Reference Values for N-Terminal Pro-B-Type Natriuretic Peptide in Umbilical Cord Blood

To the Editor:

Plasma concentrations of B-type natriuretic peptide (BNP), a 32-amino acid peptide hormone secreted by the myocardium, increase in response to myocardial stretch or strain *(1, 2)*. On secretion, proBNP, the storage form of BNP, is cleaved into the inactive N-terminal proBNP (NT-proBNP) and the endocrinologically active BNP. In patients with heart failure, plasma BNP concentrations are related to the severity of symptoms and underlying cardiac abnormality *(3)*. It is also known that neonates show transient increases in both plasma NT-proBNP and BNP in the first days of life as a result of the increased left ventricular volume load induced by the circulatory changes after birth *(4)*. After closure of the ductus arteriosus and the foramen ovale and stabilization of the forced circulatory change, plasma NT-proBNP and BNP concentrations are still increased but decrease to "adult" values in the following months *(5)*. Although a small study was published on NT-proBNP and fetal heart rate abnormalities *(6)*, little is known regarding umbilical cord blood BNP or NT-proBNP concentrations. The purpose of the present study was to establish reference values for NT-proBNP concentrations in umbilical cord blood.

Of the 71 successively born neonates enrolled in the study, 67 were delivered by vaginal delivery and 4 by cesarean section. Gestational age ranged from 32 to 42 weeks. After early clamping of the cord, blood was drawn from the arterial and venous umbilical cord vessels. NT-proBNP was measured with an electrochemiluminescence immunoassay (Elecsys® 2010; Roche).

Mean (SD) NT-proBNP concentrations were 79.5 (42.9) and 79.9 (45.0) pmol/L for arterial and venous umbilical cord blood, respectively. NT-proBNP concentrations in arterial and venous umbilical cord blood, plotted against each other, are shown in Fig. 1. There was no significant mean difference in NT-proBNP concentrations between arterial and venous umbilical cord blood (paired-sample *t*-test). In the studied group of newborns, we found no influence of gestational age, umbilical cord pH, or mode of delivery on NT-proBNP concentrations in the umbilical cord. The maternal NT-proBNP concentration was measured in eight cases and appeared to be within the adult reference interval. In all cases, large differences were found between maternal and neonatal NT-proBNP concentrations, suggesting no placental exchange of NT-proBNP. The high concentrations of NT-proBNP in umbilical cord blood compared with the reference interval for healthy adults (0–10 pmol/L) might be explained by the differences in cardiac output and ventricular stroke volume. The ventricular volume loads in fetal and neonatal life cause a constant myocardial stretch, leading to secretion of proBNP. Moreover, the growing fetal heart up-regulates the genes for the different natriuretic peptides *(1)*.

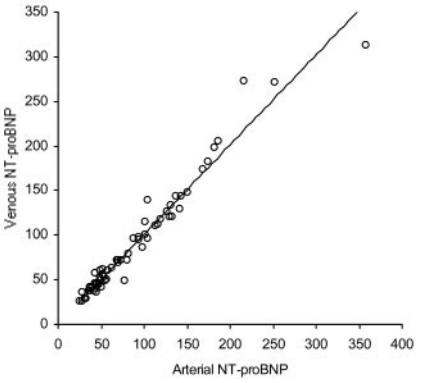

Fig. 1. Relationship between NT-proBNP concentrations (pmol/L) in arterial and venous umbilical cord blood.

Equation for the line: $y = 1.0064x + 0.2251$ pmol/L ($R^2 = 0.9587$).

This study established reference values for plasma concentrations of NT-proBNP in the umbilical artery and vein, the mean concentrations being ~80 pmol/L for both vessels when measured with an electrochemiluminescence immunoassay. Further studies will focus on BNP and NT-proBNP and the role of these natriuretic peptides in perinatal medicine.

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Jaap Bakker[1*]
Inge Gies[2]
Barbara Slavenburg[2]
Otto Bekers[1]
Tammo Delhaas[2]
Marja van Dieijen-Visser[1]

Departments of [1] Clinical Chemistry and [2] Pediatrics
University Hospital Maastricht
Maastricht, The Netherlands

* Address correspondence to this author at: Department of Clinical Chemistry, University Hospital Maastricht, PO Box 5800, NL-6202 AZ Maastricht, The Netherlands. Fax 31-43-3874667; e-mail j.bakker@klinchem.azm.nl.

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Endocrine Paradox in Heart Failure: Resistance to Biological Effects of Cardiac Natriuretic Hormones

To the Editor:

We read the interesting minireview by Goetze *(1)* concerning the bio-

chemistry of pro-B-type natriuretic peptide (proBNP)-derived peptides: the main message is the potential clinical relevance of the proBNP-derived peptides, which should be considered for current clinical interpretation of plasma BNP concentrations and be the focus of ongoing research. Accordingly, the lack of accurate studies on in vivo production/secretion mechanisms and metabolism of BNP- and proBNP-related peptides explains the incomplete knowledge of the pathophysiologic significance of this hormone system. Thus, we agree with Goetze that deeper insight into the biochemistry of these peptides could pave the way for more sensitive and disease-specific assays in the clinical setting.

We have some observations concerning the presence of an "endocrine paradox in heart failure" *(1)*. The lack of encoding and processing of the precursor peptides to the mature hormones, atrial natriuretic peptide (ANP) and BNP, which have a potent diuretic and natriuretic effect, could explain the disturbed electrolyte and fluid homeostasis occurring in chronic heart failure *(2, 3)*. However, the hypothesis of heart failure as a syndrome of cardiac natriuretic hormone (CNH) deficiency was challenged when this system was investigated in experimental animals and in humans. In fact, patients with congestive heart failure [New York Heart Association (NYHA) classes III–IV] have greatly increased plasma concentrations of CNHs compared with healthy individuals (up to 500-fold or more for plasma BNP concentration) *(1–3)*.

Goetze *(1)* tries to explain this paradox of heart failure (i.e., highly increased CNH concentrations in patients with sodium retention) by suggesting that a part of circulating CNH-related peptides (especially proBNP-related peptides) is not biologically active. We agree that increased concentrations of immunoreactive, but biologically inactive, proBNP-related peptides (as well as proANP-related peptides) are present in patients with heart failure. As also correctly pointed out in the review *(1)*, commercially available immunoassays for BNP and N-terminal proBNP (NT-proBNP) also measure the intact proBNP peptide as well as some degradation products of this peptide. Furthermore, the circulating concentrations of proBNP and its related peptides increase progressively with the progression of heart failure. Consequently, immunoassay methods tend to progressively overestimate the real biological activity of the CNH system in patients with heart failure.

Tracer kinetic studies, using radiolabeled ANP and HPLC purification of experimental plasma samples, have demonstrated that ANP kinetics are greatly altered in patients with heart failure *(4, 5)*. In particular, patients with more severe heart failure (above NYHA class II) had a significantly reduced ANP metabolic clearance rate (MCR) compared with healthy individuals or patients with lower severity of heart failure (below NYHA class II). Furthermore, MCR values are close and positively correlated to natriuresis in healthy individuals and patients with heart failure *(4, 5)*. It is interesting to note that patients in the early phase of heart failure (NYHA class I) have an increased ANP MCR and ANP production rate and natriuresis compared with patients with congestive heart failure (studied at the same sodium intake) *(5)*. These findings suggest that an increase in CNH production/secretion should be considered a compensatory mechanism, at least in the early phase of heart failure *(4)*. Unfortunately, there are no comparable tracer kinetics studies for BNP in patients with heart failure. However, ANP kinetics studies suggest that CNH metabolism is greatly disturbed in symptomatic heart failure, in accordance, at least in part, with Goetze's hypothesis *(1)*.

Previous studies have demonstrated that the pharmacologic effects (i.e., natriuresis) of infusion of CNHs [including ANP, BNP, and/or C-type natriuretic peptide (CNP)] are lower in patients or experimental animals with heart, liver, or renal failure (all sharing greatly increased plasma CNH concentrations) than those found by infusion of the same doses in control individuals or experimental animals *(6–9)*. This "blunted" natriuretic response after pharmacologic loading doses of CNHs, currently found in experimental models and in patients with chronic heart failure, has usually been interpreted as a resistance to the biological effects (natriuresis) of CNHs (i.e., a renal hyporesponsiveness to CNHs) *(2–4, 9)*.

Some studies have suggested that resistance to the biological effects of CNHs in heart failure may be attributable, at least in part, to variations in the ratio between biological and clearance-specific CNH receptors, related to an increase (up-regulation) in clearance receptors [the so-called type C receptors (NPR-C)] with a parallel decrease (down-regulation) in biological receptors [type A and B receptors (NPR-A and NPR-B)] *(2, 4, 10–13)*. Furthermore, it is well known that the clinical evolution of heart failure is characterized by predominant effects of activation of the vasoconstrictor, sodium-retentive systems (including sympathetic nervous system, renin-angiotensin-aldosterone system, antidiuretic/vasopressin hormone system, endothelins, and some cytokines), which, progressively activated with increasing severity of the disease, are only in part counterbalanced by the vasodilator natriuretic system, represented by CNHs *(2–4, 14–16)*. This has been suggested as being the most important pathophysiologic mechanism responsible for this blunted natriuretic effect *(2–4, 16)*.

In conclusion, we agree with Goetze *(1)* that it is necessary to focus more on the biology (i.e., secretion, production, and turnover) of BNP- and proBNP-derived peptides. We also agree that commercially available immunoassay methods tend to progressively overestimate the real biological activity of the CNH system in patients with heart failure. However, we believe that the endocrine paradox of heart failure is predominantly attributable to peripheral resistance to the biological actions of CNHs at the receptor level and, especially, at the postreceptor level.

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Aldo Clerico[1*]
Michele Emdin[2]

[1] Laboratory of Cardiovascular Endocrinology
and
[2] Cardiovascular Medicine Department
CNR Institute of Clinical Physiology
Pisa, Italy

*Address correspondence to this author at: Laboratory of Cardiovascular Endocrinology, CNR Institute of Clinical Physiology, Via Trieste 41, 56126 Pisa, Italy. Fax 39-0585-493601; e-mail clerico@ifc.cnr.it.

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Drs. Goetze and Rehfeld respond:

To the Editor:

In this issue of *Clinical Chemistry*, Clerico and Emdin have taken the question of an endocrine paradox in heart failure further by providing important information on the underlying pathophysiologic mechanisms. They suggest that peripheral resistance to the natriuretic peptides at the receptor and postreceptor level may be the predominant explanation. In addition, metabolic handling of natriuretic peptides in heart failure patients is grossly altered with decreased metabolic clearance rates, at least for A-type natriuretic peptide (ANP), and pharmacologic studies have shown reduced biological effects of ANP infusion in patients with heart failure.

We have hypothesized that the endocrine heart also may be implicated in the apparent paradox of increased natriuretic peptides in the presence of sodium and water retention *(1, 2)*. Like other regulatory peptides, ANP and B-type natriuretic peptide (BNP) are synthesized as propeptides that undergo cellular maturation by endoproteolytic cleavage, which in turn releases the C-terminal fragments, the bioactive hormones. However, the cellular capacity for posttranslational processing of prohormones may not always be sufficient to mature all of the synthesized prohormone, in particular in conditions with greatly increased hormone gene expression, biosynthesis, and secretion *(2)*. Thus, increased ANP and BNP gene expression could lead to higher fractions of proANP and proBNP release. Importantly, proANP and proBNP are presumed to have decreased biological potency compared with the fully processed hormone products. Measurement of the cardiac natriuretic peptides unfortunately does not always provide specific information on the molecular heterogeneity in plasma. In turn, plasma concentrations can blur such a molecular shift in disease. A change from secretion of mainly mature hormone to a release of less processed biosynthetic intermediates and prohormone is well documented in other endocrine disorders with increased expression of the hormone gene, even in highly specialized endocrine cells *(3–5)*. Laboratory and clinical medicine should therefore keep in mind that plasma concentrations may not readily be interpreted as the biological effect of the measured peptide. In particular, conditions with increased plasma BNP concentrations may be characterized by predominant release of less mature proBNP-derived peptides from the cardiac myocytes.

Congestive heart failure is characterized by reduced cardiac output. Therefore, other organs are also often affected. The metabolic handling of circulating peptides is most likely to be dramatically altered, which may have a major impact on the elimination of ANP, BNP, and their precursors. Heart failure patients often have reduced renal function attributable to the low cardiac output, which in turn could contribute to the lack of ANP and BNP effects. The local expression of natriuretic peptide receptors may also be down-regulated. Importantly, other neurohumoral mechanisms are certainly involved, which could counteract the natriuretic response to increased plasma concentrations of ANP and BNP. Our suggestion of the endocrine heart as a source of reduced natriuretic potency should therefore be