

injury (13). In patients with severe chronic heart failure (NYHA III and NYHA IV), cTnI (4, 6) and cTnT (5, 7) are often increased. In our study, none of three carriers with an increased CK-MB/CK-total ratio had dilated cardiomyopathy and none had detectable troponins. Thus a CK-MB/CK-total ratio >3% did not appear to be indicative of severe cardiac abnormalities in our study population. Two carriers had detectable cTnT; in one carrier with borderline echocardiographic abnormalities, it was slightly increased. In all other carriers, cTnT was not detectable. None of the carriers had detectable cTnI. Although 30 women (23%) among the DMD/BMD carriers showed left ventricle dilation or dilated cardiomyopathy on echocardiography, these abnormalities probably reflected cardiac dysfunction rather than cardiac cell necrosis. In our study group, no carrier had severe heart failure that was associated with increased troponins. cTnI and cTnT can be used, however, for carriers suspected of cardiac ischemia. Measurement of CK-MB alone could give a false-positive result because of high total CK.

Some authors have found a negative correlation between CK activity and age in DMD (14) and BMD carriers (15), whereas others have not (16). We found no such correlation, but we were able to demonstrate a linearly decreasing trend of total CK with increasing age groups of carriers. Surprisingly, only 53% of DMD carriers and 30% of BMD carriers had increased total CK. Only one earlier study found similar percentages (17), whereas most other studies found raised CK activity in 60–80% in DMD (16, 18–22) and 42–62% of BMD carriers (15, 20, 22). These relatively high percentages prompted clinicians in the predystrophin era to calculate the risk of being a carrier. Perhaps the difference between our study and those carried out before the 1990s is that in most of these investigations, repeat measurements of total CK were done, whereas we performed only one measurement.

In conclusion, detectable cTnI and cTnT are rare in DMD and BMD carriers and bear no relationship with disease-specific cardiac abnormalities. We cannot exclude the possibility that cTnI and cTnT will be increased in carriers with severe heart failure because no severe heart failure was present in our study group.

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**Relationship between Natriuretic Peptide Concentrations in Plasma and Posture during Blood Sampling,** Frans Boomsma,\* Jaap Deinum, and Anton H. van den Meiracker (Department of Internal Medicine, Erasmus University Medical Center, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands; \* author for correspondence: fax 31-0-10-4634531, e-mail boomsma@inw1.azr.nl)

Measurements of the plasma concentrations of one or more of the natriuretic peptides can be a valuable tool in the diagnosis, prognosis, and follow-up of patients with cardiac dysfunction (1, 2). Measurement techniques have also improved, allowing for less time-consuming direct assays than previous methods, which often required the prior extraction of peptides from plasma. However, improvement is still needed (3).

In most research settings, blood sampling is performed under carefully controlled conditions, involving the introduction of a catheter and ample bed rest before the collection of blood. Such procedures are necessary for the reliable interpretation of many neurohormone measurements. Catecholamines, for example, can fluctuate widely depending on the blood-sampling procedure and posture. Because strictly adhering to rigorous blood-sampling pro-

ocols is often impossible in everyday clinical situations, we investigated the variation in measured natriuretic peptide concentrations because of differences in blood-sampling conditions.

We have measured atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), N-terminal-pro-ANP (Nt-ANP), as well as the catecholamines noradrenaline (NOR), adrenaline, renin (REN), and aldosterone (ALDO). We studied subjects in the relevant age range (>40 years) with diseases of interest in four different blood-sampling conditions, while continuing appropriate medications for these conditions. Twenty-three subjects ( $55 \pm 10$  years; range 42–74 years) participated in the study after giving their informed consent. Six subjects were apparently healthy controls ( $46 \pm 5$  years; two females), nine were hypertensive patients ( $53 \pm 7$  years; two females), and eight ( $64 \pm 9$  years; three females) were patients with diseases known to lead to increased concentrations of natriuretic peptides: congestive heart failure (four subjects) and chronic renal failure (four subjects). All patients were taking appropriate medications for their conditions. Immediately after entering the examination room in our department, subjects sat down and a small catheter was inserted into an antecubital vein. Blood was sampled directly and then after 30 min of continued quiet sitting. Blood was sampled again both after 30 min of lying on a bed (bed rest) and after 30 min of standing and walking. The whole procedure was repeated on a second visit, at least 7 days later (median interval, 12 days) to assess intraindividual variation from one day to another. The procedure was always performed in the morning between 0900 and 1200.

Blood was collected in two different tubes: (a) one tube containing EDTA and aprotinin (1.9 mg and 100 kIU/mL of blood, respectively) for measurement of natriuretic peptides and (b) one heparin-containing tube containing 1.2 mg of glutathione/mL of blood for measurement of NOR, adrenaline, REN, and ALDO. The tubes were centrifuged (3000g) within 30 min at 4 °C for 10 min, and the plasma was separated and stored at -70 °C until assayed. ANP was determined by RIAs (Nichols Institute) after Sep-Pak C<sub>18</sub> extraction from plasma. BNP was measured with immunoradiometric assays from Shionoria, and Nt-ANP was measured with RIAs from Biotop, both

without extraction. Catecholamines were measured by HPLC with fluorometric detection after extraction from plasma and derivatization with dimedone (4). ALDO was determined with RIAs from Diagnostic Products Corporation. REN was determined by RIA of generated angiotensin I (5). All methods used had intraassay CVs in the relevant range of <10%. All samples from one subject were measured in the same assay.

Mean neurohormone concentrations of samples collected after 30 min of bed rest on the first visit are reported in Table 1, as are the concentrations for samples collected directly after the subjects arrived and the samples collected after 30 min of sitting and after 30 min of standing and walking. The concentrations in Table 1 are also expressed as percentages of the individual neurohormone concentrations after 30 min of bed rest. ANOVA analysis showed that the difference between the four procedures is significant for all neurohormones, but posttests with Bonferroni correction indicated that the differences in the natriuretic peptides were small and often (with BNP) not significant. In contrast to the well-known fact that REN and ALDO vary quite considerably under different blood-sampling conditions, natriuretic peptides appeared to be much less variable. Samples collected directly after patients arrived gave somewhat higher values, but values obtained during quiet sitting were indistinguishable from those obtained during bed rest. For Nt-ANP, the differences were the smallest, which was not surprising in view of its relatively long half-life. These findings agree with the results of Wijbenga et al. (6), who, in a study on the response of natriuretic peptides to exercise, found the highest percentage of change in ANP, a smaller percentage of change in BNP, and the smallest percentage of change in Nt-ANP. Nt-BNP, which was measured in that study (6) but not in our present study, changed more than Nt-ANP but less than BNP. For the catecholamines REN and ALDO, there were considerable differences in the values obtained directly after arrival and after 30 min of bed rest, but the differences between the values obtained after 30 min of sitting and after 30 min of bed rest were much smaller. Furthermore, Bonferroni posttests produced statistically significant results for ALDO only. The lack of statistical significance for the difference between sitting and bed rest values for NOR and adrenaline

**Table 1. Neurohormone data at first visit for the 23 subjects.**

	ANP, pmol/L	BNP, pmol/L	Nt-ANP, pmol/L	NOR, ng/L	ADR, <sup>a</sup> ng/L	ALDO, ng/L	REN, $\mu\text{g}$ Angl · L <sup>-1</sup> · h <sup>-1</sup>
Basal values (after 30 min of bed rest)							
Mean $\pm$ SD	52 $\pm$ 28	6.1 $\pm$ 7.2	448 $\pm$ 292	273 $\pm$ 127	30 $\pm$ 26	91 $\pm$ 48	19.0 $\pm$ 31.5
Range	26–122	0.7–27.0	72–1152	114–700	6–116	19–185	2.2–112.6
% of basal (mean $\pm$ SD) when sampled							
Direct sampling	124 $\pm$ 49 <sup>b</sup>	114 $\pm$ 23	108 $\pm$ 15 <sup>b</sup>	186 $\pm$ 59 <sup>b</sup>	190 $\pm$ 99 <sup>b</sup>	162 $\pm$ 55 <sup>b</sup>	126 $\pm$ 30 <sup>b</sup>
After 30 min of sitting	99 $\pm$ 20	101 $\pm$ 17	104 $\pm$ 12	127 $\pm$ 33	122 $\pm$ 37	128 $\pm$ 31 <sup>b</sup>	112 $\pm$ 18
After 30 min of standing/walking	114 $\pm$ 29	116 $\pm$ 36	110 $\pm$ 16 <sup>b</sup>	228 $\pm$ 95 <sup>b</sup>	155 $\pm$ 54 <sup>b</sup>	159 $\pm$ 90 <sup>b</sup>	129 $\pm$ 42 <sup>b</sup>
ANOVA <i>P</i> value	0.0046	0.0188	0.0066	<0.0001	<0.0001	<0.0001	<0.0001

<sup>a</sup> ADR, adrenaline; Angl, angiotensin I.

<sup>b</sup> Significantly different from basal.

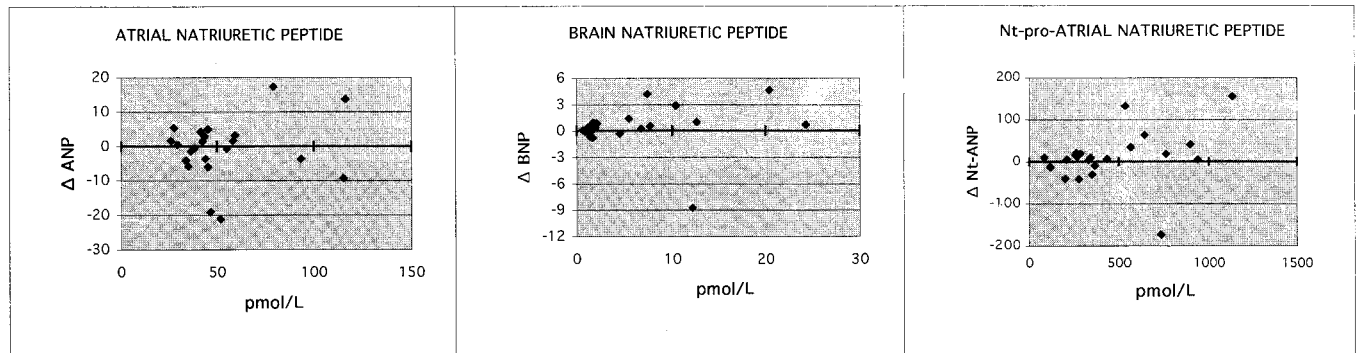


Fig. 1. Bland-Altman plots of absolute differences in ANP, BNP, and Nt-ANP between the two visits (blood sampling after 30 min sitting).

contrasts with previous findings from our laboratory (7). In that study, however, only young healthy subjects took part, whereas in the present study, subjects were older, and most had diseases associated with an activation of the sympathetic nervous system. We should mention that in younger subjects, some neurohormones may have a greater response than in the group of older subjects we studied. The effects of medication used by all patients may have also played a role.

At the second visit (again as a percentage relative to the samples collected at the first visit after 30 min of bed rest), the pattern was much the same as at the first visit. The difference between the four procedures was significant for all neurohormones, and posttests broadly showed similar results as on the first visit. Correlations between absolute values for the same blood-sampling procedure at the first visit vs those at the second visit were all significant, except for NOR after 30 min of standing and walking. A good impression of individual differences could be obtained by constructing Bland-Altman plots (8), which are shown for the natriuretic peptides in the sitting situation in Fig. 1. These indicate that although usually there was good agreement between measurements on the two different visits, occasionally a large difference was found. This could be a result of the timing of the medication used (e.g., the effects of angiotensin-converting enzyme inhibitors on REN and ALDO values), but sometimes also for no apparent reason. Here again, the differences in Nt-ANP and BNP appeared to be the smallest.

Which of the various natriuretic peptides is (are) most informative with regard to the diagnosis, course, and treatment of heart failure is still being debated. From the viewpoint of stability in blood samples, the N-terminal peptides, as well as BNP, appear to be the best (9-13). ANP is less stable, although not as unstable as has been suggested (14). In addition, because of longer half-lives, short-term fluctuations will have less of an effect on the measured concentrations of the N-terminal peptides than of the C-terminal peptides. However, clinical studies are needed to determine which is (are) the most informative.

We conclude that the measurement of natriuretic peptides in a single blood sample, obtained after 30 min of quiet sitting, can give reliable and reproducible basal

values. Measurements of the catecholamines REN and ALDO in the same blood sample are only marginally higher than values obtained after 30 min of bed rest. Especially for BNP and Nt-ANP, blood sampling directly after arrival may be a reasonable alternative when time and/or facilities are limited.

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