Evaluation and Diagnostic Potential of Serum Ghrelin in Feline Hypersomatotropism and Diabetes Mellitus

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Background: Ghrelin is a growth hormone secretagogue. It is a potent regulator of energy homeostasis. Ghrelin concentration is down-regulated in humans with hypersomatotropism (HS) and increases after successful treatment. Additionally, ghrelin secretion seems impaired in human diabetes mellitus (DM).

Hypothesis: Serum ghrelin concentration is down-regulated in cats with HS-induced DM (HSDM) compared to healthy control cats or cats with DM unrelated to HS and increases after radiotherapy.

Animals: Cats with DM (n = 20) and with HSDM (n = 32), 13 of which underwent radiotherapy (RT-group); age-matched controls (n = 20).

Methods: Retrospective cross-sectional study. Analytical performance of a serum total ghrelin ELISA was assessed and validated for use in cats. Differences in serum ghrelin, fructosamine, IGF-1 and insulin were evaluated.

Results: Ghrelin was significantly higher ($P < .001$) in control cats (mean ± SD: 12.9 ± 6.8 ng/mL) compared to HSDM- (7.9 ± 3.3 ng/mL) and DM-cats (6.7 ± 2.3 ng/mL), although not different between the HSDM- and DM-cats. After RT ghrelin increased significantly ($P = .003$) in HSDM-cats undergoing RT (from 6.6 ± 1.9 ng/mL to 9.0 ± 2.2 ng/mL) and the after RT ghrelin concentrations of HSDM cats were no longer significantly different from the serum ghrelin concentration of control cats. Serum IGF-1 did not significantly change in HSDM-cats after RT, despite significant decreases in fructosamine and insulin dose.

Conclusion and Clinical Importance: Ghrelin appears suppressed in cats with DM and HSDM, although increases after RT in HSDM, suggesting possible presence of a direct or indirect negative feedback system between growth hormone and ghrelin. Serum ghrelin might therefore represent a marker of treatment effect.

Key words: Acromegaly; Cat; Insulin growth factor 1; Pituitary adenomas; Secondary diabetes.

Feline hypersomatotropism (HS) is caused by a functional somatotropic adenoma or hyperplasia of the pars distalis of the anterior pituitary gland resulting in excessive growth hormone (GH) secretion.[1] The diabetogenic effect of excess GH leads to diabetes mellitus (DM), which is often the initial clinical presentation. In the long run, the anabolic effects of GH-induced insulin-like growth factor 1 (IGF-1) can lead to characteristic physical changes, including soft tissue growth resulting in the syndrome of acromegaly.[1,2] The diagnosis of HS remains difficult, since no single test can provide a satisfactory definitive diagnosis. Additionally, feline GH assays are not widely available commercially and therefore have currently limited value in a clinical setting despite acceptable sensitivity and specificity.[5] Although the usefulness of serum IGF-1 as a diagnostic marker for HS has been documented, false negative and false positive results do occur and positive results

Abbreviations:

BSH	British shorthair
CT	computed tomography
DLH	domestic longhair
DM	diabetes mellitus
DSH	domestic shorthair
ELISA	enzyme-linked immunosorbent assay
GH	growth hormone
Gy	gray
HS	hypersomatotropism
IGF-1	insulin growth factor 1
RIA	radioimmunoassay
RT	radiotherapy
rt	room temperature

therefore require confirmation of a pituitary lesion with advanced imaging; this is expensive and requires heavy sedation or general anesthesia.[4,6,7] Refinement of existing endocrine testing or development of alternative tests for HS is therefore desirable. Easier, more reliable and accessible diagnostics would facilitate earlier diagnosis of HS as the inciting cause of DM in cats and facilitate more rapid and appropriate treatment, increasing the chance for diabetic remission. Alternative endocrine tests might help establish the true efficacy of HS treatment, which is especially relevant after radiotherapy (after RT), since IGF-1 seems an unreliable marker for this purpose, thus rendering many cats vulnerable to potentially serious iatrogenic hypoglycemia.[7,8]

Although ghrelin was first identified as an endogenous ligand for the GH secretagogue receptor, it is now mostly known as an orexigenic peptide.[9] Little is known about the role of ghrelin in endogenous GH-secretion during normal physiological conditions. Nevertheless,

studies in humans have shown that serum ghrelin concentrations are lower when HS is present, indicating existence of a possible direct or indirect negative feedback system between GH and ghrelin.[10,11] Additionally, serum ghrelin rises after successful surgical removal of a somatotrophinoma.[10,12] Ghrelin has also been shown to suppress insulin release and modify insulin sensitivity, processes at the heart of the etiopathogenesis of type 2 DM, which currently remains incompletely understood.[13,14] Screening for dysregulation of ghrelin secretion and correlations between ghrelin and the level of glycemic control in uncomplicated or primary feline DM (non-HS-induced feline DM) is therefore of additional interest, alongside its use as a control group for HS-induced diabetic cats.

The aim of this study was to validate a serum ghrelin ELISA for use in cats and to evaluate serum ghrelin in feline HS and DM, specifically comparing serum ghrelin in cats with HS-induced DM (HSDM), cats with uncomplicated or primary DM (DM) and healthy cats, as well as assessing the effect of radiotherapy (RT) on circulating ghrelin concentration in HSDM-cats, compared to the traditionally used serum IGF-1.

Material and Methods

Sample Recruitment

Residual serum was collected from samples from three groups of cats: healthy cats (control-cats, N = 20), insulin treated cats with uncomplicated or primary DM (DM-cats, N = 20) and cats with HSDM (HSDM-cats, N = 32). All groups were age-matched; DM-cats and control-cats were weight-matched. From a subgroup (N = 13) of the HSDM-cats samples were also collected before and after RT. All cats were starved for 8–10 hours before sampling and all samples were taken before insulin administration. Serum was separated through centrifugation, separated into aliquots and stored at −80°C within 1 hour of collection. Fructosamine, IGF-1 and ghrelin were measured in samples obtained at the same time. Cats were categorized into the DM-group on the basis of clinical signs (polyuria, polydipsia, and polyphagia), clinicopathological findings consistent with DM (persistent hyperglycemia and concurrent glycosuria, serum fructosamine concentration >350 μmol/L, serum total IGF-1 concentration <700 ng/mL) and modest insulin requirements with adequate diabetic control at time of sampling and during follow-up (<1.5 IU/kg/injection). All cats had been diagnosed, and administered exogenous insulin, a minimum of 1 month before sample collection and follow-up data (minimum of 6 months) were available for all cats. Cats were categorized into the HSDM-group on the basis of a diagnosis of DM (criteria above excluding insulin requirements condition), a serum total IGF-1 concentration >900 ng/mL (all determined by the same RIA, Cambridge Specialist Laboratories Services, Cambridge, UK) and demonstration of a pituitary lesion on brain imaging studies (contrast-enhanced computed tomography [CT] or magnetic resonance imaging [MRI]). In 13 HSDM cats additional serum samples were obtained after completion of hypo-or hyper-fractionated RT (12 cats: total dose of 37 gyay (Gy) in five once-weekly fractions and 1 cat: total dose of 37 (Gy) in ten escalating fractions delivered three times weekly; median collection time after RT completion: 12 months; range 3–32 months, 25–75% percentile 5–14 months). The upper limit for the IGF-1 RIA assay used was 2000 ng/mL and values above this limit were therefore reported as 2,000 ng/mL. Residual sample from 20 age-matched cats undergoing a routine health screen were used as healthy controls; all had normal serum biochemistry (including glucose), urinalysis, and serum total thyroxine results during this health screen. Ethical approval for sample collection had been obtained previously from the Royal Veterinary College local ethical committee.

Ghrelin Assay Protocol

A competitive enzyme-linked immunosorbent assay (ELISA) kit for Rat/Mouse Ghrelin[a,b] was validated for this study. The use of this particular assay for the measurement of ghrelin in cats has previously been reported and is based on the similarities between rat and cat ghrelin.[15,16] The assay was conducted in accordance with the manufacturer's instructions. In brief, 15 μL of serum was added to 135 μL of assay buffer, vortexed and 50 μL transferred into wells primed with secondary antibody, followed by addition of primary antibody and biotinylated peptide. The plate was incubated for 2 hours at room temperature (rt) during orbital shaking (300–400 rpm) and then thoroughly washed using assay buffer. Streptavidin-peroxidase enzyme was added and left for 1 hours at rt during orbital shaking followed by washing and addition of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate; the reaction was stopped after 1 hour of incubation by adding hydrochloric acid (0.5N). The color intensity was measured immediately at 450 nm.[c] A standard curve was generated through dilution of provided standard recombinant rat/mouse ghrelin (concentrations: 0.01, 0.1, 1.0, 10, and 100 ng/mL). Based on duplicate measurements, concentrations were determined by multiplying the concentrations obtained from intra-polation of the standard curve data points with the dilution factor (1 : 10).

Ghrelin Assay—Analytical Performance

The precision of the ELISA was evaluated by calculating intra-and inter-assay coefficients of variation (CV). For determination of the intra-assay CV, three serum samples from cats in the control- and HSDM-group with a range of ghrelin concentrations were each determined ten times during one ELISA run. For determination of the interassay CV, three serum samples from cats from the same groups with a range of ghrelin concentrations were determined in duplicate in three ELISA runs. Separately frozen aliquots from the same residual sample were used to exclude interference from freeze–thaw cycles. CVs were expressed as a percentage and calculated by dividing the standard deviation (SD) by the mean of the measurements.

In the absence of purified feline ghrelin, the accuracy of the assay was evaluated indirectly by linearity under dilution and recovery studies. For linearity under dilution, serum from two of the control cats was serially diluted (2-fold) using the provided assay buffer. Additionally, to exclude matrix effects, two samples with high and low concentrations of ghrelin were mixed at different ratios to create percentage dilutions of 100%, 75%, 50%, 25%, and 0%. Feline ghrelin recovery was expressed as a percentage ([measured/expected concentration] × 100) for each dilution.[17] The limit of detection (LoD) was determined on the basis of 18 repeat measurements of the zero standard and defined as the mean plus three standard deviations.

Statistical Analysis

In order to evaluate sample matrix effects, linearity under dilution was assessed by ordinary linear regression analysis comparing measured and expected ghrelin concentrations.

Data were evaluated for normality using the Kolmogorov-Smirnov test and normally distributed data were described using mean and SD, whereas non-normally distributed data were described

using median and range. Comparison of variables between DM, HSDM and control was performed using one-way ANOVA followed by posthoc Tukey's multiple comparison test. Comparison between two groups were performed using unpaired or paired *t*-test for unpaired or paired normally distributed data and the Mann-Whitney *U*-test or paired-sample Wilcoxon signed rank test for unpaired or paired non-normally distributed data. Additionally, Bonferroni posthoc correction was performed for multiple *t*-test comparisons between paired observations (before and after RT) and controls.

Pearson (parametric) or Spearman (nonparametric) correlation analyses were used to assess for a correlation between changes in ghrelin and insulin dose, IGF-1 and fructosamine before and after RT and for a correlation between ghrelin and fructosamine concentrations in DM and HSDM cats. *P*-values of <.05 were considered statistically significant. GraphPad Software was used.[d]

Results

Ghrelin Assay—Analytical Performance

Linearity under serial dilution of feline samples was demonstrated with linear regression equations showing a correlation coefficient approximating 1.0 (Fig 1A). The mean recovery when mixing feline samples with high and low ghrelin concentration was 96.4 ± 6.9% (range 86–103%) (Fig 1B). The assay LoD was 0.64 ng/mL (mean 0.22 ± 0.14 ng/mL) and values below this were considered to be zero.

Mean intra-CV was 8.7%, 5.1%, and 11.2% for samples with low (4.3 ng/mL), medium (5.4 ng/mL), and high (14.1 ng/mL) ghrelin concentrations, respectively, and inter-CV was 7.8%, 8.3%, and 9.1% for a sample with low (3.2 ng/mL), medium (6.7 ng/mL) and high (10.2 ng/mL) ghrelin concentration, respectively.

Assessed Population

Mean body weight was significantly different between the three groups (*P* < .001). Mean body weight was not significantly different between the DM-cats and control cats, though the bodyweight of HSDM-cats was significantly higher than both other groups (*P* < .05; posthoc Tukey's multiple comparison test) (Table 1). The median insulin dose/kg in the HSDM-group was significantly higher when compared to the DM group

(*P* < .001, Mann-Whitney *U*-test). For the subgroup of HSDM-cats that underwent RT (HSDM+RT), median insulin dose and mean serum fructosamine concentrations were both significantly lower after RT when compared to pretreatment values (insulin: paired-sample Wilcoxon signed rank test, *P* = .01; fructosamine: paired *t*-test, *P* = .02). The mean serum IGF-1 concentration for the HSDM+RT-cats was not significantly lower after RT (paired *t*-test, *P* = .24). Insulin dose, IGF-1, fructosamine and ghrelin concentrations for the group of HSDM-cats that underwent RT are illustrated in Fig 2.

Feline Ghrelin Concentrations in DM-, HSDM-, and Control-Cats

The mean ± SD serum concentration of total ghrelin in the HSDM-cats was 7.9 ± 3.3 ng/mL (range 1.7–18.3 ng/mL). The mean ± SD total ghrelin concentration in the DM-cats was 6.7 ± 2.3 ng/mL (range 3.6–12.7 ng/mL), whereas the mean total ghrelin in the control-cats was 12.9 ± 6.8 ng/mL (range 2.4–32.3 ng/mL). Total ghrelin concentration was significantly different between groups (ANOVA, *P* < .001) and posthoc Tukey's analysis identified that control cats had a significantly higher total ghrelin concentration compared to HSDM and DM cats (Fig 3).

The subpopulation of HSDM-cats that underwent RT had a mean ± SD pretreatment serum ghrelin concentration of 6.6 ± 1.8 ng/mL (range 4.2–10.2 ng/mL). The after RT total ghrelin was 9.0 ± 2.3 ng/mL (range 5.4–12.7 ng/mL), which was significantly higher (paired *t*-test, *P* < .0008) (Fig 4). Total serum ghrelin in HSDM pre-RT cats (*P* = .0028), though not in HSDM after RT cats (*P* = .055), was significantly lower compared to control cats (multiple *t*-tests with Bonferroni posthoc significance level α = 0.025).

Changes in serum ghrelin before and after RT did not significantly correlate with changes in insulin dose pre- and after RT (Spearman: *r* = 0.36; *P* = .22), changes in serum IGF-1 (Pearson: *r* = 0.16; *P* = .59) or fructosamine (Pearson: *r* = 0.02; *P* = .94). Additionally, there was no significant correlation between serum fructosamine and ghrelin in the DM-cats (Pearson:
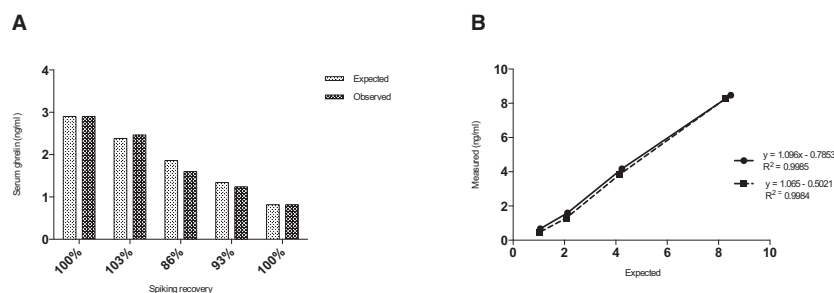

Fig 1. (A) Linearity under dilution of two control samples with ghrelin concentrations of 8.5 ng/mL (solid line) and 8.3 ng/mL (dashed line), respectively, diluted 100 : 0%, 50 : 50%, 25 : 75%, and 12.5 : 87.5%. (B) Spiking recovery expressed as percentage when mixing samples of high and low ghrelin concentration by single measurements of different ratios (100 : 0%; 75 : 25%; 50 : 50%; 25 : 75%; 0 : 100%). Recovery % = (ghrelin$_{measured}$/ghrelin$_{expected}$) × 100%.

Table 1. Physical characteristics, median (range) insulin dose and mean $\pm$ SD serum fructosamine and IGF-1 concentrations of cats with diabetes mellitus (DM), cats with hypersomatotropism and diabetes mellitus (HSDM) including the subgroup of cats that underwent RT (RT) and healthy age-matched control cats.

	DM	HSDM	RT (Pre)	RT (Post)	Controls
Number (n)	20	32	13	13	20
Age (years)	12.1 (7–17)	10 (6–14)	10 (5–13)	11 (7–13)	11 (9–17)
Breed	19 DSH/1 Abysinian	29 DSH/2 DLH/1 BSH	11 DSH/1 DLH/1 BSH		18 DSH/1 DLH/1 BSH
Sex	12 MN/6 FN/2 FE	21 MN/9 FN/2 FE	9 MN/4 FN		12MN/8 FN
BW (kg)	3.9 (2.7–6)	5.5 (3.9–9.1)[a]	5.8 (3.9–9.1)	5.4 (3.9–10.1)	4.6 (2.1–5.8)
Insulin dose (IU/kg/inj)	0.8 (0.2–1.)	2.7 (1–9.6)[b]	3.2 (1.4–9.6)	1.1 (0–4)[c]	NA
Fructosamine (μmol/L)	495 $\pm$ 95	613 $\pm$ 179[b]	623 $\pm$ 157	475 $\pm$ 174[c]	NA
IGF-1 (ng/mL)	257 $\pm$ 72	1562 $\pm$ 381	1597 $\pm$ 389	1412 $\pm$ 387	NA

[a]Significant difference compared to the DM- and control group (ANOVA; post-hoc Tukeys).
[b]Significant difference compared to the DM-group (insulin: Mann-Whitney *U*-test/fructosamine unpaired *t*-test).
[c]Significant difference compared to the before RT group (insulin: paired-sample Wilcoxon signed rank test; fructosamine: paired *t*-test).

$r = -0.08$; $P = .074$) or HSDM-cats (Pearson: $r = -0.12$; $P = .51$).

Discussion

Serum ghrelin in cats with HSDM and cats with uncomplicated or primary DM has not previously been studied. This study suggests that, in parallel to the situation in man,[10–12,18,19] serum ghrelin is suppressed in cats with HS-induced DM and in cats with DM unrelated to HS. Serum ghrelin is therefore not useful as a diagnostic for determining the presence or absence of HS in the diabetic cat. However, it might prove useful as a marker for treatment effect, since serum ghrelin increased to normal following RT in cats with HSDM.

The overlapping ranges of the ghrelin concentrations in the before and after RT group might indicate a limitation to the use of ghrelin as an indicator for treatment effect and is also encountered in human acromegaly.[20] Nevertheless, of all 13 assessed cats undergoing RT, 12 (92%) showed an increase in serum ghrelin, and in 11/12 this was of a magnitude greater than the detection limit of the assay. The rise in ghrelin concentrations was accompanied by improved glycemic control reflected by significant lowering of serum fructosamine and insulin dose, indicating treatment effect. Interestingly, serum IGF-1 concentrations were not significantly changed after treatment, supporting current belief that this represents a poor indicator of treatment effect when it concerns RT for feline HS.[8] Whether serum ghrelin can actually predict a fall in insulin requirements has yet to be determined and would require a future prospective study with several sample time points after RT before a decrease in insulin requirement. The latter will, however, be a difficult study to perform, since the RT effect might be seen 6 months to a year after treatment, if it will take effect at all. In humans suffering from acromegaly, IGF-1 concentrations attenuate very slowly after RT and maximal control of the release of GH may require more than 15 years. Radiotherapy is therefore generally reserved for tumors that have recurred or persisted after surgery.[21] On the other hand, successful hypophysectomy leads to an immediate reduction in GH and IGF-1 and both parameters are therefore used to assess biochemical control of disease, as well as predict the risk of recurrence.[22] This also highlights the superiority of hypophysectomy over RT in terms of HS-treatment efficacy in humans, rather than a mere lack of sensitivity of IGF-1 after RT. Feline case reports suggest this is also true for the hypersomatotropic cat.[3,8,23,24]

The diurnal variation in circulating ghrelin is a topic of ongoing research in humans, with some conflicting results in terms of influences on its secretion pattern.[25] Whether there is diurnal variation in feline circulating ghrelin concentrations is currently unknown. The relatively small differences between before and after radiation ghrelin concentrations could therefore also have been confounded by such unknown natural ghrelin dynamics. For instance, ghrelin is known to be influenced by meals in man.[26] Nevertheless, on 24-hour serial assessment of circulating ghrelin concentrations in humans, it was shown that samples taken before breakfast correlated strongly with 24-hour integrated area under the curve values ($r = 0.873$), suggesting that a single starved morning sample could be used as a surrogate for 24-hour profiles to estimate overall ghrelin concentrations.[27] Therefore, to minimize the effects of daily ghrelin dynamics in this study, all cat samples were taken in the early morning and before food. Additionally, the reported amplitudes of ghrelin secretion pulses in humans are usually in the range of 0.5 ± 0.1 ng/mL,[26] which therefore could only pose a modest confounding factor, given that the difference between the mean concentration of serum ghrelin before and after radiotherapy in our cats was almost 5-fold greater (2.4 ng/mL). Specific feline ghrelin physiology research is nevertheless indicated to further evaluate these issues, as well as an assessment of ghrelin stability and ideal storage conditions.
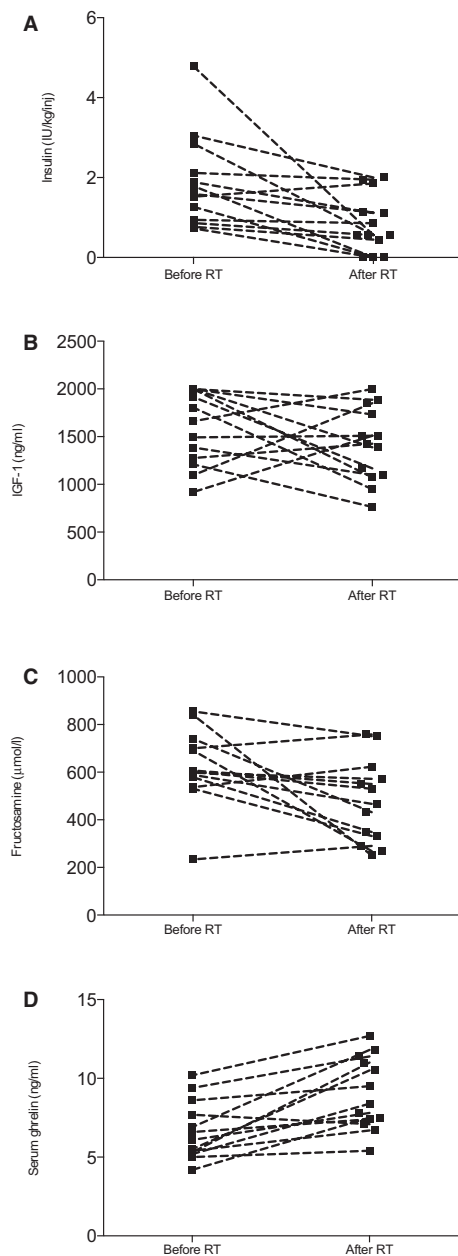
Fig 2. Insulin **(A)**, IGF-1 **(B)**, fructosamine **(C)** and ghrelin **(D)** concentrations before and after RT for the 13 cats with HSDM that underwent RT (HSDM+RT). Dashed lines connect pairs of observations before and after RT.
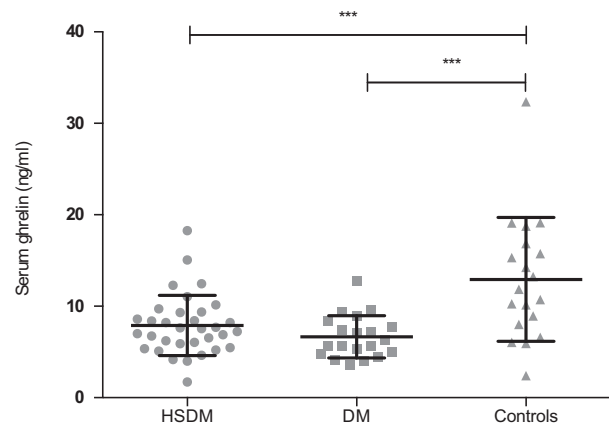

Fig 3. Serum ghrelin concentrations in cats with hypersomatotropism and diabetes mellitus (HSDM) (circles), cats with diabetes mellitus (DM) (squares) and healthy control cats (triangles). Mean values are indicated by long horizontal lines and standard deviations (SD) are indicated by short horizontal lines. ***Indicates a statistically significant difference in means between groups ($P < .05$) (ANOVA and posthoc Tukey's).
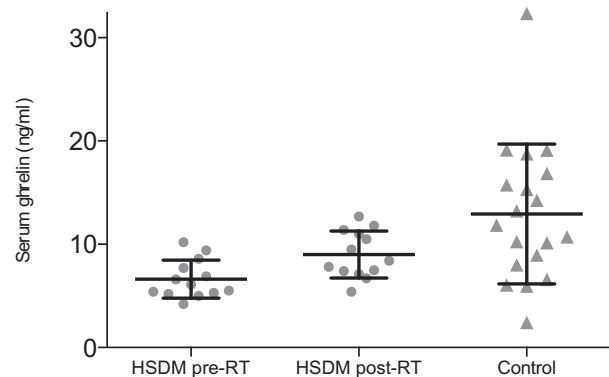

Fig 4. Serum ghrelin concentration in cats with hypersomatotropism and diabetes mellitus (HSDM) before (circles) and after (squares) RT versus control cats (triangles). Mean values are indicated by long horizontal lines and standard deviation (SD) are indicated by short horizontal lines. *Indicates significant difference, $P = .0028$ (multiple t-tests; Bonferroni posthoc significance level $\alpha = 0.025$), no significant difference was observed between post-RT and controls ($P = .055$).

The ELISA used in this study evaluated *total* ghrelin concentration, despite the existence of two major forms of ghrelin in the body. The octanoylated or "active" form of ghrelin known as acyl ghrelin can activate the GH secretagogue receptor.[9] The major form in blood is the nonoctanoylated or "inactive" form known as desacyl ghrelin. Nevertheless, increasing numbers of studies report also biological effects of desacyl ghrelin and studies in humans have shown that both forms are suppressed in acromegaly, justifying the use of a total ghrelin ELISA.[11,28,29] The study employed, after appropriate validation, a Rat/Mouse Ghrelin ELISA for measuring feline ghrelin. The absence of commercially available (recombinant) feline ghrelin posed a limitation in the assessment of accuracy. Nevertheless, the choice of the used ELISA system was based on its previous use for this purpose[16] and the fact that ghrelin has a highly conserved amino acid sequence across species. Only one amino acid difference exists between rat and feline ghrelin and synthetic rat ghrelin is able to stimulate GH secretion in cats.[15] This, in combination with the serial dilution and spiking recovery data, obtained using mixtures of feline serum, suggests an adequate level of accuracy of the chosen assay.

In humans, dogs and cats, the relationship between endogenous ghrelin secretion and pituitary GH

secretion is not fully elucidated.[15,30–32] Humans with acromegaly have lower ghrelin concentrations and these tend to increase after surgery in correlation with normalization of GH and IGF-I and improved insulin sensitivity, suggesting negative feedback to occur exerted by excess GH on ghrelin secretion.[10,12,33] Based on the before and after RT results of this study, this hypothesized negative feedback effect could also be present in the cat. The effect may be independent or could be confounded by changes in insulin secretion and sensitivity, as well as glycemic control, induced by HS and its treatment. Indeed, in humans, low ghrelin concentrations have been associated with insulin resistance and development of type 2 DM.[14,18,28,34] In addition, hyperinsulinemia, acute hyperglycemia and poor long-term glycemic control in cats with DM might impair ghrelin secretion in humans.[35,36] However, although in this study the mean insulin dose and fructosamine concentration for the HSDM+RT group were significantly lower following RT, there was no significant correlation between the changes in serum ghrelin concentration and the changes in fructosamine or insulin dose. Additionally, no correlations could be documented between fructosamine and ghrelin in the entire DM and HSDM cat populations.

In order to prevent inclusion of hypersomatotropic cats in the DM group, only cats with serum total IGF-1 concentration <700 ng/mL and modest insulin requirements with adequate diabetic control at time of sampling were included. Additionally, these cats showed no evidence of insulin resistance during a minimum of 6 months follow-up. At time of IGF-1 determination, all cats had been diagnosed with DM and had been started on exogenous insulin for a minimum of 1 month, making a false negative IGF-1 result, because of the inhibitory effect of portal insulin deficiency on hepatic IGF-1 production unlikely.[37]

Low fasting ghrelin concentrations have been consistently associated with obesity independent of the presence of type 2 DM in people and negative feedback from excess leptin produced by the numerous adipocytes has been implicated in this process.[38–42] In contrast, a previous study in obese and lean cats found that fasting serum acylated ghrelin concentrations were in fact slightly higher in an obese state (though the mean difference was only 9 pg/mL).[43] Nevertheless, because of such possible impact of obesity or body condition on ghrelin, the DM-group and control cats in this study were weight-matched; additionally, none were classified as obese by the attending clinician; furthermore, average body weight in these two groups was not strongly suggestive of underdiagnosed obesity. Body condition scores or DEXA scan results were unfortunately not available for these two groups. The HSDM-group cats were not weight-matched since weight gain and a greater stature formed part of the pathogenesis of the disease in many of this group, as evidenced by the significantly higher body weights compared to the DM- and control-cats. Nevertheless, the relationship between ghrelin concentration and weight seen in the human nonacromegaly population has been docu-

mented to be absent in human patients with acromegaly.[10] Additionally, body condition scores were available for the feline HSDM group and did not indicate presence of obesity in any of the 32 HSDM cats. Body condition scores and weight did not significantly change after RT in the assessed subpopulation of HSDM cats undergoing this treatment, rendering it unlikely that the detected significant increase in serum ghrelin after RT was caused by a change in body condition or body fat.

In conclusion, the analytical performance of a rat and mouse ghrelin ELISA was assessed to be appropriate in the cat with satisfactory inter- and intra-assay precision, linearity under dilution and recovery studies. This study also suggests that in cats with HSDM and cats with DM serum ghrelin is suppressed compared to healthy cats. Serum ghrelin increased significantly following successful RT in cats with HSDM to a level that was comparable to control cats, highlighting the potential existence of a direct or indirect negative feedback system between ghrelin and GH. Similar to the situation in human DM, the role of ghrelin in feline DM remains to be elucidated. Finally, further studies are necessary to investigate the exact utility of serum ghrelin as a diagnostic tool to assess RT effect in HSDM, whereas IGF-1 has been confirmed to be insensitive for this purpose.

Footnotes

Acknowledgments

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