Plasma amylin and insulin concentrations in normoglycemic and hyperglycemic cats

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Abstract

The recently discovered pancreatic peptide amylin is postulated to be involved in the pathogenesis of feline diabetes mellitus. However, plasma amylin concentrations in normal and diabetic cats have not yet been published. The aim of the present study was to validate a commercial amylin radioimmunoassay kit for the measurement of feline amylin in unextracted plasma, and to measure plasma amylin concentrations in normal and diabetic cats. The kit had satisfactory specificity, sensitivity, accuracy, and precision, and can be recommended for measurement of feline amylin in unextracted EDTA plasma, when nonspecific binding of plasma samples is used in the calculation of measured amylin concentration. Fasting amylin concentration in cats with normal glucose tolerance was 97 ± 4 pmol/L. Plasma amylin increased in parallel with insulin after glucose administration in cats with normal and impaired glucose tolerance. In contrast to cats with normal glucose tolerance, cats with impaired glucose tolerance had markedly delayed amylin and insulin secretion. Diabetic cats had basal hypoinsulinemia combined with hyperamylinemia. Hyperamylinemia may lead to reduced insulin secretion and insulin resistance, and contribute to the development of feline diabetes. In conclusion, feline amylin can be measured in unextracted EDTA plasma. Fasting amylin concentrations are approximately 100 pmol/L, and amylin and insulin are cosecreted in cats with normal and impaired glucose tolerance. Increased amylin concentrations may contribute to the development of feline diabetes mellitus.

Résumé

Les concentrations plasmatiques d'amyline et d'insuline chez les chats normaux et hyperglycémiques

Récemment, l'hypothèse que l'amyline, une peptide pancréatique, serait impliquée dans la pathogenèse du diabète mellitus chez le chat a été soulevés. Toutefois, aucune valeur des concentrations plasmatiques d'amyline n'ont été publiées jusqu'ici chez les chats normaux et diabétiques. Les objectifs de cette étude étaient de valider une épreuve commerciale de radioimmunologie pour mesurer l'amyline chez le chat et de déterminer les concentrations plasmatiques de cette peptide chez les chats normaux et diabétiques.

Le Kit commercial s'est avéré satisfaisant pour les épreuves de spécificité, de sensibilité, de précision et d'exactitude. Les auteurs concluent qu'il peut être recommandé pour déterminer l'amyline sérique chez le chat à partir de sérum préservé dans l'EDTA. Les concentrations d'amyline chez les chats à jeun normoglycémiques étaient de 97 ± 4 pmol/L. À la suite de l'administration de glucose, la concentration plasmatique de l'amyline a augmenté parallèlement à celle de l'insuline chez les chats ayant une tolérance normale ou altérée au glucose. Contrairement aux chats normoglycémiques, les sujets ayant une intolérance au glucose présentaient un délai marqué dans la sécrétion de l'amyline et de l'insuline. Les chats diabétiques avaient une hypoinsulinémie de base associée à une hyperamylinémie. L'hyperamylinémie peur provoquer une diminution de la sécrétion d'insuline et une résistance à l'insuline et ainsi contribuer au développement du diabète félin. En conclusion, l'amyline peut être mesurée chez le chat à partir du plasma préservé dans l'EDTA. Les concentrations chez l'animal à jeun sont environ de 100 pmol/L. L'amyline et l'insuline sont cosécrétées chez les chats normo- et hyperglycémiques. Une concentration élevée d'amyline peut contribuer au développement du diabète mellitus chez le chat.

(Traduit par docteure Thérèse Lanthier)

Can Vet J 1996; 37: 27–34

Introduction

Cats develop a type of diabetes mellitus analogous to human type 2, or non-insulin-dependent diabetes mellitus (NIDDM) (1). Although it is accepted that the main abnormalities in human type 2 diabetes are a combined beta-cell dysfunction, leading to impaired insulin secretion, and insulin resistance (2,3), the exact pathogenesis of the disease is still unknown. The only consistent histological finding in human and feline type 2 diabetics is the deposition of amyloid in pancreatic islets (4,5). The main constituent of pancreatic amyloid is the pancreatic peptide amylin, or islet amyloid polypeptide (6–8). Amylin is synthesized in pancreatic beta-cells and co-secreted with insulin in response to appropriate stimuli, such as, elevated blood glucose concentration or food intake (9–13).

It has been hypothesized that amylin is intimately involved in the development of the metabolic abnormalities associated with feline and human type 2 diabetes (14–17). In cats and humans, amylin reduces basal and glucose-stimulated insulin secretion from the pancreas and induces peripheral insulin resistance, the same metabolic abnormalities observed in type 2 diabetes (18–21).

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T.A. Lutz was a recipient of an Overseas Postgraduate Research Scholarship and a University of Queensland Postgraduate Research Scholarship.

The physiological and pathophysiological importance of amylin's effects are controversial. Increased plasma amylin concentrations have been observed in obese, insulin-resistant humans (22) and in various animal models of obesity, insulin resistance, and type 2 diabetes (23–26). Yet, it is currently unknown if elevated amylin concentrations are causative in the development of these disorders.

To our knowledge, plasma amylin concentrations in normal and diabetic cats have not been published. It was the aim of this study to measure plasma amylin concentrations in normal and diabetic cats. First, we validated a method for measuring feline amylin concentrations in unextracted plasma samples. Second, we measured plasma amylin and insulin concentrations in 17 clinically healthy cats during an intravenous glucose tolerance test. Third, we measured plasma amylin and insulin concentrations in diabetic cats and sick cats with hyperglycemia of unknown origin.

Materials and methods

Validation of amylin radioimmunoassay for use with ethylenediaminetetraacetic acid-treated plasma

The commercial amylin radioimmunoassay (RIA) kit from Peninsula Laboratories (Belmont, California) was originally designed for measurement of feline amylin in extracted plasma samples (manufacturer's instructions for the kit). This kit was validated for use with unextracted feline ethylenediaminetetraacetic acid (EDTA)-treated plasma, using 4 pools of EDTA-treated plasma obtained from 6 clinically healthy cats. The pools were obtained after a 30 h fast (pool I), 3 h after feeding (pool II), and 10 (pool III) or 20 (pool IV) min after IV injection of 1g glucose/kg body weight (BW). A proteinase inhibitor, aprotinin (Trasylol, Bayer, Leverkusen, FRG), was added (500 Kallikrein Inactivator Units/mL) to EDTA-treated plasma to decrease peptide degradation during storage (27). The plasma pools were subdivided into aliquots and frozen at −70°C until assayed.

As nonspecific binding (NSB) of plasma components with the primary or secondary antibody may be different from NSB of the standards and may, therefore, lead to inaccurate results, nonspecific binding was determined for each plasma pool by including an additional tube for each sample. This tube contained 0.2 mL of EDTA-treated plasma, 0.1 mL of radioactively labelled amylin, 0.1 mL of the secondary antibody, 0.1 mL of normal rabbit serum as supplied with the kit, and 0.5 mL of RIA buffer.

The value obtained for nonspecific binding was then used to calculate relative binding of amylin to the antibody:

$$\text{Relative \% bound} = \frac{\text{cpm (unknown sample)} - \text{NSB (unknown sample)}}{\text{cpm (zero standard)} - \text{NSB (standards)}} \times 100$$

(cpm = counts per minute)

Relative binding was used to determine measured amylin concentration in the sample by comparing with the standard curve.

Validation was performed by demonstrating specificity, sensitivity, accuracy, and precision (28,29). Specificity was assessed by investigating dilutional parallelism in serial dilutions of plasma pool III, and by determining biological specificity (30). Biological specificity was evaluated by comparing amylin concentrations in fasted cats (pool I) with those obtained after glucose administration (pools III and IV). Changes of plasma amylin concentration were also compared with those of insulin concentration using Friedman rank analysis of variance. Cross-reactivity of amylin antibodies with insulin has been investigated, and was ruled out by the manufacturer of the kit (see manufacturer's kit instructions).

Sensitivity was determined by measuring the concentration yielding at least 10% displacement of radioactively labelled amylin from the primary antibody (29). Accuracy was assessed by a recovery study where known amounts of feline amylin (provided as the standard in the RIA kit) were added to plasma pool I and the measured amylin concentration was compared with the expected concentration (28,29), using linear regression analysis. Intra and interassay precision were determined from the mean coefficients of variation (CV) of repetitive measurements of the plasma pools within one or different assays. Mean CVs below 10% were considered acceptable, between 10% and 15% marginally acceptable, and above 15% unacceptable (29).

Measured amylin concentration was compared among plasma samples using different anticoagulants and serum samples. Comparison was made in 6 blood samples collected from 6 cats. After collection, samples were immediately transferred into either EDTA, fluoride oxalate, lithium-heparin, or plain tubes. Measured amylin concentrations were compared using the Friedman rank analysis of variance.

Determination of amylin and insulin concentrations during a glucose tolerance test

Seventeen clinically healthy cats (12 castrated males, 5 females), between 18 mo and 8 y (average 4.3 y) were used in the study. All cats had fasting normoglycemia and normal fructosamine concentrations (<407 μmol/L) (31). Fructosamine (FR) was measured using a colorimetric assay (Fructosamine Test Plus, Hoffmann-La Roche, Basel, Switzerland).

The glucose tolerance test (GTT) was performed as follows. On the day before the test, an IV jugular catheter (Intracath, 19 G × 8 in with a 17 G needle; Deseret Medical, Becton Dickinson, Sydney, New South Wales, Australia) and an IV cephalic catheter (Surflo, 22 G × 25 mm, Terumo, Leuven, Belgium) were implanted under thiopentone anaesthesia after sedation with acepromazine. The catheters were flushed with a 0.9% sodium chloride solution containing 5 USP heparin units/mL to maintain patency. The cats were fasted for 12 h before the GTT. For the test, glucose (Astra Pharmaceuticals, Sydney, New South Wales, Australia), 1 g/kg BW as a 50% dextrose solution, was injected IV over 30 s into the cephalic catheter (32). Blood was collected into EDTA tubes from the jugular vein before glucose injection, and at 2, 5, 10, 15, 30, 45, 60, and 90 min after injection. Prior to obtaining each blood sample, 0.5 mL of blood was withdrawn and discarded. After obtaining the blood samples, the jugular catheter was flushed with 0.5 to 1 mL of the heparin-containing

Table 1. Assessment of specificity of an amylin radioimmunoassay kit

a) Specificity by dilutional parallelism: measured amylin concentration and percentage of expected amylin concentration following dilution

Dilution[a]	100	80	60	40	20	10
Measured amylin[b]	104	81	68	47	26	—[d]
Expected amylin[c]	(100)[c]	(97)	(108)	(113)	(125)	—

b) Assessment of biological specificity

	pool I[e]	pool II	pool III	pool IV
Amylin (pmol/L)	54	63	104	129
Insulin (μIU/mL)	6.4	7.9	23.5	21.5

correlation between insulin and amylin: $r_s = 0.92$; $P = 0.07$

[a]Dilution of plasma pool III with saline to 100%, 80%, 60%, 40%, 20%, and 10% of the original concentration
[b]Measured amylin concentration in pmol/L
[c]Amylin concentration measured (pmol/L) by the kit, expressed as percentage of that expected
[d]Not detectable
[e]EDTA-treated plasma, pooled from 6 cats; pool I: 30 h fasted cats; pool II: cats fed 3 h before bleeding; pool III: cats bled 10 min after glucose (1 g/kg BW) injection; pool IV: cats bled 20 min after glucose (1 g/kg BW) injection

saline solution to maintain patency. For volume replacement during the GTT, a volume of 0.9% NaCl solution, equal to the volume of blood collected, was injected beginning 20 min after glucose injection.

Plasma glucose, insulin, and amylin concentrations were measured during the GTT. Glucose concentration was measured using an automated chemistry analyzer (Cobas Mira Analyzer, Hoffmann-La Roche, Basel, Switzerland). Insulin concentration was measured with a RIA kit (Phadeseph, Pharmacia Diagnostics AB, Uppsala, Sweden), previously validated for measurement of feline insulin (30). Amylin was determined using the commercial RIA kit (Peninsula Laboratories), and nonspecific binding was measured for the plasma of each cat at time 0 of the GTT.

The half-time for glucose disappearance ($T_{1/2}$) from blood was determined using a statistical computer program (GraphPad InPlot, GraphPad Software, San Diego California). Linear regression analysis was used to determine the line of best fit from 2 to 90 min for the semilogarithmic plot of glucose concentration versus time. Glucose $T_{1/2}$ was then calculated. The total increase in hormone secretion above basal secretion in the first 20 min after glucose injection was calculated using a computer program (GraphPad InPlot, GraphPad Software) from the integrated area under the graph of hormone concentration versus time.

Results were compared using the unpaired Student's *t*-test, and *P* values <0.05 were considered significant. Results are presented as mean ± standard error of the mean ($\bar{x} \pm s_{\bar{x}}$).

Plasma amylin and insulin concentrations in hyperglycemic cats

Amylin, insulin, glucose, and fructosamine concentrations were measured in samples from 42 sick hyperglycemic cats (glucose >6.9 mmol/L) that were submitted to a veterinary clinical pathology laboratory (Veterinary Pathology Services, East Brisbane, Australia). Plasma glucose was measured in fluoride oxalate-treated plasma, and insulin and amylin concentrations were measured in EDTA-treated plasma. The cats were classified either as diabetic or as having hyperglycemia of unknown origin, according to the FR concentration (31), and if available, the beta hydroxybutyrate concentration. Beta-hydroxybutyrate (Ranbut, Randox RB 530, London, United Kingdom) was measured using a colorimetric assay. Cats were considered diabetic ($n = 24$) if the FR concentration exceeded 407 μmol/L (31) or plasma beta hydroxybutyrate was ≥0.5 μmol/L (normal reference range for the laboratory: 0 to <0.5 μmol/L). Cats with a FR concentration <406 μmol/L and beta hydroxybutyrate <0.5 umol/L ($n = 18$) were classified as having hyperglycemia of unknown origin, because they could have had transient stress induced hyperglycemia or diabetes (33). Because an insufficient sample volume remained after failure of 1 amylin RIA kit, amylin concentrations could only be measured in 14 of the 42 cats (10 diabetic; 4 hyperglycemia of unknown origin). Insulin was measured in all 42 cats.

Statistical comparison between the groups (diabetic; hyperglycemia of unknown origin) was performed using unpaired Student's *t*-test or ANOVA with the Bonferroni post hoc test. Results are shown as mean ± standard error of the mean ($\bar{x} \pm s_{\bar{x}}$). The level of significance was $P < 0.05$.

Results

Validation of amylin radioimmunoassay kit

The amylin RIA kit gave an acceptable result for dilutional parallelism up to and including the 20% dilution (Table 1). Biological specificity of the kit was demonstrated, as the pools obtained after glucose injection (pool III and IV) yielded markedly higher amylin concentrations than did plasma pools I or II (Table 1). In addition, amylin and insulin concentrations increased similarly after glucose injection with the correlation coefficient between amylin and insulin being $r_s = 0.92$ ($P = 0.07$). The RIA kit had a sensitivity level of 25 to 30 pmol/L. Intraassay precision was marginally acceptable (CV = 11%), and interassay precision was acceptable (CV = 10%).

Table 2. Measurement of amylin concentration (pmol/L) in serum or plasma

		Plasma treated with[b]		
Sample #	Serum[a]	Fluoride oxalate	Lithium-heparin	EDTA
1	110	111	103	95
2	111	111	107	102
3	141	159	128	89
4	153	172	145	99
5	148	171	142	105
6	120	122	92	95
Friedman analysis (rank #)				
	3	4	2 ($P < 0.001$)	1

[a]Amylin measured in serum
[b]Amylin measured in plasma

The recovery study gave a good result because the relative recovery (measured amylin concentration: total theoretical concentration) was between 93% and 107%, and the correlation between measured and expected values was highly significant ($r = 0.99$, $P < 0.001$).

Measured amylin concentrations differed significantly ($P < 0.001$) in plasma samples from the different anticoagulants and serum samples (Table 2). Amylin concentrations were highest in fluoride oxalate-treated plasma and lowest in EDTA-treated plasma.

Amylin and insulin concentrations during a glucose tolerance test

Fourteen of the 17 cats with fasting normoglycemia were defined as having normal glucose tolerance (mean glucose half-life = 56.4 ± 4.1 min; range: 29.5 to 77.2 min), based on a normal glucose half-life in plasma of ≤80 min (31,34). Three cats were defined as having impaired glucose tolerance, because the glucose half-life was markedly greater than 80 min (mean glucose half-life 125.5 ± 10.6 min; range 107.0 to 143.7 min). Glucose half-life was significantly different ($P < 0.001$) from cats with normal glucose tolerance (Figure 1).

In cats with normal glucose tolerance ($n = 14$), basal amylin concentration was 97 ± 4 pmol/L (range 70 to 133 pmol/L), basal insulin concentration was 8.2 ± 1.0 μIU/mL (range 4.2 to 20.1 μIU/mL), and basal glucose concentration was 5.6 ± 0.2 mmol/L (range 3.9 to 6.8 mmol/L). Plasma amylin concentration increased in parallel with insulin concentration in response to glucose administration (Figures 2, 3). The mean peak concentrations for amylin (184 ± 3 pmol/L) and insulin (18.5 ± 1.1 μIU/mL) were approximately double the baseline concentration and were reached after 10 to 15 min. The plasma concentration of both hormones slowly declined over the test, and at 90 min after glucose administration, were still above baseline levels. Mean glucose concentration at 90 min was also elevated above normal (14.0 ± 1.5 mmol/L) (Figure 1).

In cats with impaired glucose tolerance ($n = 3$), basal glucose (4.9 ± 0.4 mmol/L; range 4.0 to 5.8 mmol/L), amylin concentrations (101 ± 10 pmol/L; range 87 to 121 pmol/L) and insulin (9.5 ± 2.2 μIU/mL; range 6.2 to 15.4 μIU/mL) were not different from cats with normal glucose tolerance. The insulin response paralleled the amylin response to glucose injection, but both were markedly delayed in these cats compared with cats with normal glucose tolerance (Figures 2, 3). Peak hormone concentrations (amylin: 161 ± 8 pmol/L; insulin: 20.5 ± 1.7 μIU/mL) were not different from those in cats with normal glucose tolerance, but they were only reached 45 to 60 min after glucose injection. First phase secretion of insulin and amylin in the 5 to 10 min period after glucose injection (35) was almost absent in cats with impaired glucose tolerance. The total increase in insulin secretion above basal secretion in the first 20 min after glucose injection, measured as area under the curve, was 37.6 μIU/mL/20 min for cats with impaired glucose tolerance, compared with 153.7 μIU/mL/20 min for cats with normal glucose tolerance. The total increase in amylin secretion above basal secretion in the first 20 min after glucose injection was zero (calculated value: −203 pmol/L/20 min) in cats with impaired glucose tolerance, and 1290 pmol/L/20 min in cats with normal glucose tolerance. In cats with impaired glucose tolerance, insulin and amylin concentrations did not

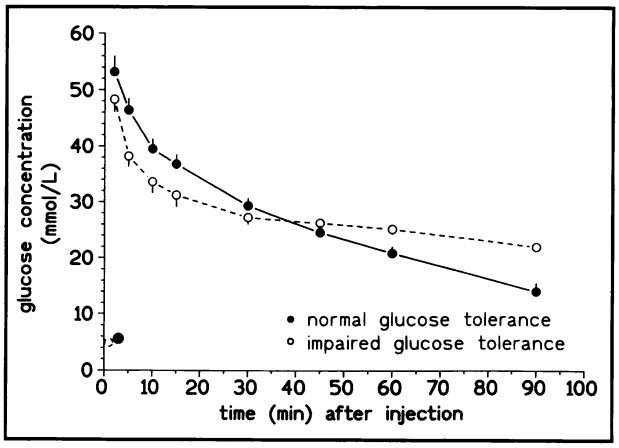

Figure 1. Plasma glucose concentration during the glucose tolerance test in cats with normal (glucose half-life <80 min; $n = 14$; solid line) and impaired (glucose half-life >80 min; $n = 3$; broken line) glucose tolerance.

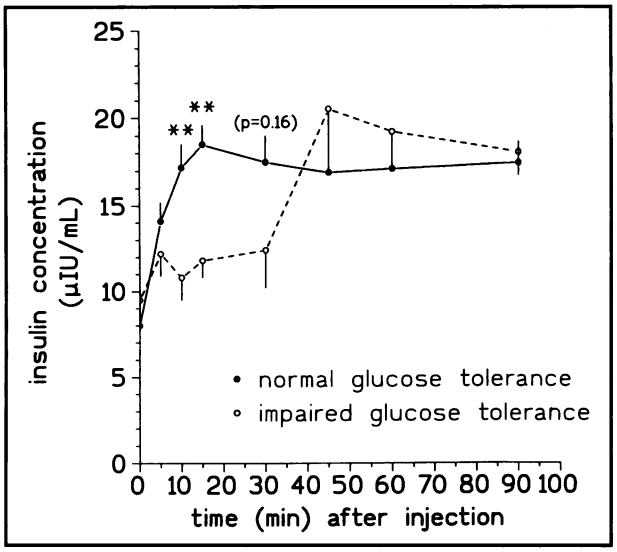

Figure 2. Plasma insulin concentration during the glucose tolerance test in cats with normal (glucose half-life <80 min; $n = 14$; solid line) and impaired (glucose half-life >80 min; $n = 3$; broken line) glucose tolerance. ** significant difference between cats with normal and impaired glucose tolerance, respectively ($P < 0.01$).

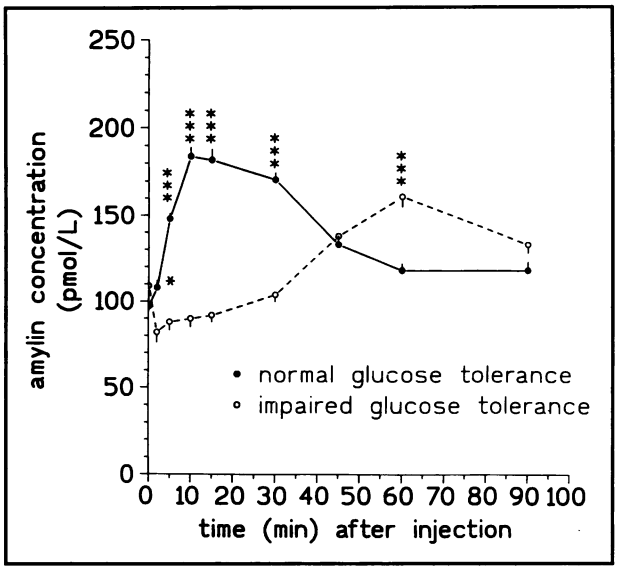

Figure 3. Plasma amylin concentration during the glucose tolerance test in cats with normal (glucose half-life <80 min; $n = 14$; solid line) and impaired (glucose half-life >80 min; $n = 3$; broken line) glucose tolerance. *,*** significant difference between cats with normal and impaired glucose tolerance ($P < 0.05$ and $P < 0.001$, respectively).

increase in the first 30 min after glucose injection, but by 45 min after glucose injection, they had increased and were not significantly different from those in cats with normal glucose tolerance.

Amylin and insulin concentrations in hyperglycemic cats

Forty-two hyperglycemic cats were included in this part of the study. The plasma glucose concentration was elevated in all cats ($\bar{x} \pm s_{\bar{x}}$ 17.5 ± 0.8 mmol/L), compared with the 17 clinically healthy cats (5.5 ± 0.2 mmol/L; $P < 0.001$) used in the previous part of the study. Cats were classified as diabetic ($n = 24$) or having hyperglycemia of unknown origin ($n = 18$), based on FR and beta hydroxybutyrate concentrations (Table 3). Plasma glucose concentration was significantly higher in diabetic cats compared with that in cats with hyperglycemia of unknown origin (Table 3).

In cats with hyperglycemia of unknown origin, basal insulin and amylin concentrations were not significantly different from those in cats with normal glucose tolerance (Table 3). However, insulin concentrations were inappropriately low considering the elevated blood glucose concentrations. In diabetic cats, basal insulin concentration (5.8 ± 0.7 μIU/mL) was significantly decreased compared to that of cats with normal glucose tolerance and cats with hyperglycemia of unknown origin. In contrast, basal amylin concentration (181 ± 13 pmol/L) was significantly higher in diabetic cats, compared with that in normal cats and cats with hyperglycemia of unknown origin (Table 3).

Discussion

Validation of the amylin radioimmunoassay kit

The RIA kit gave satisfactory results for dilutional parallelism and biological specificity, sensitivity, relative recovery (as an indication of accuracy), and precision (28,29), when used according to the protocol described in this study, and can be recommended for measurement of feline amylin in unextracted EDTA-treated plasma samples. This is consistent with a recent report of the reliable measurement of amylin concentrations in unextracted human plasma using a human specific assay (27).

The manufacturer's instructions for the commercial kit recommend the use of extracted samples, because of possible interference with the assay by unspecified factors in feline plasma. In our study, this was accounted for by determining the nonspecific binding in each plasma sample and using it in the determination of measured amylin concentration. No comparison was made of values for measured amylin in extracted and unextracted plasma samples. However, studies have demonstrated that measured amylin concentrations after extraction are lower and may have poor reproducibility. Recovery of amylin after extraction varies from 16% to 90% (27).

Because the basal plasma amylin concentrations in our normal cats were approximately 100 pmol/L, the level of sensitivity for the kit (25 to 30 pmol/L) would have been adequate for the detection of hypoamylinemia, which is a common finding in humans with type 1 and late stages of type 2 diabetes (3,36–38).

The finding that anticoagulants may influence measured amylin values is similar to a recent study that anticoagulants may also affect measured insulin concentrations in cats using certain human commercial RIA kits (30). Although the differences in absolute concentrations were modest, amylin concentrations measured in EDTA-treated plasma were always lower than those measured with other anticoagulants or in serum. Therefore, knowledge of the sample material being used is important for correct interpretation of results from different studies. The reason for the amylin concentrations measured in EDTA-treated plasma being lower than in serum or with other anticoagulants is unknown. More studies are needed to determine the

Table 3. Glucose, fructosamine, beta-hydroxybutyrate, insulin, and amylin concentrations in cats with normoglycemia, hyperglycemia of unknown origin, or diabetes mellitus

	Reference values	Diabetes	Hyperglycemia
Number of cats		24	18
Glucose (mmol/L)	3.3–6.9[a] (5.5 ± 0.2)[b]	20.4 ± 0.9*** (12.9–27.3)[d]	14.5 ± 1.0### (10.0–26.7)[d]
Fructosamine (μmol/L)	326 ± 11[c] (<407)[c]	538 ± 20*** (410–705)[d]	292 ± 15 (134–385)[d]
Beta-hydroxy-butyrate (mmol/L)	<0.5[a]	3.9 ± 0.8***	0.2 ± 0.1
Insulin (μIU/mL)	8.2 ± 1.0[b]	5.8 ± 0.7* (2.0–15.5)[d]	11.0 ± 1.9 (4.0–25.0)[d]
Amylin (pmol/L)	97 ± 4[b]	181 ± 13*** (152–203)[d] (n = 10)	99 ± 9 (83–122)[d] (n = 4)

*,***Significantly different from reference values and hyperglycemic non-diabetic cats ($P < 0.05$ and $P < 0.001$, respectively)
###Significantly different from reference values ($P < 0.001$)
[a]Reference values of the laboratory
[b]Reference values established in this study (n = 14)
[c]Reference values established in a previous study (ref. 31; n = 20)
[d]Range of observations

best method and sample material (plasma or serum) for measuring amylin concentrations in cats.

Amylin and insulin concentrations during a glucose tolerance test

To our knowledge, this is the first report of values for plasma amylin concentrations in cats. The basal amylin concentrations measured in cats with normal glucose tolerance (97 ± 4 pmol/L) were higher than the normal values reported in dogs and humans (<10 pmol/L) (39–41), but similar to those reported in rats (42). Use of unextracted plasma may explain, in part, the higher hormone concentrations in cats compared with dogs, as amylin concentrations in dogs were measured after extraction using column chromatography (40), a technique that reduces the measured hormone concentration (27,38).

Peak amylin concentration was reached 10 to 15 min after glucose injection in cats with normal glucose tolerance. This time is similar to that reported for peak insulin response to occur in cats after glucose injection (43). Both amylin and insulin concentrations were still increased above basal values 90 min after the injection of 1g glucose/kg BW, which was not unexpected, because the glucose concentration was still above normal, and both hormones are secreted in response to glucose.

In other species, amylin is reported to be cosecreted with insulin (9–13,44), and the marked increase in both insulin and amylin concentration that we observed after the injection of glucose supports the cosecretion of these hormones in cats.

Three of the 17 cats with fasting normoglycemia had a glucose half-life greater than 80 min. Based on reference values for cats and nomenclature guidelines for humans (43,45), we classified them as having impaired glucose tolerance because of the marked reduction in their first phase insulin secretion and delayed second phase secretion. These abnormalities of insulin secretion are characteristic of human type 2 diabetes, and have been previously reported in diabetic cats (3,43). Amylin secretion in these cats with impaired glucose tolerance was similarly impaired, and in agreement with findings in laboratory animals with experimentally induced obesity or diabetes (25,37).

In some humans with impaired glucose tolerance or diabetes, basal insulin concentration is increased in response to impaired insulin action in the tissues (insulin resistance) (3). This insulin resistance has been hypothesized to be due to elevated plasma amylin concentration (3,19). Basal amylin concentration was normal in the cats with impaired glucose tolerance, and this may explain why basal insulin concentrations were in the normal range.

Amylin and insulin concentrations in hyperglycemic cats

Diabetic cats had basal hyperamylinemia combined with basal hypoinsulinemia. Basal hypoinsulinemia was an interesting finding considering human type 2 diabetics often have basal hyperinsulinemia, and the similarity between human type 2 and feline diabetes mellitus. However, pancreatic beta-cell secretion in human type 2 diabetics and diabetic cats may be reduced in later stages of the disease (1,3,46). The ratio between plasma amylin and insulin concentrations has been reported as relatively constant under most conditions in normal humans and laboratory animals (37,44). In contrast, absolute hyperamylinemia, or hyperamylinemia relative

to insulinemia, has been reported in humans and animals with impaired glucose tolerance and type 2 diabetes (18,26,47–49). Amylin has been shown to reduce basal and glucose-stimulated insulin secretion in laboratory animals and cats (19,50–52). Therefore, the hyperamylinemia observed in these cats may have caused a reduction in insulin secretion from the pancreas, leading to absolute hypoinsulinemia. Alternatively, glucose toxicity may have contributed to the reduced insulin secretion (43,53–56). Due to the limited number of cats in which amylin concentrations have been measured in the present study, further studies in a larger population of normoglycemic, hyperglycemic, and diabetic cats will be required to support the findings presented here.

Exogenous amylin has been shown to produce peripheral insulin resistance in cats (19). We were unable to determine the insulin sensitivity in the diabetic cats used in this study. However, the markedly elevated basal amylin concentration suggests that the diabetic cats, in addition to having a reduced insulin secretion, also had insulin resistance.

The cats with hyperglycemia of unknown origin may have had transient stress-induced hyperglycemia or diabetes. Elevated plasma fructosamine and beta hydroxybutyrate concentrations are very useful for the diagnosis of diabetes in cats. However, they are not increased in all diabetic cats. Fructosamine may not increase with persistent hyperglycemia, if the hyperglycemia is less than 20 mmol/L, or less than 7 d duration (33). Therefore, normal FR and beta hydroxybutyrate concentrations do not exclude a diagnosis of diabetes in cats with hyperglycemia. In contrast to diabetic cats, cats with hyperglycemia of unknown origin had plasma insulin and amylin concentrations in the normal range. However, considering the degree of hyperglycemia, these cats were relatively hypoinsulinemic, which may have been due to disease-associated stress or glucose toxicity. Activation of the sympathetic nervous system during stress situations reduces insulin secretion (32,57). Glucose toxicity has been suspected to occur in diabetic cats (43,54,55) and has been documented in normal cats with experimentally induced hyperglycemia (53,56).

In summary, amylin concentration can be reliably measured in unextracted EDTA-treated plasma samples using a commerical RIA kit for feline amylin. Fasting amylin concentration for cats with normal glucose tolerance was 97 ± 4 pmol/L. Amylin and insulin concentrations increased in parallel in response to an IV injection of glucose in both normal cats and cats with impaired glucose tolerance. In cats with impaired glucose tolerance, first phase insulin and amylin secretion in response to glucose was markedly reduced, and peak hormone response was delayed. Diabetic cats in this study had basal hypoinsulinemia and basal hyperamylinemia. Hyperamylinemia may cause reduced insulin secretion and peripheral insulin resistance, and hence contribute to the development of feline diabetes mellitus.

Acknowledgments

We thank Ms. Janet Ainscow for excellent technical assistance, Veterinary Pathology Services (VPS; East Brisbane, Australia) for their invaluable support of the project, and Elizabeth Ryan from VPS for her help.

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