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Myocardial leptin transcription in feline hypertrophic cardiomyopathy

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ABSTRACT

Leptin is an adipokine, which is in humans with cardiac disease suspected to be involved in myocardial remodeling and thrombus formation. In cats, however, it is not known whether leptin plays a role in cardiac disease, i.e. hypertrophic cardiomyopathy (HCM) and the presence of an atrial thrombus (AT). The objective of the study was therefore to establish whether leptin is transcribed in the feline myocardium and to compare myocardial leptin mRNA concentrations in cats with HCM with and without AT, and in cats without cardiac diseases. Myocardial samples from 15 cats with HCM (five of these with AT), and 12 cats without cardiac diseases were investigated for leptin mRNA expression using quantitative reverse transcriptase PCR, and the transcription levels were correlated with those obtained for a range of cytokines and remodeling parameters.
Leptin mRNA expression was detected in the myocardium in all heart regions, with generally higher concentrations in the atria than in the ventricles. Cats with HCM exhibited higher atria and ventricular leptin transcription than cats without cardiac diseases, but reduced ventricular transcription levels in the presence of AT. A positive correlation between leptin, cytokine and remodeling marker transcription levels was observed.
The present study shows that leptin is constitutively transcribed in the feline myocardium. The observed increase in leptin mRNA concentrations in the myocardium from cats with HCM and the reduction when an AT is present suggests varying gene activation in different stages of the disease and a potential involvement of leptin in the feline cardiac remodeling process.

1. Introduction

Leptin is a mainly adipocyte-derived adipokine and one of the most important and widely studied factors in the control of energy balance. In humans, elevated circulating levels are observed with obesity and in cardiac disease, independent of body weight, and an association with low-grade inflammation and a pro-thrombotic state has been reported (Feijoo-Bandin et al., 2015; Ghantous et al., 2015; Schafer and Konstantinides, 2011). Leptin has been shown to promote platelet adhesion, activation and aggregation (Elbatarny and Maurice, 2005; Schafer and Konstantinides, 2011). Similarly, obese cats exhibit increased circulating leptin concentrations and an association with a increased clotting tendency was suspected (Appleton et al., 2000; Bjornvad et al., 2014; Bjornvad et al., 2012). In the heart of humans, mice and rats, cardiomyocytes, pericardial fat and vascular smooth muscle cells have been shown to produce leptin, and its receptor is abundantly expressed on cardiomyocytes (Feijoo-Bandin et al., 2015; Ghantous et al., 2015). Leptin expression by cardiomyocytes is increased by endothelin-1 and angiotensin II, which are both elevated in cardiac disease. This would suggest a potential paracrine or autocrine role of leptin in the regulation of cardiac function as well as its contribution to pathological processes in the myocardium (Rajapurohitam et al., 2006). Leptin has been shown to stimulate several factors involved in extracellular matrix deposition in vitro, including metalloproteinases and collagen profiles, and may therefore contribute to myocardial remodeling and heart failure (Ghantous et al., 2015). The recently identified presence of a leptin receptor on the mitochondria of cardiomyocytes further suggests that intracellularly synthesized leptin could directly modulate mitochondrial function (Martinez-Abundis et al., 2015). However, whether leptin has damaging or protective effects on the heart is so far not known (Feijoo-Bandin et al., 2015). Some authors report anti-apoptotic and anti-fibrotic and therefore potentially cardioprotective effects of leptin (Feijoo-Bandin et al., 2015; Ghantous et al., 2015). For women, a U-shaped association between serum leptin concentrations and cardiovascular and all-cause mortality has been reported, with increased risk

Abbreviations: AT, atrial thrombus; HCM, hypertrophic cardiomyopathy; IL, interleukin; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of matrix metalloproteinase; TGF, transforming growth factor; TNF, tumor necrosis factor.

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of death at both low and high circulating leptin levels (Mishra et al., 2015).

Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in cats (Payne et al., 2015b), and an association with an increased body condition score has been reported (Payne et al., 2015b). The disease is characterized by idiopathic left ventricular hypertrophy accompanied by myocardial fibrosis, cardiomyocyte disorientation and intramural coronary arteriosclerosis (Biasato et al., 2015; Khor et al., 2015). Cardiac thrombus formation and subsequent thromboembolic events are known complications, and are associated with reduced survival (Payne et al., 2015a). Only little is known about potential factors involved in feline cardiac remodeling and thrombus formation. Whether leptin is produced by the feline myocardium and if it might vary in cats with HCM and atrial thrombus (AT) is not known. The objective of the study was therefore to investigate whether leptin is expressed in the feline myocardium and whether its expression is altered in cats with HCM and AT in comparison to cats without cardiac diseases. We hypothesized that HCM and AT is associated with increased myocardial leptin transcription.

2. Material and methods

Fifteen cats with HCM and 12 cats without cardiac diseases were included in the study. These cats were patients that had been presented at the Universities of Helsinki and Bristol. HCM was clinically diagnosed by echocardiography, the diagnostic criterion for HCM was an idiopathic diffuse or segmental left ventricular wall thickness of ≥6 mm measured at end-diastole (Fox et al., 1995). Cats without cardiac diseases did not exhibit clinical evidence of cardiac disease, and echocardiography was not routinely carried out. The cats had been euthanized upon owner's request due to poor prognosis, impaired quality of life or financial constraints. Informed consent was obtained from owners prior to inclusion into the study and cats were assigned arbitrary numbers as identifiers. Institutional ethical approval was obtained from the University of Bristol. From each cat, the signalment including breed, sex, and age was recorded. The weight was available for 24 cats (13 cats with HCM, 11 cats with systemic diseases). The heart was removed within 1 h after death and grossly examined. Epicardial fat was removed and myocardial samples from the interventricular septum, right atrium and ventricle, and left atrium and ventricle were collected for RNA extraction and stored in RNA stabilising solution (RNAlater; Ambion, Life Technologies, Paisley, UK) at −20 °C until analysed. Hearts were subsequently fixed in 10% formalin and samples from the same sites as those for RNA extraction were prepared and routinely paraffin wax embedded for histological examination (Fonfara et al., 2015). Twenty-four cats underwent full necropsy to identify relevant disease conditions, confirm HCM in cats with clinically diagnosed HCM, and exclude cardiac diseases in cats that did not exhibit clinical signs of heart disease. Samples were collected from all major organs and any gross abnormalities, fixed in 10% formalin and routinely paraffin-embedded for histological examination. For three cats that presented without cardiac diseases (one each with nasal polyp, oesophageal stricture, and discus prolapse) owners did not give consent for a full necropsy and only dissection and further examination of the heart was permitted.

RNA extraction and real time reverse transcriptase PCR was carried out as reported previously (Fonfara et al., 2015). Primers for feline leptin (forward 5′-GCAGCTTGGCTGACAATTTAAAA-3′, reverse 5′-TCTAGTCTTGGCCCAGCTACCTAT-3′) were designed using Primer Express software (Applied Biosystems, Life Technologies), and BLAST searches performed to confirm gene specificity. Validation of the primer and PCR protocols was carried out as previously described (Fonfara et al., 2015). For the amplification of the cytokines IL-1, IL-2, IL-6, TNF-α, TGF-β, the matrix metalloproteinases (MMP)-2, -3, -9, -13 and the tissue inhibitors of matrix metalloproteinases (TIMP)-1, -2, -3 published primer pairs were applied (Fonfara et al., 2015).

Statistical analysis was carried out using SPSS. Leptin, cytokines, MMP and TIMP were log transformed to improve normality and the model assumptions necessary for parametric analysis. Due to there being no difference in transcriptions between left and right atrium (data not shown), results were combined for analysis and are reported as 'atria'. Similarly, no differences in transcriptions were observed between ventricular regions (interventricular septum, left and right ventricle; data not shown) and results were combined for analysis and are reported as 'ventricles'. Cats were grouped into cats with HCM without AT ('HCM without AT'), HCM with AT ('HCM with AT') and cats without cardiac diseases ('non-cardiac'). Cardiac regions, groups and gender were compared using 1-way ANOVA and unpaired *t*-tests as appropriate. For the investigation of the relationship between leptin and age and the markers investigated 'HCM without AT' and 'HCM with AT' cats were combined ('HCM'). Scatter plots were used to explore the association, followed by Pearson correlation test. Results are reported as mean and standard deviation. Statistical significance was defined as $p < 0.05$.

3. Results

Twenty-seven cats were included in the study, ten cats with HCM without AT ('HCM without AT'), five cats with HCM and AT ('HCM with AT'), and 12 cats without cardiac diseases ('non-cardiac'). 'HCM without AT' cats had a mean age of 6.5 years (±4.9 years) and a mean weight of 4.6 kg (±1.5 kg). Nine of these cats were male neutered, one was female entire. Four 'HCM with AT' cats were male neutered, one was female neutered; they had a mean age of 10.4 years (±4 years) and a mean body weight of 4.6 kg (±1.5 kg). 'Non-cardiac' cats had a mean age of 7 years (±3.8 years) and a mean weight of 4.2 kg (±1 kg), ten cats were female neutered, one male neutered and one male. Age and weight did not differ between 'HCM without AT' and 'non-cardiac' cats ($p = 0.580$ and $p = 0.164$, respectively); 'HCM with AT' cats were significantly older than 'HCM without AT' ($p = 0.001$) and 'non-cardiac' cats ($p < 0.001$). 'Non-cardiac' cats were diagnosed with localised disease (i.e. nasal polyp, oesophageal stricture, discus prolapse; $n = 5$), different forms of lymphoma ($n = 3$) or age-related nonspecific changes ($n = 1$) or had been euthanized due to behaviour abnormalities ($n = 3$) (Fonfara et al., 2015). None of these cats displayed histological myocardial abnormalities. Cats with HCM showed myocardial changes described for the disease (Biasato et al., 2015; Khor et al., 2015).

Leptin was transcribed in all samples investigated, with significantly higher leptin mRNA concentrations in atria than in ventricles both in 'HCM without AT' cats ($p = 0.003$) and 'non-cardiac' cats ($p < 0.001$), but not in 'HCM with AT' cats ($p = 0.083$; Table 1). A comparison between the different groups revealed higher leptin mRNA concentrations in atria ($p = 0.036$) and ventricles ($p = 0.001$) from 'HCM without AT' cats than 'non-cardiac' cats (Table 1). 'HCM with AT' cats exhibited lower ventricular leptin mRNA concentrations in comparison to 'HCM without AT' ($p = 0.003$) and 'non-cardiac' cats ($p = 0.039$). No difference was detected for atrial leptin transcription comparing 'HCM with AT' cats with 'HCM' cats ($p = 0.156$) and 'non-cardiac' cats ($p = 0.442$; Table 1). A weak negative correlation of age and leptin was observed for 'HCM' cats ($r = -0.352$, $p = 0.005$, Fig. 1), but not for 'non-cardiac' cats ($r = 0.01$, $p = 0.942$). Furthermore, 'HCM' cats showed strong positive correlations between leptin and IL-2 ($r = 0.793$, $p < 0.001$; Fig. 2a),

Table 1
Relative leptin mRNA concentrations in atria and ventricles from cats with hypertrophic cardiomyopathy (HCM; n = 10), HCM and atrial thrombus (HCM, AT; n = 5) and cats without cardiac diseases (non-cardiac; n = 12).

	Atria	Ventricles	P
HCM	3.1 (±0.53)#	2.6 (±0.41)#$	0.003
HCM, AT	2.4 (±1.2)	1.6 (±0.94)*$	0.083
Non-cardiac	2.8 (±0.37)#	2.2 (±0.42)*#	<0.001

#, *, $: significant differences between groups. AT: atrial thrombus, HCM: hypertrophic cardiomyopathy.