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Plasma NT-proBNP increases in response to LPS administration in healthy men

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[1]*Department of Medicine III, Division of Endocrinology and Metabolism, Medical University of Vienna, Austria;* [2]*Research Department, BRAHMS, Biotechnology Centre, Hennigsdorf, Germany; and* [3]*Department of Medicine II, Division of Cardiology, Medical University of Vienna, Austria*

Vila G, Resl M, Stelzeneder D, Struck J, Maier C, Riedl M, Hülsmann M, Pacher R, Luger A, Clodi M. Plasma NT-proBNP increases in response to LPS administration in healthy men. *J Appl Physiol* 105: 1741–1745, 2008. First published October 9, 2008; doi:10.1152/japplphysiol.90442.2008.—Circulating levels of B-type natriuretic peptide (BNP) and NH_2-terminal-proBNP (NT-proBNP) increase in response to volume overload and help in the differential diagnosis of acute heart failure. Elevated plasma BNP levels are observed also in sepsis and do not always correspond to left ventricular dysfunction. Here, we investigated plasma NT-proBNP fluctuations in response to human bacterial endotoxinemia, an experimental model of systemic infection and inflammation. *Escherichia coli* endotoxin (LPS) (2 ng/kg) was administered to 10 healthy volunteers in a randomized, placebo-controlled, cross-over design. Plasma NT-proBNP, C-reactive protein (CRP), COOH terminal pro-endothelin-1 (CT-proET-1), and midregional-pro-adrenomedullin (MR-proADM) were measured at hourly intervals for 6 h. LPS administration induced a continuous increase in plasma NT-proBNP that reached peak values after 6 h (40.7 ± 7.9 vs. 16.1 ± 3.2 pg/ml in placebo days, mean ± SE; $P = 0.023$). The profile of changes in NT-proBNP correlated to changes in body temperature ($P < 0.001$), heart rate ($P = 0.005$), CRP ($P < 0.001$), and CT-proET-1 ($P = 0.008$), but not to blood pressure values. Our results demonstrate that plasma NT-proBNP increases in a model of systemic infection/inflammation in healthy men with normal heart function. This finding emphasizes the necessity to consider concomitant infections when interpreting elevated circulating NT-proBNP concentrations.

B-type natriuretic peptide; C-reactive protein; bacterial endotoxin; infection; inflammation; human

B-TYPE NATRIURETIC PEPTIDE (BNP) is secreted from atria and ventricles in response to volume load and myocardial wall stress (6, 24). It promotes vasodilatation, natriuresis, and diuresis (6). In the circulation, the prohormone is cleaved into two peptides: the physiologically active BNP and the inactive NH_2-terminal-proBNP (NT-proBNP) (1). NT-proBNP has higher plasma levels and a longer half-life and therefore slower fluctuations (6). BNP and NT-proBNP are both helpful in the diagnosis of cardiac dysfunction and heart failure (16, 29) and equivalent in the prognosis of ischemic heart disease (21). Even modest elevations of the peptides are associated with increased risk of cardiovascular events, heart failure, stroke, and all-cause mortality (2, 34).

BNP and NT-proBNP plasma levels depend on age and gender, are altered in the presence of renal disease and obesity (6), and are elevated in sepsis (3, 4). NT-proBNP correlates to the Acute Physiology and Chronic Health Evaluation scores and is found to be a prognostic marker in sepsis and septic shock (3, 4). Elevated BNP levels in sepsis might be attributed to the underlying myocardial dysfunction (32). Nevertheless, BNP is elevated also in septic patients with neither clinical nor echocardiographic evidence of left ventricular systolic dysfunction (26). Moreover, increased plasma BNP is found also in patients with infections in the absence of severe sepsis or septic shock (18). The existence of a direct association between infections and BNP is supported by evidence from preclinical studies, since LPS and pro-inflammatory cytokines have a direct effect on BNP transcription and translation in cardiomyocytes in vitro (15, 30). Infection and sepsis also modulate circulating levels of other vasoactive peptides originating from the cardiovascular system, such as endothelium-derived endothelin-1 (ET-1) and adrenomedullin (12, 31), as well as their corresponding prohormone fragments C-terminal pro-endothelin-1 (CT-proET-1) and midregional pro-adrenomedullin (MR-proADM) (25, 28).

Prompted by these data, we aimed at investigating the profile of plasma NT-proBNP fluctuations in an experimental model of infection and inflammation in healthy humans with normal heart function. This was achieved by the intravenous administration of bacterial endotoxin, which contains LPS parts of the bacterial cell wall. In healthy humans, LPS induces a self-limiting process including flu-like symptoms, activation of the immune and neuroendocrine systems, tachycardia, and peripheral vasodilatation (14, 33). We present here a randomized crossover placebo-controlled study on LPS-induced changes in plasma NT-proBNP, C-reactive protein (CRP), CT-proET1, and MR-proADM.

METHODS

Experimental protocol. The study was approved by the institutional ethics committee, and written, informed consent was obtained from all subjects. Ten male volunteers aged 20–40 yr, with normal medical history, clinical examination, ECG, echocardiography, and biochemical and hematological tests, were enrolled in a randomized, placebo-controlled, crossover clinical trial. Two study protocols, lasting 6.5 h each ($t = -30$ min until $t = 6$ h) were conducted at least 3 wk apart. Isotonic saline was continuously infused at 500 ml/h for the first 30 min, 200 ml/h for the next 90 min, and then 100 ml/h till the end of the study. The subjects received either placebo or LPS (2 ng/kg body

wt National Reference *Escherichia coli* endotoxin; USP Convention, Rockville, MD) at $t = 0$ h. Body temperature was measured in the mouth (sublingually) every 15 min. Heart rate and ECG were continuously monitored (Lohmeier M607, Siemens, Munich, Germany), whereas blood pressure (BP) was measured every 30 min using a sphygmomanometer. Mean arterial pressure (MAP) was calculated as [systolic BP + (2·diastolic BP)]/3. The subjects tolerated LPS well, did not need any medications, and were discharged in good health 1–2 h after the end of the study protocol. Blood samples were obtained hourly, immediately cooled on ice, centrifuged for 10 min at 3,000 RPM at 4°C, and plasma was frozen at −80°C. Data on the LPS-induced changes in ghrelin, TNF-α, IL-6, IL-1 receptor antagonist (IL-1ra), ACTH, and cortisol obtained from this study are previously published (33).

Assays. NT-proBNP measurements were performed by Roche Diagnostics (Vienna, Austria) in heparin plasma using a sandwich immunoassay containing two polyclonal antibodies, which recognize epitopes located in the NH_2-terminal part (1–76) of the proBNP and the Elecsys 2010 immunoassay platform. The analytical sensitivity of the kit is 5 pg/ml. The intraassay CV is <5% and increases to 20% for values at the level of 50 pg/ml. CRP was determined using the Fluorokine MultiAnalyte Profiling human CRP-specific kit (R&D Systems, Minneapolis, MN). CT-proET-1 and MR-proADM were detected in EDTA-plasma using two new sandwich immunoassays (BRAHMS, Hennigsdorf/Berlin, Germany) (17, 20). All measurements were performed in duplicates. All samples were analyzed in one assay run per analyte.

Statistical analysis. Data are presented as means ± SE. Differences between LPS and placebo treatments were analyzed by repeated-measures ANOVA followed by Bonferroni-corrected paired *t*-tests if appropriate. Pearson's correlations were performed to evaluate associations between plasma NT-proBNP and other outcome parameters. SPSS release 12.0.1 was used as statistical software. All *P* values are two-sided, and levels of <0.05 were considered statistically significant.

RESULTS

Vital signs. Intravenous injection of LPS elicited a febrile response associated with tachycardia. Changes in both body temperature and heart rate became significant at 135 min and reached their peaks at 4 h (33). Peak heart rate was 83.3 ± 3.54 on LPS days and 55.7 ± 2.57 on control days ($P < 0.001$). LPS induced significant changes in MAP (ANOVA, $P = 0.001$). The maximal MAP was 91 ± 2.1 mmHg and was obtained 2 h after LPS injection (post hoc *t*-test was not significant). Then, MAP decreased continuously, reaching 81.4 ± 2.7 mmHg 6 h after LPS, whereas remaining stable at 87.9 ± 2.6 mmHg after placebo (post hoc *t*-test, $P = 0.03$). Changes in systolic BP (ANOVA, $P < 0.001$) and diastolic BP (ANOVA, $P < 0.001$) are presented in Fig. 1.

NT-proBNP, CRP, CT-proET-1, and MR-proADM. LPS administration induced a continuous increase in plasma NT-proBNP (ANOVA, $P = 0.001$) that reached a peak value of 40.7 ± 7.9 vs. 16.1 ± 3.2 pg/ml in control days at 6 h (post hoc *t*-test, $P = 0.023$; Fig. 2A). In parallel, LPS elevated the inflammatory marker CRP (1.8 ± 0.2 vs. 0.7 ± 0.1 μg/l in control days; ANOVA, $P < 0.001$) (Fig. 2B). LPS also induced significant increases in circulating CT-proET-1 (ANOVA, $P = 0.001$) and MR-proADM (ANOVA, $P < 0.001$), two endothelium-derived vasoactive peptides associated to infection and sepsis (Fig. 2, *C* and *D*).

Correlations. We tested a possible association of values of NT-proBNP with clinical and biochemical outcome measures
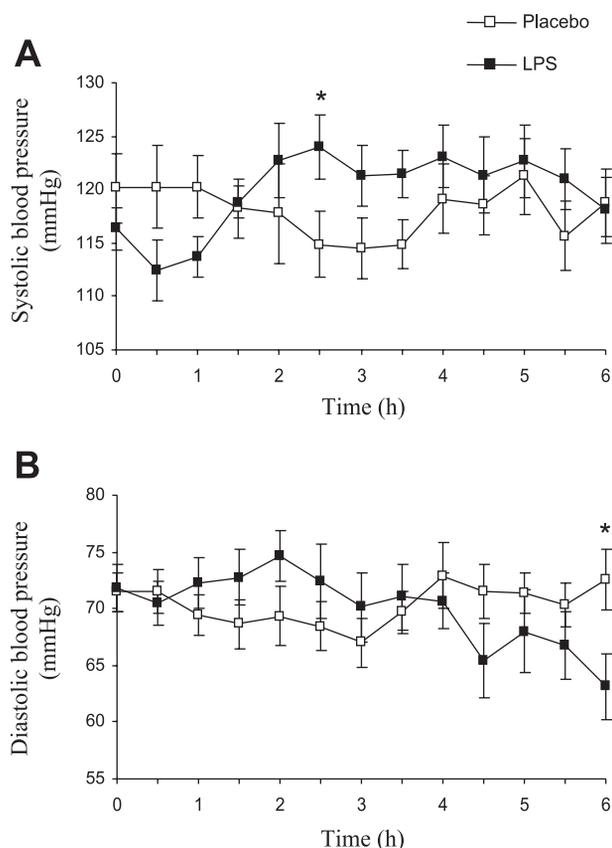

Fig. 1. Systolic blood pressure (BP; *A*) and diastolic BP (*B*) in response to placebo (open symbols) and LPS (2 ng/kg at $t = 0$ h; filled symbols) in healthy volunteers. *$P < 0.05$ (paired *t*-tests for comparison of values obtained after placebo and LPS treatment at each time point, performed only when the overall ANOVA was significant).

(Table 1). The LPS-induced increase in plasma NT-proBNP correlated with the changes in body temperature and heart rate but not with the respective values of systolic BP, diastolic BP, or MAP. NT-proBNP also correlated with CRP ($P < 0.001$), IL-1ra ($P = 0.017$), and plasma CT-proET-1 ($P = 0.008$).

DISCUSSION

This study demonstrates that systemic levels of NT-proBNP increase in response to bacterial endotoxin-induced inflammation in healthy men. The fluctuations in NT-proBNP relate to the changes in body temperature, heart rate, IL-1ra, CRP, and CT-proET-1.

Clinical studies have revealed a relationship of high plasma BNP and NT-proBNP with CRP levels in infections with or without sepsis and septic shock (3, 4, 26), but the interpretation of these results has been difficult given the compromised hemodynamic state of the patients. Here, we show that plasma NT-proBNP increases in response to the administration of parts of *E. Coli* bacterial wall that induce a mild and self-limiting inflammatory and anti-inflammatory response in the absence of an infectious source (14). This is a widely used and significant model for studying the human response to infection and inflammation. The increase in heart rate and subsequent peripheral vasodilatation are mild but significant and similar to the pattern of the hemodynamic changes obtained in systemic inflammation or sepsis. All subjects involved in the study
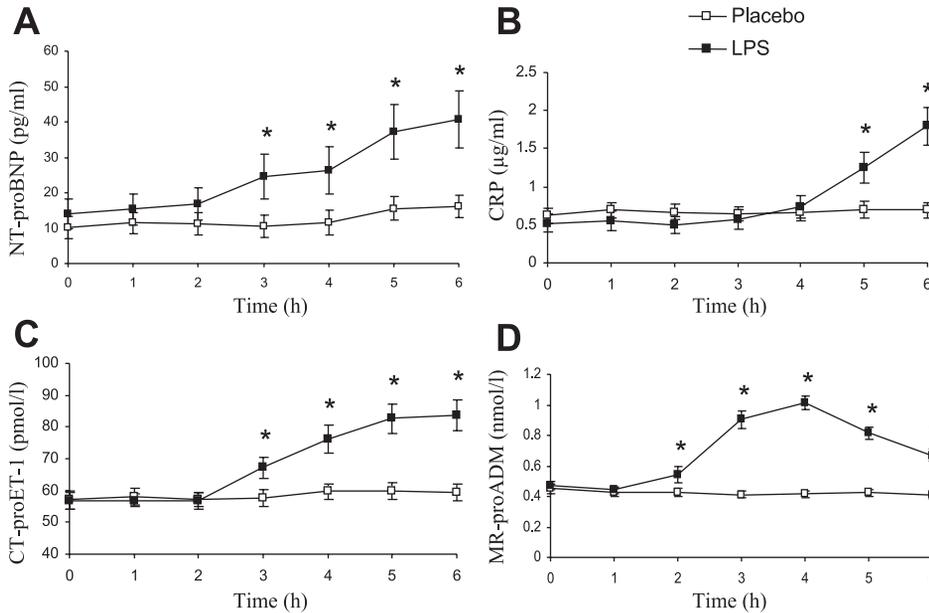
Fig. 2. NH$_2$-terminal-proBNP (NT-proBNP; *A*), C-reactive protein (CRP; *B*), COOH terminal pro-endothelin-1 (CT-proET-1; *C*), and midregional-pro-adrenomedullin (MR-proADM; *D*) plasma values after administration of placebo (open symbols) or LPS (2 ng/kg at $t = 0$ h; filled symbols) to healthy male volunteers. To convert NT-proBNP values from pg/ml to pmol/l, divide by 8.47. *$P < 0.05$ (paired *t*-tests for comparison of values obtained after placebo and LPS treatment at each time point, performed only when the overall ANOVA was significant).

tolerated LPS well and did not necessitate any medication. This study was performed only in male subjects, and we cannot comment on gender-specific effects of LPS on NT-proBNP.

NT-proBNP is released into the circulation in equimolar concentrations to those of BNP; therefore, an increase in circulating NT-proBNP may imply an increase in the release of BNP. Nevertheless, these peptides are eliminated differently from the bloodstream (6), and we have no data on the changes in plasma BNP following LPS administration in healthy men.

Volume loading increases plasma NT-proBNP levels in healthy subjects (10). Physical exercise stress induces significant elevations in heart rate and BP but only a 10% increase in plasma NT-proBNP (7). In heart failure patients, exercise-induced increases in NT-proBNP are significantly higher (36). Moreover, NT-proBNP is a good indicator of cardiac dysfunction (36). Based on this evidence, healthy subjects are not expected to increase plasma NT-proBNP in response to the mild hemodynamic changes induced by bacterial endotoxin: maximal

Table 1. *Correlations between LPS-induced changes in NT-proBNP and other primary response parameters*

	NT-proBNP	
	r	*P*
Body temperature	0.390	< 0.001
Heart rate, beats/min	0.332	0.005
MAP, mmHg		n.s.
CRP	0.495	< 0.001
TNF-α		n.s.
IL-6		n.s.
IL-1ra	0.286	0.017
ACTH		n.s.
Cortisol		n.s.
MR-proADM		n.s.
CT-proET-1	0.315	0.008

MAP, mean arterial pressure; NT-proBNP, N-terminal-pro-B-type natriuretic peptide; CRP, C-reactive protein; MR-proADM, midregional-pro-adrenomedullin; CT-proET-1, C-terminal pro-endothelin-1. Pearson correlation coefficients between NT-proBNP and clinical and biochemical parameters were obtained at time points 0-6 h after LPS administration.

mean heart rate was 82.9 ± 3.5 beats/min and maximal mean MAP was 91 ± 2.1 mmHg. In this contest, it might be assumed that factors other than hemodynamic changes contribute to the threefold elevation in NT-proBNP observed in response to LPS (Fig. 2*A*).

Studies performed in rodents indicate a direct role of LPS in the transcriptional activation and release of BNP from cardiomyocytes both in vitro and in vivo (15). The human response to bacterial endotoxin involves a series of neuroendocrine and immune changes interacting with each other (35). Some mediators of inflammation such as IL-1, IL-6, and TNF-α are stimulants of BNP secretion (24). We cannot exclude a potential role of fever, a powerful central mechanism controlling the human response to infection. Plasma NT-proBNP levels begin to rise hours after LPS administration and seem to be part of the normal human response to this challenge. We found a correlation of plasma NT-proBNP with heart rate, body temperature, and plasma IL-1ra after endotoxin. The continuous increase in NT-proBNP took place in parallel with the increase in CRP, an acute phase protein that has been related to cardiovascular disease (27).

The release of substances with vasoactive properties is part of the host response to infection/inflammation (8). Circulating levels of endothelium-derived peptides such as adrenomedullin and endothelin are increased in response to infection and sepsis (12, 31). These peptides are important mediators of vascular tone regulation: endothelin is a powerful vasoconstrictor, whereas adrenomedullin induces extreme vasodilatation and hypotension (11). Both of these peptides have a short plasma half-life, and the measurement of the more stable prohormone fragments CT-proET-1 and MR-proADM is more representative (11, 17, 20). MR-proADM is a prognostic marker in sepsis patients, even superior to procalcitonin and CRP (5). Our finding that NT-proBNP increases in response to LPS-induced systemic inflammation in healthy men suggests that natriuretic peptides might also count among the pathophysiological mechanisms underlying the vasodilatation that accompanies infection.

BNP and NT-proBNP are rapidly secreted in the presence of high intraventricular pressure and useful in the differential diagnosis of acute heart failure in the emergency care (16, 29). Although not all studies support the accuracy of BNP and NT-proBNP for clinical use outside the emergency departments (1, 19), there are data on their prognostic value in ischemic heart disease and in predicting future cardiovascular events (2, 21, 34). Among others, ischemic heart disease and atherosclerosis are associated with a low-grade inflammation (9). Highly sensitive CRP is independently associated with the incidence of coronary events, also when adjusted for age, smoking status, body mass index, diabetes, total cholesterol, HDL-cholesterol, and a history of hypertension (13, 22, 23). Nevertheless, the American Heart Association recommends only the measurement of CRP and not of other inflammatory markers (cytokines and other acute phase reactants) for determining coronary risk in clinical practice (27). The positive relation between NT-proBNP and inflammation/CRP in response to LPS in healthy men might suggest the existence of an inflammatory component strengthening the prognostic power of NT-proBNP.

This work shows that NT-proBNP increases in response to bacterial endotoxinemia in healthy young men and correlates to CRP and other acute response parameters. We stress the importance of taking into consideration concomitant infections and inflammatory conditions when interpreting elevated circulating NT-proBNP levels.

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GRANTS

DISCLOSURES

J. Struck is an employee of BRAHMS, a company that manufactures and holds patent rights on the MR-proADM assay.

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