Does Prefrontal Cortex Transcranial Direct Current Stimulation Influence the Oxygen Uptake at Rest and Post-exercise?

Abstract

The study evaluated the effect of transcranial direct current stimulation (tDCS) applied over prefrontal cortex on the oxygen uptake (VO₂) at rest and during post-exercise recovery. The VO₂ was assessed in eleven healthy subjects before, during tDCS (sham or anodal tDCS, 2 mA, 20 min), and 30-min following isocaloric aerobic exercise (~200 kcal). During tDCS, no changes were observed on VO₂ compared to baseline (P=0.95) and sham condition (P=0.85). The association between isocaloric exercise and anodal tDCS increased the VO₂ throughout 30-min recovery compared to sham condition (P<0.001). Therefore, the energy expenditure within the excess post-exercise oxygen consumption (EPOC) period, after anodal tDCS was approximately 19% higher compared to the sham condition (P<0.05). In conclusion, anodal tDCS applied on the prefrontal cortex combined with submaximal aerobic exercise increased the EPOC, enhancing the VO₂ and energy expenditure at least for 30-min of recovery.

Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive technique widely used in neurological disorders treatment [4,6,31]. It is a technically simple tool in which a continuous weak electric current is applied to the brain via 2 electrodes that are placed on the subject’s scalp. The modulator underlying effects of the stimulation seem to be able to reach sub cortical areas [19]. Anodal tDCS has been shown to induce neurophysiological changes in the cell membrane resting potential, favoring depolarization and increasing spontaneous neuronal firing rate. On the other hand, opposite effects seem to be generated by cathodal tDCS [26]. These effects may be influenced by electrode montage (i.e., bilateral or unilateral and bi-cephalic or extra-cephalic tDCS) [2] or frequency of tDCS sessions [1]. Recently, tDCS has also been applied with other purposes, such as improving physical performance [8], physical rehabilitation [36,37] or appetite regulation [24].

Little is known about the influence of tDCS on the modulation of cardiorespiratory centers and autonomic nervous system [20,30] and the available evidence is controversial. Vandermeeren et al. [41] investigated if tDCS with an extracephalic reference electrode would modulate brainstem activity as reflected by the heart rate variability (HRV), respiratory rate, blood pressure, and sympatho-vagal balance in healthy subjects. The results showed that tDCS did not significantly change the activity of brainstem autonomic centers. However, more recent studies showed that anodal tDCS may influence the autonomic nervous system modulation [23,42]. Thus, more studies are needed to verify the possible influence of tDCS on the modulation of cardiorespiratory control and autonomic nervous system. Furthermore, anodal tDCS has been shown to modulate the neuronal activation and increase regional cerebral blood flow (rCBF) at rest in the prefrontal (and motor) cortex of healthy subjects [21,43]. However, no studies investigating the magnitude of such improved cerebral oxygenation on oxygen uptake (VO₂) were found. There is evidence that aerobic submaximal exercise increases the oxygenation of the prefrontal cortex (PFC), as suggested by changes in oxyhemoglobin (O₂Hb), total Hb, and O₂ saturation [10,16,34]. Thus, the PFC activation seems to be related to VO₂ and energy expenditure (EE), as reflected by higher values of VO₂ during exercise and post-exercise recovery, when compared to baseline period [18]. Nonetheless, the tDCS influ-
ence on VO₂ at rest and after exercise remains unclear. Given that the increased EE is a desirable effect in exercise programs aiming weight management, it would be useful to know whether tDCS can influence VO₂ at rest and during recovery (i.e., excess post-exercise consumption – EPOC).

Since there is evidence indicating that the application of anodal tDCS over the scalp can stimulate cortical areas modulating the cortical activation and oxygenation at rest [21], the present study investigated whether tDCS applied on PFC would affect VO₂ magnitude at rest and during EPOC. It has been hypothesized that tDCS would increase the VO₂ after anodal stimulation both at rest and post-exercise.

Methods

Subjects

11 healthy males volunteered for the study [mean ± SD, age: 23 ± 3 yrs; body weight: 80.6 ± 16.9 kg; body mass index: 26.7 ± 4.4 kg/m²]. Exclusion criteria were: a) use of cardiovascular or metabolic medications; b) smoking or use of ergogenic substances that could affect exercise performance; c) cardiovascular, respiratory, muscle, or skeletal problems that could preclude exercise performance. The study was performed in accordance with the ethical standards required by the journal [14] and all participants signed informed consent.

Procedures

Each subject visited the laboratory 3 times. On the first visit anthropometric measurements were taken, and maximal cardiopulmonary exercise testing was performed to determine maximal HR and VO₂. The second and third visits were separated by 24–48 h and involved determination of resting VO₂ (pre and post-anodal or sham tDCS) in a counterbalanced crossover design, after which, participants performed either a continuous isocaloric exercise sessions [200 kcal] at 70 % of the oxygen uptake reserve (VO₂R, defined as the difference between maximal and resting VO₂). All tests were performed on cycle ergometer (Cateye EC-1600, Cateye™, Tokio, Japan). The ambient temperature ranged from 21–23 °C and relative humidity ranged from 55–70 %.

The resting VO₂ assessment was carried out before the isocaloric exercise sessions. Participants were instructed not to engage in any form of physical exercise in the previous 24 h, to abstain from alcohol, soft drinks and caffeine in the 24 h preceding the test and to fast for 3 h. In the laboratory, participants laid quietly for 20 min. After this rest period, the VO₂ was measured for 10 min in the sitting position, and the average of the last 2 min was regarded as resting VO₂ pre-tDCS. Subsequently the subjects remained seated and either anodal [2 mA] or sham tDCS was applied during 20 min. The average value of the last 2 min was used as resting VO₂ post-tDCS. The electric current was applied using a pair of sponges soaked in saline solution (140 mMol of NaCl dissolved in Milli-Q water) involving both electrodes (35 cm²) [9, 25]. The electrodes (anodal and reference) were connected to a constant current stimulation device with 3 power batteries (9V) presenting a maximal output of 10 mA. The batteries were regulated by a digital multimeter (Eza Ez™ 984, Shangai, China) with a standard error of ± 1.5 %.

For the anodal stimulation targeting left dorsolateral prefrontal cortex (DLPFC), the anode was placed over F3 area according to the international EEG 10–20 system [17]. The reference electrode was placed over the supraorbital contralateral area (Fp2) and fixed by elastic bands. The electrodes were placed in the same position of the anodal stimulation to perform the sham condition. However, the stimulator was turned off after 30 s [12]. Thus, the subjects reported to feel a tingling or itching sensation coming from the initial electrical stimulation, but did not receive any further current. This procedure allowed the subjects to remain ‘blind’ in respect to the type of stimulation received during the test and to assure a sham control effect [25].

The maximal heart rate (HRmax) and maximal oxygen uptake (VO₂max) were determined during maximal exercise testing using a ramp protocol. The workload increments were individualized to elicit the subject’s limit of tolerance in 8–12 min [7]. The mean ± SD predicted final power was 270 ± 36 W and the power of 0W and 25 W were used, respectively, for the 5-min warm-up period and for the initial test workload. The cadence was set at 55 revs·min⁻¹ throughout the test.

Subjects were verbally encouraged to perform maximal effort and tests were considered as maximal if the subjects satisfied at least 3 of 4 criteria: a) maximum voluntary exhaustion defined by attaining score 10 on the Borg CR-10 scale or when the cadence could not be maintained at a minimum of 50 rpm; b) 90 % of the predicted HRmax [220–age] or presence of a HR plateau (ΔHR between 2 consecutive work rates ≤ 3 beats·min⁻¹); c) presence of VO₂ plateau (ΔVO₂ between 2 consecutive work rates of less than 2.1 mL·kg⁻¹·min⁻¹); d) maximal respiratory exchange ratio (RERmax) > 1.10 [15].

Based on HR and VO₂ obtained at rest and in the maximal exercise test (HRmax and VO₂max), values corresponding to 70 % of heart rate reserve (HRR, defined as the difference between maximal and resting HR) and 70 % VO₂R were calculated to determine the intensity of the 2 isocaloric exercise bouts. The EE was calculated individually from the net VO₂ [35]. In addition, the time to achieve 200 kcal for 70 % V O₂R was calculated (see Table 1). The net VO₂ values expressed in mL·kg⁻¹·min⁻¹ were converted to L·min⁻¹ and then to kcal·min⁻¹. Each exercise bout was preceded by 5 min warm-up at 0 W and free revs·min⁻¹, and was followed by 30 min recovery at rest in sitting position. The absolute VO₂ values obtained from the % VO₂R equation were used to calculate the associated cycling power using the American College of Sports Medicine (ACSM) metabolic equation:

\[ VO₂ = \text{mL·min}^{-1} \times \text{kg}^{-1} = 3.5 \text{mL·min}^{-1} \times \text{kg}^{-1} + 122.4 \times (\text{power}) \times (\text{BW}^{-1}), \]

where power is in W and BW the body weight in kilograms [13]. Expired gases were collected during and post-exercise via metabolic cart using a Hans Rudolph face mask (Hans Rudolph™ Inc., Kansas, MO, USA). The exercise session ended when the subject had achieved a total EE of 200 kcal. During the post-exercise recovery, mean VO₂ values were obtained at 10, 20 and 30 min.

The VO₂, pulmonary ventilation (V̇E), carbon dioxide output (VCO₂), and HR were calculated, averaged, and recorded every 20 s. Gas exchanges were determined using a VO2000 analyzer (Medical Graphics™, Saint Louis, MO, USA) and HR using a cardiometer (Polar® RS-800cx, Kempele, Finland). The gas analyzers were calibrated with a certified standard mixture of oxygen (17.01 %) and carbon dioxide (5.00 %), balanced with nitrogen. The flows and volumes of the pneumotacograph were calibrated with a syringe graduated for a 3L capacity (Hans Rudolph™, Kansas, MO, USA).
Table 1 Mean ± SD values for resting heart rate (HR) and oxygen uptake (V\textsubscript{\textdia{O}}\textsubscript{2}) during the incremental exercise test and isocaloric exercise sessions. All subjects satisfied at least 3 of the 4 criteria stipulated in the ‘Procedures’ section during the incremental exercise test.

<table>
<thead>
<tr>
<th>Moment</th>
<th>Variables</th>
<th>atDCS</th>
<th>Sham Condition</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting values (pre-tDCS)</td>
<td>HR (beats·min\textsuperscript{-1})</td>
<td>68 ± 8</td>
<td>74 ± 9</td>
<td>0.03</td>
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<td></td>
<td>VO\textsubscript{2} (mL·kg\textsuperscript{-1}·min\textsuperscript{-1})</td>
<td>3.8 ± 0.6</td>
<td>3.5 ± 0.9</td>
<td>0.39</td>
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<tr>
<td>Resting values (post-tDCS)</td>
<td>HR (beats·min\textsuperscript{-1})</td>
<td>67 ± 7</td>
<td>73 ± 10</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>VO\textsubscript{2} (mL·kg\textsuperscript{-1}·min\textsuperscript{-1})</td>
<td>4.1 ± 0.8</td>
<td>3.4 ± 1.5</td>
<td>0.19</td>
</tr>
<tr>
<td>Incremental exercise test</td>
<td>HR\textsubscript{max} (beats·min\textsuperscript{-1})</td>
<td>184 ± 7</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>VO\textsubscript{O2max} (mL·kg\textsuperscript{-1}·min\textsuperscript{-1})</td>
<td>35.7 ± 4.6</td>
<td>–</td>
<td>–</td>
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<td></td>
<td>Peak power (W)</td>
<td>270 ± 36.5</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>Time to exhaustion (min)</td>
<td>9 ± 1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Isocaloric exercise sessions</td>
<td>HR\textsubscript{rest} (beats·min\textsuperscript{-1})</td>
<td>155 ± 12</td>
<td>157 ± 11</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>HR\textsubscript{mean} (beats·min\textsuperscript{-1})</td>
<td>151 ± 11</td>
<td>153 ± 11</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>VO\textsubscript{2rest} (mL·kg\textsuperscript{-1}·min\textsuperscript{-1})</td>
<td>23.0 ± 3.6</td>
<td>21.6 ± 4.1</td>
<td>0.13</td>
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<tr>
<td></td>
<td>VO\textsubscript{2mean} (mL·kg\textsuperscript{-1}·min\textsuperscript{-1})</td>
<td>21.6 ± 3.0</td>
<td>21.6 ± 4.3</td>
<td>0.98</td>
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<tr>
<td></td>
<td>Power (W)</td>
<td>155 ± 19</td>
<td>156 ± 35</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Time to achieve 200 kcal (min)</td>
<td>22 ± 3</td>
<td>22 ± 3</td>
<td>–</td>
</tr>
</tbody>
</table>

atDCS = Anodal transcranial direct current stimulation; HR = heart rate; VO\textsubscript{2} = oxygen uptake; HR\textsubscript{rest} and VO\textsubscript{2rest} = mean values observed in the last minute; HR\textsubscript{mean} and VO\textsubscript{2mean} = mean values observed during exercise session.

Fig. 1 Mean values of VO\textsubscript{2} at rest and post-exercise recovery after sham and anodal tDCS. *Significant difference between sham and anodal tDCS (10 and 30 min, P<0.001 and 20 min, P=0.002).

Statistical analyses
The effects across time and due to different tDCS (anodal vs. sham condition) on VO\textsubscript{2} in pre and post-exercise assessments were compared by 2-way ANOVA with repeated measures followed by Tukey post hoc verification in the event of significant F ratios. The Student-t test was used to compare the cardiorespiratory variables observed at rest, during exercise sessions and the energy expenditure obtained during 30-min recovery in both tDCS conditions. 2-tailed statistical significance was accepted as P ≤ 0.05. All statistical analyses were performed using Statistica 7.0 software (Statsoft\textsuperscript{TM}, Tulsa, OK, USA).

Results
The statistical power of the repeated measures ANOVA model, within-between groups interaction, was determined using the GPower version 3.1.2 (Universität Kiel, Kiel, Germany) and considering the following parameters: effect-size = 3.0; α error probability = 0.05; number of groups = 2; number of measurements = 13; correlation among repeated measures = 0.5; non-sphericity correction = 1. The actual power for N = 11 was 0.923 (effect-size = 0.30–0.768 (effect-size = 0.25), which is slightly acceptable for the type II error.

Table 1 shows the mean (SD) values for the heart rate and VO\textsubscript{2} at rest, and the variables assessed in the incremental exercise test and isocaloric exercise sessions.

Fig. 1 exhibits the VO\textsubscript{2} measured at rest (mean±SD), during 20 min of sham and anodal tDCS, and along 30-min recovery after the 200 kcal sessions (22.4 ± 3.4 min. session\textsuperscript{-1}). At rest, the VO\textsubscript{2} did not change during 20 min in both tDCS and sham conditions. After 30 min of post-exercise recovery, the VO\textsubscript{2} increased significantly, compared to baseline values in both anodal and sham conditions (P < 0.0001). However, the relative VO\textsubscript{2} in anodal tDCS remained higher than after the sham stimulation during all recovery period (P < 0.05). Fig. 2a shows the mean EE throughout the 30-min recovery following the 200 kcal sessions, and the ratio between EE during exercise and recovery (200 kcal), in both sham and anodal conditions. The absolute EE was higher after tDCS over the sham condition (P < 0.001). Fig. 2b, c exhibits the net EE post-exercise and the ratio between net EE post-exercise (calculated using the resting VO\textsubscript{2} assessed in pre and post-tDCS, respectively) and exercise. In both cases, the net EE post-exercise was greater after the anodal stimulation (post-tDCS) than before the stimulation (pre-tDCS) (P < 0.05).

Discussion
The present study investigated the effects of tDCS applied over the PFC on VO\textsubscript{2} at rest and throughout post-exercise recovery. To the best of our knowledge, this is the first study investigating the effect of tDCS associated with acute physical activity on VO\textsubscript{2} at rest and energy expenditure after isocaloric exercise. Our findings indicated that, at rest, no changes were elicited in VO\textsubscript{2} over 20 min of anodal tDCS. On the other hand, the association between anodal tDCS and aerobic exercise was able to increase the VO\textsubscript{2} throughout all post-exercise recovery when compared
to the exercise performed after sham condition. Therefore, the EE following submaximal exercise and anodal tDCS was significantly higher compared to the sham condition – actually the EE in the anodal tDCS condition was ~17–19% higher than after the sham stimulation. These data, at least in part, concur with the hypothesis that tDCS associated with exercise might be able to modulate cerebral areas related to respiratory centers (e.g., PFC). Moreover, it is feasible to think that anodal tDCS may improve the EE following exercise sessions performed at a given intensity, duration and caloric expenditure.

Previous research showed that anodal tDCS was able to modify the cerebral hemodynamic behavior reflected by significant increase in HbO2 concentration and regional cerebral blood flow, causing changes in the cortical metabolism [19,21,27,43]. Despite some tendency to increase in oxygen consumption, our results indicated that 20 min of 2 mA tDCS on PFC were not sufficient to change the resting VO2. Recent research evaluating the tDCS effects on HRV, respiratory rate, blood pressure, and sympathetic-vagal balance, showed that anodal tDCS would not modulate the activity of brainstem autonomic centers in healthy subjects [29,41]. However, other studies demonstrated effective effects of anodal tDCS on the autonomic cardiac control [23,42], reflected by changes in parasympathetic or sympathetic modulation. It can be therefore speculated that the potential increase in cerebral oxygenation due to anodal tDCS would not be enough to elicit respiratory changes directly related to the brainstem activity. Such changes would probably rely on a more complex and integrative regulation, associating different brain areas including PFC. The underlying mechanisms of stimulation on PFC and other cortical areas certainly warrant additional investigation.

The present results suggest that the association between external electric sources and exercise may affect cerebral centers regulating peripheral metabolic pathways. It has been recently demonstrated that the increase in cerebral activity induced by anodal tDCS was associated to higher systemic glucose tolerance [3], which concurs with our findings. This means that the transcranial brain stimulation not only can affect local neuronal processes, but when associated with exercise may also influence downstream metabolic systems regulated by the brain. Previous studies showed that maximal effort gradually decreases the peripheral muscle oxygenation, while increasing the PFC oxygenation in the first minutes of exercise and decreasing the workload corresponding to the second ventilatory threshold up to exhaustion [33]. In other words, it is feasible that submaximal exercise increases the PFC oxygenation in workloads below the lactate threshold, suggesting the potential role of mild exercise in enhancing cerebral blood volume [10,38]. Furthermore, when the pulmonary ventilation increases during exercise and immediately after its cessation, cortical and subcortical areas are probably hyper-activated, for instance, supplementary and pre-motor areas (SMA/PMA), cerebellum, limbic, parietal, and superior frontal cortex [11]. Interestingly, the PFC has extensive connections to most of the areas mentioned above, and it has been suggested that the one of the main functions of this area is the regulation of thoughts and actions in accordance with internal goals [22]. Since PFC receives stimuli from the brainstem arousal systems, and its function is particularly associated with its neurochemical environment [32], we speculate that anodal tDCS and submaximal exercise might have increase of psychological aspects, such as arousal, and consequently induced higher rates of VO2 and EE observed along the recovery period.
Curiously the hypo- or hyperactivity of PFC may also play a key role in the control of food and drug intake [5, 39, 40]. It has been shown through functional magnetic resonance imaging (fMRI) that the appetite activates the orbitofrontal and anterior cingulate cortex, and decreased activation of PFC [28, 39]. Moreover a recent study of our group demonstrated that anodal tDCS over PFC associated with aerobic exercise, was able to decrease the appetite sensation during post-exercise recovery period in overweight subjects [24]. The combination of improvement in appetite control and increased EPOC due to anodal tDCS, opens novel possibilities to improve the effects of exercise programs aiming weight loss, which certainly warrants future research.

The main limitation of this study was the fact that specific cortical areas activated by tDCS were not controlled. Since brain areas responsible for the respiratory and exercise control have been widely discussed, such control would allow a better understanding of the mechanisms underlying the observed effects on VO₂. Future studies using techniques to detect the brain areas involved in specific tasks are, for that reason, necessary. In conclusion, tDCS applied on the prefrontal cortex may induce favorable effects on the autonomic respiratory control by increasing the VO₂ and energy expenditure after aerobic exercise. Since it is possible that tDCS over PFC associated with aerobic exercise may also decrease appetite sensation in overweight adults, such effect may be useful in the context of exercise programs aiming weight management. Additional research should be encouraged to ratify these results and to help clarifying the physiological mechanisms underlying possible changes in the autonomic respiratory control pattern due to tDCS and, as well as to explore the potential benefits of this interesting neuro-modulation tool.

Acknowledgements
This study was supported by grants from the Brazilian Council for the Technological and Scientific Development (CNPq) and Carlos Chagas Filho Foundation for the Research Support in the State of Rio de Janeiro (FAPERJ).

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