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Increasing body condition score is positively associated interleukin-6 and monocyte chemoattractant protein-1 in Labrador retrievers

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ABSTRACT

The accumulation of excess body fat is a growing problem in dogs as well as people. Contrary to prior understanding of adipose tissue, fat is now considered to be an active endocrine organ that promotes a chronic low-grade inflammatory state often characterized by an increase in pro-inflammatory cytokines and chemokines. These have been implicated in several obesity-related disorders such as insulin resistance, cardiovascular disease, and neoplasia. The purpose of this study was to characterize fasting plasma cytokine concentrations in ninety-two healthy client-owned Labrador retriever dogs of various ages and body condition scores. The dogs were grouped according to body condition score (BCS) into three categories, lean, overweight and obese. The following cytokines and chemokines were evaluated; tumor necrosis factor-alpha, interleukin-2, interleukin-6, interleukin-8, and monocyte chemotactic protein-1 (TNF-α, IL-2, IL-6, IL-8, MCP-1). Our results indicated that fasting plasma IL-6 and MCP-1 concentrations are associated with increasing BCS. This data suggest that certain markers of inflammation increase with increasing body condition score, and that dogs, similar to humans, may be fostering a chronic inflammatory state due to obesity.

1. Introduction

In the past twenty years there have been escalating rates of obesity in the United States and other industrialized nations, with more than one-third of U.S. adults (34.9%) and approximately 17% (or 12.7 million) of children and adolescents that are obese (Ogden et al., 2014). Mirroring human trends, canine obesity rates are also a growing epidemic with estimates of 25–59% of companion dogs being considered overweight to obese (McGreevy et al., 2005; Lund et al., 2006; Courcier et al., 2010). Further compounding the obesity problem in dogs is the propensity of certain popular breeds to develop obesity, including Labrador retrievers, Beagles, and Boxers (Zoran, 2010). Companion animals such as dogs, live in similar environments as people and as monogastric mammals, broad comparisons are often drawn between the species on the pathogenesis of obesity and its associations with convergent chronic disease.

Associated with either decreased energy expenditure, increased caloric consumption, or both, obesity causes fat cells to become hyperplastic and hypertrophic, which induces an increase in inflammation (Zoran, 2010). Increased adiposity in humans has been associated with numerous pathologies including cardiovascular disease (CVD), hypertension, type 2 diabetes mellitus (T2DM), renal disease, neurologic dysfunction, and various types of neoplasias (Mohamed-Ali et al., 1998; Fortuno et al., 2003; Hotamisligil, 2003; Yudkin, 2003; Ahima, 2005). Similarly, consequences of accumulation of excess body fat in dogs has been linked with a shortened lifespan and numerous disorders, including cardiac, dermatologic, renal, respiratory, and metabolic disorders, as well as exacerbation of orthopedic disease and various types of neoplasias (Kealy et al., 2002; German, 2006; Lund et al., 2006; Adolphe et al., 2014).

Adipocytes are now recognized as an active endocrine tissue, as they produce numerous hormones, cytokines, vasoactive substances and other peptides, collectively called adipokines that communicate with the brain, leukocytes, local cells and peripheral tissues (German et al., 2010; Trayhurn, 2005). For example, adipose tissue is known to synthesize and release adipokines such as leptin,

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adiponectin, and pro-inflammatory factors such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-1, C-reactive protein and monocyte chemoattractant protein-1 (MCP-1) (Das et al., 2003; Khaodhiar et al., 2004; Christiansen et al., 2005). The vast majority of research in the human literature has corroborated this link between increased levels of pro-inflammatory mediators in the obese and an improvement in these parameters with weight loss (Das et al., 2003; Christiansen et al., 2005). In addition to the aforementioned adipokines and inflammatory cytokines, recent research indicates that interleukin-8 (IL-8), a strong neutrophil chemoattractant and activator, is also elevated in obese individuals (Carpagnano et al., 2010). Recent studies have shown that IL-8 has been positively correlated with body mass index (BMI), visceral fat, and found elevated in plasma concentrations in patients with endometrial cancer (Carpagnano et al., 2010; Ciortea et al., 2014).

The progressive accumulation of monocytes and macrophages within the adipose tissue vastly contribute to the levels of pro-inflammatory signals being released in conjunction with the adipocytes. Studies have shown that MCP-1 expression is elevated in the fat of the severely obese, which is paralleled by elevated macrophage infiltration into fat (Harman-Boehm et al., 2007). This stimulates a progressive cycle of recruitment of macrophages, production of inflammatory cytokines, and impairment of proper adipocyte function, thus creating the ideal environment for a chronic inflammation in the obese state (Trayhurn, 2005).

Although well documented in humans, it is not clearly understood if obese and overweight dogs share this chronic inflammatory state, as some of the findings in humans have mirrored what has been observed in obese dogs and others have not. There are currently discrepancies in certain adipokines and acute phase markers where contradicting research suggests that C-reactive protein is sometimes related to obesity status in dogs while others show no association (German et al., 2009; Eirmann et al., 2009; Wakshlag et al., 2011; Tvarijonaviciute et al., 2012b). Further confounding the issue is that many of the studies in obese dogs have shown that the values of common inflammatory biomarkers, such as IL-6, and TNF-α are often below the lower limit of detection (German et al., 2009; Wakshlag et al., 2011; Tvarijonaviciute et al., 2012b; Bastien et al., 2015). This inefficiency in properly detecting some canine cytokines adds to the ambiguity and disparity of results, in large part due to the difficulties in measuring cytokines in plasma or serum.

In the present study we evaluated the relationship between cytokines and obesity by measuring serum concentrations of various cytokines in client-owned Labrador retrievers of lean, overweight, and obese states. The aim of this study was to evaluate several pro-inflammatory markers including obesity-related cytokines IL-6, TNF-α, IL-8 and MCP-1. In addition, we measured IL-2 and IL-10 since these cytokines are related to possible immune status and are less likely to be influenced by obesity status.

2. Material and methods

2.1. Study groups

Each dog in the study was classified according to a body condition score (BCS), utilizing a nine-point scale (Laflamme, 1997). This is the most common and validated method of assessing body condition and subjectively quantifying the body condition in dogs. The dogs were collapsed into categories of the 9 point scale, into lean (4–5), overweight (6–7) and obese (8–9), since there were fewer dogs with the scale of 4 or 9. Age, gender and spay/neuter status of the dog were also recorded.

The criteria for inclusion in the study were Labrador retriever dogs of varying BCS that were deemed healthy after physical examination, complete blood count (CBC), blood chemistries, urinalysis and a full physical by numerous specialty services at the Cornell University Animal Hospital. Labrador retriever dogs, as a sole breed, were selected for this project in an attempt to limit genetic and breed variability in body condition assessment differences across breeds.

Table 1
Age, gender and spay/neuter status of the 92 Labradors enrolled.

Dogs ($n=92$)	Lean ($n=30$)	Overweight ($n=38$)	Obese ($n=24$)
Mean age ± SD	8.8 ± 3.3	7.8 ± 2.8	7.4 + 2.5
Median age (range)	9 (1–15)	8 (2–13)	7 (2–12)
Intact males	12	15	11
Neutered	3	3	0
Intact females	14	18	12
Spayed	2	2	1

Ninety-two client-owned Labrador retrievers were included, with 49 females (5 intact) and 43 males (6 intact). The median age was 8 years (range 2–14). Thirty dogs were in the lean range (BCS of 4 or 5), thirty-eight as overweight (BCS of 6 or 7), and twenty-four obese dogs (BCS of 8 or 9). Table 1 shows the mean and median age, gender and spay/neuter status of all dogs in the study. All procedures were approved by the Cornell University Institutional Care and Use Committee.

2.2. Blood collection and circulating marker quantification

All dogs were fasted overnight and blood was collected from the cephalic vein the following morning between the hours of 8 am and 10 am. 7 cc of whole blood was collected in a plain glass red top vacutainer and allowed to clot for 20–30 min and then centrifuged at 3800 × *g* for 10 min. Serum was then aliquoted into 3 different cryovials and then immediately placed into −80 °C for storage until the assays were performed.

2.3. Sample size calculation

The sample size for this study was based on observations from a previous study on human (Piva et al., 2013) and was based on expected changes in IL-6 between obese and lean people with a similar breakdown of body mass index into lean, overweight and obese participants by differences between obese and lean people being approximately 25 pg/mL, with a standard deviation of 30 pg/mL, a power of 80% and alpha level of 0.05. The resulting sample size was 18 dogs required per group for serum analysis, however populations closer to what was utilized in the study by Piva et al. (2013) were collected for analysis.

2.4. Canine cytokine assays

All kits were used according to the manufacturer's suggestions. The canine MCP-1 ELISA was purchased and used within one month of receiving the kits (Millipore, Temecula, CA). The interassay and intraassay coefficients of variation for the assay are 8.6% and 6.9%, respectively, with a lower limit of detection (LLOD) of 16.0 pg/mL. The canine interleukin-10 ultrasensitive assays were acquired and used within 1 month of purchase (Mesoscale Discovery, Rockville, MD). The IL-10 precision and sensitivity were: interassay CV = 19.1%; intra-assay CV 13.8%; LLOD = 5.9 pg/mL. The canine electrochemiluminescent multiplexed cytokine kit (Proinflammatory Panel 3 (4-Plex)b (Mesoscale Discovery, Rockville, MD) consisted of antibodies against canine TNF-α (interassay CV = 23.5%%; intraassay CV = 9.8%; LLOD = 0.17 pg/mL), IL-2 (inter-assay CV = 12.2%; intra-assay CV = 9.8%; LLOD = 7.6 pg/mL), IL-6 (interassay CV = 10.6%; intra-assay CV = 10.2%; LLOD = 2.4 pg/mL), and IL-8 (interassay