Myocardial leptin transcription in feline hypertrophic cardiomyopathy

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A B S T R A C T

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 an 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...
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 poly, oesophageal stricture, and 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 with AT’ 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),

27 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 poly, 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).

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**Table 1**

<table>
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<th>Atria</th>
<th>Ventricle</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>HCM</td>
<td>3.1</td>
<td>2.6</td>
<td>0.003</td>
</tr>
<tr>
<td>HCM, AT</td>
<td>2.4</td>
<td>1.6</td>
<td>0.083</td>
</tr>
<tr>
<td>Non-cardiac</td>
<td>2.8</td>
<td>2.2</td>
<td>&lt;0.001</td>
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#, *, $: significant differences between groups. AT: atrial thrombus, HCM: hypertrophic cardiomyopathy.
TGF-β (r = 0.650, p < 0.001; Fig. 2b), MMP-2 (r = 0.697, p < 0.001; Fig. 2c), MMP-3 (r = 0.851, p < 0.001), MMP-13 (r = 0.642, p < 0.001), TIMP-2 (r = 0.790, p < 0.001; Fig. 2d), and TIMP-3 (r = 0.637, p < 0.001), moderate correlations between leptin and IL-1 (r = 0.446, p = 0.001) and TNF-α (r = 0.579, p < 0.001), and a weak correlation between leptin and IL-6 (r = 0.385, p = 0.006).

4. Discussion

Leptin was transcribed in all samples investigated, indicating that the feline myocardium does produce leptin constitutively, similar to what has been reported for other species (Fonfara et al., 2011; Karmazyn et al., 2013; Purdham et al., 2004). Consistent with our hypothesis, an increase in leptin transcription was observed in cats with HCM, however, cats with HCM and AT exhibited lower ventricular mRNA concentrations in comparison to cats with HCM alone and cats without cardiac disease. This indicates that leptin transcription varies in different stages of the disease and suggests that it might be involved in cardiac remodeling (Karmazyn et al., 2013). The positive correlation of leptin and inflammatory and pro-fibrotic cytokines, MMPs and TIMPs supports an association of leptin with inflammatory, immune modulatory and remodeling parameters in cats, as reported in human medicine. For humans, a controversy about the cardiac effect of leptin exists (Feijoo-Bandin et al., 2015). The inflammatory and pro-thrombotic effects of leptin have been suggested to have a damaging influence on the heart. On the other hand, leptin was suspected to be cardioprotective by stimulating nitric oxide signaling and anti-apoptotic and anti-hypertrophic effects (Feijoo-Bandin et al., 2015). However, a pro-apoptotic influence has...
been reported under damaging conditions (Fejoo-Bandín et al., 2015), which were most likely present in the cats with HCM of the current study, considering that these had end-stage disease. Increased leptin transcription and positive correlation with several cytokines in the cats with HCM might reflect ongoing myocardial inflammation and remodeling associated with progression of the disease. Myocardial fibrosis and myocardial disorganisation are well-known pathological findings in feline HCM and an inflammatory cell infiltration has been reported (Biasato et al., 2015; Khor et al., 2015). Alternatively, the inflammatory response and the MMP and TIMP activation could indicate a cardiac remodeling process that results in impaired ventricular function, with a functional reserve sufficient to reduce the risk of thrombus formation in some cats. Consequently, the lower mRNA concentration of leptin in cats with HCM and AT might be consistent with a different form of progression of the disease, or could be a subsequent event associated with progressed remodeling processes and ventricular dysfunction that results in blood flow abnormalities which promote thrombus formation in the atria. Clinically, AT formation in cats with HCM suggests a more progressed stage of disease and is associated with an increased risk of death (Payne et al., 2015a).

The generally higher leptin mRNA concentrations in atria than in ventricles suggest regional differences in myocardial reactivity as has been reported previously for other parameters of inflammation and remodeling (Fonfara et al., 2015). A direct contribution of leptin to thrombus formation, as suggested in people (Elbatarny and Maurice, 2005; Schafer and Konstantinides, 2011), seems unlikely considering the unchanged atrial and reduced ventricular leptin transcription in cats with HCM and AT in comparison to cats with HCM alone and without cardiac diseases. However, it is possible that the results obtained for myocardial transcription are not comparable to results for circulating leptin concentrations.

The negative association of leptin and age in cats with HCM might support a potential association of leptin with impaired myocardial reactivity and activity for repair. Increased ventricular stiffness has been reported with increasing age and might contribute to the cardiac remodeling processes associated with HCM (Payne et al., 2013; Schober and Fuentes, 2001). However, this association was weak only and the absence of a negative correlation in the cats without cardiac disease suggests that the disease and not age influences this result.

Limitations of the study include the small number of cats, in particular of cats with HCM and AT, which will have limited statistical significance. A body condition score was only recorded for a few individual cats and a muscle condition score was not obtained. Obesity has been reported to increase circulating leptin concentrations in humans and cats, and might have influenced results (Appleton et al., 2000; Bjornvad et al., 2014; Fejoo-Bandín et al., 2015). However, the cats included were not obese. A gender difference was present between the groups. Higher leptin concentrations have been reported for women, however, no sex difference was found in cats (Bjornvad et al., 2014). It is therefore unlikely that the higher mRNA concentrations detected for the cats with HCM, which were primarily male, are due to gender difference. The group of cats without cardiac diseases was inhomogeneous and clinical information was not complete for all cats. Different diseases might influence cardiac leptin concentrations. However, no difference was detected comparing leptin transcription between cats with diseases that might have had an influence on cardiac function and diseases unlikely to have cardiac effects (data not shown). Furthermore, echocardiography was not obtained in these cats, however, gross examination and histology did not identify any cardiac disease. We have investigated myocardial and not circulating leptin concentrations and results are likely to differ.

5. Conclusion

The results of this study showed leptin transcription in the myocardium of cats with and without cardiac diseases. The observed increase in leptin transcription in the myocardium of cats with HCM and the reduction in cats with HCM and AT suggests varying gene activation in different stages of the disease and a potential involvement of leptin in feline cardiac remodeling processes.

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