Endurance Training Enhances Vasodilation Induced by Nitric Oxide in Human Skin

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Endurance training modifies the thermoregulatory control of skin blood flow, as manifested by a greater augmentation of skin perfusion for the same increase in core temperature in athletes, in comparison with sedentary subjects. In this study, we tested the hypothesis that a component of this adaptation might reside in a higher ability of cutaneous blood vessels to respond to vasodilatory stimuli. We recruited healthy nonsmoking males, either endurance trained or sedentary, in two different age ranges (18–35 y and >50 y). Skin blood flow was measured in the forearm skin, using a laser Doppler imager, allowing to record the vasodilatory responses to the following stimuli: iontophoresis of acetylcholine (an endothelium-dependent vasodilator), iontophoresis of sodium nitroprusside (a nitric oxide donor), and release of a temporary interruption of arterial inflow (reactive hyperemia). There was no effect of training on reactive hyperemia or the response to acetylcholine. In contrast, the increase in perfusion following the iontophoresis of sodium nitroprusside, expressed in perfusion units, was larger in trained than in sedentary subjects (younger: 398 ± 54 *vs* 350 ± 87, $p < 0.05$; older 339 ± 72 *vs* 307 ± 66, $p < 0.05$). In conclusion, endurance training enhances the vasodilatory effects of nitric oxide in the human dermal microcirculation, at least in forearm skin. These observations have considerable physiologic interest in view of recent data indicating that nitric oxide mediates in part the cutaneous vasodilation induced by heat stress in humans. Therefore, the augmentation of nitric oxide bioactivity in the dermal microcirculation might be one mechanism whereby endurance training modifies the thermoregulatory control of skin blood flow. ***Key words: exercise/laser Doppler flowmetry/nitric oxide/skin blood supply. J Invest Dermatol 121:1197–1204, 2003***

In humans, the skin is the main pathway for heat dissipation, through water evaporation, convective losses, and radiative losses. The latter two processes are markedly dependent on skin perfusion, which makes the cutaneous vascular bed an essential effector for thermoregulation. Ideally suited to this role, skin blood flow has a tremendous functional reserve, being able in selected circumstances to increase more than 10 times above the level measured in baseline conditions in a cool environment. Thermoregulation is the major process that governs skin blood flow in humans (Johnson and Proppe, 1996; Johnson, 1998). Accordingly, there is a close link between skin perfusion and core temperature, the former increasing whenever the latter rises above a certain threshold. This link results from reflexes with efferent pathways involving both the sympathetic vasoconstrictor and the sympathetic vasodilator systems (Johnson and Proppe, 1996). The relationship of skin blood flow to core temperature is modulated by many factors, including local skin temperature (Pergola *et al*, 1996), circadian rhythms, state of hydration, hormonal status in women, and, relevant to this study, endurance physical training (Roberts *et al*, 1977; Johnson and Proppe, 1996; Johnson, 1998).

The thermogenesis associated with aerobic exercise is an obvious challenge to thermoregulation. It appears that a component of the cardiovascular adaptation to regular aerobic physical activity includes an altered thermoregulatory control of skin perfusion. In the course of exercise tests in a warm environment, endurance-trained subjects have a higher skin blood flow at any given level of workload and core temperature, when compared with matched sedentary controls (Tankersley *et al*, 1991; Ho *et al*, 1997). Furthermore, the augmentation of skin blood flow induced by a pure heat stress test (whole-body warming with a water-perfused suit) is blunted after 2 wk of detraining (Crandall *et al*, 1994).

Studies on the mechanisms through which aerobic training influences the thermoregulatory control of skin blood flow have focused on potential neural pathways. For example, Thomas *et al* (1999) presented evidence that the cutaneous sympathetic vasoconstrictor system is not involved, and postulated a role for its vasodilator counterpart. Rather than or in addition to influencing neural pathways, regular physical activity could alter cutaneous vascular reactivity. In skeletal muscle and in the myocardium, endurance training is associated with an enhanced response of blood vessels

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Abbreviations: acetylcholine acetylcholine; AUC area under curve; BMI body mass index; HDL high density lipoprotein; LDI laser Doppler imager; NO nitric oxide; SNP soium nitroprusside; VO2 max maximal oxygen uptake.

to endothelium-dependent vasodilator stimuli, a fact amply documented both in animals (Koller *et al*, 1995; Mombouli *et al*, 1996; Griffin *et al*, 1999; Varin *et al*, 1999) and in humans (Higashi *et al*, 1999; Hambrecht *et al*, 2000). By contrast, the impact of training on local vasomotor reactivity remains essentially unknown in the skin.

The objective of this study was to cross-sectionally compare physically fit and sedentary healthy men for the reactivity of skin microvessels to locally applied vasodilator stimuli. Skin microvascular blood flow was measured with a laser Doppler imager (LDI). The cutaneous vasodilation caused by heat stress or exercise in a warm environment diminishes with increasing age (Tankersley *et al*, 1991; Ho *et al*, 1997; Minson *et al*, 1998; Kenney, 2001). For this reason, we prospectively recruited subjects from two different age groups.

MATERIALS AND METHODS

This study was approved by our institutional ethics committee and carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant.

Subjects Seventy-eight healthy male volunteers were prospectively recruited into four study groups based on age and amount of regular physical activity: (1) younger, trained (n = 12); (2) younger, sedentary (n = 10); (3) older, trained (n = 31); and (4) older, sedentary (n = 25). Younger and older were defined as age ranges 18 to 35 y and $\geqslant$50 y, respectively. To be considered trained, subjects of appropriate age were to have been pursuing some kind of endurance training on a regular basis ($\geqslant$4 h per week) for at least the last 3 mo before enrollment. No restriction was placed on the particular type of endurance training (**Table I**), but professional sportsmen were excluded. Sedentary subjects had not practiced any sport regularly for the preceding year or longer.

All participants had a body mass index (BMI) less than 30 kg per m^2, and had an inconspicuous medical history with no remarkable findings on clinical examination. None had ever smoked. None had been taking any nonsteroidal anti-inflammatory drug for the last 2 wk and any other medication for the last 3 mo.

We recruited a larger number of older, compared with younger individuals, because we expected that differences related to physical training would be smaller in the higher age range.

Measurements

Microvascular reactivity in the skin microcirculation The details of the methods used in this study have been previously published (Kubli *et al*, 2000, 2001; Pellaton *et al*, 2002). In essence, we determined the responses of skin blood flow to the local administration of acetylcholine, an endothelium-dependent vasodilator, and sodium nitroprusside (SNP), a donor of nitric oxide (NO), which dilates blood vessels independently of the endothelium. In addition, we measured the reaction of skin blood flow following the release of arterial inflow occlusion (reactive hyperemia).

All measurements were carried out between 15.00 h and 18.00 h in a temperature-controlled room (24°C), with the subjects quietly resting supine on a hospital bed. Skin sites on the volar face of the forearm were examined.

Measurement of dermal blood flow We used a recently developed LDI (Moor Instruments, Axminster, UK). In this device, a moving mirror directs a beam of laser light (633 nm) on to the skin. Computer-controlled rotations of the mirror allow the scanning of a square region, the size of which may be adjusted by the operator. From the analysis of the backscattered Doppler-shifted light, microvascular blood flow in each of up to 256 × 256 adjacent spots ("pixels") is calculated. The final result is a computer-generated, color-coded image of the spatial distribution of microvascular blood flow. Total flow, expressed in perfusion units (PU) according to the principle of laser Doppler flowmetry, can be calculated later by averaging the pixel values in an arbitrarily shaped region of interest within the scanned area. In comparison with usual fiberoptic laser Doppler probes, the LDI allows the measurement of microvascular blood flow in a much larger area, with no skin contact. In this study, the total distance from the front end of the laser source to the skin was 41 cm.

Table I. Main aerobic activity of trained subjects

	Younger	Older
Triathlon	3	1
Biking	9	20
Long distance running	0	6
Rapid walking	0	2
Mountain hiking	0	2
Total	12	31

Iontophoresis of vasoactive agents Iontophoresis is a noninvasive method for transdermal transfer of charged molecules locally on the skin by means of an externally applied electrical current. Coupling this method with laser Doppler flowmetry, it is possible to record the response of dermal blood flow to the local application of acetylcholine and SNP (Morris and Shore, 1996; Khan *et al*, 1997; Noon *et al*, 1998). To carry out iontophoresis, we used custom-made ring-shaped chambers fitted with a copper electrode, which was connected to an iontophoresis controller (MIC1-e, Moor Instruments). The chambers were affixed with double-sided tape to the skin on the volar face of the forearm, filled to the rim with a solution of either 1% acetylcholine or 0.1% SNP, and covered with a transparent glass slip. The internal diameter of the chamber was 10 mm, so that the exposed skin area (i.e., the window for the LDI measurement of skin blood flow) was 0.78 cm^2. An indifferent ECG electrode was placed on the wrist. Polarity was adapted to the electric charge of the vasoactive molecule (chamber positive for acetylcholine and negative for SNP).

The 1% acetylcholine solution was prepared in distilled water immediately before measurement, from the dry hydrochloride powder (Sigma, CH-9471 Buchs, Switzerland) kept at −20°C. The 0.1% SNP solution was obtained immediately before measurement, from the 10-fold dilution of a 1% stock. The latter were prepared in advance by diluting the dry substance (Nipruss, Schwarz Pharma, D-40789 Monheim, Germany) in distilled water and storing aliquots in the dark at –80°C.

The pulsed iontophoretic protocols were as described in detail (Kubli *et al*, 2000), for a total charge of 22 mC (acetylcholine, maximal instantaneous current density 256 μA per cm^2) or 38 mC (SNP, maximal instantaneous current density 320 μA per cm^2) applied in a time lapse of 8 min. With these protocols, we found in healthy nonsmokers that the delivered doses of acetylcholine and SNP were largely supramaximal, i.e., they exceeded those required for a maximal effect by a factor of at least 5 (unpublished observations).

Electrical current alone may induce a vasodilation due to the stimulation of local sensory nerves, a response that can be inhibited by local anesthesia (Wardell *et al*, 1993; Morris and Shore, 1996). In this study, acetylcholine, and SNP were therefore applied on skin pretreated for 1 h with a local anesthetic cream (EMLA cream 5%, Astra Pharmaceutica AG, Dietikon, Switzerland), under an occlusive dressing (Tegaderm, 3M Health Care Ltd, UK). The effective inhibition of current-induced vasodilatation was systematically controlled by applying the same amount of current to an adjacent control chamber filled with isotonic saline.

Practically, the EMLA patch was left in place for exactly 1 h, then removed, and the skin was gently whipped with 70% alcohol followed by deionized water, and allowed to dry. Two chambers were stuck in place. One was then filled with the appropriate drug solution, and the other with the control isotonic saline. The thin glass lid was placed in position without bubbles. The laser beam was positioned in order to scan a 4.9 × 3.1 cm rectangular area, including both chambers in slightly less than 1 min. Sixteen such scans were taken at a frequency of 1 per minute, with the appropriate iontophoretic protocol started just before the beginning of the third scan and terminated slightly after the onset of the ninth one. Thus, the first two images could be used to determine baseline flow. The background setting of the Moor LDI system was chosen so as to ensure zero flow reading on the black neoprene that made up the chambers. Their rims were thus clearly seen on each LDI image as annular areas of zero flow. Of interest, the software used for analysis excluded the pixels with backgroup value from computation. Thus, the mean flow in the exposed skin could be precisely determined (approximately 1500 pixels per chamber).

Reactive hyperemia Reactive hyperemia was assessed with the LDI in the forearm skin, at a site not exposed to local anesthesia. The arterial occlusion was achieved by a pressure cuff placed on the arm and inflated at a suprasystolic pressure (220 mmHg) for 3 min. The LDI was set for repetitive scanning of a 1.16 × 0.16 cm skin surface, corresponding to 116 pixels. Two scans were used to assess the baseline, and two others for the determination of the biologic zero during arterial occlusion. Simultaneously with cuff

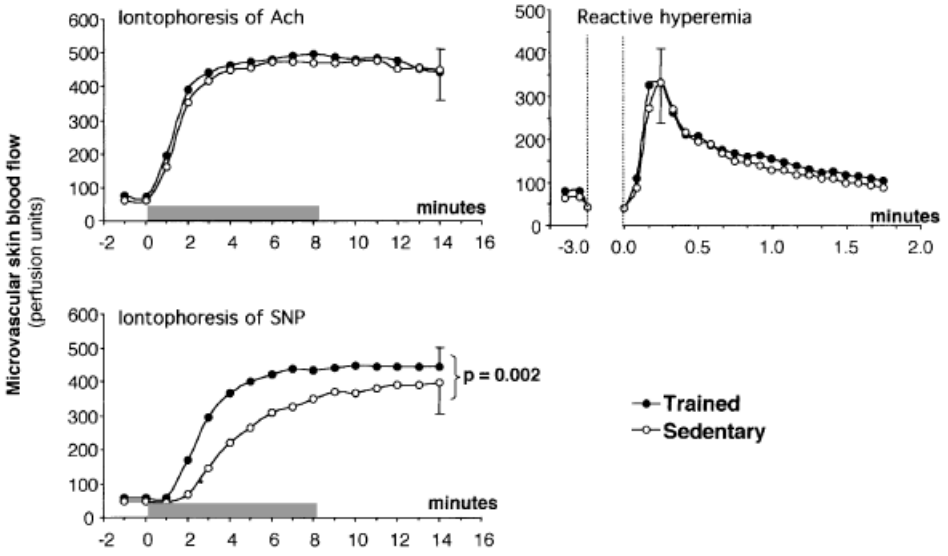

Figure 1. Younger subjects. Effects of training on the time-course of microvascular skin blood flow responses. Gray rectangles indicate application of iontophoretic current. Stippled lines delineate the 3 min arterial inflow occlusion used to trigger reactive hyperemia. Data are mean, with representative SD (error bars). Statistical comparisons of mean time courses with multivariate analysis of variance.

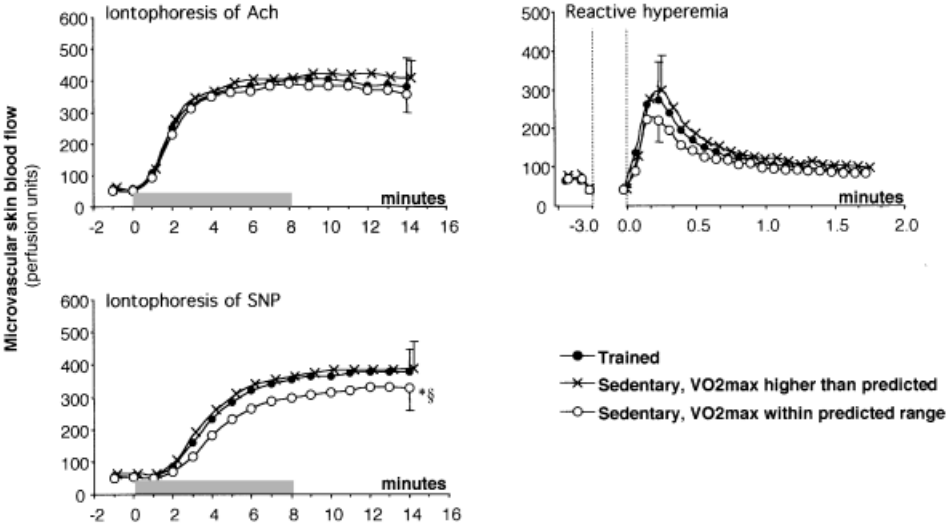

Figure 2. Older subjects. Effects of training and level of maximal oxygen uptake (VO_2max) on the time-course of microvascular skin blood flow responses. Gray rectangles indicate application of iontophoretic current. Stippled lines delineate the 3 min arterial inflow occlusion used to trigger reactive hyperemia. Data are mean, with representative SD (error bars). Statistical comparisons of mean time courses with multivariate analysis of variance. $^{*}p < 0.05$, either sedentary subgroup *versus* trained group $\S p < 0.05$ comparison between both sedentary subgroups.

deflation, repetitive scanning was launched at a frequency of one image each 5 s. This time interval was chosen so as to follow the rapid change in skin blood that followed.

Sequence for carrying out the tests of microvascular reactivity On arrival in the laboratory, the subjects reclined on an examination bed. A first EMLA patch was placed on the volar face of the forearm on the dominant side. Thirty minutes later, a second EMLA patch was placed on another area on the volar face of the same forearm.

After 1 h in place, the first patch was removed and the response to acetylcholine was recorded on this site, as described above. At the end of the test with acetylcholine, a few minutes were left to elapse, so as to remove the second patch exactly 1 h after it was put in place. The response to SNP was recorded on this second site.

Reactive hyperemia was recorded last on unanesthetized skin.

Exercise test The maximal oxygen uptake (VO_2max) was directly assessed with the CPX/MAX cardiopulmonary diagnostic system (MedGraphics, St Paul, Minnesota) during a graded exercise test on a bicycle ergometer. A constant rate of pedaling (60–80 per min) was maintained while increasing the resistance. The initial workload was 100 W in younger and 50 W in older subjects, with 30 W (younger) or 25 W increments (older) every 2 min. Some older sedentary subjects could not adapt properly to the bicycle ergometer and were tested instead on a treadmill using the Balke protocol at a speed of 5 km per hour. The exercise test was terminated when anyone of the following criteria was fulfilled: a plateau in the oxygen uptake with increasing work rate, a respiratory exchange ratio greater than 1.3, the attainment of the expected maximal heart rate, or the appearance of symptoms (exhaustion, severe shortness of breath, or marked pain in the legs). The electrocardiogram was continuously monitored in older subjects.

Study design and protocol The subjects were examined twice, at least 1 d and less than 1 mo apart, once for the exercise test and once for the assessment of skin microvascular reactivity. On this latter occasion, blood was drawn for the measurement of total and high-density lipoprotein (HDL) cholesterol. Blood pressure was determined with an oscillometric method and resting heart rate was assessed following 30 min of quiet recumbency.

Skin surface temperature was measured immediately before the testing of microvascular reactivity, using a surface thermistor probe (Yellow Springs Instruments Yellow Springs, OH) affixed close to the site used for iontophoresis.

Data analysis The time course of skin blood flow following each stimulus (see **Figs 1 and 2**) was summarized as both the maximal response and the area under curve (AUC). All these computations incorporated appropriate subtraction of the baseline blood flow recorded before application of the stimulus.

Unless stated otherwise, data were summarized as the mean $\pm$ SD, and univariate statistical analyses were carried out with two-factor ANOVA, with a model including age range, training level, and their interaction.

Table II. General characteristics of the four study groups

	Younger subjects (18–35 y)		Older subjects (⩾50 y)	
	Trained	Sedentary	Trained	Sedentary
No. of subjects	12	10	31	25
Age (y)	28.6 ± 6.3	26.0 ± 3.3	61.2 ± 9.0****	59.1 ± 7.4****
Height (cm)	178.5 ± 5.4	175.0 ± 4.4	174.1 ± 7.3	175.6 ± 6.8
Weight (kg)	71.3 ± 7.5	68.4 ± 5.5	70.4 ± 8.1**	79.5 ± 9.2****
BMI (kg per m^2)	22.3 ± 1.9	22.3 ± 2.2	23.2 ± 2.0**	25.7 ± 2.7****
Total cholesterol (mM)	4.5 ± 0.7	3.9 ± 0.4	5.4 ± 0.8****	5.6 ± 0.7****
HDL-C (mM)	1.6 ± 0.3**	1.3 ± 0.4	1.6 ± 0.4**	1.4 ± 0.3
Total chol./HDL-C	3.0 ± 0.7*	3.3 ± 1.0	3.5 ± 0.9****,*	4.1 ± 1.0****
Systolic BP (mmHg)	118.8 ± 9.2	119.3 ± 12.1	124.1 ± 12.4	127.1 ± 14.4
Diastolic BP (mmHg)	61.5 ± 7.5	59.4 ± 8.4	72.4 ± 9.7****	73.8 ± 10.1****
Resting heart rate (beats per min)	57.1 ± 9.9**	63.6 ± 9.4	58.5 ± 10.1**	69.8 ± 11.2
VO_2max (mL O_2 per min per kg)	56.9 ± 7.1**	40.3 ± 5.8	42.9 ± 7.1****,**	31.6 ± 5.9

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; BP, blood pressure. Data are mean ± SD.
*$p < 0.05$, **$p < 0.01$, effect of training at comparable age, ****$p < 0.01$ effect of age at comparable training.

When the overall F-value was significant, further comparisons between groups were carried out as follows. When the p-value associated with the interaction term was greater than 0.1, the simple main effects of training and age were used for evaluation. Otherwise, the effect of one factor was assessed separately at each level of the other factor. A similar two-way layout was employed in a multivariate ANOVA applied to the time courses of microvascular blood flow responses. With the aforementioned exception concerning the interaction term, the α value for all tests was 0.05.

All calculations were carried out with the JMP software (version 3.2.2, SAS Institute, Cary, North Carolina).

RESULTS

General characteristics of study groups The demographic variables of the four study groups are shown in **Table II**, along with lipid profile, blood pressure, resting heart rate, and VO_2max. All the younger subjects were lean (BMI < 25 kg per m^2), normotensive (blood pressure < 140/85 mmHg), and normocholesterolemic (i.e., total cholesterol < 5.2 mM or 200 mg per dL; HDL cholesterol ⩾1 mM). In contrast, the majority of older sedentary volunteers were overweight, although not obese (BMI 25–30 kg per m^2), such that body weight and BMI were significantly higher in this group, compared with all other three. Furthermore, marginal (total cholesterol > 5.2 but < 6.2 mM) or clear hypercholesterolemia (6.2–7.2 mM) was present in 45% and 23% of older subjects, respectively. Finally, diastolic blood pressure was significantly higher in older than younger study participants, with an upper decile ranging from 85 to 95 mmHg.

Within each age range, trained and sedentary subjects were comparable for age, height, total plasma cholesterol, and blood pressure. According to expectation (Williams *et al*, 1982; McArdle *et al*, 1991), HDL cholesterol was slightly higher and the ratio of HDL to total cholesterol slightly lower in the trained, compared with the sedentary subjects of any age.

Per protocol, trained and sedentary subjects were distinguished on the sole basis of history. This method of enrollment generated groups which highly differed with respect to aerobic fitness, as shown by the lower resting heart rate and markedly higher VO_2max in the trained *versus* the sedentary study participants of comparable age. Indeed, VO_2max was on the average 41.2% higher in the younger trained subjects than in their sedentary contemporaries, the corresponding figure for the older study groups being 35.6% ($p < 0.0001$, no significant interaction of age with training status). Whatever the training status, VO_2max declined with age.

Table III. Two subcategories of older sedentary subjects: general characteristics

	VO_2max > predicted	VO_2max within predicted range
No. of subjects	9	16
Age (y)	59.7 ± 8.0	58.8 ± 7.3
Height (cm)	174.3 ± 5.2	176.3 ± 7.7
Weight (kg)	72.2 ± 4.3****	83.8 ± 8.7**
BMI (kg per m^2)	23.6 ± 2.1****	27.0 ± 2.2**
Total cholesterol (mM)	5.3 ± 0.6	5.8 ± 0.7
HDL-C (mM)	1.4 ± 0.3	1.5 ± 0.3
Total chol./HDL-C	3.9 ± 0.9	4.2 ± 1.1
Systolic BP (mmHg)	124.1 ± 15.0	128.8 ± 14.3
Diastolic BP (mmHg)	69.9 ± 11.3	76.0 ± 8.9
Resting heart rate (beats per min)	64.9 ± 9.5	72.6 ± 11.3**
VO_2max (mL O_2 per min per kg)	37.3 ± 5.1*, ****	28.4 ± 3.4**

Older sedentary subjects were subcategorized according to whether their maximal oxygen uptake (VO_2max) was above or within the range expected for healthy men of the same age (see text). BMI, body mass index, HDL-C, high-density lipoprotein cholesterol; BP, blood pressure. Data are mean ± SD. The two subcategories were compared with one another and with the older trained group (i.e., column 3 in **Table II**) by means of a simple ANOVA.
*$p < 0.05$, **$p < 0.01$, either subcategory *versus* older trained group, ****$p < 0.01$ comparison between both subcategories.

The VO_2max of each study participant was categorized as within or above the range predicted for a sedentary male of that age, based on tables from Jackson and Ross (1986; Murray, 1990). The number of individuals with a VO_2max above this predicted range was as follows: younger trained: 12 of 12, younger sedentary, two of 10, older trained: 30 of 31, older sedentary nine of 25 ($p < 0.001$, χ^2-test with continuity correction). These counts suggest an heterogeneity of aerobic fitness in the sedentary groups, most notably in the older age range. Therefore, we carried out a *post hoc* analysis of data from older volunteers, splitting those identified as sedentary into two subcategories as shown in **Table III**. It may be seen that the 16 subjects with a VO_2max within the predicted range entirely accounted for the differences in body weight, BMI, and resting heart rate noted between the third and last column of **Table II**. In contrast, the nine volunteers with a VO_2max higher than predicted were essentially comparable with the older trained individuals in terms of these variables.

Table IV. Responses of skin blood flow in the four groups of subjects

	Younger subjects (18–35 y)		Older subjects (≥50 y)	
	Trained	Sedentary	Trained	Sedentary
No. of subjects	12	10	31	25
Skin temperature (°C)	32.9 ± 1.0	32.7 ± 1.1	32.4 ± 1.0	32.7 ± 1.0
Iontophoresis of vasoactive agents				
Baseline blood flow (PU)	66.5 ± 17.3	54.0 ± 7.2	55.9 ± 11.2	57.5 ± 12.2
Response to acetylcholine				
Maximal (PU)	450.2 ± 65.8	439.9 ± 92.9	363.3 ± 90.3****	358.9 ± 67.4****
AUC (PU*min)	5328 ± 715	5276 ± 1108	4168 ± 1062****	4105 ± 741
Response to SNP				
Maximal (PU)	397.6 ± 54.2*	349.7 ± 87.2	338.6 ± 72.1****,*	306.9 ± 66.4****
AUC (PU times min)	4311 ± 737**	3261 ± 954	3295 ± 783****	2947 ± 836
Current control. acetylcholine				
Maximal (PU)	15.2 (9–33)	27.7 (13–45)	7.8 (2–22)	8.7 (3–19)
AUC (PU times min)	37.4 (−26–137)	130.7 (36 to 175)	17.5 (−70–103)	67.5 (−15–153)
Current control. SNP				
Maximal (PU)	57.8 (5–320)	24.2 (−2–241)	10.3 (1–63)	41.6 (10–200)
AUC (PU times min)	309.5 (−21–2789)	51.9 (−116–1814)	30.1 (−56–255)	227.2 (30–1055)
Reactive hyperemia				
Baseline blood flow (PU)	80.2 ± 15.3	65.8 ± 8.3	67.4 ± 14.1	73.1 ± 19.8
Maximal (PU)	272.8 ± 79.1	267.7 ± 87.5	221.4 ± 92.3****	189.9 ± 76.6****
AUC (PU times min)	156.9 ± 42.2	156.5 ± 67.7	122.6 ± 62.7****	99.9 ± 40.1****

All responses are quantified both as the maximal increase above baseline and as the area under curve (AUC). SNP, sodium nitroprusside, PU, perfusion units. Data are mean ± SD, with the exception of the current controls which, because of a highly skewed distribution, are expressed as the median, with the interquartile range in parentheses.

*$p < 0.05$, **$p < 0.01$, effect of training at comparable age, ****$p < 0.01$ effect of age at comparable training status.

Skin microvascular reactivity Results from the primary analysis of skin blood flow responses in the four groups of subjects are shown in **Table IV**. The conditions of skin temperature under which microvascular reactivity was examined were comparable between groups. Whether measured on skin pretreated with EMLA cream for subsequent iontophoresis or on the untreated skin (reactive hyperemia), the baseline blood flow was slightly higher in the younger trained volunteers compared with all other groups, but this difference did not reach statistical significance.

The responses of skin blood flow to the application of the iontophoretic current alone were not completely suppressed by surface anesthesia in some subjects, but were always markedly lower than their counterpart observed in presence of the active substance (current controls, **Table IV**, presented as median and interquartile range due to a highly asymmetric distribution). None of the current control responses varied significantly among groups (Kruskall–Wallis test).

Irrespective of training status, the maximal responses were clearly lower in the older than in the younger age groups, by roughly 20% for the iontophoresis of acetylcholine, 15% for the iontophoresis of SNP, and 25% for reactive hyperemia. The same was true when the responses were quantified as the AUC, except concerning the iontophoresis of SNP. In this latter case, the decline of AUC with age reached statistical significance in trained subjects only (interaction of age with training status $p = 0.09$, effect of age in trained subjects $p < 0.001$, effect of age in sedentary subjects $p = 0.30$).

The comparative time courses of the various skin blood flow responses in the trained and sedentary younger subjects are shown in **Fig 1**. Reactive hyperemia and the response to iontophoresis of acetylcholine were not affected by training. In contrast, the increase in skin blood flow elicited by the iontophoresis of SNP was both brisker and of higher amplitude in the trained, compared with the sedentary group. This effect of training was statistically significant, whether considering the maximal response (14% higher in younger trained *vs* younger sedentary subjects, $p = 0.028$, **Table IV**), the AUC (32%, $p = 0.004$, **Table IV**), or the detailed time course (multivariate ANOVA $p = 0.002$, **Fig 1**).

In the older, as in the younger, groups there was no effect of training on the response to acetylcholine or on reactive hyperemia (**Table IV**). The maximal response to SNP was 10% higher in older trained, compared with older sedentary subjects ($p = 0.028$), but the corresponding difference in AUC did not reach significance ($p = 0.12$) (**Table IV**). With multivariate ANOVA, the effect of training on the time course of the response to SNP was not statistically detectable ($p = 0.18$).

***Post hoc* analysis** The skin microvascular reactivity data of all older volunteers were reanalyzed, so as to take into account the aforementioned heterogeneity of aerobic fitness among the older sedentary participants. As shown in **Table V** and **Fig 2**, this new analysis had no impact on conclusions regarding reactive hyperemia and response to acetylcholine. In contrast, older sedentary volunteers whose VO_2max was within the predicted range had a blunted response to SNP, when compared with either their trained contemporaries (maximal response $p = 0.019$, AUC $p = 0.016$, multiple ANOVA $p = 0.020$) or with the older sedentary subjects of better aerobic fitness (maximal response $p = 0.069$, AUC $p = 0.036$, multivariate ANOVA $p = 0.019$). Among the latter, the response to SNP was the same as in the older trained participants (maximal response $p = 0.93$, AUC $p = 0.73$, multivariate ANOVA $p = 0.47$).

DISCUSSION

In this study, we hypothesized that endurance training could be associated with an enhanced responsiveness of the skin microcirculation to local vasodilatory stimuli in healthy humans. This hypothesis was tested in two different age groups of male nonsmoking volunteers in apparent health. We made the highly novel finding that endurance training is associated with a selective enhancement of the vasodilation induced in the skin by the local administration of a NO donor.

Table V. Two subcategories of older, sedentary subjects: responses of skin blood flow

	VO_2max > predicted	VO_2max within predicted range
No. of subjects	9	16
Skin temperature (°C)	33.1 ± 0.8	32.5 ± 1.0
Iontophoresis of vasoactive agents		
Baseline blood flow (PU)	64.4 ± 12.5	53.6 ± 10.4
Response to acetylcholine		
Maximal (PU)	371.7 ± 41.5	351.6 ± 78.8
AUC (PU times min)	4317 ± 489	3986 ± 842
Response to SNP		
Maximal (PU)	340.6 ± 66.7	287.9 ± 60.2*
AUC (PU times min)	3395 ± 880***	2695 ± 719*
Current control. acetylcholine		
Maximal (PU)	7.5 (2–41)	1.5 (3–17)
AUC (PU times min)	68 (−53–358)	65 (−18–132)
Current control. SNP		
Maximal (PU)	59.7 (20–268)	21.2 (5–200)
AUC (PU times min)	255 (61–2046)	199 (−3–656)
Reactive hyperemia		
Baseline blood flow (PU)	79.7 ± 17.4	69.5 ± 20.6
Maximal (PU)	226.4 ± 83.4	169.4 ± 66.6
AUC (PU times min)	123.8 ± 37.6	86.5 ± 35.9

Older sedentary subjects were subcategorized according to whether their maximal oxygen uptake (VO_2max) was above or within the range expected for healthy men of the same age (see text). All responses are quantified both as the maximal increase above baseline and as the area under curve (AUC). PU, perfusion units. Data are mean ± SD, with the exception of the current controls which, because of a highly skewed distribution, are expressed as the median, with the interquartile range in parentheses. The two subcategories were compared with one another and with the older trained group (i.e., column 3 in **Table IV**) by means of a simple ANOVA. The ANOVA was parametric, except for current control responses for which a Kruskall–Wallis test was used.

*$p < 0.05$, **$p < 0.01$, either subcategory *versus* older trained group

***$p < 0.05$, ****$p < 0.01$ comparison between both subcategories.

Effects of age on skin microvascular reactivity All vasodilatory responses examined in this study were lower in the older than in the younger age groups (**Table IV**). Although very little information is available in the literature concerning the effect of age on the specific responses measured here, this demographic variable has a clear negative correlation with the maximal cutaneous vasodilation elicited by local warming, ascribed to the structural modifications of the microcirculation known to occur with advancing age (Rooke *et al*, 1994; Martin *et al*, 1995; Kenney *et al*, 1997). On histologic examination, the skin of aged subjects appears thinner and less vascular than the skin of younger persons (Kligman, 1979; Montagna and Carlisle, 1979). More recently, an *in vivo* study using capillaroscopy has shown an impressively lower density of papillary capillary loops in older, compared with younger humans (Kelly *et al*, 1995). These morphologic alterations were observed at two different sites, including the volar face of the forearm tested for microvascular reactivity in this study. In addition to the structure of dermal microcirculation, the two age groups might also have differed with respect to hemorheology. The older subjects had a higher BMI, diastolic blood pressure, and total cholesterol (**Table I**). These variables and age itself have all been found independently and positively associated with whole blood viscosity (de Simone *et al*, 1990). Finally, the dermal microcirculations of older and younger subjects might also have differed functionally. In that respect, it is presumed on the basis of observations in other vascular beds that reactive hyperemia is partly endothelium dependent and acetylcholine-induced vasodilation is largely endothelium dependent (Hirooka *et al*, 1994; Engelke *et al*, 1996; Meredith *et al*, 1996; Dakak *et al*, 1998), although the response to SNP is not. Thus the blunting of the former two reactions in older volunteers might in part reflect an age-related decline in vascular endothelial function, consistent with observations made in other vascular beds (Zeiher *et al*, 1993; Corretti *et al*, 1995).

Effects of training on skin microvascular reactivity Among the three measured microvascular skin responses, only the vasodilation induced by the iontophoresis of SNP was dependent on training status, being enhanced in trained compared with sedentary volunteers. This pattern was especially evident in the younger subjects (**Table IV**, **Fig 1**). Findings in their elders were complicated by the likely heterogeneity of the sedentary aged group (**Table III**). The *post hoc* subdivision of the older sedentary volunteers was based on individual values of VO_2max, and might have been influenced by errors made on this measurement; however, a validity cross-check was provided by the independent assessment of resting heart rate, which, as expected, was highest in the subcategory with the lowest VO_2max (**Table III**). The simplest, although undocumented explanation for the heterogeneous aerobic fitness of the older sedentary group is variability in the amount of physical activity in daily life. Akin to observations made in the younger age groups, the subcategory of older sedentary subjects with the lowest VO_2max featured a selective blunting of the response to SNP, whether compared with the older trained group or to the better fit older sedentary volunteers (**Fig 2**, **Table V**). Thus, there appears to be an age-independent link of aerobic fitness with the reactivity of skin microvessels to transdermal local administration of SNP.

To the best of our knowledge, there are two other studies concerning the impact of endurance training on skin microvascular reactivity. Kvernmo *et al* (1998) characterized the responses of skin blood flow to the iontophoresis of acetylcholine and SNP in young male adults who were either highly trained long distance runners (competing in national events for more than 5 y, range of VO_2max 62–73 mL per min per kg, n = 9) or less well trained controls (soldiers exercising 1–7 h per wk, VO_2max 44.4–61.4 mL per min per kg, n = 9). Wang *et al* (2002) made a similar comparison between elderly volunteers who either practiced Tai Chi Chuan, a particular form of aerobic training (VO_2max 23 ± 0.7 mL per min per kg, mean ± SEM, n = 10) or were sedentary (VO_2max 17 ± 0.9 mL per min per kg, n = 10). In apparent contradiction with our present data, both studies found that the responses to acetylcholine but not SNP were highest in the most aerobically fit subjects. In addition to marked differences in the investigated populations, several technical considerations could contribute to this discrepancy. In each of the two cited reports, dermal blood flow was measured with conventional laser Doppler flowmetry, i.e., by means of an optical fiber with the tip affixed to the skin, whereas we used the LDI, which captures blood flow over a larger surface area while avoiding any direct contact of the skin with the probe. In comparison with the former, the latter method has been shown to reduce the variability of the response to acetylcholine iontophoresis (Morris and Shore, 1996). In that context, it is notable that we enrolled more than twice the number of subjects totaled by previous studies (Kvernmo *et al*, 1998; Wang *et al*, 2002); furthermore, our iontophoretic protocols markedly differed from those employed by these authors. As explained in the *Materials and Methods*, we used surface anesthesia in order to inhibit current-induced hyperemia (Morris and Shore, 1996; Kubli *et al*, 2000) and only assessed the effects of a supramaximal dose of each agent, administered relatively quickly (8 min). In the cited studies by contrast, three (Wang *et al*, 2002) or four (Kvernmo *et al*, 1998) sequentially increasing doses were applied, with a 5 min interval between each, on unanesthetized skin. The dose–response curves thus constructed did not reach a plateau. It might therefore be that any effect of aerobic training on acetylcholine responsiveness in the skin microcirculation would only be detectable with submaximal doses of this agent, and thus would have been missed using our conditions. Indeed, in Kvernmo *et al*'s study, although not in

Wang *et al*'s, the difference between athletes and controls in acetylcholine responsiveness was only observed with the lowest dose.

Contrary to our data (**Table IV**) and due to the lack of surface anesthesia, the responses to SNP recorded by Kvernmo *et al* (1998) and Wang *et al* (2002) must have been heavily contaminated by current-induced hyperemia. This phenomenon, due to the stimulation of local sensory nerves (Wardell *et al*, 1993), is particularly intense with the cathodic polarity required for the iontophoresis of the nitroprusside anion (Morris and Shore, 1996). In the precise protocol used by Kvernmo *et al* it amounts to approximately 70% of the response to the highest dose of SNP (Andreassen *et al*, 1998). Even in our conditions, we could not avoid some modest current-induced hyperemia with the cathodic polarity (**Table IV**), but this occurred equally in all four groups and thus cannot account for the higher responses to SNP of trained compared with sedentary subjects.

The effects of endurance training on vascular reactivity have been well characterized in conduit arteries and in the muscular skeletal vascular bed. An impressive amount of concordant evidence, both in animals (Koller *et al*, 1995; Mombouli *et al*, 1996; Sakamoto *et al*, 1998; Griffin *et al*, 1999), and in humans (Green *et al*, 1994; Kingwell *et al*, 1996; Kingwell *et al*, 1997; Bank *et al*, 1998; Clarkson *et al*, 1999; Higashi *et al*, 1999), indicates that at these locations, training essentially enhances endothelium-dependent vasodilation, as induced by acetylcholine, but has no impact on the response of vascular smooth muscle to donors of NO. Therefore, the findings of this study are quite surprising and need critical scrutiny. Laser Doppler flowmetry is sensitive, not to blood flow *per se*, but to erythrocyte flux. As such, its results are dependent in part on particular rheologic conditions (e.g., local hematocrit) in the explored dermal microvascular network. These might not necessarily be comparable between trained and sedentary subjects. Such rheologic differences, however, should equally affect all measurements made with the same laser Doppler apparatus in the same subject, and thus cannot account for a selective variation of the response to only one of several applied stimuli. We might also construe that training favored either the transdermal penetration of SNP or the intradermal generation of NO from the nitroprusside anion. These possibilities are hard to exclude entirely, but appear very unlikely considering the fact that the delivered dose of SNP was largely supramaximal (see *Materials and Methods*). In view of these considerations, our observations imply that aerobic training enhances either the bioavailability of NO or the sensitivity of vascular smooth muscle to the relaxant effect of this molecule in the dermal microcirculation. At present, these two alternatives cannot be resolved. The former one would be consistent with the known stimulation of anti-oxidant defenses by physical training (Ji *et al*, 1998; Brites *et al*, 1999; Fukai *et al*, 2000), although the skin remains unexplored in this respect.

If aerobic fitness promotes the bioactivity of NO but leaves invariant the vasodilatory response to acetylcholine (**Figs 1 and 2**), is that not a contradiction in terms? That would definitely be the case if in the considered vessels endothelial release of NO was a proven major mediator of the vasorelaxation induced by acetylcholine, as is well known for conduit arteries or the vascular bed of skeletal muscle (Linder *et al*, 1990; Taddei *et al*, 2000; Higashi *et al*, 2001); however, the dermal microcirculation behaves differently in that respect. Although the intradermal administration of acetylcholine by microdialysis may locally increase NO production, this effect has only been documented with unusually high acetylcholine concentrations (1 M) in the perfusate (Crandall and MacLean, 2001). In two concordant studies, the dermal vasodilation caused by the iontophoresis of acetylcholine in the forearm was not influenced by the concomitant administration of N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of NO production, although it was partially blocked by a cyclooxygenase inhibitor (Khan *et al*, 1997; Noon *et al*, 1998).

In conclusion, we provide evidence that endurance training enhances the vasodilatory effects of NO in the human dermal microcirculation, at least in forearm skin. This impact of training occurs at any age. These observations have considerable physiologic interest in view of recent data indicating that NO mediates in part the cutaneous vasodilation induced by heat stress in humans (Kellogg *et al*, 1998; Shastry *et al*, 1998). Therefore, the augmentation of NO bioactivity in the dermal microcirculation might be one mechanism whereby endurance training modifies the thermoregulatory control of skin blood flow (Tankersley *et al*, 1991; Ho *et al*, 1997).

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