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Obesity and sex influence insulin resistance and total and multimer adiponectin levels in adult neutered domestic shorthair client-owned cats

C.R. Bjornvad [a,*], J.S. Rand [b], H.Y. Tan [b], K.S. Jensen [a], F.J. Rose [c], P.J. Armstrong [d], J.P. Whitehead [c]

[a] Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, 1870 Frederiksberg, Denmark
[b] Centre for Companion Animal Health and Immunology and Metabolic Medicine, University of Queensland, Brisbane, Queensland, Australia
[c] Diamantina Institute for Cancer, Immunology and Metabolic Medicine, University of Queensland, Brisbane, Queensland, Australia
[d] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

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ABSTRACT

In this study, we estimated insulin sensitivity and determined plasma concentrations of total-, low-molecular-weight (LMW), and high-molecular-weight (HMW) adiponectin and leptin in 72 domestic shorthair, neutered, client-owned cats. Glucose tolerance was assessed with an intravenous glucose tolerance test and body fat percentage (BF%) was measured with dual-energy x-ray absorptiometry. Total adiponectin was measured with 2 different ELISAs. Low-molecular-weight and HMW adiponectin plasma concentrations were determined by Western blot analysis after sucrose-gradient velocity centrifugation, and the adiponectin multimer ratio [S_A = HMW/(HMW + LMW)] was calculated. Differences in glucose tolerance, leptin, total adiponectin, and multimer ratio among lean (BF% <35; n = 26), overweight (35 <BF% <45; n = 28), and obese (BF% >45; n = 18) cats as well as between male (n = 34) and female (n = 38) neutered cats were evaluated by linear regression and 2-way ANOVA. Sex and age were included as covariates for analysis of BF%, whereas BF%, fat mass, and lean body mass were covariates for analysis of sex differences. Increased BF% was negatively correlated with multimer ratio (S_A, $r = -45$; $P < 0.002$), whereas no differences were found in total adiponectin concentrations among BF% groups ($P > 0.01$). Male cats had indices of decreased insulin tolerance and significantly lower total adiponectin concentrations than did female cats (mean ± SEM, 3.7 ± 0.4 vs 5.4 ± 0.5 μg/mL; $P < 0.02$). Altered S_As could contribute to an obesity-associated decreasing glucose tolerance in cats, and low total adiponectin concentrations may relate to increased risk of diabetes mellitus in neutered male cats.

1. Introduction

In cats and humans, obesity and physical inactivity predispose to insulin resistance and type 2 diabetes [1–6]. As in humans, the incidence of both feline obesity and diabetes is increasing worldwide and is becoming a serious problem in veterinary practice [1,3]. Obesity-induced insulin resistance has shown to be partly relayed by adipokines, hormones produced primarily in adipose tissue [7]. Circulating concentrations of the proinflammatory adipokine, leptin, increase with obesity and have been shown to promote insulin resistance in cats, humans, and rodents [8–10]. Adiponectin is the most abundant circulating adipokine, which, in contrast to leptin and all other known adipokines, has anti-inflammatory properties and attenuates insulin resistance [11]. Paradoxically, however, the production of adiponectin

* Corresponding author. Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlaegevej 16, 1870 Frederiksberg C, Denmark. Tel.: +45 40 22 46 82; fax: +45 35 33 29 29.
E-mail address: crb@sund.ku.dk (C.R. Bjornvad).

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seems to decrease with increasing body fat (BF) mass in humans and cats [12–14]. Studies in humans indicate that adiponectin may be independently associated with a reduced risk of type 2 diabetes in apparently healthy persons [15–17] but also that the adiponectin isoform distribution is important for insulin-sensitizing and anti-inflammatory effects [18].

In humans and rodents, adiponectin circulates as trimers, hexamers (both denoted as low-molecular-weight [LMW] multimers) and high-molecular-weight (HMW) multimers that are functionally different [19,20]. High-molecular-weight adiponectin is more closely associated with insulin sensitivity and diabetes risk than is total or LMW adiponectin, and it is selectively decreased in obese humans [20,21]. Few studies have examined the role of total and multimer adiponectin in species other than humans and laboratory animals. In dogs, a species in which obesity-related insulin resistance occurs, but type 2 diabetes does not develop [22], investigators found a higher circulating concentration of both total and HMW adiponectin than with humans, and no correlations were found between adiponectin concentrations and obesity or insulin sensitivity [23]. In cats, obesity-related changes in total adiponectin concentrations have been studied in purpose-bred research cats and in spontaneously obese pet cats [12,14,24,25]. Most studies have shown decreasing total adiponectin with increasing body weight. Whether these decreased adiponectin concentrations in cats reflect decreased HMW, LMW, or both is unknown, and, further, no studies to date have assessed whether adiponectin is associated with insulin sensitivity in cats, as it appears to be in humans and laboratory rodents. Currently, only one previous report examined adiponectin multimers in cats, and that study was performed in lean cats to investigate changes in circulating concentrations associated with dietary change [26].

The purpose of this study was to investigate associations between obesity and sex and plasma concentrations of total, LMW, and HMW adiponectin, adiponectin multimer ratio (S_A), and leptin in naturally lean, overweight, and obese, physically inactive, neutered, client-owned domestic shorthair cats. In addition, this study estimated the correlations of adipokines and multimers with measures of glucose homeostasis and BF.

2. Materials and methods

2.1. Animals, clinical examination, blood sampling, and handling

Client-owned domestic shorthair cats (n = 101) that were neutered before 1 yr of age and confined indoors were recruited through advertisements in a university magazine and a local weekly newspaper. Informed consent was obtained from all owners, and study procedures were approved by the National Committee on Animal Experimentation in Denmark. Food was withheld for 10 to 12 h before admission, when the cats underwent a thorough health examination, including physical examination, measuring body weight and abdominal circumference at the level of L4 (girth), body condition scoring (BCS; 9-point scale [27,28]), and urine and blood analyses. Blood was collected by jugular venipuncture, using minimum stasis and a 21-gauge butterfly needle. Blood samples were collected into one 4-mL serum, two 3-mL citrated, and two 2-mL EDTA plastic tubes (Vacutainer; GreinerBio-One International AG, Frickenhausen, Germany). Serum and EDTA blood samples were immediately kept on ice, centrifuged (4,400 × *g* for 3 min at 4°C) within 1 h and either frozen for later analysis (−80°C) or used for the hematologic (ADVIA 120; Siemens Medical Solutions Diagnostics, Deerfield, IL) and biochemical blood profiles, including thyroxine measurement (ADVIA 1800 and Immulite 2000; Siemens Medical Solutions) and testing for feline immunodeficiency virus (FIV) and feline leukemia virus (Witness FeLV-FIV; Synbiotics Corporation, Lyon, France). Twenty-nine cats were excluded from the study at this stage because of intolerance to handling (n = 12), abnormal urinalysis or biochemistry results (n = 7), audible heart murmur (n = 5), hyperthyroidism (n = 2), blood sampling/analysis problems (n = 2), or a positive FIV test (n = 1). Remaining cats (n = 72; 34 neutered males and 38 neutered females) were each fitted with 2 intravenous catheters, one in the cephalic vein (22 gauge) and one in the medial saphenous vein (20 gauge). To minimize stress-induced hyperglycemia, cats were rested for at least 3 h in a cat-specific cage area before continuation with the intravenous glucose tolerance test (IVGTT).

2.2. Intravenous glucose tolerance test

After a 3-h rest and approximately 16 h of fasting, a glucose bolus (1 g/kg body weight) was injected through the cephalic catheter and 1-mL blood samples were collected in ice-chilled EDTA plastic tubes from the medial saphenous catheter at times 0, 5, 10, 15, 30, 45, 60, 90, and 120 min. To avoid coagulation in the catheter, 0.3 mL of heparinized saline was injected after each sample collection. To avoid sampling of diluted blood, 1 mL of blood was collected in a separate syringe at each blood sampling, and this fraction was re-injected after sampling. Glucose concentrations were measured immediately (Reflotron; Roche, Basel, Switzerland), whereas plasma was collected after centrifugation (4,400 × *g* for 3 min at 4°C) and frozen (−80°C) for later determination of insulin concentrations.

2.3. Dual-energy x-ray absorptiometry

After completion of the IVGTT, cats were hospitalized overnight, offered a meal, and maintained on intravenous fluids (Ringer Acetate; Fresenius Kabi, Uppsala, Sweden) to avoid dehydration. Food was withheld after midnight. The next day, cats were anaesthetized with the following standard protocol: sedation with butorphanol 0.2 mg/kg intramuscularly (Torbugesic; 10 mg/mL, Fort Dodge, Valle de Bianya, Spain) and diazepam 0.2 to 0.3 mg/kg intravenously (Stesolid emulsion, 5 mg/mL; Actavis, Gentofte, Denmark), followed by intravenous injection of propofol (Rapinovet; Shering Plough Animal Health, Ballerup, Denmark) for induction (4–6 mg/kg) and maintenance (10 mg/kg/h). Body composition and BF% were assessed with dual-energy x-ray absorptiometry (DEXA; Small