Acute Stress Hyperglycemia in Cats Is Associated with Struggling and Increased Concentrations of Lactate and Norepinephrine

Jacqueline S. Rand, Emily Kinnaird, Anthony Baglioni, Judith Blackshaw, and Jan Priest

We characterized the changes in blood glucose concentrations in healthy cats exposed to a short stressor and determined the associations between glucose concentrations, behavioral indicators of stress, and blood variables implicated in stress hyperglycemia (glucose, lactate, insulin, glucagon, cortisol, epinephrine, and norepinephrine concentrations). Twenty healthy adult cats with normal glucose tolerance had a 5-minute spray bath. Struggling and vocalization were the most frequent behavioral responses. There was a strong relationship between struggling and concentrations of glucose and lactate. Glucose and lactate concentrations increased rapidly and significantly in all cats in response to bathing, with peak concentrations occurring at the end of the bath (glucose baseline 83 mg/dL, mean peak 162 mg/dL; lactate baseline 6.3 mg/dL, mean peak 64.0 mg/dL). Glucose response resolved within 90 minutes in 12 of the 20 cats. Changes in mean glucose concentrations were strongly correlated with changes in mean lactate ($r = .84; P < .001$) and mean norepinephrine concentrations ($r = .81; P < .001$). There was no significant correlation between changes in mean glucose concentrations and changes in mean insulin, glucagon, cortisol, or epinephrine concentrations. Struggling and lactate concentrations were predictive of hyperglycemia. Gluconeogenesis stimulated by lactate release is the likely mechanism for hyperglycemia in healthy cats in this model of acute stress. Careful handling techniques that minimize struggling associated with blood collection may reduce the incidence of stress hyperglycemia in cats.

**Key words:** Behavior; Blood glucose; Blood variables; Stress hormones.

Stress hyperglycemia describes the transient increase in blood glucose in sick cats or in cats displaying signs of fear. Stress hyperglycemia poses a challenge for veterinarians using blood glucose concentrations for diagnosis and management of diabetes mellitus in cats. Although fasting blood glucose greater than 200 mg/dL is consistently found in cats with diabetes mellitus, blood glucose as high as 360–613 mg/dL has been reported to result from stress hyperglycemia. A study of 320 cats with transient hyperglycemia concluded that transient hyperglycemia associated with illness occurs more often in cats (3.2%) than does diabetes mellitus (0.57%). The diseases associated with transient hyperglycemia include urinary tract disease, viral and bacterial infections, gastrointestinal diseases, nephropathy, renal insufficiency, and cardiomyopathy.

In addition to illness, psychological factors are recognized to be strong indicators of stress in humans and in animals. The term "white coat hyperglycemia" has been used in the human literature to describe the alterations in blood glucose associated with the stress of visits to medical facilities. Although psychological stress is difficult to define in animals, changes in behavior such as escape attempts, vocalization, or immobility indicate anxiety or fear associated with psychological stress. Link and Rand found that glucose tolerance test (GTT) results were abnormal in cats displaying these behaviors.

The absence of glycosuria despite hyperglycemia has been used as a marker for stress hyperglycemia; however, this is not reliable. Walter Cannon, the originator of the fight or flight theory, produced "emotional glycosuria" in cats immobilized and given water by stomach tube. The animals that appeared to be frightened or enraged developed glycosuria more quickly than those that were calm.

Release of epinephrine has been implicated as one of the initiators of stress-associated hyperglycemia in sick cats. Increased cortisol secretion was documented in cats chronically stressed with changes in management routine and complex stressors (eg, travel and intermittent restraint). These cats exhibited behavioral changes such as increased hiding, decreased play, and decreased exploratory activity.

There is little well-documented data on transient hyperglycemia associated with stress in cats. It is unknown what maximum increase in blood glucose concentrations occurs in cats with normal glucose tolerance when exposed to the stress of blood sampling or when associated with illness. Previous studies have not investigated whether cats with stress hyperglycemia had underlying abnormalities of glucose tolerance that amplified the hyperglycemia. Stress hormone concentrations (glucagon, epinephrine, norepinephrine, and cortisol) during stress hyperglycemia have been poorly documented. Because of the problems associated with the use of blood glucose concentrations for the diagnosis and monitoring of glycemic control in diabetic cats, it would be advantageous if stress hyperglycemia could be identified by behavioral analysis or by measurement of a biochemical or hormonal parameter in a blood sample.

The aim of this study was to determine the behavioral responses and changes in concentrations of glucose, glucagon, epinephrine, norepinephrine, cortisol, insulin, and lactate in response to a standardized 5-minute stressor in cats with normal glucose tolerance, and to determine if there were any parameters, either behavioral or biochemical, that could be used to predict stress hyperglycemia.
Materials and Methods

Cats

Twenty randomly sourced, mixed-breed, neutered cats (10 females and 10 males) were used in this study. All cats were clinically healthy and had negative feline immunodeficiency virus antibody and feline leukemia virus antigen test results. Estimated ages ranged from 9 months to 5 years. Body weight ranged from 3.4 to 5.6 kg, and body condition scores ranged from 2 to 4 on a 5-point scale.11

Cats were randomly divided into 2 groups. One group had a stress test (5-minute spray bath), followed by a GTT 7 days later. The other group had this order reversed. The GTT was performed to ensure that cats in the study had normal glucose tolerance. Because hyperglycemia after sugar ingestion is associated with changes in stress hormone concentrations in humans,14,15 concentrations of glucagon, epinephrine, norepinephrine, and cortisol were also measured during the GTT to provide information on the effect of glucose itself on these hormones.

Catheter Placement

A jugular catheter was placed 24 hours before the 1st test for blood sampling during both procedures. An 18-gauge, 8-cm single-lumen indwelling jugular catheter was placed as previously described and capped with a Luer lock injection site cap.15a Catheter tips were flushed daily with 3 mL heparinized saline (5 IU/mL) until removal at the end of the 2nd test. Food was withheld for 18-20 hours before each test.

Spray Bath Test

After removing the cats from their cages, a bathing harness was placed on each cat. Cats were then put into a carrier, moved to the bathing area, and removed from the carrier, and the bathing harness was fastened to the tub table with the attached suction cup. Cats were thoroughly sprayed with water for 5 minutes. An assistant provided restraint as required, to keep the cats in the tub. After bathing, the cats were towel dried for 2 minutes, placed in the carrier, and transported back to their cage.

Blood Sampling

Jugular blood (2.5 mL) was collected in the cage immediately before removing the cat (t1), after placement on the tub table (t2); at the end of the 5-minute spray bath (t3), after placement of the cat in its cage (t4); and every 15 minutes for 2 hours after replacement in the cage (t5, 10 t6).

Before blood sampling, a 0.5-mL mixture of blood and heparinized saline was removed from the jugular catheter and retained. A 2.5-mL sample of blood was collected, and 2.0 mL was placed in a sterile chilled EDTA tube containing 1,000 KIU aprotinin for measurement of glucose, lactate, glucagon, cortisol, and insulin. The remaining 0.5 mL of whole blood was placed in a chilled tube containing 2 mg sodium metabisulfite for measurement of catecholamines. Both tubes were centrifuged immediately to separate red blood cells and plasma. Plasma was removed and stored at −70°C until analysis. After each collection, the retained blood-saline mixture was reintegrated into the jugular catheter, and the catheter was flushed with 2.0 mL of heparinized saline (5 IU/mL). After the final sampling, red blood cells remaining in the EDTA tubes after removal of plasma were washed, resuspended in saline, and reintegrated through the jugular catheter to maintain red blood cell mass.17

Measurement of Behavioral Response

Point sampling techniques were used to record behavior during the experiment.18 Behavioral responses were observed (Table 1) and recorded at each blood sampling point (t5 to t6). The same observer (EK) assessed all cats in the study. Defined behavioral responses were graded on a 4-point scale (absent = 0, mild = 1, moderate = 2, and severe = 3).

Table 1. Definitions of the behavioral responses recorded and graded on a 4-point scale during the spray bath and glucose tolerance tests.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Struggling</td>
<td>Any vigorous body movements requiring restraint</td>
</tr>
<tr>
<td>Vocalization</td>
<td>Any noise made by the cat, with the exception</td>
</tr>
<tr>
<td></td>
<td>of hissing, including meowing, moaning,</td>
</tr>
<tr>
<td></td>
<td>screaming, and howling</td>
</tr>
<tr>
<td>Aggression</td>
<td>Behavior directed against the handler,</td>
</tr>
<tr>
<td></td>
<td>including hissing, clawing, or biting</td>
</tr>
<tr>
<td>Immobility response</td>
<td>Crouched position with no attempts to move</td>
</tr>
<tr>
<td>Hypersalivation</td>
<td>Increased salivation resulting in dripping of</td>
</tr>
<tr>
<td></td>
<td>saliva from the cat’s mouth</td>
</tr>
<tr>
<td>Mouth breathing</td>
<td>Open-mouthed position without vocalization</td>
</tr>
</tbody>
</table>

Behavioral responses were also recorded at 1-minute intervals during the bath (t1 to t6). Each cat was given a bath score for struggling and vocalization based on the sum of points assigned to the specified behavior at each minute during the bath from t5 to t6.

The GTT

Thirty minutes before the test, a 22-gauge cephalic catheter was placed for injection of glucose19 (0.5 g/kg) and was then flushed with heparinized saline (5 IU/mL). Blood was sampled from the jugular catheter before and 2, 5, 10, 15, 30, 45, 60, 90, and 120 minutes after glucose injection. Struggling and vocalization were recorded at each sampling time with the 4-point scale. A GTT score for struggling and vocalization was based on the sum of points assigned to each sampling time over the duration of the test.

The glucose disappearance (Ke glu/dis) and half-time for glucose disappearance (T1/2) values were calculated for each cat as previously reported.18 Cats were defined as having normal glucose tolerance if values for T1/2, Ke glu/dis, and blood glucose concentrations at T0 and T60 were within the range for the laboratory. The upper limit of the range used for the study of T60 was 86.4 minutes, and the lower limit for Ke glu/dis was 0.5%/min.18 The upper limits of the reference ranges used for fasting glucose and glucose at 90 minutes postglucose injection were 177 and 281 mg/dL, respectively (corrected for methodology).19

Analysis of Samples

Plasma concentrations of glucose and lactate were measured with immobilized enzyme sensors.20 Plasma concentrations of cortisol,16 insulin,15 and glucagon were measured by radioimmunoassay. All radioimmunoassay kits were previously validated for use in cats.20

Epinephrine and norepinephrine were measured by reverse-phase isocratic high-performance liquid chromatography (HPLC). Alumina extraction was used for sample purification. Acetic acid was used to elute the catecholamines from the alumina. After injection into the HPLC system, a Novapak C18 reverse-phase column separated the individual catecholamine peaks and the internal standard peak. The catecholamines were quantified via electrochemical detection. The assay was validated for feline samples and for variation in handling and storage of the plasma sample. The limit of quantitation for epinephrine and norepinephrine was 0.0015 ng/mL, and coefficients of variation were 4.0 and 3.8%, respectively.21 No change in concentrations occurred in samples stored at −70 for 60 days or in samples stored and refrozen once.21
Table 2. Summary of behavioral responses at times $t_0$ to $t_{105}$ in 20 cats during the spray bath test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time (minutes)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>105</th>
</tr>
</thead>
<tbody>
<tr>
<td>Struggling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean score</td>
<td></td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
<td>0.55</td>
<td>0.15</td>
<td>0</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>0–1</td>
<td>0–1</td>
<td>0–2</td>
<td>0–2</td>
<td>0–1</td>
<td>0</td>
<td>0</td>
<td>0–1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of cats exhibiting behavior</td>
<td></td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vocalization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean score</td>
<td></td>
<td>0.05</td>
<td>0.25</td>
<td>0.35</td>
<td>0.3</td>
<td>0.15</td>
<td>0</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>0–1</td>
<td>0–2</td>
<td>0–1</td>
<td>0–1</td>
<td>0–1</td>
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<td>0–1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of cats exhibiting behavior</td>
<td></td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Behavioral responses were graded on a 4-point scale (absent = 0, mild = 1, moderate = 2, and severe = 3).

Statistical Analysis

All analyses were conducted by SPSS for Windows, version 7.0.* Results in the text and tables are expressed as means ± standard error of the mean. Differences were considered statistically significant at $P < .05$.

Blood Parameters

All variables resulting from analysis of glucose, lactate, cortisol, insulin, glucagon, epinephrine, and norepinephrine were reviewed for normality of distribution by the EXAMINE procedure. Kolmogorov-Smirnov tests for normality were applied, and the variables were examined for skewness and kurtosis. Non-normally distributed variables were log or square root transformed to improve normality, and then parallel analyses were run with transformed and nontransformed variables. Data were examined for trends, including mean baseline, mean peak, time to peak, and return to baseline.

Short- and long-term relationships between blood parameters in the bath test and the GTT were evaluated via repeated-measures analysis of variance. Pearson's product moment correlation was used to determine the strength of relationships between blood variables in the bath test and the GTT. In the bath test, cats were divided into 2 groups on the basis of a median split of peak glucose concentrations. Independent sample t-tests for equality of means compared behavioral scores between the 2 groups. Blood variables from the high glucose group were compared with variables from the low glucose group at $t_{60}$ and peak concentration.

Behavior

Data for struggling scores and vocalization scores were examined for normality of distribution by the SPSS EXAMINE procedure. Histograms were used to demonstrate normality. Behavioral data were examined for frequency and distribution. The relationship between behavior scores and blood glucose concentrations was evaluated by Spearman's rank correlation.

Stress hormones (cortisol, glucagon, and catecholamines) were analyzed for change over time by repeated-measures analysis of variance. Cortisol data from the bath test were compared with data from the GTT test by paired-sample t-tests.

Results

Spray Bath Test

Behavioral Responses. Struggling (19 cats) and vocalization (10 cats) were the most frequent behaviors observed throughout the study. Mouth breathing (6 cats), immobility (4 cats), aggression (2 cats), and salivation (1 cat) occurred less frequently. They did not occur at any period outside the bathing interval, except for 1 incidence of aggression associated with placement back in the kennel.

Struggling. Struggling was the most common behavioral response (Table 2). More cats struggled (19 cats) and with greater intensity during the bath than outside the bath interval (11 cats) (Fig 1). Struggling associated with blood collection alone was most frequent during initial blood collection (4 cats), resulting in mild struggling scores even at $T_0$ (Table 2). After the bath, struggling was most frequent when cats were placed back in the kennel ($t_{60}$; n = 9) (Table 2). The struggling scores during the bath were used for statistical analysis because of the low frequency of struggling outside the bath interval.

Vocalization. Vocalization was the next most frequent behavioral response during the bath test, with half of the cats vocalizing. However, the vocalization scores were lower than the struggling scores at nearly every point in the study (Table 2). After the bath, vocalization was most common after replacement in the kennel ($t_{60}$; n = 6). Vocalization rarely occurred with blood collection alone (Table 2).

Glucose Response. Changes in glucose concentrations during the experiment were rapid and significant ($P < .001$) (Fig 2). Glucose concentrations were already higher than baseline before the bath, when the cats were on the bath.

![Image of graph showing struggling score over time](image_url)

**Fig 1.** Mean struggling scores for 20 cats during a 5-minute spray bath and at each blood sampling point from $t_0$ to $t_{105}$ of the spray bath test. Struggling was scored on a 4-point scale (0–3), with a score of 3 indicating severe struggling. **(a)** Blood collection in the kennel. **(b)** Placement of the cat onto the bathing table. **(c)** Spray bath. **(d)** Replacement of cat in kennel.
Fig. 2. Lactate and glucose concentrations (X ± SEM) in 20 cats during the spray bath test. Asterisks (*) indicate data significantly different from baseline (P < .05). The 5-minute spray bath is indicated by the 2 arrows. (a) Blood collection in the kennel. (b) Placement of the cat onto the bathing table. (c) End of spray bath. (d) Replacement of cat in kennel.

Table 1 (t1) (P = .02), but the greatest increase in glucose concentrations occurred during the bath (Fig 2). Mean glucose concentrations doubled during the 1st 10 minutes (t0 to t10) of the study, with peak concentrations occurring at the end of the bath (t90: baseline glucose 83 mg/dL, mean peak 162 mg/dL ± 11). Although the mean increase in glucose overall was only 74 mg/dL, the increase was as high as 194 mg/dL in individual cats.

Glucose concentrations remained high during the interval when the cats were towel dried and moved back to their cages (t10 to t30) (Fig 2). Group glucose concentrations decreased rapidly after the cats were returned to their cages but did not return to baseline concentrations at the end of the test (Fig 2).

Glucose concentrations showed large interindividual variability (Fig 3). Analysis of individual cat trends revealed that glucose concentrations rose before the bath (t1) in 5 cats. Glucose concentrations were increased in all cats by the end of the bath (t90) and peaked at the end of the bath or 5 minutes postbath (t100 or t150) in 19 of 20 cats. Most cats in the study had a very transient glucose response, with glucose concentrations returning to baseline by 30 minutes. However, 8 cats still had high glucose concentrations at the conclusion of the test. In general, cats with high glucose concentrations at test conclusion also had higher peak glucose concentrations (Fig 3).

Lactate Response. Lactate concentrations increased rapidly during the experiment (Fig 2). Significant changes over time occurred in group lactate concentrations (P < .001), with increased concentrations occurring before the start of the bath (t1; P < .001) and remaining significantly high until t100. Mean lactate concentrations increased 10-fold between the initial blood collection and blood collection at the end of the bath, whereas the increase in lactate concentrations during the bath (t1 to t90) was 4-fold (mean peak 64.0 mg/dL ≥ 6.5).

Lactate concentrations showed little interindividual variability (Fig 2). Analysis of data for individual cats revealed that most cats (13 cats) had increased lactate concentrations by t2. All cats had increased lactate concentrations by the end of the bath (t90). Peak lactate concentrations occurred at the end of the bath (n = 13) or 5 minutes postbath (t150; n = 7). Lactate concentrations had returned to baseline by the end of the testing period (t150) in most cats (n = 17).

Comparisons Between Glucose and Lactate. Mean lactate concentrations correlated with mean glucose concentrations when compared from t1 to t150 (r = .84; P < .001). Both glucose and lactate increased before the bath (t1), and both parameters peaked by the end of the bath (t90) (Fig 2). However, the increase in lactate concentrations was 5 times greater than the increase in glucose concentrations. Both mean glucose and mean lactate concentrations began to decrease at t150, with lactate decreasing more rapidly than glucose between t90 and t150 (Fig 2). Lactate concentrations returned to baseline by t150, whereas glucose concentrations remained above baseline through the end of the experiment. From t90 to t150, when glucose concentrations were almost horizontal but significantly increased, the only consistent association between glucose and the other blood variables was still with lactate (P < .05: t90, t100, t150; P < .1: t90).

Comparisons Based on a Median Split of Peak Glucose Concentrations. Cats were split into 2 groups on the basis of a median split of peak blood glucose concentrations (149.5 mg/dL). Cats in the high peak glucose group had higher lactate concentrations at the end of the bath (t90; P = .05) and 5 minutes after the bath (t150; P = .04), and they also had higher peak lactate concentrations (P = .04). There were no differences between the high and low peak glucose groups when other blood parameters were compared.

Comparisons Between Glucose, Lactate, and Behavior. There was a strong relationship between struggling and high concentrations of glucose and lactate (Table 3). Also, cats in the high peak glucose group had higher struggling scores during the bath than cats in the low peak glucose group (P = .02). There was no relationship between bath scores for struggling and any other blood parameter.

Neither vocalization nor other measured behaviors were associated with changes in glucose, lactate, insulin, or stress hormones. In addition, vocalization scores during the bath...
Table 3. Correlation between bath scores for struggling and plasma glucose and lactate concentrations during the spray bath test.*

<table>
<thead>
<tr>
<th>Plasma Variable</th>
<th>Time</th>
<th>Spearman’s Rank Coefficient</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Peak</td>
<td>( r = .59 )</td>
<td>( P = .01 )</td>
</tr>
<tr>
<td></td>
<td>( t_{50} )</td>
<td>( r = .47 )</td>
<td>( P = .04 )</td>
</tr>
<tr>
<td></td>
<td>( t_{75} )</td>
<td>( r = .70 )</td>
<td>( P = .001 )</td>
</tr>
<tr>
<td>Lactate</td>
<td>Peak</td>
<td>( r = .33 )</td>
<td>( P = .3 )</td>
</tr>
<tr>
<td></td>
<td>( t_{50} )</td>
<td>( r = .47 )</td>
<td>( P = .04 )</td>
</tr>
<tr>
<td></td>
<td>( t_{75} )</td>
<td>( r = .60 )</td>
<td>( P = .01 )</td>
</tr>
<tr>
<td></td>
<td>( t_{90} )</td>
<td>( r = .57 )</td>
<td>( P = .01 )</td>
</tr>
<tr>
<td></td>
<td>( t_{95} )</td>
<td>( r = .03 )</td>
<td>( P = .90 )</td>
</tr>
</tbody>
</table>

* Bath score was calculated from the sum of struggling scores observed at 1-minute intervals during the bath \( t_{50} \) to \( t_{95} \) and ranged from 0 to 11 (mean, 4.5 \( \pm \) 3.2).

were not different between the peak glucose groups \( (P = .1) \).

**Insulin Response.** Analysis revealed that group insulin concentrations changed over time \( (P < .001) \); however, significant changes did not occur until \( t_{50} \) \( (P = .02) \) (Fig 4). Group insulin concentrations fell below baseline concentrations at \( t_{75} \) and did not return to baseline values at the end of the experiment \( (P = .02) \). There was wide interindividual variability in insulin data, including baseline insulin concentrations, peak insulin concentrations \( (7.0–24.0 \mu U/mL) \), and time to peak concentrations (Fig 4).

There was no significant correlation between changes in insulin concentrations and changes in glucose concentrations \( (P > .10) \). Mean insulin concentrations did not change significantly from \( t_{50} \) to \( t_{90} \), the time of greatest change in mean glucose concentrations. No relationship was observed between struggling during the bath (bath score) and insulin concentrations. Insulin concentrations were not associated with any other blood variable.

**Glucagon.** There was wide interindividual variability in glucagon concentrations at all sampling times, making interpretation of trends difficult (Fig 4). Time to peak also varied among cats. There was no clear modal time to peak; peak scores occurred at 7 of the 10 sampling intervals, including \( t_{50} \) and \( t_{90} \).

Group glucagon concentrations increased during the experiment \( (P < .001) \), with a significant increase 1st occurring at the end of the bath \( (t_{90}, P = .02) \) (Fig 4). Mean glucagon concentrations increased 1.8 times above baseline, and peak concentrations occurred after the cats were returned to their cages at \( t_{90} \) (Fig 4). Increased concentrations persisted through \( t_{90} \) \( (P = .02) \). However, at \( t_{90} \) glucagon returned to baseline concentrations and remained there for the remainder of the sampling period.

There was no significant correlation between the change in mean glucagon concentrations and the change in mean glucagon concentrations \( (r = .48; P > .05) \) (Fig 4). Nor was any relationship observed between struggling during the bath and glucagon concentrations. There was an association between glucagon and norepinephrine \( (r = .77; P = .02) \) but not with any other blood variable.

**Cortisol Response.** Cortisol concentrations changed over time, with a significant increase occurring at the end of the bath \( (P < .001) \) (Fig 5). Mean cortisol concentrations rose 5-fold during the experiment, with peak concentrations at \( t_{30} \), 15 minutes after the cats were replaced in their cages. Cortisol remained high through the final testing time \( (P < .001) \).

There was no significant correlation between changes in mean glucagon concentrations and changes in mean cortisol concentrations \( (r = .3; P > .10) \). The increase in glucagon occurred earlier \( (t_{75} \) versus \( t_{90} \)) than the increase in cortisol (Fig 5). However, the increase in cortisol was of greater magnitude \( (5\text{-fold versus 2\text{-fold})} \) and was more prolonged than the glucagon response. After the bath, glucose decreased quickly, whereas mean cortisol concentrations continued to rise, reaching a peak at \( t_{50} \). At no time when cortisol was increased \( (t_{50} \) to \( t_{90} \)) were cortisol concentrations associated with glucagon concentrations. Nor was any relationship observed between struggling during the bath and cortisol concentrations. Cortisol was not correlated with any other blood variable.

**Norepinephrine.** Group norepinephrine concentrations changed significantly over the testing period \( (P < .001) \)
Fig 6. Plasma norepinephrine and glucose concentrations (± SEM) in 20 cats during bathing experiment. Asterisks (*) indicate data significantly different from baseline (P < .05). (a) Blood collection in the kennel. (b) Placement of the cat onto the bathing table. (c) End of spray bath. (d) Replacement of cat in kennel.

Fig 7. Plasma epinephrine and glucose concentrations (± SEM) in 20 cats during bathing experiment. Asterisks (*) indicate data significantly different from baseline (P < .05). (a) Blood collection in the kennel. (b) Placement of the cat onto the bathing table. (c) End of spray bath. (d) Replacement of cat in kennel.

>0.59%/min during the GTT and normal glucose concentrations at baseline and 90 minutes after the glucose challenge (Table 4).6

Behavioral Responses. Few very behavioral responses occurred during the GTT. No cats showed aggression, mouth breathing, salivation, or immobility at any measurement point. Struggling (14 cats) and vocalization (7 cats) were the most common behavioral responses during the GTT, but these responses were mild and infrequent compared to those during the bath test (Tables 2, 4). The majority of behavioral responses were seen at the first sample time after the glucose injection (t1) and the following 2 sample times: t2 and t3 (Table 4).

Glucose and Insulin. As expected, glucose and insulin increased significantly from baseline by t1 (Fig 8), and there was a significant association between insulin and glucose (r = .76; P < .01). Peak glucose concentrations in the GTT were more than 2.5 times higher than in the spray bath test. Glucose and insulin concentrations returned to baseline by 120 minutes.

Lactate. Lactate did not change from baseline, but there was a very large interindividual variability (Table 4). In the spray bath test, lactate was associated with struggling (r = .60; P < .01) but not with vocalization.

Glucose and Lactate. In contrast with the spray bath test, glucose and lactate were not associated during the GTT.

Norepinephrine and Epinephrine. Norepinephrine and epinephrine did not change from baseline during the GTT, and there was wide interindividual variability in both parameters (Table 4). There was no association between glucose, lactate, or behavior and norepinephrine or epinephrine.

Glucagon. As expected, glucagon decreased significantly from baseline beginning at t1, and remained below baseline to the end of the test (Table 4). There was a significant negative association with cortisol (r = -.79; P < .01).

Cortisol. There was a pronounced cortisol response during the GTT (Fig 8; P < .001). Cortisol concentrations began to rise at t1 (P = .04). Mean cortisol concentrations rose until t3 and then remained high through the end of the
test ($P < .001$). Peak cortisol concentrations were approxi-
mately 3 times baseline concentrations (Table 4). Modal
time to peak was $t_{60}$ (7 cats), but individual cats had peak
cortisol concentrations occurring from $t_{60}$ (4 cats) to $t_{150}$ (2
cats). When baseline values were excluded, there was a
strong negative association with glucose ($r = -0.92; P =
.0005$). Cortisol concentrations were not associated with
struggling or vocalization scores.

**Cortisol Response during GTT Compared with Cortisol Response during Bath Test.** Comparisons of mean cortisol response between the bath test (Fig 6) and the GTT (Fig 8) revealed that cats had a similar cortisol responses during both procedures. Paired $t$-tests showed that cortisol concentra-
tions during the GTT did not differ significantly from
cortisol concentrations during the spray bath test at any
measured point.

**Discussion**

The key finding in this study is the strong relationship
between increased glucose concentrations, struggling,
and increased lactate production. Cats that struggled during the
bath had higher peak concentrations of glucose and lactate.
This relationship was not seen with vocalization or other
measured behaviors such as mouth breathing.

Struggling commonly occurs in cats during blood collec-
tion. Struggling and attempts to escape are fear responses
that may aid an animal in its escape attempts from a pred-
ator. As such, they are common and predictable behavioral
responses to physical restraint and stress. The association
between struggling and glucose is an important one for cli-
nicians, who need to be aware that increases in blood glu-
cose concentrations are likely in cats that struggle. Careful
handling techniques that minimize the stress and struggling
associated with blood collection may reduce the incidence
of stress hyperglycemia in cats.

Even the mild stress associated with initial blood collec-
tion and placement on the bathing table was sufficient to
cause struggling and increased glucose concentrations in
some cats. As expected, the increase in glucose concentra-
tions was greatest during the bath, with the highest glucose
concentrations occurring at the end of the bath. Although
glucose concentrations had normalized within 90 minutes
in more than half of the cats in this study, some cats still
had glucose concentrations in the diabetic range ($>200$ mg/
dL) 90 minutes after the stressor. Therefore, clinicians
should be aware of the potential for hyperglycemia of at
least 90–120 minutes in duration in healthy cats that have
been restrained or exposed to other types of short-term
stress that induce struggling.

There is confusion in the literature about the maximum
blood glucose concentrations reached during stressful situ-
ations in healthy cats. In this study, peak glucose ranged
from 94 to 285 mg/dL. Blood glucose concentrations of
288–396 mg/dL have been reported for cats stressed by
visits to veterinary hospitals. Several factors in these studi-
es were different and may account for the differences in
blood glucose concentrations. It is likely that subject selection
fluenced behavioral responses, as cats chosen for this study
were easily handled. All cats had been acclimatized to rou-
tine handling procedures and group housing. Poorly social-
ized cats or cats unaccustomed to routine handling pro-
dures (eg, pet cats subjected to blood collection) may be
have differently in response to restraint.

It is also likely that the choice of stressor influenced be-
avioral response and severity of hyperglycemia. The bath
stressor was chosen because it could be standardized in time
and intensity, because it was repeatable, and because it was
an ethically acceptable stressor. Although restraint and con-
tact with water would be expected to elicite more active
responses in the majority of cats, the stressor used in this
study was probably of shorter duration than the stressors
associated with a visit to a veterinary hospital. Longer du-
rations of stress potentially result in higher glucose concen-
trations.

Hyperglycemia associated with illness is reported to re-
sult in higher glucose concentrations than those found in
this study of healthy cats. Illness is a chronic stressor, and
the mechanisms producing hyperglycemia in illness may be
quite different from those responsible for the transient hy-
perglycemia produced in this study. When hyperglycemia
from struggling during blood collection is superimposed on
the hyperglycemia of illness, it is conceivable that glucose
may go higher than with either influence alone. It is also
unknown whether cats with marked hyperglycemia associ-
ated with illness have normal underlying beta-cell function,
because there are no reports of these cats being tested once
hyperglycemia resolved. It is possible that some sick cats
have underlying beta-cell dysfunction and reduced insulin
secretion, contributing to the marked hyperglycemia. There-
fore, the magnitude of the glucose increase observed in this
study is only relevant to healthy cats subjected to acute
stress that results in struggling (eg, physical restraint for a
diagnostic procedure or blood sampling).

Lactate concentrations increased rapidly and dramatically
in all cats by the end of the bath. The association between
lactate and struggling during this experiment is consistent
with studies in other species showing that lactate production
increases during exercise. Measurement of lactate produc-
tion has been used as an indication of the intensity of ex-
ercise and the associated anaerobic glycolysis. Even mild
restraint stress has been shown to increase lactate concen-
trations in pigs. Although struggling was infrequent and
mild during the GTT, lactate concentrations were still asso-
ciated with struggling.

Research in other species has shown that the excess lac-
tate that accumulates as a result of vigorous exercise is
oxidized to carbon dioxide and water or is used for the
gluconeogenic production of glucose and tissue glycogen.
Lactate is the main gluconeogenic precursor in nor-
mal humans made hypoglycemic with insulin. Hyper-
glycemia occurring in cats associated with blood collec-
tion has been attributed to psychological stress or the
"white coat" effect. Results of this study suggest that
struggling and release of lactate contribute to hypergly-
cemia by stimulating increased gluconeogenesis.

Stress hyperglycemia is reported to be associated with
insulin resistance, decreasing peripheral glucose uptake.
However, in a recent study of cats tested with the same
bath test, peripheral insulin resistance did not occur.
Our hypothesis, that acute stress hyperglycemia is the result
of increased gluconeogenesis, rather than peripheral insulin
Table 4. Summary of glucose, lactate, insulin, glucagon, cortisol, epinephrine, and norepinephrine concentrations collected at times t₀ to t₁₅₀ in 20 cats during the glucose tolerance test. Glucose (0.5 g/kg) was injected IV immediately after t₀. Bathing occurred between 5- and 10-minute samples.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>87 ± 2</td>
<td>404 ± 14</td>
<td>326 ± 11</td>
<td>292 ± 11</td>
<td>258 ± 11</td>
<td>220 ± 11</td>
</tr>
<tr>
<td>Lactate (mg/dL)</td>
<td>6.9 ± 0.02</td>
<td>7.7 ± 0.8</td>
<td>10.9 ± 3.4</td>
<td>11.3 ± 3.0</td>
<td>7.9 ± 1.0</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(2.7–15.2)</td>
<td>(7.5–18.9)</td>
<td>(3.3–13.3)</td>
<td>(3.0–73.0)</td>
<td>(2.5–24.1)</td>
<td>(4.6–16.4)</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>8.9 ± 0.7</td>
<td>15.9 ± 1.4</td>
<td>16.5 ± 1.3</td>
<td>18.6 ± 1.5</td>
<td>17.9 ± 1.4</td>
<td>16.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>(5.0–15.3)</td>
<td>(5.6–27.8)</td>
<td>(7.2–28.5)</td>
<td>(7.8–30.6)</td>
<td>(7.5–30.6)</td>
<td>(8.4–30.6)</td>
</tr>
<tr>
<td>Glucagon (pg/mL)</td>
<td>351 ± 39</td>
<td>342 ± 52</td>
<td>304 ± 43</td>
<td>290 ± 37</td>
<td>286 ± 42</td>
<td>285 ± 44</td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
<td>1.23 ± 0.3</td>
<td>1.12 ± 0.2</td>
<td>1.16 ± 0.2</td>
<td>1.41 ± 0.2</td>
<td>1.85 ± 0.3</td>
<td>2.50 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>(0.18–4.6)</td>
<td>(0.3–3.9)</td>
<td>(0.3–4.0)</td>
<td>(0.3–4.1)</td>
<td>(0.4–4.6)</td>
<td>(0.3–5.9)</td>
</tr>
<tr>
<td>Epinephrine (ng/mL)</td>
<td>0.13 ± 0.05</td>
<td>0.15 ± 0.06</td>
<td>0.12 ± 0.04</td>
<td>0.15 ± 0.05</td>
<td>0.14 ± 0.04</td>
<td>0.15 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>(0.02–0.77)</td>
<td>(0–0.82)</td>
<td>(0–0.68)</td>
<td>(0.01–0.73)</td>
<td>(0.01–0.51)</td>
<td>(0–0.55)</td>
</tr>
<tr>
<td>Norepinephrine (ng/mL)</td>
<td>0.48 ± 0.12</td>
<td>0.53 ± 0.11</td>
<td>0.48 ± 0.10</td>
<td>0.69 ± 0.14</td>
<td>0.52 ± 0.1</td>
<td>0.49 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>(0.01–1.9)</td>
<td>(0.01–1.39)</td>
<td>(0.01–2.00)</td>
<td>(0.06–2.10)</td>
<td>(0.07–1.53)</td>
<td>(0.06–1.75)</td>
</tr>
</tbody>
</table>

*Values given are mean ± SEM with range in parentheses.
*Number of cats exhibiting behavior.

resistance, is physiologically logical. The flight or fight response is associated with increased metabolic rate. It is to be expected that this response would be facilitated by increased glucoseogenesis, providing glucose for muscle and brain activity. Conversely, peripheral insulin resistance decreases glucose availability to muscle, impairing the capacity for a flight or fight response. Although peripheral insulin resistance does not appear to be the mechanism of hyperglycemia in acute stress in cats, chronic stress may result in reduced insulin sensitivity. In chronic illness in humans, insulin sensitivity is decreased. This is an appropriate adaptation, so that blood glucose is maintained and used for essential metabolic processes, rather than utilized for muscle metabolism.

Although increased lactate concentrations were associated with the initial rapid increase in blood glucose concentrations during the 1st 15 minutes of the experiment, lactate concentrations returned to baseline more quickly than glucose, suggesting that the rapid initial rise in glucose concentrations may be produced by mechanisms different from those maintaining high glucose concentrations. High concentrations of glucagon, norepinephrine, and cortisol and decreased insulin concentrations would be expected to contribute to maintenance of hyperglycemia. However, the only consistent association with glucose concentrations after the 1st 15 minutes of the experiment was with lactate, suggesting it was still the major factor in maintaining hyperglycemia after the bath.

Data from measurements of cortisol, insulin, glucagon, and catecholamines showed wide interindividual variation and non-normal distributions. For this reason, results of analyses for these hormones must be interpreted with caution.

Although cortisol has been implicated as a major contributor to the stress response, recent literature reports limitations in the usefulness of cortisol in assessing the effect of stressors on animals. Studies in cats have shown that even a change in husbandry routines results in increased urinary excretion of cortisol. In this study, although cortisol concentrations increased, they were not reliable predictors of hyperglycemia because cortisol concentrations increased later than glucose concentrations. In fact, the greater increase in cortisol concentrations in both the bath test and the GTT occurred as glucose concentrations decreased. Because cortisol response was similar in both tests, despite minimal behavioral evidence of stress in the latter, it is further evidence that cortisol is a poor predictor of stress hyperglycemia in restrained, healthy cats.

Insulin concentrations did not increase as expected in response to increased glucose concentrations and, in fact, decreased significantly 60 minutes after the bath. Suppression of insulin has been reported in response to some types of stressors. Inhibition of insulin secretion during stress has been shown to be mediated by sympathetic activity of the adrenal glands. However, in this study, decreased insulin concentrations were not associated with increased stress hormone concentrations. Although the decrease in insulin concentrations later in the experiment may have contributed to the persistence of hyperglycemia in some cats, the effect was not statistically significant at P < .05.

Glucagon is reported to be one of the rapid counterregulatory hormones because of its ability to increase blood.
Table 4. Extended

<table>
<thead>
<tr>
<th>Time (minutes)*</th>
<th>45</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>180 ± 11</td>
<td>150 ± 11</td>
<td>108 ± 7</td>
<td>88 ± 4</td>
<td>(88-249)</td>
</tr>
<tr>
<td>6.4 ± 0.5</td>
<td>7.6 ± 0.8</td>
<td>5.9 ± 0.50</td>
<td>5.6 ± 0.5</td>
<td>(3.7-11.6)</td>
</tr>
<tr>
<td>16.5 ± 1.4</td>
<td>14.8 ± 1.3</td>
<td>11.4 ± 1.1</td>
<td>9.0 ± 0.7</td>
<td>(8.6-28.6)</td>
</tr>
<tr>
<td>283 ± 39</td>
<td>277 ± 35</td>
<td>283 ± 35</td>
<td>278 ± 31</td>
<td>(109-757)</td>
</tr>
<tr>
<td>2.57 ± 0.4</td>
<td>2.97 ± 0.4</td>
<td>2.75 ± 0.4</td>
<td>2.57 ± 0.3</td>
<td>(0.3-5.7)</td>
</tr>
<tr>
<td>17 ± 0.06</td>
<td>13 ± 0.03</td>
<td>1.4 ± 0.04</td>
<td>0.14 ± 0.04</td>
<td>(0.02-0.45)</td>
</tr>
<tr>
<td>0.48 ± 0.09</td>
<td>0.56 ± 0.11</td>
<td>0.54 ± 0.10</td>
<td>0.53 ± 0.12</td>
<td>(0.07-1.19)</td>
</tr>
<tr>
<td>0.1</td>
<td>0.15</td>
<td>0.05</td>
<td>0</td>
<td>(0-1)</td>
</tr>
<tr>
<td>2*</td>
<td>3*</td>
<td>1*</td>
<td>0*</td>
<td>(0-1)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
<td>(0)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>(0-1)</td>
</tr>
<tr>
<td>0*</td>
<td>0*</td>
<td>1*</td>
<td>0*</td>
<td></td>
</tr>
</tbody>
</table>

Glucose concentrations within a few minutes. In this study, increased glucagon concentrations were not predictors of stress hyperglycemia. Although glucagon concentrations rose significantly above baseline, glucagon response occurred after the increase in glucagon concentrations, indicating that glucagon secretion was not primarily responsible for the increase in glucose concentrations. In addition, there was no significant difference in glucagon concentrations between cats with high peak glucose concentrations and cats with lower peak glucose concentrations.

There is a substantial body of literature that supports the involvement of catecholamines in the production of stress hyperglycemia in humans and animals. Studies in cats with altered glucose tolerance or stress hyperglycemia have indicated that release of catecholamines during testing is responsible. It has been suggested that measurement of catecholamines may be valuable in the assessment of hyperglycemic cats. In our bath study, there was an association between norepinephrine and glucose concentrations. However, there was wide inter- and intrasubject variation, and comparisons of norepinephrine concentrations between cats in the high peak glucose group and the low peak glucose group revealed no differences.

Epinephrine concentrations varied widely among individual cats, but grouped data did not rise significantly during the bath, and no difference was found in epinephrine concentrations between cats with high and low peak glucose concentrations.

Studies in humans have shown that changes as minor as rising from a sitting position can increase catecholamine concentrations within seconds. This rapid change in catecholamine concentrations with a minor stimulus may explain the large variation in catecholamine concentrations among individual cats, and within cats over time, that was observed in this study. Therefore, it is unlikely that measurement of catecholamines is useful when assessing blood glucose concentrations in a clinical situation. In addition, measurement of catecholamines is not available as a routine test at most laboratories.

In this study, the increase in lactate associated with struggling in the GTT was insufficient to affect mean group glucose concentrations. However, the number of cats struggling was small and the severity mild. On the basis of this bath study, any cat with marked struggling during catheter placement or blood collection for a GTT may have spuriously high glucose concentrations and glucose half-life. It is recommended that at least 3 hours elapse between catheter placement and the start of a simplified GTT, to minimize the effect of stress hyperglycemia on results.

In summary, blood glucose did not increase above 285 mg/dL in this model, suggesting that higher blood glucose concentrations should not be attributed solely to the acute stress of blood collection in clinically normal cats. On the basis of reports in the literature, sick cats may develop higher glucose concentrations associated with stress. Lactate may be a useful predictor of stress hyperglycemia if measured within 5–10 minutes of the stressor occurring. However, before lactate can be recommended for use as an indicator of stress hyperglycemia, further studies are needed in newly diagnosed diabetic and nondiabetic sick cats to determine if lactate is associated with glucose concentrations and struggling. Although lactate measurement may be useful, observation is an inexpensive and invaluable tool. If the cat struggles before or during blood sampling, then glucose concentrations are likely to be affected by stress hyperglycemia.

Footnotes

*a Catheter, Cook, Brisbane, Qld, Australia
*b Luer lock injection site cap, Tuta Laboratories, Sydney, NSW, Australia
*c Stay-N-Wash Noose, Pet Network Pty Ltd, Seaford, Victoria, Australia
*d Trasylol, Bayerwerk, FRG, Leverkusen, Germany
*e Catheter, Becton Dickinson, Franklin Lakes, NJ
References


