



# Assessment of brain-derived neurotrophic factor and osteopontin in a healthy pediatric population

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## Abstract

Biomarkers are routinely used for noninvasive identification or monitoring of disease processes in clinical practice, as well as surrogate end points for drug development. There is a significant lack of data regarding biomarkers in children. An understanding of biomarker levels in a healthy pediatric cohort is essential as more studies begin to apply noninvasive biomarkers to pediatric populations. Brain-derived neurotrophic factor (BDNF) functions in neuronal survival and plasticity and is associated with exercise capacity and inflammatory disease processes. Osteopontin (OPN) plays a regulatory role in inflammation and may be a clinically useful biomarker of cardiovascular disease processes, ventricular remodeling, and skeletal muscle regeneration. This study describes our initial experience with a cohort of healthy pediatric patients and seeks to provide normal values of BDNF and OPN with correlation to age, gender, and cardiovascular and fitness measures. Serum BDNF and plasma OPN were measured using enzyme-linked immunosorbent assay in 33 healthy pediatric subjects. Subjects underwent complete cardiac evaluation, including echocardiography, exercise stress testing, and health risk assessment. The 5th–95th percentile was 5.63–37.86 ng/ml for serum BDNF and 4.9–164.9 ng/ml for plasma OPN. Plasma OPN correlated with number of days of exercise per week ( $r = 0.46$ ,  $p = 0.008$ ). No other correlations were significant. This study provides the initial data on serum BDNF and plasma OPN in children and begins to explore the relationships of BDNF and OPN to cardiovascular health and fitness in the pediatric population.

## Keywords

Pediatric, reference range, biomarker, cardiovascular, brain-derived neurotrophic factor, osteopontin

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## Introduction

Biomarkers are routinely used for noninvasive identification or monitoring of disease processes in clinical practice, as well as surrogate end points for drug development. Despite their widespread use in clinical practice and research, there remains a lack of data regarding biomarkers in children, let alone normal values.<sup>1</sup> Additionally, given the inherent differences in disease pathogenesis in children, as well as the potential effect growth and development can have on biologic processes, specific attention must be paid to the pediatric population in the development of biomarkers.<sup>2</sup>

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Brain-derived neurotrophic factor (BDNF) was initially identified in 1981 and its function in promoting neuronal survival, plasticity, and efficacy has been well established.<sup>3–6</sup> Recent publications support a role for BDNF outside of the central nervous system and its association with exercise capacity and inflammatory disease processes, including cardiovascular disease and asthma.<sup>7–12</sup> Osteopontin (OPN) is an extracellular matrix protein with a regulatory role in inflammation and biomineralization through its effects on cellular adhesion, migration, differentiation, and survival.<sup>13</sup> Increasing evidence has suggested that OPN has the potential to be a clinically useful biomarker of cardiovascular disease processes, ventricular remodeling, and skeletal muscle regeneration.<sup>14–21</sup>

Given their role in inflammatory and cardiovascular disease processes, BDNF and OPN have significant potential as biomarkers both in adult and pediatric populations. Indeed, our pilot data demonstrated the potential for both BDNF and OPN for assessing cardiovascular disease severity in patients with Duchenne muscular dystrophy.<sup>22</sup> There are limited data on their normal ranges in healthy adults and currently no data available specifically in children. An understanding of biomarker levels in a healthy pediatric cohort is essential as more studies begin to apply noninvasive biomarkers to pediatric populations. This study describes our initial experience with a cohort of healthy pediatric patients and seeks to provide normal values of BDNF and OPN with correlation to age, gender, and cardiovascular and fitness measures.

## Materials and methods

### Patient enrollment

The Vanderbilt University Institutional Review Board approved the study protocol. Appropriate consent and assent were obtained for all participants. Healthy pediatric subjects aged 8–18 years who were referred to a pediatric cardiologist and underwent treadmill testing for chest pain, syncope, palpitations, or tachycardia were recruited. Exclusion criteria were: (1) abnormal treadmill test, (2) presence or concern for structural or functional cardiovascular disease (congenital heart disease, cardiomyopathy, or any secondary cardiovascular disease), (3) abnormal echocardiogram, and (4) arrhythmia or clinical concern for arrhythmia.

### Cardiac evaluation

Participants underwent a thorough cardiac evaluation as indicated by clinical presentation, including a history and physical by a pediatric cardiologist and an electrocardiogram in all participants. Echocardiograms and Holter monitors were ordered as deemed necessary. All patients were determined to be healthy by the primary pediatric cardiologist. All clinic notes, electrocardiograms, echocardiograms,

treadmill tests, and Holter monitors were reanalyzed by study author (JHS) to ensure all subjects meet the inclusion/exclusion criteria.

### Health risk assessment and blood sample

After consent/assent was obtained, and prior to treadmill testing, a health risk assessment was completed by the participant in order to assess their general health and activity levels (Online Supplemental Material). General health was self-reported as a five-point ordinal variable ranging from excellent to poor. The number of days of aerobic exercise was assessed as a multiple choice question with responses from 0 to 7 days per week. A 20 ml blood sample was then obtained, with 10 ml aliquoted into a vacutainer with ethylenediaminetetraacetic acid for plasma and 10 ml aliquoted into a vacutainer with clot activator for serum. Blood samples were centrifuged, and plasma or serum was separated and stored in  $-80^{\circ}\text{C}$  until used for biomarker measurements.

### Treadmill testing

Participants underwent a treadmill stress test using the standard Bruce protocol as part of their clinical evaluation.<sup>23</sup> Oxygen consumption ( $\text{VO}_2$ ) was measured during the treadmill stress test. Treadmill data collected included duration of exercise,  $\text{VO}_2$ , predicted  $\text{VO}_2$ , anaerobic threshold, and predicted anaerobic threshold. Treadmill tests were interpreted by the primary cardiologist and reviewed by a study author (JHS) and were normal in all participants.

### Imaging analysis

All clinically indicated echocardiograms performed at our institution were reviewed by a study author (JHS) and confirmed to be normal. Functional analysis of echocardiograms was performed by the same reader (JHS) based on American Society of Echocardiography guidelines and consisted of: (1) left ventricular chamber dimensions and corresponding  $z$ -scores measured on M-mode images in the short axis at the level of the papillary muscles; (2) fractional shortening calculated from the left ventricular internal dimensions in systole and diastole; (3) left ventricular ejection fraction (LVEF) measured using the 5/6 area length method from the short axis at the level of the papillary muscles and the apical four-chamber views; (4) left atrial dimension and corresponding  $z$ -score measured in the long axis on two-dimensional imaging; (5) mitral E and A wave velocities; and (6) E' tissue Doppler velocities measured at the septum and free wall.<sup>24–27</sup>

### Biomarker analysis

BDNF was measured from serum using the Quantikine BDNF enzyme-linked immunosorbent assay (ELISA) kit

(R&D systems, Minneapolis, Minnesota, USA; catalog no. DBD00). Standards were generated with a 1:2 serial dilution of reconstituted standard. Samples were prepared by diluting 20-fold with a calibrator diluent for serum samples. Assay was read using BioTek (Winooski, Vermont, USA) Epoch microplate reader software. Four quality control (QC) samples were included on each plate. All assays were run in duplicates and the average % coefficient of variation (CV) was 5% or less.

OPN was measured from plasma using the Quantikine ELISA kit (R&D catalog no. DOST00). Standards were generated by a 1:2 serial dilution of reconstituted standard in the kit. Samples were diluted 25-fold with appropriate diluent. Assay was read using BioTek Epoch microplate reader software. Four QC samples were included on each plate. All assays were run in duplicates and the average %CV was less than 7.5%.

### Statistical analysis

Differences in biomarker levels between groups were assessed using Mann–Whitney *U* test. Correlations between continuous variables were evaluated using Spearman's rho. Analyses were performed using Stata Statistical Software: Release 14 (StataCorp LP, College Station, Texas, USA). Study data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at Vanderbilt University Medical Center.<sup>28</sup> REDCap is a secure, web-based application designed to support data capture for research studies, providing (1) an intuitive interface for validated data entry; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for importing data from external sources. We report the results of all analyses performed and make no formal adjustment for multiple comparisons.

### Results

Thirty-three participants with median age 14.7 years (interquartile range (IQR) 13.4–16.0) were included in the study. Eighteen participants (54.5%) were male. The remaining demographic data, including reason for referral, are present in Table 1. Twenty-eight participants underwent an echocardiogram at our institution. Each echocardiogram was normal, including normal functional indices, chamber sizes, mitral inflow velocities, and left ventricular E' Doppler velocities (Table 2). Two additional participants had an echocardiogram, which was unavailable for review, performed at a different institution; both of these were reported as normal. Thirty-two participants completed treadmill stress testing, with 25 meeting their predicted VO<sub>2</sub> maximum and anaerobic threshold. Four participants reached their predicted anaerobic threshold but failed to reach their predicted VO<sub>2</sub> maximum; three participants failed to reach

**Table 1.** Baseline demographics.<sup>a</sup>

Sex	
Male	18 (54.5%)
Age (years)	14.7 (13.4–16.0)
Race and ethnicity	
Non-Hispanic Caucasian	21 (63.6%)
Non-Hispanic African American	8 (24.2%)
Hispanic Caucasian	2 (6.1%)
Unknown	2 (6.1%)
BMI (kg/m <sup>2</sup> )	20.3 (19.8–24.0)
BSA (m <sup>2</sup> )	1.7 (1.4–1.8)
Reason for cardiology referral	
Chest pain	18 (54.5%)
Syncope	7 (21.1%)
Palpitations	3 (9.1%)
Shortness of breath	2 (6.1%)
Tachycardia	2 (6.1%)
Premature ventricular complexes	1 (3.0%)

BMI: body mass index; BSA: body surface area.

<sup>a</sup>N = 33. Variables presented as median (interquartile range) or N (%).

**Table 2.** Echocardiographic and treadmill test results.

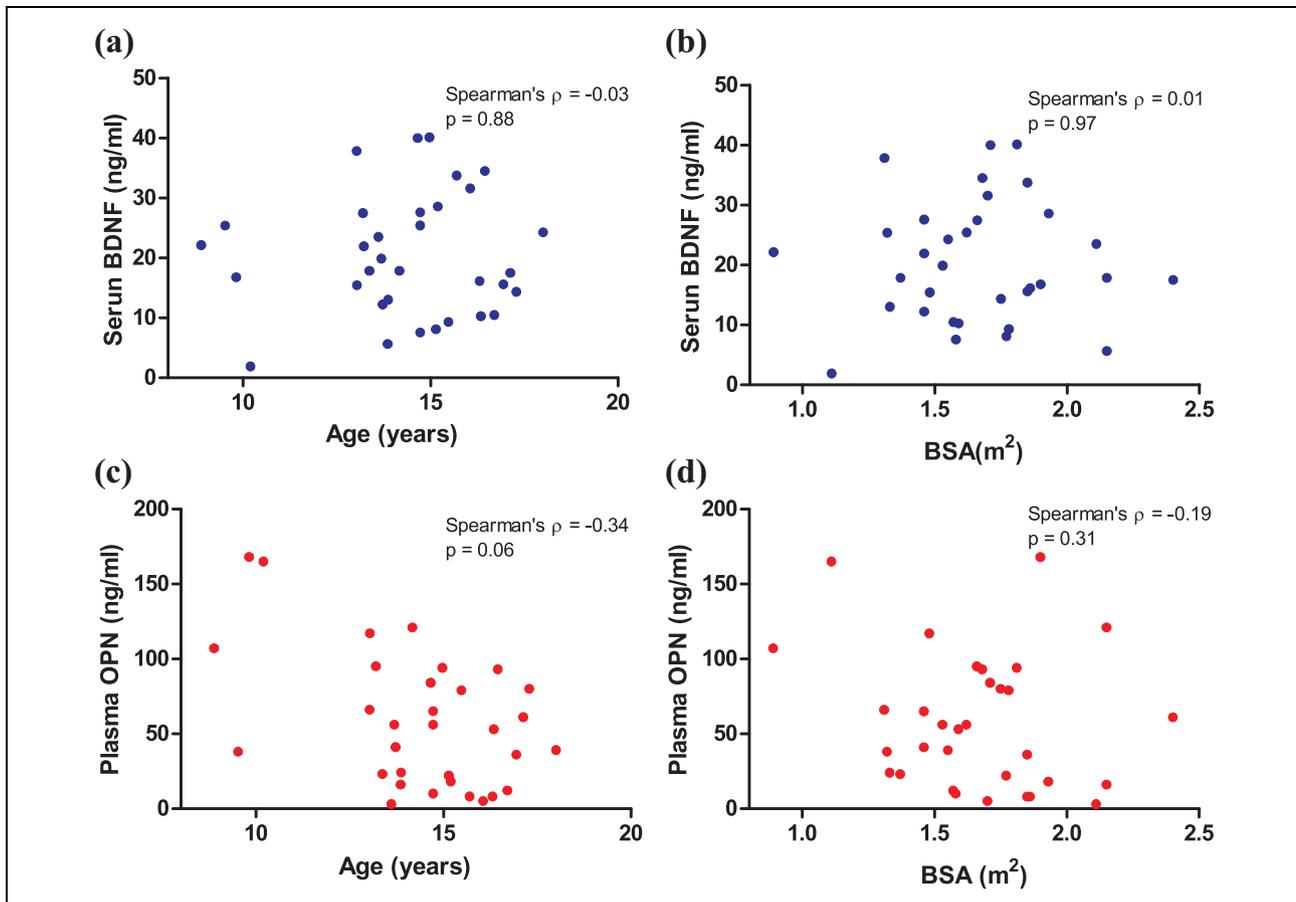
	Median (IQR)
Echocardiographic measures	
LV ejection fraction (%)	61.7 (58.6–63.8)
Shortening fraction (%)	39.5 (36.8–42.2)
LV internal dimension—diastole (cm)	4.8 (4.5–5.1)
LV internal dimension—systole (cm)	3.0 (2.6–3.2)
Left atrial dimension (cm)	2.9 (2.7–3.3)
Mitral E wave velocity (cm/s)	88.3 (78.5–94.3)
Mitral A wave velocity (cm/s)	39.0 (34.1–47.2)
LV free wall E' tissue Doppler (cm/s)	17.5 (16.1–19.4)
LV septum E' tissue Doppler (cm/s)	12.9 (11.8–14.1)
Treadmill test	
Duration of exercise (min)	12.7 (10.7–14.0)
VO <sub>2</sub> maximum(ml/kg/min)	40.7 (35.3–49.1)
Anaerobic threshold (ml/kg/min)	29.2 (25.3–31.8)

IQR: interquartile range; LV: left ventricular; VO<sub>2</sub>: oxygen consumption.

both. Median duration of exercise, VO<sub>2</sub> maximum, and anaerobic threshold are summarized in Table 2.

### Health risk assessment

The majority of participants rated their health as comparable to that of their peers (*N* = 27, or 81%) with 30 participants (91%) rating their health as “very good” or “excellent.” Participants were relatively active and exercised a median of 5 days per week (IQR 4–7). Five participants self-reported mild asthma, three requiring medications, and one self-reported increased blood pressure. One participant smoked less than 10 cigarettes per day and two others reported smoking in the past. As expected, the peak VO<sub>2</sub> and anaerobic thresholds correlated with the number of days of reported exercise (*r* = 0.53, *p* = 0.002 and *r* = 0.42, *p* = 0.016). Fractional shortening



**Figure 1.** Association of BDNF and OPN with age and BSA. (a) Association of BDNF with age. (b) Association of BDNF with BSA. (c) Association of OPN with age. (d) Association of OPN with BSA. BDNF: brain-derived neurotrophic factor; OPN: osteopontin; BSA: body surface area.

correlated weakly with number of days exercised per week ( $r = 0.38$ ,  $p = 0.049$ ).

### Biomarker indices

There was no statistical difference between sex for BDNF or OPN ( $p = 0.11$ ,  $p = 0.12$ , respectively). Additionally, BDNF and OPN did not differ based on status of reaching predicted  $\text{VO}_2$  maximum ( $p = 0.48$ ,  $p = 0.71$ , respectively) or anaerobic threshold ( $p = 0.97$ ,  $p = 0.89$ , respectively). No significant bivariate correlations between BDNF or OPN levels with age, body mass index (BMI), body surface area (BSA), LVEF, shortening fraction, duration of exercise,  $\text{VO}_2$  maximum, and anaerobic threshold were found (all  $|r| < 0.34$ ; Figure 1 and Table 3). Plasma OPN correlated with number of days of exercise per week ( $r = 0.46$ ,  $p = 0.008$ ). There were no differences in BDNF or OPN levels in participants with or without asthma. BDNF was lower in subjects that rated their general health as excellent as compared with those who rated it as very good or worse ( $p = 0.012$ ). Patients with a history of smoking had increased BDNF ( $19,300 \text{ pg/ml} \pm 9300$  vs.  $35,100 \text{ pg/ml}$

**Table 3.** Association of serum BDNF and plasma OPN with anthropometric, health risk assessment, echocardiographic, and treadmill stress test characteristics.

	Serum BDNF		Plasma OPN	
	Spearman's $r$	$p$ Value	Spearman's $r$	$p$ Value
Age (years)	-0.03	0.88	-0.34	0.06
BMI ( $\text{kg/m}^2$ )	0.09	0.61	-0.27	0.14
BSA ( $\text{m}^2$ )	0.01	0.97	-0.19	0.31
Days of exercise per week	-0.10	0.57	0.46	0.01
LVEF	-0.23	0.24	0.21	0.31
FS	-0.32	0.09	-0.16	0.41
Duration of exercise (min)	0.19	0.28	0.32	0.07
$\text{VO}_2$ maximum ( $\text{ml/kg/min}$ )	0.20	0.28	0.33	0.07
Anaerobic threshold ( $\text{ml/kg/min}$ )	0.13	0.48	0.18	0.33

BDNF: brain-derived neurotrophic factor; BMI: body mass index; BSA: body surface area; LVEF: left ventricular ejection fraction; FS: shortening fraction; OPN: osteopontin;  $\text{VO}_2$ : oxygen consumption.

**Table 4.** Serum BDNF and plasma OPN values of healthy pediatric population.

	Median	Interquartile range	5th–95th percentile
Serum BDNF (ng/ml)	17.67	12.21–25.39	5.63–37.86
Plasma OPN (ng/ml)	54.5	19.8–88.4	4.9–164.9

BDNF: brain-derived neurotrophic factor; OPN: osteopontin.

$\pm 4400$ ,  $p = 0.018$ ) but no difference in OPN; these three patients were removed from the median values reported in Table 4. The median values, IQR, and 5th–95th percentile ranges for the population are presented in Table 4.

## Discussion

This is the first study to provide data on serum BDNF and plasma OPN levels in healthy children and to evaluate their association with cardiovascular and fitness measures. There are currently no published reference ranges for either serum BDNF or plasma OPN in adults, let alone children. Our study provides a foundation for future use of BDNF and OPN as biomarkers in the pediatric population. The ranges for serum BDNF and plasma OPN in our study are comparable to published studies in adults.<sup>29–43</sup> Our cohort did not demonstrate any associations between BDNF or OPN and demographic or anthropometric data, though there was a trend toward correlation between OPN and age. Subset analysis demonstrated a correlation between OPN and age in females, and further investigation will be needed to more fully delineate any possible age-dependent effect on BDNF and OPN levels.

Prior studies have found lower levels of BDNF associated with depression.<sup>44,45</sup> Given these previous findings, it is possible that the self-assessed general health was acting as a surrogate marker of depression in our cohort. However, our data demonstrated lower BDNF in those with self-reported excellent health and higher BDNF in patients with only very good or worse health. This direction is opposite from what would be expected if this were due to depression. Of note, the majority of respondents recorded their health as very good or excellent. We hypothesize that this finding is not clinically significant given our sample size and the limited heterogeneity of responses to self-reported general health.

The complex relationship of BDNF with exercise continues to stimulate debate despite extensive work in the field. Although the positive effect of acute aerobic exercise on serum BDNF levels is fairly well established, the association of BDNF with chronic physical activity and cardiorespiratory fitness is not well understood.<sup>46</sup> Prior studies in healthy adults have demonstrated a significant negative correlation of  $VO_2$  maximum with serum BDNF.<sup>32,34,36</sup> Interestingly, in our cohort, BDNF did not significantly

correlate with  $VO_2$  maximum, anaerobic threshold, or duration of exercise during treadmill stress test. Additionally, the trend was toward a positive correlation between BDNF and the measured markers of cardiorespiratory fitness. It is unclear if this represents a difference in BDNF between children and adults or the fact that our study included only healthy children with a relatively narrow range of  $VO_2$  maximum.

Our study did not demonstrate relationships between BDNF or OPN with any of the evaluated echocardiographic markers of cardiac function. This finding is not unexpected despite the body of evidence supporting their roles as biomarkers in cardiovascular disease. The children included in our cohort were all healthy with normal cardiac evaluations; therefore, the evaluated echocardiographic measures demonstrated little variation within the cohort. It is possible that a relationship between BDNF or OPN and markers of cardiovascular function would become evident with the evaluation of a more diverse population that included children with cardiovascular disease.

Given the previously demonstrated relationships with various disease processes, BDNF and OPN have promise as clinically useful biomarkers in children. Both biomarkers have demonstrated associations with cardiovascular disease and inflammatory processes. Therefore, we postulate that diseases characterized by chronic ventricular dysfunction and inflammation, such as myocarditis, cardiomyopathies, and Kawasaki disease, might warrant investigation using BDNF and OPN.

## Limitations and future research

The predominant limitation of our study is the small sample size, which precluded the precise determination of 95th percentile clinical reference following Clinical and Laboratory Standards Institute (CLSI) guidelines.<sup>47</sup> In addition, the range of normal values reported here is specific to the Quantikine kits and may not translate to other methods of determining BDNF and OPN levels. A second limitation is that all patients were undergoing treadmill testing as part of their clinical evaluation and some patients could have had undetected cardiovascular disease or as yet undiagnosed chronic illnesses. However, we consider this unlikely, as all participants were selected based on a low likelihood of having disease and all cardiovascular studies were reviewed by two cardiologists to ensure normal results. This study only evaluated patients aged 8–18 years old. Future studies in younger children should be pursued in order to evaluate normal levels in children less than 8 years of age. Further studies are also necessary to evaluate the relationship of BDNF to both acute and chronic exercise, as well as cardiorespiratory fitness, in children. Additional work is required to establish the utility of BDNF and OPN as biomarkers for cardiovascular disease in children.

## Conclusions

This study provides the initial data on serum BDNF and plasma OPN in children and begins to explore the relationships of BDNF and OPN to cardiovascular health and fitness in the pediatric population.

## Authors' note

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Declaration of Conflicting Interests

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## Supplemental Material

Supplemental material for this article is available online.

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