# Determination of reference values for glucose tolerance, insulin tolerance, and insulin sensitivity tests in clinically normal cats

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**Objective**—To determine reference values and test variability for glucose tolerance tests (GTT), insulin tolerance tests (ITT), and insulin sensitivity tests (IST) in cats.

**Animals**—32 clinically normal cats.

**Procedure**—GTT, ITT, and IST were performed on consecutive days. Tolerance intervals (ie, reference values) were calculated as means ± 2.397 SD for plasma glucose and insulin concentrations, half-life of glucose ($T_{1/2glucose}$), rate constants for glucose disappearance ($K_{glucose}$ and $K_{itt}$), and insulin sensitivity index ($S_I$). Tests were repeated after 6 weeks in 8 cats to determine test variability.

**Results**—Reference values for $T_{1/2glucose}$, $K_{glucose}$, and fasting plasma glucose and insulin concentrations during GTT were 45 to 74 minutes, 0.93 to 1.54 %/min, 37 to 104 mg/dl, and 2.8 to 20.6 μU/ml, respectively. Mean values did not differ between the 2 tests. Coefficients of variation for $T_{1/2glucose}$, $K_{glucose}$, and fasting plasma glucose and insulin concentrations were 20, 20, 11, and 23%, respectively. Reference values for $K_{itt}$ were 1.14 to 7.3%/min, and for $S_I$ were 0.57 to 10.99 × $10^{-4}$ min/μU/ml. Mean values did not differ between the 2 tests performed 6 weeks apart. Coefficients of variation for $K_{itt}$ and $S_I$ were 60 and 47%, respectively.

**Conclusions and Clinical Relevance**—GTT, ITT, and IST can be performed in cats, using standard protocols. Knowledge of reference values and test variability will enable researchers to better interpret test results for assessment of glucose tolerance, pancreatic β-cell function, and insulin sensitivity in cats. (*Am J Vet Res* 2001;62:630–636)

The primary metabolic abnormality in cats with diabetes mellitus is hyperglycemia associated with impaired insulin secretion and insulin resistance.[1,2] To identify cats with impaired glucose tolerance or insulin insensitivity, validated reference ranges for glucose tolerance and insulin sensitivity tests need to be established. Specific test protocols also need to be established, and the repeatability for the tests determined.

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**Glucose tolerance tests (GTT)** have routinely been used to determine glucose tolerance status in cats.[3-5] Although classification criteria for glucose tolerance in healthy cats have been described,[3,6,7] these studies were performed under differing conditions and test procedures, making comparison of results between studies difficult. In humans, glucose tolerance in healthy individuals is classified on the basis of **glucose half-life ($T_{1/2glucose}$)**, the **rate of disappearance for glucose ($K_{glucose}$)**, and absolute plasma glucose concentrations at 0, 60, 90, and 120 minutes during a standardized GTT.[8,9] Reference values for $T_{1/2glucose}$, $K_{glucose}$, and plasma glucose concentrations during GTT have also been established for cats.[3] However, the testing protocol and method for determining glucose concentrations in the study reported here differed from those used in previous studies.[3,6,7]

To the authors' knowledge, use of the **insulin tolerance test (ITT)** has not been reported in cats. This test allows general assessment of insulin sensitivity by determining the decrease in plasma glucose concentration following an IV insulin injection.[10] Despite some limitations, results of ITT provide a reliable index of insulin sensitivity in humans[11-13] and have been used in other animals, including dogs,[14] pigs,[15] and calves.[16]

Results of **insulin sensitivity tests (IST)**, using minimal model analysis of the frequently sampled glucose tolerance test, have been described at least twice in cats.[2,17] However, sample size (n = 5 and 10) was limited in both studies, and each study used a different protocol. The IST is simple to perform, compared with the more established euglycemic clamp method, and provides a measure of insulin sensitivity that correlates well with values derived from clamp studies in both cats and humans.[17,18]

The purposes of the study reported here were to establish reference values for GTT, ITT, and IST, using standardized protocols, and determine the degree of variability within these tests in clinically normal cats.

## Materials and Methods

**Animals**—Thirty-two (18 spayed females, 14 castrated males) cats were used for this study. Cats were determined to be clinically normal on the basis of results of physical examinations and routine hematologic and serum biochemical analyses and judged to be nonobese on the basis of weight, body mass index, and body condition scores. Accurate ages of the cats were not known; however, all were estimated to be between 1 and 5 years old.

Body mass index was determined with the cats under general anesthesia, using the following formula:

$$\text{Body mass index} = \text{Body weight}/\text{body length} \times \text{height}.^{5}$$

Body length was measured from the point of the shoul-

der to the tuber ischium, and height was measured from the point of the shoulder to the paw with the cat positioned in lateral recumbency. Body condition scores (1 to 5) were determined from a body condition score chart.[19]

Cats were initially quarantined and housed in a group facility for approximately 8 weeks. For the 2 weeks preceding the study, cats were acclimatized to the test holding facilities and fed a commercially available extruded diet[a] containing 32.0% protein, 9.9% fat, 2.6% crude fiber, 6.7% ash, and 8.0% moisture at maintenance requirements recommended for moderately active adult cats (70 kcal/kg of body weight/d).[20] Ingredients in this diet consisted of chicken meal, corn, rice, beet pulp, egg, vitamins, and minerals. Cats were allowed free access to water at all times. All protocols were approved by the Animal Experimentation Ethics Committee of the University of Queensland.

**Experimental protocol**—At least 24 hours prior to the first test, cats were anesthetized with propofol[b] (initial dose, 6 to 7 mg/kg, IV), and an IV catheter[c] was placed in a single jugular vein. Catheters were flushed twice daily with heparinized[d] saline (20 U of heparin/ml in saline [0.9% NaCl] solution) to maintain patency. Blood was collected from each cat for CBC and serum biochemical analyses.

Food bowls were removed at least 12 hours prior to testing. Glucose tolerance tests, ITT, and IST were performed on separate days in each cat over 4 days. Insulin sensitivity tests were performed in the mornings and ITT and GTT in the afternoons. Cats were fed immediately after each test was completed. No cat underwent more than 1 test in a 24-hour period.

Tests were repeated after an interval of 6 weeks in 8 cats. During this period, these cats were housed in the same facilities and fed the same extruded diet. However, feeding was decreased to 60 kcal/kg/day because some cats gained weight. Weight gain was restricted to less than 10% of body weight over the 6-week test period.

Blood samples were collected at various times during each test. Prior to each sample collection, 1 ml of blood was removed to clear the catheter of saline-diluted blood. After each collection, the 1 ml of saline-diluted blood was reinjected. To maintain blood volume and to flush the catheter, a volume of saline solution equal to the volume of blood removed was injected immediately after sampling. To maintain RBC mass, RBC were autotransfused instead of saline at certain collection times. To prepare RBC for autotransfusion, cells remaining in tubes after plasma was removed were washed with a volume of saline solution equal to the original blood sample and centrifuged for 3 minutes. The saline solution was aspirated, and RBC were resuspended in fresh saline solution (half the volume of the original blood sample). Red blood cells were then injected over a 15- to 30-second period, depending on volume. This was followed by injection of 2 ml of saline solution to flush the catheter.

Blood samples from each test were handled similarly. Samples were placed into vacuum tubes containing EDTA and aprotinin[e] (0.05 ml/ml of blood). After collection, samples were kept on ice for 15 to 30 minutes and centrifuged for 8 minutes at 1,500 × g to separate plasma. Two plasma samples were stored in 500-µl vials at –70 C until assayed for glucose and insulin concentrations.

**Glucose tolerance test**—Blood samples (4 ml) were collected prior to (0 minutes) and 2, 5, 10, 15, 30, 45, 60, 90, and 120 minutes after glucose[f] administration. Glucose (0.5 g/kg) was administered via the jugular vein catheter as a bolus dose over 30 seconds. Immediately after glucose injection, 3 ml of saline solution was infused to flush the catheter. Red blood cells from samples collected at 0, 2, 5, and 10 minutes were autotransfused after collection of the 60-minute sample, and cells from samples collected at 15, 30, and 45 minutes were autotransfused after collection of the 90-minute sample. Red blood cells from samples collected at 60 and 90 minutes were autotransfused within 20 minutes of collection of the 90-minute sample, and cells from the 120-minute sample were autotransfused 20 minutes after the test was completed.

**Insulin tolerance test**—Blood samples (2 ml) were collected prior to (0 minutes), and 2, 5, 10, 15, 30, 45, 60, 90, and 120 minutes after regular human insulin[g] (0.1 U/kg) was administered via the jugular vein catheter. Immediately after the insulin injection, 3 ml of saline solution was infused to flush the catheter. Red blood cells from samples collected at 0, 2, 5, and 10 minutes were autotransfused after collection of the 45-minute sample, and cells from samples collected at 15, 30, and 45 minutes were autotransfused after collection of the 60-minute sample. Red blood cells from samples collected at 60, 90, and 120 minutes were autotransfused within 20 minutes of collection of each respective sample.

**Insulin sensitivity test**—Four baseline blood samples (1 ml) were collected over the 15 minutes immediately preceding glucose administration (–15, –10, –5, and –1 minutes). Glucose (0.3 g/kg) was injected as a bolus via the jugular vein catheter. Immediately following glucose injection, 3 ml of saline solution was infused to flush the catheter. Twenty-seven 1-ml blood samples were collected over the next 3 hours at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes. Twenty minutes after glucose injection, regular human insulin (0.05 U/kg) was injected via the jugular catheter, followed immediately by 3 ml of saline solution. Red blood cells from the first 12 samples were autotransfused after collection of the 70-minute sample, and cells from the next 8 samples were autotransfused after collection of the 80-minute sample. Red blood cells from samples collected at 40, 50, 60 and 70 minutes were autotransfused after collection of the 100-minute sample, and those from the next 3 samples were autotransfused after collection of the 140-minute sample. Red blood cells from the remaining 4 samples were autotransfused within 20 minutes of collection of each respective sample.

**Determination of plasma glucose and insulin concentrations**—Plasma glucose concentrations were determined, using an automated glucose analyzer.[h] This analyzer incorporates immobilized enzyme technology to measure glucose concentrations. A thin membrane containing immobilized glucose oxidase is placed over a platinum anode. Glucose diffuses into this membrane and produces hydrogen peroxide, which is measured at the platinum anode. Precision of replicate analyses is $< \pm 2\%$.

Plasma insulin concentrations were determined, using a commercially available radioimmunoassay.[i] This assay is based on a double antibody solid phase technique and allows for quantitative determination of insulin in human and feline serum and plasma.[21]

**Test calculations**—Rate of disappearance for glucose and $T_{1/2glucose}$ were calculated by use of linear regression analysis of the semilogarithmic plot of glucose concentration versus time between 15 and 90 minutes after glucose administration. Between these time points, the glucose disappearance curve is most rectilinear.[22] Glucose concentrations in 0-, 60-, 90-, and 120-minute samples were also used as criteria for determining glucose tolerance.[3] **Area under the glucose concentration versus time curve** ($AUC_{glucose}$) for the entire 120-minute test was calculated by use of the trapezoidal method.[23] Incremental **area under the insulin concentration versus time curve** ($AUC_{insulin}$) was calculated by use of the trapezoidal method after subtracting nadir values.

During the ITT, insulin sensitivity was estimated from the **rate constant for glucose ($K_{itt}$)**. This rate constant was estimated from the slope of the regression line of the logarithm of plasma glucose concentration versus time between 2 and 15 minutes of the test. During this period, glucose concentration decreases linearly without the influence of counter-regulatory hormones.[11,13,24] **Mean residence time for insulin ($MRT_{insulin}$)** was calculated in minutes.[25] The $AUC_{glucose}$ for the entire 120-minute test was calculated by use of the trapezoidal method.[23] The $AUC_{insulin}$ was calculated by use of the trapezoidal method after subtracting nadir values.

Insulin sensitivity was determined, using a mathematical model of glucose disappearance to estimate insulin sensitivity (ie, the minimal model method).[26] This method is based on the frequently sampled GTT modified to include an injection of insulin.[27,28] The model provides an **insulin sensitivity index ($S_I$)**, defined as the dependence of fractional glucose disappearance on plasma insulin concentration.[26] **Glucose effectiveness ($S_G$)** indicates the effect of glucose itself, at baseline insulin concentrations, to promote its own disposal through uptake into tissues and suppression of endogenous glucose production.[29] The theoretical **glucose concentration at time zero ($G_0$)**, is the glucose concentration that would be obtained immediately after glucose injection if there was instantaneous mixing of glucose in the extracellular fluid.[30]

**Statistical analyses**—For normally distributed data, reference values were established by calculation of tolerance intervals comprising results for 95% of the population with a probability of 0.90.[31] Tolerance intervals from data for all 32 cats were calculated as mean values ± 2.3971 SD.[31] Data were analyzed for normality by the use of the Shapiro-Wilk test of normality.[j] When not normally distributed, data were logarithmically transformed prior to calculating tolerance intervals.[31] When data remained nongaussian before and after log transformation, observed reference values were recorded. Results of tests repeated at a 6-week interval in 8 cats were compared by use of a 1-way repeated measures ANOVA.[k] Body weight and body mass index were added as covariates. **Coefficients of variation (CV)** between results of each test were calculated as the SD divided by the mean then multiplied by 100.

Data were tested for outliers by use of the Grubbs T-statistic, with suspected values rejected when T was greater than its critical value of 2.75 for n = 16 or 3.128 for n = 32 ($P < 0.01$).[32] All values are reported as mean ± SD and calculated tolerance intervals.

## Results

Mean body weight, body mass index, and body condition score for the 32 cats in the study were 4.45 ± 0.79 kg, (range, 3.3 to 6.25 kg), 60.3 ± 6.7 kg/m$^2$, (51 to 75 kg/m$^2$) and 3 ± 0.44 (2 to 4), respectively. Mean body weight of the 8 cats that were tested twice was 4.2 kg initially and 4.5 kg after 6 weeks. Mean weight gain during the 6-week period was 6.7%. Initial body weight was significantly ($P < 0.01$) less than final body weight; however, body weight exerted no influence when used as a covariate.

Of the 8 cats that were tested twice, plasma calcium, creatinine, and urea concentrations were significantly higher after the 6-week interval, compared with initial values. However, all values remained within the laboratory's reference ranges.

**Glucose tolerance tests**—During the GTT, mean $T_{1/2glucose}$ was 56.8 ± 8.8 minutes (observed range, 45 to 74 minutes), $K_{glucose}$ was 1.25 ± 0.2%/min (observed range, 0.93 to 1.54%/min), $AUC_{glucose}$ was 17,989 ± 4,118 mg/dl/min (tolerance interval, 8,114 to 27,863 mg/dl/min), and $AUC_{insulin}$ was 1,205 ± 408 µU/ml/min (tolerance interval, 510 to 2,559 µU/ml/min). Reference values for plasma glucose and insulin concentrations at each time point during the GTT were also determined for 32 clinically normal cats (**Table 1**).

Glucose half-life, $K_{glucose}$, $AUC_{glucose}$, $AUC_{insulin}$, plasma glucose concentrations at all time points, and plasma insulin concentrations at most time points did not significantly ($P = 0.43$) differ between the 2 tests performed at a 6-week interval in 8 cats. Coefficients of variation for $T_{1/2glucose}$, $K_{glucose}$, $AUC_{glucose}$, and $AUC_{insulin}$ were 19.6, 20.6, 20.6, and 13.3%, respectively. Coefficients of variation for plasma glucose and insulin concentrations ranged from 11 to 56%, with the greatest variation at 90 and 120 minutes.

**Insulin tolerance tests**—During the ITT in 32 clinically normal cats, mean $K_{itt}$ was 3.83 ± 1.67%/min

Table 1—Results of glucose tolerance tests for 32 clinically normal adult cats

| | Time (min) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **0** | **2** | **5** | **10** | **15** | **30** | **45** | **60** | **90** | **120** |
| Glucose (mg/dl) | 71 ± 14 (37–104) | 356 ± 76 (173–539) | 289 ± 72 (118–461) | 263 ± 65 (108–418) | 245 ± 49 (126–363) | 208 ± 51 (86–329) | 163 ± 40 (68–258) | 134 ± 37 (47–223) | 88 ± 31 (12–163) | 69 ± 16 (30–108) |
| Insulin (µU/ml) | 8.4 ± 4.4 (2.8–20.6) | 18 ± 5.6 (8.9–34.1) | 19 ± 6.3 (8.6–36.4) | 22 ± 7.9 (10–44.8) | 22 ± 7.3 (10.2–44) | 19 ± 6.5 (8.7–36.8) | 20 ± 6.4 (9–40) | 21 ± 7.3 (8.2–45.4) | 13 ± 6.0 (3.8–38.4) | 8.9 ± 4.3 (3–22) |

Data reported as mean ± SD (tolerance interval).

Table 2—Results of insulin tolerance tests for 32 clinically normal adult cats

| | Time (min) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **0** | **2** | **5** | **10** | **15** | **30** | **45** | **60** | **90** | **120** |
| Glucose (mg/dl) | 71 ± 14.1 (37–158) | 70 ± 16.9 (30–141) | 65 ± 12.4 (35–148) | 52 ± 11.6 (24–111) | 44 ± 10.5 (19–89) | 38 ± 9.15 (16–77) | 48 ± 12.4 (18–90) | 55 ± 12.8 (25–115) | 62 ± 15.9 (24–119) | 65 ± 14.4 (30–137) |
| Insulin (µU/ml) | 9.4 ± 4.48 (2.5–27.3) | 672 ± 191 (342–1229) | 325 ± 103 (145–665) | 125 ± 48 (51–271) | 65.3 ± 31 (22.2–161) | 19.4 ± 9.5 (6.3–49.2) | 10.8 ± 4.7 (3.9–45.6) | 8.1 ± 3.6 (2.9–19) | 6.8 ± 2.9 (2.3–16.8) | 7.3 ± 3.4 (2.4–18.4) |

Data reported as mean ± SD (tolerance interval).

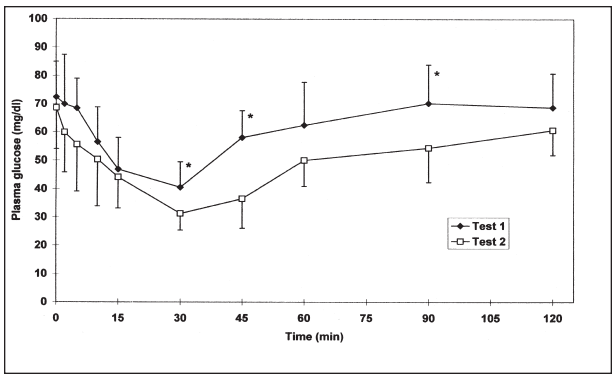


Figure 1—Mean (± SD) plasma glucose concentrations determined during 2 insulin tolerance tests (ITT) performed 6 weeks apart in 8 clinically normal adult cats. *Significantly ($P < 0.05$) different from value determined during test 2.

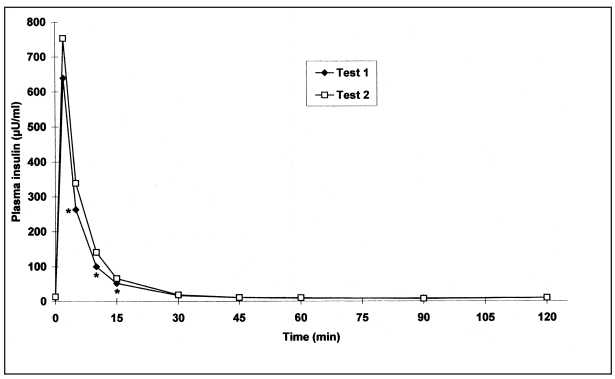


Figure 2—Mean (± SD) plasma insulin concentrations determined during 2 ITT performed 6 weeks apart in 8 clinically normal adult cats. *See* Fig 1 for key.

(observed range, 1.14 to 7.3%/min). The $AUC_{glucose}$, $AUC_{insulin}$, and $MRT_{insulin}$ were 6,553 ± 1,269 mg/dl/min (tolerance interval, 3,509 to 9,596 mg/dl/min), 4,503 ± 1,520 µU/ml/min (2,039 to 9,028 µU/ml/min), and 9.1 ± 3.2 minutes (6 to 13.5 minutes), respectively. Reference values for plasma glucose and insulin concentrations at each time point during the ITT were also determined (**Table 2**).

Mean residence time for insulin, $K_{itt}$, $AUC_{insulin}$, and plasma glucose and insulin concentrations at most time points did not significantly differ between the 2 tests performed in 8 cats (**Figs 1 and 2**). However, $AUC_{glucose}$ was significantly ($P < 0.01$) greater in the first test (7,279 ± 926 mg/dl/min), compared with the second test (5,811 ± 816 mg/dl/min). Coefficients of variation for plasma glucose concentration ranged from 15.3 to 31.1%, whereas CV for plasma insulin concentrations ranged from 11.7 to 51.2%, with the greatest variation at 90 and 120 minutes. Coefficients of variation for $MRT_{insulin}$, $AUC_{glucose}$, and $AUC_{insulin}$ were 23.4, 11.4, and 14.1%, respectively. Coefficient of variation for $K_{itt}$ was high (60.1%); however, $K_{itt}$ did not significantly differ between tests.

**Insulin sensitivity tests**—Mean $S_I$ determined for all 32 cats was 2.98 ± 1.84 × $10^{-4}$ min/µU/ml (tolerance interval, 0.57 to 10.99 × $10^{-4}$ min/µU/ml). Mean $S_G$ was 2.52 ± 0.77 × $10^2$/min (0.67 to 4.38 × $10^2$/min), and mean $G_0$ was 299 ± 64.3 mg/dl (145 to 453 mg/dl).

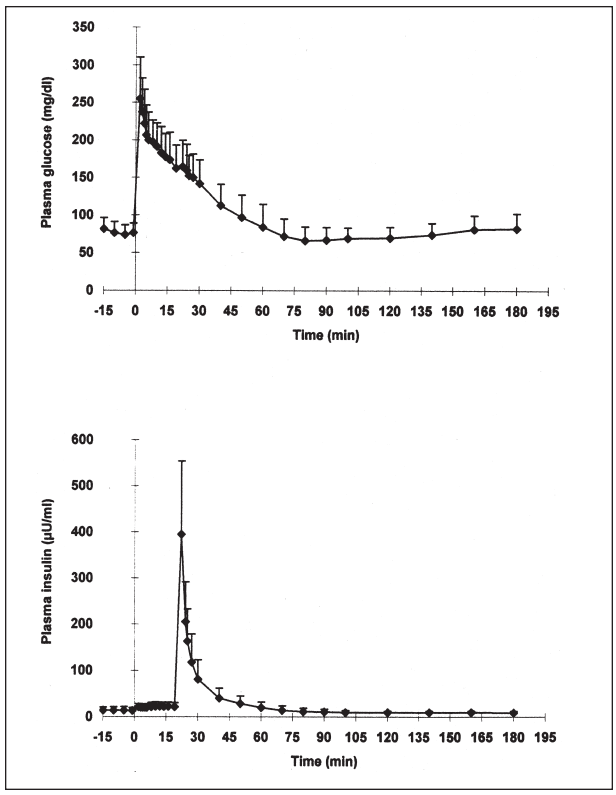


Figure 3—Mean (± SD) plasma glucose concentrations (top) and insulin concentrations (bottom) determined during insulin sensitivity tests in 32 clinically normal adult cats.

Plasma glucose and insulin concentration versus time curves were also calculated from data collected from these 32 cats (**Fig 3**).

Insulin sensitivity index determined during the initial IST in 8 cats was not significantly different, compared with results of the second test. However, CV was high, because $S_I$ determined in the second test for 1 cat was more than 5-fold greater than the initial value. When this high $S_I$ value was tested and rejected as an outlier, the CV for $S_I$ decreased to 47%. Coefficients of variation for $S_G$ and $G_0$ were 38 and 21.5%, respectively. Plasma glucose concentrations at all time points and insulin concentrations at most time points did not significantly differ between tests.

## Discussion

Glucose tolerance is the result of the interplay of insulin secretion from pancreatic β-cells in response to glucose, insulin delivery to target cells, and sensitivity of target cells to the metabolic action of insulin. The term impaired glucose tolerance refers to a metabolic stage intermediate between normal glucose homeostasis and diabetes mellitus.[8] Cats with impaired glucose tolerance have fasting blood (or serum) glucose concentrations within or slightly greater than the normal reference range as well as high blood (or serum) glucose concentrations and $T_{1/2glucose}$ during a GTT.[3,33,34] These cats also have higher serum insulin concentrations relative to glucose concentrations, compared with clinically normal cats, which indicates that they are insulin resistant.[33] However, cats with impaired glu-

cose tolerance do not have clinical signs of diabetes mellitus. Humans with impaired glucose tolerance develop clinical signs of diabetes mellitus at a higher rate than humans with normal glucose tolerance.[9]

The test routinely used to diagnose impaired glucose tolerance in cats is the intravenous GTT.[5-7,33] There is a lack of standardization in testing procedures, making the development of standard reference values extremely difficult. The dose of glucose administered varies from 0.05 to 1.0 g/kg. Methods used to determine plasma glucose and insulin concentrations and derive $T_{1/2glucose}$, $K_{glucose}$, $AUC_{glucose}$, and $AUC_{insulin}$ also vary from study to study. The preferred method of blood collection for GTT is via a catheter placed in the jugular vein. This ensures that blood is collected with minimal stress to the cat. By placing catheters in cats 24 hours prior to testing, we minimized both the stress of handling[1] and the influence of chemical restraining agents on glucose concentrations.[35]

In the present study, we described a standard procedure for GTT and established reference values and expected variations for the relevant plasma hormone concentrations and glucose tolerance indices in clinically normal cats. Mean $T_{1/2glucose}$ and $K_{glucose}$ (56.8 ± 8.8 minutes and 1.25 ± 0.2%/min, respectively) are in agreement with results of other studies evaluating clinically normal nonobese cats.[5,6] In other studies, however, $T_{1/2glucose}$ was only 30.2,[33] 35,[36] or 40.9 minutes.[37] These lower values may be attributable to the differing dose of glucose administered[33,37] or assay methods.[33,36,37] Although age of cats was not reported in most of these studies, cats may have been younger than those in our study; age is the most clinically relevant risk factor for development of diabetes mellitus in cats.[38] Increased body weight has been shown to reduce glucose tolerance in cats.[5,36] Mean body weight of cats in our study was greater than that reported in other studies.[4] In addition, it is possible that genetic differences between cat populations exist. In another study, the upper limit of the reference range for $T_{1/2glucose}$ in cats was 201.8 minutes,[7] which was higher than our upper limit (74.2 minutes). This higher upper limit may be a result of differing sampling techniques. In the previous study, collection of blood samples commenced immediately after a catheter was placed in the cephalic vein. Chemical restraint was not used. Plasma glucose concentration increases rapidly in response to stress related to struggling in cats.[1] Although cats in the previous study were believed to be accustomed to restraint and blood collection, the effects of stress on glucose metabolism may have still been evident.

The intraindividual CV for $T_{1/2glucose}$, determined from results of 2 GTT performed 6 weeks apart in 8 cats, was 19.6%, which indicated reasonable reproducibility for this variable with acceptable variation in individual cats. In humans, CV for blood (or plasma) glucose concentration at 120 minutes during oral and intravenous GTT are between 20 and 35%.[39-42] In the present study, the CV for plasma glucose concentration at this time point was 39.5%. In humans, normal glucose tolerance is often defined on the basis of the 120-minute plasma glucose concentration determined during GTT.[8] The large CV for 120-minute plasma glucose concentration in both humans and cats, however, suggests that normal or impaired glucose tolerance should not be defined on the basis of a single test result.

In animals with insulin resistance, more insulin is required to obtain the same decrease in plasma glucose concentration, compared with that required in clinically normal animals.[43] In the ITT, $K_{itt}$ is used as an index of insulin resistance.[44] Values for $K_{itt}$ that we determined in clinically normal cats (mean ± SD, 3.83 ± 1.67%/min; observed range, 1.14 to 7.3%/min) were similar to those documented in humans (mean ± SD, 4.4 ± 1.2%/min; range, 1.7 to 7.4%/min)[44] and dogs (mean, 6.76%/min).[14] The CV for $K_{itt}$ was 60%, which is higher than that reported in humans (26%).[44] In our study, $K_{itt}$ varied more than 7-fold among clinically normal cats. This result agreed with the reported 4-fold variation for $K_{itt}$ in humans.[44] Variability in $K_{itt}$ in humans or cats could not be accounted for by changes in body weight or body mass index.

The ITT has several limitations. The major criticism of this test is that the insulin-induced decrease in plasma glucose concentration elicits hypoglycemia, which in turn triggers a counter-regulatory hormone response characterized by the release of catecholamines, cortisol, glucagon, and growth hormone.[11,13,45,46] This counter-regulatory response may result in a decrease in $K_{itt}$, which indicates that the rate of disappearance of glucose from plasma is not a simple function of insulin action.[47] Mild hypoglycemia also developed in our cats. In humans, plasma concentrations of counter-regulatory hormones do not significantly change during the first 15 minutes of the ITT, despite the development of mild hypoglycemia.[12,13,24] Thus, decreases in glucose concentration during the first 15 minutes after insulin administration should reflect only the action of insulin, because counter-regulatory hormones remain at baseline concentrations during this period.[12,13,24] To avoid the potential for hypoglycemia and subsequent release of counter-regulatory hormones, some researchers have decreased the duration of the ITT to 15 minutes.[11,24] A further drawback of the ITT is that the amount of insulin injected (0.1 U/kg) is a pharmacologic dose and results in plasma insulin concentrations in excess of physiological concentrations commonly encountered in clinically normal cats.[48] Insulin administered at this dose suppresses hepatic glucose output, which allows assessment of glucose uptake by peripheral tissues.[45] However, the relevance of such high plasma insulin concentrations to normal metabolism has not been established.[48] This dose of insulin also may cause hypoglycemia.

Despite these limitations, insulin sensitivity derived from results of ITT in humans is closely correlated with that derived from euglycemic and hyperglycemic clamp techniques.[11,12,24] The advantage of performing an ITT rather than an IST for determination of insulin sensitivity is that only 10 blood samples are required for the former technique, compared with 31 for the latter technique. The need for fewer blood samples reduces the cost and time required to determine insulin sensitivity. Although the site of insulin resistance (ie, liver or muscle) cannot be determined, the

ITT has been shown to provide a relevant index of insulin sensitivity in humans[11,12,45] and has been used in other animals, including dogs,[14] pigs,[15] and calves.[16]

The minimal model technique incorporates computer modeling to analyze plasma glucose and insulin concentration dynamics during an intravenous, frequently sampled GTT.[26] The $S_I$ calculated from the model represents the extent to which a given plasma insulin response accelerates the decrease in plasma glucose concentration after a glucose injection.[49] The index is defined as the increase in fractional glucose disappearance per unit of insulin increase.[29] The larger the index is, the more insulin sensitive the subject is.

Results of the standard minimal model technique (ie, standard IST) are highly correlated with results from clamp-based studies in dogs[30] and cats.[17] A limitation to the standard minimal model technique is that it cannot be used to calculate insulin sensitivity in animals that are insulin resistant or when endogenous insulin release is minimal.[29] To overcome this limitation, a modified protocol was developed that incorporates an injection of tolbutamide to stimulate endogenous insulin release 20 minutes after administration of glucose.[18] In humans, results of the tolbutamide-modified minimal model technique correlate well with results of clamp-based studies.[29] However, both the standard and modified minimal model techniques require endogenous insulin. The technique was further modified; tolbutamide was replaced by insulin, which enabled a more accurate prediction of insulin sensitivity in patients with diabetes mellitus and clinically normal subjects.[27,28] The insulin-modified minimal model technique was the protocol we used in the present study.

Reference values for $S_I$ described in the present study are in agreement with those in a previous study; mean $S_I$ in 10 clinically normal cats was $3.2 \pm 0.37 \times 10^{-4}$ min/μU/ml (range, 1.71 to $5.23 \times 10^{-4}$ min/μU/m).[2] However, in a second study of 5 clinically normal cats, mean $S_I$ was $12.41 \pm 6.89 \times 10^{-4}$ min/μU/ml.[17] The discrepancy in results among studies may be attributable to the protocols used in each study. The standard protocol without insulin modification was used in the latter study. In humans, the insulin-modified IST yields lower $S_I$ values than those obtained by use of the standard test, and results from the 2 protocols cannot be directly compared.[50]

Regardless of the method used to measure insulin sensitivity in humans, insulin sensitivity is influenced by independent variables susceptible to daily variation. These include composition of diet and amount of physical activity.[51] It is likely that these and other variables influence insulin sensitivity in cats. In addition, rapid and pronounced changes in insulin sensitivity may develop in cats to maintain normoglycemia.[m] Taking these factors into consideration, it is reasonable to expect wide variations of insulin sensitivity in cats. Moreover, because studies describing the use of IST in cats have used different test procedures, direct comparison of results between studies is difficult. Results of single tests should be interpreted with caution in individual cats.

There is evidence that blood glucose concentrations in client-owned cats increase in response to the stress of visiting a veterinary clinic.[3] Reference values established in the present study have been determined in clinically normal cats acclimatized to their environment over several weeks and may not be representative of values obtained from client-owned cats with stress-induced hyperglycemia. However, in a research setting, the standard test protocols and reference ranges described in this study may allow better interpretation of test results for defining normal and impaired glucose tolerance and diagnosing pancreatic β-cell dysfunction and insulin insensitivity in cats.

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[a]Eukanuba veterinary diets, Restricted-calorie formula/feline, The Iams Co, Lewisburg, Ohio.

[b]Diprivan (Propafol, 10 mg/ml), ZENECA Ltd, Macclesfield, Cheshire, UK.

[c]18 gauge × 8 cm polyurethane jugular catheters, Cook Veterinary Products, Brisbane, Queensland, Australia.

[d]Multiparin (heparin sodium, 5,000 U/ml), Fisons Pty Ltd, Thornleigh, New South Wales, Australia.

[e]Trasylol (Kallikrein Inactivator 10,000 U/ml), Bayer Australia Ltd, Pymble, New South Wales, Australia.

[f]50% glucose injection BP, Astra Pharmaceuticals, North Ryde, New South Wales, Australia.

[g]Actrapid (regular human insulin, 100 U/ml), Nova Nordisk A/S, Bagsvaerd, Denmark.

[h]YSI 2300 Stat Plus, YSI Bioanalytical Products, Yellow Springs Instrument Co, Yellow Springs, Ohio.

[i]Phadeseph insulin RIA, Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden.

[j]Proc Univariate, SAS Institute, Cary, NC.

[k]SigmaStat, SPSS Inc, Chicago, Ill.

[l]Kinnaird ER, Rand JS, Baglioni AJ Jr, et al. Stress hyperglycaemia in cats (abstr), in *Proceedings*. 16th Annu Meet Vet Med Forum 1998;162.

[m]Link KRJ, Rand JS. Arginine and phentolamine response tests in cats (abstr). *J Vet Intern Med* 1996;10:185.

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