Serum N-Terminal Type III Procollagen Propeptide: An Indicator of Growth Hormone Excess and Response to Treatment in Feline Hypersomatotropism

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Background: N-terminal type III procollagen propeptide (PIIINP) is a biomarker of soft tissue proliferation. Hypersomatotropism (HS) is associated with soft tissue proliferation.

Hypothesis: Serum PIIINP is increased in cats with HS and decreases with effective treatment, and may be an additional tool in the diagnosis and treatment of feline HS.

Animals: Cats with uncomplicated diabetes mellitus (DM; n = 30) and with HS-induced DM (HSDM; n = 30). Pre- and post-treatment samples were available from 5 cats undergoing radiotherapy (RT) and 16 cats undergoing hypophysectomy (HPX).

Methods: Retrospective and prospective cross-sectional study. Analytical performance of a serum PIIINP ELISA was assessed and validated for use in cats. PIIINP and insulin-like growth factor 1 (IGF-1) radioimmunoassays (RIA) were performed pre- and post-treatment in cats with DM and HSDM. PIIINP and IGF-1 were compared between cats treated by RT and HPX.

Results: Serum PIIINP concentrations were significantly higher ($P < .001$) in HSDM cats (median, 19.6 ng/mL; range, 1.7–27.9) compared to DM cats (median, 5.0 ng/mL; range, 2.1–10.4). A cut-off of 10.5 ng/mL allowed differentiation between DM and HSDM cats with 87% sensitivity and 100% specificity (area under the curve [AUC], 0.91; 95% confidence interval [CI], 0.82-1). After RT, PIIINP increased significantly ($P = .043$) with no significant change in IGF-1 concentrations. After HPX, serum PIIINP ($P = .034$) and IGF-1 concentrations ($P < .001$) decreased significantly.

Conclusion and clinical importance: PIIINP concentrations are increased in cats with untreated HSDM compared to those with DM, demonstrating the effect of excess GH on soft tissue. PIIINP concentrations decreased after HPX in most HSDM cats.

Key words: Acromegaly; Cat; Hypophysectomy; Insulin-like growth factor 1; Procollagen.

Hypersomatotropism (HS), excess production of growth hormone (GH) by a functional somatotrophic adenoma, carcinoma or hyperplasia of the pituitary gland, and the resulting syndrome acromegaly are thought to be relatively common among diabetic cats.[1–3] Feline HS now is hypothesized to cause diabetes in as many as 1 in 4 diabetic cats.[3] A subtle or initially unremarkable phenotype and difficult and expensive diagnostic process likely have contributed to the previous underestimation of its prevalence.[1,3,4] Given the pulsatile nature of GH secretion and the absence of an easily accessible commercial GH assay, a presumptive diagnosis currently is most commonly based on measurement of circulating GH-induced production of

Abbreviations:

AUC	area under the curve
BSH	British shorthair
CI	confidence interval
DLH	domestic longhair
DM	diabetes mellitus
DSH	domestic shorthair
ELISA	enzyme-linked immunosorbent assay
GH	growth hormone
HPX	hypophysectomy
HSDM	hypersomatotropic diabetic cats
HS	hypersomatotropism
IGF-1	insulin-like growth factor 1
IGFBP	insulin-like growth factor binding protein
LoD	limit of detection
PIIINP	N-terminal type III procollagen propeptide
RIA	radioimmunoassay
RT	radiotherapy

insulin-like growth factor 1 (IGF-1), rather than GH itself.[1,2,4–10]

Initial suspicion of HS mostly has been based on the screening of poorly controlled diabetic cats for the presence of increased IGF-1 concentrations[1] (>1000 ng/mL). However, non-acromegalic diabetic cats can have increased IGF-1 concentrations (false positive) and newly diabetic acromegalic cats can have normal IGF-1 concentrations (false negative).[1,2,4,11] More advanced, expensive, and invasive diagnostic tests, specifically intracranial imaging under sedation or general anesthesia, are therefore often necessary to allow confirmation of the diagnosis.[12] Nevertheless, this approach also can

prove falsely negative in cats with microadenoma or acidophilic hyperplasia.[1,2] Definitive diagnosis of HS therefore is made by pituitary gland histopathology. This process involves hypophysectomy (HPX) or postmortem examination, which is not performed routinely. Therefore, development of additional, easily accessible tools for diagnosis of HS is desirable.

Earlier diagnosis and treatment could be facilitated by additional diagnostic tests, improve chances of diabetic remission[13,14] and decrease the impact of excess GH on the patient's body.[15] Development of markers other than serum IGF-1 also would be beneficial to understand the wider impact of HS on the cat's physiology and compare the beneficial effects of various treatment modalities. Indeed serum IGF-1 concentration merely reflects the ability of GH to directly stimulate IGF-1 production (mainly in the liver). However, IGF-1 production also is influenced by other factors, including availability of portal venous insulin, which in turn depends on remaining beta-cell function or exogenous insulin provision. Finally, serum IGF-1 concentration has been shown to be a poor marker of treatment success after radiotherapy (RT) in cats with diabetes and HS.[16]

Growth hormone stimulates bone and soft tissue turnover resulting in some of the acromegalic changes typical of chronic HS.[17,18] Assessment of a marker that reflects the tissue impact of HS is therefore of interest. Collagen is synthesized with propeptides at both ends of the molecule, and cleavage of these propeptides promotes formation of collagen fibrils and fibrosis.[19] The propeptides are either retained in the matrix or released into the circulation, and the latter can be measured in serum. One such propeptide is N-terminal type III procollagen propeptide (PIIINP), which has been shown to correlate in a dose-dependent manner with serum concentrations of GH in humans.[20,21] Indeed, serum PIIINP concentrations consistently have been shown to be increased in humans with active acromegaly and decrease after successful treatment.[22,23] Measurement of PIIINP therefore represents an opportunity to assess a different aspect of the impact of HS and its treatment than its glycemic impact, on which most clinicians focus on when evaluating a cat with HS.

The aims of our study were therefore to: a) validate a human N-terminal propeptide of collagen alpha-1 (III) chain (PIIINP) enzyme-linked immunosorbent assay (ELISA) for use in cats; b) describe and compare PIIINP concentrations in cats with HS-induced diabetes mellitus (HSDM) and primary diabetes mellitus (DM); and, c) compare PIIINP as a measure of soft tissue turnover after RT and HPX.

Materials and Methods

Study Population

The study included serum samples from 30 DM cats and 30 HSDM cats as defined by the following criteria: DM—clinical signs consistent with DM, evidence of pathologic hyperglycemia and glucosuria, inappropriately high serum fructosamine concentration, lack of evidence of insulin resistance (defined as >1.5 iu/kg/injection on a q12h protocol), low serum IGF-1 concentration (<600 ng/mL); and HSDM—diabetic cats (with a history of DM and insulin treatment) with confirmed HS based on identification of a pituitary lesion on intracranial imaging (pituitary dorsoventral height >4 mm) and an IGF-1 concentration >1000 ng/mL[3] or histopathologic evidence of a somatotrophinoma. One HSDM cat undergoing HPX had an initial IGF-1 concentration >1000 ng/mL and a repeated IGF-1 concentration <1000 ng/mL immediately before HPX. This cat was included in the HSDM group based on pituitary histopathology confirming the diagnosis. The groups were matched by preferentially selecting samples for age, sex, and breed (in that order), and by confirming absence of a statistical difference (Mann-Whitney U-test, Table 2). Confirmed HSDM cats that underwent RT or HPX with sufficient residual serum available for analysis were recruited both retrospectively and prospectively from the Acromegalic Cat Clinic from May 2007 to May 2015.

Sample Collection

These samples, as well as additional samples from cats in the treatment group, had been collected previously as part of an ongoing, prospective diabetic cat screening project, as well as through the Royal Veterinary College Acromegalic Cat Clinic, which diagnoses and treats cats with HS. Samples from HSDM cats undergoing treatment were collected from residual blood both retrospectively and prospectively. All serum samples were stored at −80°C until analysis. The interval between therapeutic intervention and sample procurement for measurement of posttreatment IGF-1 and PIIINP concentrations varied because of owner availability. However, a minimum time period of 1 month was chosen for all groups. The Ethics Committee of the Royal Veterinary College approved collection of all samples.

Human N-Terminal Propeptide of Collagen Alpha-1 (III) and IGF-1 Measurement

A competitive ELISA kit for human N-terminal propeptide of collagen alpha-1 [III] chain was validated.[a] The assay was performed according to the manufacturer's instructions at room temperature.[a] In brief, 50 μL of sample or standard containing PIIINP was added to each well, primed with monoclonal antibody specific to the N-terminal propeptide of collagen alpha-1 (III) chain, followed by addition of a fixed amount of competing biotin-labeled PIIINP. After a 1 h incubation, plates were washed with provided solution, 100 μL avidin conjugated to horseradish peroxidase added, incubated for 45 min, 90 μL tetramethylbenzidine (TMB) substrate solution added, incubated for another 20 min, and finally 50 μL sulfuric acid added. All samples were analyzed within 5 min and in duplicate. Color change was measured by spectrophotometric analysis at a wavelength of 450 nm. The concentration of PIIINP was calculated by comparing the measured optical density to the standard curve. The standard curve consisted of diluted human PIIINP (concentrations of 0, 0.31, 0.62, 1.25, 2.5, 5.0, 10.0 and 20.0 ng/mL) using zero standard. Where sample values fell outside of the standard curve, they were diluted until they fell within the standard curve. The PIIINP concentrations were calculated by multiplying the concentrations obtained from intrapolation with the corresponding dilution.

ELISA Validation

Parallelism with the standard curve was evaluated and confirmed by serial dilution of feline serum containing high PIIINP concentrations using zero standard. Assay accuracy was further evaluated by assessing recovery of serially mixing samples with

high PIIINP concentration with those containing low PIIINP concentration (percentage mixtures: 100 : 0; 75 : 25; 50 : 50; 25 : 75; 0 : 100) and comparing expected with measured concentrations. Assay precision was established by calculation of inter- and intra-assay coefficients of variation (CV). Intra-assay CV was determined by repeated measurement of 2 feline serum samples with known low (4.1 ng/mL) and high (38.5 ng/mL) PIIINP concentrations during the same assay run (at least 4 times) with the same ELISA plate. Inter-assay CV was determined by repeated measurement of PIIINP concentrations in 3 consecutive ELISA runs on different days with different plates. The different PIIINP concentrations were achieved by dilutions 1 : 1, 1 : 4, 1 : 8, 1 : 10 of a serum sample from a HSDM cat to simulate dilutions likely required to bring the HSDM cat samples onto the standard curve. Aliquots of the dilutions were used in the 3 runs. The lower limit of detection (LoD) was calculated by performing 12 consecutive measurements of zero standard in the same ELISA and was defined as the mean plus 2 standard deviations.[24]

Impact of freeze-thaw cycles was assessed by creating aliquots of residual serum samples from a single cat. The serum samples were stored frozen at −80°C and underwent 3 cycles of thawing at room temperature and refreezing. The impact of icterus, hemolysis, lipemia and azotemia were not specifically assessed, but all samples with macroscopic signs of hyperbilirubinemia, hemolysis, or lipemia were excluded.

Serum IGF-1 concentrations were measured simultaneously in all used samples, employing a previously validated RIA.[1,3] For the purpose of our study, concentrations in excess of 2000 ng/mL (upper LoD of the assay) were defined as equal to 2000 ng/mL, and concentrations <15 ng/mL as equal to 15 ng/mL (the assay's lower LoD).

Statistical Analysis

All analyses were performed using commercial statistical software packages should be.[b,c] Data distributions were assessed by visual assessment of histograms and the Shapiro-Wilk normality test. Dilutional parallelism was evaluated by calculation of a correlation coefficient during linear regression. If PIIINP concentrations were not normally distributed, nonparametric tests were used and data presented as median and range. If data was normally distributed, parametric tests were used and data were presented as mean and standard deviation. Comparisons of data between 2 groups were performed with the Mann-Whitney test. For comparisons of data among multiple groups, the Kruskal-Wallis ANOVA was used. Wilcoxon signed rank tests were used for comparisons of IGF-1 and PIIINP concentrations within groups (eg, pre- versus posttreatment). Correlations between variables were evaluated by calculation of Spearman's rank correlation coefficient. Results were deemed significant if $P < .05$. To assess performance of PIIINP as a potential diagnostic biomarker, a receiver-operating characteristic (ROC) curve was constructed.

Results

PIIINP Assay Validation

Parallelism with the standard curve was confirmed during serial dilution of feline serum containing high PIIINP concentration with zero standard. A correlation coefficient of 0.98 was determined based on linear regression (Fig 1A). Intra-assay coefficients of variation for high PIIINP concentration and low PIIINP concentrations were 7.3 and 7.8%, respectively. Inter-assay coefficients of variation for high PIIINP concentration

and low PIIINP concentrations are presented in Table 1. Because of the inter-assay variability demonstrated, all samples analyzed subsequently for comparison (DM versus HSDM and pre- and posttreatment) were performed during the same assay run and all samples were appropriately diluted. The lower LoD was determined to be 0.7 ng/mL.

Repeated freeze-thaw cycles did not significantly affect PIIINP concentrations (Kruskal-Wallis; $P = .37$). Recovery studies performed by mixing high and low concentration PIIINP samples at serial ratios (100 : 0, 75 : 25, 50 : 50, 25 : 75 and 0 : 100) showed an average of 16% variability between expected and observed PIIINP concentrations (Fig 1B).

Study Population

No statistically significant differences in age, breed, or sex between DM and HSDM cats were found. Cats with DM had a median age of 133 months (range, 70–198) and HSDM cats 120 months (range, 63–186). The HSDM cats had significantly higher body weight (mean, 5.93 kg; ±1.32 versus mean, 4.77 kg; ±1.64, $P = .007$); (Table 2), insulin requirements (median, 1.4 iu/kg/injection (range, 0.5–4.5) versus 0.6 iu/kg/injection (range, 0.2–1.4), $P < .005$), and serum fructosamine concentration (median, 579 μmol/L (range, 214–910) versus 481 μmol/L (range, 218–1241), $P = .007$). None of the HSDM cats in the study had unequivocal phenotypical signs of acromegaly.

Serum PIIINP Concentrations in HSDM and DM Cats

Serum PIIINP concentration did not correlate significantly with body weight when considering both groups together (Spearman's rho, $P = .098$) or when evaluating both groups separately (Spearman's rho; DM cats only $P = .59$; HSDM cats, $P = .79$). Serum PIIINP concentrations were significantly different between HSDM cats (median, 19.6 ng/mL; range, 1.7–27.9) and DM cats (median, 5.0 ng/mL; range, 2.1–10.4; Mann-Whitney U-test, $P < .001$; Fig 2). The area under the ROC curve was 0.91 (95% CI, 0.8-1). Using a cut-off value of 10.5 ng/mL, PIIINP concentration had a sensitivity of 86.7% and specificity of 100% for differentiation between DM and HSDM cats.

PIIINP after RT and HPX

Samples from 5 cats were recruited for comparison of pre- and post-RT PIIINP concentrations. Post-RT samples were taken a median of 6 months after treatment (range, 4–8 months). The IGF-1 concentrations did not change significantly post-RT in these cats with HSDM (median pre, 1915 ng/mL; range, 1087–2000; median post, 1263 ng/mL; range, 645–2000; Wilcoxon signed rank test; $P = .068$; Fig 3C), whereas serum fructosamine concentration (median pre, 691 μmol/L; range, 464–740; median post, 412 μmol/L; range, 298–590; Wilcoxon signed rank test; $P = .04$; Fig 3B) and
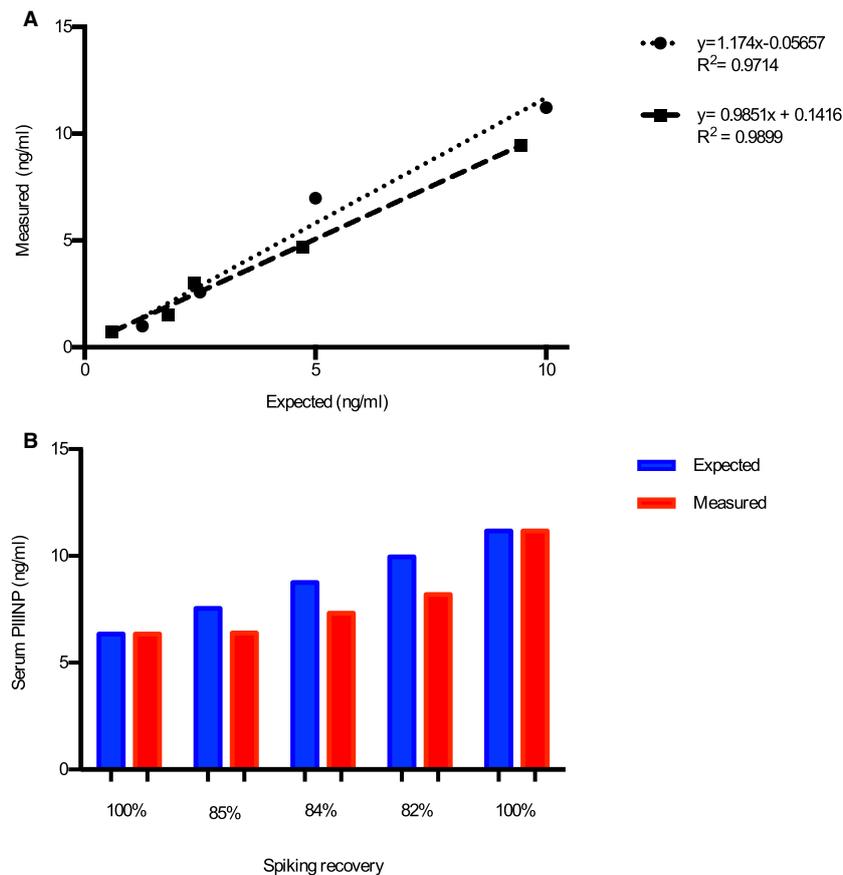
Fig 1. **(A)** Demonstration of dilutional parallelism of feline serum containing 9.5 ng/mL PIIINP (square) and the standard human sample containing 20 ng/mL PIIINP (circle) diluted at 1:1 (shown for feline sample only), 1 : 2, 1 : 4, 1 : 8, and 1 : 16, respectively using zero standard. **(B)** Demonstration of spike and recovery expressed as a percentage when mixing different samples of high and low PIIINP concentrations by single measurements of different ratios (100 : 0, 75 : 25, 50 : 50, 25 : 75, 0 : 100%). Recovery = (PIIINPmeasured/PIIINPexpected) $\times$ 100%.

Table 1. Inter-assay coefficients of variation (%CV) of low (mean, 1.6 ng/mL; 1.8 ng/mL), intermediate (mean, 3.1 ng/mL), and high (mean, 8.6 ng/mL) PIIINP concentrations. The different PIIINP concentrations were achieved by dilutions 1 : 1, 1 : 4, 1 : 8, and 1 : 10 of serum of a HSDM cat.

Measured concentration 1 (ng/mL)	Measured concentration 2 (ng/mL)	Measured concentration 3 (ng/mL)	Mean concentration (ng/mL)	Standard deviation	% CV
11.9	6.3	7.7	8.6	2.9	33.9
3.0	2.9	3.5	3.1	0.3	9.8
1.7	1.6	2.0	1.8	0.2	9.4
1.2	1.3	2.3	1.6	0.6	39.4

exogenous insulin dose decreased significantly (median pre, 1.30 IU/kg/injection; range, 0.77–3.05); median post, 1.02 iu/kg/injection; range, 0.49–2.26; Wilcoxon signed rank test; P = .03; Fig. 3A). The PIIINP concentration increased significantly post-RT in HSDM cats (median pre-RT, 13.5 ng/mL; range, 10.5–19.8; median post-RT, 15.0 ng/mL; range, 12.7–21.5; Wilcoxon signed rank test; P = .043; Fig. 3D).

Samples from 16 HSDM cats that underwent HPX were recruited. Post-HPX samples were taken a median of 5 months after treatment (range, 1–13 months).

Serum IGF-1 concentration was significantly decreased post-HPX in HSDM cats (median pre-HPX, 1705 ng/mL; range, 590–2000; median post-HPX, 53 ng/mL; range, 15–1819; Wilcoxon signed rank test; P < .001; Fig 4C). Serum fructosamine concentrations and exogenous insulin dosages decreased significantly and are shown in Figures 4A and 4B. Serum PIIINP concentrations also changed significantly post-HPX (median pre-HPX, 26.46 ng/mL; range, 14.59–99.61; median post-HPX, 20.87 ng/mL; range, 8.7–34.42; Wilcoxon signed rank test; P = .034; Fig 4D).

Table 2. Characteristics of cats with diabetes mellitus (DM), cats with hypersomatotropism and diabetes mellitus (HSDM) including the subgroup of cats that underwent radiation therapy (RT) and hypophysectomy (HPX).

	DM	HSDM	RT (Pre)	RT (Post)	HPX (Pre)	HPX (Post)
Number (n)	30	30	5	5	16	16
Age (months)	133 (70–198)	120 (63–186)	106 ± 25	–	126 ± 46	–
Breed	23 DSH/4 DLH/1 Burmese/1 Siamese/1 Maine Coon	27 DSH/1 DLH/2 BSH	4 DSH/1 DLH	4 DSH/1 DLH	14 DSH/1 DSH/1 BSH	14 DSH/1 DSH/1 BSH
Sex	15 MN/13 FN/2 FE	23 MN/6 FN/1 ME	4 MN/1 FN	4 MN/1 FN	13 MN/3 FN	13 MN/3 FN
BW (kg)	4.77 ± 1.64	5.93 ± 1.32[a]	5.9 (3.92–9.1)	6.07 (5.44–10.1)	5.73 ± 1.33	5.79 ± 1.14
Insulin (iu/kg/inj)	0.60 (0.2–1.4)	1.40 (0.5–4.5)[a]	1.30 (0.77–3.05)	1.02 (0.49–2.26)[b]	1.40 (0.6–2.6)	0 (0–0.9)[c]
Fructosamine (μmol/L)	481 (218–1241)	579 (214–910)[a]	691 (464–740)	412 (298–590)[b]	539 (380–743)	299 (197–676)[c]
IGF-1 (ng/mL)	331 (97–589)	1804 (1050–2000)[a]	1915 (1087–2000)	1263 (645–2000)	1705 (590–2000)	53 (15–1819)[c]
PIIINP (ng/mL)	5.0 (2.1–10.4)	19.6 (1.7–27.9)[a]	13.5 (10.5–19.8)	15.0 (12.7–21.5)	26.46 (14.59–99.61)	20.87 (8.7–34.42)[c]

[a]Significant difference compared to the DM group.
[b]Significant difference compared to the Pre-RT group.
[c]Significant difference compared to the Pre-HPX group.
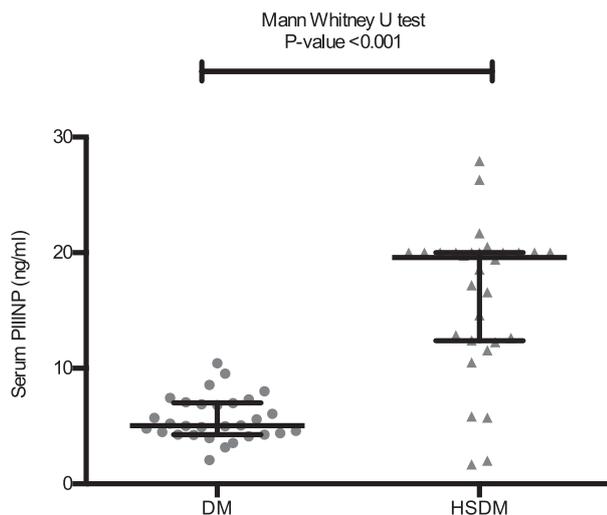
Fig 2. Serum PIIINP concentration in cats with diabetes mellitus (DM) (circles) and hypersomatotropism and diabetes mellitus (HSDM) (triangles). Median values are indicated by long horizontal lines and interquartile ranges (IQR) are indicated by short horizontal lines.

Discussion

Serum PIIINP was found to be significantly increased in diabetic cats with HS compared to those without. This observation emphasizes the marked body-wide impact excess GH has in cats with HS beyond its impact on insulin sensitivity, mirroring findings in human acromegalic patients.[17,23] Our study also suggests that there is merit in exploring the potential for serum PIIINP concentration as a diagnostic tool, along with other established tools, showing a sensitivity of 86.7% and specificity of 100% when using a cut-off value of 10.5 ng/mL for the diagnosis of HS. Finally,

the significant decrease in both IGF-1 and PIIINP concentrations after HPX but not RT, further indicates the necessity for studies into possible differences in effectiveness of these treatment modalities, beyond the ability to ameliorate the HS-associated diabetic state.
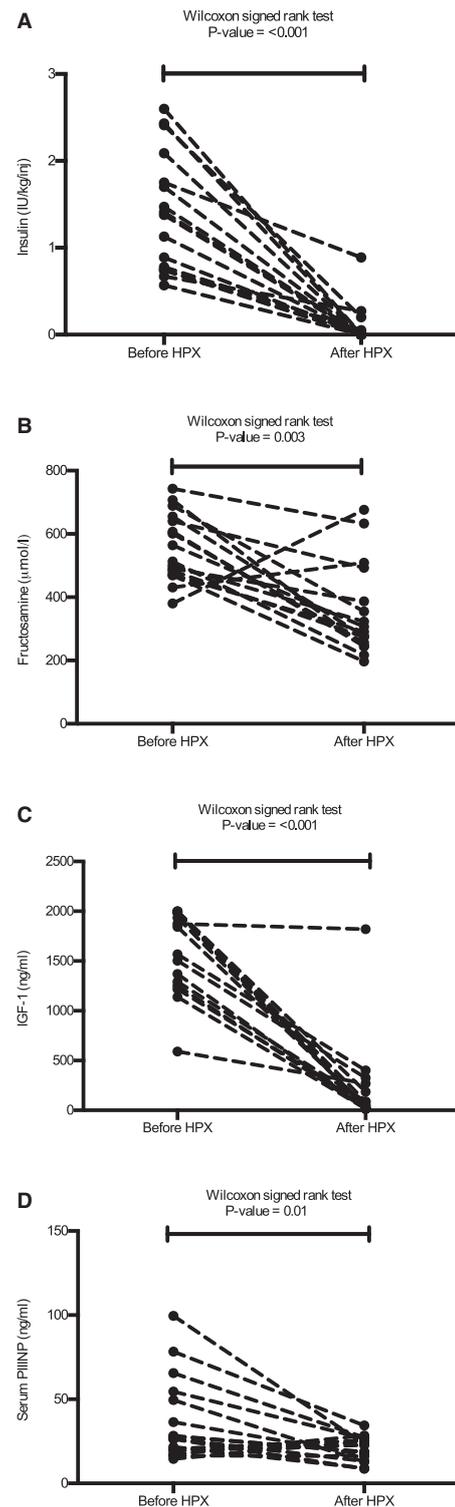
Serum PIIINP concentration depends on the rate of PIIINP production in the tissue of origin (mainly connective tissue), release into the bloodstream and degradation and elimination by the liver and kidneys.[25] In general, an ideal biomarker should be measurable in the target species, stable, obtained easily and non-invasively, have good sensitivity and specificity for the disease process, and allow rapid turnaround of results.[26] Unlike GH, PIIINP does not exhibit pulsatile secretion; it also has a short half-life of 1 hour in circulation.[27] The lack of commercial availability of a recombinant feline PIIINP could have posed a limitation in its assessment. However, good cross-reactivity between feline and human PIIINP was expected because collagen synthesis and metabolism have been shown to be well preserved among mammals.[28] The assay validation process highlights the current level of accuracy and precision for the chosen ELISA. The stability of PIIINP, as evidenced by the lack of freeze-thaw effect in our study and others,[29] suggests the possibility of clinical use with submission of samples in the practice setting. The higher inter-assay CV for low and high concentrations should be born in mind. For high concentrations, this vulnerability could be circumvented by appropriate dilution of the samples. The validation process could be strengthened further by including a more extensive range of concentrations for the purpose of CV calculations.

The signalment of cats was matched on the basis of breed, sex, and age (in that order) to prevent impact of signalment-associated confounding factors. Cats were not matched on weight, and HSDM cats had significantly higher body weights. This observation could be
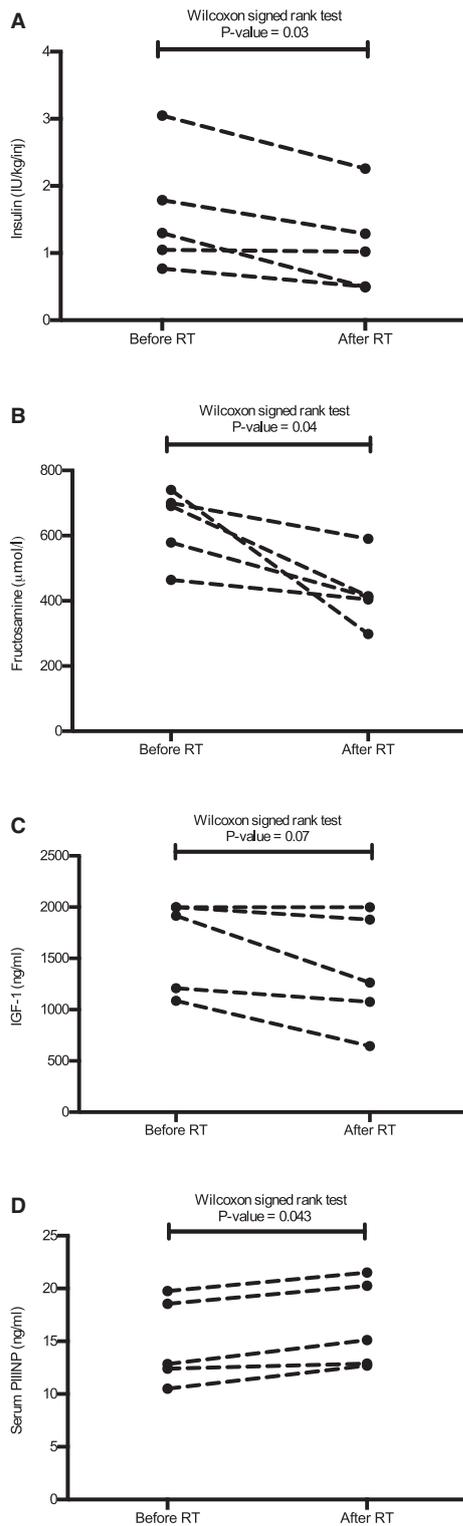
Fig 3. Insulin **(A)**, fructosamine (B), IGF-1 (C), IGF-1 **(C),** and PIIINP **(D)** concentrations before and after radiation therapy (RT) for the 5 cats with HSDM that underwent RT. Dashed lines connect pairs of observations before and after RT.

Fig 4. Insulin **(A)**, fructosamine **(B)**, IGF-1 **(C),** and PIIINP **(D)** concentrations before and after hypophysectomy (HPX) for the 16 cats with HSDM that underwent HPX. Dashed lines connect pairs of observations before and after HPX.

because of the nature of HS, where excess GH and IGF-1 can result in an increase in body mass. Nevertheless, no correlation could be detected between weight

and PIIINP in the cats of our study, making it unlikely that differences in weight accounted for the difference in PIIINP concentrations between the 2 groups. This

conclusion is further substantiated by the fact that in cats that showed an objective response to treatment of HS (defined as decreased IGF-1 concentration[23] or decrease in insulin requirement to control clinical signs), this response led to a significant decrease in PIIINP, but not body weight. Body weight may be a poor marker of PIIINP-secreting tissues. Therefore, use of this test alone, as compared to incorporating results of feline body mass index calculation and dual-energy X-ray absorptiometry (DXA) in all cases is a study limitation. Another limitation is the lack of confirmatory pituitary histopathology for all HSDM cases (available for 14/30 cats). Nevertheless, histopathology was available for 14/30 cats, and all 30 cats had macroadenoma and increased IGF-1, which have been considered to be sufficient for a clinical diagnosis of HS in both in human and veterinary medicine.[3,30] The insulin requirement of cats with confirmed HS varied based on the time of referral. Not all HSDM cases were insulin-resistant (if defined as an insulin requirement >1.5 IU/kg/injection on a q12h protocol) at the time of assessment. This observation is in agreement with recent literature that describes confirmed HSDM cases as having a wide range of insulin requirements, and demonstrating that insulin resistance does not necessarily need to be present for a diagnosis of HSDM to be made.[1,3,31]

The PIIINP testing could have different characteristics to offer as a screening test, which might enhance the value of current IGF-1 screening. Indeed, non-acromegalic diabetic cats can have increased IGF-1 concentrations, and newly diabetic acromegalic cats may have normal IGF-1 concentrations. Lack of portal venous insulin, not uncommon to diabetics, has been implicated in the latter.[11] Measurement of serum PIIINP concentration may help identify cats with false-positive or false-negative IGF-1 concentrations. Future studies of these clinically challenging groups are warranted.

The occasional inaccuracy of serum IGF-1 concentration is also illustrated by the current study, where 1 HSDM cat in the HPX group, with histologically confirmed somatotrophinoma, had an IGF-1 concentration >2000 ng/mL at time of initial diagnosis, but an IGF-1 concentration of 590 ng/mL just before HPX. In human medicine, the variability of IGF-1 concentrations is recognized, but not completely understood. Some suggestions include an effect of insulin-like growth factor binding proteins (IGFBP), or fragments of these, after sample extraction that may interfere with the assay, leading to falsely decreased or increased total IGF-1 concentration. In addition, the integrity of IGF-1 may be altered in vitro, leading to inconsistencies in measurement.[32] Interestingly, the PIIINP concentration in the sample described above (IGF-1 590 ng/mL) was increased (26.6 ng/mL). In humans and cats, other disease states may affect IGF-1 concentration, depending on the type of assay used.[33] Concurrent hepatopathies decreased IGF-1 production, whereas renal failure and solid tumors increased the concentration of IGFBP and thus plasma IGF-1 concentration in humans.[34] Hyperthyroidism increased and lymphoma decreased IGF-1 concentrations in cats in 1 study, suggesting that

interpretation of results becomes more difficult if these conditions are present in cats with DM.[33] In turn, several disease processes other than HS have been reported to affect PIIINP concentrations in humans, including chronic hepatitis and fibrosis,[35,36] pulmonary hypertension,[37] diabetic retinopathy,[38] and hyperthyroidism.[39] This observation emphasizes that PIIINP is by no means a specific marker for HS or any other specific disease but merely identifies abnormalities in collagen type III metabolism.

We aimed to exclude other such disease processes by ensuring that thorough physical examinations and histories were performed and performing screening tests on HSDM cats when appropriate, including serum total T4 concentration and abdominal imaging (eg, ultrasound examinations or computed tomography), as well as by following the progress of cats after their initial visits. Two previous studies demonstrated use of PIIINP concentration in dogs.[40,41] Future studies assessing the impact of common disease processes and varying physiological states in the cat also are indicated, but were beyond the scope of our current study. Using diabetic cats as a control group likely makes the findings of our study stronger, given the known high prevalence of comorbidities in diabetic cats,[42] all with potential to affect PIIINP concentrations. Regardless, pretreatment HSDM and DM cats still could be differentiated in most cases on the basis of serum PIIINP concentration, suggesting a strong correlation between substantially increased PIIINP concentration and the effects of excess GH caused by HS.

As in previous studies, no significant difference was found in IGF-1 concentration pre- and post-RT in HSDM cats.[16,43] Ideally, more cats would have been recruited into this group. However, given the fact that HPX was available to owners as an equally expensive alternative with greater success rate,[d,e,5,44,45] this was not possible. The low statistical power for cats undergoing RT could have prevented detection of a significant difference after this therapeutic modality. In 2 of the 5 cats undergoing RT, we could not accurately measure pre-RT IGF-1 concentration because it exceeded the upper LoD (Fig 3C). The decrease in IGF-1 post-RT therefore may have been more significant than identified in our study. Interestingly, PIIINP concentrations increased significantly after RT. This finding could indicate a lack of consistent efficacy of RT as a treatment for HS. Indeed, even in cases which see diabetes resolved after RT, ongoing soft tissue changes have been observed.[43] Alternatively, RT may be associated with a late onset of treatment effect.[5,16] Cats therefore may not have yet fully responded to RT, and GH concentration may still have been high, leading to ongoing body-wide increased soft tissue turnover. This hypothesis would be consistent with the fact that IGF-1 concentrations also did not decrease in these cats. However, the cats did show a clinical response to RT with all cats requiring less insulin after RT. A delay in the maximum RT-response nevertheless could explain a lack of change in PIIINP, but it does not explain why concentrations actually increased. The latter could have

been a consequence of RT-induced damage and resulting fibrosis.

The human literature on serum PIIINP concentrations after RT, however, does not indicate a consistent demonstrable systemic increase. For instance, in women receiving RT for breast cancer, when samples were taken 10–96 months (mean 26) after RT, local skin collagen turnover increased, although serum PIIINP concentrations did not.[46] This is a period of time similar to that in the studied cats. An additional study in humans assessing serum PIIINP concentrations weekly during and after a 5-week period of high-dose hemi-thorax RT for pleural mesothelioma or nonsmall cell lung cancer also did not identify consistently increased PIIINP concentrations.[47] Studies on the effects of RT on serum PIIINP concentrations in cats, aside from our study, are not available. The PIIINP concentrations might have decreased if samples had been taken even later after RT. Indeed, in people with acromegaly, a decrease in IGF-1 can be identified up to 15 years after RT.[48] Unfortunately, samples were only available over a more limited time period and their recruitment heavily relied on owners returning for the suggested re-evaluations.

In contrast to RT, in people, serum, IGF-1 concentrations rapidly decrease after HPX. Normalization of GH and IGF-1 is used to assess biochemical control of acromegaly and acts as a predictor of long-term survival post-surgery.[30] Markers of collagen synthesis, including PIIINP, and IGF-1 also have been shown to improve after successful medical management of acromegaly in humans with the GH receptor antagonist pegvisomant.[22] The same would be expected with other treatment modalities, including HPX. This improvement was encountered in the HSDM cats that underwent HPX in our study, where both IGF-1 and PIIINP concentrations decreased significantly in 12 of 16 cats. Alternative explanations for the increase in PIIINP post-HPX in 4 of the 16 cats include the effect of surgical trauma and collagen formation from healing soft tissue and scar formation. These 4 cats had follow-up samples taken 1, 3, and 13 months post-treatment, compared to a median of 5 months in those with decreased PIIINP concentrations (range, 1–10). In the human literature, serum PIIINP concentration has been shown to peak 2–3 weeks after orthopedic surgery and to remain increased for up to 60 days.[49] In animal models, changes in serum PIIINP concentration mirror collagen formation also supporting this hypothesis.[50] Despite the increase in PIIINP concentration, 3 of 4 of these cats no longer required insulin treatment at the time of assessment. Diabetic remission was common among the HPX group with 12 of 16 cats not requiring insulin treatment post-HPX, compared to all 5 RT cats requiring insulin post-RT.

It is not known how some medications would affect serum PIIINP concentrations in cats. This consideration could be relevant because all HPX cats received hormone replacement after pituitary gland removal, which included 2.5 mg hydrocortisone q24h,[5,e] 0.1 mg levothyroxine q24h,[51,e] and a decreasing amount of synthetic antidiuretic hormone (desmopressin acetate, q8h initially, then tapered)[e]. The effect of these drugs on serum PIIINP concentrations is unknown, but all are used for physiologic replacement of lost endogenous hormone function.

In conclusion, serum PIIINP concentrations are significantly increased in cats with HSDM compared to those with DM, likely indicating increased soft tissue turnover. The potential for serum PIIINP concentration as an additional diagnostic biomarker for HS in cats should be further evaluated. In addition, serum PIIINP concentration appears to decrease after HPX.

Footnotes

[a] Human N-terminal propeptide of collagen alpha-1 (III) chain ELISA kit, EIAab Science Co., LTD, Wuhan, China
[b] GraphPad Prism version 5.0 a for Windows, GraphPad Software, San Diego, CA
[c] SPSS Statistics 22, SPSS Inc, Chicago, IL, USA
[d] Kenny PJ SC, Keyte SV, Swan JW, Fowkes RC, Church DB, Forcada Y, Niessen SJM. Experiences of a newly established hypophysectomy clinic for treatment of feline hypersomatotropism. J Vet Intern Med 2015;29:1271
[e] Kenny PJ SC, Keyte SV, Swan JW, Fowkes RC, Church DB, Forcada Y, Niessen SJM. Treatment of feline hypersomatotropism - efficacy, morbidity and mortality of hypophysectomy. J Vet Intern Med 2015;29:127

Acknowledgments

References

1. Niessen SJ, Petrie G, Gaudiano F, et al. Feline acromegaly: An underdiagnosed endocrinopathy? J Vet Intern Med 2007;21:899–905.

2. Niessen SJ, Khalid M, Petrie G, et al. Validation and application of a radioimmunoassay for ovine growth hormone in the diagnosis of acromegaly in cats. Vet Rec 2007;160:902–907.

3. Niessen SJ, Forcada Y, Mantis P, et al. Studying cat (Felis catus) diabetes: Beware of the acromegalic imposter. PLoS ONE 2015;10:e0127794.

4. Niessen SJ. Feline acromegaly: An essential differential diagnosis for the difficult diabetic. J Feline Med Surg 2010;12:15–23.

5. Niessen SJ, Church DB, Forcada Y. Hypersomatotropism, acromegaly, and hyperadrenocorticism and feline diabetes mellitus. Vet Clin North Am Small Anim Pract 2013;43:319–350.

6. Peterson ME, Taylor RS, Greco DS, et al. Acromegaly in 14 cats. J Vet Intern Med 1990;4:192–201.

7. Peterson ME. Acromegaly in cats: Are we only diagnosing the tip of the iceberg? J Vet Intern Med 2007;21:889–891.

8. Berg RI, Nelson RW, Feldman EC, et al. Serum insulin-like growth factor-I concentration in cats with diabetes mellitus and acromegaly. J Vet Intern Med 2007;21:892–898.

9. Norman EJ, Mooney CT. Diagnosis and management of diabetes mellitus in five cats with somatotrophic abnormalities. J Feline Med Surg 2000;2:183–190.

10. Greco DS. Feline acromegaly. Top Companion Anim Med 2012;27:31–35.

11. Starkey SR, Tan K, Church DB. Investigation of serum IGF-I levels amongst diabetic and non-diabetic cats. J Feline Med Surg 2004;6:149–155.

12. Elliott DA, Feldman EC, Koblik PD, et al. Prevalence of pituitary tumors among diabetic cats with insulin resistance. J Am Vet Med Assoc 2000;216:1765–1768.

13. Roomp K, Rand J. Intensive blood glucose control is safe and effective in diabetic cats using home monitoring and treatment with glargine. J Feline Med Surg 2009;11:668–682.

14. Gostelow R, Forcada Y, Graves T, et al. Systematic review of feline diabetic remission: Separating fact from opinion. Vet J 2014;202:208–221.

15. Borgeat KNS, Scudder C, Gostelow R, et al. Feline hypersomatotropism is a naturally occurring, reversible cause of myocardial remodelling. J Vet Intern Med 2015;29:1263.

16. Dunning MD, Lowrie CS, Bexfield NH, et al. Exogenous insulin treatment after hypofractionated radiotherapy in cats with diabetes mellitus and acromegaly. J Vet Intern Med 2009;23:243–249.

17. Piovesan A, Terzolo M, Reimondo G, et al. Biochemical markers of bone and collagen turnover in acromegaly or Cushing's syndrome. Horm Metab Res 1994;26:234–237.

18. Ezzat S, Melmed S, Endres D, et al. Biochemical assessment of bone formation and resorption in acromegaly. J Clin Endocrinol Metab 1993;76:1452–1457.

19. Prockop DJ, Kivirikko KI, Tuderman L, et al. The biosynthesis of collagen and its disorders (first of two parts). N Engl J Med 1979;301:13–23.

20. Sartorio A, Agosti F, Marazzi N, et al. Combined evaluation of resting IGF-I, N-terminal propeptide of type III procollagen (PIIINP) and C-terminal cross-linked telopeptide of type I collagen (ICTP) levels might be useful for detecting inappropriate GH administration in athletes: A preliminary report. Clin Endocrinol (Oxf) 2004;61:487–493.

21. Guha N, Erotokritou-Mulligan I, Burford C, et al. Serum insulin-like growth factor-I and pro-collagen type III N-terminal peptide in adolescent elite athletes: Implications for the detection of growth hormone abuse in sport. J Clin Endocrinol Metab 2010;95:2969–2976.

22. Parkinson C, Kassem M, Heickendorff L, et al. Pegvisomant-induced serum insulin-like growth factor-I normalization in patients with acromegaly returns elevated markers of bone turnover to normal. J Clin Endocrinol Metab 2003;88:5650–5655.

23. Brabant G. Insulin-like growth factor-I: Marker for diagnosis of acromegaly and monitoring the efficacy of treatment. Eur J Endocrinol 2003;148(Suppl 2):S15–S20.

24. Wilkinson ADMA. IUPAC. Compendium of Chemical Terminology, 2nd ed. New Jersey: (the "Gold Book") Blackwell Scientific Publications; 1997.

25. Risteli J, Risteli L. Analysing connective tissue metabolites in human serum. Biochemical, physiological and methodological aspects. J Hepatol 1995;22:77–81.

26. Strimbu K, Tavel JA. What are biomarkers? Curr Opin HIV AIDS 2010;5:463–466.

27. Jensen LT. The aminoterminal propeptide of type III procollagen. Studies on physiology and pathophysiology. Dan Med Bull 1997;44:70–78.

28. Jones SR. Structure, biosynthesis and disorders of collagen: A review. Vet Clin Pathol 1976;5:4–16.

29. Jensen LT, Henriksen JH, Risteli J, et al. Fate of circulating amino-terminal propeptide of type III procollagen in conscious pigs. Am J Physiol 1993;265:R139–R145.

30. Barkan AL. Biochemical markers of acromegaly: GH vs. IGF-I. Growth Horm IGF Res 2004;14(Suppl A):S97–S100.

31. Scudder CJ, Gostelow R, Forcada Y, et al. Pasireotide for the Medical Management of Feline Hypersomatotropism. J Vet Intern Med 2015;29:1074–1080.

32. Janssen JA, van der Lely AJ, Lamberts SW. Circulating free insulin-like growth-factor-I (IGF-I) levels should also be measured to estimate the IGF-I bioactivity. J Endocrinol Invest 2003;26:588–594.

33. Tschuor F, Zini E, Schellenberg S, et al. Evaluation of four methods used to measure plasma insulin-like growth factor 1 concentrations in healthy cats and cats with diabetes mellitus or other diseases. Am J Vet Res 2012;73:1925–1931.

34. Jenkins PJ, Mukherjee A, Shalet SM. Does growth hormone cause cancer? Clin Endocrinol (Oxf) 2006;64:115–121.

35. Giustina G, Fattovich G, De Paoli M, et al. Serum procollagen type III peptide in chronic hepatitis B. Relationship to disease activity and response to interferon-alpha therapy. Int J Clin Lab Res 1996;26:33–36.

36. Plebani M, Burlina A. Biochemical markers of hepatic fibrosis. Clin Biochem 1991;24:219–239.

37. Agrinier N, Thilly N, Boivin JM, et al. Prognostic value of serum PIIINP, MMP1 and TIMP1 levels in hypertensive patients: A community-based prospective cohort study. Fundam Clin Pharmacol 2013;27:572–580.

38. Arkkila PE, Ronnemaa T, Koskinen PJ, et al. Biochemical markers of type III and I collagen: Association with retinopathy and neuropathy in type 1 diabetic subjects. Diabet Med 2001; 18:816–821.

39. Faber J, Horslev-Petersen K, Perrild H, et al. Different effects of thyroid disease on serum levels of procollagen III N-peptide and hyaluronic acid. J Clin Endocrinol Metab 1990;71:1016–1021.

40. Hezzell MJ, Boswood A, Chang YM, et al. Associations among serum N-terminal procollagen type III concentration, urinary aldosterone-to-creatinine ratio, and ventricular remodeling in dogs with myxomatous mitral valve disease. Am J Vet Res 2012;73:1765–1774.

41. Schuller S, Valentin S, Remy B, et al. Analytical, physiologic, and clinical validation of a radioimmunoassay for measurement of procollagen type III amino terminal propeptide in serum and bronchoalveolar lavage fluid obtained from dogs. Am J Vet Res 2006;67:749–755.

42. Kraus MS, Calvert CA, Jacobs GJ, et al. Feline diabetes mellitus: A retrospective mortality study of 55 cats (1982–1994). J Am Anim Hosp Assoc 1997;33:107–111.

43. Littler RM, Polton GA, Brearley MJ. Resolution of diabetes mellitus but not acromegaly in a cat with a pituitary macroadenoma treated with hypofractionated radiation. J Small Anim Pract 2006;47:392–395.

44. Kenny PJSC, Keyte SV, Swan JW, et al. Experiences of a newly established hypophysectomy clinic for treatment of feline hypersomatotropism. J Vet Intern Med 2015;29:1271.

45. Kenny PJSC, Keyte SV, Swan JW, et al. Treatment of feline hypersomatotropism – efficacy, morbidity and mortality of hypophysectomy. J Vet Intern Med 2015;29:1271.

46. Riekki R, Jukkola A, Sassi ML, et al. Modulation of skin collagen metabolism by irradiation: Collagen synthesis is increased in irradiated human skin. Br J Dermatol 2000;142:874–880.

47. Maasilta P, Salonen EM, Vaheri A, et al. Procollagen-III in serum, plasminogen activation and fibronectin in bronchoalveolar

lavage fluid during and following irradiation of human lung. Int J Radiat Oncol Biol Phys 1991;20:973–980.

48. Swearingen B, Barker FG 2nd, Katznelson L, et al. Long-term mortality after transsphenoidal surgery and adjunctive therapy for acromegaly. J Clin Endocrinol Metab 1998;83:3419–3426.

49. Joerring S, Jensen LT. Changes in collagen metabolites in serum after cemented hip and knee arthroplasty. Arch Orthop Trauma Surg 1993;112:139–141.

50. Jensen LT, Garbarsch C, Horslev-Petersen K, et al. Collagen metabolism during wound healing in rats. The aminoterminal propeptide of type III procollagen in serum and wound fluid in relation to formation of granulation tissue. Apmis 1993;101:557–564.

51. Ramsey I. BSAVA Small Animal Formulary, 8th ed. Gloucester: BSAVA; 2014.