Analysis of Adiopokine mRNAs in Canine Cranial Cruciate Ligament and Their Associations with Cartilage Matrix Markers

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Introduction: Cranial cruciate ligament rupture (CCLR) is one of the most important causes of hindlimb lameness in dogs and morbidity in comparative species such as man. CCLR can lead to articular cartilage damage, meniscal tears, and the development of osteoarthritis (OA). Obesity is characterised by an expansion of white adipose tissue mass that can lead to adverse health effects including joint disease and OA. Adipocytes synthesise and release an array of chemical mediators name adipokines, including acute phase proteins, chemokines, and cytokines; these can result in inflammation and increased breakdown of body tissues. However, the precise mechanism underlying the association between obesity and CCLR in dogs has not yet been clarified. The purpose of this study was to assess gene expression for key adipokines, inflammatory mediators, and cartilage degradation markers in the stifle joint tissues from dogs with CCLR with different body condition scores, and to determine any relationship with disease.

Methods: 25 ruptured cranial cruciate ligaments (CCLs) and 13 torn medial meniscal samples were collected from dogs referred for surgical treatment of unilateral CCLR. Gene expression for key adipokines (adiponectin, leptin, visfatin), inflammatory cytokines (MCP-1, TNF-α, IL-6) and cartilage degradation biomarkers (AGC-1, COL-1, MMP-13) in CCL and meniscus samples were determined by quantitative reverse transcription polymerase chain reaction. Data were normalized to the control gene β-actin, and the fold change in gene expression level was calculated using deltaC_T (C_T Target - C_T Reference). The relationship of gene expression with clinical measures including age, weight, body condition score and severity of lameness was determined. Software R version 3.0.3 was used to determine correlation between gene expression of each cytokines and clinical parameters, significance level was set at P < 0.05.

Results: The most highly expressed adipokines in ligament were MCP-1 and visfatin. We found significant correlations with adipokines and cartilage degradation biomarkers (adiponectin vs AGC-1, r² = 0.90 and P = 0.03; leptin vs COL-1, r² = 0.62 and P = 0.004; and visfatin vs MMP-13, r² = 0.34 and P = 0.02). Moreover, positive correlations of lameness score and AGC-1 expression (r² = 0.23 and P = 0.02), bodyweight and TNF-α (r² = 0.07 and P = 0.03), and between age and MMP-13 (r² = 0.14 and P = 0.04) were also identified (Figure 1). No statistically significant correlation between body condition score and expression of the genes examined was found in this study.
**Discussion:** Our data suggest an association between gene expression for key adipokines and markers of cartilage degradation. The presence of cartilage like tissue in CCLs and whether it represents a pathological or adaptive state is still not clearly defined. Our data indicates that canine ligament contains cells that can express adipokines and that a relationship exists between this expression and the expression of cartilage markers. Moreover, the results suggest there is a relationship linking both cartilage markers and cytokines with clinical measures. For instance, TNF-α may be associated with canine obesity and possibly involved in CCLR. A larger scale study is now underway to take this work forward.

**Significance:** Elucidating a mechanical, systemic and biochemical link between canine obesity and dogs with naturally occurring CCLR will have huge significance in their pre- and post-operative management in terms of weight control and nutrition. This will lead to the development of prophylactic management regimes to prevent the onset of naturally occurring canine CCLR and novel therapeutic intervention.