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Alternation of Physical Indexes and Plasma Biochemical Makers in Overweight Dogs Induced by High-Fat Diet Feeding

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ABSTRACT

The aims of this study were to investigate the effect of high-fat diet feeding for 30 days on Body Weight (BW), Body Fat (BF), Pelvic Circumference (PC), plasma metabolite, hormone and cytokine levels and to demonstrate correlations between BF and biochemical makers in dogs. All subject dogs which were diagnosed clinically healthy and Body Condition Score (BCS) 3 were four female Beagles. All dogs were fed with high-fat diet containing 30% higher calorie compared to the usual diet. At this examine, BF was determined using morphometric measurement with simple calculations and non clinical laboratory equipment. As a result, BUN concentrations decreased significantly and plasma concentrations of adiponectin, as key factor for obesity and insulin resistance, decreased 19%, whereas visfatin increased 11%. BW, BF, PC, glucose, AST, insulin and Cholesterol Ester Transfer Protein (CETP) levels to assess metabolic conditions increased significantly in overweight dogs. Body fat, was calculated by simple formula without specific laboratory equipment, showed strongly positive correlation with plasma ALT, TNF-α and insulin levels and appeared to be a useful indicator to assess physical and metabolic condition of overweight dogs.

Key words: Adiponectin, body fat, visfatin

INTRODUCTION

Common symptoms of overweight are heterotopic accumulation of lipid and increased level of circulating lipid such as hyperlipidemia (Watson and Barrie, 1993). In obese individuals, concentrations of various hormones and adipocytokines which are released from adipose tissue, changed and aberration of those adipocytokines contributed to insulin resistance (Kahn *et al.*, 2006). Circulating levels of adiponectin (APN) which is one of adipocytokines activating insulin sensitivity, decreased significantly in obese dogs (Ishioka *et al.*, 2006). Obesity is also characterized by infiltration of macrophages into adipose tissue promoting low-grade inflammation state implicated in the development of various complications (Swarbrick *et al.*, 2008). Adipocytokines and inflammatory cytokines such as IL-6, IL-8, TNF-α and visfatin play important roles in development of insulin resistance and cardiovascular disease (Sommer *et al.*, 2008). In particular, circulating visfatin concentrations have been reported to be higher in obese human subjects than in lean human subjects (Catalan *et al.*, 2010). Serum full-length visfatin was significantly higher in subjects with diabetes compared to non-diabetic (Retnakaran *et al.*, 2008) in human. Visfatin was expressed in visceral fat (Revollo *et al.*, 2007), whereas several studies have noted no differences in visfatin expression and between visceral and subcutaneous adipose tissue (Varma *et al.*, 2007; Berndt *et al.*, 2005). In small animals, small adipocytes represented markedly greater percentage of the total adipocyte population in Zucker Obese rats than Zucker lean rats. In Zucker Obese rat, small adipocytes had 4-fold increased visfatin (Liu *et al.*, 2010). Unfortunately changes in plasma visfatin concentrations have not been inspected in normal and obese dogs. There are various methods to assess human physical condition (Lukaski, 1987). For dogs various methods including MRI, CT, deuterium oxide dilution and dual-energy X-ray absorptiometry are used to assess their physical conditions. However, most of them are not actually used in clinical practice for dogs because of their high cost and complications in use. In contrast, morphometric measurements for Body Fat (BF) could be used easily in clinical practice without specific clinical laboratory equipments (Burkholder *et al.*, 1997). The aims of this study were (1) to investigate the early effect of high-fat diet feeding on Body Weight (BW), BF (%) and Pelvic Circumference (PC) and plasma metabolite, hormone and cytokine levels and (2) to demonstrate the correlations between BF and biochemical makers in overweight dogs.

MATERIALS AND METHODS

Four female Beagles (3-8 years), maintained for examination in the common laboratories at Department of Veterinary Science of Nippon Veterinary and Life Science University and diagnosed clinically healthy and Body Condition Score (BCS) 3 which was determined on a five point scale: 1. very thin; 2. underweight; 3. ideal; 4. overweight; 5. obese, were used. This examination, approved by Nippon Veterinary and Life Science University Animal Research Committee, started from the 18th August and finished 17th September 2010.

All dogs were fed with high-fat diet for 1 month which is equivalent to 6 months in human term. At first Resting energy requirement (RER = 70×body weight$^{0.75}$) were calculated for each dogs and multiplied it by moderate work coefficient (3.0) in order to calculate total amount of calories. We made a high-fat diet containing 30% higher calorie compared to usual diet (usual diet, 314.5 kcal/100 g; high-fat diet, 411.7 kcal/100 g). Nutrient profiles of the high-fat diet were ash 6.9%, crude fat 24.3%, crude fiber 1.86%, protein 22.9% and moisture 8.4%. Diet included corn, wheat flour, bean curd refuse, defatted soybean, fish flour, gluten meal, beer yeast, soy oil, beet pulp, poultry by product meal, vitamin E, salt, oxygen water, beef fat, zinc carbonate as detail ingredient composition. Dogs were fed the diet with a half amount of designated diet twice at 6:00 am and 2:00 pm and water was constantly available during the examination. BF was determined using morphometric measurement with simple calculations and non clinical laboratory equipment and correlated highly with dual- energy X-ray absorptiometry (Mawby *et al.*, 2004). There are gender-specific and either gender formulas on morphmetric measurement. BF (%) was calculated according to gender-specific formula:

$$\text{Female body fat (\%)} = -1.7\times\text{HS (cm)}+0.93\times\text{PC (cm)}+5$$

where, HS is length from hock joint to back knee and PC is length around bony pelvis.

Blood samples were taken from the fore limb vein of animals were obtained and collected into heparinized plastic tubes before breakfast at before and after high-fat diet feeding for one month. Plasma was recovered by centrifugation at 1200 g for 5 min at 4°C and subsequently stored at -25°C. Alkaline phosphatase(ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase

(AST) and Lactate Dehydorgenase (LDH) activities and Blood Urea Nitrogen (BUN), Creatinin (CRE), Glucose (GLU), Total Cholesterol (T-Cho), Total Protein and Triglyceride (TG) concentrations were measured using auto analyzer (AU680, Olympus Corporation, Tokyo) with the manufacturer's reagents. Free fatty acid (FFA), Adiponectin (APN), Insulin (ISN), Cholesterol Ester Transfer Protein (CETP), Phospholipid Transfer Protein (PLTP), TNF-α and visfatin were determined with the following commercial kits: NEFA-C test Wako (Wako Pure Chemical Industries, Inc., Tokyo), Dog Adiponectin ELISA Kit and Mouse Visfatin/PBEF ELISA Kit (CircuLex Co., Ltd. Nagano), Lbis Dog Insulin kit (SHIBAYAGI Co., Gunma), CETP Activity Assay Kit and PLTP Activity Assay Kit (BioVision Research Products, Mountain View, CA), Canine TNF-α/TNF SF1A Immunoassay (R and D Systems, Inc., MN).

Data are presented as Means±SD. The Student's t-test for paired groups was performed on the various data in order to determine significance using Graph Pad Prism software version4.0 (Graph Pad Soft ware, San Diego, CA). Differences were considered significant when the p value was <0.05. Pearson's product moment correlation was used to identify significant correlations between BF and biochemical makers.

RESULTS

All dogs gained 2.8±0.5 kg as BW after the high-fat diet feeding. Three physical indexes, BW, BF (%) and PC increased significantly after the high-fat diet feeding (Table 1). Dogs showed

Table 1: Changes in physical indexes and plasma biochemical markers in female beagles before and after high-fat diet feeding

	Female beagles (n = 4)	
Physical and biochemical parameters	Before	After
Age (year)	6.5±2.4	
Weight (kg)	13.7±0.4	16.5±0.3[a]
Body fat by morphometric measurements (%)	27±4	34±2[a]
Pelvic circumference (cm)	48±4	56±3[a]
GLU (mg dL^{-1})	94±4	109±5[a]
TG (mg dL^{-1})	48±6	64±36
T-Cho (mg dL^{-1})	191±38	191±39
BUN (mg dL^{-1})	16.1±2.8	8.9±1.4[a]
CRE (mg dL^{-1})	0.56±0.10	0.50±0.06
TP (g dL^{-1})	6.2±0.5	6.6±0.1
LDH (U L^{-1})	38±10	51±17
AST (U L^{-1})	31±7	40±11[a]
ALT (U L^{-1})	67±34	118±93
ALP (U L^{-1})	22±26	39±41
FFA (mEq L^{-1})	0.52±0.03	0.85±0.50
APN (μg mL^{-1})	15.2±7.6	12.4±10.3
Visfatin (ng mL^{-1})	269±339	300±280
CETP (pmol μL^{-1} sample h^{-1})	19±7	30±5[a]
PLTP (pmol μL^{-1} sample h^{-1})	9±4	6±2
TNF-α (pg mL^{-1})	24.3±21.1	22.1±29.5
ISN (ng mL^{-1})	1.0±0.6	1.8±1.1[a]

Values are presented as Mean±SD. [a]Indicates significance when compared against before value (p<0.05 paired t-tes). ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, APN: Adiponectin, AST: Aspartate aminotransferase, CETP: Cholesterol ester transfer protein, CRE: Creatinin, FFA: Free fatty acid, GLU: Glucose, ISN: Insulin, LDH: Lactate dyhydrogenase, PLTP: Phospholipid transfer protein, T-Cho: Total cholesterol, TG: Triglyceride, TP: Total protein, BUN: Blood urea nitrogen

Table 2: Correlation between body fat (%) and biochemical markers in female beagles before and after high-fat diet feeding

Biochemical parameter	Before	After
BUN	-0.59	-0.75
T-Cho	0.37	0.61
ALT	0.54	0.97
ALP	0.73	0.71
TNF-α	1.00	0.96
ISN	0.92	1.00

Pearson's product moment correlation coefficients, r, are displayed. BUN: Blood urea nitrogen, T-Cho: Total choresterol, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, ISN: Insulin

significant differences in GLU, BUN, AST, ISN and CETP as biomedical markers. BUN concentrations after the diet feeding were significantly lower than the basal concentrations values before the feeding but concentrations of CRE as a main maker of kidney failure were constant. Adiponectin as maker of which is one of adipocytokines activating insulin sensitivity was on a declining trend. Whereas Visfatin, TG, FFA, TP, ALP and LDH in obese dogs with fed high-fat foods also were on the rise and T-cho was stable. Table 2 shows the correlations between BF and some biomedical makers. BUN showed a strongly negative correlation with BF after gaining weight (before r = -0.59, after r = -0.75). ALT and T-Cho correlated positively with BF (%) in overweight dogs (T-Cho r = 0.61, ALT r = 0.97). ALP, TNF-α and insulin had a strongly positive correlations with BF (ALP, before r = 0.73 after r = 0.71; TNF-α, before r = 1.00 after r = 0.96; ISN, before r = 0.92 after r = 1.00).

DISCUSSION

Three physical indexes and some biomedical makers at this examination distinctly altered after one month high-fat feeding. The rise rates of the physical indexes were 20% for BW, 26% for BF and 16% for PC compared to the values before the feeding. As body composition assessment is a tool for diagnosis as it may be significantly altered in obesity and many disorders (Shah *et al*., 2009). Therefore not only BW but also BF and PC should be regularly measured at clinical checkup for dogs. Particularly BF using morphometric measurement with simple calculations and non clinical laboratory equipment was easily and cost less. Significant increases in glucose and insulin after high-fat feeding as biochemical makers appeared to indicate early stage of insulin resistance. Decreased BUN concentrations may indicate not a decline in renal function but decrease of urea synthesis in liver for compulsive metabolism of high fat diet. AST activities, as a fatty liver maker, significantly increased after the feeding. Therefore, it was expected that high-fat diet feeding induced early hepatic failure and inflammation in dogs. ALP also increased after high-fat feeding. Raja *et al*. (2011) supported this results by their evidence that increased ALP activity has also diagnostic value in the hepatic diseases in dogs. Recent research has implicated the innate immune system in the obesity-related liver damage (Maher *et al*., 2008; Szabo *et al*., 2007). HDL dominant animals including dog show fundamentally very low activity of CETP which transfer cholesteryl ester from HDL-cholesterol to LDL-cholesterol (Guyard-Dangremont *et al*., 1998). However, the after high-fat diet feeding, CETP activity was significantly elevated in overweight dogs, so plasma LDL-cholesterol concentrations might income. Plasma concentrations of adiponectin decreased 19%, whereas visfatin increased 11% after the high-fat diet feeding. Precious evidence that decreased adiponectin plays causative role in the development of insulin resistance (Assal *et al*., 2007) was agreed with the present result with significant increased insulin. Visfatin in obese dogs obviously increased as obese human and obese rats. If dogs were feeding high-fat for further long period, adiponectin and visfatin may make apparent changes as human obesity. There was some evidence that the primary mechanism by which adiponectin enhance insulin sensitivity appears to be through fatty acid oxidation and inhibition of hepatic glucose production (EL-Ghaffar *et al.*, 2006). It would be accurate to say that adiponectin and insulin were great interested markers for metabolic disorder commencing with obesity. Abdominal obesity, recognized by increased waist circumference, is the criterion listed for metabolic syndrome in human by the American Diabetic Association (Mahajan *et al.*, 2010). In near further, if the criterion for metabolic syndrome dogs set up, BF (%), had strong correlations with main metabolic parameters, BUN, ALT ALP, total-cholesterol, TNF-α and insulin, may have a significant role to play at diagnosis list.

CONCLUSION

High-fat diet feeding induced significant increases in physical indexes, BW, BF (%), PC and plasma glucose, AST, CETP and insulin levels as biochemical makers in female beagle dogs. In overweight dogs with high-fat diet feeding, plasma BUN concentrations decreased significantly. BF showed strongly positive correlation with plasma ALT, TNF-α and insulin levels in over weight dogs. BF was calculated by simple formula without specific laboratory equipment and appeared to be useful indicator to assess physical and metabolic condition of overweight dogs. The usefulness of body fat as a clinical index to assess metabolic condition of whole body should be farther studied in more breeds dogs (e.g. characterized with short legs: Miniature dachshund) with various physical conditions.

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