

# 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

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**Objective**—To evaluate 4 methods used to measure plasma insulin-like growth factor (IGF) 1 concentrations in healthy cats and cats with diabetes mellitus or other diseases.

**Animals**—39 healthy cats, 7 cats with diabetes mellitus, and 33 cats with other diseases.

**Procedures**—4 assays preceded by different sample preparation methods were evaluated, including acid chromatography followed by radioimmunoassay (AC-RIA), acid-ethanol extraction followed by immunoradiometry assay (AEE-IRMA), acidification followed by immunochemiluminescence assay (A-ICMA), and IGF-2 excess followed by RIA (IE-RIA). Validation of the methods included determination of precision, accuracy, and recovery. The concentration of IGF-1 was measured with all methods, and results were compared among cat groups.

**Results**—The intra-assay coefficient of variation was < 10% for AC-RIA, A-ICMA, and AEE-IRMA and 14% to 22% for IE-RIA. The linearity of dilution was close to 1 for each method. Recovery rates ranged from 69% to 119%. Five healthy cats had IGF-1 concentrations > 1,000 ng/mL with the AEE-IRMA, but < 1,000 ng/mL with the other methods. Compared with healthy cats, hyperthyroid cats had significantly higher concentrations of IGF-1 with the A-ICMA method, but lower concentrations with the IE-RIA method. Cats with lymphoma had lower IGF-1 concentrations than did healthy cats regardless of the method used.

**Conclusions and Clinical Relevance**—Differences in the methodologies of assays for IGF-1 may explain, at least in part, the conflicting results previously reported in diabetic cats. Disorders such as hyperthyroidism and lymphoma affected IGF-1 concentrations, making interpretation of results more difficult if these conditions are present in cats with diabetes mellitus. (*Am J Vet Res* 2012;73:1925–1931)

The GH-IGF-1 axis plays an important role in regulating cell growth and differentiation. Insulin-like growth factor-1 is a major mediator of the growth-promoting effects of GH; thus, circulating IGF-1 concentrations are often used to assess the status of the GH axis.<sup>1-6</sup> Increased IGF-1 is useful in supporting a diagnosis of acromegaly in diabetic cats, and its measurement is currently used by most clinicians for this purpose.<sup>5,6</sup>

Insulin-like growth factor-1 is a small, single-chained polypeptide, most of which is bound to IGFFBPs. In addition, most bound IGF-1 circulates in

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## ABBREVIATIONS

A-ICMA	Acidification immunochemiluminescence assay
AC-RIA	Acid chromatography radioimmunoassay
AEE-IRMA	Acid-ethanol extraction immunoradiometric assay
CV	Coefficient of variation
DM	Diabetes mellitus
GH	Growth hormone
IE-RIA	Insulin-like growth factor-2 excess radioimmunoassay
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor-binding protein

a ternary complex composed of IGF-1, IGFBP-3, and a protein identified as the acid-labile subunit. Because IGFBPs are known to interfere with the measurement of IGF-1 resulting in falsely high values, most immunoassays are preceded by methods that

remove IGFBPs from the samples.<sup>7</sup> Acid chromatography is a reliable method to remove IGFBPs before IGF-1 measurement in humans<sup>7</sup> and has been used in studies<sup>1,3</sup> of diabetic cats. However, acid chromatography is time-consuming and labor-intensive and has therefore been largely replaced by other methods, such as acidification and acid-ethanol extraction or displacement.<sup>7,8</sup> Whether these newer methods are effective in removing IGFBPs from feline plasma samples is unknown. Studies evaluating IGF-1 concentrations in nonacromegalic diabetic cats yielded conflicting results; 1 study<sup>2</sup> found higher IGF-1 concentrations, whereas 2 other studies<sup>1,4</sup> found lower IGF-1 concentrations in cats with newly diagnosed DM, compared with results for healthy cats. Some of those discrepancies may have been attributable to procedural problems, such as incomplete removal of IGFBPs, which have been found in studies of humans with DM.<sup>8</sup>

A number of chronic diseases affect IGF-1 concentrations in humans.<sup>9</sup> Whether diseases other than DM affect IGF-1 concentrations and account for some of the conflicting results in studies of cats has not been investigated.

The objective of the study reported here was to compare 4 methods for IGF-1 measurement in healthy cats, cats with newly diagnosed DM, and cats with diseases known to increase or decrease IGF-1 in humans.

## Materials and Methods

**Healthy cats**—Thirty-nine healthy cats were enrolled in the study and allocated to 2 groups according to age. One group consisted of 19 young castrated male cats with a median age of 3 years (range, 2 to 4.5 years) and a median body weight of 4 kg (range, 3 to 4.5 kg). The other group consisted of 20 middle-aged or older cats with a median age of 10 years (range, 6 to 14 years) and a median body weight of 5 kg (range, 4 to 6 kg). Of the 20 cats, 3 were spayed females and 17 were castrated males. All of the cats were considered healthy on the basis of results of physical and laboratory examinations; blood glucose concentrations were within the reference range. Blood samples were collected during routine health examinations.

**Cats with diabetes mellitus**—Seven cats with DM were enrolled in the study. They consisted of 5 castrated male and 2 spayed female cats with a median age of 12 years (range, 7 to 15 years) and a median body weight of 6 kg (range, 3 to 10 kg). A diagnosis of DM was based on clinical signs (including polyuria, polydipsia, and weight loss), hyperglycemia (nonfed blood glucose > 9 mmol/L), and high fructosamine concentrations (> 340 μmol/L). The cats had no concurrent diseases.

**Cats with various diseases**—Thirty-three cats with various diseases other than DM were included in the study. All cats underwent a complete physical examination and laboratory testing to identify the underlying disorder.

Hyperthyroidism was diagnosed in 7 cats on the basis of clinical signs and high total thyroxine concentrations (> 3.5 μg/dL). There were 4 castrated male and 3 spayed female cats with a median age of 11 years (range, 7 to 17 years) and a median body weight of 3 kg (range, 2 to 4 kg).

Malignant lymphoma was diagnosed in 5 cats on the basis of histologic or cytologic examination of tissue samples by a board-certified pathologist. All cases were multicentric lymphoma. There were 3 castrated male and 2 spayed female cats with a median age of 8 years (range, 5 to 17 years) and a median body weight of 4.5 kg (range, 2 to 5 kg).

Chronic renal failure was diagnosed in 7 cats on the basis of history, clinical signs, laboratory examinations (International Renal Interest Society stage II to IV), and ultrasonographic examination of the kidneys. There were 3 castrated male and 4 spayed female cats with a median age of 15 years (range, 7 to 20) and a median body weight of 3 kg (range, 2 to 4 kg).

Anorexia of unknown cause and lasting for at least 3 days was diagnosed in 7 cats. There were 6 castrated male cats and 1 spayed female cat with a median age of 8 years (range, 4 to 15 years) and a median body weight of 5 kg (range, 4 to 6 kg).

Obesity was diagnosed in 7 cats on the basis of a body condition score > 6 (scale, 1 to 9). There were 5 castrated male and 2 spayed female cats with a median age of 8 years (range, 4 to 15 years) and a median body weight of 6 kg (range 6 to 11 kg).

**Plasma samples**—Blood samples were collected from 79 cats on admission via jugular venipuncture into tubes containing EDTA and centrifuged for 15 minutes at 1,600 × g. The plasma was divided into aliquots in 4 plastic tubes and stored at -80°C until analysis. The samples were shipped to the laboratories on dry ice.

**Methods**—Four methods for measurement of plasma IGF-1 were evaluated. The AC-RIA, which was used by the authors in previous studies,<sup>1,10</sup> was performed at the University Hospital of Zurich as described. The AEE-IRMA<sup>a</sup> and A-ICMA<sup>b</sup> were performed at commercial laboratories.<sup>c,d</sup> For the AEE-IRMA, samples were extracted at a 1:10 extraction ratio of plasma to acid-ethanol with an HCl-ethanol kit component. The acidified sample was incubated at 22°C for 30 minutes and then centrifuged for 3 minutes at 8,000 × g. The supernatant was neutralized with a neutralization buffer (1:2 ratio of supernatant to neutralization buffer) and

Table 1—Intra-assay variation of 4 methods used in 4 samples to measure plasma IGF-1 concentrations in cats.

Sample	AC-RIA		A-ICMA		AEE-IRMA		IE-RIA	
	Mean ± SD (ng/mL)	CV (%)						
I	19.1 ± 1.9	9.9	108.2 ± 1.9	1.8	328 ± 14.3	4.4	17.6 ± 3.9	21.9
II	152 ± 11.1	7.3	232.0 ± 4.7	2.0	464.6 ± 32.9	7.1	321 ± 55.5	17.2
III	385 ± 27.0	7.0	330.2 ± 11.7	3.5	896.1 ± 40.8	4.5	442.1 ± 62.0	14.0
IV	367 ± 26.3	7.2	1,160.6 ± 22.6	1.9	1,169.8 ± 11.2	1.0	1,184 ± 202	17.0

diluted to a final 1:100 dilution of the original sample. After this sample preparation, the IRMA was performed. For the A-ICMA, plasma IGF-1 was measured with a solid-phase, enzyme-labeled CLIA. In this assay, a murine anti-IGF-1 antibody coated to a solid phase (capture antibody) and a polyclonal rabbit anti-IGF-1 antibody conjugated to alkaline phosphatase (detection antibody) were used. To avoid IGFBP interferences, samples were prediluted and acidified (pH, 3.1) to separate IGF-1 and IGFBP-3. Once the sample was neutralized again (ie, restoring pH to 7), IGFBP binding sites were blocked by adding an excess of IGF-2 to prevent reaggregation of IGF-1 and IGFBP-3. Thereafter, the ICMA was performed. The IE-RIA was performed in-house with a commercial kit.<sup>c</sup>

**Validation of methods and statistical analysis—**Validation included determination of precision by evaluation of intra-assay variability and determination of accuracy by dilutional parallelism and standard recovery. Intra-assay variability was calculated by evaluating 4 plasma samples 10 times within the same run of the method, including 2 blood samples from healthy cats and 2 from diabetic cats. Coefficients of variation were

calculated for each run. Dilutional parallelism was assessed by diluting 2 plasma samples from healthy cats to 75%, 50%, 25%, and 12.5% of their original concentration. The expected values were plotted against the observed values. Recovery was determined by mixing plasma samples with different known concentrations of IGF-1 (as measured by AC-RIA). The ratio of observed and expected values and a correlation coefficient ( $r^2$ ) were calculated.

In addition to determining precision and accuracy, the reliability of the methods was further investigated by comparing the results of healthy cats obtained during validation of AC-RIA (the method previously used by the authors<sup>1,3</sup>) with those of the 3 other methods via regression equations and correlation coefficients.

In addition, IGF-1 concentrations of plasma samples of the following cat groups were measured via the 4 methods: healthy cats, healthy cats allocated into young and middle-aged to older cats, and cats with DM lymphoma, hyperthyroidism, chronic renal failure, obesity, or anorexia. Various comparisons were made between the IGF-1 concentrations obtained for different groups of cats and the IGF-1 concentrations obtained by use of the different methods. Plasma IGF-1 concentrations obtained with each method were

compared between healthy young cats and healthy middle-aged to older cats. Plasma IGF-1 concentrations were compared in healthy middle-aged to older cats and cats with the various diseases; healthy middle-aged to older cats were used to ensure that cats of similar ages were compared because diseases are more likely to occur later in life. Healthy middle-aged to older cats were also compared with obese and anorexic cats. All of the comparisons were performed with the Friedman test followed by the Dunn multiple comparisons test. A value of  $P < 0.05$  was considered significant.

## Results

**Precision—**The intra-assay CVs of the 4 methods ranged from 1.0% to 21.9% (Table 1). The CVs of AC-RIA, A-ICMA, and AEE-IRMA were  $< 10\%$ , whereas the CV of the IE-RIA varied from 14.0% to 21.9%.

**Accuracy—**The linearity of dilution of the 4 methods was determined (Figure 1). The slopes of the regression equations and the  $r^2$  were close to 1 for each method. Recovery rates after mixing 2 plasma samples of known IGF-1 concentration were determined results of o:E ratio, range and correlation were shown in Table 2. The AC-RIA and AEE-IRMA yielded recoveries of 80.8% to 115.0%, whereas A-ICMA and IE-RIA had recoveries of 68.9% to 118.6%.

**Comparison with AC-RIA—**The values from AC-RIA, which is the method previously used by the authors as a gold standard,<sup>1,3</sup> were plotted against the observed values of the other methods (Figure 2). The AEE-IRMA method yielded the highest correlation with AC-RIA ( $r^2 = 0.839$ ).

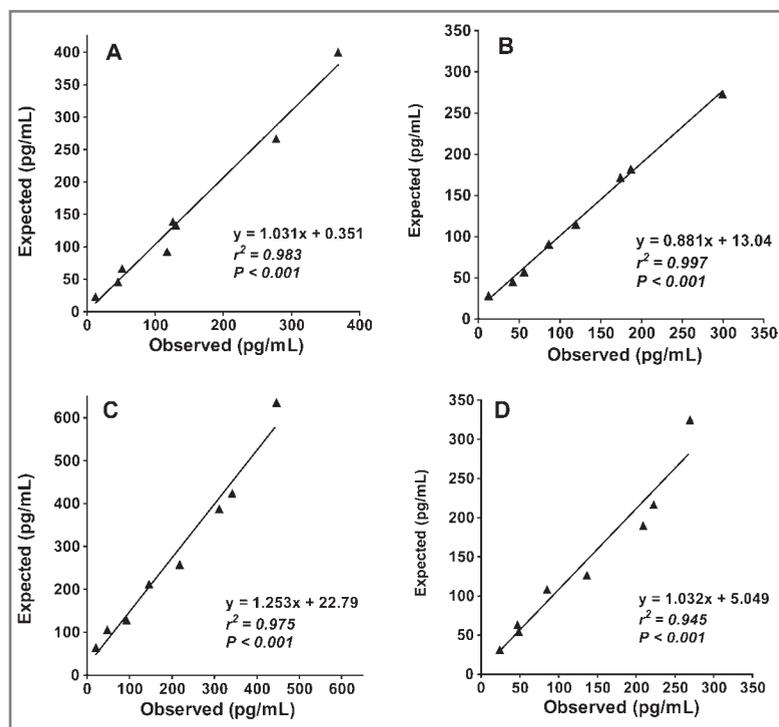


Figure 1—Linearity of dilution of methods (AC-RIA [A], A-ICMA [B], AEE-IRMA [C], and IE-RIA [D]) used to measure plasma IGF-1 concentrations in cats.

Table 2—Accuracy of 4 assays of IGF-1 determined with known feline plasma concentrations.

Assay	O:E ratio (%)	Range (%)	Correlation ( $r^2$ )
AC-RIA	101.8	96.9–108.0	0.996
A-ICMA	97.0	53.9–102.8	0.961
AEE-IRMA	102.5	80.8–115.0	0.985
IE-RIA	97.0	68.9–118.6	0.956

O:E ratio = Ratio of observed concentrations to expected concentrations.

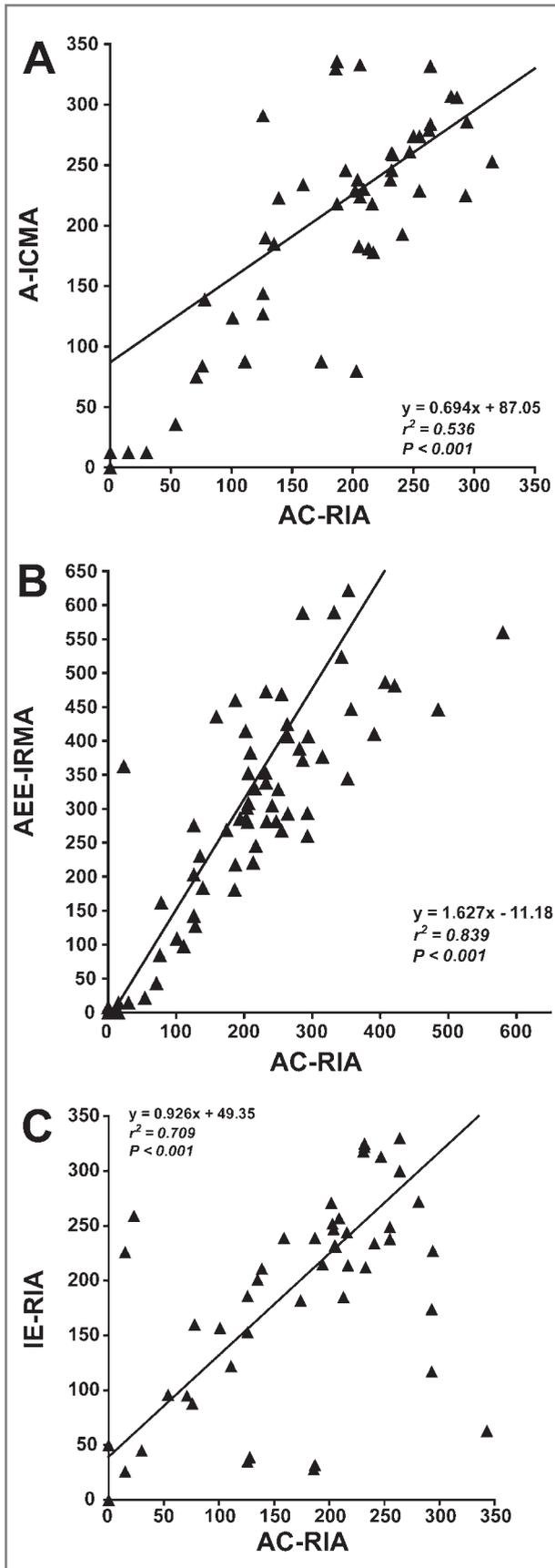


Figure 2—Analysis of correlation between results of AC-RIA and IE-RIA (A), A-ICMA (B), and AEE-IRMA (C).

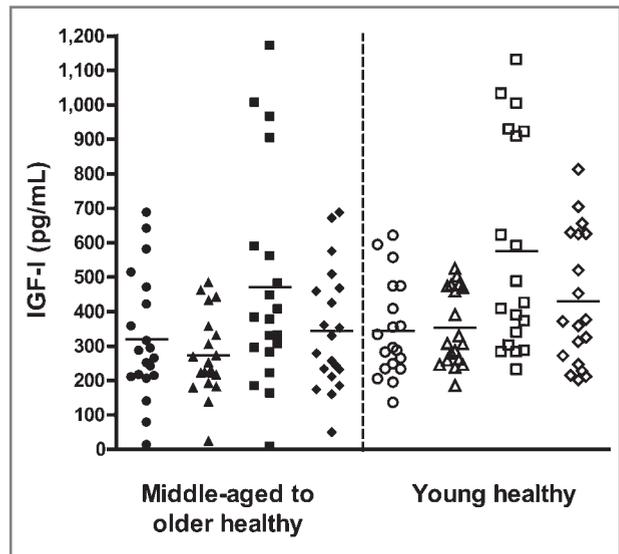


Figure 3—Scattergram of plasma IGF-1 concentrations of healthy middle-aged to older and young cats measured via AC-RIA (circles), A-ICMA (triangles), AEE-IRMA (squares), and IE-RIA (diamonds). Horizontal lines indicate median values.

**Comparison of IGF-1 measurements in healthy cats**—In healthy cats, the median IGF-1 concentration measured with the AEE-IRMA was 407 ng/mL (range, 7.7 to 1,172), which was significantly higher than values obtained with the other methods; the median IGF-1 concentration was 286 ng/mL (range, 13 to 687 ng/mL) for the AC-RIA, 279 ng/mL (range, 12.5 to 525 ng/mL) for the A-ICMA, and 353 ng/mL (range, 50 to 813 ng/mL) for the IE-RIA. The IGF-1 concentrations were compared between healthy young cats and healthy middle-aged to older cats (Figure 3). In both groups, the AEE-IRMA resulted in significantly higher values, compared with results of the other methods. Moreover, the IGF-1 concentrations in 5 healthy cats (2 middle-aged to older cats and 3 young cats) were in the range considered to be consistent with acromegaly (> 1,000 ng/mL).

In healthy cats, the difference between age groups was significant ( $P = 0.018$ ) only with the A-ICMA, although some young cats had higher IGF-1 concentrations than did middle-aged to older cats.

**Comparison of IGF-1 measurements in cats with DM and cats with other diseases**—In cats with DM, the AEE-IRMA yielded some higher values, compared with results of the other methods; however, because of the large overlap, the differences were not significant. Cats with hyperthyroidism had significantly higher IGF-1 concentrations with the A-ICMA, compared with the AC-RIA ( $P = 0.041$ ) and the IE-RIA ( $P = 0.027$ ). In addition, the IE-RIA results were significantly ( $P = 0.031$ ) lower, compared with the AEE-IRMA results. Cats with lymphoma had higher IGF-1 concentrations when measured with the IE-RIA, compared with the AC-RIA and the A-ICMA ( $P = 0.032$  and  $0.026$ , respectively). In cats with chronic renal failure and obesity, results of the AEE-IRMA were significantly higher than those of the IE-RIA ( $P = 0.031$  and  $P = 0.042$ , respectively). Moreover, when IGF-1 was measured in anorectic cats,

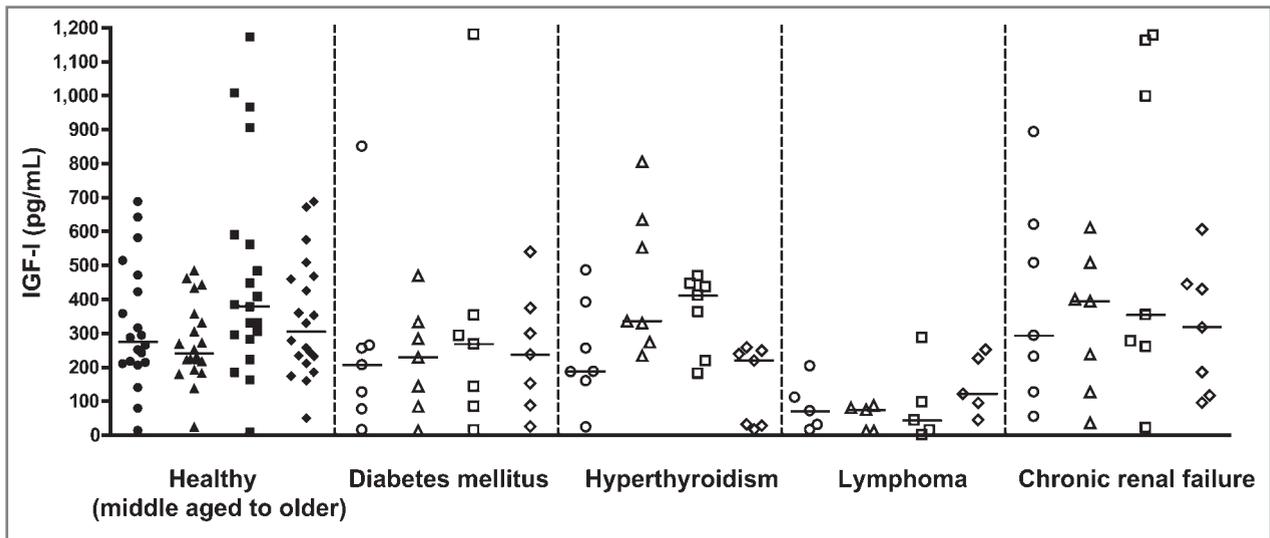


Figure 4—Scattergram of plasma IGF-1 concentrations in healthy middle-aged to older cats and cats with DM, hyperthyroidism, lymphoma, and chronic renal failure measured via AC-RIA (circles), A-ICMA (triangles), AEE-IRMA (squares), and IE-RIA (diamonds). Horizontal lines indicate median values.

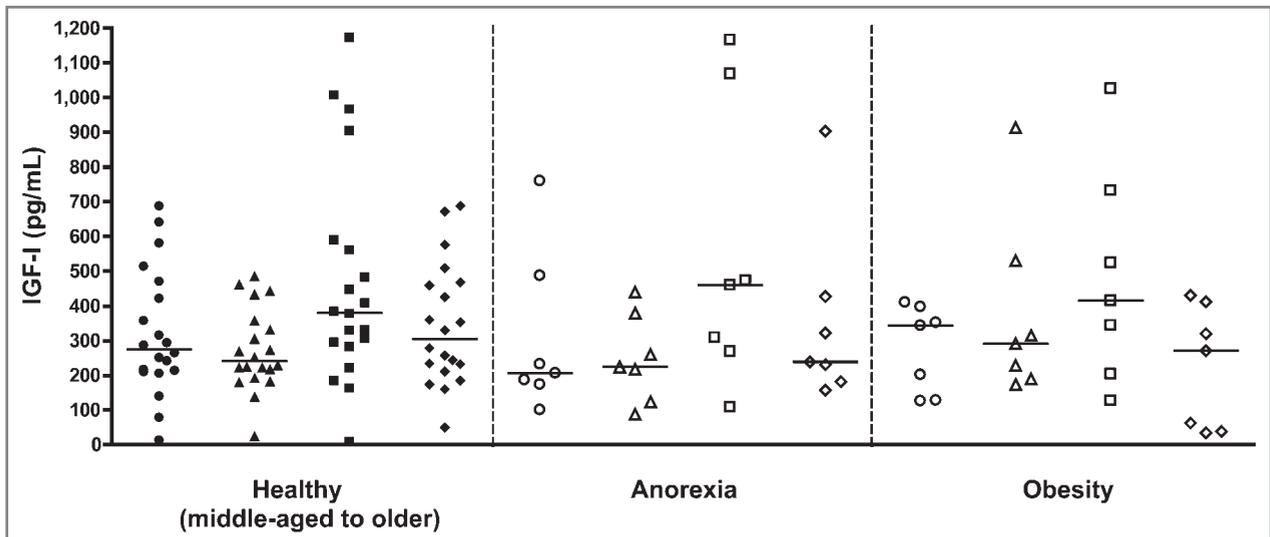


Figure 5—Scattergrams of plasma IGF-1 concentrations in healthy middle-aged to older cats and anorectic or obese cats measured via AC-RIA (circles), A-ICMA (triangles), AEE-IRMA (squares), and IE-RIA (diamonds). Horizontal lines indicate median values.

the AEE-IRMA yielded significantly higher results, compared with all other methods ( $P = 0.031$ , [AC-RIA],  $0.029$  [A-ICMA], and  $0.038$  [IE-RIA], respectively).

Concentrations of IGF-1 in healthy middle-aged to older cats and cats with DM, hyperthyroidism, lymphoma, or chronic renal failure were determined (Figure 4). Cats with DM had lower IGF-1 concentrations than did healthy middle-aged to older cats; the difference was significant ( $P = 0.045$ ) for the AC-RIA. Results from cats with hyperthyroidism were conflicting among the 4 methods. The A-ICMA yielded significantly higher values in hyperthyroid cats than in healthy middle-aged to older cats, whereas the IE-RIA yielded significantly lower values in cats with hyperthyroidism, compared with healthy middle-aged to older cats. Cats with malignant lymphoma had significantly ( $P = 0.015$ ) lower concentrations of IGF-1 than did healthy middle-aged to older cats regardless of the method used.

Cats with chronic renal failure had IGF-1 concentrations similar to those of healthy middle-aged to older cats. Concentrations of IGF-1 in healthy middle-aged to older cats were also compared with those of anorectic and obese cats (Figure 5), but no significant differences were detected regardless of method used.

## Discussion

In previous studies,<sup>1,3</sup> the authors determined that the AC-RIA method yielded low concentrations of IGF-1 in untreated diabetic cats, whereas other studies<sup>3,4</sup> have found both lower and higher concentrations of IGF-1, compared with values in healthy cats. We felt that this discrepancy may have been attributable to technical differences and performed the present study to compare 4 commercially available assays for plasma IGF-1. Results obtained with the 4 methods

varied considerably. All techniques yielded good results for linearity by dilution, and the intra-assay CVs were good to excellent for all assays except the IE-RIA. Recovery was good with the AC-RIA and AEE-IRMA and acceptable with the A-ICMA and IE-RIA. The AEE-IRMA yielded significantly higher IGF-1 concentrations, compared with the other methods.

The IE-RIA had lower precision and accuracy than the other methods, which was surprising because the displacement method used in this procedure yields reliable results in humans.<sup>8</sup> The IE-RIA is also currently used in veterinary medicine and is assumed to be a good assay. We have no explanation for this finding.

The AEE-IRMA had the best agreement with AC-RIA, the technique previously used by our group,<sup>1,3</sup> even though it overestimated IGF-1 concentrations more than the other methods. In human medicine, several studies<sup>7,11,12</sup> revealed that the AEE-IRMA is not entirely efficient in removing IGFBPs and recovering IGF-1. It is possible that the overestimation of IGF-1 concentrations observed in cats was partially attributable to the same mechanism. One of the studies<sup>2</sup> in diabetic cats used the AEE-IRMA, which may explain the conflicting results.

Compared with middle-aged to older cats, IGF-1 concentrations were occasionally higher in young cats, although the difference was not significant. Insulin-like growth factor-1 is the major determinant of the growth-promoting effects of GH<sup>8</sup>; it would therefore be expected that IGF-1 concentrations are higher in young cats than in middle-aged to older cats. Higher IGF-1 concentrations in childhood and during growth in humans have been well documented.<sup>13-15</sup>

Results of the present study were in agreement with those of previous studies,<sup>1,4</sup> which revealed that cats with newly diagnosed DM have lower IGF-1 concentrations than do healthy cats. Low concentrations were also consistently found in humans with type 1 DM.<sup>16-18</sup> This phenomenon is explained by the fact that high insulin concentrations are required in the portal vein for expression and function of GH receptors on hepatocytes and that this mechanism is impaired in insulin-deficient states. Results in humans with type 2 DM have been conflicting. A more recent study<sup>8</sup> found decreased IGF-1 concentrations in type 2 diabetic patients, whereas older studies<sup>19,20</sup> found concentrations similar to those of healthy controls. These discrepancies were thought to be due to different methodologies.<sup>8</sup>

There is a complex relationship between thyroid function, IGF-1 concentration, and IGF-1 binding proteins. In humans with hyperthyroidism, IGF-1 concentrations are reported to be within reference range or increased in most studies, and patients with thyrotoxicosis consistently have high concentrations.<sup>21,22</sup> In the present study, some hyperthyroid cats had higher concentrations than did healthy cats, although the difference was not significant.

Cats with malignant lymphoma had significantly reduced IGF-1 concentrations, compared with healthy cats, and there was no overlap between the 2 groups. This finding is noteworthy and has been observed with

different hematopoietic tumors in humans. In particular, children with non-Hodgkin's lymphoma and leukemia have severely suppressed IGF-1 concentrations.<sup>23</sup> The reason for this seems to be related to increased breakdown of IGF-1 protein, which occurs during severe catabolic stress in tumor-bearing patients.<sup>24</sup> Whether the same mechanism occurs in cats with malignant lymphoma is not known and deserves further investigation. In contrast to lymphoma, chronic renal failure, obesity, and anorexia did not appear to affect IGF-1 concentrations in cats.

It is important to mention the limitations of the present study. To better understand the variation in the results of the different IGFBP separation procedures, it may be helpful to include all of the separation methods (acid-ethanol extraction, acidification, IGF-2 excess, and acid chromatography) with each assay (radioimmunoassay, immunoradiometric assay, and immunochemiluminescent assay). This would provide more information about precision and effectiveness of IGFBP separation. It is also possible that the performance of the different IGF-1 assays differed, in addition to the variations in IGF-1 concentrations attributable to IGFBP separation. The potential limits of IGFBP separation and assay performance may collectively influence IGF-1 results in plasma samples of cats.

Precision and accuracy of the assays were tested by use of samples from healthy and diabetic cats, but not from cats with acromegaly, which are expected to have increased plasma IGF-1 concentration. Including samples with high concentrations of IGF-1 would have helped to better assess reliability of the 4 methods evaluated in the study.

The number of samples used to measure IGF-1 concentration in some diseases, such as chronic renal failure, obesity, and anorexia, may have been too small to detect differences; these 3 disorders are known to be associated with abnormal IGF-1 concentrations in humans.<sup>8,9</sup> Children with chronic renal failure have IGF-1 concentrations within reference range or greater, for unknown reasons.<sup>25</sup> In adult humans, IGF-1 concentrations do not appear to be influenced by kidney function,<sup>26</sup> similar to the cats in our study. In anorexic humans, IGF-1 concentrations are decreased, whereas in obese patients, they are increased.<sup>27</sup> Compared with healthy cats, the concentration of IGF-1 in anorexic and obese cats did not differ.

In conclusion, the methods used for separation of IGFBPs and measurement of circulating IGF-1 concentrations in cats yielded results that differed from each other. Compared with the AC-RIA, which has been used by the authors in the past,<sup>1,3</sup> the AEE-IRMA overestimated IGF-1 concentrations the most. The performance of the A-ICMA was good, whereas that of the IE-RIA was slightly inferior. Therefore, the AC-RIA and A-ICMA seem reliable for use in clinical and research settings in cats. In addition, the present study revealed that IGF-1 concentrations can be influenced by specific disease states such as hyperthyroidism and lymphoma.

- a. DSL-5600 ACTIVE IGF-1-IRMA-Assay, Diagnostic Systems Laboratories, Webster, Tex.
- b. DPC-Immulite 2000, Diagnostic Products, Los Angeles, Calif.

- c. Vet Med Labor, Idexx GmbH, Ludwigsburg, Germany.
- d. Unilabs, Dr. Weber, St Gallen, Switzerland.
- e. IGF-R20, Mediagnost, Reutlingen, Germany.

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