J Vet Clin 29(4) : 277-282 (2012)

Journal of Veterinary Clinics
www.ksvc.or.kr

The Relationship between Homocysteine, Obesity, Glucose and Lipid Profiles in Small-Breed Dogs

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(Accepted: August 20, 2012)

Abstract : This study was conducted to evaluate whether plasma homocysteine levels were related to obesity or its contributing factors (e.g., lipids, insulin, glucose, glucagon, and fructosamine) in dogs without systemic diseases such as diabetes or renal failure. For achieving our study goal, 100 client-owned dogs without systemic diseases were enrolled in this study. Fasting glucose concentration; lipid profile (i.e., total triglycerides [TG], total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], and low-density lipoprotein cholesterol [LDL-C]); and fructosamine, insulin, and glucagon levels were determined. The dogs were subdivided by the body condition score (BCS). The median levels of homocysteine were considerably higher in obese dogs than in lean and normal dogs. Interestingly, not only was homocysteine positively associated with the level of HDL-C, but also found to have a significant positive association with TG, TC, plasma glucagon levels, and fructosamine. In contrast, LDL-C, fasting glucose and insulin did not show any association with homocysteine. The findings presented, suggest that elevated levels of homocysteine may play a biological role in obesity in dogs.

Key words **:** homocysteine, obesity, dog, fructosamine, BCS.

Introduction

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine (14). Hyperhomocysteinemia harms the vascular endothelium, induces endothelial dysfunction, and contributes to the development of atherosclerosis (10,32). Thus, homocysteine is considered an independent risk factor for atherosclerotic and thromboembolic vascular diseases in humans (10,32). Normally, serum homocysteine is maintained at a low level by continuous turnover to either methionine or cysteine. The established determinants of plasma homocysteine levels include folate and vitamin B-12 status, serum creatinine concentration, and renal function (7,24,37). In addition, researchers have suggested that obesity and obesity-related characteristics (e.g., hyperlipidemia, hyperinsulinemia, and insulin resistance) are linked to increased levels of homocysteine (13,40,41). In humans, obesity is considered one of the most important health concerns (30) because it is closely linked to insulin resistance, hypertension, hyperlipidemia, and heart disease (3,11,34,41). Similar to humans, obese dogs and cats have a decreased life span and multiple metabolic disorders such as insulin resistance (5,15). Moreover, previous studies have also shown that homocysteine has detrimental effects on the cardiovascular system of dogs (1). However, in contrast to human studies, investigations of homocysteine in veterinary medicine, dogs in particular are scarce (36). Therefore, the aim of this study was to identify whether plasma homocysteine levels were related to obesity or its contributing factors (e.g., lipids, insulin, glucose, glucagon, and fructosamine) in dogs without diabetes or renal failure.

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Materials and Methods

Study population

The study population was comprised of 100 small-breed healthy dogs presented at either the Chung-Hyun Animal Medical Center or Veterinary Teaching Hospital of Kangwon National University between 2010 and 2011. The approval of the animal ethics committee of Kangwon National University was obtained for blood sampling prior to the commencement of this study. Additionally, informed written consent and relevant blood collection were obtained from all dog owners. History, physical examination and routine laboratory tests (blood work) were performed to select healthy dogs, and to exclude dogs with other systemic diseases (e.g. chronic inflammatory diseases, renal failure, and diabetes mellitus). The body condition score (BCS) system (8,39) was used to assess the degree of obesity in the study subjects. The system involves detailed assessment of each dog by a veterinarian for body

outline, fat coverage, and ability to palpate the ribs (8,39). The dogs were categorized according to the established 9-point scale and divided into 3 groups according to BCS: lean group (BCS 1-3), normal group (BCS 4-6), and obese group (BCS 7-9).

Blood tests

All blood samples were taken from the jugular vein after a 12-hour overnight fast. The blood was collected into tubes containing potassium EDTA for the analysis of plasma triglycerides (TG) and cholesterol (including total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], and high-density lipoprotein cholesterol [HDL-C]) and in heparinized tubes for analysis of plasma glucose, fructosamine, and insulin levels. Blood samples were centrifuged within 60 minutes of collection, and the plasma was stored at −20°C before overnight shipment at 4°C for analysis. The blood samples were shipped to commercial laboratories for the accurate measurement of plasma concentrations of homocysteine, lipid, and glucose profiles. Homocysteine concentrations were determined with a direct chemiluminescent immunoassay by using a homocysteine reagent and the ADVIA Centaur (ADVIA Centaur® XP Immunoassay System; Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA), which has a proven track record of reliability in assessing canine samples. This method of determining homocysteine was first used and validated on human samples (38). The plasma TC, HDL-C, and TG levels were determined using conventional enzymatic methods. An enzymatic assay kit (Pureauto S TG-N; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) was used for total cholesterol, and the ADVIA 2400 (ADVIA® 2400 Chemistry System; Siemens Healthcare Diagnostics Inc., Deerfield, IL) was used for HDL-C and TG. LDL-C was calculated using the modified Friedewald equation (30): LDL-C (mg/dL) = TC (mg/dL) – HDL-C (mg/dL) – 1/6(TC [mg/dL]). The amino acid sequences of human and canine insulin have been reported to have a high homology, the plasma concentrations of insulin were measured using a commercially available immunoassays used in human medicine. The insulin levels were measured using a test assay kit (ADVIA Centaur™ Insulin Lite Reagent & Solid Phase; Siemens, NY) and detected by an immunoassay system (ADVIA Centaur™ Insulin Lite Reagent & Solid Phase; Siemens, NY). The use of these immunoassays does not influence the results of insulin determination in dogs (35). Moreover, this immunoassay was previously validated for use in dogs. Fructosamine was measured by a colorimetric assay (ADVIA® 2400 Chemistry System; Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) and glucose was measured using an automated chemical analyzer (FUJI DRI-CHEM 3500i; Fuji Film Corporation, Japan).

Statistical analysis

Statistical analyses were performed using commercial statistical software (SPSS version 19.0 for Windows; SPSS Inc., San Diego, CA). Continuous variables were described by the mean ± standard deviation (SD). The statistical methods used were 1-way analysis of variance (ANOVA), Kruskal-Wallis Test (Non-parametric independent group comparisons), and Pearson's coefficient of correlation. Differences in homocysteine concentration among groups were evaluated by ANOVA. The Pearson's coefficient of correlation was used to test the strength of the association between homocysteine and lipid profile (TC, TG, HDL-C, and LDL-C), and between homocysteine and glucose profile (glucose, fructosamine, insulin, and glucagon). For all comparisons, $P < 0.05$ was considered statistically significant, unless stated otherwise.

Table 1. Demographic characteristics of the study population including 100 dogs classified by BCS for obesity

	BCS		
	Lean (BCS 1-3)	Normal (BCS 4-6)	Obese (BCS 7-9)
n (101)	19	53	28
Age	10.9 ± 2.4	11.2 ± 2.5	11.8 ± 2.0
Sex (M:F)	8:11	15:39	10:18
BW	5.6 ± 2.4	4.2 ± 1.7	6.0 ± 2.8
Breeds			
Cocker spaniel	1	2	
CKCS	1		1
Chihuahua	1	2	
Crossbreed	1	3	2
Maltese	8	23	9
Poodle		3	2
Pekingese	1	8	1
Pomeranian	1	2	
Schnauzer	2	1	6
Shih-Tzu	3	6	5
Yorkshire terrier		3	2

All data are expressed as mean ± SD.
BW, body weight; CKCS, Cavalier King Charles spaniel.

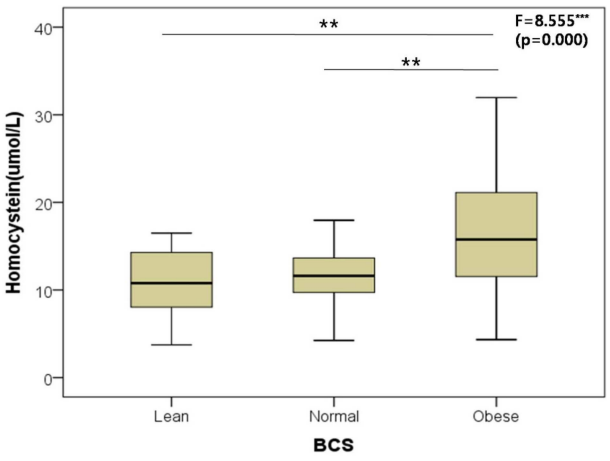

Fig 1. Homocysteine levels in BCS (B) subgroups. **p < 0.001.

Results

The composition of each group was listed in Table 1. Maltese and old dogs were well represented in this study population. Because most dogs enrolled in this study were small dog breeds, the body weight of each population was less than 10 kg.

Median levels of homocysteine were considerably higher in obese dogs (BCS 7-9) compared to lean and normal dogs (Fig 1). Interestingly, we found that homocysteine was posi-

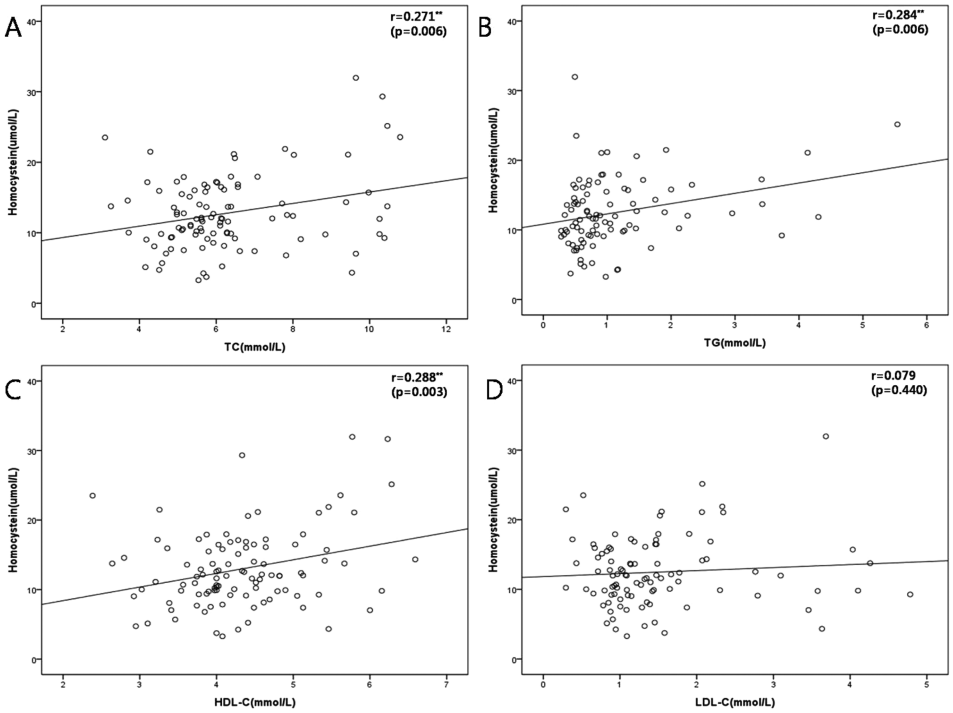

Fig 2. Correlations of plasma concentrations of homocysteine with (A) TC, (B) TG, (C) HDL-C, and (D) LDL-C in dogs.

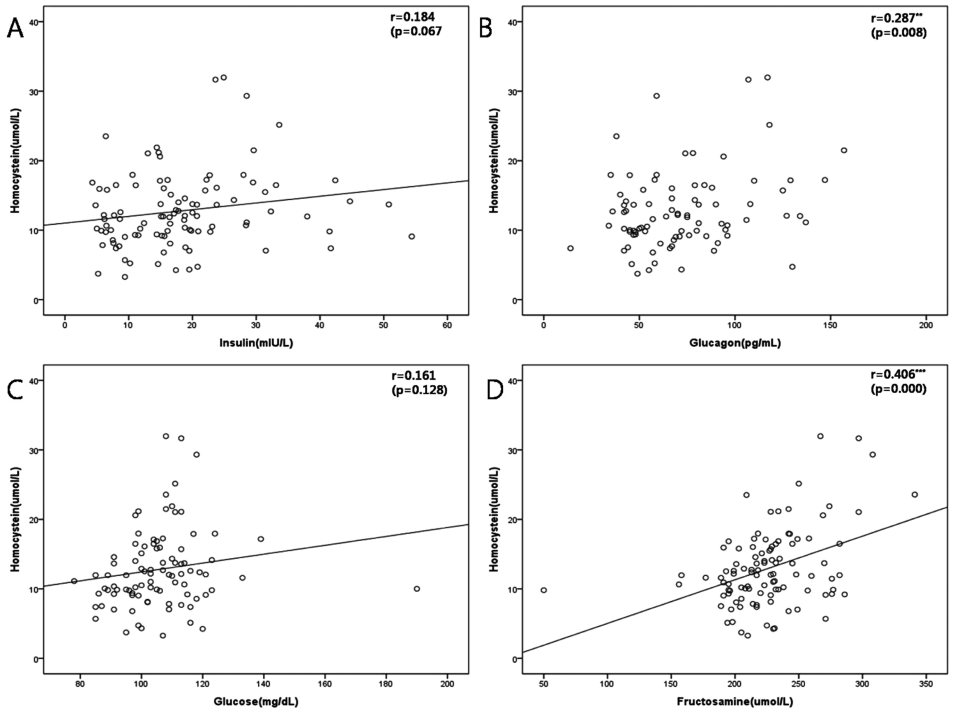

Fig 3. Correlations of plasma concentrations of homocysteine with (A) insulin, (B) glucagon, (C) glucose, and (D) fructosamine in dogs.

tively associated with the level of HDL-C, and a significant association with TG, TC (Fig 2), glucagon (Fig 3B), and Fructosamine (Fig 3D). In contrast, LDL-C did not show any association with homocysteine. In contrast, fasting glucose (Fig 3C) and Insulin levels (Fig 3A) showed no association with homocysteine.

Discussion

Because multiple detrimental effects of hyperhomocysteinemia on the cardiovascular system have been proven in human medicine as well as in dogs and cats (1,8,30,35,36,39) studies have focused on identifying factors associated with hyperhomocysteinemia in patients with or without heart failure (29,30,40). Obesity together with other factors has received a lot of attention in the human literature (14,26,40). Therefore, obesity is considered a causative factor of hyperhomocysteinemia in humans (14,40). The present study also showed a significant difference between obese and normal dogs with respect to homocysteine concentrations. Although the available literature on homocysteine in dogs is scarce (18,36), the medical literature recommends that homocysteine should be monitored in patients with a history of atherothrombotic vessel disease, diabetes, hyperlipidemia, and renal dysfunction as well as in obese subjects and elderly people (14,40). The pathophysiological mechanisms by which high levels of homocysteine are produced in obese individuals have not been completely elucidated. However, many studies in human medicine have shown that factors (e.g., hyperinsulinemia, hyperlipidemia, and high fasting glucose) closely related to metabolic syndrome due to obesity have significant associations with hyperhomocysteinemia (11,14,23,33,41); this indicates that hyperhomocysteinemia is indirectly linked with obesity. The detrimental effects of hyperhomocysteinemia have already been demonstrated in dogs (1), which suggest that homocysteine levels in obese dogs should be monitored to minimize obesity related complications.

Human studies have shown that hyperhomocysteinemia has statistically significant associations with serum triglyceride and cholesterol levels (20,26,42). Liao *et al*. showed that hyperhomocysteinemia decreases circulating HDL-C by inhibiting apolipoprotein A-I protein synthesis and promoting HDL-C clearance (25). Mikael *et al*. insisted that hyperhomocysteinemia may increase the levels of serum triglycerides via multiple biochemical pathways (20). Our data support these previous studies with respect to the association between serum homocysteine and triglyceride, in that homocysteine levels were found to be positively associated with triglyceridemia. In contrast, we observed that HDL-C was positively associated with the serum concentration of homocysteine in dogs. Generally, HDL-C is considered "good" cholesterol because it helps remove cholesterol from artery walls and deliver it to the liver for recycling (21,27). Moreover, many human studies have suggested that high levels of LDL-C and low levels of HDL-C may play important roles in the development of hyperhomocysteinemia in the cardiovascular system (17,38,41,42). Previous studies have also showed an inverse association between hyperhomocysteinemia and HDL-C in mice (42), this is in stark contrast to the results of this study. Thus far, there are few reports that clearly explain this paradox. Nonetheless, the fact that dogs are considered HDL mammals compared to humans, who are considered LDL mammals, may provide a starting point for future studies (16,19,21).

High insulin levels cause a reduction in cystathionine synthase levels, leading to homocysteine accumulation (9,12,22, 33). Therefore, patients with diabetes mellitus have a relatively high incidence of hyperhomocysteinemia, which appears to be related to a prognosis of diabetes. In contrast, we found that plasma homocysteine levels were not related to insulin levels but rather, glucagon levels. In contrary to the role of insulin in modulating plasma homocysteine levels, glucagon is known to decrease homocysteine levels by activating cystathionine beta synthase; accordingly, its administration can reduce homocysteine levels (6). However, the results of only a few human studies are consistent with our data. Therefore, there are limited studies to explain our paradoxical data and therefore, can be addressed by further studies on homocysteine metabolism in dogs.

Fructosamine together with glucose are useful indicators in evaluating glucose imbalances in dogs suspected of having diabetes (2), their levels generally reflect average blood glucose concentration over the past 2-3 weeks (31). Thus, increased fructosamine levels in obese dogs mean that they showed more episodes of hyperglycemia than controls throughout this period. Veiga *et al*. showed that plasma fructosamine levels and not glucose levels were increased in obese dogs as compared to control dogs (23); this may explain the positive association between homocysteine and fructosamine observed in our study.

Although vitamin B12 levels are important determinants of plasma homocysteine levels (36), we did not determine vitamin B12 levels in this study population. In addition, dietary cholesterol and fat might contribute to hyperhomocysteinemia (4); therefore, a major weakness of our study is the lack of data regarding dietary habits and calorie intake of the study dogs, because we could not completely control their diet.

In conclusion, we found that homocysteine levels are significantly higher in obese dogs than in normal and lean dogs. In addition, among candidate factors related to obesity, homocysteine was positively associated with lipids (i.e., TC, TG, and HDL-C), glucagon, and fructosamine. These results suggest that elevated levels of homocysteine may play a biological role in linking obesity and cardiovascular disease in obese dogs. Consequently, homocysteine may be considered a risk factor for cardiovascular disease in obese dogs.

Acknowledgements

This study was supported from the Institute of Veterinary

Science, Kangwon National University. The authors would like to thank Dr. Lopeti Lavulo (Bioline, Australia) for advice on manuscript preparation.

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소형견종에서 Homocysteine과 비만, 당 관련 인자, 지방 관련인자의 상관관계에 대한 연구

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요 약 : 본 연구는 건강한 개에서 혈장 homocysteine농도가 비만이나 비만 관련인자(예, 지방, 인슐린, 혈당, 글루카곤, fructosamine)들과 어떤 상관관계가 있는지를 확인하기 위해 실시되었다. 이를 위해, 절식시 혈당, 지방지수(예, total triglycerides [TG], total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C]과 low-density lipoprotein cholesterol [LDL-C]), fructosamine, insulin 및 glucagon 농도를 각각 측정하였다. 실험에 사용된 개들은 body condition score (BCS)에 따라 분류하였다. 평균 혈장 homocysteine 농도는 비만한 개 집단이 정상이나 마른 개 집단에 비해 상당히 높았다. 또한 혈장 homocysteine농도는 HDL-C농도 뿐아니라 TG, TC, 혈장 glucagon 및 fructosamine 농도와도 밀접한 상관관계를 가지고 있었다. 반대로 LDL-C 농도, 절식시 혈당농도 및 insulin농도는 아무런 상관관계가 확인되지 않았다. 본 연구 결과, homocysteine의 농도 상승이 비만견에서 생물학적으로 중요한 역할을 하는 것으로 추정된다.

주요어 : homocysteine, 비만, 개, fructosamine, BCS