Carbohydrate level and source have minimal effects on feline energy and macronutrient metabolism

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ABSTRACT: The carnivorous nature of the domestic cat makes feline metabolism of carbohydrates unique. The cats' glycemic response has been previously studied, with variable outcomes in response to carbohydrate level and source, but is an important response to understand how to control glycemia. The objectives of this study were to determine the glucose and insulin responses of cats fed 3 commercial diets differing in carbohydrate content and source, and to investigate the effects of diet on RQ, energy expenditure (EE), and glycemic response. Domestic shorthair cats (=19, 10 males, 9 females) of similar age (4.3 ± 0.48 yr, mean $\pm$ SD) and of ideal body condition score were used. Cats were fed, once a day, 1 of 3 commercial diets that differed in their perceived glycemic response (PGR; 36.8%, 30.7%, and 23.6% starch for high, medium, and low PGR, respectively) with cats cycling through all diets in 3 periods in 6 complete and 1 incomplete 3×3 Latin square. Each period

consisted of 8 d of adaptation to the diet, followed by 21-h calorimetry measurements, and real-time interstitial glucose measurements on day 9. On day 10, sequential blood sampling was completed to determine blood glucose and insulin. BW and ME intake did not differ among treatments. EE in the fasted state did not differ among treatments ($P = 0.160$), whereas postprandial EE was highest for the high PGR diet compared with the medium PGR and low PGR diets ($P < 0.001$). In conclusion, cats revealed a prolonged postprandial glucose and insulin response compared with other monogastric animals, yet diet effects were minimal. Overall, interstitial glucose measures were less variable than serum glucose measurements and followed a parallel pattern to RQ. Therefore, going forward, calorimetry and continuous interstitial glucose monitoring should be considered as less invasive alternatives to repeated blood sampling.

Key words: calorimetry, carbohydrate, feline, glycemic response, interstitial glucose

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INTRODUCTION

Dry commercial diets formulated for domestic cats include carbohydrates to ensure product stability and limit microbial growth (Forrester and Kirk, 2009). However, as domestic cats are obligate carnivores (Verbrugghe and Hesta, 2017), their mechanisms of starch digestion, absorption,

and metabolism are not entirely understood. A majority of the literature investigating the glycemic responses of cats has been conducted in obese or diabetic subjects, and therefore, there exists a gap in research regarding carbohydrate metabolism in healthy individuals (Appleton et al., 2004; Hoenig et al., 2007). Additionally, the specific effects of carbohydrate ingredient source on feline energy and macronutrient metabolism are not well understood. Although work has been done regarding the effects of macronutrient level on energy expenditure (EE) and macronutrient oxidation,

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there is limited research concerning ingredient-specific effects on these same parameters.

The postprandial glycemic response measures the rate of glucose appearance, and consequential insulin release, into the bloodstream in response to consumption of a meal (Jenkins et al., 1981) and is often measured in cats with repeated blood sampling. Although these methods are exercised commonly in feline research, they are invasive and stressful to cats (Rand et al., 2002). Furthermore, measures of blood glucose taken at interval time points cannot accurately capture a real-time glucose response (Rebrin et al., 1999). As a less invasive alternative to repeated blood sampling, continuous interstitial glucose monitoring systems have been evaluated in other species and have been found to correlate with whole blood glucose levels (Wiedmeyer et al., 2003).

Considering the limited research regarding the effects of carbohydrates on the feline glycemic response, the present study discusses the perceived glycemic response (PGR) which refers to the expected theoretical glycemic response that would result from consumption of each diet (Asaro et al., 2017). The objectives of the present study were to measure effects of 3 diets differing in their PGR and ingredient composition on 1) RQ and EE in healthy adult cats and 2) interstitial glucose and serum glucose and insulin in healthy adult cats. We hypothesized that 1) EE would not differ between cats fed similar daily amounts of dietary energy that are different in carbohydrate composition; 2) RQ will be greatest for cats fed the high PGR diet; and 3) cats fed the high PGR diet will exhibit the highest glycemic response relative to the medium and low PGR diets.

MATERIALS AND METHODS

All procedures were reviewed and approved by Procter & Gamble Pet Care's Institutional Animal Care and Use Committee (AUP 013-9127) and were in accordance with USDA and AAALAC guidelines.

Animals, Diets, and Experimental Design

Domestic shorthair cats ($n = 19$, 10 males, 9 females) of similar age (4 to 5 yr) and body condition score (3 to 4.5 on a 9-point scale) were used. Cats were provided by and housed at the Pet Health and Nutrition Centre (PHNC) at Procter & Gamble Pet Care (Lewisburg, OH) and received veterinary exams to ensure health before and during the study. Cats had

been acclimated to calorimetry chambers (Gooding et al., 2012) and also trained to accept repeated (up to 8 per d) saphenous vein blood draws (Lockhart et al., 2011). Cats were assigned to diets in a 3×3 Latin square, with cats cycling through all diets in 3 periods in 6 complete and 1 incomplete 3×3 Latin square.

The term PGR was selected to characterize the diets in the present study. This estimate was determined by looking at feline glycemic responses to the main carbohydrate containing ingredients included in each diet (Carciofi et al., 2008). However, although the glycemic response is suggestive of glycemic index (GI), it is not determined against a known control in cats, and consequently no GI can be calculated in the current study. Therefore, to predict the relative differences in glycemic response between these ingredients, GI values, quantified in human nutrition research, were also considered when selecting diets. Diets were selected for containing ingredients that are predicted to elicit high, medium, and low glycemic responses, and named based on their respected PGR. Three diets were studied (Table 1): high PGR: Purina ONE Chicken and Rice (Nestlé, St. Louis, MO), medium PGR: Iams Kitten Proactive Health (Procter & Gamble, Cincinnati, OH), and low PGR: Innova Dry Adult Cat Food (Procter & Gamble, Cincinnati, OH). Purina ONE Chicken and Rice was chosen as the high PGR diet due to its high inclusion of Brewer's rice, a high GI grain (Jenkins et al., 1981; Carciofi et al., 2008). Iams Kitten Proactive Health was comprised of ingredients including corn meal and sorghum that elicit a lower glycemic response than ingredients such as brewer's rice and were termed medium PGR (de-Oliveira et al., 2008). Innova Dry Adult Cat Food was predicted to have the lowest PGR because of the use of barley, a low GI carbohydrate (Jenkins et al., 1988). Cats were fed to maintain BW using individual historical data of energy intake. Dietary ME was calculated using the modified Atwater calculation, though it was later determined that the Atwater equation consistently underestimated true ME of the experimental diets (AAFCO, 1997; Asaro et al., 2017). Cats followed an 8-d prefeeding schedule followed by calorimetry and glucose measures. Food intake and orts were recorded daily, and BW was recorded on day 9 of each period. After the first period, cats were then switched to their next diet. The same schedule was used for all 3 periods of the study.

Experimental Procedures

Cats were housed in free-living group environments with room enrichment including perches,

Table 1. Analyzed nutrient composition on an as-fed basis of the 3 experimental diets differing in perceived glycemic response (PGR)

Item	High PGR ¹	Medium PGR ²	Low PGR ³
Moisture, %	7.16	6.76	5.31
Ash, %	6.36	6.31	6.38
CP ⁴ , %	38.0	35.9	42.1
Crude fat, %	10.8	20.0	20.4
NFE ⁵ , %	34.1	29.5	23.6
Starch ⁶ , %	36.8	30.7	23.6
Crude fiber, %	1.17	1.78	2.58
ADF, %	1.88	2.95	2.43
NDF, %	7.36	12.58	10.57
Available lysine, %	1.62	1.91	2.80
GE, Mcal/kg	4.92	5.25	5.46
Calculated ME ⁷ , Mcal/kg	3.75	4.08	4.14

Each diet was analyzed in triplicate. Results (except moisture) presented on a DM basis.

¹High PGR was Purina ONE Chicken and Rice (Nestlé, St. Louis, MO) contained as main ingredients: chicken, brewer's rice, corn gluten meal, poultry byproduct meal, wheat flour, animal fat preserved with mixed-tocopherols, whole grain corn, soy protein isolate, fish meal, animal liver flavor, KCl, H₃PO₄, CaCO₃, caramel color, choline chloride, and salt.

²Medium PGR was Iams Kitten Proactive Health (Procter & Gamble, Cincinnati, OH) contained as main ingredients: chicken, chicken byproduct meal, corn meal, chicken fat preserved with mixed tocopherols, dried beet pulp, ground whole grain sorghum, dried egg product, natural flavor, fish oil preserved with mixed tocopherols, KCl, fructooligosaccharides, choline chloride, CaCO₃, brewer's dried yeast, DL-Met, and salt.

³Low PGR was Innova (Procter & Gamble, Cincinnati, OH) contained as main ingredients: turkey, chicken, chicken meal, whole grain barley and whole grain brown rice, chicken fat preserved with mixed tocopherols, peas, natural flavors, apples, herring, flaxseed, eggs, blueberries, pumpkin, tomatoes, sunflower oil, KCl, DL-Met, carrots, pears, cranberries, menhaden oil, cottage cheese, taurine, green beans, alfalfa sprouts, parsnips, and salt.

⁴Crude protein = $N \times 6.25$.

⁵NFE (%) = $100 - \text{moisture (\%)} - \text{protein (\%)} - \text{fat (\%)} - \text{fiber (\%)} - \text{ash (\%)}$.

⁶Determined using AOAC Official Method 979.10.

⁷Calculated with modified Atwater equation (AAFCO, 1997): ME (kcal/kg) = $[3.5 \times \text{CP (\%)} + 3.5 \times \text{NFE (\%)} + 8.5 \times \text{crude fat (\%)}] \times 10$.

toys, beds, scratching posts, and climbing apparatus. Cats were able to choose to go outside through a swinging door during daylight hours and the swinging door was locked from 16:00 to 07:00. Lighting schedule followed a pattern of 12-h light beginning at 06:30 and 12-h darkness beginning at 18:30 although the room also had a full wall of windows so they also had natural light. Room temperature was kept at 22 °C, and relative humidity was 50% to 60%. Water was provided freely via automatic waterers for the duration of the study. Surfaces were disinfected weekly with Nolvasan (Allivet, St. Hialeah, FL) and daily cleaning was performed.

Cats were divided into 4 groups of 5 and 1 group of 4 to accommodate rotation through the 5 calorimetry chambers. Cats began the study with an 8-d prefeeding period on 1 of the 3 diets. Day 1 for each group of 4 cats was staggered to ensure each group received exactly 8 d of prefeeding before indirect calorimetry measurements on day 9. Cats were placed in individual cages for feeding at 07:00 each morning and given 60 min to eat before they were removed and any left-over food was weighed.

Early in the morning (6 a.m.) on day 9 (Table 2), the first group of cats was implanted with subcutaneous interstitial glucose sensors (Guardian Continuous Monitoring Devices, Medtronic Diabetes, Northridge, CA) placed between the ninth and last rib. Sensor cannulae were 14 mm, with an attached 4.17- × 3.56- × 0.94-cm transmitter that lies flat on top of the skin. Cats had a patch of fur shaved the day before to accommodate the sensor, which was secured with Polyskin II transparent dressing (Tyco Healthcare, Schaffhausen, Switzerland). Cats wore bodysuits to prevent scratching or licking the sensor or the dressing over the sensor. After interstitial glucose sensor placement, the first group of cats was placed in the calorimetry chambers for a 22-h measurement of respiratory gases. The calorimetry chambers (Qubit Systems, Kingston, ON, Canada) were composed of Plexiglas and measured 53.3 × 53.3 × 76.2 cm. Each contained a shelf, feeder, water bowl, hammock, litter box, toy, and free area with a fleece bed. Water was given freely from water bowls. The chamber was large enough to provide enough separation between areas used for feeding, sleeping, and elimination (Gooding et al., 2012). Indirect calorimetry techniques were used to measure EE and RQ and indirect calorimetry conduct and calculations for EE were performed as described by Gooding et al. (2014). On a daily basis, the chambers and water bowls were disinfected, and the litter, litter boxes, toys, hammock, and beds were removed and cleaned. Analyzers (CO₂ and O₂) were calibrated at 06:45 and 18:00 (Table 2). Interstitial glucose measurements were automatically recorded from transmitters to a computer, recording glucose every 5 min. These real-time measurements were used to create a curve of interstitial glucose concentrations throughout the day for each of the cats. Calibration of interstitial glucose monitors occurred 3 times per day at 08:00–08:30, 13:30–14:00, and 18:00–19:00 (Table 2). Calibration consisted of pricking each cat's paw pad and reading the blood glucose level using an Alphasat 2 monitor (Abbot Animal Health, Abbott Park, IL). This reading was then

Table 2. Timeline of calorimetry and interstitial glucose procedures on day 9 up to 600 min postprandial

Time of day	06:00	06:45	07:00	07:30	08:00	10:00	13:30	18:00
Time Postprandial (min)			–60	–30	0	120	330	600
Calorimetry timeline	Cat placed in chamber	Calibration	Fasting	Fasting	Food provided	Fed	Fed	Calibration
Interstitial glucose timeline	Implantation of interstitial sensor				Calibration	Begin recording	Calibration	Calibration

programmed into the interstitial glucose monitor. All interstitial glucose measurements were in the fed state, due to the 2-h calibration period required after sensor placement (Table 2). The detection limit was 400 mg/L and this value was used when the measurement was on or below this limit.

On day 10, sequential blood sampling was completed. Blood samples (1 mL) were taken 15 min before feeding (06:45, fasting) and at 30, 60, 120, 240, 360, 480, and 600 min after feeding. Sampling was started on the right rear leg, moving upwards for each subsequent blood draw, and switched to the left rear leg on sample 4, or sooner if needed depending on the individual cat. Cats were kept in individual cages in a procedure room for the duration of the blood draws (05:45–16:15) for constant monitoring. Blood was obtained using the BD Safety-Lok Vacutainer, 23 gauge with 1.91-cm needle and 30.5-cm tubing without anticoagulant. Samples were kept on ice for up to 1 h immediately after they were obtained and were centrifuged using a Thermo Scientific IEC Centra GP8 centrifuge at 1862 g and –4 °C for 15 min after clotting. Samples were stored at –20 °C until analysis. Serum glucose analysis was completed through colorimetric measurements (UV/vis spectrometry) with a Beckman Coulter AU480 automated chemistry analyzer (Indianapolis, IN). Serum insulin analysis was completed using a feline ELISA kit (Winston Salem, NC). Peak glucose and insulin were calculated by averaging serum concentrations among cats at each time period, with the greatest average indicating peak mean glucose and insulin.

Chemical Analyses

Proximate analyses were completed in triplicate for each of the 3 experimental diets using AOAC procedures (AOAC, 1997). Ether extract was analyzed after acid hydrolysis (954.02), and DM was determined by vacuum drying at 100 °C for 24 h (934.01). Dietary nitrogen was determined by oxidation using a crude protein/nitrogen (CP/N) analyzer (990.03; Leco Corp., St Joseph, MI) and CP

calculated. Crude fiber (CF) was analyzed through a ceramic fiber filtration method (962.09). Acid detergent fiber (973.18) and NDF (2002.04) were subsequently analyzed. Starch was determined using AOAC method 979.10. nitrogen-free extract (NFE), calculated as follows:

$$NFE(\%) = 100 - \text{protein}(\%) - \text{fat}(\%) - \text{fiber}(\%) - \text{ash}(\%) - \text{moisture}(\%).$$

Ash (979.10) was measured after exposure at 550 °C for 4 h (942.05), phosphorus (P) through spectrophotometry (964.06), and calcium (Ca) by atomic absorption spectrometry with electrothermal furnace (968.08) (AAFCO, 1997).

Statistical Analyses

Statistical power was calculated before the study using SAS version 9.3 and for the population size of 19 cats was found to be 97.4% for EE (Shoveller et al., 2010), serum insulin, and glucose concentrations (de-Oliveira et al., 2008).

Data were analyzed using the proc MIXED function of SAS version 9.3 with cat as the experimental unit, cat and period as the random effects, diet as the fixed effect, and time as the repeated measure. BW was included as a covariate in the serum glucose and insulin analyses. Repeated measures analyses were performed for RQ, EE, serum glucose, serum insulin, and interstitial glucose over time. The covariance structure used in the interstitial glucose analysis was Autoregressive Moving Average Model (ARMA 1.1) because it was found to have the lowest value for Akaike Information Criterion (AIC).

When the effect of diet was significant (α of 0.05), means were separated using the least significant difference. If $0.05 < P < 0.10$, a trend was described. Data were reported as least-squares means $\pm$ SEM.

RESULTS

On day 7 of period 3, blood was detected in the urine of 1 cat. The cat was given 150 mL of IV fluid

and did not show other symptoms, and was allowed to continue the study after veterinary examination. On day 9 of period 2, the interstitial glucose sensor of 1 cat lost signal at around 21 h postmeal feeding. For data analyses, this time point became the end point of interstitial glucose measurement for all cats. The measurement period was thus shortened to 20 h instead of the planned 22 h.

In period 2, data were not recorded past the 10.5-h calibration for 4 cats due to software malfunction. Two of these cats were fed the low PGR diet: 1 was fed the medium PGR diet, and 1 was fed the high PGR diet. The data that were collected in the 12 h previous to calibration were included in our analyses. In period 3, the calorimetry software did not record the first 12 h of data for 1 cat fed the low PGR diet. Therefore, the sample size was reduced to 18 cats for our calorimetry measurements.

On an as-fed basis, daily food intake was greater ($P = 0.02$; Table 3) for the high PGR diet than the medium PGR or low PGR diets. Cats were fed isocalorically among the 3 diets; therefore, ME intake (kcal/d) based on calculated ME did not differ ($P = 0.98$) among diets.

Fasted RQ was greatest ($P = 0.004$; Table 4) for cats fed the high PGR diet than cats fed the

medium PGR and low PGR diets. Immediately after meal feeding, the RQ increased until approximately 360 min, plateaued, and then declined from 660 min to the end of the calorimetry period (Figure 1). The RQ in the fed state was greater ($P < 0.001$) for cats fed the high PGR diet than cats fed the medium PGR or low PGR diets. The EE in the fasted state did not differ ($P = 0.166$) among diets. In the fed state, EE was greatest ($P < 0.05$) in the high PGR than the medium PGR diet or low PGR diets ($P < 0.001$).

Overall interstitial glucose was greater ($P = 0.003$; Table 5) for the high PGR diet than the medium or low PGR diet (Figure 2). Occasionally, sensors recorded a reading of 40-mg glucose/dL with little variation during the entire period. This reading was due to interstitial glucose levels of the individual cat being below the threshold of 40 mg/dL. This occurred for 4 cats in period 1, 2 cats in period 2, and 1 cat in period 3. Of these, 4 instances were for cats fed the low PGR diet, 2 for cats fed the medium PGR diet, and instance for a cat fed the high PGR diet. However, low glucose readings were consistent among the same cats, and as such, were not a concern.

Serum glucose concentrations were greatest in the fasted state and decreased immediately

Table 3. Body weight and intake of energy for cats consuming 3 experimental diets

Variable	High PGR	Med PGR	Low PGR	SEM ¹	P value
BW, (kg)	4.99	4.95	4.94	0.34	0.99
Intake, (g/d)	45.6 ^a	37.9 ^b	40.0 ^b	2.68	0.02
Calculated ME ² intake, (kcal/d)	155.8	154.5	154.3	10.08	0.98
True ME intake ³ , (kcal/d)	230.7 ^a	173.9 ^b	196.2 ^b	12.71	<0.001

^{a-c}Within row, means without a common superscript differ ($P < 0.05$).

¹Means were based on 19 cat observations per diet.

²Calculated with modified Atwater equation (AAFCO, 1997): ME (kcal/kg) = $[3.5 \times \text{CP} (\%) + 3.5 \times \text{NFE} (\%) + 8.5 \times \text{crude fat} (\%)] \times 10$.

³Using analyzed ME from Asaro et al. (2017).

Table 4. Indirect calorimetry measurements of cats fed experimental diets at different time intervals

Variable	High PGR	Med PGR	Low PGR	SEM ¹	P-value
RQ					
Fasting ²	0.77 ^a	0.75 ^b	0.75 ^b	0.007	0.004
Postprandial ³	0.79 ^a	0.76 ^b	0.76 ^b	0.003	<0.001
EE, ⁴ (kcal kg ⁻¹ d ⁻¹)					
Overall	43.3 ^a	41.8 ^b	41.6 ^b	0.28	<0.001
Fasted	42.1	40.9	39.8	1.18	0.160
Postprandial	43.3 ^a	41.8 ^b	41.6 ^b	0.29	<0.001

Fasting measurements were taken at $t = -60$ and -30 min, and fed measurements were taken at $t = 0$ min onward.

^{a-c}Within row, means without a common superscript differ ($P < 0.05$).

¹Means were based on 18 cat observations per diet.

²Fasting measurements were taken at $t = -60$ and -30 min.

³Fed (postprandial) measurements were taken at $t = 0$ min onward.

⁴EE = Energy Expenditure.

Figure 1. Respiratory quotient of cats consuming 3 experimental diets differing in perceived glycemic response (PGR): high, medium, and low PGR. The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet. Measurements were taken in 30-min intervals. Cats ($n = 19$) were fed at $t = 0$ min and equipment calibration occurred at $t = 630$ min (vertical dashed line).

Table 5. Interstitial glucose and sequential blood sampling measurements of cats fed diets with high, medium, and low predicted glycemic response (PGR)

Variable	High PGR	Med PGR	Low PGR	SEM ¹	<i>P</i> value
Interstitial glucose					
20 h, (mg/L d ⁻¹)	719 ^a	643 ^b	590 ^b	26.4	0.003
Serum glucose					
600 min, (mg/L d ⁻¹)	952	960	946	13.1	0.559
Fasting, ² (mg/L d ⁻¹)	1,008	1,051	1,073	24.6	0.173
Fed, ³ (mg/L d ⁻¹)	944	947	928	13.3	0.298
Peak, (mg/L d ⁻¹)	1,008	1,051	1,073	25.7	0.074
Serum insulin					
600 min, (μIU/mL d ⁻¹)	10.4 ^a	7.2 ^b	9.5 ^a	0.68	<0.001
Fasting, (μIU/mL d ⁻¹)	6.3	5.3	6.5	0.77	0.468
Fed, (μIU/mL d ⁻¹)	10.9 ^a	7.5 ^b	9.8 ^a	0.75	<0.001
Peak, (μIU/mL d ⁻¹)	13.6 ^a	9.2 ^b	14.7 ^a	1.26	0.004

^{a-c}Within a row, means without a common superscript differ ($P < 0.05$).

¹Means were based on 19 cat observations per diet.

²Fasting measurements were taken at $t = -60$ and -30 min before meal feeding.

³Fed measurements were taken at $t = 0$ min onward.

postmeal feeding and then reached plateau for the 3 diets (Figure 3). Serum glucose concentration did not differ among treatments (Table 5; $P < 0.559$) or between the fasted or fed states. Peak serum glucose did not differ among diets. Greater BW resulted in greater serum glucose ($P < 0.001$; data not shown). Time, included as a covariate, also affected serum glucose ($P < 0.001$). Diet and time did not interact ($P = 0.112$).

Serum insulin generally increased postprandially, followed by a plateau (Figure 4). In the fasted state, serum insulin did not differ among diets ($P = 0.468$). In the fed state, serum insulin was greater for the high PGR than for the medium PGR diet ($P < 0.001$) and did not differ from the low PGR diet. Greater BW resulted in greater serum insulin ($P < 0.05$; data not shown). Time, included as a covariate, also affected serum insulin ($P < 0.001$).

Figure 2. Interstitial glucose concentrations of cats consuming 3 experimental diets differing in perceived glycemic response (PGR): high, medium, and low PGR, over a measurement period of 20 h. The PGR refers to the expected theoretical glycemic response that would result from consumption of each diet. Cats ($n = 19$) were fed at $t = 0$ min, but monitors did not start recording until 120 min post feeding (vertical dashed line).

Diet and time interacted ($P < 0.001$). Average peak time for insulin was 60-min postprandial for the low PGR diet, followed by the medium PGR (240 min) and high PGR diets (360 min). Peak insulin of the high PGR and low PGR diets was greater than that for the medium PGR diet ($P = 0.004$).

DISCUSSION

The results from the present study indicate that healthy cats fed a diet high in starch content (36.75%) exhibit a greater RQ than cats fed diets with lower starch content (30.72% and 23.56%). Second, consumption of a high starch diet (36.75%) with significant contributions from brewer's rice, elevated interstitial glucose but not serum glucose, compared with cats fed diets lower in starch, and comprised of ingredients predicted to elicit a lower glycemic response. Third, carbohydrate source and level have a minor effect on postprandial glucose and insulin responses in cats. Last, EE was greatest in diets predicted to have a high glycemic response when compared with diets predicted to have a low glycemic response. Overall, the current results support the value of using interstitial glucose monitors or calorimetry to examine glucose metabolism and that carbohydrate content and ingredient selection

may alter the thermic effect of feeding and alter the net energy available.

Our observed differences in both fasted and fed RQ between diets were similar to those reported in the literature. Previously, mean fasted RQ was greater in cats fed a high-carbohydrate diet than those fed a high-fat diet (Gooding et al., 2014). Furthermore, baseline RQ was greater in cats fed a high-carbohydrate diet with 38.1% carbohydrate and 27.5% protein than cats fed a high-protein diet with 24.7% carbohydrate and 45.2% protein (Hoenig et al., 2007). Additionally, cats had lower RQ and therefore increased levels of fat oxidation with greater dietary fat (Lester et al., 1999; Gooding et al., 2014). The lower RQ of cats fed the medium and low PGR diets in the present study suggest that our cats adapt comparably to previous studies, by increasing fat oxidation in response to greater dietary fat content and fat intake. Similarly, the greater mean fed RQ in cats fed the high PGR diet implies increased carbohydrate oxidation compared with cats fed the medium or low PGR diets. This fluctuation of RQ with diet composition is consistent with the literature and indicates that lean healthy cats can adapt macronutrient utilization to alterations in dietary macronutrient intake (Hoenig et al., 2007; Gooding et al., 2015).

Figure 3. Serum glucose concentrations of cats consuming 3 experimental diets differing in perceived glyceimic response (PGR): high, medium, and low PGR, after 8 sequential blood draws ($P < 0.559$). The PGR refers to the expected theoretical glyceimic response that would result from consumption of each diet. Cats ($n = 19$) were fed at $t = 0$ min.

Figure 4. Serum insulin concentrations of cats consuming 3 experimental diets differing in perceived glyceimic response (PGR): high, medium, and low PGR, after 8 sequential blood draws ($P < 0.001$). The PGR refers to the expected theoretical glyceimic response that would result from consumption of each diet. Cats ($n = 19$) were fed at $t = 0$ min.

The RQ for all diets followed a biphasic response, peaking twice throughout the calorimetry period (Figure 1). A similar pattern has been observed in dogs and pigs; however, the response seen in the present study is significantly delayed in contrast to other species. In dogs, a peak in RQ is observed within the first 30 min of feeding,

and again at 75 min postprandial (Diamond and LeBlanc, 1987). The first peak in RQ may be attributed to stimulation of the sympathetic nervous system, coupled with increased respiratory rate that may have occurred with the excitement of the feeding process (Diamond and LeBlanc, 1987). The second peak in RQ observed in dogs coincides with the

digestive phase, indicating an increase in carbohydrate oxidation when food is digested and absorbed (Diamond and LeBlanc, 1987). Furthermore, in pigs, RQ began to increase 25 min postprandial and remained elevated up to 80 min postfeeding (Stombaugh and Grifo, 1977). Because the cats in the present study did not exhibit a rapid increase in RQ directly after feeding, it is likely that first peak in RQ coincides with the digestive phase, similar to that of dogs. The second peak in RQ coincides with calibration of the chambers; therefore, it is possible that this peak is attributed to excitement of cats when researchers entered the room (Rand et al., 2002). This is notable, as disruptions were minimal when cats were in chambers, indicating that human interaction can significantly affect rates of glucose oxidation and EE especially in well-socialized cats.

The lack of difference in fasted EE among diets is consistent with previous research, both in cats and other species. However, the differences in postprandial EE observed in the present study were not anticipated, nor consistent with the limited previous research. In cats, EE was not altered by differences in macronutrient intake when fed isocaloric provisions (Lester et al., 1999; Gooding et al., 2014). Similarly, in dogs fed 2 diets, both low and high in carbohydrate content relative to fat, postprandial EE was not different throughout the course of the 22-h calorimetry period (McKnight et al., 2014). Meal size and frequency, not dietary macronutrient content, are factors that can influence EE. Previously, EE decreased due to overfeeding (Pouteau et al., 2000), whereas feeding multiple times daily increased dietary-induced thermogenesis in dogs (Diamond and LeBlanc, 1987). Because cats in the present study were fed once daily, and provisions were restricted to 95% maintenance requirements, we did not expect to see differences in postprandial EE. Differences in postprandial EE may be explained by increased lipogenesis in response to high-carbohydrate intake. The correlation between carbohydrates and lipogenesis has been observed in multiple species. In omnivorous fish, increased lipid biosynthesis is related to increased dietary carbohydrate intake (Hemre et al., 2002). Similarly, in human infants, metabolic rate was positively correlated with carbohydrate intake, presumably as a consequence of increased fat synthesis (Sauer et al., 1986). It is probable that differences in carbohydrate content and resulting intake of the experimental diets in the present study stimulate variable lipid synthesis, and therefore different postprandial EE among treatments. Furthermore, we previously determined that the calculated ME

from which feeding recommendations were developed was inaccurate (Asaro et al., 2017). When caloric intake was reanalyzed using the true ME of the diets, we found that cats were unintentionally offered nonisocaloric provisions, with more energy being consumed by cats fed the high PGR diet (Table 3). It is likely that these unintended differences contributed largely to the observed differences in postprandial EE among diets and supports the fact that postprandial EE is in part related to the total calories consumed (Apfelbaum et al., 1971).

Interstitial glucose is not well-studied in the domestic cat. Feline glycemic responses are usually measured using repeated blood sampling or requiring the insertion of catheters (Reineke et al., 2010). These methods are not only invasive and stressful to cats, but also cannot report values in real-time (Rebrin et al., 1999). Stress has been found to cause temporary hyperglycemia in cats (Rand et al., 2002), and therefore, blood glucose concentrations may be overestimated due to stress-induced changes in blood glucose (Wiedmeyer et al., 2003). Continuous interstitial glucose monitoring systems were first evaluated by Wiedmeyer et al. (2003) and were well-correlated with whole blood glucose levels (Wiedmeyer et al., 2003). Additionally, interstitial glucose has been found to mimic blood glucose in multiple species, including rabbits, rats, and humans (Rebrin et al., 1999). As expected, interstitial glucose followed a parallel pattern to RQ in the present study, peaking twice throughout the total calorimetry period. The first peak in interstitial glucose begins approximately 6 h postprandial and corresponds with the first peak in RQ. Similarly, the second peak in interstitial glucose coincides with the second peak in RQ. As discussed previously, it is possible that the first peak is due to the effect of feeding and digestion, whereas the second peak is likely due to the excitement of having people enter the room (Rand et al., 2002). Although not statistically correlated, the relationship between RQ and interstitial glucose may be an alternative, less invasive technique to measure glycemic responses. Therefore, interstitial glucose measures should be considered as an alternative to determine feline glycemic response.

The preprandial elevation in serum glucose observed in the present study was unexpected. Based on glucose response studies conducted in multiple monogastric species, we anticipated serum glucose to peak postprandially (Appleton et al., 2004; Galgani et al., 2006; Carciofi et al., 2008; de-Oliveira et al., 2008; Farrow et al., 2013). In dogs (Carciofi et al., 2008) and humans (Galgani

et al., 2006), serum glucose peaked at approximately 60 and 45 min postprandially, respectively, and returned to baseline (measures taken at time 0) levels by 180 min postprandial. Previously, blood glucose peaked at 10 h (rice) and 18 h (sorghum/corn) postprandial for cats fed 2 diets of similar starch content (33%) with significant starch contributions from either rice or sorghum and corn (Appleton et al., 2004). Furthermore, blood glucose has remained elevated for upwards of 24 h in cats fed a high-carbohydrate diet (Farrow et al., 2013). These studies indicate that cats seem to reach peak glucose concentrations much slower than other monogastric omnivorous species (Farrow et al., 2013). Our 10-h blood sampling period may not have been long enough to capture peak serum glucose concentrations in the cats. Additionally, it is likely that similar to our interstitial measurements, serum glucose peaked preprandially due to meal anticipation, resulting in transient hyperglycemia (Rand et al., 2002). However, it is worth noting that our measures of serum glucose among all diets did not deviate more than 15 mg/dL between baseline and peak measurements. This agrees with previous literature that cats have a low and more gradual glucose response compared with other monogastric species (Appleton et al., 2004).

Our measurements of serum insulin are inconsistent with the literature. Previously, consumption of either a low-carbohydrate or high-carbohydrate diet did not affect serum insulin in the fed and fasted states in healthy adult cats (Coradini et al., 2011). Additionally, serum insulin did not differ between cats fed high-fat and high-carbohydrate diets (Gooding et al., 2014). However, in the present study, mean serum insulin, but not serum glucose, was different among cats fed dietary treatments. We expected these 2 parameters to be dependent on one another (de-Oliveira et al., 2008; Farrow et al., 2013). Our results suggest that other factors besides starch levels and ingredient source affect insulin release in cats.

The findings that serum glucose and serum insulin did not increase with PGR were unexpected. Our postprandial glucose and insulin measurements suggest that differences in the glycemic response due to changes in starch level and source are not as distinct in cats compared with other species. Similar to our results, in cats fed 6 diets that differed in their starch ingredient source (corn, brewer's rice, sorghum, peas, lentils, and cassava flour), only the corn diet stimulated a significant increase in postprandial glucose from baseline values and is consistent with previous literature (de-Oliveira et al., 2008).

Furthermore, previous studies similar to ours agree that starch has a minimal effect on the glucose and insulin responses of cats (Kienzle, 1994; Bouchard and Sunvold, 2000). This metabolic anomaly may be associated with cats' enzymatic ability to preferentially use amino acids for energy (Kienzle, 1994; Morris, 2001). However, the exact mechanisms of carbohydrate metabolism in cats are unknown and require further investigation.

The limitations of the present study lie in the nutrient differences between experimental diets, therefore leaving us unable to measure the effect of individual macronutrients on the feline glycemic response. However, previous research has suggested that diet composition has a strong influence on insulin secretion, and subsequently, glucose uptake (Holt et al., 1997). Farrow and colleagues (2013) found that cats consuming high-protein diets tended to reach peak insulin faster than those fed high-starch or high-fat diets. Similarly, in the present study, serum glucose peaked fastest in cats fed the low PGR diet, which also contained the largest proportion of protein. This pattern may be explained due to the fact that certain amino acids, such as arginine, leucine, and alanine, have been found to stimulate insulin release in cats (Yasuda et al., 2011). Conversely, consumption of low-protein diets has been found to decrease time to peak insulin compared with low-starch diets in cats (Verbrugghe et al., 2010).

It has also been suggested that dietary fat can substantially alter the glycemic response (Simpson et al., 1985). Oral fat administration has previously been found to reduce the rate of gastric emptying and subsequent rate of passage through the small intestine in humans, therefore leading to a flatter glucose response (Gulliford et al., 1989). Furthermore, it has been found that hepatocytes may increase glucose oxidation and insulin sensitivity when exposed to an increase in short-chain fatty acids (Venter et al., 1990). This evidence supports the results observed in the present study, as the glucose responses to the medium PGR and low PGR diets, which contained higher fat content (20.0% and 20.4%, respectively), were less pronounced than the high PGR diet, which contained significantly less fat (10.8%).

There are very little data in the literature on the effect of fiber on the feline glycemic response. However, the effect of dietary fiber on postprandial glucose concentrations has been studied in dogs. Nelson and colleagues (1991) found that diets high in insoluble-fiber and soluble-fiber both lower postprandial glucose concentrations in diabetic dogs.

Conversely, Monti et al. (2016) concluded that a range in soluble and insoluble fiber intake does not alter the postprandial glycemic response in healthy nonobese dogs. In the present study, there did not appear to be a relationship between dietary soluble or insoluble fiber and the glycemic response in our cats; however, this area clearly requires further research especially in light of the propensity for cats to develop type 2 diabetes (Henson and O'Brien, 2006).

In conclusion, although RQ, EE, and interstitial glucose behaved as expected, serum glucose and insulin presented variability that emphasizes their lack of scientific value. Our results indicate that cats have a prolonged glucose and insulin response compared with that of other monogastric species, when consuming diets with starch ranging from 24% to 36%. Furthermore, the present study suggests that starch source and inclusion level have minimal effects on postprandial glucose and insulin responses in adult lean cats. Overall, interstitial glucose proved to be less variable than serum measurements and should be considered a noninvasive measure that additionally can more robustly define the real-time glucose response to a meal. For these reasons, we suggest that calorimetry and interstitial glucose measurements are superior to blood glucose measures, as they require less animals to investigate metabolism, and should be considered going forward.

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