

Comparison of the Plasma Insulin and Adiponectin Concentrations as Metabolic Markers in Clinically Healthy Dogs with Ageing

Nobuko Mori, Koh Kawasumi and Toshiro Arai

The Laboratory of Veterinary Biochemistry, Department of Veterinary Science,
School of Veterinary Medicine, Nippon Veterinary and Life Science University,
1-7-1 Kyonancho, Musashino, 180-8602 Tokyo, Japan

Abstract: Plasma metabolite concentrations reflect changes in the energy metabolic and physical conditions of animals with metabolic disorders. A reduced adiponectin level in humans and rodents is the critical factor associated with the pathogenesis of obesity-associated atherosclerosis, insulin resistance and type 2 diabetes mellitus. Appropriate plasma metabolic markers appear to be useful for the early diagnosis of latent metabolic disorders with no clinical signs in animals. The aim of this study was to assess changes in the values of plasma metabolic markers in healthy ageing dogs. Significant differences were observed in the plasma insulin and total cholesterol concentrations in healthy dogs of different genders and ages. Adiponectin concentrations peaked at a young age and decreased with ageing. Apparently, insulin increased according to age. Plasma insulin concentrations were particularly influenced significantly by age as well as glucose and triglyceride levels whereas adiponectin concentrations were affected by gender on multiple linear regressions for all factors. No criteria exist for the early diagnosis of latent metabolic disorders without remarkable signs regarding age and gender in dogs. Thus, in case of dogs, it may be necessary to set up improved age-dependent criteria for metabolic disorders with changes in plasma insulin concentrations.

Key words: Adiponectin, dog, insulin, metabolic disorder, plasma, Japan

INTRODUCTION

In recent years, the prevalence of metabolic disorders such as hyperlipemia, obesity and diabetes mellitus has increased in dogs (German *et al.*, 2007; Lund *et al.*, 2006). Plasma metabolite concentrations reflect changes in the energy metabolic and physical conditions of animals with metabolic disorders (Downs *et al.*, 1997; Pitkanen *et al.*, 1999). In the plasma of dogs with type 1 diabetes mellitus the insulin concentration decreased and glucose and Free Fatty Acid (FFA) concentrations increased significantly (Magori *et al.*, 2005). Adiponectin is an adipokine involved in modulating whole-body metabolism and other vital functions related to inflammation and immune responses (Berg and Scherer, 2005; Saltiel, 2001; Mori *et al.*, 2011; Ghazanfari *et al.*, 2011). It is a unique adipokine because it is the only one having anti-atherogenic effects.

These effects can not only be attributed indirectly in part to its insulin-enhancing action that decreases the atherogenic burden associated with insulin resistance but also to its direct anti-atherogenic effects on the arterial wall where it reduces monocyte adhesion to the

endothelium thereby inhibiting smooth muscle cell proliferation and foam cell formation (Berg *et al.*, 2001; Yamauchi *et al.*, 2002). Coincidentally, mounting evidence in humans and rodents suggests that a reduced adiponectin level is the critical factor associated with the pathogenesis of obesity-associated atherosclerosis, insulin resistance and type 2 diabetes mellitus (Chandran *et al.*, 2003; Kumada *et al.*, 2004; Weyer *et al.*, 2001).

Appropriate plasma metabolic markers appear to be useful for the early diagnosis of latent metabolic disorders with no clinical signs in animals. The aim of this study was to assess changes in the values of the plasma metabolic markers in healthy dogs with age.

MATERIALS AND METHODS

Researchers examined plasma metabolic markers in 704 (Female = 338; Male = 366) blood samples of dogs from 7 veterinary clinics in the residential streets in the centre and suburb of big cities with a large population of >3 million, i.e., Tokyo, Osaka and Fukuoka. All the dogs were examined for the filariasis antigen from March

Corresponding Author: Toshiro Arai, The Laboratory of Veterinary Biochemistry, Department of Veterinary Science,
School of Veterinary Medicine, Nippon Veterinary and Life Science University,
1-7-1 Kyonancho, Musashino, 180-8602 Tokyo, Japan

2008-2010. In total, 223 dogs (Female = 111; Male = 112) with abnormal values of Triglyceride (TG>165 mg dL⁻¹) and/or Total cholesterol (T-cho>242 mg dL⁻¹) or high values of Lactate Dehydrogenase (LDH>300 IU L⁻¹), Aspartate Aminotransferase (AST>150 IU L⁻¹) and Alanine Aminotransferase (ALT>120 IU L⁻¹) were excluded as metabolic disorder dogs with hyperlipidemia and liver failure. Another 481 dogs (F = 227; M = 254; 1-17 years of age) including Golden Retrievers (F = 6; M = 6), Labrador retrievers (F = 14; M = 9), Border collies (F = 3; M = 3), Welsh Corgis (F = 7; M = 5), Beagles (F = 4; M = 10), Cavaliers (F = 4; M = 2), Shibas (F = 22; M = 27), Pugs (F = 3; M = 4), Shih tzus (F = 11; M = 9), miniature Dachshunds (F = 31; M = 34), Toy poodles (F = 12; M = 7), Yorkshire terriers (F = 7; M = 5), Maltese (F = 4; M = 2), Papillons (F = 3; M = 6), Pomeranians (F = 2; M = 6), Chihuahuas (F = 9; M = 19) and Mongrels (F = 71; M = 72) were used as healthy dogs. All the dogs were divided into 4 categories by age: infant (0-2 years), young (3-5 years), middle age (6-10 years) and old age (>11 years). Blood samples obtained from fore-limb veins were transferred into heparinised plastic tubes and plasma was recovered by centrifugation at 4°C and stored at -25°C until it was assayed. Plasma Glucose (GLU), protein, TG and T-Cho concentrations as well as LDH, AST and ALT activities were measured using an auto analyser (AU680, Olympus Corp., Tokyo, Japan). FFA and adiponectin and insulin concentrations were measured using commercial kits (FFA: NEFA-C test Wako, Wako Pure Chemical Industries, Inc., Tokyo, Japan; adiponectin: Mouse/Rat Adiponectin ELISA kit, Otsuka Pharmaceutical Co., Tokyo; Insulin: Lbis Dog Insulin kit, Shibayagi Co., Shibukawa, Japan).

The Adiponectin kit was validated previously for use in dogs by Ishioka *et al.* (2006) and Asako *et al.* (2004). Statistical significance was determined using the Holm-Sidak one-way Analysis of Variance (ANOVA) to compare age and gender and a three-way ANOVA and linear regression analysis was performed to determine the effect of adiponectin and insulin.

All tests were performed using Sigmaplot Version 11.2, Build 11.2.0.5 (Systat Software Inc., San Diego, CA, USA). A p<0.05 was considered significant.

RESULTS AND DISCUSSION

Table 1 shows plasma metabolic marker values by age and gender. Significant differences in plasma T-cho and insulin concentrations were observed with increasing age. The plasma T-cho concentrations in male old age dogs were significantly higher than those in infant male dogs. Insulin concentrations in middle and old age dogs of both genders were significantly higher than those in infant dogs. No significant difference were observed for plasma GLU, TG, protein and FFA concentrations or LDH, AST and ALT activities among all age groups and both genders.

Figure 1a and b shows the transitional changes in insulin and adiponectin concentrations with increasing age in both genders. Adiponectin concentrations peaked at a young age and decreased with age. Adiponectin concentrations in males of each age group were consistently about 10 µg mL⁻¹ higher than those in females of the same age group (Fig. 1b). Insulin concentration increased significantly with age (Fig. 1a). Table 2 shows p-values for individual factors that may have influenced plasma adiponectin and insulin concentrations. Age, GLU and TG had a significant effect on insulin concentrations (p≤0.001, 0.007, 0.007, respectively). Moreover, ALT influenced insulin concentrations (p≤0.052).

The gender of dogs had a significant effect on adiponectin concentrations (p≤0.02). Plasma markers such as metabolites, lipids, hormones and enzymes are used frequently as diagnostic indicators of metabolic disorders (Neumann *et al.*, 2008). Increases in plasma TG and ALT levels are diagnostic characteristics indicating fat accumulation in the liver of animals (Hsiao *et al.*, 2007;

Table 1: Comparison of metabolic markets in clinically healthy dogs of different ages and genders

Age (year)	Gender (n)	Glucose (mg dL ⁻¹)	Triglyceride (mg dL ⁻¹)	Total cholesterol (mg dL ⁻¹)	Protein (g dL ⁻¹)	FFA (mEq L ⁻¹)	Insulin (ng mL ⁻¹)	Adiponectin (µg mL ⁻¹)	LDH (U L ⁻¹)	AST (U L ⁻¹)	ALT (U L ⁻¹)
Infant (0-2)	F 50	96±2	52±3	171±6	5.9±0.1	0.26±0.03	1.1±0.2	24±3	97±15	29±2	40±3
	M 60	95±2	51±4	153±6	5.9±0.1	0.29±0.04	1.2±0.1	34±4	89±9	30±1	47±3
Young (3-5)	F 58	90±2	56±4	174±6	5.9±0.1	0.28±0.03	1.2±0.2	27±3	136±23	29±1	40±2
	M 68	90±2	48±3	151±5	6.0±0.1	0.24±0.03	1.7±0.2	38±6	120±17	28±1	45±3
Middle (6-10)	F 88	90±2	60±3	164±5	5.9±0.1	0.30±0.03	1.9±0.2*	24±4	108±11	27±1	38±3
	M 87	90±2	52±3	164±5	6.1±0.1	0.38±0.06	1.8±0.2*	32±4	102±10	26±1	45±2
Old (11>)	F 31	87±2	54±3	169±7	6.0±0.2	0.25±0.04	2.4±0.3*	12±2	112±17	26±1	56±7
	M 39	91±2	58±5	180±7*	6.3±0.1	0.25±0.03	2.2±0.3*	25±4	109±15	28±2	58±8
Dog total	F 227	91±1	57±2	169±3	5.9±0.1	0.28±0.02	1.6±0.1	23±2	113±8	28±1	41±2
	M 254	91±1	52±2	161±3	6.1±0.1	0.30±0.02	1.7±0.1	33±2	104±7	28±1	47±2

Values are presented means±SE; F = Female; M = Male; *Indicates the significant difference when compared against the same gender in the infant group (Hold-Sidak one-way analysis of variance; p<0.05)

Table 2: Multiple linear regressions for insulin and adiponectin

Factors	Insulin	Adiponectin
Gender	0.709	0.020 ^b
Age	<0.001 ^a	0.209
BCS	0.068	0.219
Glucose	0.007 ^a	0.678
Triglyceride	0.007 ^a	0.217
Total cholesterol	0.741	0.177
Protein	0.214	0.557
FFA	0.597	0.735
LDH	0.858	0.185
AST	0.182	0.190
ALT	0.052	0.505

The numbers are p-values for each factor; ^aIndicates the significant difference in insulin (p<0.05), ^bIndicates the significant difference in adiponectin (p<0.05)

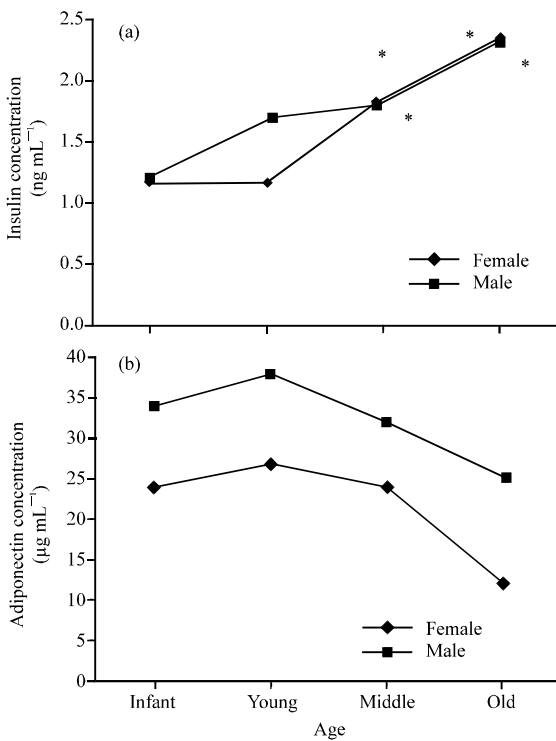


Fig. 1: Transitions in the plasma insulin and adiponectin concentrations in dogs of different ages; a) insulin concentration and b) adiponectin concentration; Infant, 0-2 years; Young, 3-5 years; Middle, 6-10 years; Old, >11 years; *Indicates the significant difference when compared with the same gender in the Infant group (Hold-Sidak one-way analysis of variance; p<0.05)

Iacobellis *et al.*, 2008). Adiponectin is recognised as a protein synthesised and secreted from the adipose tissue and one of the key adipocytokines responsible for obesity leading to atherosclerosis and insulin resistance (Kondo *et al.*, 2002). In a previous report, plasma adiponectin concentrations decreased significantly in

obese dogs (IDF, 2005) whereas more recent studies have reported that dog plasma adiponectin concentrations do not decrease in overweight and obese dogs and are not associated with obesity-associated insulin resistance (Verkest *et al.*, 2011). Researchers speculate that the discrepancy in adiponectin concentrations may be because of changing adipose status. Adiponectin is secreted from small adipose cells. Adipose cells secrete reduced amounts of adiponectin as they expand in size and as lipid accumulates in subcutaneous and visceral areas of the body (Pasarica *et al.*, 2009). In short, adiponectin may be an adipose status maker rather than an adiposity or obesity marker.

In humans, there are clear criteria for check-up examinations such as the definition of metabolic syndrome (IDF, 2005): central obesity (defined as a particular waist circumference with ethnic specific values) and any two of the following four factors: reduced high density lipoprotein cholesterol (M<40 mg dL⁻¹; F<50 mg dL⁻¹) or specific treatment for a lipid abnormality; increased blood pressure (>130 or diastolic BP >85 mm Hg) or treatment for previously diagnosed hypertension and Increased fasting plasma glucose (>100 mg dL⁻¹) or previously diagnosed type 2 diabetes. These criteria are effective for the early diagnosis of latent metabolic disorders in humans. Unfortunately, no criteria have been developed for an early diagnosis of latent metabolic disorders in dogs of different ages and genders. Thus, it may be necessary to set up improved age-dependent insulin criteria for dogs.

CONCLUSION

The findings indicate that insulin and T-Chol significantly differed by gender and age in healthy dogs. Plasma insulin, GLU and TG concentrations were particularly significantly influenced by age whereas adiponectin concentrations were affected by gender.

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