

Effects of an Acute Exercise on Endothelium Function in Athletics Young Subjects: A Case Control Study

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Abstract

Aim: to investigate vascular reactivity on forearm skin blood flow (FSBF) in response to iontophoresis of acetylcholine (ACh) after an acute exercise.

Materials and method: 49 healthy male subjects 29 trained and 20 sedentary (recruited according to their oxygen consumption) participated in this case control study (mean age: 15 ± 1 year). Incremental exercise testing was performed by means of an individualized and personalized exercise test protocol. FSBF was measured before and after exercise using a laser Doppler flowmeter in response to local iontophoresis of a cumulative dose of ACh.

Results: Although groups were not different in age, height or body mass index, weight, resting heart rate, maximum oxygen consumption was significantly different (sedentary 40.05 ± 4.53 and trained 51.65 ± 5.76 ml.min⁻¹.kg⁻¹) (p<0.05; p<0.001; p<0.001, respectively). The mean response of FSBF to ACh was significantly increased after incremental exercise in the two groups (349%). Maximal FSBF response to ACh was significantly greater in trained (987% ± 78) than in sedentary (638% ± 42) (p=0.001) after a single acute exercise.

Conclusion: Response of FSBF to ACh-induced endothelium dependent relaxation was affected by the level of training whether before or after an acute exercise.

Keywords: Exercise; Endothelial dysfunction; Forearm skin blood flow; Laser doppler flowmetry; Iontophoresis

Abbreviations: FSBF: Forearm Skin Blood Flow; Ach: Acetylcholine; CVD: Cardiovascular Disease; ED: Endothelial Dysfunction; EDR: Endothelium Dependent Relaxation; NO: Nitric Oxide; LDF: Laser Doppler Flowmetry; EF: Endothelia Function; SD: Standard Deviation; SKBF: Stimulated Skin Blood Flow; BMI: Body Mass Index; VO₂: Oxygen Consumption; VO₂max: Maximum Oxygen Consumption; VCO₂: Carbon Dioxide Output; VCO₂/VO₂: Respiratory Exchange Ratio; VE: Global Ventilation; HR: Heart Rate; Spo2: Oxygen Saturation; Enos: Endothelial Nitric Oxide Synthase; T: Trained Group; S: Sedentary Group

Introduction

The prevalence of cardiovascular disease (CVD) has markedly increased in many countries (i.e., in Tunisia its prevalence was 28.9%) and it's considered as a leading cause of death [1] with a rate ranging between 27 to 30% every year [2]. CVD in adults are laid in childhood and accelerated by the presence of comorbid conditions, such as obesity, diabetes, hypertension and dyslipidemia [3]. Furthermore, young Tunisian people are exposed earlier to CVD risk factors [4]. One of the CVD risk biomarker in healthy subjects is endothelial dysfunction (ED). In fact, the importance of the endothelium in maintaining normal vascular function is well recognized [5]. An ED alters the endothelium dependent relaxation (EDR) through the reduction of nitric oxide (NO). Besides, the loss of the anti-atherogenic and antithrombotic properties of endothelium may have a multiplicity of causes [6]. Exercise training induces adaptation of the cardiovascular system, resulting in improvement of oxygen intake by both cardiac and skeletal muscles [7]. The impact of exercise training on the endothelium is dependent on different factors, namely the duration, frequency, intensity or volume [8,9]. While aerobic exercise (high intensity and long duration) is associated with a transient reduction of endothelium-dependent vasodilation in peripheral arteries [10-12], moderate aerobic

exercise appears to enhance its function. The durations and frequencies conditions of this type of exercise are noticeably effective [13-15]. Other studies have shown also that exercise preserves endothelial function NO-dependent [16-18]. Training realized with variable intensities of exercise allows observing that moderate intensities potentiate the EDR whereas raised intensities in animals and humans decrease the EDR with the increase of oxidant stress [8,12]. In addition, Kingwell et al. (1997) [19] indicate that exercise may prevent ED and improve its function in normal subjects. In studies including human, noninvasive methods are usually favored because invasive studies were limited to a very small number of patients and were subject to ethical problems. However, there is a noninvasive measurement method of microcirculatory blood flow used to assess the endothelial function. This method consists of inducing changes in regional blood flow by Laser Doppler Flowmetry (LDF) in responses to local infusion of vasoactive substances like ACh [20,21]. This method was previously used to assess whether the endothelial function is affected by type and intensity of exercise in healthy and pathologic subjects. However, it is not clear how exercise acutely affects endothelial function (EF). Many studies resulting from varied exercise protocols [8] present conflicting results and ambiguity in data analysis after exercise. To determine

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whether exercise is associated with a beneficial effect on endothelial NO release, we investigated the endothelium-dependent and -independent vasodilation by measuring noninvasively the change in forearm skin blood flow (FSBF) in response to graded infusion of ACh, using combined LDF and heating probe, in two groups of healthy males trained and sedentary.

The purpose of the study was to investigate vascular reactivity on FSBF in response to ACh iontophoresis before and after an acute exercise among trained and sedentary young people. The null hypothesis is that there is no difference between mean values of their FSBF data.

Method

Type of study

This was a case-control study spread over one year (from February 2010 to January 2011). It was conducted in the Department of Physiology and Functional Exploration at Farhat Hached Hospital in Sousse, Tunisia. The study was conducted in accordance with the Declaration of Helsinki, participants provided written consent and the study protocol were approved by the ethics committee of the Hospital.

Sample size

The null hypothesis [22] was $H_0: m_1=m_2$ and the alternative hypothesis was $H_a: m_1=m_2+d$, where d is the difference between two means and n_1 and n_2 are the sample sizes for the trained (T) and sedentary (S) groups, such $N=n_1+n_2$. The total sample size was estimated using the following formula [22] is $N = \left[(r+1)(z_{\alpha} + z_{1-\beta})^2 \sigma^2 \right] / rd^2 \cdot z_{\alpha}$ the normal deviate at a level of significance=1.64 (5% level of significance), $Z_{1-\beta}$ is the normal deviate at $1-\beta\%$ power with $\beta\%$ of type II error (0.84 at 80% statistical power); "r" equal to n_1/n_2 is the ratio of sample size required for two groups ($r=0.67$) gives the sample size distribution as 1:1.5 for two groups. σ and d are the pooled standard-deviation (SD) and difference of FSBF means of two groups. These two values were obtained from a previous study based on a similar hypothesis [23]. These researchers found that the mean baseline was stimulated with an increased temperature of the skin (the skin blood flow SKBF). The total sample size for the study was 47 males (30 T and 18 S).

Subjects general characteristics

49 healthy male subjects, among whom 29 are trained and 20 are sedentary (recruited according to their oxygen consumption), participated in this investigation (mean age: 15 ± 1 year). All subjects were in the same stage of maturation, as determined from questionnaire, which corresponded to Tanner Stage 4 [24]. Body mass was measured to the nearest 0.1 kg with the subjects in light clothing without shoes. Height was determined to the nearest 0.5 cm with a measuring tape fixed to the wall. The body mass index (BMI) was calculated as the ratio of mass (kg) to height² (m²). All the athletes participated in regional or national competitions, and were regularly trained. Selection was based firstly on the regular training (hour.week⁻¹) and secondly on the value of maximum oxygen consumption (VO₂max: beyond 45 ml.min⁻¹.kg⁻¹ subjects is considered trained). A complete medical examination was performed, including measurements of resting arterial blood pressure. History of familial or individual CVD and treatments were collected for each participant. Subjects with a history of CVD, hypercholesterolemia, liver disease, renal disease, or a smoking habit were excluded.

Maximal test

In order to determine VO₂max and so select groups, a maximal

aerobic exercise test was performed on a separate day (8 days before doing experimentation (D0)). Incremental exercise testing was performed on an electronically braked cycle ergometer (Ergoline, Bitz, Germany) based on an individualized and personalized exercise test protocol (American Thoracic Society/American College of Chest Physicians, 2003). Before each experiment, volume and gas analyzers were calibrated. During the test, VO₂ per kilogram of body mass, carbon dioxide (VCO₂) output, O₂ pulse, respiratory exchange ratio (VCO₂/VO₂), global ventilation (VE) and its components tidal volume and breathing frequency were recorded continuously using a breath-by-breath automated exercise metabolic system (ZAN 600 Ergotest, ZAN Mebgeraˆte GmbH, Germany). The ventilatory equivalents for O₂ and CO₂ were then calculated. Heart rate (HR) was monitored continuously using a 12-lead electrocardiogram (ZAN ECG, 800). The anaerobic threshold was determined by the ventilatory equivalents method (American Thoracic Society/American College of Chest Physicians, 2003). Oxygen saturation (SpO₂) was recorded by pulse oximetry (Model 9847, Nonin Medical, Inc., Minneapolis, MN). Mean drop in SpO₂ was computed as the individual drop in SpO₂ from baseline. Achievement of the exercise test was accepted when subjects fulfilled at least three of the following criteria: a plateau in VO₂ despite an increase in exercise intensity, a maximal HR above 90% of the predicted maximal theoretical HR ($220 - \text{age in year}$) [25], a respiratory exchange ratio greater than 1.1, a blood lactate concentration higher than 8.0 mmol.L⁻¹ and the apparent exhaustion of the subject. The adolescents were thoroughly familiarized with all testing equipment and procedures. Subjects were asked to rest the day before all testing and be well hydrated.

Laser doppler flowmetry combined with iontophoresis of ACh

Endothelium-dependent and independent vasodilatation of the forearm skin microcirculation was evaluated by iontophoresis and skin heating in combination with LDF [26] before and after a maximal test. A laser beam penetrates the skin and a fraction of the light is backscattered by moving blood cells and undergoes a frequency shift according to the Doppler principle, generating a signal proportional to tissue perfusion. Forearm skin blood perfusion was measured by means of a LDF apparatus (Periflux PF5001, Perimed, Stockholm, Sweden). The skin temperature was monitored throughout and maintained at 32°C by the same LDF heating probe. The basal perfusion index was measured during the first five minutes at rest, without infusion of ACh and without heating the skin (skin temperature=32°C). Baseline skin blood perfusion was defined as the mean value recorded during a four minutes time period. In order to investigate the endothelium-dependent vasodilatation, iontophoresis of graduated doses of ACh, was undertaken. Iontophoresis is a noninvasive standard method of drug application that allows the local transfer of electrically charged substances across the skin by using a small electric current. The electrical potential difference actively causes ions in the solution to migrate according to their electrical charge. ACh (diluted at 2% solution) was used to fill the chamber of the electrode. We used a delivery current of 10 mA and administered three successive doses of acetylcholine for ten seconds with an interval of two minutes between each dose in order to achieve a plateau of the response following each delivery of ACh. Lastly, the laser probe was heated to 44°C for five minutes and we recorded the maximal response to local skin heating, i.e., the endothelium-independent maximal vasodilatation. In order to eliminate baseline variability, the maximum skin perfusion value following iontophoresis was expressed as maximum percent change from the baseline [26].

Perfusion index after the third dose of ACh iontophoresis was the maximal endothelial response (arbitrary unit). Maximum perfusion index after heat hyperemia and without ACh infusion (skin temperature=44°C, arbitrary unit) [27]. Results are expressed as ACh-induced percentage changes in perfusion index *versus* basal values.

Drug

The drug used is the ACh chloride (Sigma-Aldrich, Switzerland) and was obtained from commercially available source. It was dissolved in deionized sterile water to 2% solution before starting the experimental protocol. It was kept on ice at -4°C.

Statistical analysis

Variables distributions were normal and results are presented as mean ± SD. Statistical analysis was performed using Student's *t* test for paired and unpaired data. Comparisons of variables were carried out by the software *SigmaStats 3.1* statistical package for windows. Comparisons between baseline and cumulative evaluation were made with a paired student's *t*-test. Differences were considered statistically significant when *P*<0.05.

Comparisons of variable were carried out by the software *SigmaStats 3.1* statistical package for windows.

Results

Subject characteristics

Table 1 summarizes the analysis of the baseline values of physical and clinical characteristics. Age, height, BMI, systolic and diastolic pressure were similar in the two groups. As expected, S group showed significantly higher values for body mass than T group (*p*<0.05). The resting HR values and the VO₂ peak was significantly higher in T group than S group (*p*<0.001).

Effects of iontophoresis delivery of ACh on FSBF

The iontophoresis delivery of ACh, increased FSBF in a dose-dependent manner was observed in the two groups. No difference in the FSBF response to iontophoresis delivery of ACh (expressed as percentage change from the baseline) registered during resting conditions between groups (Figure 1).

These results indicate that skin infusion of ACh, increased FSBF in a dose-dependent manner in the two groups. The T group responds significantly higher than sedentary one after exercise (*P*<0.001).

For the two groups, whatever before or after exercise, maximum

response to the ACh-induced endothelium-dependent relaxation was observed in the 3rd dose (48 µg/ml) and was significantly higher than the other doses in T group (*p*<0.001).

Unlike the endothelium-dependent vasodilator ACh, which induced a significant impact on the FSBF of the T group after exercise, elevation of skin temperature at 44°C, an endothelium-independent vasodilator, did not significantly affect the FSBF response of the groups, whatsoever before or after exercise, as shown in Figure 2.

Discussion

The present study, demonstrated that the FSBF response to ACh-induced endothelium-dependent relaxation was significantly increased on trained young subjects compared to control after a single acute exercise. So the null hypothesis, that there is no difference between the FSBF data of the two groups is rejected. Present data show also that the training in healthy young men influences significantly the EDR by an increase of FSBF response. Physical and clinical characteristics indicates that, in T group, lower values of body mass compared to S one, these differences was due to the regular training; indeed exercise training decrease fat mass [5]. As expected, we observed, a significantly increase in VO₂ peak and a significantly decrease in resting HR values, these results are in agreement with literature [8,28]. Therefore, CVD in adults are laid in childhood and accelerated by the presence of comorbid conditions such as sedentarity, uncontrolled alimentation, obesity and smoking [3].

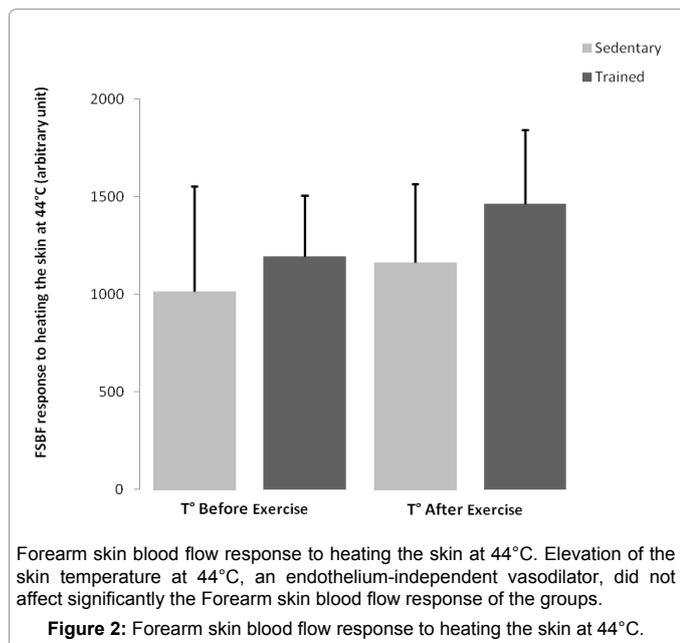
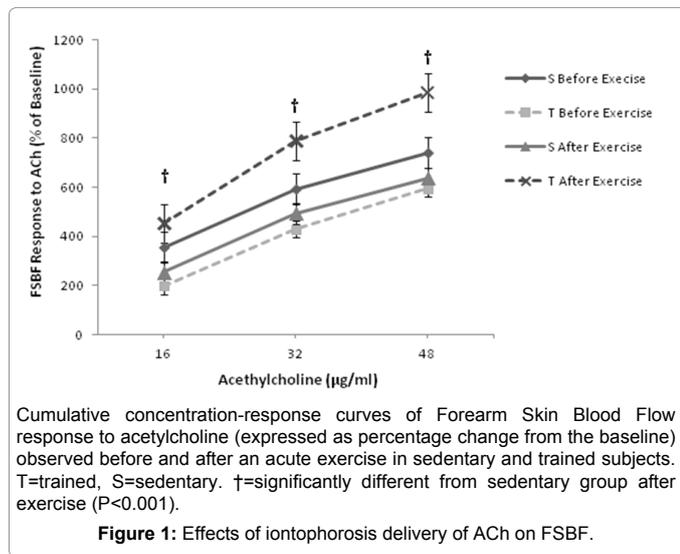
Laser Doppler Flowmetry

Clinical assessment of endothelial function is an insightful exploratory process by which one could detect the upstream events that may lead to the prevention of cardiovascular complication for subjects at high risk. In this context, pharmacological methods, which consist of evaluating the endothelium-mediated flow-(in)dependent dilation by LDF along with iontophoresis [29,30] have recently been used to report a significant transient increase of cutaneous blood flow signal in response to local infusion of vasoactive substances like ACh. This method has been tested on healthy subjects as well as on people with disease states affecting their endothelial reactivity [21,31]. As it provides a direct assessment of microvascular endothelial function, LDF is considered to be reliable non-invasive investigative technique for human clinical exploration. Moreover, the fact that the intra-subject measurements resulting from the LDF are highly reproducible without the intervention of the investigator's subjectivity is a clear indication that this technique provides strict standardization of the recording site [32]. Dose-response curves were obtained by ACh iontophoresis.

Variable	Sedentary n=20	Trained n=29	<i>P</i> value between groups S vs. T
Age (years)	15 ± 1	15 ± 1	<i>Ns</i>
Height (cm)	178 ± 7	180 ± 7	<i>Ns</i>
Weight (kg)	74 ± 10	68 ± 7	<0.05
BMI (kg/m ²)	23.1 ± 2	21.8 ± 2	<i>Ns</i>
HR rest (bpm)	83.1 ± 10	72.2 ± 7	<0.001
HR peak (bpm)	182.2 ± 10	185.51 ± 10	<i>Ns</i>
Resting systolic Blood Pressure (mm Hg)	120.38 ± 9	117.4 ± 5	<i>Ns</i>
Resting diastolic Blood Pressure (mm Hg)	80.4 ± 2	78.2 ± 4	<i>Ns</i>
VO ₂ peak (ml.min ⁻¹)	3.322 ± 0.652	3.78 ± 3.786	<0.05
VO ₂ peak (ml.min ⁻¹ .kg ⁻¹)	40.05 ± 4.53	51.65 ± 5.76	<0.001

Data are Average of values ± SD. BMI: Body Mass Index; R. HR: Resting Heart Rate; HR peak: peak Heart Rate; VO₂ peak: peak of oxygen consumption; sedentary group vs. trained group. *Ns*: not significant.

Table 1: General characteristics of sedentary and trained subjects.



Other studies indicated that ACh-induced vasodilatation is not only dependent on the production of NO but also the action of prostaglandin in the skin vessels [33].

Effects of Iontophoresis Delivery of ACh on FSBF

Before exercise

We evaluated vasoreactivity by comparing the changes in FSBF response to a cumulative concentration of ACh an endothelium-dependent vasodilator. Since the original data of Furchgott and zwadevski, 1980 who have explained the vasodilator effects of ACh via NO releasing on healthy rabbit aorta, many others studies showed and confirm this finding [12,34,35]. So our study joined these many studies who confirmed the changes in blood flow response with vasodilator effects of ACh through two mechanisms, both of which are dependent on intact endothelium [12,30]. Our data showed an increase in the FSBF response in both S and T groups after an ACh iontophoresis at rest.

In fact, it's well known that ACh directly binds muscarinic receptors on the endothelium and causes the NO liberation which diffuses to the media and induces relaxation of the vascular smooth muscle cells [34]. The second mechanism is called Lewis triple-flare response, or nerve axon reflex-mediated vasodilatation, and is caused by the action of ACh on C-nociceptive fibers. ACh sensitizes the C-nociceptive fibers and augments the release of substance-P and calcitonin gene-related peptide. This peptide directly causes vasodilatation [36], and substance-P induces a release of mast-cell histamine. This indirectly leads to vasodilatation and increased vascular permeability. So, this first result of our studied groups was in accordance with literature.

After exercise

The data obtained from the comparison of groups demonstrate that FSBF response to ACh-induced endothelium-dependent relaxation was significantly increased on trained young subjects compared to the sedentary one after a single acute exercise. This result was in agreement with other studies that showed beneficial effects of exercise training on endothelium-dependent vasodilation [8,36,37]. In this population, our data constituted original data because this study is the first who involves young people (15 ± 1 year).

Endothelium released NO in response to both endocrine mediators (e.g., ACh and bradykinin) [34,38] and mechanical stimuli (e.g., changes in blood flow velocity and endothelial shear stress) [39]. The fact that there are no differences between systolic and diastolic blood pressure and heart rate peak does not mean that the duration of submission to shear stress is the same in sedentary and trained when VO₂max is reached. Indeed T takes longer than S to reach their VO₂max. Therefore, their resistance vessels are subjected during longer time to shear stress in T. thus, the effect of endothelium-dependent vasodilatation induced by the shear stress is more important in trained. It is obviously noticed that exercise training provides direct beneficial effects to the vasculature which may contribute to decrease cardiovascular disease risk factors [40]. Although, there have been lots of evidence implying the beneficial effect of exercise on endothelial function. It's well known that exercise training induces increase in blood flow that causes vasodilation [41]. Many studies showed that exercise training improve endothelial function with a better NO release and/or bioavailability, a decreased oxidant stress, an increased functional capacity of circulating angiogenic cells [42]. Laufs et al. (2004) [43] showed that exercise training improves endothelial function by increasing vasodilatory NO release and endothelial nitric oxide synthase (eNOS) activity in endothelial cells and they also noted that physical inactivity causes endothelial dysfunction in part through impairment of eNOS.

In conclusion, cutaneous microvascular endothelium-dependent vasodilatation data show that training of young subjects potentiates the vasodilators effects of ACh after an acute exercise compared to the sedentary subjects. The training in healthy young men influences significantly the EDR by an increase of FSBF response. It is necessary to pursue a better understanding of the mechanisms of ED in the future. Consequently, it would be interesting to explore the consequences of other types of isolated exercises and training in young subjects with regard to molecular interactions and the metabolism of NO by measuring more critical parameters, which may modulate endothelial function. It will be intriguing to pursue these investigations particularly in pathological subjects (e.g., cardiovascular and obesity).

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