Short Communication

Evaluation of plasma non-esterified fatty acid levels in dogs

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Abstract:

We have previously proposed the new criteria for detecting hyperlipidemia at an early stage in dogs. Hyperlipidemia dogs were detected based on the any two of the following three factors, namely elevated plasma triglyceride (TG) ≥165 mg dL^{-1}, total cholesterol (TC) ≥200 mg dL^{-1} and non-esterified fatty acid (NEFA) ≥ 1.5 mEq L^{-1} levels. Since plasma NEFA levels are regarded as an important diagnostic marker of lipotoxicity, in this study, we focused on the significance of plasma NEFA levels and investigated it. 14 (21.2%) of 66 dogs with ≥1.5 mEq L^{-1} NEFA levels indicated significantly higher total cholesterol (TC), low-density cholesterol (LDL-C), triglyceride (TG) concentrations and LDL-C/HDL-C ratio compared to those of dogs with 0.7 mEq L^{-1} NEFA levels, respectively. We verified the usefulness of the criteria of the raised plasma NEFA levels ≥1.5 mEq L^{-1} in dogs.

Keywords: dog, lipid metabolism, non-esterified fatty acid (NEFA)

Introduction

In dogs, an occurrence of glucose and lipid metabolism disorders such as obesity and diabetes has increased markedly in recent years[1]. In our previous study, we have proposed the new criteria for detecting hyperlipidemia at an early stage in dogs and preventing the hyperlipidemia progresses to metabolic syndrome (MS) and severe metabolic disorders in dogs[2]. Hyperlipidemia dogs were detected based on the any two of the following three factors, namely elevated plasma TG ≥165 mg dL^{-1}, TC ≥200 mg dL^{-1} and NEFA ≥1.5 mEq^{-1} levels .

Since NEFA induced lipotoxicity[3] and oxidative stress[4], plasma NEFA levels are regarded as an important diagnostic marker of lipid metabolic disorders. In this study, we focused on plasma NEFA levels and investigated relation between plasma NEFA levels and lipid metabolic parameter levels in dogs.

Materials and Methods

Sixty-six client-owned (volunteered) dogs (31 female, 35 male, 1–16 years old) of 5 veterinary clinics in Kanto district in Japan were used to evaluate the importance of plasma NEFA levels. The degree of obesity was assessed by body condition score (BCS) on the following five-point scale: very thin, underweight, ideal, over weight and obese. Blood samples were taken from jugular veins of dogs fasted overnight (without any nutri-

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ent for over 8 hours after the last meal) in heparinized tubes. Plasma was recovered by centrifugation at 4℃ and stored at −25℃ until use. Glucose (GLU), triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprrptein (LDL-C) levels were measured using an autoanalyzer (JCA-BM2250, JEOL Ltd., Tokyo, Japan) with manufacture's reagents at blood chemistry service corporation, FUJIFILM Monolith Co., Ltd. Tokyo, Japan. Plasma insulin (ISN) and adiponectin concentrations were measured with commercial ELISA kits, Lbis dog insulin kit (SHIBAYAGI Co., Gunma, Japan), Mouse/Rat adiponectin kit (Otsuka Pharmaceutical Co., Ltd. Tokyo, Japan), respectively. NEFA was measured using commercial kit, NEFA-C test (Wako Pure Chemical Industries, Inc., Tokyo, Japan). Results are presented as mean±95% C.I. Statistical significance was determined by student's t-test. The significance level was set at $p<0.05$.

Results and Discussion

14 (21.2%) of 66 dogs with ≥1.5 mEq L^{-1} NEFA levels indicated significantly higher TC, LDL-C, TG concentrations and LDL-C/HDL-C % compared to those of dogs with ≤0.7 mEq L^{-1} NEFA levels, respectively. Moreover, there were significant differences between the dogs with 0.7–1.5 mEq L^{-1} NEFA levels group and dogs with ≥1.5 mEq L^{-1} NEFA levels group in TC and TG levels. There were no significant differences between the dogs with <0.7 mEq L^{-1} NEFA levels group and dogs with 0.7–1.5 mEq L^{-1} NEFA levels group in all plasma biomarkers. Based on these findings, we verified the usefulness of the criteria of the raised plasma NEFA levels ≥1.5 mEq L^{-1} in dogs.

Our criteria for diagnosis of hyperlipidemia at an early stage, previously proposed (Kawasumi et al., 2012), showed lower plasma TG and TC concentrations compared to those for clinicians. Hyperlipidemia is diagnosed clinically as over 300 mg dL^{-1} of plasma TC concentration in dogs[5], while plasma triglyceride (TG) concentrations of 150 to 400 mg dL^{-1} are considered as mild elevation for clinical assessment in dogs[5]. Based on these findings, 14 (21.2%) of 66 dogs with ≥1.5 mEq L^{-1} NEFA levels might have passed through the early stage of hyperlipidemia. Hyperlipidemia includes pancreatitis, liver disease, atherosclerosis, ocular disease and seizures as possible complications[6] and induces insulin resistance[7].

NEFA leads to the accumulation of reactive oxy-

Table 1 Comparison of plasma NEFA levels associated with plasma biomarker levels in dogs.

	NEFA (mEq L^{-1})		
	< 0.7 (n=29)	≥ 0.7–1.5 < (n=23)	1.5 ≤ (n=14)
BCS	3.6±0.3	3.8±0.3	3.6±0.4
Age (year old)	10±1	9±2	8±2
GLU (mg dL^{-1})	100±8	108±13	132±56
TC (mg dL^{-1})	270±39	266±38	454±127*1,*2
TG (mg dL^{-1})	106±26	174±92	496±262*1,*2
HDL-C (mg dL^{-1})	210±24	209±21	232±27
LDL-C (mg dL^{-1})	33±13	57±68	147±118*1
LDL-C/HDL-C (%)	13±4	26±31	60±48*1
ADN (μg mL^{-1})	18±6	25±15	12±6
Insulin (ng mL^{-1})	2.9±2.0	1.6±0.6	2.1±0.8

Data are expressed as mean±95% C.I.
The numbers in parentheses indicate the number of animals examined.
*1 Significant ($p<0.05$) when compared against <0.7 mEq L^{-1} NEFA level group
*2 Significant ($p<0.05$) when compared against ≥0.7–1.5 mEq L^{-1} < NEFA level group

gen species[8] and excess reactive oxygen species react with lipid. Therefore, it is supposed that examination of plasma malondialdehyde (MDA), lipid peroxide marker, will be needed to detect canine early stage hyperlipidemia more definitely.

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短報

犬における血漿非エステル型脂肪酸値の評価

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和文要約：

我々は以前，犬における高脂血症の早期を検出する新しい診断基準を提唱した。その診断基準では，高脂血症犬は以下の 3 つの条件，すなわち血漿トリグリセリド（TG）値 165 mg dL^{-1} 以上，総コレステロール（TC）値 200 mg dL^{-1} 以上，非エステル型脂肪酸（NEFA）値が 1.5 mEq L^{-1} 以上のうち，2 つが該当したことに基づき，検出された。血漿 NEFA 値は脂肪毒性の重要な診断マーカーとされている。今回，我々は血漿 NEFA 値の重要性について検討した。NEFA 値が 1.5 mEq L^{-1} 以上を示した 66 頭中 14 頭（21.2%）は，総コレステロール値，LDL コレステロール値，TG 値および LDL-C/HDL-C 比が NEFA 値 0.7 mEq L^{-1} の犬に比べて著しく高値を示した。我々は NEFA 値 1.5 mEq L^{-1} 以上の診断基準の有用性を立証した。

キーワード：犬，脂質代謝，非エステル型脂肪酸（NEFA）

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