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Adiponectin mRNA Expression in the Cat (*Felis domesticus*)

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Abstract: Problem statement: Adiponectin is a hormone expressed from adipose tissue in people, rodents and dogs. Adiponectin has anti-inflammatory action with beneficial effects on cardiovascular health and insulin sensitivity. With increasing fat mass, adiponectin concentrations paradoxically decrease. Adiponectin's role in metabolism and diabetes mellitus is of interest in feline medicine because cats are susceptible to developing type II diabetes with weight gain. This study determined relative amounts of adiponectin mRNA expression from various body tissues and organs in domestic cats. **Approach:** Two intact male cats and one intact female cat were evaluated post-mortem. All cats were estimated to be young adults and had lean body conditions. Tissues samples from inguinal subcutaneous adipose, visceral mesenteric adipose, liver, skeletal muscle, cardiac muscle, aorta, stomach fundus, duodenum, pancreas, thyroid gland, adrenal gland (cortex and medulla) and renal cortex were collected and frozen. Following RNA extraction, adiponectin mRNA expression of each tissue was detected using Reverse Transcriptase (RT) real-time (Q) PCR. **Results:** Visceral adipose tissue had the highest level of expression, averaging 12% higher than subcutaneous adipose. All other tissues had negligible levels of expression compared to adipose samples. **Conclusion:** This study provided a valuable step for adiponectin research in cats by determining which tissues express this hormone. Cats differ from human beings by expressing higher levels of adiponectin in visceral compared to subcutaneous fat. The metabolic impact of this expression pattern is not known and provides a basis for future research.

Key words: Adiponectin, obesity, feline, diabetes, adipokine

INTRODUCTION

The metabolic role of adipose tissue is a rapidly expanding area of research. Discovery of cell-signaling proteins secreted by adipocytes, termed adipokines, dispelled the belief that fat is a passive tissue functioning solely for energy storage and insulation. Adiponectin is the most abundantly secreted adipokine with serum concentrations in the $\mu g\ mL^{-1}$ range, which is three orders of magnitude higher than leptin (Whitehead *et al*., 2006). One of the most intriguing aspects of adiponectin is its paradoxical decline with increases in body fat mass. Adiponectin is closely associated with insulin sensitivity and has beneficial cardiovascular effects (Matsuzawa, 2005; Weyer *et al*., 2001; Trujillo and Scherer, 2005; Kern *et al*., 2003; Hara *et al*., 2006; Tarquini *et al*., 2007). Adiponectin's role in metabolism and diabetes mellitus is of interest in feline medicine because cats are susceptible to developing insulin resistance and Type-2 diabetes in response to weight gain (Appleton *et al*., 2001). Only one study has evaluated the mRNA expression of feline adiponectin and it was limited in the scope of tissues examined (Hoenig *et al*., 2007; Zini *et al*., 2009).

Although adiponectin is secreted predominately from adipocytes in the species studied, small amounts have been found in preadipocytes from bone marrow (Yokota *et al*., 2002), cultured cardiac myocytes and portal endothelial cells (Kaser *et al*., 2005). In dogs, adiponectin mRNA expression has only been detected in adipose tissue (Ishioka *et al*., 2006). In a single study of cats, adiponectin expression was examined in adipose tissue and skeletal muscle (Hoenig *et al*., 2007; Zini *et al*., 2009). Visceral adipose appeared to have higher tissue expression of adiponectin than subcutaneous adipose tissue. This is in contrast to studies in human beings where subcutaneous adipose tissue secretes 25-60% more adiponectin than visceral

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adipose (Hernandez-Morante *et al*., 2007; Lihn *et al*., 2004; Fisher *et al*., 2002; 2005). Findings in human beings coincide with research linking visceral adiposity to lower levels of circulating adiponectin and higher risks of cardiovascular disease and T2DM (Ryo *et al*., 2004; Hanley *et al*., 2007).

To fully understand adiponectin's impact on feline metabolism, it is important to determine the tissues from which it is derived. It is not uncommon for hormones and adipokines to have dramatic species differences in their secretion patterns. For example, resistin is a hormone secreted almost exclusively from adipose tissue in rodents, but it is predominately found in bone and lung tissues of human beings (Steppan and Lazar, 2004; Patel *et al*., 203). The goal of this study was to quantify adiponectin's expression in various tissues and determine the primary areas of adiponectin production. By knowing which tissues exert the greatest influence on circulating adiponectin, we may better understand which areas of the body impact insulin sensitivity. As a result, new preventive and therapeutic measures may be implemented to increase circulating adiponectin levels and subsequently improve insulin sensitivity in cats.

MATERIALS AND METHODS

Sample collection: Three feline cadavers were obtained from a local humane society. Cats were euthanized for reasons unrelated to the study and samples were collected immediately after expiration. The cats' physical parameters are described in Table 1. The following tissues were harvested using sterilized instruments treated to remove DNA contamination: (DNA Away, Molecular Bioproducts, San Diego, CA) inguinal subcutaneous adipose, visceral mesenteric adipose, liver from the left lateral lobe, skeletal muscle from the gastrocnemius, left ventricular cardiac muscle, aorta, stomach fundus, duodenum, pancreas, thyroid gland, adrenal gland (cortex and medulla) and renal cortex. Care was taken to remove surrounding fat from tissues and new instruments were used for the collection of each sample. Tissues were immediately placed in a RNA preservative (RNAlater, Qiagen, Valencia, CA) and frozen to -80°C.

Table 1: Description of sample cats

	Sex	Age (years)	Breed	Body weight (kg)	Body condition score
Cat A	Female	<3	Domestic short hair	3.14	4/9
Cat B	Male	1-2	Domestic short hair	4.45	5/9
Cat C	Male	1-2	Domestic short hair	3.96	5/9

Note: Description of cats from which tissue samples were collected

RNA extraction and PCR: Messenger RNA was extracted from adipose tissue using a lipid-specific extraction kit to improve RNA yield (RNeasy lipid tissue mini kit, Qiagen, Valencia, CA). All other tissues were extracted using a standard mRNA extraction kit (RNeasy minikit, Qiagen, Valencia, CA). On-column DNase digestions were performed during the extraction (RNase-free DNase set, Qiagen, Valencia, CA) and samples were stored at -80°C. An additional DNase treatment was performed to remove residual DNA before samples were reverse transcribed (TURBO DNA-free, Ambion, Austin, TX).

Reverse-Transcription (RT) was performed on each sample according to the SuperScript™ II Reverse Transcriptase manufacturer protocol (Superscript II Reverse Transcriptase, Invitrogen, Carlsbad, CA). The reverse priming and quantitative, real-time (Q) PCR were performed with the following primers and FAM-labeled probe designed for this study (Taqman custom gene expression assay, Applied Biosystems, Foster City, CA). They included forward primer (18 µM): 184 -CCGGGTGAAAAGGGTGAG-201, probe (5 µM): 222-AACAAGACCTGGATCTCCT-204 and reverse primer (18 µM): 245-TCACCAGTGTCACCCTTAGGA-225. cDNA sequence locations correspond to GenBank accession number AB115956. FAM-labeled probes are more specific than cyber green markers used previously for feline adiponectin PCR and the specificity of the primers and probe were confirmed through DNA sequencing of the PCR product (Zini *et al*., 2009). For Q-PCR, cDNA (2 µL) was combined with the primer and probe mixture (1 µL), (Taqman custom gene expression assay, Applied Biosystems, Foster City, CA) Taq polymerase (2x concentration, 10 µL), (Taqman Universal PCR master mix, Applied Biosystems, Foster City, CA) and water (7 µL). The total reaction volume was 20 µL. The amplification profile consisted of 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. The ubiquitously expressed housekeeping gene, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) was used as a control to assess the relative quantity of mRNAextracted from different tissues. RT and Q-PCR using the same protocol as above with GAPDH specific primers and probes were performed for each sample. Extracted mRNA from tissue samples that had not undergone an RT reaction was also subjected to Q-PCR using the adiponectin primers to assess potential DNA contamination in the samples.

Statistical methods: To confirm linearity of each Q-PCR assay performed, a standard curve of adiponectin

DNA using ten fold dilutions ranging from 1×10^1 to 1×10^5 was measured. To obtain a consistent number of adiponectin DNA copies, a plasmid with an adiponectin gene insert was constructed (TA cloning kit (pCR 2.1), Invitrogen, Carlsbad, CA). Standard curve equations were developed using SPSS v. 15 (SPSS v. 15, SPSS Inc., Chicago, IL).

RESULTS

The efficiency of RNA tissue extraction using GAPDH showed all tissues had Q-PCR CT values well below comparable adiponectin levels. Mean GAPDH and adiponectin CT values for all tissues were 14.9 (±3.8) and 25.4 (±6.3), respectively. The amount of DNA remaining in tissues was measured using untranscribed mRNA in the Q-PCR reaction. Cats A and C had no detectable DNA contamination. Cat B had small amounts of DNA detected in the pancreas and adrenal gland. Adiponectin levels measured in these two tissues were negligible compared to adipose tissue; therefore, the DNA contamination did not affect overall results. To compare adiponectin expression of various tissues, the number of DNA copies of the adiponectin-inserted plasmid was calculated (9.89×0^{19} mL^{-1}). The standard curves created from the diluted plasmid DNA fit a logarithmic curve ($R^2>0.97$) and the CT values from the various tissues were then fit to the curves. Once the PCR values were standardized to the curve, the relative number of DNA copies of each tissue was compared to visceral adipose tissue (Table 2). Expression of adiponectin was highest in visceral adipose tissue in cats A and C and equal to subcutaneous adipose in cat B. Both types of adipose tissue had high levels of adiponectin expression compared to other tissue types.

Table 2: Relative amounts of adiponectin mRNA expression

Tissue type	Cat A	Cat B	Cat C	Mean
Renal cortex	<0.1	<0.1	<0.1	<0.1
Skeletal muscle	0.3	<0.1	<0.1	0.1
Liver	<0.1	<0.1	<0.1	<0.1
Thyroid gland	<0.1	0.1	1.1	0.4
Aorta	<0.1	0.7	2.4	1.0
Fundus	0.2	<0.1	0.6	0.3
Cardiac muscle	<0.1	<0.1	0.3	0.1
Adrenal gland	<0.1	0.1	6.5	2.2
Duodenum	<0.1	<0.1	0.1	<0.1
Pancreas	<0.1	0.3	0.3	0.2
Subcutaneous adipose	87.0	100.0	75.0	87.3
Visceral adipose	100.0	100.0	100.0	100.0

Note: The expression of adiponectin mRNA relative to visceral adipose. Note the high levels of expression in the aortic and adrenal tissue from Cat C. This is most likely due to contamination with adipocytes given the small amounts detected in the other 2 cats

With the exception of subcutaneous adipose, adrenal tissue and aorta, adiponectin expression was less than 1% of visceral adipose in all samples. Expression of adiponectin in all tissues was less than 3% of adipose tissue expression.

DISCUSSION

Based on a prevalence rate of 0.0124%, there are over one hundred thousand diabetic cats in the United States (Prahl *et al*., 2007). This number is expected to rise as the rate of feline obesity increases. Therefore, researchers and veterinarians need to look for new ways to combat and treat feline obesity and diabetes. Adiponectin is an attractive target for early diabetic screening and new drug therapies and understanding its physiology is critical for therapeutic applications in felids. The twelve types of tissue sampled for this study were chosen either for their involvement in carbohydrate and lipid metabolism or for their known endocrine functions. Although adipose and skeletal muscle had been previously evaluated in the cat, many other tissues hold the potential to secrete adiponectin. As stated previously, small amounts of adiponectin expression have been detected in several human and rodent tissue types and adipokines can have dramatic species differences in their secretion patterns (Yokota *et al*., 2002; Kaser *et al*., 2005; Steppan and Lazar, 2004; Patel *et al*., 2003; Berner *et al*., 2004). Our results show that adiponectin is secreted almost exclusively from adipose tissue in the cat. In comparison to visceral adipose, most other tissues had expression values of less than one percent. The aorta and adrenal gland tissue from cat C had expression levels of 2.4 and 6.5%, respectively. Cats A and B had levels below 1% for these tissues. Despite efforts to minimize contamination of samples by adipose tissue, the adrenal and aorta samples from cat C may have been contaminated by surrounding fat and this would falsely increase adiponectin mRNA levels. Although we cannot rule out the possibility that aortic and adrenal tissues secrete small amounts of adiponectin, the amounts of adiponectin mRNA detected in the non-fat samples are considered clinically and biologically irrelevant and are probably the result of adipocyte contamination within the tissues.

Identifying the tissues that secrete adiponectin in cats is an important first step in characterizing this hormone. This study demonstrates cats are similar to other species in that adiponectin is secreted almost exclusively from adipose tissue. However, cats differ from other species in their relative expression of adiponectin from visceral and subcutaneous adipose. We found visceral adipose samples express

approximately 12% more adiponectin mRNA than subcutaneous samples. The finding that visceral adipose expresses more adiponectin mRNA than subcutaneous adipose is in agreement with another study in cats in which visceral adipose expressed 50% more adiponectin than subcutaneous adipose (Zini *et al*., 2009). This is an intriguing finding because humans secrete 25-60% less adiponectin from visceral fat (Hernandez-Morante *et al*., 2007; Lihn *et al*., 2004; Fisher *et al*., 2002). Since humans secrete more adiponectin from subcutaneous adipose, a person with more visceral fat will have less adiponectin than a person with the same body fat content who has more fat distributed subcutaneously. This is a possible mechanism for the deleterious cardiovascular and metabolic affects associated with visceral adiposity in people (Bergman *et al*., 2007). The opposite pattern may be seen in cats. A cat with larger deposits of visceral fat would be expected to have more circulating adiponectin than a cat of the same body fat mass with more subcutaneous adipose tissue. Perhaps visceral adiposity may not be as detrimental to cats as it is in humans and maybe subcutaneous adipose depots may contribute more to the development of insulin resistance and diabetes in cats.

The results of this study are intriguing and provide the opportunity for many additional projects. It would be interesting to compare adiponectin expression patterns in adipose tissue of cats with different genders, ages, breed and body condition scores. For example, could adiponectin expression hold the key to understanding the increased incidence of diabetes mellitus in male cats (Prahl *et al*., 2007). From the results of this study, it appears adiponectin is an adipokine expressed only from fat tissue in cats and future research efforts can concentrate on how adiposity patterns influence adiponectin and if this relationship impacts insulin sensitivity.

CONCLUSION

The results of this study demonstrate adiponectin is expressed from only adipose tissue in cats. Unlike humans, cats appear to have more mRNA expression from visceral compared to adipose tissue.

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