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Diagnosis of prediabetes in cats: glucose concentration cutpoints for impaired fasting glucose and impaired glucose tolerance

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ABSTRACT

Diabetes is typically diagnosed in cats once clinical signs are evident. Diagnostic criteria for prediabetes in cats have not been defined. The objective of the study was to establish methodology and cutpoints for fasting and 2-h blood glucose concentrations in healthy client-owned senior cats (≥ 8 yrs) using ear/ paw samples and a portable glucose meter calibrated for feline blood. Of the 78 cats, 27 were ideal (body condition score (BCS) 4 or 5 out of 9),

31 overweight (BCS 6 or 7) and 20 obese (BCS 8 or 9); 19 were Burmese and 59 non-Burmese. Following an 18 - 24 h fast and an ear/paw blood glucose measurement using a portable glucose meter, glucose (0.5 g/kg bodyweight) was administered IV and blood glucose measured at 2 min and 2 h. Cutpoints for fasting and 2-h glucose concentrations were defined as the upper limits of 95% reference intervals using cats with BCS 4 or 5. The upper cutpoint for fasting glucose was 6.5 mmol/L. Of the overweight and obese cats, one (BCS 7) was above this cutpoint indicating evidence of impaired fasting glucose. The cutpoint for 2-h glucose was 9.8 mmol/L. A total of 7 cats (4 with BCS 8 or 9 including 1 Burmese; 3 with BCS 6 or 7, non-Burmese) were above this cutpoint and thus had evidence of impaired glucose tolerance. In conclusion, the methodology and cutpoints for diagnosis of prediabetes are defined for use in healthy cats 8 yrs and older with a range of body condition scores.

1. Introduction

In cats, 0.2% to 1% [1-3] are reported to be diabetic compared to 4 [4] to 10% [4,5] of humans. Humans with blood glucose concentrations above normal but below diabetic for fasting or at 2-h in a glucose tolerance test are classed as having impaired fasting glucose or impaired glucose tolerance respectively. They are considered prediabetic and develop diabetes at a rate of 5 - 10% per yr [6,7]. It is estimated that over 50% of humans in the USA with diabetes are undiagnosed [8], and the number with undiagnosed prediabetes is 3 to 4 times greater than with undiagnosed diabetes [8]. There are no corresponding data for cats in the veterinary literature. As in humans, there is a genetic predisposition for feline diabetes. Burmese cats from the United Kingdom and Oceania are approximately 4 times more likely to develop diabetes than other breed [9], with one in 50 affected [2].

Diagnostic criteria for subclinical and pre-diabetes in cats have not been defined, and cats are not typically diagnosed until clinical diabetes is evident. In obese cats, mild fasting or

postprandial hyperglycemia is reported to be the only early sign of diabetes, prior to onset of classical signs of diabetes such as polyuria [10]. Reported upper limits for normal fasting blood glucose in cats vary from 6.1 mmol/L [11] to 9 mmol/L [12-14]; this variability is due at least in part to a lack of standardization of the test protocol.

Intravenous (IV) glucose tolerance tests are used to assess glucose tolerance in cats [15]. The 'gold standard' test requires multiple samples and interpretation can be difficult because of the complex calculations required to generate the necessary statistics such as glucose half-life, glucose clearance time and area under the curve. Veterinarians need screening tests for impaired fasting glucose and impaired glucose tolerance that are inexpensive, non-invasive, and easy to perform and interpret in a clinical setting. A standardized IV glucose tolerance test would need a standardised glucose dose rate, fasting period, sampling times, and an established reference range applicable to all cats, lean, overweight and obese.

Numerous portable blood glucose meters calibrated for human blood are used for glucose monitoring in cats [16-18]. Although precise, they are less accurate, typically measuring 0.5 to 2.2 mmol/L lower than a serum chemistry analyser [19]**.** A meter validated for feline blood, requiring a 0.3 uL blood sample is now commercially available [20], facilitating successful blood sampling from the ear or foot pad and more accurate measurements. A simplified protocol for IV glucose tolerance testing in cats using this glucose meter has been reported using a glucose dose of 1g/kg [7], but from a practitioner's perspective, the volume to be infused can be problematic. A glucose dose of 0.5 g/kg is typically used in cats for assessing glucose tolerance whereas 1 g/kg is used for assessing maximal insulin secretory capacity.

Administering an IV glucose dose to overweight and obese cats based on bodyweight spuriously affects some measures of glucose tolerance [21]. This is presumed to occur

because blood volume does not increase linearly with the increase in body weight due to obesity [22]. As a result, peak (2-min) glucose concentration is higher in obese cats, which subsequently increases 2-h glucose concentration when glucose is dosed on bodyweight [21]. This can be overcome by adjusting either the glucose dose or measured 2–h blood glucose concentration based on body condition score, so that one reference interval can be used for lean, overweight and obese cats. To the authors' knowledge, these adjustments have not been applied to cats in the age group at risk of diabetes (≥ 8 yrs).

The aims of this study were to establish methodology and cutpoints for fasting and 2–h blood glucose concentration in healthy client-owned, senior cats of varying body condition using ear/ paw samples and a portable glucose meter calibrated for feline blood, to compare these between Burmese and non-Burmese cats, to apply adjustment equations to 2–h blood glucose concentrations in overweight and obese cats.

2. Materials and Method

1.1. Study overview:

The protocol for these studies and the care and handling of these animals were approved by the Animal Experimentation Ethics Committee of the University of Queensland approval number SVS/040/10/NC/ABBOTT. In 78 client-owned cats, fasting blood glucose was measured from a paw or ear sample using a portable glucose meter then an IV glucose tolerance test performed using a glucose dose of 0.5g/kg. This was repeated in 8 of these cats 23 to 57 d later to determine variability over time. An IV glucose tolerance test using the same protocol but a glucose dose rate of 1g/kg was also subsequently performed in 11 of the 78 cats.

1.2. *Animals:*

Clinically healthy client-owned cats ≥ 8 yrs (n = 90) were recruited though veterinary clinics, advertisements and radio interviews between May 2011 and November 2012. Cats were tested at the University of Queensland Small Animal Clinic and a private specialist cat clinic. All cats included in the study appeared clinically healthy during examination. The cats were not on any medications except routine flea and worming control. Exclusions were based on haematological and biochemical panels, body condition score (BCS) of ≤ 3 out of a 9 point scale [23] and behaviour of the cats. Exclusions (n = 12) were for stress/ aggressive behaviour (n = 3), suspected pancreatitis based on increased fPLI of > 3.5 ug/L in line with the general interpretive guidelines of our reference laboratory (n = 2), hyperthyroidism (n = 3), ongoing health issues (n = 2), pancreatic cancer (n = 1) and BCS ≤ 3 out of 9 (n = 1). Remaining cats (n = 78) were classified as non-Burmese (n = 59) or Burmese (n = 19). Body condition scores of the cats (out of 9) [23] included in the study were all assessed by one person (MRJ), and were 4 (8 cats), 5 (19 cats), 6 (14 cats), 7 (17 cats), 8 (14 cats) and 9 (6 cats). Data was collected on diets of the study cats and consisted of a variety of supermarket, premium and home cooked dry and tinned food.

1.3. Protocol:

Cats were admitted to the hospital the d before the glucose tolerance tests and all cats stayed overnight. On admission, a 5-mL venous blood sample was collected for a routine health screen performed by a commercial veterinary diagnostic laboratory (Idexx Laboratories, Brisbane Australia). The following morning, after food was withheld for 18 to 24 h, a jugular venous blood sample (4 mL) was collected for hormone assays and then a 22 - gauge catheter (Surflo 22G x1” intravenous catheter, Terumo Europe, Belgium) was placed in the cephalic vein and flushed (2 mL 0.9% sodium chloride (Baxter)). To allow for resolution of stress hyperglycemia, fasting blood glucose was measured 3 h after catheter placement [24]. A

120 portable glucose meter calibrated for feline blood (Abbott Alpha Trak©) was used and the
121 sample obtained from the paw or ear. Glucose (undiluted 50% glucose injection BP; Astra
122 Pharmaceutical) (0.5g/kg) was then administered IV over 30 sec via the catheter. A timer was
123 started halfway through the infusion and blood samples were taken at 2 min, 2 h and then
124 hourly until glucose returned to below our laboratory's upper limit of normal fasting glucose
125 concentration of 6.5 mmol/L [25]. On completion, the catheter was removed, cats were fed
126 and discharged.

127

128 Blood samples from syringes from 3 cats were analysed 20 times with 2 different portable
129 glucose meters of the same brand within 1 h of collection to assess intra and inter-meter
130 variability. The interassay CV for the glucose meter was 2 % and the intra-assay 3.3 %. To
131 determine repeatability, fasting blood glucose assessments and glucose tolerance tests were
132 repeated in 8 cats 23 to 57 d after their first admission (median 42 days). To compare the
133 previously-derived adjustment equations with those derived from this population of cats, a
134 glucose tolerance test using the same protocol but a glucose dose rate of 1g/kg was also
135 performed in 11 of the 78 cats (BCS 4 n = 3; 5 n = 3; 7 n = 4; 8 n = 1) 38 to 365 d later
136 (median 60 d), depending on client availability, after their first glucose tolerance test.

137

138 *1.4. Statistical analyses:*

139 Reference intervals for fasting and 2-h glucose concentration were calculated using published
140 method used in humans, whereby data are transformed as necessary and outliers identified
141 and excluded from analysis [26]. This methodology results on average in a 10 % narrower
142 reference interval than if outlier detection was not used [27]. Data were entered into a
143 spreadheet (Microsoft Excel, Reference Interval Draft Version, Copyright 2005, University
144 of Cincinnati**),** transformed to approximate a normal distribution using the Box-Cox

transformation, and outliers excluded from subsequent calculations. Diagnositc cutpoints were defined as the upper limits of the 95% reference intervals. Associated 90% confidence intervals (CI) for the upper limits of the reference intervals were estimated using bootstrapping with 1000 replications. Based on *a priori* knowledge that some overweight and obese cats have abnormal glucose tolerance [15], only lean cats (BCS of 4 or 5) were used for estimating fasting and 2–h reference intervals. Data from Burmese were pooled with non-Burmese to determine reference intervals for fasting and 2-h glucose concentrations as the median glucose concentrations and interquartile ranges were similar (median fasting Burmese and non-Burmese 4.6 and 4.7 mmol/L, respectively, and 0.7 and 1.1 mmol/L respectively; median 2-h Burmese and non-Burmese 6.2 and 5.7 mmol/L respectively, and interquartile range 2.6 and 3.1 mmol/L, respectively.

Repeatability was established using repeatability coefficients calculated using specialized software (the Pairs etc module (version 3.57) of the WinPepi software (version 11.62; www.brixtonhealth.com)).

Repeatability coefficients were calculated:

based on the within-cat variance. Approximate 95% CIs were obtained by substituting confidence limits for the within- cat variance, estimated by the method described by Zar [28] (formula 7.16).

Associations between breed (Burmese or non-Burmese) and each of 2-min and 2-h glucose concentrations were assessed using linear regression with body condition score, age (both fitted as continuous variables) and sex (fitted as covariates). Associations between body condition score and 2-min glucose concentration, 2-min and 2-h glucose concentration and fasting and 2-h glucose concentrations were each assessed using univariable linear regression.

Homoscedasticity of residuals were assessed using plots of residual versus fitted values. The effects of glucose dose on 2-h glucose concentration were also assessed using linear regression, with cat-time as the unit of analysis, with cat fitted as a random effect; maximum likelihood estimation was used. Interactions between dose and each of breed (Burmese or non-Burmese) and body condition score (fitted as a continuous variable) were also assessed. Regression analyses were performed using a commercial software program (Stata (version 12, StataCorp, College Station, Texas, USA)) .

1.5. Adjustments of measured 2-h glucose:

We used two previously developed algorithms (Reeve-Johnson *et al*, unpublished data), to compensate for the spurious effect on 2-h glucose concentration that arises from dosing on a bodyweight basis (rather than using total blood volume), as previously demonstrated in obese dogs [22]. Using one algorithm, observed 2-h glucose concentration was adjusted downward by 0.1 mmol/L for every unit of BCS above 5. Using the other algorithm, the difference between the observed 2-min blood glucose concentration and the mean 2-min blood glucose concentration of lean cats (17.5 mmol/L) was calculated, and multiplied by 0.09. The measured 2-h blood glucose concentrations were then adjusted downwards by subtracting the calculated product; this was done for all cats with values above the upper cutpoint.

2. Results

2.1. *Fasting blood glucose concentrations*

The upper cutpoint for fasting blood glucose concentration in cats with BCS 4 and 5 (n = 27) was 6.5 mmol/L based on the upper limit of the 95% reference interval (Table 1). When the statistical power was increased by including all 78 study cats (BCS varied from 4 to 9), the

upper cutpoint was 6.3 mmol/L and the 90% CI 6.0 to 6.5 mmol/L. Only 1 of the 51 cats (2%) with BCS 6 to 9 was classed as having impaired fasting glucose (> 6.5 mmol/L) based on this cutpoint (BCS 7; non-Burmese), as well as one of the lean cats (BCS 5; non-Burmese). The lower limit of the 95% reference interval for cats with BCS 4 and 5 was 3.9 mmol/L (90% CI 3.6 to 4.2 mmol/L), and when all 78 cats were included, was 3.4 mmol/L (90% CI 3.2 to 3.5 mmol/L).

When 8 lean cats were retested 23 to 57 d later, the repeatability coefficient for fasting blood glucose concentration was 1.1 mmol/L (95% CI 0.7 to 2.2 mmol/L) when data from 7 of the 8 cats were used. One cat had an initial value of 4.6 mmol/L, and a value of 12.3 mmol/L after a further 43 d. At the first and second tests, fasting blood glucose concentrations for the other 7 cats ranged from 3.6 to 5.6 mmol/L and 4.1 to 5.7 mmol/L, respectively. When this cat was included in the data, the repeatability coefficient was 5.4 mmol/L (95% CI 3.7 to 10.4 mmol/L). As the 95% CI for these repeatability coefficients was wide, this estimate should be interpreted with caution. The second value for this latter cat was inconsistent with fasting concentrations in healthy cats and may have been the result of stress hyperglycemia or laboratory error such as a bubble in the blood sample. These results indicate that when cats are tested twice 23 to 57 d apart, glucose concentrations differ within cats by up to about 1.1 mmol/L for most cats.

2.2. *2-h blood glucose concentrations*

The cutpoint for 2-h blood glucose concentration in an IV glucose tolerance test using 0.5 g/kg glucose estimated from cats with BCS 4 or 5 (n = 27) was 9.8 mmol/L. This was the upper limit of the 95% reference interval (90% CI 8.5 to 10.7 mmol/L) (Table 1). The

repeatability coefficient for 2-h blood glucose concentration was 3.8 mmol/L (95% CI 2.6 to 7.2 mmol/L).

2.3. Adjustment for effect of BCS on interpretation of glucose tolerance test results

The measured 2-h blood glucose concentration for cats in the present study was adjusted in overweight and obese cats (BCS > 5) using 2 previously established algorithms (Reeve-Johnson et al, unpublished data), and the adjusted values compared to the upper cutpoints established in the present study. A total of 7 cats had 2-h glucose concentrations above the diagnostic cutpoint reported above of 9.8 mmol/L (4 obese (BCS 8 or 9), 3 overweight (BCS 6 or 7); 5 domestic, 1 Burmese and 1 British Blue). Adjusted 2-h blood glucose concentrations from both algorithms for these 7 cats were all above the upper limit of the reference range, and thus all were considered to be glucose intolerant (data not shown).

2.4. Effect of breed on fasting and 2-h blood glucose concentration

Although Burmese cats are overrepresented amongst diabetic cats, after adjusting for BCS, sex and age, Burmese cats (n = 19) did not have significantly differing fasting and 2-h glucose concentrations compared to non-Burmese (n = 59) cats. After adjusting for BCS, sex and age, the estimated difference in mean 2-h blood glucose concentrations (Burmese minus non-Burmese) was -0.6 mmol/L (95% CI of difference -1.4 to 0.2; $P = 0.140$). After adjusting for BCS, sex and age, the estimated difference in mean 2-h blood glucose concentrations (Burmese minus non-Burmese) was 0.1 mmol/L (95% CI of difference -1.1 to 1.3; $P = 0.856$).

2.5. Associations between body condition score and 2-min glucose concentration, and 2-min glucose and 2-h glucose concentrations

There tended to be a positive association between 2-min glucose concentration and body condition score; for every 1 unit increase in body condition score, 2-min glucose concentration increased by 0.8 mmol/L (95% CI -0.1 to -1.7mmol/L; $P = 0.078$). There was no significant association between 2-min and 2-h glucose concentrations ($P = 0.396$) but the point estimate was consistent with a positive relationship; for every 1 mmol/L increase in 2-min glucose concentration, 2-h glucose concentration increased by 0.04 mmol/L (95% CI -0.054 to -0.14). Although, these point estimates were not significantly associated, they were of similar magnitude to previously determined adjustments in another cohort of cats (Reeve-Johnson *et al*, unpublished data).

2.6. Effect of glucose dose rate on 2-h blood glucose concentrations

We evaluated the effect of glucose dose (0.5 versus 1.0 g/kg bodyweight) on 2-h blood glucose concentrations in lean, overweight and obese cats (n = 11; BCS 4 n = 3; 5 n = 3; 7 n = 4; 8 n = 1). Increasing the dose rate from 0.5 g/kg to 1 g/kg increased 2-h glucose in non-Burmese cats by an estimated 1.4 mmol/L (95% CI -0.1 to 2.8; $P = 0.031$). However in Burmese, relative to 0.5 g/kg, 1 g/kg had a much larger effect; 2-h glucose was 6.4 mmol/L higher than for the lower glucose dose (95% CI 4.6 to 8.1; $P < 0.001$; P for interaction 0.001). Mean 2-h glucose concentration for Burmese was estimated to be 0.7 mmol/L lower than for non-Burmese (95% CI 1.2 lower to 2.6 higher; $P = 0.483$) at 0.5 g/kg but 5.6 mmol/L higher (95% CI 3.7 to 7.5; $P < 0.001$) at 1 g/kg. No significant interaction was detected between dose and BCS (P for interaction 0.334). Increasing the dose rate from 0.5 g/kg to 1 g/kg increased 2-h glucose by an estimated 2.2 mmol/L (95% CI -0.4 to 4.9; $P = 0.098$) where BCS was 4, and by an estimated 4.5 mmol/L (95% CI 1.4 to 7.7; $P = 0.005$) where BCS was 8.

2.7. Associations between fasting glucose concentration and 2-h glucose concentrations

We assessed whether there was an association between fasting glucose and glucose concentrations at 2-h in an IV glucose tolerance test, because cats with impaired fasting glucose might be expected to also have impaired glucose tolerance. For every unit increase in fasting glucose, 2-h glucose increased by 0.5 mmol/L (P = 0.0064; 95% CI 0.2 to 0.9). Two cats of BCS 5 and 7 had high fasting glucose concentrations (>10 mmol/L) and this positive relationship between fasting and 2-h glucose was almost entirely due to these cats.

3. Discussion

In this study of cats 8 yrs or older, we established a standardized clinical protocol for diagnosing impaired fasting glucose and glucose tolerance using a portable glucose meter. The upper cutpoint for normal fasting glucose concentration was 6.5 mmol/L and for 2-h glucose concentration following a simplified IV glucose tolerance test (delivering 0.5 g/kg glucose dose) was 9.8 mmol/L. When applied to cats with a range of body condition scores, 3% were classed as having impaired fasting glucose and 9% as glucose intolerant. In contrast, 12 to 26 % [29] of human populations in USA, Europe and Australia have impaired fasting glucose and 7 to 28% are reported to be glucose intolerant [30,31]. However, reported rates of overweight and obesity are typically higher in these human populations (66-75%) than are reported from feline studies (14 [32] - 63% [33]), although the rate in cats varies with the population studied, and how body condition was measured [33,34]. In the absence of more accurate data on the frequency of prediabetes in the feline population 8 yrs of age or older, it is unknown if more stringent cutpoints should be applied, for example, 90% reference intervals or lower. For fasting glucose, the 90% interval would result in an upper cutpoint of 6.2 mmol/L. In humans, a link between microvascular disease such as

retinopathy and glucose concentrations [35] is well accepted. As this link has not been established in cats, we have chosen to use the 95% reference intervals.

Currently, there is no accepted cutpoint between impaired fasting glucose and diabetes in cats and various values have been suggested ranging from 9.5 [36] to 16 mmol/L, with the latter approximately representing the renal threshold [14]. In humans, cutpoints were established in part based on the association with renal and microvascular complications [6]. There is an urgent need for these cutpoints to be established in cats, especially for fasting glucose, because this measurement is easily evaluated in clinical practice. The prevalence of undiagnosed diabetes in adults in a U.S. population was 2.8%, increasing to 5.8% by the age of 60 yrs [37]. It is unknown how many cats have undiagnosed diabetes. Until the cutpoint for diabetes is established, the authors suggest using 6.5 mmol/L as the upper cutpoint for impaired fasting glucose, and unstressed cats with glucose concentrations of $\geq$10 mmol/L that are confirmed with repeated measurements be considered diabetic [38].

Humans with impaired fasting glucose or impaired glucose tolerance are considered prediabetic [6,29,30], because they are at high risk of developing diabetes, with 5-10% of individuals progressing to diabetes per yr [35]. Evidence-based cutpoints are important for diagnosing pre-diabetes in at risk cats, such as obese and Burmese cats. Because cats with impaired fasting glucose or glucose intolerance are at increased risk of diabetes [7], prediabetic cats need to be identified, and management regimes implemented including weight loss and dietary intervention.

3.1. Repeatability of fasting blood glucose concentrations

Repeatability coefficients describe repeatability from a clinical perspective, ie. if the same animal is sampled on different day, how much variation is likely between two results. This incorporates both the within lab precision plus the biological variation within the same animal. Repeatability studies showed that fasting glucose concentrations differed within cats over 3-7 weeks by approximately 1.0 mmol/L for most cats. The group size, the heterogeneity and the lack of acclimatization would have contributed to the relatively large variation. Diagnosis of impaired fasting glucose or impaired glucose tolerance in humans is based on the mean of two values measured no more than 3 months apart [6,30], and a similar recommendation would be prudent for cats.

3.2. Reference values for 2-h blood glucose concentrations

Our upper cutpoint for 2-h glucose concentration of 9.8 mmol/L was similar to 9.5 mmol/L established previously by Link et al [14] , but higher than 6.0 mmol/L calculated from Appleton's raw data [39] (data not shown), and likely higher than estimated from Hoenig's [15] lean cats (mean concentration estimated from graph was 5.6 mmol/L. The latter two studies used acclimatized research cats, and inserted jugular catheters under general anesthesia prior to obtaining blood samples, decreasing the probability for stress hyperglycaemia. They also used automated analysers which delayed sample analysis and might have contributed to lower glucose concentrations. Link et al [14] used human portable glucose meters calibrated for whole blood which are biased to lower readings than meters calibrated for cat blood that provide plasma-equivalent measurements [20] . Appleton's cats were much younger (1-5 yrs old) and there is some evidence glucose tolerance decreases with age in cats [40].

Results from an IV glucose tolerance test is more sensitive (but slightly less specific) than fasting blood glucose for identifying people at high risk of diabetes [30]. Reflecting this higher test sensitivity, impaired glucose tolerance is more prevalent than impaired fasting glucose in human populations [30]. Similarly in our study, 9% of all cats and 20% of obese cats had impaired glucose tolerance, whereas only 3% of overweight cats (BCS 6–7), and no obese cats had impaired fasting glucose. We tested only cats ≥ 8 yrs old and recruited a large proportion (65%) that were overweight or obese, because this age group and body condition are at greatest risk of developing diabetes. Also, glucose tolerance decreases with age and increasing body condition [15,41]. The prevalence of abnormal glucose homeostasis would be expected to be lower if all ages or more lean cats had been included.

3.3. Repeatability for 2-h blood glucose concentrations

Based on our results, there is a 95% expectation that two measurements would differ within cats by less than 3.8 mmol/L but by as much as 7.2 mmol/L. Caution is necessary when interpreting a single test result in client-owned cats because compared to acclimatized cats, non-acclimatized cats have a longer glucose half-life, attributed to stress [42]. Struggling 10 min prior to blood sampling is reported to increase blood glucose by as much as 10 mmol/L in cats [24]. We recommend retesting cats with glucose concentrations above the cutpoints, based on the variability of glucose tolerance test results in humans [43-45] and cats [42], although owner compliance may limit retesting for client-owned cats.

3.4. Effect of breed on fasting and 2-h blood glucose concentrations and dose

Neither fasting nor 2-h blood glucose concentrations were higher in Burmese compared to non-Burmese cats. Despite this, Burmese are 3 to 4 times more likely to develop diabetes than non-Burmese cats [46]. Because Burmese had significantly higher 2-h blood glucose

363 concentrations at the higher dose rate, it could suggest relative intolerance to glucose at
364 higher doses and this warrants further investigation.

365

366 3.5. *Protocol standardization*

367 The glucose dose rate used for a glucose tolerance test depend on the measurements of
368 interest. In cats, 1 g/kg is more sensitive than 0.5 g/kg for determining abnormalities in
369 insulin secretory patterns and maximum insulin secretory capacity [15]. However, a lower
370 glucose dose rate (i.e. 0.5 g/kg) is used when investigating insulin action [14,39]. Our study
371 used a glucose dose rate of 0.5 g/kg. The higher dose of 1 g/kg was observed to cause nausea
372 and distress in some cats (personal observations Reeve-Johnson and Gottlieb) and the lower
373 dose rate (and therefore volume of injection) was considered more user-friendly for
374 practitioners. However, at 1 g/kg, the significantly higher 2-h glucose concentrations in
375 Burmese compared to non-Burmese cats raises the question whether a higher glucose dose
376 can better differentiate cats with impaired glucose tolerance.

377 Our aim was to establish reference intervals for use in veterinary practice. Our protocol
378 decreases technical and laboratory variability reported to affect measured blood glucose
379 concentrations [15]. The same type of portable glucose meter can be used in each veterinary
380 practice to measure glucose immediately after blood collection, avoiding the variable time
381 delay in measuring glucose using a variety of serum chemistry analysers in external
382 laboratories. Postprandial glucose concentrations can be strongly influenced by diet [47] and
383 thus blood glucose should be measured in fasted cats. This requires a 14-h fast if less than
384 50% of the daily energy requirement is consumed, and a 24-h fast after 100% of the daily
385 energy requirement is consumed [48]. In our study, cats were fasted for 18-24 h and

hospitalized overnight to avoid owner non-compliance and to minimize confounding of blood glucose measurement by stress.

3.6. Associations of 2-min and 2-h glucose concentrations and adjustment for obesity

Adjustment for the spurious effects of obesity on glucose measurements following glucose dosing based on body weight was further evaluated in this study. While the associations between 2-min and 2-h glucose concentrations were not significant in the present study compared to our previous study (Reeve-Johnson et al, unpublished data), the calculated values for adjustment were very similar to those previously reported (0.05 versus 0.09 mmol/L per unit of body condition above 5; $P = 0.282$ versus $P = 0.006$ respectively). Hence, any cat with a BCS ≥ 6 which is persistently just above the cutpoint at 2 h should have the observed glucose concentration adjusted downward by 0.1 mmol/L per unit of BCS above 5. The 2-min blood sample following the glucose injection was difficult to obtain with accurate timing using a lancing device on the ear using one veterinarian and one handler. Adjusting on BCS is more precise (Reeve-Johnson et al, unpublished data), and it is therefore recommended.

4. Conclusions

We have established the methodology and cutpoints for fasting glucose and glucose tolerance in a simplified intravenous glucose tolerance test for identifying prediabetic cats in clinical practice with lean or obese body condition. We recommend 6.5 mmol/L for the cutpoint between normal and impaired fasting glucose, and 9.8 mmol/L for the 2-h glucose cutpoint between normal and impaired glucose tolerance when using a glucose dose of 0.5g/kg with blood glucose measured from ear or pad samples using a portable glucose meter calibrated for feline blood and performed after an overnight fast and hospitalization. Impaired fasting

glucose and glucose intolerance should be confirmed by repeat measurements, to minimize the probability of incorrectly diagnosing a cat with stress hyperglycemia as prediabetic. Using the criteria established, 20 % of obese cats 8 yrs of age or older are glucose intolerant. Prospective studies are required to determine the relative risk of diabetes in cats with glucose concentrations above these cutpoints. It is recommended that measured 2-h glucose concentration be adjusted downward by 0.1 mmol/L for every BCS above 5, and tests be repeated to confirm abnormal glucose tolerance.

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562 **Table 1:** Descriptive statistics and upper limits of 95 % reference intervals (90 % confidence
563 intervals) in mmol/L after fasting, and 2 min and 2 h after a glucose infusion of 0.5 g/kg
564 bodyweight iv for all cats (n = 78) and various sub-groups; BCS was assessed using a 9 point
565 scale.

Sub-group of cats	Variables	Fasting blood glucose (mmol/L)	2-min blood glucose (mmol/L)	2-h blood glucose (mmol/L)
BCS 4 or 5	n=	27	27	27
	Mean	5.1	23.3	5.8
	Median	4.9	23.4	5.4
	SEM	0.3	1.2	0.3
	SD	1.6	6.2	1.5
	Range	2.4-12.3	12.8-35.9	3.4-9.6
	95% reference interval upper limit	6.5	36.7	9.8
	Upper limit 90% CI	6.0-6.7	33.7-38.6	8.5-10.7
BCS 4 or 5; Burmese only	n=	6	6	6
	Mean	4.5	20.7	6.1
	Median	4.7	23	6
	SEM	0.4	2.6	0.9
	SD	1.1	6.4	2.2
	Range	2.4-5.4	12.8-28.7	3.4-9.6
	95% reference interval upper limit	[1]	[1]	[1]
	Upper limit 90% CI	[1]	[1]	[1]
BCS 6 or 7	n=	31	31	31

	Mean	4.9	24.6	6.4
	Median	4.4	25.1	5.7
	SEM	0.3	1	0.5
	SD	1.5	5.7	2.6
	Range	3.6-12.4	13.7-35.9	3.4-15.7
	95% reference interval upper limit	9.1	36.4	13.3
	Upper limit 90% CI	6.0-10.8	33.3-38.9	10.4-15.6
BCS 8 or 9	n=	20	20	20
	Mean	4.6	25.4	7.9
	Median	4.6	24.8	7.9
	SEM	0.2	1.2	0.6
	SD	0.9	5.5	2.7
	Range	3.2-6.3	17.3-38.7	3-12.9
	95% reference interval upper limit	6.6	39.1	14.1
	Upper limit 90% CI	6.0-7.1	33.8-42.9	12.1-15.8
All cats (BCS 4-9)	n=	78	78	78
	Mean	4.9	24.4	6.6
	Median	4.7	24.7	5.8
	SEM	0.2	0.7	0.3
	SD	1.4	5.8	2.4
	Range	2.4-12.4	12.8-38.7	3.0-15.7
	95% reference interval upper limit	6.3	36.3	12.8
	Upper limit 90% CI	6.1-6.5	34.4-37.9	11.5-13.9

1 Number of cats was insufficient to estimate reference interval

DAE-15-175R2 Highlights

- Aim: to establish methodology and cutpoints for fasting and 2-h blood glucose concentrations in healthy client-owned senior cats (≥ 8 years) using ear/ paw samples and a portable glucose meter calibrated for feline blood.
- Cutpoints for fasting and 2-h glucose concentrations were defined (upper limits of 95% reference intervals using cats with BCS 4-5 (n = 27)).
- The upper cutpoint for fasting glucose was 6.5 mmol/L.
- The cutpoint for 2-h glucose was 9.8 mmol/L.