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Canine adiponectin: cDNA structure, mRNA expression in adipose tissues and reduced plasma levels in obesity

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Abstract

Adiponectin is a protein synthesized and secreted by adipocytes. Decreased adiponectin is responsible for insulin resistance and atherosclerosis associated with human obesity. We obtained a cDNA clone corresponding to canine adiponectin, whose nucleotide and deduced amino acid sequences were highly identical to those of other species. Adiponectin mRNA was detected in adipose tissues, but not in other tissues, of dogs. When 22 adult beagles were given a high-energy diet for 14 weeks, they became obese, showing heavier body weights, higher plasma leptin concentrations, but lower plasma adiponectin concentrations. The adiponectin concentrations of plasma samples collected from 71 dogs visiting veterinary practices were negatively correlated to plasma leptin concentrations, being lower in obese than non-obese dogs. These results are compatible with those reported in other species, and suggest that adiponectin is an index of adiposity and a target molecule for studies on diseases associated with obesity in dogs.

Keywords: Adipocytokine; Adiponectin; Diabetes mellitus; Dogs; Leptin

1. Introduction

The adipose tissue has long been thought to be the site for energy storage and a target of various hormones. However, recent studies have revealed that the adipose tissue itself is an active endocrine organ which produces and secretes many polypeptides, such as adipsin, leptin, tumor necrosis factor, and plasminogen activator inhibitor-1. These are collectively called as adipocytokines and implicated in the regulation of a wide variety of physiological functions (Matsuzawa et al., 1999). For example, leptin is secreted from adipose tissue in response to changes in energy balance, acts on the hypothalamus to regulate food intake and the neuroendocrine mechanisms controlling energy expenditure (Friedman and Halaas, 1998). Adiponectin is an adipocytokine found at almost three-order higher concentrations in blood than other adipocytokines, and its plasma concentration decreases with body fat accumulation (Chandran et al., 2003). It is now recognized as one of the key adipocytokines responsible for obesity-associated atherosclerosis (Ouchi et al., 2001) and insulin resistance (Kondo et al., 2002).

In companion animal medicine, as in human medicine, obesity is the most common nutritional disorder

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(Edney and Smith, 1986; Lund et al., 1996). However, in contrast with growing interest in the pathophysiology of obesity itself and obesity-related metabolic and endocrine diseases in human medicine, there have been limited information on these subjects, particularly on adipocytokines in the dog and cat. In a series of studies on adipocytokines in companion animal medicine, we have shown the molecular structure of canine leptin, its mRNA expression in adipose tissues (Iwase et al., 2000a), and a positive correlation between the plasma concentration and body fat content (Ishioka et al., 2002a; Sagawa et al., 2002). Thus, plasma leptin is a good index of adiposity, and may be useful for quantitative assessment of obesity in dogs. While adiponectin is also involved in many obesity-related diseases, its complete amino acid sequence and relationship with obesity have not been published in the dog.

In the present study, we focused on canine adiponectin, determined its deduced amino acid sequence by cDNA cloning, examined mRNA expression in peripheral tissues, and measured the plasma concentration by enzyme-linked immunosorbent assay (ELISA) focusing on relationship with obesity.

2. Materials and methods

2.1. RNA extraction

An adult beagle (male, 3 years old) was euthanatized by intravenous administration of an over dose of pentobarbital, and 5–10 g tissue specimens were collected quickly from subcutaneous adipose tissue, mesenteric adipose tissue, lung, heart, liver, spleen, kidney and skeletal muscle (*m. gastrocnemius*). Total RNA was extracted by homogenizing individual tissue samples in TRIzol (Gibco BRL, Gaithersburg, MD, USA).

2.2. cDNA cloning of canine adiponectin

Total RNA extracted from mesenteric adipose tissue was reverse-transcribed (RT) using Reverse transcriptase (Gibco BRL, Gaithersburg, MD, USA) and an oligo-dT primer. Polymerase chain reaction (PCR) was performed with a cDNA template and primers based on the reported sequences of human (Accession No., D45371), murine (Accession No., BC028770) and bovine (Accession No., AF269230) adiponectin, including untranslated regions (Forward, 5′-CACACCW GAGGGGCTCAGG-3′; Reverse, 5′-GTCATGTA TRTGAARCTCCCCAG-3′). PCR was conducted for 30 cycles at 94 °C for 1 min, at 60 °C for 30 s and at 72 °C for 1 min in a solution (30 μl) containing 20 pmol of each primer and LA Taq DNA polymerase (TaKaRa, Otsu, Japan). The PCR product was ligated to a vector plasmid pGEM, cloned in *E. coli*, and sequenced using DNA sequencer (ABI PRISM 310 capillary DNA sequencer; PERKIN ELMER Applied Biosystems, Foster City, CA, USA).

2.3. Tissue distribution of adiponectin mRNA

Total RNA extracted from various peripheral tissues was treated with DNase (Gibco BRL, Gaithersburg, MD, USA) at 37 °C for 30 min, reverse-transcribed using Reverse transcriptase with an oligo-dT primer, and subjected to PCR. The primer for PCR were: canine adiponectin (Forward, 5′-TCCCCAATGTTC-CCATTCGCT-3′; Reverse, 5′-AAGCCCGTAAAGG-TGGAGTCATTGA-3′), canine leptin (Forward, 5′-TTCCTGTGGCTTTGGCCCTAT-3′; Reverse, 5′-GC-CACCACCTCTGTGGAGTA-3′), and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Forward, 5′-ACCACAGTCCATGCCATCAC-3′; Reverse, 5′-TC-CACCACCTGGTTGCTGTA-3′). Nucleotide sequence of canine GAPDH is highly identical to rat GAPDH, and these primers were confirmed to detect canine GAPDH before the study.

2.4. Blood sampling

Twenty-two healthy beagles (castrated 6/spayed 16; 1–3 years old; body weight, 7.5–13.6 kg) were kept in a climate-controlled room, fed on a high-energy diet (p/d dry; Hill's Pet Nutrition Inc., Topeka, KS, USA) at 3500–5300 kJ/day and water *ad libitum* for 14 weeks. Blood was taken after overnight fasting, before and after 14 weeks of treatment, and plasma samples were stored at −80 °C until adiponectin and leptin assays.

Plasma samples were also collected after overnight fasting from 71 dogs visiting four animal hospitals in Japan. The breed, age, sex, body weight, feeding condition, and present illness of individual dogs were confirmed from a record sheet kept by each practice. They were summarized as follows: male/female, 34/37; age, 5 months–16 years old; breed, Beagle, Bichon Frise, Cocker Spaniel, Golden Retriever, Maltese, Miniature Dachshunds, Miniature Schnauzer, Pekingese, Pomeranian, Shetland Sheepdog, Shiba Inu, Shih Tzu, Siberian Husky, Tosa Inu, Yorkshire Terrier, Welsh Corgi, and mixed breeds; most dogs studied were brought to hospitals for health examination, dental treatment, castration and spray. The body condition score (BCS) was assessed by clinical doctors in the hospitals and expressed as a five point scale: 1, lean; 2, underweight; 3, optimal; 4, overweight; 5, obese (Lund et al., 1996).

2.5. Measurement of plasma adiponectin and leptin concentrations

Plasma adiponectin concentrations were measured using a murine/rat adiponectin ELISA Kit (Otsuka,