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Opinion Paper

Pathophysiological and Clinical Relevance of Circulating Levels of Cardiac Natriuretic Hormones: Are They Merely Markers of Cardiac Disease?

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Several specific and sensitive markers for myocardial injury as well as diagnostic tests for the assessment and stratification of cardiovascular risk have been recently introduced in clinical laboratories. However, until a few years ago, there were no laboratory tests for diagnosis, stratification and follow-up of patients with heart failure. The assay for cardiac natriuretic hormones (CNH) fills this gap. Heart failure is not only the most frequent "final common pathway" in cardiovascular disease, but is also the most common primary hospital discharge diagnosis, as well as the most common cause of death in patients over 50 years of age in Western countries; therefore, CNH assay may be destined to assume a growing relevance in clinical cardiology. However, to consider CNH assay only as a general and functional indicator of cardiac structural disease, without recalling that atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are powerful hormones, may lead to underestimation of the physiological role they play in healthy subjects as well as in patients with heart failure. Indeed, the circulating levels of CNH should be always interpreted taking into account not only hemodynamic factors and myocardial performance, but also their relationship with the counter-regulatory neuroendocrine system (including renin-angiotensin-aldosterone system, sympathetic system, endothelins, cytokines and vasopressin), as well as other hormones (such as sex steroid hormones, thyroid hormones and glucocorticoids). Clin Chem Lab Med 2002; 40(8):752–760

Key words: ANP; BNP; Natriuretic hormones; Female sex steroid hormones; Aging; Cardiac failure.

Abbreviations: AMI, acute myocardial infarction; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNH, cardiac natriuretic hormones; CNP, C-type natriuretic peptide.

Introduction

Human cardiomyocytes produce and secrete a family of related peptide hormones (cardiac natriuretic hormones, CNH) which have potent diuretic, natriuretic and vascular smooth muscle-relaxing effects as well and interact with the hormonal and nervous systems, as recently reviewed (1–6). CNH include atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and their related peptides, while other natriuretic peptides, such as C-type natriuretic peptide (CNP) and urodilatin, structurally related to the ANP/BNP peptide family, are produced and secreted not by cardiomyocytes but by other tissues (1–6).

CNH greatly increase in diseases characterized by an expanded fluid volume (2), especially in heart failure (1–7). In particular, the importance of measuring circulating levels of CNH for the classification and screening of patients with heart failure and/or for predicting their mortality/survival rates was reported (1–6). As a result, the assay of circulating levels of CNH is now considered to be a useful marker of myocardial function (1–8). In particular, the important role of BNP assay in screening for heart disease (9), stratification of patients with congestive heart failure (10), detecting diastolic dysfunction (11) and differential diagnosis of dyspnea (12, 13) has been confirmed even more recently. Finally, the CNH assay was included in the first step of the algorithm for the diagnosis of heart failure along with ECG and X-ray examination, as suggested by the Task Force of the European Society of Cardiology for the Diagnosis and Treatment of Chronic Heart Failure (14).

ANP and BNP are powerful hormones with important physiological effects; consequently, by considering their level only as a marker for cardiac disease may result in a misinterpretation or underestimation of their biological action as well as of their pathophysiological role in cardiovascular and other diseases.

In the present article, after a brief summary of the biochemical characteristics and biological effects of CNH, we will discuss clinical and physiological conditions where the interpretation of increased levels of CNH may be difficult or provoke misunderstanding.

Biochemical Characteristics of CNH

CNH are a family of peptides derived from common precursors, prepro-hormones (*i.e.* preproANP and preproBNP), which contain a signal peptide sequence at the amino-terminal end (Figures 1 and 2). The pro-hormones (proANP and proBNP) are produced by cleavage of the signal peptide and stored in the secretory granules in the atria. The pro-hormone peptide is further split into a longer amino-terminal fragment (N-

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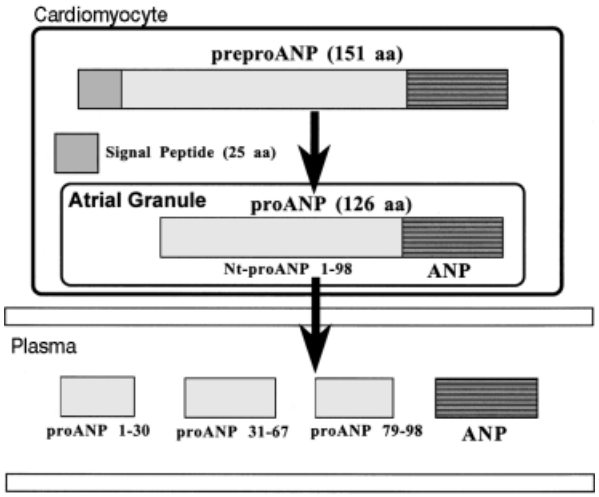

Figure 1 Synthesis and secretion of ANP. Atrial natriuretic peptides are a family of peptides derived from a common precursor, called preproANP, which in humans contains 151 amino acids and has a signal peptide sequence at its amino-terminal end containing 25 amino acids. The pro-hormone is stored in the atrial granule as a 126-amino acid peptide, $proANP_{1-126}$, which is produced by cleavage of the signal peptide. When appropriate signals for hormone release are given, $proANP_{1-126}$ is further split into an N-terminal fragment, $proANP_{1-98}$, and the C-terminal peptide $proANP_{99-126}$ (generally called ANP), which is considered to be the biologically active hormone. Moreover, N-terminal $proANP_{1-98}$ can be degraded *in vivo* with the production of at least three different peptides (*i.e.*, N-terminal $proANP_{1-30}$, $proANP_{31-67}$ and $proANP_{79-98}$).

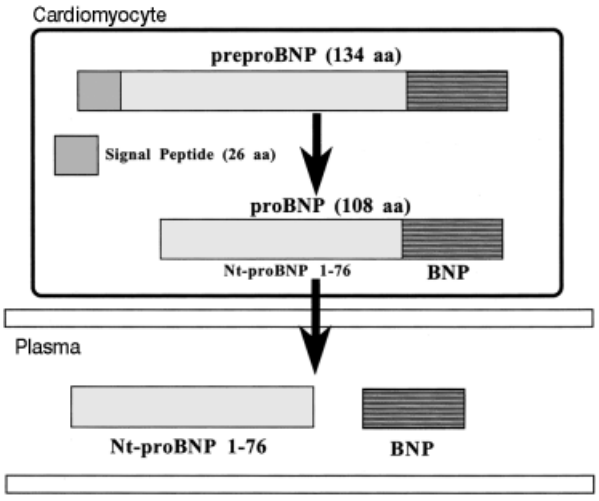

Figure 2 Synthesis and secretion of BNP. BNP derives from a precursor, called preproBNP, which in humans contains 134 amino acids and has a signal peptide sequence at its amino-terminal end containing 26 amino acids. The pro-hormone containing 108 amino acids ($proBNP_{1-108}$) is produced by cleavage of the signal peptide, when appropriate signals for hormone release are given. $proBNP_{1-108}$ is further split into an N-terminal fragment, $proBNP_{1-76}$, and the C-terminal peptide $proBNP_{77-108}$ (generally called BNP), which is considered to be the biologically active hormone. BNP is preferentially produced and secreted in the ventriculum without a storage in the granules, although some secretory granules can contain this hormone. For this reason, in contrast to ANP, regulation of BNP synthesis and secretion occurs mainly at the level of gene expression (5, 15).

Table 1 Normal values of CNH and related peptides.

Peptide	Number of subjects studied	Mean ± SD (pmol/l)	Range (pmol/l)
ANP	220	5.4±3.5	0.2–16.0
BNP	220	2.7±2.5	0.1–12.4
ProANP1–98	67	731.0±628.0	43.0–1052
Mid-proBNP	67	117.5±100.3	0.2–368
Nt-proBNP	67	246.8±120.1	64.0–488

ANP, BNP and proANP 1–98 were assayed by IRMA methods (16–19), while the peptides related to N-terminal proBNP (named Mid-proBNP and Nt-proBNP, respectively) were assayed by RIA methods (19, 20). The results are expressed in pmol/l to better illustrate the relative increase of N-terminal pro-peptides compared to biologically active peptides (*i.e.*, ANP and BNP).

terminal proANP or N-terminal proBNP), and a shorter carboxyl terminal peptide (ANP or BNP), which are secreted in the blood in equimolar amounts. However, ANP and BNP have shorter plasma half-lives compared to N-terminal propeptides (proANP and proBNP) and consequently they also have lower plasma concentrations (Table 1).

Studies on structure-activity relationships have shown the importance of the central ring structure of CNH formed by a disulfide bridge between the two cysteine residues; because this cysteine bridge is necessary for the binding to the specific receptors. Disruption of this ring by hydrolytic cleavage leads to loss of biological activity. For this reason, only ANP and BNP, which present the disulfide bridge in the peptide chain, are biologically active hormones, while the N-terminal proANP and proBNP and their related peptides are not.

Physiological Considerations for Circulating Levels of CNH

After secretion into blood by cardiomyocytes, CNH reach the target tissues in the periphery, where, like all the other peptide hormones, they bind to specific receptors on cell membranes. The circulating levels of CNH can be regulated or modified by several physiological factors (such as circadian rhythm, age, exercise, body posture and water immersion), eating habits (especially sodium intake), clinical conditions (especially cardiac and renal insufficiency) and drugs (including glucocorticoids, sex steroid hormones, thyroid hormones, diuretics, ACE inhibitors and adrenergic agonists) (2).

Upon secretion, ANP is rapidly distributed and degraded (its metabolic clearance rate is on average about 2000 ml/min in healthy subjects); in humans about 50% of the ANP secreted into the right atrium is extracted by the peripheral tissues during first pass throughout the body (21–23). Furthermore, circulating ANP represents only a small fraction of the total body pool (no more than 1/15) in normal subjects (2, 21–23)

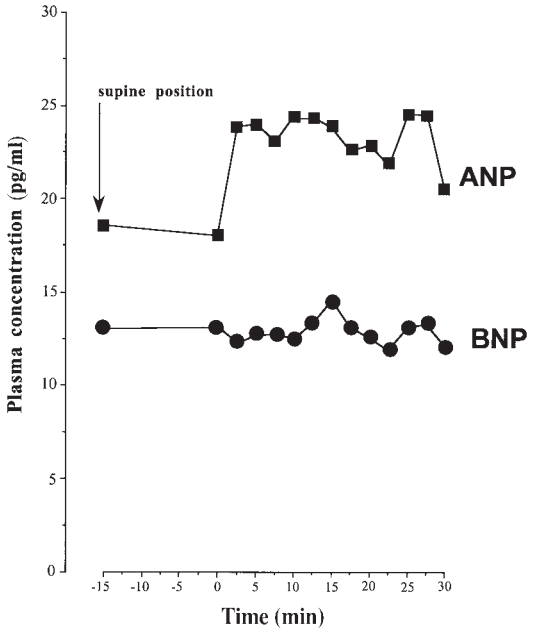

Figure 3 Variations of plasma ANP and BNP in a normal subject (woman, aged 23 years) over 45 min at rest in recumbent position. The supine position at rest was attained at –15 min; starting from time 0, blood samples (5 ml) were drawn every 2.5 min for the following 30 min. Saline infusion was used to flush the catheter and to replace the volume of fluid withdrawn.

and plasma ANP concentration shows rapid, wide fluctuations in healthy subjects, even at rest in recumbent position (Figure 3).

The biological action, metabolic pathways, and turnover parameters of BNP are not as well-known as those of ANP. However, it is commonly believed that the BNP turnover is less rapid than that of ANP (2, 5); indeed, circulating levels of BNP are more stable than those of ANP (Figure 3).

The turnover data suggest that circulating levels of CNH may not accurately reflect their disposal, and therefore the activity of the CNH system, as is implicitly accepted in physiological or clinical studies where only the plasma concentration of the hormone is measured, without an estimation of turnover rate. However, it was demonstrated that ANP clearance is constant in the presence of rapid and large changes in endogenous ANP plasma levels induced by atrial and/or ventricular pacing. This indicates that, at least for studies lasting only few hours, changes in the circulating level of ANP may provide a reliable estimate of production rate variations (24).

Pathophysiological and Clinical Interpretations of Circulating Levels of CNH

CNH, including CNP, have several physiological actions, the most important being: a) decrease in blood pressure; b) increase in natriuresis and diuresis; c) regulation of the sympathetic nervous system and inhibition of the release or action of several hormones (including renin-angiotensin-aldosterone system, sympathetic system, endothelins, cytokines and vasopressin); d) regulation of pathophysiological mechanisms responsible for ventricular and vascular hypertrophy and remodeling (1–6).

Because of these actions, it may be suggested that CNH play a protective or beneficial role in the cardiovascular system. An important pathophysiological mechanism in cardiovascular diseases is the imbalance between the vasoconstrictive/anti-natriuretic action of some neuroendocrine factors (including renin-angiotensin-aldosterone system, vasopressin, sympathetic nervous system) and the counter-regulatory vasodilatory/natriuretic response of other factors, such as cardiac natriuretic peptides (7, 21, 25). Indeed, the activation of neurohumoral mechanisms may be involved in the progression of clinical symptoms in patients with heart failure (7, 21, 25). As cardiac performance declines, all neurohormonal systems are progressively stimulated in an attempt to maintain circulatory homeostasis. However, the activation of neurohumoral mechanisms may worsen the hemodynamic abnormalities in heart failure or may have a direct adverse effect on myocardial function (7, 21, 25). The large increase in circulating levels of CNH in heart failure is secondary to activation of the neuroendocrine system, and therefore should be considered to be an adaptive and potentially protective response mechanism in cardiovascular disease.

CNH, as well as CNP, also share a protective action with respect to endothelium through regulation of the action of some neurohormones, as well as inhibition of the vascular remodeling and restenosis after percutaneous transluminal coronary angioplasty (26, 27). Table 2 summarizes the main beneficial effects of CNH and CNP in endothelial dysfunction secondary to the atherosclerotic process.

It is important to note that some studies have suggested that intravenous administration of CNH or their analogues (such as anaritide or besiritide) may have beneficial effects in patients with heart failure (28–31). Furthermore, a new pharmacological approach was recently developed in order to increase circulating levels of CNH by means of inhibition of enzymes which degrade CNH; this therapy should prove to be particularly useful in patients with arterial hypertension and/or heart failure (28–31).

Since ANP and BNP are hormones with important physiological actions, their circulating levels should be interpreted according to the complex interrelationships between the several neurohormone systems

Table 2 Beneficial effects of CNH in endothelial dysfunction.

1. Shear stress decrease.
2. Inhibition of vascular remodeling and restenosis mechanism.
3. Inhibition of renin-angiotensin-aldosterone system and endothelin activity.
4. Regulation of coagulation and fibrinolysis. Inhibition of platelet activation.

which regulate fluid and electrolyte homeostasis as well as cardiovascular hemodynamics (15).

Even if CNH are greatly increased in patients with acute myocardial infarction (AMI) during the acute phase, this increase is probably due to several factors, including alterations in hemodynamics and ischemia, which can increase the synthesis (especially in the peri-infarction zone), as well as the release, of CNH from necrotic myocardium, especially in the early phase (1, 3, 5). Unlike markers of acute myocardial necrosis (such as troponin, CK-MB or myoglobin), increased levels of CNH in patients with AMI should be more related to stretching of the atrial and/or ventricular wall and/or to the degree of activation of the neuroendocrine system, due to the myocardial dysfunction, rather than to the amount of myocardial tissue injured by the disease (1, 3, 5). Persistently increased levels of CNH after the early phase of AMI suggest a sustained neurohormonal activation and are strongly associated with manifest heart failure (myocardial dysfunction), morbidity and mortality (1, 5).

Thus, the CNH assay should be fundamentally considered as a functional index, not a marker of cardiac disease or injury. This statement is in agreement with the very recent suggestion of Struthers that BNP should be considered a general indicator of raised intracardiac pressure (8). This author also correctly suggested that BNP assay "could become a general screening test for all echocardiogram requests, irrespective of whether the echocardiogram request is to look for the causes of symptoms, or whether the it is being done as a screening procedure to look for asymptomatic killers such as left ventricular hypertrophy" (8).

However, to consider CNH assay only as a general functional indicator of cardiac structural disease, without recalling that ANP and BNP are powerful hormones produced and secreted by the human heart, may lead to an underestimation (or even misinterpretation) of the important pathophysiological role played by CNH in several physiological and clinical conditions, as suggested by the following examples.

Relationship between Circulating Levels of CNH, Gender and Age

A good example of the possible difficulties in the interpretation of physiological relevance of CNH measurement is the variation of CNH levels in adult normal subjects in relation to age and gender. The relationship between CNH and female steroid sex hormones and/or age has been little investigated in humans (32), although these data are evidently fundamental for the development of accurate reference values for CNH assay, as well as for the interpretation of physiological or clinical studies concerning the CNH system.

This lack of studies specifically dedicated to the influence of aging and sex hormones on the ANP/BNP system may be due to methodological problems. First, it is difficult to find a large number of truly healthy subjects over 65 years of age, since approximately 85% of the people over the age of 65 suffer from one or more chronic conditions (33). Other methodological problems are related to the very low plasma concentrations of CNH in normal adults (on average 10–20 pg/ml), often very similar to the sensitivity levels of immunoassay methods used to measure these hormones, especially for BNP assay (34).

Recent studies from our laboratory (32, 35) indicate independent stimulatory effects of aging and female sex hormones on the CNH system in healthy adults. However, a more complete interpretation of circulating levels of ANP (Figure 4) and BNP (Figure 5) throughout

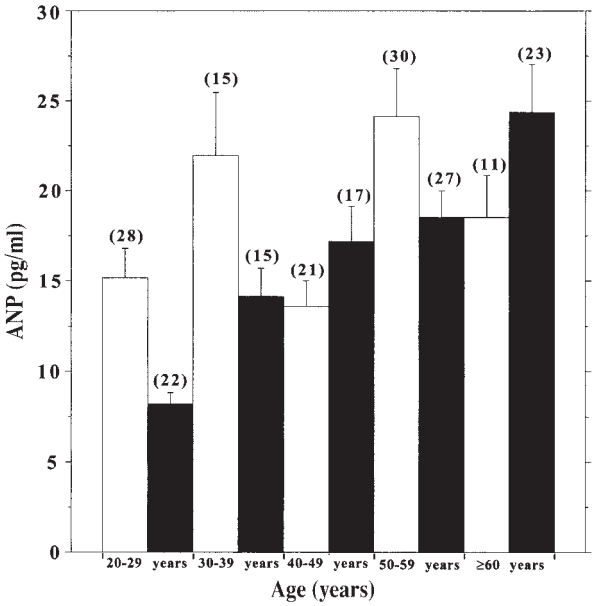

Figure 4 Circulating levels of ANP measured in healthy subjects divided according to gender and age (mean ± SEM). The number of subjects included in each age group is indicated in parentheses. □ women, ■ men.

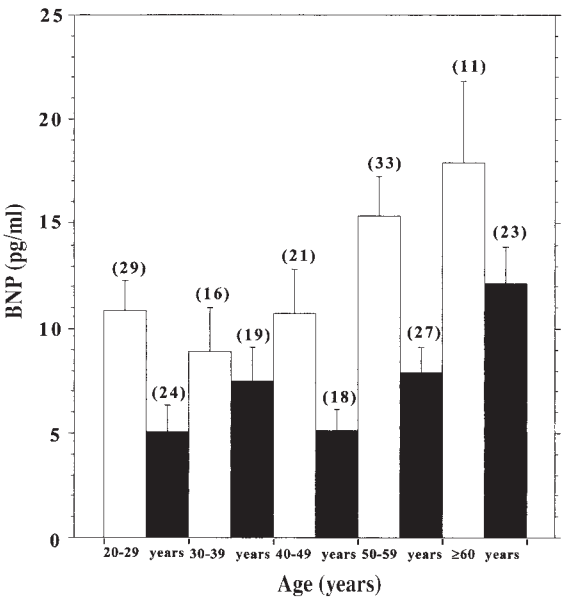

Figure 5 Circulating levels of BNP measured in healthy subjects divided according to gender and to 5 age groups (mean ± SEM). Number of subjects in each age group is indicated in parentheses. □ women, ■ men.

adult life in healthy men and women requires more complex analysis. In order to explain the significant variations of circulating levels of cardiac natriuretic peptides which occur with aging, as well as the different patterns found in men and women, the effects of three distinct pathophysiological mechanisms should be taken into account (32).

1. The modification of the cardiovascular system with aging. These modifications can induce dilatation of cardiac chambers (atria and/or ventricles) and/or hypertrophy of ventricle walls. These alterations may increase progressively with aging by producing different stimulatory patterns of ANP and BNP (see point 2) in both men and women. Heart rate declines and systolic blood pressure increases with increasing age (33, 36, 37). In men, the decline in resting heart rate is compensated by an age-associated increase in resting cardiac volumes (*i.e.* end-diastolic, end-systolic and stroke volumes) (36, 37), so that systolic dysfunction is more often seen in men aged about 60 years (38, 39). In contrast, resting cardiac volumes in healthy women vary less with aging than in men (36, 37), while diastolic dysfunction with concentric cardiac hypertrophy is more common in elderly women, aged 70 years and older (33, 36–39). Very recently, Luchner *et al.* (39) suggested a gender-specific control of myocardial adaptation to hemodynamic overload and a more rapid induction of left ventricular hypertrophy during myocardial dysfunction in men than in women.
2. The difference in synthesis/secretion sites for ANP and BNP. ANP is prevalently produced and secreted in the atria by all conditions which induce stretching of the atrial wall, while BNP is preferentially produced and secreted in the ventricles after stretching of the ventricle wall (15, 40). Furthermore, BNP is generally considered to be a reliable and sensitive index of cardiac hypertrophy (1–5, 15, 38, 40), even without dilation of the ventricle chambers.
3. The possible effects of sex steroid hormones on the ANP/BNP system. Some studies suggest that female steroid hormones have a stimulating effect on the ANP/BNP system (35, 41); evidently, the maximum effect is observed in healthy cycling women, with a progressive decrease after the menopause. Furthermore, the action of female steroid hormones is probably greater on plasma BNP than on ANP levels (35). Only scarce and contradictory data have been reported concerning the action of male steroid hormones on the ANP/BNP system (41–44).

The combined action of these three mechanisms may explain different patterns of circulating levels of ANP and BNP found in both sexes throughout adult life. While the significant differences between CNH levels in young adult men and cycling women could be explained by the effect of female steroid hormones, it is more difficult to explain the gender differences in hormone levels after menopause.

To better illustrate the possible influence of old age and the effects of the decrease in the levels of female sex hormones after menopause, we divided 222 healthy subjects into four groups according to age and sex (two groups included 61 men and 67 women aged <50 years; the other two groups included 47 men and 47 women aged ≥50 years) (Table 3). The cut-off of 50 years was chosen because this value is usually considered to be the mean age of menopause, at least in Western European countries.

Table 3 Circulating levels of ANP and BNP in 222 healthy subjects divided into four groups according to sex and age (see reference 32 for more details).

	Men	Women	p-Value*
ANP (pg/ml)	Mean (±SD)	Mean (±SD)	
Age < 50 years	12.8± 6.9	16.1±10.0	0.2696
Age ≥ 50 years	21.4±11.0	22.1±12.7	0.7714
p-Value*	0.0002	0.0015	
BNP (pg/ml)			
Age < 50 years	5.9±6.0	10.1± 8.3	<0.0001
Age ≥ 50 years	10.1±7.8	15.6±11.2	0.0099
p-Value*	0.0001	0.0080	

*Fisher's protected least significant difference (PLSD) test after ANOVA using the logarithmic transformation of original set of data.

Taking into account the data reported in Figure 4 and in Table 3, plasma ANP values tend to be higher in healthy cycling women compared to men of the same age owing to the stimulating effects of female steroid hormones; with menopause this action decreases, but the effects produced by the alteration of the cardiovascular system induced by aging progressively increase, inducing a similar increase in ANP levels in both sexes (32). Our data suggest that women have a biphasic pattern of ANP throughout adult life with a decline occurring in the age group of 40–49 years, probably because some women enter menopause at this age (Figure 4). On the contrary, a progressive and linear increase of circulating levels of ANP was found in men (Figure 4) (32).

Our findings also suggest that plasma BNP levels (Figure 5 and Table 3) are higher in healthy cycling women than in men of the same age. After menopause, the effect of steroid hormones decreases, but the increased thickness of the interventricular septum and ventricular wall with aging, more frequent and more accentuated in women (33, 36–39), may induce a progressive increase in BNP, also greater in women. Consequently, women show higher BNP values than men throughout adult life (Figure 5).

In conclusion, according to the mechanisms reported above, the higher CNH values of women compared to those of men during the fertile adult period could be explained by the physiological stimulation by female sex steroid hormones. The increase in circulating levels of CNH caused in women by female sex steroid hormones during the cycling period (32), as well as by hormone replacement therapy after menopause (35), should not be considered an index of myocardial dysfunction; on the contrary, this stimulation

may explain the lower incidence of cardiovascular disease in women before menopause and should therefore be considered a cardioprotective effect (35, 41). On the other hand, it can be hypothesized that the increase in CNH with age may be due to the decline in myocardial function typical of senescence (33, 36) and/or to a decrease in CNH clearance related to the aging process. Indeed, a recent study reported that the amount of C-type receptors in platelets was significantly reduced in elderly healthy subjects compared to the younger group; moreover, a significant inverse relationship between the amount of receptors per cell and the subject's age was observed (45). Further studies are necessary to test these hypotheses.

Comparison between CNH Assay and that of CNH-Related Pro-Hormone Peptides

The circulating levels of N-terminal proANP and proBNP are closely correlated with the respective levels of ANP and BNP, since these two forms of peptides are equimolar upon release (34) (Figures 1 and 2). While ANP and BNP are biologically active peptides, the respective N-terminal pro-hormones (*i.e.* proANP and pro-BNP-related peptides) have no biological action, since they lack the cysteine ring in the peptide chain which binds to the specific receptors. Consequently, only the circulating levels of ANP and BNP should be considered a reliable and direct index of hormonal status (activity) of the CNH system.

Owing to their lower turnover rates, N-terminal proANP and proBNP concentrations are more stable and thus are less affected by the acute variations in hemodynamic conditions and/or fluid and electrolyte homeostasis than ANP and BNP (34). Consequently, the assay of N-terminal proANP and proBNP could be less informative in conditions characterized by rapid variations in cardiovascular hemodynamics and/or myocardial function, and, on the contrary, they could be more clinically useful in the follow-up of patients with chronic (rather than acute) disease than ANP and BNP.

Theoretically, setting up an immunoassay for N-terminal peptide fragments of proANP and proBNP should be easier than that for ANP and BNP, because these peptides have higher plasma concentrations (Table 1) (34). However, immunoassays for N-terminal peptide fragments of proANP and proBNP may be affected by several analytical problems, mainly related to different assay specificities. Consequently, very different results are produced by different methods and a large bias is observed (34).

Table 4 summarizes the respective advantages of the assay of biologically active peptide hormones (ANP and BNP) compared to the assay of N-terminal peptide fragments of proANP and pro BNP. The measurement of plasma concentration of N-terminal proANP or proBNP, which are inactive peptides, fits better the definition of a disease marker than the measurement of circulating levels of ANP or BNP, which can be considered a more reliable index of the activation of the CNH system.

Taking into account these physiological characteristics, it is theoretically conceivable that ANP is a better marker of acute overload and/or rapid cardiovascular hemodynamic changes than BNP, and especially than intact N-terminal proANP or proBNP. For example, it is well-known that the circulating levels of ANP decrease more than those of BNP during hemodialysis session in patients with chronic renal failure, while plasma levels of intact N-terminal $proANP_{1-98}$ do not significantly change (19, 34, 46, 47). Furthermore, ANP increases to a greater extent than N-terminal $proANP_{1-98}$ during rapid ventricular pacing (48).

Resistance to the Biological Effects of CNH in Patients with Heart Failure

Deficiencies in the activity of the CNH system could explain the disturbed fluid and electrolyte homeostasis in chronic heart failure. However, the attempt to treat heart failure as a syndrome of CNH deficiency was challenged when this system was investigated in experimental animals and in humans. In fact, patients with chronic heart failure show greatly increased plasma levels of CNH compared to normal subjects (1–6) (Figure 6). Moreover, a blunted natriuretic response after pharmacological loading doses of ANP and BNP (or structurally related and biologically active substances, such as anaritide or besiritide) was observed in experimental models and in patients with chronic heart failure, thus suggesting a resistance to the biological effects of CNH, such as natriuresis (2, 23, 28, 49). The presence of this resistance syndrome was demonstrated by an *in vivo* turnover study using radioactive tracers in patients with heart failure (21, 23, 28, 49).

Some data suggest that resistance to the biological effects of CNH in heart failure may be due, at least in part, to variations in the ratio between biological and

Table 4 Relative adavantages of ANP and BNP assay compared to that of N-terminal propeptides.

ANP/BNP	N-terminal proANP/BNP
Close correlation between hormonal and immunological activity	Higher and more stable circulating levels
Better correlation between physiological or clinical conditions in acute changes of hemodynamic parameters or myocardial function	Lesser degradation *in vivo* and *in vitro*

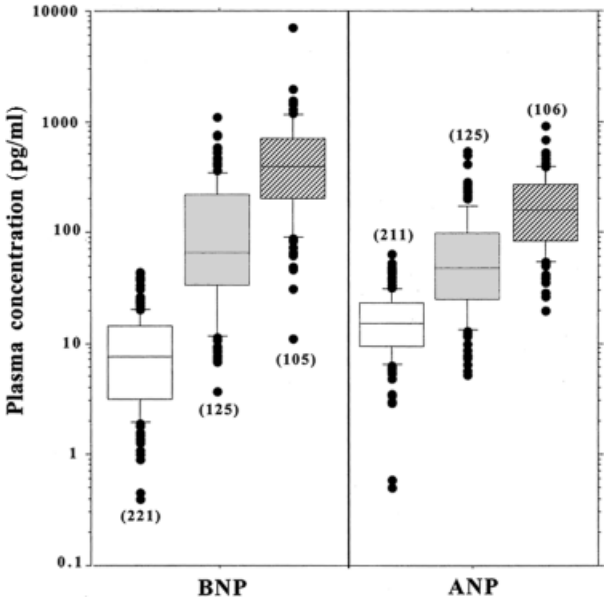

Figure 6 Circulating levels of ANP and BNP measured in healthy subjects and in patients with heart failure (HF), divided according to the severity of disease (mild HF: NYHA class I and II; severe HF: NYHA class III and IV). The number of subjects included in each group is indicated in brackets. The results are expressed as boxes with 5 horizontal lines, displaying the 10th, 25th, 50th (median), 75th, and 90th percentiles. All values above the 90th percentile and below the 10th percentile (outliers) are plotted separately (as circles). □ normal subjects, ▩ mild HF, ▨ severe HF.

clearance-specific CNH receptors, related to an increase (upregulation) of clearance receptors (the so called type C-receptors) with a parallel decrease (downregulation) of biological receptors (type A- and B-receptors) (50–53). Type A- and B-receptors mediate all known hormonal actions of the CNH, so their downregulation induces a deactivation of the CNH system. The upregulation of C-receptors, which do not mediate any biological effects but only contribute to the clearance of the biologically active peptides, further increase the resistance to CNH in patients with heart failure.

Since increased activity of the neurohormonal system may cause and accelerate the deterioration of left ventricular function that occurs over time, drugs which can inhibit the detrimental action of neurohormonal systems have now assumed a fundamental role in treatment of heart failure (2, 3, 23, 25, 28). The drugs commonly used in heart failure (such as ACE inhibitors and β-blockers) tend to "normalize" the turnover and kinetics parameters and increase their biological activity (23, 28). Furthermore, this therapeutic approach increases the natriuretic effects of the administration of ANP or BNP analogues or, in other words, decreases resistance to the biological effects of CNH (23, 28). These data indicate that the estimation of the degree of resistance to the biological effects of CNH may be clinically useful in patients with heart failure.

According to the above hypothesis, the measurement of circulating levels of ANP and BNP may allow a more reliable and earlier assessment of this hormonal system than the measurement of the N-terminal proANP and proBNP-related peptides, since these levels are promptly and finely regulated by even small changes in the activation of the counter-regulatory neurohormonal system and in the number and affinity of C-receptors. However, further studies are necessary to confirm this hypothesis.

Discussion and Conclusions

Up to 30 years ago, clinical laboratories could offer only a very few diagnostic tests dedicated to the prevention and diagnosis of cardiovascular disorders and to the follow-up of patients with cardiac disease (54). In recent years, this field has grown at a tremendous rate with the introduction of more specific and/or sensitive markers for myocardial injury (such as myoglobin, CK-MB, myosin, and especially troponin I and T) and with the development of an increasing number of diagnostic tests for the assessment and stratification of cardiovascular risk, in particular related to the presence of the atherosclerotic disease associated with inflammation and thrombus formation (55). However, until a few years ago, there were no laboratory tests for diagnosis, stratification and follow-up of patients with heart failure; the CNH assay fills this gap.

It is important to emphasize that heart failure is not only the most frequent "final common pathway" in cardiovascular disease, but is also the most common primary hospital discharge diagnosis, as well as the foremost cause of death in patients over 50 years of age in the Western countries (36, 37). For these reasons, CNH assay may be destined to assume a growing relevance in clinical cardiology.

However, to consider CNH assay solely as a general and functional marker of cardiac structural disease, without recalling that ANP and BNP are powerful hormones, may lead to underestimation of the physiological role they play in healthy subjects as well as in patients with heart failure or other conditions. Indeed, circulating levels of CNH should be interpreted taking into account not only hemodynamic parameters and myocardial performance but also their relationship with the counter-regulatory neuroendocrine system (including the renin-angiotensin-aldosterone system, sympathetic system, endothelins, cytokines and vasopressin) as well as other hormones (such as sex steroid hormones, thyroid hormones and glucocorticoids).

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