Circulating Brain Natriuretic Peptide Values in Healthy Men Before and After Exercise

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Circulating brain natriuretic peptide (BNP) has recently served as a marker of left ventricular dysfunction, while treadmill exercise has been used clinically for assessing cardiac problems. The current study was undertaken to investigate the possible effect of exercise on circulating BNP concentrations. A total of 138 blood samples from 23 healthy men aged 23 to 27 years (mean, 25) was analyzed. All subjects maintained a similar diet and physical activity a week before the test. Plasma samples were drawn at baseline and immediately, 1 hour, 4 hours, 24 hours, and 48 hours after exercise. Every subject completed exercise according to the Bruce protocol with normal electrocardiogram (EKG) results. Specimens were simultaneously analyzed for concentrations of plasma BNP and other biochemical parameters including aldosterone (Aldo), adrenocorticotropic hormone (ACTH), cortisol, creatine phosphokinase (CPK), triiodothyronine (T_3), and thyroxine (T_4). Hematocrit (Hct), red blood cell count (RBC), and hemoglobin (Hgb) were analyzed immediately after each sampling. A transient increase in plasma BNP was found immediately after exercise (8.21 *v* baseline value, 3.38 pg/mL, $P < .01$). Twenty-two percent (5/23 subjects) had values above the normal limit (18.2 pg/mL). The Hct-corrected concentrations of plasma BNP were also significantly increased immediately after exercise compared with the baseline values (0.17 ± 0.04 *v* baseline, 0.07 ± 0.01, $P < .01$), but returned rapidly to baseline. Weak, but significantly positive, relationships were found between plasma BNP and T_3 and T_4. Our study demonstrates that circulating BNP values increase immediately after treadmill exercise in young adults. The elevation did not result from exercise-induced hemoconcentration. BNP concentration, however, returned to normal levels within 1 hour after exercise. Thus, we suggest that plasma samples should not be taken immediately after exercise to avoid possible artifacts.

BRAIN (B TYPE) natriuretic peptide (BNP) has biologic activities similar to those of atrial natriuretic peptide (ANP), including diuretic, natriuretic, hypotensive, and smooth muscle relaxant activities, as well as the ability to inhibit the renin-aldosterone axis.[1] It is mainly produced and released into the circulation by the ventricle in response to increased ventricular wall pressure or stretching.[2,3] Currently, BNP is the only Food and Drug Administration (FDA)-cleared marker for the diagnosis of congestive heart failure (CHF).[4] High plasma BNP concentrations have been observed in disease states characterized by fluid overload.[2,3] BNP serial profiles also suggest that in contrast to transient BNP increases, persistent elevations are associated with potentially decompensatory states.[5] Circulating BNP appears to be superior to ANP as a prognostic marker in patients with CHF[6] and as an early responsive "emergency" gene to stress in the myocardium.[7] BNP is expressed earlier and at higher levels than ANP.[8] Diastolic stretch and neuroendocrine mechanisms, especially angiotensin, may initiate the cardiac growth process and result in BNP gene expression.[9] Physiologic increases of BNP may also decrease plasma renin activity and the urinary aldosterone excretion rate.[1]

In practice, there is increasing recognition of the importance of BNP in the pathophysiology, diagnosis, and treatment of certain cardiac disorders.[5] In addition, BNP may facilitate the early diagnosis of heart failure by detecting asymptomatic left ventricular dysfunction or myocardial ischemia and may be used as an objective blood test for these indications.[10,11] Moreover, Cowie et al[12] found that BNP was the most useful natriuretic peptide for routine identification of patients with cardiac dysfunction who might require further investigation.

Treadmill exercise testing by the Bruce protocol is a well-established method for clinical evaluation of cardiac problems. It is unknown whether treadmill exercise can influence BNP measurement. Acute exercise may cause hemoconcentration, which may alter serum hormone values. In this study, we measured plasma BNP along with other hormones to clarify possible artifacts of BNP measurement of the treadmill exercise.

MATERIALS AND METHODS

A total of 138 blood specimens from 23 healthy men ranging from 23 to 27 years of age (average, 25) was collected for measurement of plasma BNP, aldosterone (Aldo), adrenocorticotropic hormone (ACTH), cortisol, creatine phosphokinase (CPK), triiodothyronine (T_3), and thyroxine (T_4). All of the subjects were healthy, normotensive volunteers free from cardiovascular disease. They consumed identical diets and maintained similar activity for a week before the study. Plasma samples were obtained at baseline, immediately, 1 hour, 4 hours, 24 hours, and 48 hours after treadmill exercise.

The standard Bruce treadmill protocol was used[13]. All subjects finished the protocol with normal electrocardiogram (EKG) results. The study was approved by the ethical review board of our hospital. All patients gave written informed consent before participation.

Specimens were collected into tubes containing EDTA. After separation, plasma were stored at –70°C. Samples were simultaneously analyzed for concentrations of plasma BNP and other biochemical parameters. Plasma BNP concentrations were determined by a commercial immunoradiometric assay (IRMA) kit (SHIONORIA BNP, CIS BIO-international, Osaka, Japan; normal mean, 5.5; normal reference, < 18.2 pg/mL for men). The averaged intra- and interassay coefficients of variation were 2.6% and 4.4%, respectively. The detection limit was assessed as 2.0 pg/mL. Cross-reactions were human ANP, less than 10^{-5}%; CNP, less than 10^{-5}%; albumin, less than 6.7

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$\times 10^{-6}$%; and immunoglobulin G (IgG), less than 1.6×10^{-7}%. The average recovery ranged from 80% to 110%.

Plasma Aldo, ACTH, cortisol, CPK, thyroid-stimulating hormone (TSH), T_3, and T_4 were measured with commercial kits. Reference ranges were 37 to 240 pg/mL for Aldo; 9 to 52 pg/mL for ACTH, 2.5 to 25 μg/dL for cortisol; 5 to 70 IU/L for creatine kinase (CK); 86 to 187 ng/dL for T_3, and 4.5 to 12.5 μg/dL for T_4.

The hematocrit (Hct), hemoglobin (Hgb), and red blood cell count (RBC) were measured by routine automatic methods. BNP values were also corrected by the corresponding Hct and expressed as BNPc.[14]

Statistical Analysis

The results are presented as mean values with the standard errors of the mean indicated. Because the distributions of plasma biochemical parameters were highly skewed, we log transformed the values to improve normality. We used generalized estimating equations with an identity link function to model the change in BNP levels over time by contrasting with the baseline level with or without adjusting for other covariates assuming an exchangeable correlation structure between the repeated measures.[15] Spearman rank-correlation coefficients were used to indicate the linear association between BNP and the other plasma biochemical parameters. All reported *P* values are with the statistical significance set at less than .05.

RESULTS

The plasma values of BNP, biochemical parameters, and hematologic profiles of the studied subjects are shown in Table 1. Hct, Hgb, and RBC significantly increased immediately after exercise as compared with the baseline values (50.1% *v* 46.6%, 16.7 *v* 15.8 mg/dL, and 5.59 *v* 5.30 M/dL; $P < .05$, respectively). The plasma BNP, Aldo, ACTH, and CPK values also significantly increased immediately after exercise as compared with the baseline values (8.21 *v* 3.38 pg/mL for BNP, 228 *v* 152 pg/mL for Aldo, 141 *v* 52 pg/mL for ACTH, and 69.2 *v* 54.4 IU/L for CPK; $P < .01$, respectively). Among the parameters, Aldo, cortisol, ACTH, CPK, T_3, and T_4 changes positively correlated with Hct ($r = .30$, .30, .56, .21, .29, and .21; $P < .01$, respectively, except for T_4 at $P < .05$).

While the mean BNP values were within the reported normal range, 5 of 23 volunteers (22%) had elevated values immediately after exercise. They also had higher baseline values (6.42 ± 1.41 *v* 2.61 ± 0.28 pg/mL; $P = .05$). There was no significant change in BNP values at any other postexercise time as compared with the baseline (1 hour, 3.77 ± 0.53; 4 hours, 4.12 ± 1.16; 24 hours, 3.0 ± 0.29; and 48 hours, 3.60 ± 0.80 pg/mL) as shown in Fig 1 and Table 1. The Hct and Hgb values demonstrate significant hemoconcentration immediately after exercise (Table 1). However, the Hct adjusted BNP concentration (either using BNPc or using multiple regression analysis) values was still significantly increased immediately after exercise as compared with the baseline values (BNPc, 0.17 ± 0.04 *v* 0.07 ± 0.01; $P < .01$). No significant change in BNPc was found at any other postexercise time as compared with the baseline (1 hour, 0.08 ± 0.01; 4 hours, 0.09 ± 0.03; 24 hours, 0.07 ± 0.01; and 48 hours, 0.08 ± 0.02) (Table 1). Interestingly, a weak, but significant, correlation was found between plasma BNP values and T_3 and T_4 ($r = .17$; $P = .046$ and $r = .21$; $P = .013$). There was no significant relationship between plasma BNP values and those of Aldo, ACTH, or CPK with or without controlling for Hct.

Table 1. Values of Circulating Biochemical Parameters Before and After Treadmill Exercise

	Pre	Imm	1 Hour	4 Hours	24 Hours	48 Hours
Hct (42-52 %)	46.6 ± 0.5*	50.1 ± 0.5	44.7 ± 0.5*	44.1 ± 0.4*	44.0 ± 0.4*	43.5 ± 0.4*
Hgb (14-18 mg/dL)	15.8 ± 0.2*	16.7 ± 0.2	15.4 ± 0.2*	15.1 ± 0.2*	15.0 ± 0.2*	14.9 ± 0.2*
RBC (4.7-6.1 M/dL)	5.30 ± 0.08*	5.59 ± 0.08	5.11 ± 0.07*	5.06 ± 0.05*	5.01 ± 0.07*	4.97 ± 0.08*
BNP (<18.2 pg/mL)	3.38 ± 0.50*	8.21 ± 2.02	3.77 ± 0.53†	4.12 ± 1.16*	3.0 ± 0.29*	3.60 ± 0.80*
BNPc	0.07 ± 0.01*	0.17 ± 0.04	0.08 ± 0.01†	0.09 ± 0.03†	0.07 ± 0.01*	0.08 ± 0.02*
CPK (5-70 IU/L)	54.4 ± 5.0*	69.2 ± 6.2	56.3 ± 5.0*	57.2 ± 5.0*	56.9 ± 4.5*	50.3 ± 2.9*
ACTH (9-52 pg/mL)	52.3 ± 4.6*	141.4 ± 23.8	35.9 ± 3.4*	30.3 ± 2.5*	33.1 ± 2.3*	34.1 ± 1.9*
Cortisol (2.5-25 μ g/dL)	12.2 ± 0.8	11.9 ± 0.8	9.82 ± 0.77†	6.94 ± 0.53*	8.78 ± 0.58*	7.69 ± 0.54*
Aldosterone (37-240 pg/mL)	151 ± 10*	228 ± 13	219 ± 12	135 ± 12*	149 ± 11*	141 ± 10*
T_3 (86-187 ng/dL)	113 ± 7†	120 ± 4	101 ± 3*	99.0 ± 3.6*	98.8 ± 3.4*	101 ± 6*
T_4 (4.5-12.5 μ g/dL)	7.34 ± 0.26	7.70 ± 0.26	6.97 ± 0.27†	7.14 ± 0.33†	7.27 ± 0.23	7.06 ± 0.27†

NOTE. Data are expressed as mean ± SEM; parentheses represent normal reference values.

Abbreviations: Pre, pre-exercise; Imm, immediately after exercise; 1, 4, 24, and 48 hours, 1, 4, 24, and 48 hours after exercise; Hct, hematocrit; Hgb, hemoglobin; RBC, red cell count; BNP, brain natriuretic peptide; BNPc, Hct corrected BNP; CPK, creatine phosphokinase; ACTH, adrenocorticotropic hormone; T_3, triiodithyronine; T_4, thyroxine.

*$P < .01$ *v* Imm.

†$P < .05$ *v* Imm.

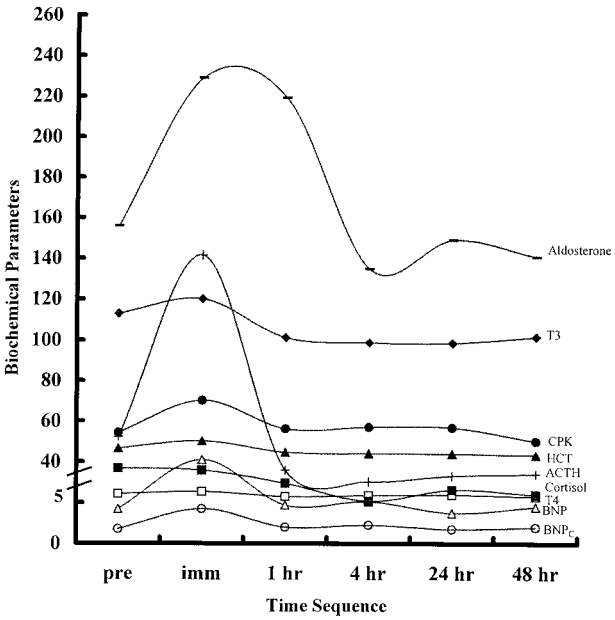

Fig 1. Changes of circulating biochemical parameters in healthy men before and after treadmill exercise. Abbreviations are defined in Table 1.

DISCUSSION

Hemoconcentration is a common phenomenon encountered in subjects after acute exercise. Circulating hormonal values after exercise may reflect acute transcapillary movements of water, which resolve shortly after exercise ceases.[14,16] Therefore, any biochemical measurements, particularly hormone values, should be properly corrected for such acute changes. In the current study, significant increases in Hct, Hgb, and RBC immediately after exercise implied the presence of hemoconcentration. Positive correlations between BNP, Aldo, ACTH, T_3, and T_4 values with Hct suggested that hemoconcentration did, in fact, affect the concentrations of circulating hormones. However, a significant increase in BNP values immediately after exercise, even after correction for Hct, was also found in the current study, suggesting that factor(s) other than hemoconcentration might contribute to the secretion of BNP immediately after treadmill exercise.

The effect of exercise on changes in plasma BNP concentration have been related to angiotensin-converting enzyme (ACE) gene polymorphism and cardiovascular disorders (CVD).[17-20] Increased BNP levels with training were found to be dependent on ACE genotype. Exercise-induced BNP increase also occurred in individuals with CVD.[21] Treatment with ACE inhibitors to improve left ventricular (LV) function may also decrease BNP in hypertensive patients with LV hypertension (LVH).[21] These results suggest that factors, such as mass size, volume overload, wall stretch, and hemodynamics, as well as neurohormones, may contribute to exercise-induced increases in BNP.[20,22] On the other hand, it was also found that increased BNP might serve as a protective and compensatory mechanism against further deterioration.[1,18,23] It appears that increased levels of BNP are a natural physiologic response to hypertrophic signals in the heart.[24]

A transient increase in plasma BNP concentration was also found in normal subjects after exercise.[25] Systolic blood pressure and plasma epinephrine values were found to be related to BNP release, suggesting that sympathetic stimulus may also play a role.[25] Recently, Ohba et al[26] found that prolonged strenuous exercise in healthy men increased BNP and ANP levels, which correlates with the acute increase of BNP found in our study.[26] Nicholson et al[18] found a nonsignificant trend of increasing plasma BNP and Aldo after treadmill exercise in a small group of older subjects. Plasma BNP values could also be related to volume-related stimuli, such as a high-sodium diet, passive leg raising, or the sitting position.[1,27]

The renin-aldosterone axis is important for the overall regulation of body fluid and cardiovascular homeostasis in men.[1] The ACTH-cortisol axis plays a major role in modulation of the stress response.[28] Creatine phosphokinase is found mainly in skeletal and cardiac muscle and is commonly elevated by acute physical exertion. Therefore, we assessed the relationship between these hormonal levels and plasma BNP levels. However, although plasma BNP, Aldo, and ACTH significantly increased immediately after exercise, changes in these hormonal levels do not appear to be attributable to changes in BNP. It should be noted that infusion of synthetic BNP usually decreases Aldo levels.[1,22,29] The increased Aldo levels found in our exercised subjects could be partially due to hemoconcentration.

Interestingly, a weak, but significant, correlation was found between plasma BNP values and T_3 and T_4 concentrations. The action of thyroid hormones (THs) on both heart contractility and the systemic vascular system is rapid.[30] The consequences of increased heart rate, reduced systemic vascular resistance, and enhancement of diastolic function result in increased preload and cardiac output.[31] T_3 is thought to be the final mediator presumably involving T_3 nuclear receptors in the heart.[32] There is only minimal conversion of T_4 into T_3 in the heart; the predominant source of T_3 comes from T_3 in the circulation.[33] However, the prohormone-T_4 also stimulated atrial ANP expression in a rat model.[34] Likewise, Kohno et al[21] found that THs were able to stimulate BNP release from the heart.[21] Plasma BNP was also positively correlated with T_4 levels in both humans and rats.[35] The magnitude of the T_4 influence on plasma BNP is unknown. Although we have shown a relationship between plasma BNP and T_4 ($P = .013$), the T_4 values in our volunteers were all within normal ranges.

Curiously, we found that those subjects with elevated BNP values immediately after exercise also had higher pre-exercise (baseline) BNP values (6.42 *v* 2.61 pg/mL; $P = .05$). Whether this is due to individual characteristics or some kind of subclinical abnormality requires further clarification.

In conclusion, measurement of circulating BNP values and treadmill exercise are both clinically available methods for evaluating patients with suspected cardiovascular dysfunction. A significant increase in plasma BNP was found in healthy subjects immediately after treadmill exercise, which returned to normal within 1 hour. Thus, we suggest that for clinical measurement of BNP, plasma samples should not be taken immediately after exercise to avoid possible physiologic variations.

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