signal was transmitted to the computer using an A/D conversion board

(National Instruments, Co., Autix, TX, USA) for off-line analysis. All data analyses were performed by the same investigator, who showed an intra-subject variability (coefficient of variation) of 1.4 and 1.8% for resting and RH measurements, respectively. Heart rate and blood pressure were monitored using the digital infrared photoplethysmographic Finapres device (model 2050, Ohmeda, Englewood, CO, USA) and mean arterial pressure (MAP) was calculated by the standard equation: $\text{MAP} = \text{diastolic blood pressure} + (\text{systolic blood pressure} - \text{diastolic blood pressure}/3)$.

After baseline evaluation, subjects performed an exercise test on a treadmill (Inbramed, Porto Alegre, RS, Brazil) following a progressive, maximal intensity, and individualized ramp protocol that was designed to last approximately 10 min, based on the subject's age and gender. The volunteers performed the procedures exactly as described above (resting FBF and RH) 10, 60, and 120 min after completing the exercise test. To assure that the actual effects of exercise were observed, an additional time-control group was used (N = 5). All individuals participated in the exercise experiment that was performed at least one week before the time-control test. However, due to technical limitations, data for two individuals were excluded from the exercise experiment. The same procedures were done as in the exercise test day except that, after the baseline values were recorded, the subjects stood for 10 min on the treadmill, with no movements, and the 10-, 60-, and 120-min evaluations were performed thereafter.

Results were analyzed statistically by one-way analysis of variance (ANOVA) for repeated measures. When ANOVA revealed significant differences, the *post hoc* Bonferroni or Dunnett test was used to determine which specific time points were different from pre-exercise data taken as reference. Data are reported as means \pm SEM. Statistical significance was set at $P < 0.05$.

Results

The demographic and exercise-related results for the exercise and control group are shown in Table 1.

The AUC for baseline FBF and RH for the different intervals of analysis are shown in Figure 1. Compared to pre-exercise values, baseline FBF (upper panel) was significantly increased at 10 min ($P = 0.019$), but not at 60 min or 120 min after exercise. FBF during RH (Figure 1, lower panel) was higher at 10 min ($P = 0.013$) and 60 min ($P = 0.05$)

Table 1. Demographic and exercise-related results.

	Exercise group (N = 9)	Control group (N = 5)
Age (years)	28 \pm 2	34 \pm 3
Gender (female)	5	3
Weight (kg)	69.4 \pm 5	65.9 \pm 4
Height (cm)	1.73 \pm 0.03	1.71 \pm 0.02
Exercise time (min)	13.5 \pm 0.7	-
METs	12.8 \pm 0.6	-

Data are reported as mean \pm SEM. METs = metabolic equivalents.

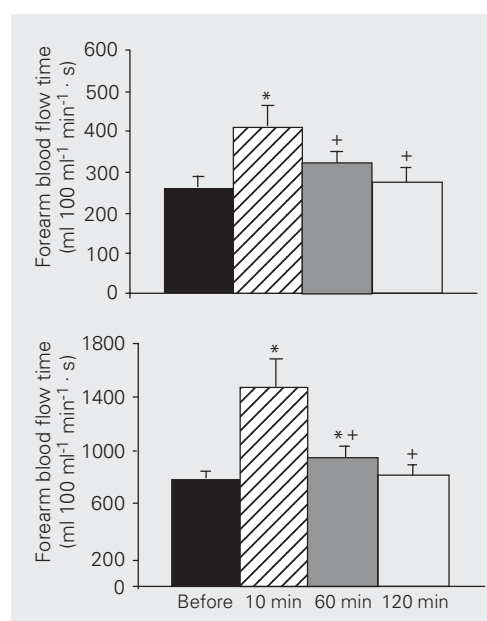


Figure 1. Forearm blood flow during baseline and reactive hyperemia calculated as the area under the curve (AUC). AUC for flow vs time was measured before and after the exercise test. Flow was higher for the baseline measurement only at 10 min after physical effort (upper panel). Values for reactive hyperemia were higher at 10 and 60 min after exercise (lower panel). * $P < 0.05$ vs before exercise; + $P < 0.05$ vs 10 min after exercise (ANOVA for repeated measures with the *post hoc* Bonferroni test).

after exercise and returned to values close to baseline at 120 min ($P > 0.05$). There was no significant correlation between FBF or HR and the peak exercise intensity achieved during the test.

Analysis of the whole temporal response to RH during the 3-min interval of recording (Figure 2, upper panel) showed that FBF was

increased throughout all but the last measurements obtained at 10 min post-exercise compared to the pre-exercise. FBF was also increased 60 min after the exercise test at several time points (Figure 2, upper panel). When vascular reactivity was analyzed as AUC of excess flow (Figure 2, lower panel), exercise caused an increase at 10 min ($P = 0.015$) and 60 min ($P = 0.02$), but not at 120 min.

There were no significant changes in MAP in the 10-min post-exercise period or at the other times (data not shown). Therefore, the subacute flow changes provoked by exercise were due to increases in vascular conductance and not simply to higher driving pressure.

When the results of the time-control group were evaluated, a progressive decrease in baseline FBF and RH was observed although without statistically significant changes. While the baseline FBF values increased by 73% in the post-exercise interval for the experimental group, the time control group showed a decrease of 15 to 25% at the 10-, 60-, and 120-min measurements compared to the pre-exercise values (data not shown). For the RH analysis, the decrease over time was of 11-21%, contrasting to the 25% increase in the 10-min post-exercise interval in the experimental group. These findings reflect the effect of time on blood flow measurements and served as an internal control to support the view that the enhanced blood flow found in the experimental group was due to the physical test stimulus.

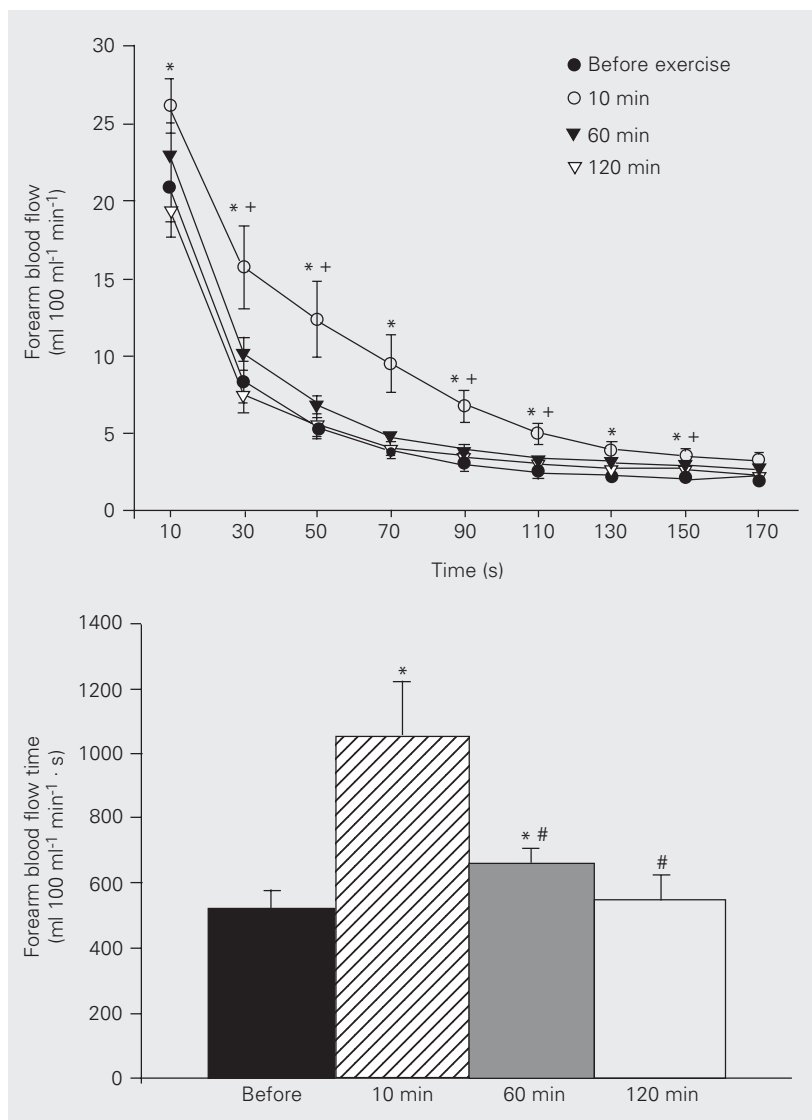


Figure 2. Temporal response and total excess blood flow during reactive hyperemia. Forearm blood flow increased during reactive hyperemia at 10 min and 60 min after the exercise test compared to before exercise (upper panel). Total excess flow (blood flow during reactive hyperemia minus baseline flow; lower panel), an indicator of vascular reactivity, was increased up to 1 h after cessation of exercise. * $P < 0.05$ for 10 min vs before exercise; + $P < 0.05$ for 60 min vs before exercise; # $P < 0.05$ vs 10 min (ANOVA for repeated measures with the *post hoc* Bonferroni test).

Discussion

The main finding of the present study was that an acute maximal aerobic exercise test enhances baseline FBF and vascular reactivity during RH. Vascular reactivity was still increased 60 min after the end of exercise. Although many studies have investigated the chronic adaptations to exercise training, little is known about the acute, and

more specifically the subacute effects of a single bout of exercise on the vascular reactivity of healthy sedentary subjects.

The chronic effects of exercise training on endothelial function and vascular reactivity have been studied extensively. Eight weeks of combined aerobic and resistance training enhanced the vasodilation in response to RH and infusion of acetylcholine into individuals with type 2 diabetes (4). In healthy individuals, Maeda et al. (10) showed increased levels of plasma nitrite/nitrate and decreased levels of endothelin-1 after eight weeks training on a leg ergometer. Interestingly, this effect lasted until the 4th week after cessation of exercise training and the levels of nitric oxide and endothelin-1 returned to basal levels (before training) in the 8th week after completion of exercise training. These data suggest that the chronic effects of physical training develop over the weeks as an adaptation to the acute stress of exercise bouts.

Exercise acutely reduces triglycerides (11), increases HDL-cholesterol (11) as well as insulin sensitivity and ability to resynthesize the glycogen of skeletal muscle (12), and produces an acute blood pressure reduction (13). In fact, these effects could be classified as subacute since the reduction of triglycerides is not immediate but occurs 18-24 h after exercise and lower levels of blood pressure may persist for 24 h (14). Baynard et al. (8) investigated the vasodilatory response of endurance- and resistance-trained individuals before and after an acute bout of maximal aerobic exercise. The findings included a greater ability to respond to RH in endurance-trained individuals and a larger vasodilatory response following RH, upon completion of acute exercise, in both endurance- and resistance-trained men. However, the authors evaluated the vasodilatory capacity in a physically active population and just before and immediately after the execution of physical exercise on a treadmill. More importantly, Baynard et al. (8) did not report

the baseline blood flow after exercise. Thus, it is not possible to determine whether blood flow increased during RH because of an enhanced reactive vasodilation or because of increased enhanced overall vascular conductance.

In the present study, we evaluated the vasodilatory response of healthy sedentary individuals before and 10, 60 and 120 min after an exercise test on a treadmill. In agreement with other studies, we observed higher FBF at baseline and in response to RH in the post-exercise period (15). When the results were analyzed using the AUC, baseline values post-exercise were still higher compared to pre-exercise but an increased RH response could be detected not only in the post-exercise interval but also after 60 min of cessation of exercise test. In addition to the increased peak vasodilatory flow during RH immediately after the exercise test, we observed that the values obtained for the temporal response to RH maintained upper levels for most of the measurement period compared to the pre-exercise evaluation. Again, this profile was observed in the 60-min post-exercise measurement. Baynard et al. (8) showed a biphasic FBF response to RH following an acute exercise bout. After the initial 30 s after cuff deflation, FBF was exaggerated for about 1 min, followed by a gradual decline towards baseline values, and this response was especially evident in the endurance-trained group.

An original finding of our study was that, although baseline values returned to the normal range in the 60-min post-exercise period, an increased vascular reactivity was observed, reflected by higher values of total excess flow. It may be assumed that the maximal exercise test evoked a subacute effect of increased vascular reactivity and increased vascular conductance, as the MAP did not change in the post-exercise period. Several mechanisms have been suggested to clarify the physiological processes involving enhanced subacute vasodilatory re-

sponses mediated by exercise. It has been demonstrated that the increased post-occlusion brachial artery vasodilation observed after ischemic handgrip exercise is at least partly mediated by nitric oxide since infusion of L-NMMA, a nitric oxide synthase inhibitor, evoked lower post-occlusion vasodilation compared with infusion of NaCl (16). Joyner et al. (17) investigated the separate and combined contribution of nitric oxide and vasodilating prostaglandins as mediators of RH in the human forearm and observed that L-NMMA reduced baseline FBF at rest and had a modest effect on peak forearm vascular conductance and flow. Ibuprofen, a cyclooxygenase inhibitor, did not change FBF at rest and reduced peak FBF. When L-NMMA was infused after ibuprofen, a reduced FBF and no effect on peak flow was observed, suggesting that nitric oxide synthase inhibition has a modest effect on peak vasodilation during RH in contrast to the prostaglandins. Another potential mechanism for the increased vascular conductance after exercise is a diminished sympathetic activity leading to lower vasoconstrictor tone, as previously shown (18).

This could increase overall vascular conductance, but it is unlikely that decreased sympathetic vasoconstriction would cause a specific effect on vascular reactivity.

To exclude the temporal influence on the vasodilatory response, a time control group was submitted to the same protocol except for the exercise test. A progressive decrease in the values of baseline FBF and RH response was observed, which contrasted with the higher levels of those parameters observed in the group that performed exercise. This confirmed the subacute effect of the exercise test on the vasodilatory response.

In conclusion, a maximal aerobic exercise test provoked a subacute effect on vascular function that lasted 60 min, with increased vascular conductance and vasodilatory reactivity in healthy young subjects.

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