Many similarities exist between type 2 diabetes mellitus in cats and in humans. Both conditions typically have an onset in middle age or later, are associated with obesity, and are characterized by defective insulin secretion, insulin resistance, loss of pancreatic β cell mass, and islet amyloid deposition. Islet amyloid is derived from IAPP (also called amylin), a normal secretory hormone product of β cells that is copackaged and cosecreted with insulin. The amino acid sequence of the midportion of IAPP is amyloidogenic in cats, humans, and macaques, and type 2 diabetes mellitus can develop in these species. Intracellular formation of small oligomeric aggregates of IAPP is an early step in amyloid formation. These aggregates are toxic to β cells and are associated with apoptosis; therefore, they may be linked to the loss of β cells that develops in cats and humans with type 2 diabetes mellitus. Results of an earlier study by our laboratory group indicated that cats with impaired glucose tolerance have increased β cell IAPP content and an increased incidence of islet amyloidosis, compared with that of clinically normal cats. However, it is not known whether obesity in cats is associated with increased circulating concentrations of IAPP and insulin in nondiabetic cats. (Am J Vet Res 2011;72:1052–1058)
linked to increased circulating concentrations of IAPP or, if so, at what stage of obesity these concentrations increase. Circulating concentrations of insulin and IAPP are increased in overweight, glucose-intolerant human patients and in those with early type 2 diabetes mellitus, whereas these values are decreased in patients with advanced type 2 diabetes mellitus that require insulin treatment. Therefore, the purpose of the study reported here was to evaluate plasma concentrations of IAPP in nondiabetic cats classified according to BCS (as an estimate of obesity) and in cats with naturally acquired diabetes mellitus after food was withheld from all cats for 12 hours; we also sought to compare plasma concentrations of IAPP with serum concentrations of insulin and glucose in these same cats.

Materials and Methods

Animals—One hundred nine client-owned cats that were either nondiabetic or had naturally acquired diabetes mellitus were enrolled in the study with informed consent of the owners. Nondiabetic cats (n = 82) were determined to be healthy on the basis of history, physical examination, and results of analysis of a blood sample obtained after food was withheld for 12 hours (serum glucose < 152 mg/dL or serum fructosamine < 340 µmol/L). Each diabetic cat was evaluated by means of physical examination, CBC, serum biochemical analysis, FeLV antigen anti–FIV antibody testing, measurement of serum thyroxine concentrations, urinalysis (via cystocentesis), and aerobic microbial culture of a urine sample. Glycosuria and ketonuria were evaluated via a dipstick test. Diabetes mellitus was diagnosed in affected cats ≤ 1 week prior to enrollment in the study; cats were excluded if they had received any diabetogenic drug treatment (eg, corticosteroids, progestogens, or anticonvulsants) within 3 months before diagnosis. Diagnosis of nonketoadipose diabetes mellitus (DM group; n = 21 cats) was made on the basis of clinical signs (polyuria, polydipsia, and weight loss) with hyperglycemia and concurrent glycosuria on at least 2 days without ketonuria. Cats were excluded from this group if they were determined to have a history of treatment for diabetes (other than a change in diet) or any of the following conditions: ketonuria, dehydration, debilitation, poor appetite, or physical or biochemical abnormalities other than those attributable to untreated diabetes (eg, hepatomegaly and hypercholesterolemia). Diagnosis of ketoacidotic diabetes mellitus (DKA group; 6 cats) was made on the basis of clinical signs with concurrent hyperglycemia, acidosis (total CO₂ < 12 mmol/L), glycosuria, and ketonuria. Ketoacidotic cats were excluded if they had a history of diabetogenic drug therapy (eg, corticosteroids or progestogens) within the preceding 3 months or if they had any clinically important abnormality other than conditions commonly associated with untreated diabetes such as urinary tract infection.

A BCS estimate was made for each cat by 1 investigator (MSH) according to a scale from 1 to 9 (1 = emaciated, 5 = ideal, and 9 = morbidly obese). For purposes of the present study, only cats with BCS ≥ 5 were included. Nondiabetic cats were then further grouped according to BCS for various analyses. The study was approved by the University of Minnesota Animal Care and Use Committee and conducted in accordance with the US Animal Welfare Act and the NIH Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training.

Experimental protocol—After food was withheld for 12 hours (with free access to water), a blood sample (10 mL) was collected from a jugular or medial saphenous vein for measurement of plasma concentration of IAPP and serum concentrations of glucose and insulin. For nondiabetic cats, food was withheld at home by the owners for the prescribed period of time. Samples from cats in the DM group were collected prior to any treatment with insulin or oral hypoglycemic agents. Samples from cats in the DKA group were collected after ketoacidosis was resolved by means of rehydration with intravenous fluid treatment (saline [0.9% NaCl] solution supplemented with potassium chloride and potassium phosphate) and regular insulin administration; insulin was then withheld for 12 hours prior to sample collection.

Sample handling and analysis—Blood samples were divided among 2 tubes. Samples for serum insulin and glucose analysis were allowed to clot for 15 minutes at room temperature (21°C) and then centrifuged for 10 minutes at 500 × g. Serum was transferred to polypropylene tubes and stored at −70°C until assay. Glucose measurements were performed by use of the glucose oxidase method. Blood samples for IAPP analysis were immediately transferred to chilled (0°C) 7-mL tubes containing EDTA and the protease inhibitor aprotinin (500 kallikrein U/mL) and centrifuged at 4°C for 10 minutes at 500 × g. The plasma was then transferred to polypropylene tubes and frozen at −70°C until extraction.

Radioimmunoassay for insulin—Serum insulin concentrations were determined by use of a commercially available radioimmunoassay kit for measurement of porcine insulin according to the manufacturer’s instructions. All samples were assayed in duplicate and means reported. The minimum detectable insulin concentration that could be distinguished from 0 was 12 pmol/L. The intra-assay coefficient of variation (measurement precision) determined by means of repeated measurement (5 tests/pool) of insulin concentration in each of 3 pools of feline serum that contained 510, 306, or 90 pmol of insulin/L (determined by use of the same assay) was 2.6%, 3.1%, and 0.9%, respectively. The interassay coefficient of variation for insulin measurement determined by use of these same 3 pools in 6 separate assays was 3.6%, 3.9%, and 8.9%, respectively. Assay linearity was demonstrated by measuring insulin concentrations in 2 samples of pooled feline serum that contained 330 and 486 pmol of insulin/L, respectively, prepared at various dilutions (undiluted, 3 parts serum:1 part diluent, 1:1, and 1:3) with the manufacturer-provided zero standard used as diluent. Percentages of expected concentrations at the 4 dilutions were 100%, 93%, 96%, and 96%, respectively, for the first pooled sample (330 pmol/L) and 100%, 112%, 116%, and 132%, respectively, for the second pooled
sample (486 pmol/L). The percentage of assay recovery was determined by adding various concentrations (0, 45.6, 50, and 252 pmol/L) of porcine insulin to feline serum (pooled sample from study cats) and measuring insulin concentration in each of these 4 test conditions. For this analysis, 4 separate aliquots of each test sample were assayed in duplicate. Mean percentage recovery for the test samples was 100%, 87%, 103%, and 114%, respectively.

Radioimmunoassay for IAPP—The IAPP assay was performed essentially as described elsewhere. Samples were analyzed in duplicate, and mean values were reported. For preparation of calibrators and controls, a 12.8-µg vial of synthetic feline IAPP reconstituted with water to make a 0.01 g/L stock solution. Calibrators were immediately prepared via further dilution of the stock solution with a freeze-drying solution (50 g of lactose/L, 2.5 g of bovine serum albumin/L, and 4.25 mL of formic acid/L in water) at 4°C, then aliquoted, lyophilized, and stored at −70°C. Each calibrator was used only once because 1 freeze-thaw cycle of IAPP in buffer (60 mmol of phosphate/L; pH, 7.4; with 0.1% Triton X-100 and 0.1% bovine serum albumin) resulted in a 39.7% reduction in measured peptide concentrations. The remaining stock solution was aliquoted and stored at −70°C. The concentration of IAPP in the stock solution was confirmed via amino acid analysis.

Because of the large volume of plasma required for validation studies and assay controls, plasma from multiple healthy study cats was pooled; to make the matrices of the 3 pooled samples as different as possible, each pooled sample consisted of plasma from cats with particular BCs; one contained plasma from cats with BCs of 5 or 6, another contained plasma from cats with a BC of 7, and the remaining pooled sample contained plasma from cats with a BC of 8. Concentrated spiking solutions for controls and for recovery and dilution experiments were prepared from thawed stock solution (predicted concentration corrected for expected freeze-thaw loss) with buffer added at 4°C to maintain the same proportional volume and a spiking percentage < 4%. Spiked pooled samples were aliquoted and stored in polypropylene tubes at −70°C until extraction. For dilution experiments, independent fractional dilutions in buffer solution were made after reconstitution of the lyophilized samples immediately prior to assay. Solid-phase extraction and radioimmunoassays were performed as previously described. For calculations, plasma IAPP concentrations below the minimal detectable concentration of 2.5 pmol/L were recorded at the arbitrary level of half that concentration.

Statistical analysis—Mean ± SD circulating concentrations of IAPP, insulin, and glucose were summarized for nondiabetic cats grouped according to BCs and for diabetic cats grouped as ketoacidotic or nonketoadetic (DKA and DM groups, respectively) on the basis of clinicopathologic findings. Box-and-whiskers plots were generated for graphic evaluation of plasma IAPP and serum insulin and glucose concentrations for each group.

A Shapiro-Wilk test was used to test for normality of data, and ANOVA and F tests were performed to evaluate whether mean insulin, IAPP, and glucose concentrations were significantly different among groups. Because the variance of IAPP, insulin, and glucose concentrations was not constant across all groups, weighted least squares with weights that were inversely proportional to the variance in each group were used to yield the most precise estimates. To assess differences among means, multiple comparisons were performed with Tukey-Kramer adjustment for the P values and 95% confidence limits were calculated for the differences of means to account for multiple tests within a trait. Values of P ≤ 0.05 were considered significant.

Linear regression analysis was used to examine the relationship between circulating concentrations of IAPP and insulin. Spearman correlation coefficients for plasma IAPP and serum insulin concentrations were calculated for each group. Spearman correlation coefficients were also calculated to evaluate relationships between BCs and IAPP concentration and between BCs and insulin concentration.

Results

Physical and clinical characteristics of cats—Signalment was evaluated for nondiabetic cats grouped according to BCs and for diabetic cats assigned to DM or DKA groups on the basis of clinicopathologic findings.

### Table 1—Physical characteristics and mean ± SD concentrations of plasma IAPP and serum insulin and glucose for 82 nondiabetic cats grouped according to BCs and 27 diabetic cats with and without ketoacidosis (DKA and DM groups, respectively).

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cats</th>
<th>Age (BCS)</th>
<th>Sex</th>
<th>Concentration*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>M</td>
<td>IAPP (pmol/L)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Median (range)</td>
<td>F</td>
<td>Insulin (pmol/L)</td>
</tr>
<tr>
<td>Nondiabetic (any BC)</td>
<td>82</td>
<td>6.1 ± 0.7</td>
<td>45</td>
<td>9 ± 5</td>
</tr>
<tr>
<td>BCS 5</td>
<td>24</td>
<td>7.0 ± 0.7</td>
<td>13</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>BCS 6</td>
<td>12</td>
<td>5.3 ± 0.7</td>
<td>5</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>BCS 7</td>
<td>19</td>
<td>5.0 ± 0.7</td>
<td>11</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>BCS 8</td>
<td>22</td>
<td>6.7 ± 0.7</td>
<td>11</td>
<td>10 ± 6</td>
</tr>
<tr>
<td>BCS 9</td>
<td>5</td>
<td>5.6 ± 0.7</td>
<td>5</td>
<td>11 ± 6</td>
</tr>
<tr>
<td>DM</td>
<td>21</td>
<td>9.3 ± 0.7</td>
<td>15</td>
<td>11 ± 8</td>
</tr>
<tr>
<td>DKA</td>
<td>6</td>
<td>12.4 ± 0.7</td>
<td>6</td>
<td>2 ± 2.3</td>
</tr>
</tbody>
</table>

Only cats with a BCS ≥ 5 on a scale of 1 to 9 were included in the study. Nondiabetic cats were further grouped according to BCs for data analysis and for each analysis, P values indicate significant differences among values within a column: *P = 0.001, +P < 0.001, and †P = 0.002.

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Breeds of nondiabetic cats included domestic shorthair (n = 46), domestic medium-hair (7), domestic longhair (11), Siamese (14), Himalayan (1), Manx (1), and 2 cats for which breed was not recorded. All nondiabetic cats in the study were neutered with the exception of 1 sexually intact female that had a BCS of 7. All laboratory values were within reference limits for the 82 nondiabetic cats (data not shown).

The 21 cats in the DM group were neutered males and females. Breeds included domestic shorthair (n = 13), domestic medium-hair (1), domestic longhair (5), Siamese (1), and Persian (1). Body condition scores for these cats were as follows: 5 (n = 1), 6 (4), 7 (4), 8 (10), and 9 (2). In addition to hyperglycemia and glycosuria, cats in the DM group had mild increases in serum concentrations of cholesterol (3/21 cats; mean, 288 ± 26.5 mg/dL; reference range, 119 to 264 mg/dL) and globulin (4/21 cats; mean, 5.75 ± 0.4 g/dL; reference range, 2.5 to 5.3 g/dL) and activities of alanine aminotransferase (7/21 cats; mean, 138 ± 15.7 U/L; reference range, 17 to 95 U/L) and aspartate aminotransferase (15/21 cats; mean, 56 ± 20.2 U/L; reference range, 13 to 29 U/L). All other laboratory variables were normal in these cats.

The 6 cats in the DKA group were neutered males. Breeds in this group included domestic shorthair (n = 3), domestic medium-hair (1), and domestic longhair (2). Body condition scores for these cats were as follows: 5 (n = 4), 6 (1), and 8 (1). In addition to clinicopathologic abnormalities associated with ketoacidosis, 1 cat had a urinary tract infection and 1 was recovering from perineal urethrostomy surgery. All cats in the DM and DKA groups tested negative for FeLV antigen and anti-FIV antibody, and all had serum thyroxine concentrations within the laboratory reference range.

IAPP, insulin, and glucose concentrations—Plasma concentrations of IAPP and serum concentrations of insulin and glucose were evaluated in samples obtained from all cats after food was withheld for 12 hours (Table 1). A significant (P = 0.007) difference in mean IAPP concentrations was detected between nondiabetic cats with BCSs of 5 (6 ± 3 pmol/L) and 7 (10 ± 4 pmol/L; Figure 1). A significant (P < 0.001) difference was also detected in mean insulin concentrations between nondiabetic cats with BCSs of 5 (55 ± 50 pmol/L) and 8 (124 ± 53 pmol/L; Figure 2). Although mean IAPP and insulin concentrations appeared to be increased in the remaining cats with BCSs ≥ 6, compared with those in cats with a BCS of 5, these differences were not significant. There were no significant differences in mean glucose concentrations among nondiabetic cats with various BCSs (Figure 3).

Cats in the DM and DKA groups had significantly (P < 0.001) higher mean serum glucose concentrations than did nondiabetic cats of any BCS (Table 1; Figure 3). Cats in the DM group had a mean plasma IAPP concentration similar to those of nondiabetic cats with a
BCS of 8 or 9; mean serum insulin concentration was lower in cats of the DM group, compared with that of nondiabetic cats, but this difference was not significant. Cats in the DKA group had a significantly (P < 0.001) lower mean IAPP concentration (3 ± 2 pmol/L) than did nondiabetic cats with BCSs of 6 (9 ± 3 pmol/L), 7 (10 ± 4 pmol/L), or 8 (10 ± 6 pmol/L) and cats in the DM group (11 ± 8 pmol/L; P = 0.002). Although mean insulin concentration in cats of the DKA group (46 ± 9 pmol/L) was lower than in cats of the DM group (53 ± 21 pmol/L), this difference was not significant. The mean glucose concentration was higher in cats of the DKA group (368 ± 113 mg/dL) than in cats of the DM group (269 ± 53 mg/dL), but this difference was also nonsignificant.

Results of linear regression analysis with plasma IAPP concentration as the dependent variable and serum insulin concentration and group as independent variables indicated that, after adjusting for group, insulin concentrations were significantly (P < 0.003) associated with IAPP concentrations; as insulin concentration increased by 1 pmol/L, IAPP concentration increased by 0.032 pmol/L. In addition, calculation of Spearman correlation coefficients revealed significant correlations between plasma IAPP and serum insulin concentrations in nondiabetic cats with a BCS of 5 (P < 0.003) or 7 (P = 0.005) but not in those with a BCS of 6, 8, or 9 or in cats of the DM or DKA groups (Figure 4; Table 2). In nondiabetic cats overall, BCS and plasma IAPP concentration were significantly (P < 0.001) and positively correlated (r = 0.38; Figure 5). Similarly, BCS and serum insulin concentration were significantly (P < 0.001) and positively correlated in these cats (r = 0.54; Figure 6).

**Discussion**

Although the pathogenesis of type 2 diabetes mellitus in humans and cats is far from being resolved, there is increasing evidence that pathological consequences of IAPP misfolding and subsequent aggregation into toxic oligomers and amyloid fibrils play an important role in the molecular pathogenesis of the disease. Evidence from studies of type 2 diabetes mellitus in transgenic rodents, humans, and cats has suggested an important role for increased synthesis and secretion of IAPP in this process. In this regard, evidence from human studies has shown that circulating insulin and IAPP concentrations are increased in overweight glucose-intolerant individuals and those with early type...
2 diabetes, while they are decreased in patients with advanced type 2 diabetes who are being treated with insulin. Our group has also previously shown that cats with impaired glucose tolerance had increased IAPP content in their pancreatic β cells with early development of islet amyloid deposits.14

Results of the present study added to those earlier findings in cats and revealed that nondiabetic moderately obese cats (BCS, 7) had significantly increased plasma IAPP concentrations, compared with those of cats of ideal body weight (BCS, 5). Furthermore, a significant positive correlation was identified between BCS and plasma IAPP concentrations in nondiabetic cats. Although mean IAPP concentrations were also greater in the more severely obese groups of cats (BCS, 8 or 9) than in cats with ideal body weight, the differences among the groups were not statistically significant.

Because obesity is established as a predisposing factor in the development of type 2 diabetes, the findings of the present study indicate a potential link between obesity, with the associated increase in circulating IAPP concentrations, and the development of type 2 diabetes mellitus in cats. The pathogenetic connection between increased circulating IAPP concentrations and type 2 diabetes mellitus is linked to the fact that IAPP-derived amyloidosis is a characteristic feature of all species that develop type 2 diabetes mellitus, including humans, cats, and macaques.1,10 Furthermore, it has long been known that concurrent with the development of islet amyloidosis in these species there is significant loss of islet β cells and that this process occurs prior to the development of type 2 diabetes mellitus. Islet amyloid polypeptide amyloidogenesis has been linked to β-cell apoptosis via the generation of toxic intermediate-sized oligomers, which are formed early in the pathway and eventually lead to the formation of amyloid fibrils. A factor that has been implicated in the pathogenesis of islet amyloidosis is increased production of IAPP by β cells. The positive correlation between plasma concentrations of IAPP and BCS and the increased mean concentration of IAPP in cats of the DM group, compared with that of nondiabetic cats of ideal body weight in the present study, are therefore of pathogenetic interest because increased IAPP may potentially serve as a prelude to the eventual initiation of amyloidogenesis in susceptible cats.

Although obesity (or other causes of insulin resistance) appears generally to be necessary for the development of type 2 diabetes, obesity and increased IAPP values alone are not sufficient to explain the transition to diabetes. The factors that allow formation of toxic IAPP oligomers and subsequent development of type 2 diabetes mellitus in only a small proportion of obese cats, humans, or macaques are unknown and are the subject of considerable ongoing research.

In the study reported here, mean serum insulin and plasma IAPP concentrations measured in samples obtained after food was withheld for 12 hours were positively and significantly correlated in cats with ideal body weight (BCS, 5) consistent with a tightly regulated ratio of production and secretion between insulin and IAPP, as previously reported.7,21 However, correlation coefficients were reduced with increasing obesity in most remaining BCS groups, and a significant correlation was detected only for cats with a BCS of 7. Similarly, the correlation between serum insulin and plasma IAPP concentrations in cats of the DM and DKA groups was not significant. This was also reflected in the high mean IAPP concentrations detected in cats of the DM group, despite the fact that mean circulating insulin concentrations appeared to be decreased, compared with those in nondiabetic cats. Therefore, these data suggest that obesity and diabetes are associated with loss of correlation between circulating IAPP and insulin concentrations, which may highlight an underlying fundamental alteration in pancreatic β-cell function that begins prior to development of clinical diabetes and continues in the disease state. It should also be noted that cats at the time of diagnosis of type 2 diabetes mellitus typically have an approximately 50% reduction in β-cell mass,25 which implies that the remaining β cells must have extremely high IAPP production to maintain the described high circulating concentrations of IAPP. As mentioned, increased IAPP production has been hypothesized to be a contributing factor in the cascade of events leading to misfolding of the IAPP molecule and consequent formation of toxic IAPP oligomers ultimately leading to β-cell death.15

Another key feature in the pathogenesis of type 2 diabetes mellitus in cats and humans is the development of insulin resistance with attendant high insulin concentrations, which is most often secondary to obesity.23–25 Results of the present study revealed that markedly obese cats (BCS, 8) had significantly higher serum insulin concentrations, compared with cats with an ideal BCS, despite normoglycemia, consistent with an insulin-resistant state. Furthermore, a significant positive correlation was detected between BCS and serum insulin concentrations, indicating that this metabolic state can occur early in the development of obesity. Thus, our data are consistent with previous findings in nondiabetic humans in which obesity was associated with hyperinsulinemia and high circulating concentrations of IAPP and also show that even mild obesity may be associated with perturbations in insulin and IAPP regulation in cats. These findings highlight the potential importance of maintaining normal body weight in the long-term health of domestic cats and other species at risk of development of type 2 diabetes mellitus.

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