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Methodological considerations to determine the effect of exercise on brain-derived neurotrophic factor levels

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ABSTRACT

Objectives: Physical exercise up-regulates brain-derived neurotrophic factor (BDNF) in the brain and blood. However, there is yet no consensus about the adequate blood processing conditions to standardize its assessment. We aimed to find a reliable blood sample processing method to determine changes in BDNF due to exercise.

Design and methods: Twelve healthy university students performed an incremental cycling test to exhaustion. At baseline, immediately after exercise, and 30 and 60 min of recovery, venous blood was drawn and processed under different conditions, i.e. whole blood, serum coagulated for 10 min and 24 h, total plasma, and platelet-free plasma. BDNF concentration was measured by ELISA.

Results: Exercise increased BDNF in whole blood and in serum coagulated for 24 h when corrected by hemoconcentration. We did not find effects of exercise on BDNF in serum coagulated for 10 min or in plasma samples. Plasma shows heterogeneous BDNF values in response to exercise that are not prevented when platelets are eliminated while homogeneous BDNF levels were found in whole blood or serum coagulated for 24 hour samples.

Conclusions: In exercise studies, BDNF levels should be adjusted by hemoconcentration. Our data highlight the importance of blood sample selection since the differences between each one affect significantly the BDNF factor changes due to exercise.

Introduction

Neurotrophic factors are able to induce several trophic changes in the nervous system, such as neurogenesis in specific areas of the brain or growth, differentiation, repair, and survival of preexisting neurons. Nerve growth factor, insulin-like growth factor-1, neurotrophins 3 and 4/5, and especially the brain-derived neurotrophic factor (BDNF) are among the most important neurotrophic factors. BDNF was first identified in 1982 by Yves Barde and Hans Thoenen [1], and its importance as a therapeutic agent in neuropsychiatric disorders [2] and in improving plasticity and cognition in healthy conditions has been well documented [3].

Rosenfeld et al. originally showed that BDNF could be detected in human serum or plasma [4]. Later, a positive correlation between brain and plasma levels of BDNF was found [5]. Since then, numerous studies performed in humans have analyzed circulating BDNF levels [6,7]. However, because of the poor pharmacokinetic profile, poor delivery and short half-life of BDNF [8], the direct treatment with recombinant BDNF has not been proved to be successful [9–11]. Thus, several research efforts have focused on analyzing which type of interventions can up-regulate the endogenous BDNF, e.g., antidepressants [12], antipsychotics [13], euthymic drugs [14], or diet [15]. Physical exercise has emerged as one of the most potent inductors of BDNF both in health [6,7] and in disease [16].

Although changes in circulating BDNF are good surrogates of the changes occurring in the brain, differences in blood processing conditions (e.g. plasma, serum or whole blood; and timing of collection; among others) make difficult compare studies. There is no consensus about the most adequate blood processing conditions for studying exercise-induced changes in circulating BDNF and therefore researchers use the different blood conditions indistinctly [6].

The main purpose of this study was to evaluate exercise-induced changes in BDNF levels under the different blood processing conditions more frequently used in the literature [6]. To the best of our knowledge this is the first study in which BDNF is determined in blood samples obtained using five different procedures: whole blood (WB), serum coagulated for 10 min (S10m) or 24 h (S24h), total plasma (TP), and

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platelet-free plasma (PFP), collected from the same subjects before and after performing one bout of exhaustive physical exercise. BDNF should be measured either in WB or in S24h and not in plasma and S10m samples.

Materials & methods

Ethics statement

The study complies with the World Medical Association Declaration of Helsinki—Ethical Principles for Medical Research Involving Human Subjects. The experimental protocol was approved by the Ethics Research Committee of the Faculty of Medicine, University of Valencia (Spain).

Subjects

Twelve healthy male university students (24.7 ± 5.6 years; 1.77 ± 0.06 m of height; and 74.5 ± 5.7 kg of weight) from the University of Valencia participated in this study after providing their written informed consent. Inclusion criteria were: non-smokers, disease-free (especially neuropsychiatric disorders), not taking any medication, vitamin complex or dietary supplementation (for at least 6 months), alcohol or caffeine (for at least 48 h). During the first visit, they received a full explanation of the procedures and measurements.

International Physical Activity Questionnaire

Participants filled the self-reported Spanish version of the International Physical Activity Questionnaire (short form, IPAQ-S), which provides information of all dimensions of physical activity in the adult population (18–65 years old) [17]. The IPAQ-S test is widely used in population studies and has been validated in a series of international and national studies carried out by the World Health Organization and EU Eurobarometers, among others [18].

Exercise protocol

The maximal exercise test was performed on a magnetic brake bicycle ergometer (Monark, Sweden). After a 5-minute warm-up, the incremental cycling test started at 75 W and the workload was increased by 50 W every 3 min for a total of 9 min. Thereafter, workload was incremented by 25 W/min until volitional exhaustion. Participants' heart rate (Sigma PC 3, Germany) as well as capillary blood lactate (Lactate pro™ Netherlands) were determined before, during exercise, immediately after exercise, and during recovery at 30 and 60 min.

Collection and processing of the samples

Participants arrived at the laboratory (15:00 p.m.) and remained sitting for 30 min. After that, baseline blood samples (pre) were collected. Then, participants performed the incremental cycling test. Blood samples were drawn immediately after exercise (post) as well as at 30 and 60 min of recovery. The blood collections were performed by using an Abocat® catheter from the ante-cubital vein. Two K3-EDTA containing tubes and three additive-free tubes Vacutainer® were used in order to obtain all samples analyzed in the study (see below).

Whole blood samples (WB)

Blood was collected in K3-EDTA containing tubes and immediately frozen at −20 °C. After several days each sample was defrosted at 4 °C for 1.5 h. After that, Triton X-100 (9:1 v/v) was added to each sample and was incubated during 1.5 h at 4 °C. Thereafter, all samples were sonicated and centrifuged at 12,000 *g* for 10 min at 4 °C. Supernatant was collected and frozen at −20 °C [19].

Serum samples

Serum samples were collected in additive-free tubes under two different conditions: samples coagulated at 4 °C during 10 min (S10m) or during 24 h at 4 °C (S24h). Then samples were centrifuged at 1500 *g* for 15 min at 4 °C and supernatants were frozen at −20 °C.

Plasma samples

Blood was collected in K3-EDTA containing tubes and centrifuged at 1500 *g* for 15 min at 4 °C. Supernatants for the total plasma (TP) condition were frozen at −20 °C and those for the platelet-free plasma (PFP) samples were centrifuged again at 10,000 *g* for 10 min at 4 °C. Finally supernatants were frozen at −20 °C [20].

Biochemical measurements

BDNF levels in all the samples were measured using the ELISA kit – ChemiKine™, Millipore, Temecula, CA, USA; Ref. CYT306 – following the manufacturer's instructions. Hematocrit (Hc), hemoglobin (Hb), platelet and leukocyte counts were assessed using a Sysmex XE-2100 (Roche Diagnostics S.L., Barcelona, Spain).

Calculations

Plasma and blood volume modifications during exercise were estimated by Dill's equation [21]. Accordingly, whole blood post-exercise BDNF measurements were corrected by blood volume loss during exercise and plasma and serum post-exercise BDNF measurements were corrected by plasma volume loss due to exercise.

Statistical methods

Statistical analysis was performed using SPSS 21 (IBM Corporation, Armonk, NY, USA). Data are expressed as mean ± standard deviation (SD) and analyzed for normality by the Shapiro–Wilk test. Two subjects were removed from the study because of problems with their samples processing and analysis. Therefore, BDNF variables were log-transformed and reported as median and (25th–75th) quartiles. A one-way ANOVA for repeated measures (sampling time: pre-exercise, post-exercise, 30′-recovery and 60′-recovery) was performed to assess the effect of exercise on the measured variables. When the main effect was significant, post hoc comparisons with Bonferroni's correction were used to compare values between different sampling times. $\eta^2_{partial}$ values were reported as an estimation of effect size. Correlation between circulating BDNF measurement methods was performed by Spearman's correlation coefficient (ρ). Results were considered to be statistically significant when $P < 0.05$.

Results

The daily energy expenditure of the participants, obtained from the IPAQ-S questionnaire, was 7 ± 2 MET/min per week. Moreover, the majority of the energy (56%) was spent in high intensity activities. The incremental cycling test lasted an average of 34 ± 1 min and the maximal heart rate was 184 ± 8 beats per minute. Blood lactate levels were higher immediately after exercise (11.8 ± 2.6 mmol/L) than in baseline conditions (3.1 ± 2.3 mmol/L; $P < 0.001$). Lactate levels were restored 60 min after exercise (2.8 ± 1.1 mmol/L; $P < 0.001$). Platelet count rose to 49 ± 18% after exercise ($P < 0.001$) (see Table 1). Taking into account the Hc and Hb values, we calculated that exercise resulted in a concentration of 5.3 ± 2.6% in whole blood and 9.7 ± 3.8% in plasma volume [21]. After adjusting for plasma hemoconcentration, platelet count did not significantly change between pre- and post-measurement.

Fig. 1 shows that exercise increases BDNF levels corrected by hemoconcentration in WB ($P = 0.008$) and in S24h ($P = 0.003$). However, in both S10m ($P = 0.772$) and plasma samples (FP: $P = 0.070$; TP: