Kasetsart J. (Nat. Sci.) 45 : 622 - 628 (2011)

Biochemical Alterations and Their Relationships with the Metabolic Syndrome Components in Canine Obesity

Tippayaporn Tribuddharatana[1], Yuwadee Kongpiromchean[2], Kosit Sribhen[3] and Choosri Sribhen[4]*

ABSTRACT

Metabolic changes accompanying obesity have been intensively studied in humans but are rarely reported in animals. The present study aimed to investigate alterations of glucose metabolism and biochemical parameters in renal, lipid and liver profiles and to study their metabolic relationship determined in 31 obese dogs compared with 31 non-obese control dogs. The results showed that with the exception of creatinine and aspartate aminotransferase, all other parameters measured in obese animals exhibited significantly higher levels than those in the control group. In contrast to non-obese dogs, significant associations of glucose with several lipid parameters and liver enzymes alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) were observed in obese dogs. These enzymes, on the other hand, displayed significant correlations with total cholesterol and low-density lipoprotein cholesterol, but not with high-density lipoprotein cholesterol. The results indicated that ALT and GGT may have major pathophysiological roles in obesity-related metabolic alterations and should be included as biochemical criteria of metabolic syndrome in canine obesity.

Keywords: biochemical alteration, relationship, metabolic syndrome, canine obesity

INTRODUCTION

Obesity is an escalating global health problem both in humans and domestic animals. The incidence of obese dogs and cats has recently been estimated to be between 20 and 40%, and there is an increasing trend in the incidence of obesity in the pet population (Scalett *et al*., 1994; McGreevy *et al*., 2005). The clinical problem of obesity in animals lies in the fact that it is frequently associated with several metabolic abnormalities (dyslipidemia, insulin resistance, metabolic syndrome) and the development of morbidities including endocrinopathies (diabetes mellitus, hypothyroidism), orthopedic disorders (osteoarthritis, intervertebral disc disease), and neoplasia (German, 2006). Therefore, early recognition of the pathophysiology of obesity with its metabolic alterations and correlates is essential in the management of obese animals.

1 Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
2 Division of Animal Health Science, Faculty of Agriculture Bangpra, Rajamankala University of Technology Tawanok, Chon Buri 20110, Thailand.
3 Department of Clinical Pathology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.
4 Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: fvetcss@nontri.ku.ac.th

Received date : 03/12/10 Accepted date : 03/03/11

Biochemical changes that can be observed in the early course of obesity development with the accumulation of fat in visceral organs include alterations in the lipoprotein and carbohydrate metabolism as well as renal dysfunction. The constellation of these biochemical abnormalities represents criteria of the "metabolic syndrome" that, in humans, was reported to be associated with insulin resistance and the development of type 2 diabetes mellitus and cardiovascular disease (Blaha *et al*., 2008). However, these criteria have not been well defined in other mammalian species. Therefore, the aim of the present study was to investigate changes in glucose levels and alterations of biochemical parameters in the lipid and renal profiles of dogs with obesity. Since fat accumulation occurs in the liver, parameters in the liver profile were also measured. Associations of these biochemical changes were studied in correlational analyses.

The results obtained may represent the basis for future investigations of the treatment effect in canine obesity.

MATERIALS AND METHODS

Study animals

Sixty-two dogs of various breeds, consisting of 31 non-obese control dogs and 31 obese dogs, matched for sex (17 males and 14 females in each group) were used in the present study. The poodle was the most frequent breed of animal studied in both groups. A 5-point body condition scoring (BCS) system based on inspection and palpation was used to define obesity (BCS 5) and included the following observations for BCS 5: the ribs are very difficult to feel under a thick fat cover; the tailbase appears thickened and is difficult to feel under a prominent layer of fat; the bony prominences are covered by a moderate to thick layer of fat; dogs over six months of age have a pendulous ventral bulge and no waist when viewed from the side due to extensive fat deposits; the back is markedly broadened when viewed from above; and a trough may form when epaxial areas bulge dorsally (Thatcher *et al*., 2000). The non-obese dogs with BCS 1 to 4 (lean to overweight) represented the control group.

Laboratory analysis

Venous blood samples were collected from the saphenous veins. The serum, obtained after clotting and centrifugation, was kept frozen at -20°C until analysis. The glucose (GLU) concentration was measured by the glucose oxidase method. Levels of blood urea nitrogen (BUN) and creatinine (CRT), as well as of total cholesterol (TC) and triglycerides (TG) were determined using standard enzymatic methods and enzymatic colorimetric tests, respectively. The high density lipoprotein (HDL) cholesterol concentration was measured with a heterogenous immunoassay. The low density lipoprotein (LDL) cholesterol level was calculated using the Friedewald equation (LDL cholesterol = total cholesterol – HDL cholesterol – triglycerides / 5). The activities of the liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gammaglutamyl transferase (GGT) were determined by standard enzymatic methods. Measurements of all these variables were performed using a fully-automated blood chemistry analyzer (Cobas Integra 700, Roche Diagnostics, Switzerland).

Statistical analysis

Laboratory test results were reported as the mean ± standard deviation. Differences in the means between the non-obese and obese groups were analyzed by 2-tailed Student's t-test or the Mann-Whitney test as appropriate. Pearson or Spearman rank correlation coefficients were used to assess the relationship among measured parameters. A value of $P < 0.05$ was considered to indicate statistical significance. All data analyses

were performed using SPSS, version 11 (SPSS Inc., Chicago, IL, USA).

RESULTS

Comparisons of the mean levels of glucose and biochemical parameters in the renal, lipid and liver profiles in non-obese and obese dogs are presented in Table 1. On average, obese animals exhibited significantly higher glucose and BUN concentrations than those in the non-obese control animals. On the other hand, there was no statistically significant difference in the creatinine levels between the two groups. With regard to the lipid profile, all variables measured (total cholesterol, triglycerides, HDL cholesterol) and calculated LDL cholesterol showed significantly higher values in the obese group compared to the control group. Obese dogs also displayed higher activities of all liver enzymes measured, with statistically significant differences observed for ALT, ALP and GGT (Table 1).

Metabolic associations between the biochemical components of metabolic syndrome (glucose, lipids and lipoproteins) and parameters in the renal and liver profiles in non-obese and obese dogs are presented in Tables 2 and 3, respectively. In non-obese animals (Table 2), there were significant correlations between BUN and creatinine as well as among several lipid parameters. Strong associations between the activity of the liver enzymes AST and ALT as well as between ALP and GGT were also observed.

In dogs with obesity (Table 3), a different pattern of metabolic association compared with non-obese dogs was found. Notably, the glucose levels in obese dogs showed, in contrast to non-obese dogs, significant correlations with concentrations of several parameters in the lipid profile and with the activity of the enzymes ALT and GGT. These enzymes, on the other hand, exhibited significant associations with each other and with several lipid parameters including TC and LDL cholesterol (LDL-C). The strong correlation between TC and HDL cholesterol (HDL-C) seen in non-obese dogs, however, lost its significance in obese dogs.

Table 1 Levels (mean ± SD) of biochemical components of metabolic syndrome and activity levels of liver enzymes in non-obese and obese dogs.

	Non-obese dog (n = 31)	Obese dog (n = 31)	*P* value
Glucose (mg/dL)	75.13 ± 8.12	84.81 ± 10.59	< 0.001
BUN (mg/dL)	14.42 ± 6.97	21.87 ± 9.58	< 0.01
Creatinine (mg/dL)	0.84 ± 0.26	0.80 ± 0.26	ns
Total cholesterol (mg/dL)	137.32 ± 43.09	244.00 ± 75.51	< 0.001
Triglycerides (mg/dL)	70.39 ± 50.42	146.06 ± 91.36	< 0.001
LDL cholesterol (mg/dL)	31.81 ± 26.79	77.32 ± 76.03	< 0.01
HDL cholesterol (mg/dL)	90.45 ± 29.14	137.39 ± 29.56	< 0.001
AST (U/L)	21.06 ± 8.52	28.77 ± 20.54	ns
ALT (U/L)	25.39 ± 14.69	74.48 ± 49.25	< 0.001
ALP (U/L)	31.81 ± 18.19	149.52 ± 97.93	<0.001
GGT (U/L)	2.84 ± 2.30	7.23 ± 5.62	< 0.001

BUN = blood urea nitrogen; LDL = low density lipoprotein; HDL = high density lipoprotein; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyltransferase; ns = not significant.

Table 2 Correlation coefficients among measured parameters in non-obese dogs.

	GLU	BUN	CRT	TC	TG	LDL-C	HDL-C	AST	ALT	ALP	GGT
GLU	1.0	0.06	0.17	0.28	0.02	0.28	0.16	0.03	0.25	0.07	0.15
BUN	-	1.0	0.39*	0.26	0.48**	0.41*	0.04	0.01	0.27	0.12	0.05
CRT	-	-	1.0	0.24	0.22	0.02	0.27	0.37*	0.16	0.13	0.40*
TC	-	-	-	1.0	0.38*	0.67***	0.70***	0.04	0.20	0.35	0.18
TG	-	-	-	-	1.0	0.07	0.01	0.10	0.12	0.12	0.23
LDL-C	-	-	-	-	-	1.0	0.22	0.03	0.10	0.03	0.21
HDL-C	-	-	-	-	-	-	1.0	0.03	0.13	0.45*	0.36*
AST	-	-	-	-	-	-	-	1.0	0.54**	0.04	0.06
ALT	-	-	-	-	-	-	-	-	1.0	0.08	0.30
ALP	-	-	-	-	-	-	-	-	-	1.0	0.52**
GGT	-	-	-	-	-	-	-	-	-	-	1.0

GLU = glucose; BUN = blood urea nitrogen; CRT = creatinine; TC = total cholesterol; TG = triglycerides; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyltransferase.

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Table 3 Correlation coefficients among measured parameters in obese dogs.

	GLU	BUN	CRT	TC	TG	LDL-C	HDL-C	AST	ALT	ALP	GGT
GLU	1.0	0.20	0.25	0.37*	0.38*	0.36*	-0.22	0.07	0.58**	0.32	0.52**
BUN	-	1.0	0.44*	0.04	0.15	0.08	0.20	0.40*	0.06	0.03	0.03
CRT	-	-	1.0	0.01	0.01	0.08	0.19	0.07	0.22	0.25	0.20
TC	-	-	-	1.0	0.27	0.92***	0.23	0.17	0.38*	0.34	0.52**
TG	-	-	-	-	1.0	0.11	-0.21	0.04	0.22	0.35	0.17
LDL-C	-	-	-	-	-	1.0	-0.31	0.23	0.39*	0.30	0.59***
HDL-C	-	-	-	-	-	-	1.0	-0.06	-0.30	-0.10	-0.28
AST	-	-	-	-	-	-	-	1.0	0.46*	0.25	0.28
ALT	-	-	-	-	-	-	-	-	1.0	0.45*	0.64***
ALP	-	-	-	-	-	-	-	-	-	1.0	0.55**
GGT	-	-	-	-	-	-	-	-	-	-	1.0

GLU = glucose; BUN = blood urea nitrogen; CRT = creatinine; TC = total cholesterol; TG = triglycerides; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyltransferase;

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

DISCUSSION

The results indicated that obese dogs had significantly higher levels of blood glucose and BUN as well as biochemical parameters in the lipid profile than non-obese dogs. Several of the metabolic abnormalities represent biochemical components of metabolic syndrome and surrogate markers of insulin resistance (Brehm *et al.*, 2004; Mclaughlin *et al.*, 2005). Obese animals also exhibited significantly higher activities of the liver enzymes ALT, ALP and GGT compared to non-obese animals. Similar results were obtained from a previous study on beagle dogs for glucose, total cholesterol, triglycerides, LDL cholesterol and the enzyme ALP, but not ALT (Yamka *et al.*, 2006).

In the present study, correlational analyses revealed significant associations among several of these parameters which may indicate their pathophysiological relationship in canine obesity.

The pathophysiological mechanism of metabolic abnormalities linking obesity to insulin resistance has only recently been elucidated. It has been reported that in obese individuals, intra-abdominal adipose tissue release increased the amounts of non-esterified fatty acids (NEFAs), hormones (adiponectin, leptin) and proinflammatory cytokines (tumor necrosis factor ∝, interleukin-6), factors that are involved in the development of insulin resistance. In the insulin resistance state, the observed β-cell dysfunction with a defect in insulin release results in decreasing suppression of hepatic glucose production and reduces the efficiency of glucose uptake in muscle which, in turn, leads to increased plasma glucose levels (Kahn *et al*., 2006). However, the pathophysiological associations between obesity and renal dysfunction are less well understood but may involve obesity-related excess excretory load, excess renal sodium retention and lipotoxicity (Bagby, 2004). With regard to the lipid profile, NEFAs overload provides substrate for triglyceride synthesis in the liver and for triglyceride-rich very low density lipoprotein assembly and secretion. Furthermore, increased hepatic lipase activity (usually found in the state of insulin resistance) reduces HDL cholesterol levels (Bailhache *et al*., 2003). Nevertheless, HDL cholesterol may not represent a biochemical component of metabolic syndrome in dogs since HDL is, in contrast to humans, the principal lipoprotein found in several animal species (Jerico *et al*., 2009; Xenoulis and Steiner, 2010). In addition, an increased HDL cholesterol level was observed in the obese dogs in the present study, in contrast to a decreased level of this lipoprotein reported in obese humans (Blaha *et al*., 2008). Furthermore, the variable associations between triglycerides and BUN found in non-obese and obese dogs indicate that both lipid parameters may not have a major pathophysiological role in canine obesity and insulin resistance.

In recent years, accumulated data from several trials have shown that hepatic insulin resistance plays a dominant role in the pathophysiologic cascade initiated by abdominal obesity and development of the metabolic syndrome. It has been demonstrated in a canine obesity model that free fatty acids (FFAs) are among the most important products of visceral adipocytes to cause insulin resistance, and that the anatomical position of the visceral adipocyte depot (that is, portal drainage into the liver) plays an important role in the pathogenesis of the metabolic syndrome (Kim *et al*., 2003; Bergman *et al*., 2007). In addition, increased delivery of FFAs to the liver observed in obese adolescents was reported to be associated with the development of hepatic steatosis and metabolic complications of nonalcoholic fatty liver disease (NAFLD) (Fabrini *et al*., 2009). Patients with NAFLD, on the other hand, are usually obese, and fat accumulation in the liver may be the primary event leading to hepatic insulin resistance (Utzschneider and Kahn, 2006). In this context, it has been proposed that NAFLD should be considered a component of the metabolic syndrome (Musso *et al*., 2008).

Biochemical alterations frequently found in individuals with NAFLD include mild to moderate elevations of liver enzyme levels. A strong relationship between hepatic enzymes (ALT and GGT, in particular) and metabolic syndrome has also been reported (Chen *et al*., 2008; Saely *et al*., 2008). In addition, significant correlations between ALT and several biochemical components of metabolic syndrome were found in both the study by Chen *et al*. (2008) and in the present study. Of interest was the observation that moderate to severe hypertriglyceridemia, a component of metabolic syndrome in humans, was reported to be associated with high serum ALP, AST, ALT and GGT activities in healthy Miniature Schnauzers (Xenoulis *et al*., 2008). However, although a higher mean level of triglycerides was

found in obese dogs compared to non-obese dogs in the present study, the correlations between triglyceride levels and the activity levels of all liver enzymes measured did not reach statistical significance. Since the strongest association with hypertriglyceridemia found in the study by Xenoulis *et al*. (2008) was with ALP and not ALT activity, it was suggested that the underlying liver condition in Miniature Schnauzers with hypertriglyceridemia differs from NAFLD in humans. In this context, it should be noted that both ALP and AST are found in significant amounts in tissues other than the liver, thus limiting their specificity for hepatic injury (Hoffmann and Solter, 2008). The enzymes ALT and GGT, on the other hand, were reported to have a greater specificity to hepatic tissue, and ALT was shown to have a strong correlation with directly measured liver fat content (Schindhelm *et al*., 2006). It was hypothesized that increased ALT activity may be specifically reflecting hepatic insulin resistance (Hanley *et al*., 2007). In light of this body of evidence and the fact that both ALT and GGT are inexpensive and routinely measured clinical variables in most laboratories, the addition of these enzymes to existing clinically based metabolic risk definition may improve the identification of animals with insulin resistance and metabolic syndrome.

CONCLUSION

Metabolic alterations constantly observed in canine obesity include a relative increase in the glucose level and increased concentrations of biochemical parameters in the lipid profile, many of which represent biochemical criteria of the metabolic syndrome. In addition, the activity levels of liver enzymes are significantly increased. Several of these parameters exhibited strong and significant correlations with each other, which indicated their pathophysiological relationship in dogs with obesity. Since the hepatic specific enzymes ALT and GGT are inexpensive and available for clinical use in virtually all laboratories, measurements of these enzymes may provide cost-effective means for the recognition of a pathophysiological process in the liver and identification of metabolic syndrome in canine obesity.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand.

LITERATURE CITED

Bagby, S.P. 2004. Obesity-initiated metabolic syndrome and the kidney: A recipe for chronic kidney disease? **J. Am. Soc. Nephrol.** 15: 2775–2791.

Bailhache, E., P. Nguyen, M. Krempf, B. Siliart, T. Magot and K. Ouguerram. 2003. Lipoproteins abnormalities in obese insulin-resistant dogs. **Metabolism** 52: 559–564.

Bergman, R.N., S.P. Kim, I.R. Hsu, K.J. Catalano, J.D. Chiu, M. Kabir, J.M. Richey and M. Ader. 2007. Abdominal obesity: Role in the pathophysiology of metabolic disease and cardiovascular risk. **Am. J. Med.** 120(2A): S3–S8.

Blaha, M.J., S. Bansal, R. Rouf, S.H. Golden, R.S. Blumenthal and A.P. DeFilippis. 2008. A practical "ABCDE" approach to the metabolic syndrome. **Mayo Clin. Proc.** 83: 932–943.

Brehm, A., G. Pfeiler, G. Pacini, H. Vierhapper and M. Roden. 2004. Relationship between serum lipoprotein ratios and insulin resistance in obesity. **Clin.Chem.** 50: 2316–2322.

Chen, Z., L. Chen, H. Dai, J. Chen and L. Fang. 2008. Relationship between alanine aminotransferase levels and metabolic syndrome in nonalcoholic fatty liver disease. **J. Zhejiang Univ. Sci. B.** 9: 616–622.

Fabbrini, E., D. deHaseth, S. Deivanayagam, B.S. Mohammed, B.E. Vitola and S. Klein. 2009. Alterations in fatty acid kinetics in obese

adolescents with increased intrahepatic triglyceride content. **Obesity** 17: 25–29.

German, A.J. 2006. The growing problem of obesity in dogs and cats. **J. Nutr.** 136: 1940S–1946S.

Hanley, A.J.G., L.E. Wagenknecht, A. Festa, R.B. D'Agostino, Jr. and S.M. Haffner. 2007. Alanine aminotransferase and directly measured insulin sensitivity in a multiethnic cohort. **Diabetes Care** 30: 1819–1827.

Hoffmann, W.E. and P.F. Solter. 2008. Diagnostic enzymology of domestic animals, pp. 351–378. *In* J.J. Kaneko, J.W. Harvey and M.L. Bruss, (eds.). **Clinical Biochemistry of Domestic Animals.** Elsevier. Amsterdam.

Jerico, M.M., F.D.C. Chiquito, K. Kajihara, M.A.B. Moreira, R. Gonzales, F.L.A. Machado, V.S. Nunes, S. Catanozi and E.R. Nakandakare. 2009. Chromatograplic analysis of lipid fractions in healthy dogs and dogs with obesity or hyperadrenocorticism. **J. Vet. Diagn. Invest.** 21: 203–207.

Kahn, S.E., R.L. Hull and K.M . Utzschneider. 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. **Nature** 444: 840–846.

Kim, S.P., M. Ellmerer, G.W. Van Citters and R.N. Bergman. 2003. Primacy of hepatic insulin resistance in the development of the metabolic syndrome induced by an isocaloric moderate-fat diet in the dog. **Diabetes** 52: 2453–2460.

McGreevy, P.D., P.C. Thomson, C. Pride, A. Fawcett, T. Grassi and B. Jones. 2005. Prevalence of obesity in dogs examined by Australian veterinary practices and the risk factors involved. **Vet. Rec.** 156: 695–707.

McLaughlin, T., G. Reaven, F. Abbasi, C. Lamendola, M. Saad, D. Waters, J. Simon and R.M. Krauss. 2005. Is there a simple way to identify insulin-resistant individuals at increased risk of cardiovascular disease? **Am. J. Cardiol.** 96: 399–404.

Musso, G., R. Gambino, S. Bo, B. Uberti, G. Biroli, G. Pagano and M. Cassader. 2008. Should nonalcoholic fatty liver disease be included in the definition of metabolic syndrome? **Diabetes Care** 31: 562–568.

Saely, C.H., A. Vonbank, P. Rein, M. Woess, S. Beer, S. Aczel, V. Jankovic, C. Boehnel, L. Risch and H. Drexel. 2008. Alanine aminotransferase and gamma-glutamyl transferase are associated with the metabolic syndrome but not with angiographically determined coronary atherosclerosis. **Clin. Chim. Acta** 397: 82–86.

Scarlett, J. M., S. Donoghue, J. Saidla and J. Wills. 1994. Overweight cats: Prevalence and risk factors. **Int. J. Obes. Relat. Metab. Disord.** 18: S22–S28.

Schindhelm, R.K., M. Diamant, J.M. Dekker, M.E. Tushuizen, T. Teerlink and R.J. Heine. 2006. Alanine aminotransferase as a marker of non-alcoholic fatty liver disease in relation to type 2 diabetes mellitus andcardiovascular disease. **Diabetes Metab. Res. Rev.** 22: 437–443.

Thatcher, C.D., M.S. Hand and R.L. Remillard. 2000. Small animal clinical nutrition: An iterative process, pp. 1–19. *In* M.S. Hand, C.D. Thatcher, R.L. Remillard and P. Roudebush, (eds.). **Small Animal Clinical Nutrition.** Walsworth Publishing Company. Marceline, MO, USA.

Utzschneider, K.M. and S.E. Kahn. 2006. Review: The role of insulin resistance in nonalcoholic fatty liver disease. **J. Clin. Endocrinol. Metab.** 91: 4753–4761.

Xenoulis, P.G., J.S. Suchodolski, M.D. Levinski and J.M. Steiner. 2008. Serum liver enzyme activities in healthy Miniature Schnauzers with and without hypertriglyceridemia. **J. Am. Vet. Med. Assoc.** 232: 63–67.

Xenoulis, P.G. and J.M. Steiner. 2010. Lipid metabolism and hyperlipidemia in dogs. **Vet. J.** 183: 12–21.

Yamka, R.M., K.G. Friesen and N.Z. Frantz. 2006. Identification of canine markers related to obesity and the effects of weight loss on the markers of interest. **Intern. J. Appl. Res. Vet. Med.** 4: 282–292.