Plasma proANP Decreases after Meal Intake

To the Editor:

Cardiac natriuretic peptides and their molecular precursor fragments are sensitive plasma markers of congestive heart failure and left ventricular dysfunction. Most studies on their value as markers in cardiac diseases have also addressed the effects of aging and sex, in which increasing age and female sex are associated with higher concentrations per se and thus are recommended to be included in establishing reference intervals. Biological variation has also been associated with the circadian pattern of cardiac natriuretic peptide mRNA contents in mice and the concomitant plasma concentrations in humans.

New findings have demonstrated that natriuretic peptides and their receptors are involved in fat and muscle metabolism (1). These data, combined with the observation that obese people display lower plasma concentrations than individuals of normal weight, suggest that cardiac natriuretic peptides are also involved, for instance, in the metabolic syndrome and perhaps even in diabetes. No study thus far has tested whether plasma concentrations of pro–atrial-type natriuretic peptide (proANP)1 and pro–brain-type natriuretic peptide (proBNP) may be affected by meal intake, a standard stimulus for the gastrointestinal output of hormones involved in glucose metabolism. Therefore, in this study the 3-h effect of meal intake in healthy young individuals was tested.

Six healthy young individuals between 20 and 30 years of age (2 men and 4 women) were included. None of the participants reported having any known disease, and all were free of medication. All individuals gave informed consent to participate, and the study was performed according to the Helsinki Declaration. The participants fasted overnight and met at 0800 for the test. An intravenous catheter was inserted into the antecubital vein for blood sampling. The meal consisted of 250 g roast beef, 300 g oven-baked potatoes in cream sauce, 1 bread roll, and 250 mL orange juice (1500 calories total). Water was administered ad libitum. Ten milliliters of blood was collected into EDTA-containing tubes every 10–15 min, kept on ice, and then immediately centrifuged (2500 g) for 10 min. The plasma was then stored at −80 °C until analysis. Plasma proANP was measured with an in-house assay, a so-called processing-independent assay (2). The performance of the assay and its correlation with other proBNP methods have been published (5).

The results of proANP measurements are shown in Fig. 1. The fasting mean plasma concentration was 176 pmol/L (95% CI, 134–218 pmol/L; n = 6). A decrease in plasma proANP was observed at 60 min and in hour 3 of the experiment. The statistical significance of these changes were tested with one-way ANOVA, followed by the Newman-Keuls multiple-comparison test (P < 0.0018). Although not statistically significant, a minor decrease was also noted 10 min after the start of the meal. In contrast, no changes were seen in plasma proBNP over the whole course of the 180-min experiment (data not shown).

The present study revealed that plasma proANP concentrations decrease dramatically in healthy young individuals after meal intake. In contrast, no such effect was observed for proBNP in plasma. To the best of the author’s knowledge, this decrease in proANP after food intake has not been reported previously, but it may have direct clinical implications. No rules for fasting before blood sampling for proANP analysis have been recommended thus far. In a worst-case scenario, the results of the present study imply that an increase in

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1 Nonstandard abbreviations: pro-ANP, pro–atrial-type natriuretic peptide; pro-BNP, pro–brain-type natriuretic peptide.
proANP as a marker for heart failure could be missed with a nonfasting sample. If the clinical presentation is unclear, such an occurrence could have dire consequences for the patient with values that are otherwise in the diagnostically “gray zone.” For now, the recommendation is that proANP measurements be made on fasting patients. Future studies must also examine the effects of meal intake on the active hormones (ANP and BNP), for which their profiles with respect to food intake are completely unknown. Finally, investigations for mechanistic insights into this phenomenon should address the possible relationship between insulin and natriuretic peptide secretion to explain the current finding and perhaps the reportedly low plasma proANP and proBNP concentrations in obese persons.

In conclusion, plasma proANP concentrations, but not proBNP concentrations, are affected by meal intake, with a decrease of 50% after 2 h. This observation needs to be confirmed in clinical studies of proANP. If the results are confirmed, the findings may provide new biological insight into insulin-resistant persons or patients with diabetes.

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References

Jens P. Goetze*
Department of Clinical Biochemistry
Rigshospitalet
University of Copenhagen
Copenhagen, Denmark

*Address correspondence to the author at: Department of Clinical Biochemistry
Section 3014, Rigshospitalet
Blegdamsvej 9, DK-2100
Copenhagen, Denmark
Fax +45-3545-2880
E-mail JPG@dadmderk.dk

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Comparison of Bleomycin-Detectable Iron and Labile Plasma Iron Assays

To the Editor:

Non–transferrin-bound iron (NTBI)1 is detected in the plasma of patients with diseases in which the transferrin-binding capacity for iron is exceeded by a massive iron overload, such as in certain forms of thalassemia and hemochromatosis, repeated blood transfusions, or bone marrow failure. NTBI may occur in plasma as insoluble polynuclear ferrhydrate species, as Fe(III) citrate or acetate complexes, or bound to certain flavonoids, amino acids, albumin, or modified albumin. Excess iron is harmful because it causes oxidative stress, accumulates in and damages tissues, and enhances the growth of pathogens. Therefore, the measurement of NTBI is of clinical importance, because NTBI may be used as an indicator of systemic iron overload and iron toxicity (1, 2).

We compared 2 NTBI assays: a labile plasma iron (LPI) assay that uses desferrioxamine as the iron chelator (3) and a microwell modification of a bleomycin-detectable iron (BDI) assay (2). The BDI assay is an indirect method based on the formation redox-active complexes between NTBI in the sample and the bleomycin reagent. The Fe(II)–bleomycin complex then degrades DNA in the sample mixture via a free-radical reaction, which can be measured photometrically. The detection limit of our BDI assay was 0.05 μmol/L, and the interassay CV was 18% and 9.8% at 0.2 μmol/L and 1.5 μmol/L, respectively (2). The LPI assay is based on the measurement of the redox-active and readily chelatable fraction of serum NTBI. The assay measures iron-catalyzed radical generation in the presence of a low ascorbate concentration. Radical generation is measured with the fluorogenic redox-sensitive probe dihydrorhodamine 123 (Sigma-Aldrich), and iron-catalyzed radical generation is calculated by subtracting the radical generation in the presence of 100

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1 Nonstandard abbreviations: NTBI, non–transferrin-bound iron; LPI, labile plasma iron (assay); BDI, bleomycin-detectable iron (assay); TS, transferrin saturation.