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 exercise) 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; VO₂/ VCO₂: 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 it’s considered as a leading cause of death [1] with a rate ranging 6607.1000163

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whether exercise is associated with a beneficial effect on endothelial 
NO release, we investigated the endothelium-dependent and – 
independent vasodilatation 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 
and difference of FSBF means of two groups. These two values were 
compared to the null hypothesis that 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 H₀: m₁=m₂ and the alternative 
hypothesis was Hₐ: m₁≠m₂. The total sample size was estimated 
using the following formula [22] is 

\[ N = \frac{(r+1)(\bar{z}_α + \bar{z}_β)^2 \sigma^2}{d^2 z^2} \]

where \(r\) is the ratio of sample size for each group, \(\sigma\) is the 
standard deviation, and \(d\) is the difference of mean values. 
The total sample size for the study was 47 males (31 T and 16 S).

Subjects general characteristics

49 healthy male subjects, among whom 29 were 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 within 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) and secondly on the value 
of maximum oxygen consumption (VO₂max) beyond 45 ml.min⁻¹.kg⁻¹. 
All the participants were familiarized with all testing equipment 
and be well hydrated.

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/VVO₂), 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 Ergostest, 
ZAN Medgera’te 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 – 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 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.

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**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 versus 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 were indicated that, in T group, lower values of body mass compared to S one, these differences were 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 sedentary, 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 [21,31,32]. 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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sedentary n=20</th>
<th>Trained n=29</th>
<th>P value between groups S vs. T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
<td>Ns</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178 ± 7</td>
<td>180 ± 7</td>
<td>Ns</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74 ± 10</td>
<td>68 ± 7</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.1 ± 2</td>
<td>21.8 ± 2</td>
<td>Ns</td>
</tr>
<tr>
<td>HR rest (bpm)</td>
<td>83.1 ± 10</td>
<td>72.2 ± 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR peak (bpm)</td>
<td>182.2 ± 10</td>
<td>185.51 ± 10</td>
<td>Ns</td>
</tr>
<tr>
<td>Resting systolic Blood Pressure (mm Hg)</td>
<td>120.38 ± 9</td>
<td>117.4 ± 5</td>
<td>Ns</td>
</tr>
<tr>
<td>Resting diastolic Blood Pressure (mm Hg)</td>
<td>80.4 ± 2</td>
<td>78.2 ± 4</td>
<td>Ns</td>
</tr>
<tr>
<td>VO2 peak (mL.min⁻¹)</td>
<td>3.322 ± 0.652</td>
<td>3.78 ± 3.788</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VO2 peak (mL.min⁻¹.kg⁻³)</td>
<td>40.05 ± 4.53</td>
<td>51.65 ± 5.76</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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.
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 vasodilatation [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 $V_{O2}\text{max}$ is reached. Indeed T takes longer than S to reach their $V_{O2}\text{max}$. 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 vasodilatation [41]. Many studies showed that exercise training improve endothelial function with a better NO release and/or biodisponibility, 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).
References


