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Urinary BDNF-to-creatinine ratio is associated with aerobic fitness

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HIGHLIGHTS

- Urinary BDNF level is positively associated with frequency of aerobic exercise.
- Performance on a sub-maximal Step Test improves as basal BDNF level increases.
- Urinary BDNF level may be a viable peripheral biomarker of central BDNF activity.

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ABSTRACT

Circulating levels of brain-derived neurotrophic factor (BDNF) are known to be affected by aerobic exercise. As the previous research focus in humans has been to examine peripheral BDNF levels through blood, serum, and platelet assay, the present study investigated the association between basal urinary BDNF concentration and indices of aerobic fitness in a sample of young adults ($n = 52$). Aerobic fitness was evaluated with self-report of exercise habits and heart rate (HR) assessment during a sub-maximal Step Test. BDNF concentration was determined by enzyme-linked immunosorbent assay and adjusted for creatinine. Results indicated that the basal $BDNF_{log}$/creatinine ratio was positively associated with greater frequency of exercise and, during aerobic challenge, a quicker rise in HR upon exercise, lower peak HR during exercise, and lower HR during the recovery period, each indicative of enhanced fitness. These results highlight the utility of urine capture as a non-invasive technique to assess for exercise-mediated changes in peripheral BDNF.

1. Introduction

Brain-derived neurotrophic factor (BDNF), a neurotrophin that promotes neuroplasticity in humans [1], is a candidate of interest for investigations of the biological mediators of exercise-induced affective (e.g., antidepressant, anxiolytic) [2] and cognitive (e.g., improved long-term memory and executive functioning) changes [3–5]. Research indicates that aerobic exercise modulates peripheral levels of BDNF across age groups, fitness levels [4,6–8], and medical conditions [for review, see 9], which is important given the notion that BDNF is protective against neurodegenerative decline [10].

BDNF bi-directionally crosses the blood–brain barrier, suggesting that peripheral BDNF acts as a reserve for the brain [11]. Furthermore, the positive correlations between peripheral and central BDNF concentrations in animals [12] and peripheral BDNF concentrations and cortical integrity in humans [13] suggest that peripheral levels are good biomarkers for central BDNF functioning. To date, however, analysis of peripheral BDNF in conjunction with exercise-related variables has focused exclusively on serum, plasma, and more recently, platelet values [6,14]. To our knowledge, no study has investigated whether urinary BDNF concentration relates to aerobic fitness profiles in a similar fashion.

The purpose of this study was, therefore, to determine the relation between urinary BDNF concentration and indices of aerobic fitness in a healthy young adult sample while taking into account reported gender effects in peripheral BDNF levels [15]. It was expected that urinary BDNF concentration, adjusted for individual differences in overall hydration via creatinine normalization [16], would be positively associated with aerobic fitness via both self-report of aerobic exercise habits and heart rate assessment during a sub-maximal Step Test.

2. Materials and methods

2.1. Participants

52 young adults (32 women; mean age = 19.2 ± 1.3 years) participated for partial college course credit. Participants were screened

Abbreviations: BDNF, brain-derived neurotrophic factor; bpm, beats-per-minute; ELISA, enzyme-linked immunosorbent assay; HR, heart rate.

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for conditions known to affect circulating BDNF levels, including chronic ketamine use [17], current or recent use of antidepressant and other psychotropic medication [18], and presence of bladder conditions such as overactive bladder [19] as well as any physical conditions that would interfere with a Step Test (e.g., recent lower limb injury). 67.3% of the sample self-reported their ethnicity as White/Caucasian, 19.2% as Asian/Asian-American, 5.8% as Latino/Hispanic, 3.9% as Black/African/African-American, and 3.8% as bi- or multi-racial. The protocol was consistent with ethical guidelines of the Declaration of Helsinki and was approved by the local Institutional Review Board.

2.2. Procedure

The protocol included provision of written informed consent, urine capture, completion of exercise lifestyle questionnaires, and an aerobic challenge. Due to potential circadian rhythms of peptides in urine, all data were collected between 1130 and 1330 h. After a period of rest and prior to the exercise challenge, urine was collected following a mid-stream capture protocol in 120 mL sterile cups and promptly stored at −80 °C.

2.3. Urinalysis

BDNF urinalysis involved acidification, extraction, and enzyme-linked immunosorbent assay. In the acidification step, 0.8 mL of thawed urine was treated with 0.4 mL of 1.5% trifluoroacetic acid (TFA) and then centrifuged after 15 min at 4000 RPM at 4 °C (Beckman Coulter Danvers, MA, USA). In the extraction step, Strata-XL 60 mg cartridges (Phenomenex, Torrance, CA, USA) were equilibrated with 1 mL of methanol (MeOH) and 1 mL of distilled water. The urine supernatant was loaded into each column and allowed to drain. The columns were then washed with 0.1% TFA, followed by vacuum suction (Fisher Scientific, St. Louis, MO, USA) for 10 min to rid the columns of any residual fluids. Samples were eluted with 80% acetonitrile, and the resulting eluent was evaporated using a lyophilizer (Labconco, Kansas City, MO, USA). The BDNF ELISA was then completed with the ab99978 BDNF Human ELISA Kit (Abcam, Cambridge, MA, USA). The lyophilized sample was reconstituted with a prepared buffer solution and then analyzed following manufacturer's suggestions. Absorbance levels of each sample were measured using a SPEC 20 (BioTek, Winooski, VT, USA) at 450 nm wavelength, with concentration calculated according to a standard curve. Samples were run in duplicate, with the two values averaged to generate one concentration value per participant. Intra- and inter-CV were 7.3% and 15.0%, respectively.

A second ELISA was performed to determine the amount of creatinine in each sample. Urine was first diluted 1:20 with distilled water and then assayed following the manufacturer's protocol (Cayman Chemical Company, Ann Arbor, MI, USA). Absorbance levels per sample were measured at 500 nm wavelength using a SPEC 20 (BioTek, Winooski, VT, USA), with concentration calculated according to a standard curve. Samples were run in duplicate, with the two absorbance levels averaged to generate one creatinine concentration value per participant. Intra- and inter-CV were 2.8% and 4.8%, respectively.

2.4. Assessment of exercise habits and aerobic fitness

A self-report questionnaire was administered to assess the frequency (number of sessions per week and last three days) and duration of exercise sessions (min) and to characterize the type of exercise as either aerobic or anaerobic in nature. Only aerobic activities were included in data analysis. Aerobic fitness level was assessed via heart rate (HR) during the Step Test, an exercise in which the subject steps onto and off of a 16-inch raised platform as often as possible in 2 min, using a HR monitor (Garmin, Forerunner 410). HR data were collected continuously during a 1-min rest (baseline), during the 2-min aerobic challenge, and for 2 min post-challenge in order to assess return to baseline. In light of evidence that aerobic fitness is associated with a quicker initial rise in HR upon exercise, lower maximum HR during exercise, and a quicker drop in HR upon rest [for review, see 20], variables were selected to reflect these distinct aspects of HR behavior. In order to characterize immediate HR reactivity to exercise, we calculated the length of time (s) from onset of exercise until HR reached 100 bpm (a shorter time interval reflects a steeper rise in HR). Three variables were chosen to quantify HR during exercise: average HR during the first minute of aerobic challenge (bpm), average HR during the second minute of aerobic challenge (bpm), and peak HR (bpm). Finally, to index the rapidity and nature of HR decline upon rest, we calculated the length of time (s) from offset of exercise until HR returned to 100 bpm (a shorter time interval reflects a steeper HR decline) as well as the average HR during the recovery period (bpm). The relation of these discrete variables to HR continuous data is visualized in Fig. 1.

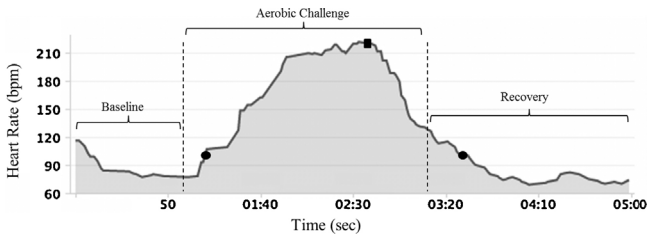

Fig. 1. An example of Step Test HR data collected continuously during a 1-min rest (baseline), during a 2-min aerobic challenge, and during a 2-min post-challenge relaxation period (recovery). The point at which the individual's HR reached 100 bpm upon initiation of exercise is represented by the left-hand circle, and the point at which HR slowed to 100 bpm after cessation of exercise is represented by the right-hand circle. Peak HR is represented by the square. Individuals with greater aerobic fitness, such as shown here, tend to have a quicker rise in HR upon exercise, lower maximum HR during exercise, and a quicker drop in HR upon rest compared to unfit individuals.

2.5. Data analytic strategy

Analyses were computed using SPSS software version 21.0 for Windows (Armonk, NY, USA). BDNF concentration was standardized based on creatinine concentration, resulting in a single value per participant that reflected the ratio of BDNF to creatinine (BDNF/Cr). Normality of data was checked using Kolmogorov–Smirnov Goodness of Fit tests as well as by inspection of skew and kurtosis values.

One-way analyses of variance (ANOVA) were used to determine if the BDNF/Cr ratio and any exercise-related variables differed as a function of gender. Collapsing across gender to make use of the full sample, we used two-tailed partial correlations to determine if the BDNF/creatinine ratio was associated with self-report of exercise habits and Step Test outcome data while controlling for gender. An alpha value of 0.05 was used as the significance threshold for analyses except when a Bonferroni correction for multiple comparisons was warranted.

3. Results

3.1. Descriptive statistics

Descriptive statistics for all variables are shown in Table 1. Of the 52 participants, 2 BDNF/creatinine data points were determined to be outliers and therefore excluded from subsequent analysis. Similar to findings reported elsewhere [21,22], concentration levels