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The effects of weight loss on adipokines and markers of inflammation in dogs

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Abstract

Evidence suggests that adipose tissue-derived adipokines induce mild inflammation and may play a role in insulin resistance associated with diabetes. The present study was designed to examine a series of adipokines and markers of inflammation in dogs before and after a successful weight loss. The study included fasting serum samples from twenty-five dogs before and after a weight-loss programme. Serum C-reactive protein (CRP) and monocyte chemoattractant protein-1 (MCP-1) were measured as indicators of chronic inflammation, while serum adipokines including total adiponectin, high-molecular-weight (HMW) adiponectin, resistin and leptin were also examined. Medians for CRP (before, 10·0 (interquartile range 5·4–15·0) μg/ml; after, 5·6 (interquartile range 3·8–7·0) μg/ml) and MCP-1 (before, 212 (interquartile range 157–288) ng/ml; after, 185 (interquartile range 143–215) ng/ml) decreased significantly after weight loss. Medians for resistin showed a mild, yet significant reduction (before, 67·1 (interquartile range 44·4–88·5) pg/ml; after, 60·5 (interquartile range 32·3–67·1) pg/ml), while leptin showed a dramatic decrease after weight loss (before, 18·9 (interquartile range 10·8–35·4) ng/ml; after, 6·6 (interquartile range 3·9–10·2) ng/ml). Serum total adiponectin and HMW adiponectin were unchanged on all analyses performed. These data suggest that weight loss can decrease chronic inflammation; however, the clinical implications of this decrease are not well elucidated in dogs. Surprisingly, there was no increase in total or HMW serum adiponectin after weight loss, as observed previously in human subjects. The lack of change in total and HMW adiponectin might explain why insulin resistance and type 2 diabetes are less prevalent in obese dogs when compared with humans and cats.

Key words: Adiponectin: Obesity: C-reactive protein: Resistin: Leptin: Monocyte chemoattractant protein-1

Estimates in the US dog population consistently show that approximately 35–40 % of the canine population is overweight to obese, thus making obesity the number one chronic health concern in our canine companions[1,2]. Many proactive measures to induce weight loss can be taken to address this problem through nutrition and/or pharmacological intervention. Although the health implications for dogs may not be as well established as in human medicine, the limited evidence suggests that conditions such as osteoarthritis and other orthopaedic problems, renal disease and cancer may be more prevalent in overweight dogs[3–5].

Advances in obesity research suggest that adipose is not an inert tissue, but rather it releases a variety of adipokines that drive the chronic inflammatory response in peripheral tissues, thereby exacerbating many disease processes[6,7]. Recent investigations have shown that obese pet dogs show mild insulin resistance and have increased serum haptoglobin concentrations, an acute-phase protein. Additionally, obese dogs display higher serum leptin, as well as increased serum TNF-α; all of which indicate mild chronic inflammation due to obesity[8]. However, other markers of chronic inflammation including C-reactive protein (CRP), resistin and adiponectin have exhibited mixed results, which may be due to small sample sizes or variable populations being studied[8–12]. For example, adiponectin data are conflicting, with two studies suggesting a rise in adiponectin in lean dogs[11,12], while another study has reported no change in adiponectin after weight loss in dogs[8]. The differences observed may be due to populations, experimental conditions, study design and/or breeds represented. The objective of the present study was to quantify increases or decreases in adipokines and markers

Abbreviations: BCS, body condition score; CRP, C-reactive protein; HMW, high molecular weight; MCP-1, monocyte chemoattractant protein-1; TBST, Tris-buffered saline + 0·1 % Tween 20.

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of chronic inflammation before and after a successful weight-loss programme in a group of dogs.

Materials and methods

All animals used for the present study were client-owned, and clients signed a consent form before initiation of the study protocol. The protocol was approved by the Cornell University Institutional Animal Care and Use Committee, Ithaca, NY, USA. The protocol included the acquisition of whole blood before and after weight loss in twenty-five dogs. A previously defined weight-loss programme was used[13], in which, at the end of a 2-week weight maintenance period, the relative daily maintenance energy requirement was calculated based on any weight gain or weight loss on a therapeutic weight-loss diet (Purina OM Veterinary Therapeutic Diet, Nestle Purina Pet Care, St Louis, MO, USA). This initial maintenance energy requirement was entered into a clinically proven weight management system (Veterinary Weight Management Program 2.1.0, Nestle Purina, St Louis, MO, USA), which provides a predicted number of energy allowed each day to achieve 2 % weight loss weekly[13].

Fasting blood drawn via the cephalic vein was performed at each initial visit of dogs and, again, at completion of the weight-loss programme. Blood (5 ml) was collected in a coagulation tube, and all samples were allowed to clot, and were refrigerated for a minimum of 30 min and a maximum of 2 h before centrifugation. The samples were centrifuged at 3800 ***g*** at room temperature for 10 min, and serum was equally divided into aliquots into two different cryovials and immediately frozen at −80°C.

Serum assays

On the same day, four colorimetric assays and two fluorescence-based ELISA were performed. Serum samples were taken from storage and thawed on ice. Serum adiponectin and resistin were performed together using the custom-assembled luminex-based total adipokine multiplex kit (Milliplex Canine Adiponectin Kit; Millipore, Concord, MA, USA), which was performed according to the manufacturer's instructions. If values were below the lower limit of detection, we used approximately five times the serum required in the initial dilution step to get numbers within the detectable limits. Total adiponectin and resistin assays were performed according to the manufacturer's instructions with an intra-assay CV of 7·9 and 9·2 % and inter-assay CV of 9·3 and 16·6 %, with lower limits of detection at 22·1 and 1·5 pg/ml, respectively. The canine leptin ELISA (Canine Leptin ELISA Kit; Millipore, Concord, MA, USA) was performed according to the manufacturer's instructions with an intra-assay CV of 6·7 % and an inter-assay CV of 5·9 % and a lower limit of detection at 0·78 ng/ml. The monocyte chemoattractant protein-1 (MCP-1) assay (Canine MCP-1 ELISA Kit; R&D Systems, Minneapolis, MN, USA) was a canine-specific assay, which was performed according to the manufacturer's instruction with an intra-assay CV of 4·5 % and an inter-assay CV of 6·6 % and a lower limit of detection at 1·2 pg/ml. In addition to these canine-specific assays, a human high-molecular-weight (HMW) assay (HMW adiponectin ELISA Kit; Millipore, Concord, MA, USA) was used to assess canine HMW complexes. Although not validated in canine serum, the assay-generated values within the standard curve were thus reported.

Non-denaturing PAGE and immunoblotting

Serum from thawed samples was mixed in a one-to-one mixture with 2 × non-denaturing, non-reducing loading buffer (Bio-Rad, Hercules, CA, USA). The samples were then mixed thoroughly, and 2 μl of each sample were loaded onto a 4–20 % Tris-glycine polyacrylamide gel (Invitrogen, Inc., Carlsbad, CA, USA). The gel was resolved, transferred onto a polyvinylidene difluoride membrane and blocked with 10 % non-fat dried milk in Tris-buffered saline + 0·1 % Tween 20 (TBST) for 1 h at room temperature on a platform rocker. The membrane was then incubated in a 1:1000 dilution of a primary rabbit polyclonal anti-rat adiponectin antibody (Biovisions, Mountain View, CA, USA) overnight on a platform rocker at 4°C. Membranes were washed three times with TBST and then incubated at room temperature for 1 h using a 1:5000 dilution of a anti-rabbit horseradish peroxidase-labelled secondary antibody (Cell Signalling, Danvers, MA, USA). Blots were again washed three times for 10 min each with TBST and treated with Western lighting reagent (Millipore,

Table 1. Body weight, body condition score (BCS), serum adipokines and markers of inflammation for all twenty-five dogs before and after weight loss

(Medians, ranges and 25th and 75th interquartile ranges)

	Before			After			
	Median	Range	Interquartile range	Median	Range	Interquartile range	*P*
Body weight (kg)	43·1	14·9–61	36·5–48·5	33·8	10·5–44	28·8–37·8	<0·001
BCS	8	7–9	7·5–9	5	5–6	5–5·5	<0·001
Leptin (ng/ml)	18·9	3·8–48	10·8–35·4	6·6	10·8–16·8	3·9–10·2	<0·001
Adiponectin (pg/ml)	38·1	4·0–170·1	23·5–58·8	29·5	10·6–323·2	21·3–58·2	1·0
Resistin (pg/ml)	67·1	7·4–144	44·4–88·5	60·5	7·1–90·0	32·2–67·1	0·003
HMW adiponectin (ng/ml)	52·0	11·3–133	35·3–77·2	54·3	10·4–142·6	37·6–72·7	0·86
MCP-1 (ng/ml)	202	84–448	157–288	170	78–632	143–215	0·038
CRP (μg/ml)	10	1·9–34·1	5·4–15·0	5·6	1·7–45·2	3·8–7·0	0·005

HMW, high molecular weight; MCP-1, monocyte chemoattractant protein-1; CRP, C-reactive protein.

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Inc., Concord, MA, USA) and visualised using the Biospectrum 400 (UVP Systems, Upland, CA, USA). Densitometry was performed comparing the adiponectin pixel values after weight loss with the values before weight loss for percentage change.

Statistical analysis

All physical examination parameters including body weight and body condition score (BCS) were statistically evaluated using paired Wilcoxon's rank-sum test due to the abnormal distribution of data. Nearly all ELISA values and pixel densitometric values of before and after weight loss show abnormal distribution, therefore non-parametric paired Wilcoxon's rank-sum tests were performed for all assays with a P value set at 0·05.

Results

Dogs showed a significant decrease in BCS and weight, achieving a 23% weight loss over an average of 26 weeks (range 12–40 weeks) time span (Table 1). Of the twenty-five dogs, nine were spayed females, one was an intact female and fifteen were neutered males. Breeds of dogs represented were eight Labrador Retrievers, eight mixed breed dogs, three Beagles, three Bernese Mountain dogs, two Golden Retrievers and one Cocker Spaniel. Serum analysis for MCP-1 (P=0·038) and CRP (P=0·005) as markers of inflammation showed a decrease in both of these parameters with weight loss (Table 1). Adipokines that decreased with weight loss were resistin (P=0·003) and leptin (P<0·001) as expected, while total serum adiponectin (P=1·0) showed no increase. Further examination of HMW adiponectin (P=0·86) showed no differences before and after weight loss. Additionally, Western blot analyses and densitometry revealed no significant change in serum concentration of all (60, 90 and 150 kDa) molecular weight forms of adiponectin examined (Fig. 1).

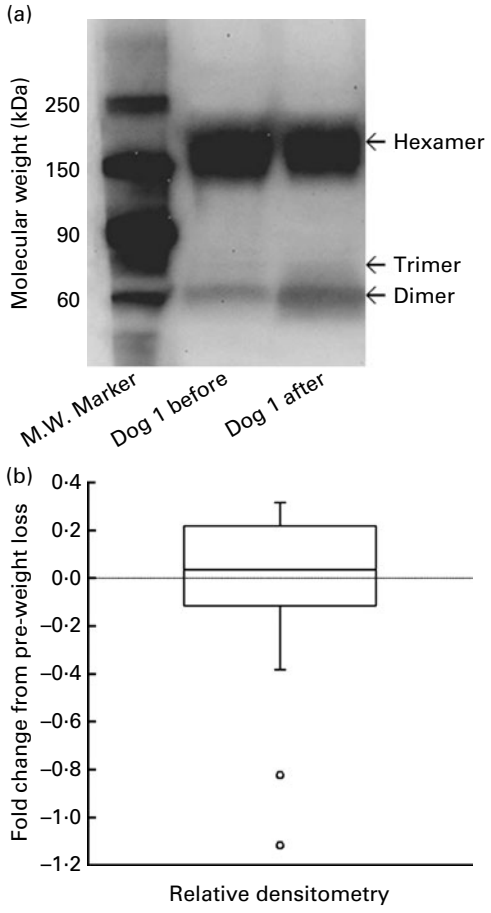

Fig. 1. (a) Western blot depicting various isoforms of adiponectin including dimers, trimers and abundant hexamers typically observed in non-denaturing, non-reducing PAGE before and after weight loss. (b) Graph depicts relative densitometric changes after weight loss compared with densitometric results before weight loss. Before weight loss value was set at 0, with after weight-loss change reflected as fold increase or decrease; no significant difference was observed.

Discussion

Markers of chronic inflammation and adipokines have been assessed in human obesity and weight loss, with evidence showing a decrease in pro-inflammatory adipokines, such as leptin and resistin, and an increase in the insulin-sensitising hormone adiponectin, particularly the HMW form of adiponectin. These adipokine changes associated with weight loss correlated with the health benefits that included a decreased risk in CVD and improved insulin sensitivity[6–8]. Although CVD is rare in dogs, a previous report has shown that weight loss improved insulin sensitivity[8]. However, that study showed no changes in total adiponectin after weight loss, while other studies have suggested that lean dogs have increased serum total adiponectin when compared with obese dogs[11,12]. Additionally, German *et al.*[8] showed decreases in the serum acute-phase proteins haptoglobin and CRP, as well as trends in TNF-α and leptin reduction in serum after weight loss. Other studies examining markers of chronic inflammation have been less convincing. CRP, for example, has been shown to be higher in lean dogs in one study, while another study in laboratory dogs has suggested no change in serum CRP after acute weight gain[9,10]. Yet, a recent field study has shown decreases in serum CRP in separate populations of obese and lean dogs[11].

These discrepancies led us to evaluate similar markers of inflammation and adipokines in a group of dogs undergoing a weight-loss protocol designed to achieve 2% weight loss per week, which culminated in patients decreasing their BCS from 8 to 5 and losing about 23% of their body weight. There were very few surprises when evaluating the serum parameters of inflammation as serum CRP and MCP-1 decreased, while adipokines including resistin and leptin decreased as well. Leptin has been well studied, often showing a strong correlation between BCS, which our data support[14–16]. Furthermore, the leptin concentrations that we observed were lower than those observed in other studies examining dogs with a BCS of 5–6[15,16]. This lower-than-expected serum leptin concentration may have been due to our population having their last blood draw at the end of their weight-reduction protocol while still being energy restricted.

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Assessment of adiponectin was more perplexing. Adiponectin is an enigmatic serum adipokine, which has relatively different bioactivity depending on its multimerisation. Typically, monomers and trimers have little biological activity, and higher-molecular-weight multimers of hexameric form (low molecular weight) and larger multimers are biologically active enhancing insulin sensitivity[17], and the HMW form in human subjects has been shown to increase during weight loss[18]. Our total canine adiponectin assay showed no significant difference before and after weight loss. Therefore, we chose to use a human ELISA kit to assess HMW adiponectin. This kit provided quantities in the nanogram range which was expected; however, the HMW adiponectin ELISA showed no significant differences in serum concentrations before and after weight loss.

A previous study examining canine adiponectin has shown that a HMW isoform does exist; however, this isomer cannot be detected using non-denaturing, non-reducing gel electrophoresis techniques[17]. The lack of detectable species above 150 kDa makes the study of HMW isoforms difficult in dogs using electrophoresis. The use of non-denaturing, non-reducing conditions and adequate resolution of adiponectin dimers, trimers and hexamers allows for electrophoresis and immunoblotting to resolve multiple species of adiponectin by densitometry for total adiponectin evaluation[17]. The present densitometric results showed no appreciable difference, suggesting the possibility that total adiponectin does not decrease with adiposity and that basal concentrations of total adiponectin are higher in dogs than in other species. Higher basal concentrations of total adiponectin and its hexameric form might enhance insulin sensitivity during obesity.

In summary, chronic inflammation associated with obesity exists in dogs, and it is evident that weight loss decreases this inflammation as observed by decreases in CRP and MCP-1 after weight loss. The serum adipokines resistin and leptin as adipocyte-released hormones also decrease with weight loss, suggesting less inflammation in fat and a pronounced decrease in hypothalamic signalling by leptin that probably results in increased hunger in dogs undergoing weight loss. The typical increase in serum adiponectin that is observed after weight loss in human subjects was not observed in dogs, and dogs may be different by having relatively constant serum adiponectin concentrations. The clinical ramifications of this decrease in inflammatory mediators and changes in serum adipokines have yet to be elucidated, but future investigation into insulin resistance focusing on adiponectin in dogs may provide a better understanding of adiponectin and its role in type 2 diabetes in companion animals.

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