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Adiponectin: Why Dogs Are Different

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Abstract

The role of adiponectin in obesity-induced insulin sensitivity was examined in dogs. Leptin was strongly associated with changes in both adiposity and homeostasis model assessment (HOMA) insulin sensitivity, but adiponectin was not associated with either in 104 client-owned dogs. The proportion of adiponectin present as high molecular weight (HMW) multimers (S_A) was significantly higher in dogs than in humans, and there was no significant difference between male and female dogs, unlike in humans. S_A was not decreased in client-owned obese dogs compared with matched lean dogs and was not associated with MINMOD insulin sensitivity. We conclude that the role of adiponectin in obesity-induced changes in insulin sensitivity differs substantially between dogs and humans.

Glossary of Abbreviations
GLP-1: Glucagon-Like Peptide-1
HMW: High Molecular Weight
HOMA: Homeostasis Model Assessment
LMW: Low Molecular Weight

Background

Obesity is a risk factor for the development of some types of diabetes mellitus in humans, including type 2 diabetes. Dogs, like humans and domestic cats, develop obesity-induced insulin resistance. However, unlike humans and cats, dogs maintain fasting and first-phase insulin secretion and do not appear to progress from obesity-induced insulin resistance to type 2 diabetes mellitus. This implies that there is either a mechanism that protects dogs from type 2 diabetes or dogs lack a pathophysiological process crucial to the development of type 2 diabetes.

The elucidation of the pathophysiology of type 2 diabetes mellitus, an obesity-associated disease, has progressed rapidly since the discovery of hormones secreted by adipocytes (adipokines). Uniquely among adipokines, adiponectin concentrations are decreased with increasing body fat mass. Since its discovery over a decade ago, adiponectin has been found to be important in the pathophysiology of obesity-induced insulin resistance and a predictor of the development of type 2 diabetes in humans and laboratory rodents.

Low adiponectin concentrations in humans are associated with reduced insulin sensitivity measured by fasting methods such as HOMA,[1] by dynamic methods such as frequently sampled glucose tolerance tests[2] and hyperinsulinaemic glucose clamps,[3] and with higher glucose concentrations after a meal[4] or oral glucose tolerance test.[3] Low adiponectin concentrations predict progression from insulin resistance to type 2 diabetes.[5] Adiponectin circulates as low molecular weight (LMW) trimers and hexamers and HMW multimers. HMW adiponectin is more closely associated with insulin sensitivity and diabetes risk than total or LMW adiponectin and is selectively decreased in obesity.[6] Canine adiponectin has approximately 90% sequence homology with human adiponectin but lacks the TTT motif that is associated with posttranslational sialylation in humans (Figure 1). The sialylated TTT region is associated with adiponectin half-life in humans and rodents.[7]

Beta-cell failure is the *sine qua non* of diabetes. As long as beta cells compensate adequately, insulin resistance does not progress to diabetes. Studies with adiponectin

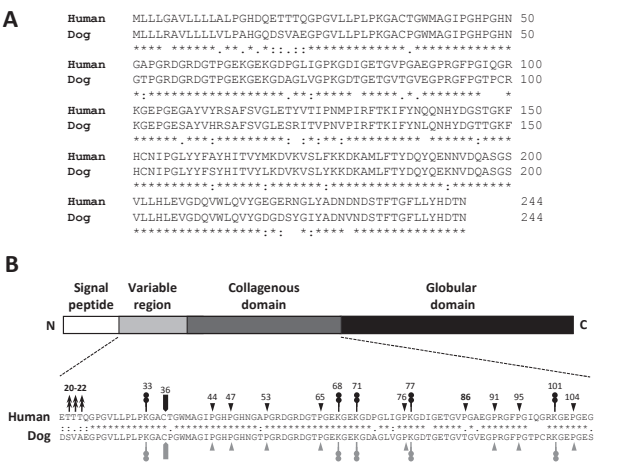

Figure 1. Sequence alignment of human and dog adiponectin. (a) Clustal W alignment of human and dog adiponectin showing identical (*), highly conserved (:) and conserved (.) amino acids. (b) Schematic showing structural organization of adiponectin and experimentally determined (black) and inferred (gray) post-translational modifications of human and dog adiponectin.

and other adipokines have altered the understanding of the process of beta-cell failure as well as compensation. Although beta cells secrete insulin in response to increases in glucose concentrations, glucose-stimulated insulin secretion is altered by many other hormones and nutrients. Adipokines, including adiponectin and leptin, alter beta-cell function and may be involved in mediating or permitting compensatory hyperinsulinaemia.

Dogs develop obesity-related insulin resistance but have never been shown to develop type 2 diabetes mellitus. We hypothesized that differences in the physiology of adipokines might help explain species differences in susceptibility to type 2 diabetes.

Investigation of the Physiology of Adipokines in Dogs

Fasting measures of insulin sensitivity and beta-cell function, recently validated for use in dogs,[8] were applied in the first study reported using path analysis in small animal medicine or veterinary endocrinology. Further investigation of the role of adiponectin in obesity in dogs was then facilitated by adaptation of the established method of sucrose gradient followed by SDS-PAGE and Western blotting for use in dogs, which allowed separate measurement of LMW trimers and hexamers and HMW multimers. Total adiponectin concentration was measured using a mouse/rat adiponectin ELISA validated for use in dogs,[9] with additional validation performed to confirm the results.

Approaches and Results

Three studies were performed that examined aspects of the physiology of adipokines in client-owned dogs. Study 1 was a cross-sectional study examining the relationships of three hormones with obesity and obesity-associated changes in insulin secretion and beta-cell function in 104 lean, overweight and obese dogs.[10] Body condition score was assessed by one veterinarian, and a 24-hour fasting blood sample was collected for measurement of serum concentrations of glucose, insulin, leptin, total adiponectin, and glucagon-like peptide-1 (GLP-1). Fasting insulin sensitivity and beta-cell function were assessed using HOMA, validated for use in dogs.[8] Path analysis was used to test possible causal interrelationships. A null path model with all biologically plausible relationships was constructed (Figure 2), and multivariable linear regression was used to support or refute the existence of the postulated relationships. A final path model was constructed to include all confirmed relationships (Figure 3). This study found that leptin was strongly associated with obesity-induced changes in insulin sensitivity, but there was no association between obesity and adiponectin nor between adiponectin and either insulin sensitivity or beta-cell function (Figure 3).

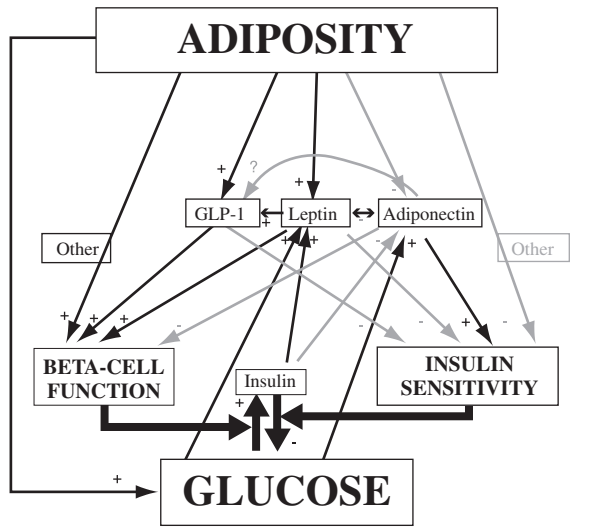

Figure 2. The null path model — the causal relationships considered plausible prior to data analyses. Black arrows denote relationships that were hypothesized *a priori* to be positive associations. Gray arrows denote relationships that were expected to be negative associations. Relationships shown in bold black were not considered in the path analyses because plasma glucose concentrations were used to calculate beta-cell function and insulin sensitivity and because these relationships are already well-documented. (From Verkest et al.[10])

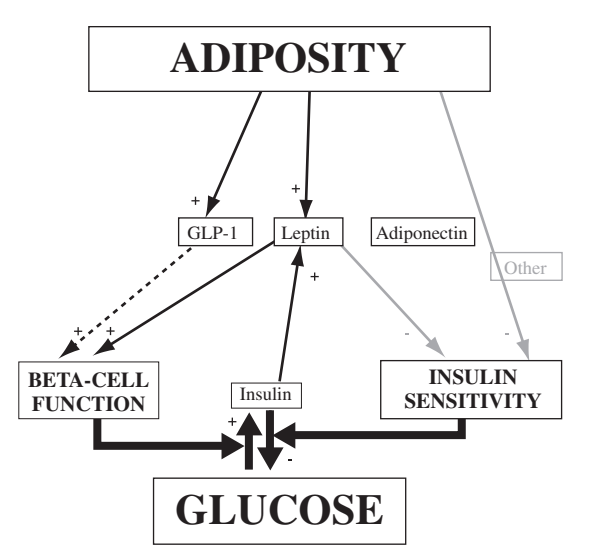

Figure 3. The final path model showing the relationships that were statistically significant after regression analysis. The final path model when no direct causal relationships between leptin and adiponectin concentrations were assumed *a priori* in the null path model. (Adapted from Verkest et al.[10])

Study 2 was a cohort study of total and HMW adiponectin in six lean and six naturally occurring obese dogs.[11] Total and HMW adiponectin and S_A, the ratio of HMW to total adiponectin, were measured in fasting blood samples, and insulin sensitivity was measured using

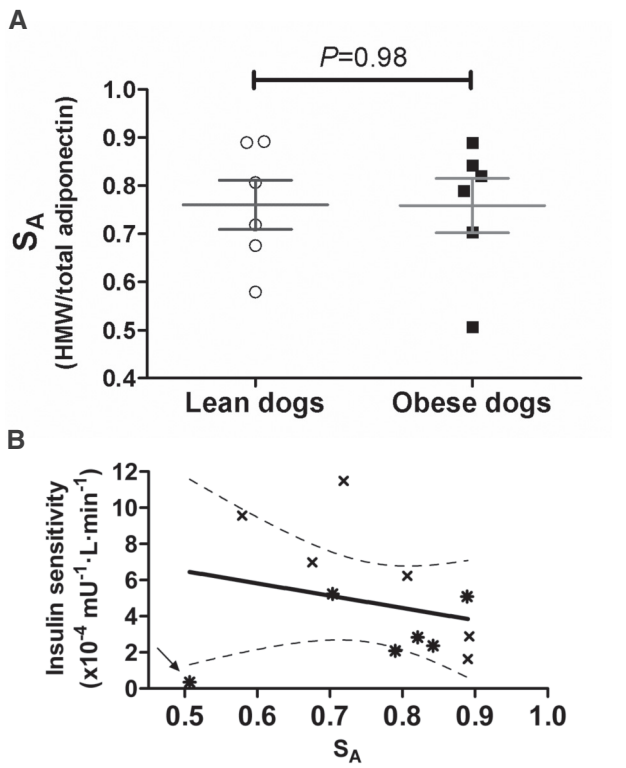

Figure 4. Comparison of adiponectin profiles in lean and obese dogs. (a) Graph showing S_A in age- and gender-matched lean and obese dogs (n=6 in each group). Lines show mean ± SEM. (b) There was no significant association in dogs between insulin sensitivity and S_A (regression coefficient = -6.5 (95% CI -22 to 9.1), P=0.37) (lean dogs shown as crosses, obese dogs as stars). When one outlying obese dog (arrow) was excluded, there was a significant negative association between S_A and insulin sensitivity; the association in humans and rodents is positive. Regression line (solid) with 95% confidence intervals (dashed) are shown. (From Verkest et al.[14])

MINMOD analysis of frequently sampled intravenous glucose tolerance tests. No differences were found in S_A between lean and obese dogs (Figure 4a), no significant decrease in total adiponectin concentration was associated with obesity, and no association between either total adiponectin concentration, HMW adiponectin or S_A, and insulin sensitivity. Thus, the biologically active form of adiponectin, the HMW multimers, is not selectively decreased in severely obese dogs and is not associated with insulin sensitivity (Figure 4b).

Study 3 was also a cohort study of HMW adiponectin in 10 sexually intact dogs, 10 neutered dogs and 10 non-diabetic humans. All dogs and humans were lean, young adults. Since differences in assay binding might cause spurious differences in total adiponectin, S_A was compared between species as well as total adiponectin. Dogs were found to have higher proportions of adiponectin circulating as HMW adiponectin than humans (Figure 5). Although dog adiponectin was expected to have lower affinity for the assay antibody, which was developed for a different species, and hence lower adiponectin concentrations using ELISA, dogs, in fact, had three to four times higher total and HMW adiponectin concentrations than humans. Adiponectin also was not decreased in intact male dogs compared with neutered or female dogs, suggesting that adiponectin is not suppressed by testosterone in dogs as in humans and laboratory rodents.

Discussion

These studies established that neither total nor HMW adiponectin is decreased in overweight or obese dogs and that total and HMW adiponectin are not associated with fasting or MINMOD-derived insulin sensitivity in dogs. This supports two recent studies that showed no effect of weight loss on total adiponectin concentrations in naturally occurring obese dogs.[12,13] It appears that adiponectin does not have a strong role in the pathophysiology of obesity-associated insulin resistance in dogs. Leptin may be involved in the development of obesity-associated fasting insulin resistance and/or compensatory beta-cell function, but this remains to be confirmed. The function of adiponectin in dogs remains to be found. In humans and laboratory rodents, adiponectin has some endocrine functions, but it has poor affinity for its receptor and circulates in quantities that are vastly larger than those of any other hormone. This suggests that adiponectin has non-endocrine functions and has acquired endocrine functions that are peripheral to its primary role in some species.

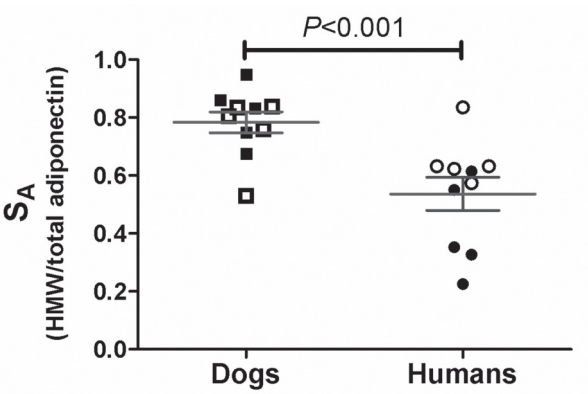

Figure 5. Comparison of adiponectin profiles in dogs and humans and the association between S_A and insulin sensitivity in dogs. (a) The ratio of HMW to total adiponectin concentrations (S_A) in dogs was significantly higher than in humans (males shown as filled symbols, females as open symbols). Lines show mean ± SEM. (From Verkest et al.[14])

Conclusion

There are important differences between dogs and species that develop type 2 diabetes. Adiponectin and HMW adiponectin are not decreased in obese dogs, insulin-resistant dogs or intact male dogs. These observations may explain why insulin-resistant obese dogs maintain adequate fasting and first-phase insulin secretion and do not progress to develop beta-cell failure and type 2 diabetes mellitus.

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