Editorial Commentary

Does impaired processing of pro-B-type (or brain) natriuretic peptide cause decreased plasma BNP levels in obese heart failure patients?

Toshio Nishikimi[1,2], Yasuaki Nakagawa[1]

[1]Department of Cardiovascular Medicine, Kyoto University Garduate School of Medicine, Kyoto, Japan; [2]Department of Internal Medicine, Wakakusa-Tatsuma Rehabilitation Hospital, Osaka, Japan

Correspondence to: Toshio Nishikimi, MD, PhD. Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. Email: nishikim@kuhp.kyoto-u.ac.jp.

Provenance: This is an invited article commissioned by our Guest Section Editor Dr. Ying Zhao (Department of Laboratory Medicine, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China).

Comment on: Lewis LK, Raudsepp SD, Prickett TCR, *et al.* ProBNP That Is Not Glycosylated at Threonine 71 Is Decreased With Obesity in Patients with Heart Failure. Clin Chem 2019. [Epub ahead of print].

Submitted Aug 04, 2019. Accepted for publication Aug 11, 2019.
doi: 10.21037/atm.2019.08.56
View this article at: http://dx.doi.org/10.21037/atm.2019.08.56

The natriuretic peptide system consists of three distinct endogenous peptides: atrial natriuretic peptide (ANP), brain (or B-type) natriuretic peptide (BNP), and C-type natriuretic peptide (CNP), as well as three receptors: natriuretic peptide receptor-A (NPR-A or guanynyl cyclase-A), natriuretic peptide receptor-B (NPR-B or guanynyl cyclase-B) and natriuretic peptide receptor-C (NPR-C or clearance receptor) (1). ANP and BNP are cardiac hormones, with ANP produced mainly in the atria and BNP produced mainly in the ventricles. CNP is produced in the central nervous system, bone, and vascular endothelial cells. Although BNP was discovered in porcine brain extract, subsequent studies demonstrated that BNP is a cardiac hormone in humans, that plasma BNP levels are increased in proportion to the severity of heart failure, and that the magnitude of the change in BNP is greater than the change in ANP (1). Accordingly, BNP measurements are now used as a diagnostic biomarker for heart failure all around the world, and current guidelines used worldwide recommend measurement of BNP and N-terminal proBNP (NT-proBNP) as a useful diagnostic biomarker for acute and chronic heart failure (2).

Up to about 10 years ago, proBNP, the precursor of BNP, was thought to be processed to BNP and NT-proBNP intracellularly and to be secreted from the heart into circulation at a 1:1 molar ratio. However, recent studies have revealed that precursor proBNP as well as BNP and NT-proBNP are all increased in heart failure, but that the relative ratio of BNP to proBNP is altered depending on the specific pathophysiological conditions of the heart failure (3). Previous studies also revealed that current BNP immunoassay kits cross-react with proBNP (4). Because proBNP has little biological activity, increasing proBNP levels may do little to compensate heart failure. Moreover, mass spectrometry analyses showed that there is very little BNP1-32 (mature BNP) in the plasma of heart failure patients, but there are higher levels of BNP fragments, such as BNP3-32, BNP4-32, and BNP5-32, and that current BNP assay kits cross-reacts with those too (4). In addition, the N-terminal portion of proBNP is *O*-glycosylated as a posttranslational modification (5), and high concentrations of *O*-glycosylated proBNP and NT-proBNP circulate in the plasma of patients with heart failure (6).

One should be aware that various conditions can lead to the overestimation or underestimation of BNP levels when they are used as a diagnostic test for heart failure. The pathophysiological conditions in which BNP is overestimated include renal failure, atrial fibrillation, inflammation, cancer, hyperthyroidism, use of LCZ696 (sacubitril/valsartan), and macroproBNP. The pathophysiological conditions in which BNP is underestimated are obesity, constrictive pericarditis,

pericardial effusion, and flash pulmonary edema (7). BNP is eliminated from the blood through metabolization by neprilysin (neutral endopeptidase), by binding to NPR-A and NPR-C, and by excretion into the urine. It is known that neprilysin is highly expressed in the brush border of renal tubular cells (8), and that NPR-A is highly expressed in renal tubular cells and glomerular cells. As a result, BNP elimination is decreased in renal failure, leading to increased plasma of BNP. In addition, increased body fluid levels in renal failure may contribute to increased cardiac production of BNP. In atrial fibrillation, plasma BNP increases through overproduction of BNP, probably in the atria. Plasma BNP levels are increased in atrial fibrillation, even without heart disease. Consequently, the cutoff point for diagnosis for heart failure should be elevated in heart failure patients with atrial fibrillation. Inflammatory cytokines directly stimulate *BNP* gene expression (9), and plasma BNP levels are greatly increased in myocarditis. Furthermore, if cardiac rejection occurs in patients who have received heart transplantation, plasma BNP increases even when intracardiac pressure is normal. Thyroxin also directly stimulates *BNP* gene expression. In fact, in patients with thyrotoxicosis, plasma BNP levels are high, and with treatment normalizing thyroid hormone levels, plasma BNP levels are also normalized (10). As mentioned above, BNP and ANP are metabolized in part by neprilysin. Recently, the PARADIGM-HF study reported that LCZ696, a formulation containing antagonists of both neprilysin and angiotensin receptor, clearly improves mortality rates and reduces the occurrence of heart failure, as compared to enalapril, the conventional first choice drug for chronic heart failure. Because one possible mechanism of LCZ696's beneficial effects is inhibition of ANP and BNP degradation (mainly ANP), the concentration of BNP may increase in patients taking LCZ696. In the PARADIGM-HF study, the proportion of patients whose plasma BNP concentration was higher than the pretreatment level after 8 to 10 weeks of LCZ696 treatment was larger than the proportion exhibiting increases in NT-proBNP. Nonetheless, plasma BNP levels were well correlated with plasma NT-proBNP levels, and the prognostic accuracy of plasma BNP was nearly the same as plasma NT-proBNP (11).

There are also cases in which plasma BNP levels are underestimated. Patients with constrictive pericarditis have lower BNP levels than those with restrictive cardiomyopathy, though the hemodynamic indices are similar in the two groups (7). It is also known that plasma BNP and ANP levels are lower in patients with pericardial effusion, and that BNP and ANP levels start to increase after pericardial drainage to remove the effusion (12). Because atrial and ventricular stretch is an important stimulus for ANP and BNP release, suppression of stretch by massive effusion or thickening of the pericardium may lead to reduced BNP release from both the atria and ventricles. Flash pulmonary edema is caused by a sudden loss of cardiac function due to ischemic heart disease, such as acute myocardial infarction. ANP is stored in atrial granules and is immediately secreted in response. This is a so-called regulatory pathway. By contrast, BNP is controlled via gene expression, which is a so-called constitutive pathway. It therefore takes time for blood levels of BNP to rise, even in response to a strong stimulus. For example, BNP levels are not increased within 30 min after the onset of acute myocardial infarction (13). On the other hand, one hour after treatment of acute heart failure, BNP levels are reportedly changed significantly in parallel with the hemodynamic changes. Thus, in cases of rapid hemodynamic change, changes in plasma BNP may be delayed, so caution is required.

In patients with obesity-related heart failure, plasma BNP levels are lower than one would expect from the severity of the heart failure; however, the precise mechanism has not been elucidated (14). Adipocytes strongly express NPR-A, and BNP and ANP can induce lipolysis via NPR-A (15). This finding may be associated with the cardiac cachexia observed in severe heart failure patients. In addition, adipocytes also strongly express NPR-C (15). Because BNP binds to both NPR-A and NPR-C expressed in adipocytes, the plasma BNP levels in obese patients are thought to be decreased as a result of trapping by these receptors. In the Framingham study, obese subjects (BMI >30) with lower BNP levels also showed lower levels of NT-proBNP (16). NT-proBNP and BNP are processed from proBNP at a 1:1 molar ratio, but NT-proBNP cannot bind to NPRs and has no bioactivity. Consequently, the lower plasma NT-proBNP levels seen in obese subjects cannot be explained by trapping by NPRs expressed in adipocytes. This suggests cardiac production of BNP is reduced in obese patients. Mizuno *et al.* (17) reported that plasma BNP levels in the coronary sinus are lower in obese heart failure patients than non-obese patients, and that plasma BNP levels in obese heart failure patients correlate inversely with HOMA-R, an index of insulin resistance. Taken together, these results suggest that lower plasma BNP levels in obese heart failure patients may be due not only to trapping by NPR-A and NPR-C expressed in

adipocytes, but also to reduced cardiac BNP production.

In the article "ProBNP That Is Not Glycosylated at Threonine 71 Is Decreased With Obesity in Patients with Heart Failure" published in *Clin Chem*, Lewis *et al*. (18) reported interesting new findings that may explain why plasma BNP is decreased in patients with obesity-related heart failure. They developed three immunoassay systems to measure total proBNP (non-glycated proBNP + glycosylated proBNP), Thr71-nonglycosylated proBNP, and central region-nonglycosylated proBNP. They then tested plasma from obese and non-obese heart failure patients using these three immunoassays and a conventional NT-proBNP assay. The obese patients had lower NT-proBNP and lower Thr71-nonglycosylated proBNP levels than the non-obese patients, while there were no differences in central region-nonglycosylated proBNP or total proBNP levels between the two groups. This finding suggests that levels of Thr71-glycosylated proBNP are higher in obese than non-obese heart failure patients. Given that NT-proBNP levels are also low in obese heart failure patients, it may be that in patients with obesity-related heart failure, the increased Thr71-glycosylated proBNP impairs proBNP processing and leads to decreases in NT-proBNP.

It was revealed for the first time in 2006 that proBNP is an *O*-linked glycopeptide (5). *O*-glycosylation is a post-translational modification known to abundantly occur in the N-terminal region of proBNP at Thr36, Ser37, Ser44, Thr48, Ser53, Thr58, and Thr71 (2). A more recent study showed that *O*-glycosylation also occurs at Ser4 and Thr14 or Thr15 of proBNP (19). Glycosylation is generally known to be involved in protein stabilization by making them more water soluble and less susceptible to metabolism, among other effects. Glycosylation is also known to be involved in peptide processing. For example, the glycosyltransferase ppGalNacT3 (GALNT3) mediates *O*-glycosylation of the bone derived hormone fibroblast growth factor 23 (FGF23), which protects the hormone from proteolysis-mediated inactivation upon its secretion (20). If the biallelic GALNT3 gene or homozygous FGF23 is mutated such that FGF23 cannot be glycosylated, processing of FGF23 is increased. The resultant elevated levels of cleaved, inactive c-terminal FGF23 and decreased levels of active FGF23 underlie the pathogenesis of tumoral calcinosis. Glycosylation to proBNP is also closely related to processing (21). When we transfected a proBNP gene encoding a nonglycosylated mutant into cardiomyocytes, proBNP was nearly completely processed into BNP and NT-proBNP, whereas 40% of proBNP was not processed after transfection of wild-type human proBNP (21). When Semenov *et al*. inserted the human proBNP gene or its mutant into HEK 293 cells, they found that glycosylation at Thr71 nearly completely inhibited processing of proBNP, suggesting that *O*-glycosylation at Thr71 is important for processing of proBNP (22). In fact, recent studies have reported that the proBNP/total BNP ratio is decreased in patients with acute heart failure due to a reduction in glycosylation at Thr71 and increased proBNP processing (23,24). Because the degree of proBNP glycosylation depends on the cells into which proBNP gene has been inserted, the transfected cells may alter the level of proBNP glycosylation and, thereby, affect processing efficiency. Therefore, experiments using cardiomyocytes that endogenously express proBNP are preferable to using HEK 293 cells, which show strong glycosylation of proBNP. Nakagawa *et al*. (25) inserted human proBNP gene or its mutant into rat cardiomyocytes and found that glycosylation at both Thr71 and Thr48 plays an important role in proBNP processing and that glycosylation at these two sites cooperatively inhibits proBNP processing. They also showed that GALNT 1 and 2 are involved in the glycosylation of proBNP. In a study from Lewis *et al.*, the percentage of Thr71-nonglycosylated proBNP is extremely low in obese heart failure patients, suggesting an increase in plasma Thr71-glycosylated proBNP in these patients. Because plasma nonglycosylated-central proBNP levels are very low, these results suggest an increase in both Thr71- and central-glycosylated proBNP in obese heart failure patients, leading to an increase in the proBNP/NT-proBNP ratio (18). It remains unclear, however, why the pattern of proBNP glycosylation is altered and proBNP processing is impaired in the hearts of obese heart failure patients.

In their paper, Lewis *et al.* showed the significance of proBNP glycosylation in patients with obesity-related heart failure. This finding sheds new light on the pathophysiology of obese heart failure patients. However, there are several limitations in this study. First, it remains unclear whether GALNT1 and/or 2 are increased and are related to Thr71 glycosylation in the hearts of obese heart failure patients. There may also be involvement of other GALNT types. In addition, transfer of sialic acid after *O*-glycosylation may also be involved in proBNP processing. Second, this study did not present plasma BNP levels measured using a conventional BNP immunoassay. Because the current BNP measurement system cross-reacts with proBNP (4), it is thought that the decrease in plasma BNP levels seen in obese heart failure patients may be due to a decrease in

the BNP1-32 level, given that the proBNP level does not change. Taken together, these results suggest that there are two mechanisms active in obese heart failure patients: reduced cardiac production of proBNP (which may be related to insulin resistance) and impaired processing due to increased Thr71-glycosylation of proBNP. Altered glucose metabolism in the hearts of obesity heart failure patients, activation of a certain type of GALNT, and reduced proBNP gene expression may be involved in the pathogenesis of heart failure in obese patients. Further studies will be necessary to elucidate that mechanism in the future.

Acknowledgments

Funding: This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science 18K08103 and 15K09138 (T Nishikimi), 19K08580 and 16K09498 (Y Nakagawa).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Nishikimi T, Kuwahara K, Nakao K. Current biochemistry, molecular biology, and clinical relevance of natriuretic peptides. J Cardiol 2011;57:131-40.
2. Maisel AS, Nakao K, Ponikowski P, et al. Japanese-Western consensus meeting on biomarkers. Int Heart J 2011;52:253-65.
3. Nishikimi T, Minamino N, Ikeda M, et al. Diversity of molecular forms of plasma brain natriuretic peptide in heart failure--different proBNP-108 to BNP-32 ratios in atrial and ventricular overload. Heart 2010;96:432-9.
4. Kuwahara K, Nakagawa Y, Nishikimi T. Cutting Edge of Brain Natriuretic Peptide (BNP) Research - The Diversity of BNP Immunoreactivity and Its Clinical Relevance. Circ J 2018;82:2455-61.
5. Schellenberger U, O'Rear J, Guzzetta A, et al. The precursor to B-type natriuretic peptide is an O-linked glycoprotein. Arch Biochem Biophys 2006;451:160-6.
6. Nishikimi T, Ikeda M, Takeda Y, et al. The effect of glycosylation on plasma N-terminal proBNP-76 levels in patients with heart or renal failure. Heart 2012;98:152-61.
7. Daniels LB, Maisel AS. Natriuretic peptides. J Am Coll Cardiol 2007;50:2357-68.
8. Ozaki J, Shimizu H, Hashimoto Y, et al. Enzymatic inactivation of major circulating forms of atrial and brain natriuretic peptides. Eur J Pharmacol 1999;370:307-12.
9. Nakagawa Y, Nishikimi T, Kuwahara K. Atrial and brain natriuretic peptides: Hormones secreted from the heart. Peptides 2019;111:18-25.
10. Kato K, Murakami H, Isozaki O, et al. Serum concentrations of BNP and ANP in patients with thyrotoxicosis. Endocr J 2009;56:17-27.
11. Myhre PL, Vaduganathan M, Claggett B, et al. B-Type Natriuretic Peptide During Treatment With Sacubitril/Valsartan: The PARADIGM-HF Trial. J Am Coll Cardiol 2019;73:1264-72.
12. Minai K, Komukai K, Arase S, et al. Cardiac tamponade as an independent condition affecting the relationship between the plasma B-type natriuretic peptide levels and cardiac function. Heart Vessels 2013;28:510-3.
13. Omar HR. Acute cardiogenic pulmonary edema with normal BNP: the value of repeat BNP testing. Am J Emerg Med 2015;33:605.e5-6.
14. Mehra MR, Uber PA, Park MH, et al. Obesity and suppressed B-type natriuretic peptide levels in heart failure. J Am Coll Cardiol 2004;43:1590-5.
15. Nishikimi T, Iemura-Inaba C, Akimoto K, et al. Stimulatory and Inhibitory regulation of lipolysis by the NPR-A/cGMP/PKG and NPR-C/G(i) pathways in rat cultured adipocytes. Regul Pept 2009;153:56-63.
16. Wang TJ, Larson MG, Levy D, et al. Impact of obesity on plasma natriuretic peptide levels. Circulation 2004;109:594-600.
17. Mizuno Y, Harada E, Katoh D, et al. Cardiac production of B-type natriuretic peptide is inversely related to the plasma level of free fatty acids in obese individuals - possible involvement of the insulin resistance -. Endocr J 2013;60:87-95.
18. Lewis LK, Raudsepp SD, Prickett TCR, et al. ProBNP That Is Not Glycosylated at Threonine 71 Is Decreased With Obesity in Patients with Heart Failure. Clin Chem 2019. [Epub ahead of print].
19. Halfinger B, Hammerer-Lercher A, Amplatz B, et al. Unraveling the Molecular Complexity of O-Glycosylated Endogenous (N-Terminal) pro-B-Type Natriuretic

Peptide Forms in Blood Plasma of Patients with Severe Heart Failure. Clin Chem 2017;63:359-68.
20. Chefetz I, Sprecher E. Familial tumoral calcinosis and the role of O-glycosylation in the maintenance of phosphate homeostasis. Biochim Biophys Acta 2009;1792:847-52.
21. Nishikimi T, Nakagawa Y, Minamino N, et al. Pro-B-type natriuretic peptide is cleaved intracellularly: impact of distance between O-glycosylation and cleavage sites. Am J Physiol Regul Integr Comp Physiol 2015;309:R639-49.
22. Semenov AG, Postnikov AB, Tamm NN, et al. Processing of pro-brain natriuretic peptide is suppressed by O-glycosylation in the region close to the cleavage site. Clin Chem 2009;55:489-98.
23. Vodovar N, Séronde MF, Laribi S, et al. Post-translational modifications enhance NT-proBNP and BNP production in acute decompensated heart failure. Eur Heart J 2014;35:3434-41.
24. Takahama H, Takashio S, Nishikimi T, et al. Ratio of pro-B-type natriuretic peptide (BNP) to total BNP is decreased in mild, but not severe, acute decompensated heart failure patients: A novel compensatory mechanism for acute heart failure. Int J Cardiol 2018;258:165-71.
25. Nakagawa Y, Nishikimi T, Kuwahara K, et al. MiR30-GALNT1/2 Axis-Mediated Glycosylation Contributes to the Increased Secretion of Inactive Human Prohormone for Brain Natriuretic Peptide (proBNP) From Failing Hearts. J Am Heart Assoc 2017. doi: 10.1161/JAHA.116.003601.

Cite this article as: Nishikimi T, Nakagawa Y. Does impaired processing of pro-B-type (or brain) natriuretic peptide cause decreased plasma BNP levels in obese heart failure patients? Ann Transl Med 2019;7(Suppl 6):S221. doi: 10.21037/atm.2019.08.56

