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Relationships between NT-proBNP and Obesity, Glucose and Lipid Profiles in Dogs with Chronic Mitral Valve Insufficiency

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Abstract : In humans, N-terminal pro-B-type natriuretic peptide (NT-proBNP) was shown to be inversely related to obesity; in addition, its association with contributing factors for obesity such as insulin, lipids, and glucose profiles has been demonstrated in the literature. However, this association between NT-proBNP and the severity of obesity has not been investigated in veterinary medicine. Our study hypothesis is that plasma levels of NT-proBNP may be related to body condition score (BCS) and contributing factors to obesity in dogs with heart diseases. To achieve our study goal, we collected blood samples from 73 client-owned dogs of small breeds at different stages of heart failure due to chronic mitral valvular insufficiency (CMVI). Fasting glucose concentrations, lipid profiles (i.e., total triglycerides [TG], total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C]), fructosamine, insulin and NT-proBNP concentrations were measured. The insulin/glucose ratio was also determined. NT-proBNP showed not only a significant correlation with the severity of CMVI related heart failure but also an inverse relationship to body condition scores (BCS), insulin plasma levels and fructosamine concentrations. We found the presence of an inverse relationship between plasma levels of NT-proBNP and the severity of obesity. In addition, NT-proBNP was associated with lower levels of contributing factors to obesity such as fructosamine and insulin, creating a possible link between the obesity and NT-proBNP in dogs with heart disease. This is also the first report demonstrating an inverse association between obesity and NT-proBNP in dogs with heart failure.

***Key words* :** NT-proBNP, impaired fasting glucose, obesity, insulin, heart failure.

Introduction

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is released in response to hemodynamic stress (e.g. volume expansion and pressure overload) on cardiac chambers (1,10, 27,36). The role of NT-proBNP as a cardiac biomarker has been well documented in both human and veterinary medical literature (1,2,5,6,22,23) where it has been validated as a practical and reliable marker of cardiac wall stress in dogs, cats, and humans (6,22,23). Currently, the major clinical challenge is to elucidate the factors that influence variations in circulating levels of NT-proBNP during heart failure (33). Causative factors that are known to influence plasma levels of brain natriuretic peptides (BNPs), including NT-proBNP, can be largely classified into 2 categories; cardiogenic factors, such as heart disease which can cause an abnormal increase in myocardial stress, and non-cardiogenic factors, such as obesity, metabolic syndrome, impaired renal function, sex hormone levels, and old age (26,33).

Although the reliability of NT-proBNP testing for dogs and cats with heart failure has been well documented (22,26,28), except for renal clearance, the factors that influence the circulating levels of NT-proBNP, is not well understood (26). Obesity has been known to influence on NT-proBNP levels in humans (10,16,21). However, a relationship between the levels of NT-proBNP, and the degree of obesity has yet to be evaluated in dogs, especially with heart failure, until this study. Therefore, we evaluated NT-proBNP, obesity and heart failure in a study population of dogs. This was accomplished by assessing NT-proBNP levels, contributing factors to obesity such as lipid profiles (e.g. total triglycerides [TG], total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C]), and insulin resistance (e.g. serum insulin, insulin/glucose ratio, and fasting glucose).

Materials and Methods

Study population

Informed written consent for collecting samples and patient history were obtained from each dog owner before the study commenced. The study population consisted of 73 client-

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owned dogs at different stages of heart failure due to CMVI. CMVI was diagnosed by echocardiographic evidence of regurgitant flow into the left atrium (LA) at the systolic period, enlarged LA, myxomatous degeneration of the mitral valve leaflet, and the presence of mitral valve prolapse in addition to thoracic radiography, physical examination, laboratory test to discriminate the patients with other concurrent diseases such as chronic inflammatory disease, systemic disease, and other types of heart diseases. The stage of heart failure was classified by the classification system used in the report of the American College of Veterinary Internal Medicine (ACVIM) Specialty of Cardiology Consensus Panel (8). Some dogs were medicated to control clinical signs of heart failure.

Evaluation of NT-proBNP, obesity and contributing factors to obesity

BCS for each dog was evaluated by a method used in previously (3). All blood samples were taken from the jugular vein after a 12-hour overnight fast. The blood was collected into tubes containing either potassium EDTA for NT-proBNP, TG, cholesterol (including TC, LDL-C and HDL-C) analysis, or heparin for glucose, fructosamine and insulin analysis. Blood samples were centrifuged within 60 minutes after collection, and serum was stored at −20ºC prior to overnight shipment at 4ºC for analysis. The blood samples were analyzed by commercial laboratories for the accurate measurement of serum concentrations of NT-proBNP and the determination of lipid and glucose profiles. All assays were validated for use in dogs. Plasma TC, HDL-C and TG were determined by conventional enzymatic methods. Total cholesterol was measured by an enzymatic assay kit (IDEXX laboratories, USA) for cholesterol. HDL-C and TG were measured using ADVIA 2400 (Pureauto S TG-N; Daiichi Pure Chemicals Co, Ltd, Tokyo). LDL-C was calculated by a modified Fredewald equation (17): LDL-C(mg/dL) = TC(mg/dL) − HDL-C(mg/dL) − 1/6 (TC[mg/dl]).

The levels of insulin were measured by the test assay kit (ADVIA® 2400 Chemistry System, Siemens Healthcare diagnostics Inc, Deerfield, IL), which was detected by an immunoassay system (ADVIA Centaur™ Insulin Lite Reagent & Solid Phase, Siemens, NY, USA). Fructosamine was measured by a colorimetric assay (ADVIA Centaur® XP Immunoassay System, Siemens Healthcare diagnostics Inc, Deerfield, IL). Glucose levels were assayed by a chemistry analyzer (VetTest chemistry analyzer, IDEXX Laboratories Inc. FUJI DRY-CHEM 3500i, Fuji Film Corporation, Japan).

Statistical analysis

Statistical analyses were performed using commercially available statistical software (SPSS version 19.0 for Windows, SPSS Inc., San Diego, CA, USA). Continuous variables are presented as mean ± standard deviation (SD). The nonparametric Spearman's coefficient of correlation was used to test the strength of the association between NT-proBNP level and the severity of heart failure and body condition score (BCS). Correlations of NT-proBNP level with glucose profiles (fasting glucose, insulin/glucose, insulin, and fructosamine) and lipid profiles (TC, TG, HDL-C, LDL-C, and HDL-C/LDL-C) were determined by Pearson's correlation analyses. Unless stated otherwise, $p < 0.05$ was considered statistically significant.

Results

Seventy-three aged and toy dogs at different stages of heart failure due to CMVI were enrolled in this study (Table 1). Maltese (45.2%) and Shih Tzu (13.7%) were well-represented in this study population. The mean body weight in this study population was 4.1 ± 2.5 kg. Female dogs (n = 43) were also well-represented in this study population (Table 1).

Not surprisingly, a strong positive association was seen between NT-proBNP and the severity of heart failure (r = 0.596, n = 73, $p < 0.001$; Fig 1a). Dogs with a more severe stage of heart failure had higher serum NT-proBNP levels. The level of NT-proBNP was inversely associated with BCS in our study population (r = −0.300, n = 73, $p < 0.01$, Fig 1b). Dogs with lower BCS had higher serum NT-proBNP levels. However there was no correlation between the severity of heart failure and the BCS in his study population (Fig 2). Furthermore, there was no association between the concentrations of TG, TC, HDL-C and LDL-C and the level of NT-proBNP in this study population (Fig 3). In contrast, the plasma concentration of NT-proBNP was inversely correlated with insulin levels and fructosamine levels (r = −0.360, n = 47, $p < 0.02$; r = −0.503, n = 47, $p < 0.001$, respectively, Fig 4). However, there was no association between serum glucose concentration and insulin/glucose ratio and the level of NT-proBNP in this study population (Fig 4).

Table 1. Demographic characteristics of the study population including 73 dogs classified by American College of Cardiology/American Heart Association classification system[36]

Demographic Characteristics		American College of Cardiology/American Heart Association classification system					Total
		Grade A (n = 5)	Grade B1 (n = 15)	Grade B2 (n = 20)	Grade C (n = 22)	Grade D (n = 11)	n = 73
Sex							
	Male	60.0% (3/5)	20.0% (3/15)	30.0% (6/20)	54.5% (12/22)	54.5% (6/11)	41.1% (30/73)
	Female	40.0% (2/5)	80.0% (2/15)	70.0% (14/20)	45.5% (10/22)	45.5% (5/11)	58.9% (43/73)
Age		9.6 ± 2.3	9.9 ± 2.0	11.0 ± 2.1	12.1 ± 2.3	12.4 ± 2.1	11.17 ± 2.31
BW		5.4 ± 2.7	4.5 ± 2.2	5.9 ± 2.9	5.1 ± 2.4	4.6 ± 2.5	4.1 ± 2.5

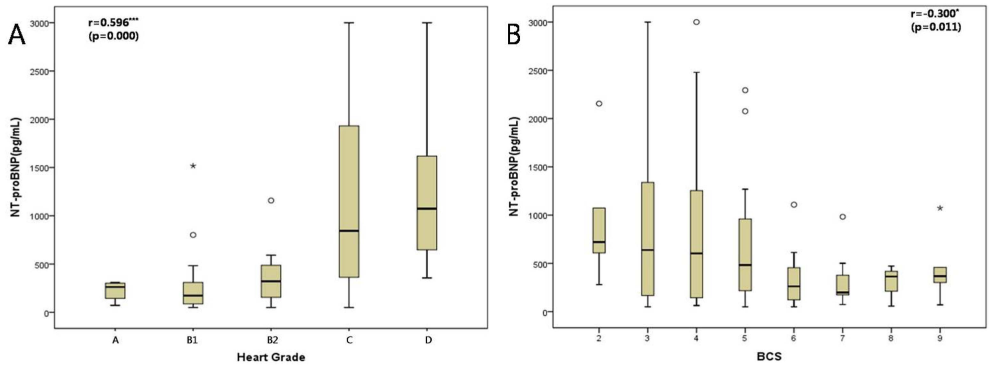

Fig 1. Correlation between plasma concentrations of NT-proBNP and the severity of heart failure (A), and those between NT-proBNP and BCS (B) in dogs with chronic mitral valve insufficiency. Association of the NT-proBNP with BCS or the stages of heart failure are marked in the upper edge of the box plot. The lines inside the boxes represent the median; the lower and upper edge of each box represents the 25th and 75th percentile, respectively; the lowest and highest lines represent the 10th and 90th percentiles, respectively. Significant association was considered at a value of P < .05.

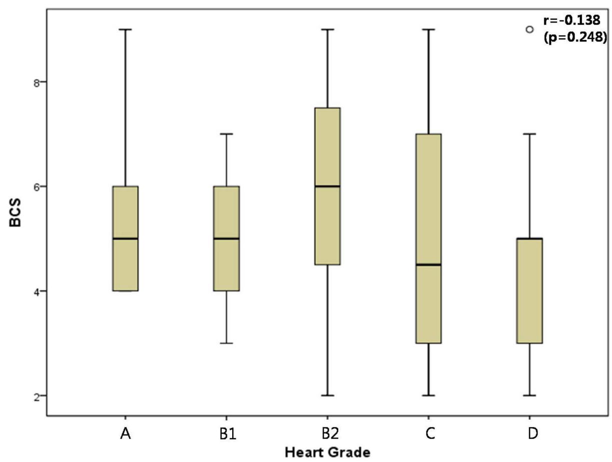

Fig 2. Correlations between the scores of BCS and the severity of heart failure in dogs with chronic mitral valvular insufficiency. Associations of the BCS and the severities of heart failure are marked in the upper edge of the box plot. The lines inside the boxes represent the median; the lower and upper edge of each box represents the 25th and 75th percentile, respectively; the lowest and highest lines represent the 10th and 90th percentiles, respectively.

Discussion

The level of NT-proBNP was found to be inversely associated with BCS and the serum levels of insulin and fructosamine in this study population. The inverse association of natriuretic peptides (NT), including NT-proBNP, with obesity has been demonstrated in human studies (4,27,35). Early human studies suggested that obesity itself might be the major causative factor for elevating NT-proBNP (35), although later studies found that the increased degradation and clearance of BNP by natriuretic peptide clearance receptors (NPR-Cs) and neutral endopeptidases (NEPs) in adipose tissue could significantly reduce NT-proBNP levels in obese patients (27,33). However, recent studies found that the amount of NT-proBNP cleared by NPR-Cs and NEPs were relatively minimal (26). Moreover, because NT-proBNP is structurally different from BNP, the NT-proBNP may not be cleared by NPR-Cs and NEPs (26). Therefore, recent studies suggested that there might be other causative factors (e.g. altered neurohormonal interactions or sex steroid hormones) involved in reducing NT-proBNP levels (27,33). This relationship between the level of NT-proBNP and obesity in humans is still under investigation. Furthermore this relationship has rarely been studied in small animals, to date.

In humans, several studies have been conducted to clarify the relationships between NT-proBNP levels and contributing factors to obesity (e.g. insulin, fasting glucose, and lipid profiles) (7,10,29,33), as hyperinsulinemia, increased fasting glucose, and dyslipidemia are common in obese men (8,20). Most studies found that NT-proBNP level was lower in humans with higher serum triglyceride and insulin levels (10,16), although there were some controversial findings related to relationship between NT-proBNP level and lipid profiles (e.g. TG, TC, LDL-C, and HDL-C) (7). In dogs, serum insulin, fasting glucose, fructosamine, and lipid profiles) were also closely related to obesity (11,17,21,25,30). Similar to human studies (10,16), we found that NT-proBNP level was lower in dogs with higher serum insulin and fructosamine levels, although the fasting glucose level was not consistent with NT-proBNP levels. The reason why NT-proBNP level was inversely related to the levels of serum insulin and fructosamine has yet been clarified. This inverse relationship may either be the direct effect the NT-proBNP levels and obesity and, or an independent influence from insulin, considering insulin has an negative effect on lipolysis (12) against NT-proBNP (15,29,31). This relationship between serum insulin/fructosamine and NT-proBNP levels will require future investigation.

Although lipid profiles (e.g. TC, TG, or HDL-C) are closely related to obesity in human and dogs, the relationship between serum levels of lipid profiles and NT-proBNP was not estab-

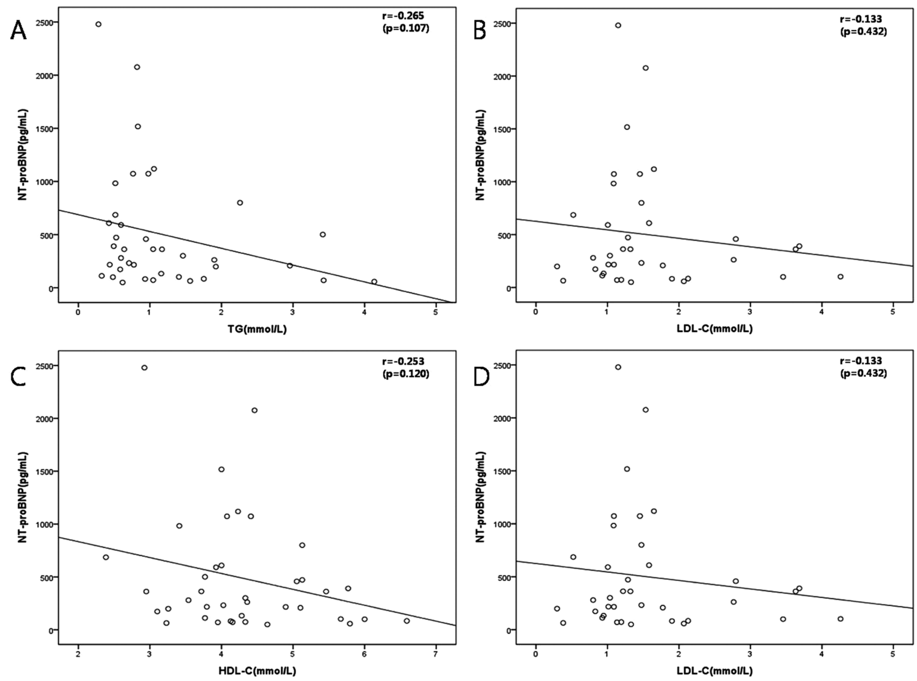

Fig 3. Correlation between plasma concentrations of NT-proBNP and lipid profiles including TG (A), TC (B), HDL-C (C) and LDL-C (D) in dogs with chronic mitral valve insufficiency. Associations of the NT-proBNP with lipid profiles are marked in the upper edge of the box. See abbreviations for TG, TC, HDL-C, LDL-C. Significant association was considered at a value of P < .05.

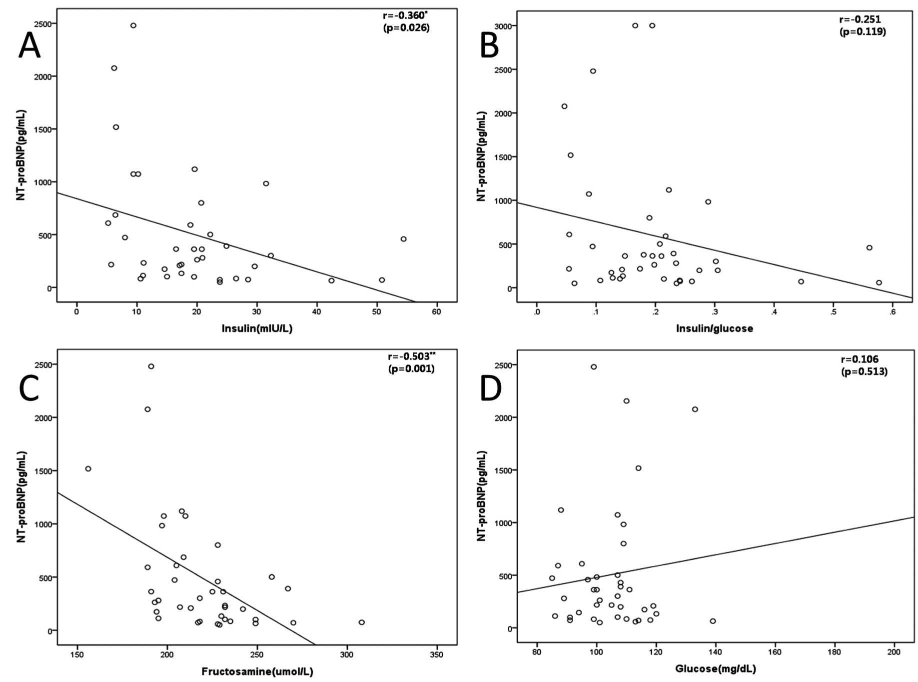

Fig 4. Correlations between plasma concentrations of NT-proBNP and glucose profiles including insulin (A), insulin/glucose ratio (B), fructosamine (C) and glucose (D) in dogs with chronic mitral valve insufficiency. Associations of the NT-proBNP with glucose profiles are marked in the upper edge of the box. Significant association was considered at a value of P < .05.

lished in this study. This is similar to previous studies in humans, where some studies found the serum levels of lipid profiles were lower with higher NT-proBNP levels, whilst other studies found the opposite result (9,10,16,29). However, it is generally accepted in human studies that a reduced natriuretic peptide signaling could promote lipid accumulation in adipose tissue and possibly lead to dyslipidemia (13). Interestingly, dogs are considered as HDL-C mammals and humans are considered as LDL-C mammals (14,18,19). This critical difference between dogs and humans in the composition of these two main lipoprotein fractions, lead to the question of whether or not these species have similar metabolic and/or transport systems for lipids.

There were several study limitations. Firstly, we did not directly measure the adiposity of the dogs in this study, although BCS can be a reliable method for measuring obesity in dogs. Therefore, further studies should be directed to use more accurate measurement on the adiposity of each individual. Secondly, the relationship between BCS and NT-proBNP level should be more clearly confirmed in healthy control dogs, although dogs with the grade A of CMVI can be regarded as normal. In addition, there was no direct relation between the BCS and the severity of heart failure in this study population. Lastly, known non-cardiac factors influencing NT-ProBNP levels can be more meticulously considered to recruit the study population, although we restricted the study population to small aged dogs. The renal clearance impaired by heart failure or dehydration should be more carefully assessed in further studies, although it may not affect our major findings in this study.

In conclusion, the level of NT-proBNP was inversely associated with BCS and serum concentrations of insulin and fructosamine. This is the first report demonstrating an inverse association between obesity and NT-proBNP in dogs with heart failure.

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이첨판 패쇄부전증에 이환된 개에서 NT-proBNP농도, 비만, 당 관련 인자 및 지방 관련인자간에 상관관계에 대한 연구

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요 약 : 사람에 연구에 따르면 심부전 진단에 널리 사용되는 NT-proBNP농도는 비만, 비만 관련인자들과 밀접한 상관관계가 있는 것으로 알려져 있다. 그러나 이러한 상관관계는 아직까지 수의분야에서는 입증된 바가 없다. 따라서 본 연구에서는 다양한 정도의 이첨판 패쇄부전증(CMVI)에 이환된 73마리 개 집단에서 NT-proBNP와 비만 및 비만 관련 인자들간의 상호관계를 조사하였다. 절식시 혈당 농도, 지방관련 인자(i.e., total triglycerides [TG], total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C]), fructosamine, insulin 및 NT-proBNP 농도를 측정하였다. Insulin/glucose ratio 역시 계산하였다. NT-proBNP 농도는 CMVI에 의한 심부전의 정도에 따라 농도변화가 관찰되었으며, body condition scores (BCS), insulin 및 fructosamine 농도와도 관련이 있었다. 또한 본 연구를 통해 비만과 혈중 NT-proBNP농도 간에 상관관계 역시 확인되었다. 또한 NT-proBNP농도는 비만과 관련 있는 인자인 fructosamine과 insulin농도와도 관련이 있었다. 본 연구는 개에서 NT-proBNP농도와 비만 정도와 상관관계를 입증한 첫 보고이다.

주요어 : NT-proBNP; 절식시 혈당; 비만, 인슐린, 심부전