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Introduction of adult cats to indirect calorimetry respiration chambers causes increased energy expenditure and respiratory quotient that decrease following acclimation

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OBJECTIVE
To replicate a previously defined behavioral procedure to acclimate adult cats to temporary restriction in indirect calorimetry chambers and measure energy expenditure and respiratory quotient changes during acclimation.

ANIMALS
8 healthy adult cats (4 spayed females, and 4 neutered males; mean ± SEM age, 2.5 ± 1.5 years; mean body weight, 4.8 ± 1.8 kg).

PROCEDURES
Cats underwent a 13-week incremental acclimation procedure whereby cats were acclimated to the chambers in their home environment (weeks 1 to 3), to the study room (weeks 4 to 6), and to increasing lengths of restriction within their home environment (weeks 7 to 8) and the chambers (weeks 9 to 13). Cat stress score, respiratory rate, fearfulness (assessed with a novel object test), energy expenditure, and respiratory quotient were measured. Data were analyzed by use of a repeated-measures mixed model.

RESULTS
Stress, based on cat stress scores, fearfulness, and respiration, peaked at weeks 4, 9, and 10 but returned to baseline levels by week 11. Energy expenditure and respiratory quotient peaked at weeks 10 and 11, respectively, but were reduced significantly by weeks 11 and 13, respectively. All cats returned to baseline by the end of the study and were deemed fully acclimated.

CLINICAL RELEVANCE
Changes in perceived stress level, energy expenditure, and respiratory quotient at various stages of the acclimation procedure suggest that stress should be considered a significant variable in energy balance measurements when indirect calorimetry is used in cats. An incremental acclimation procedure should therefore be used to prepare cats for the temporary space restriction necessary for indirect calorimetry studies.

Feeding recommendations for pets are established to ensure that their energy requirements are met; however, these recommendations often utilize predictive equations that require knowledge of energy expenditure (EE). The gold standard for predicting EE is indirect calorimetry,[1] a procedure that measures the consumption of O_2 and production of CO_2 in order to calculate resting EE (REE) and respiratory quotient (RQ). In order to accurately measure these gases, subjects must be placed in chambers with a total volume appropriate for their relative body size. The acute exposure to this novel space-restricted environment may cause an increase in stress, which not only raises welfare concerns but also may affect the accuracy of data collected, as stress can cause physiological and behavioral changes.[2] Gooding et al[3] reported that a procedure for incremental acclimation to calorimetry chambers resulted in cats with lower levels of stress, as perceived by means of reduced latencies to approach a novel object during a novel object test (NOT) and lower cat stress scores (CSSs). Unlike unacclimated cats, the cats that were subjected to the acclimation procedure returned to baseline stress measures after full acclimation to temporary space restriction. However, while Gooding et al[3] reported that cats could be acclimated to indirect calorimetry, they did not report the effects of this stress on energy and macronutrient metabolism; as such, it is unclear whether stress in cats may be associated with changes in these parameters.

Stress affects several behavioral and neuroendocrine responses that modify energy and macronutrient metabolism. Cortisol and glucose concentrations increase in response to stress and may cause an increase in EE as well as a shift in macronutrient metabolism from mixed macronutrient usage toward carbohydrate use.[4] These stress-induced increases in EE may lead to an overestimation of their caloric requirements, and the inaccuracy of these estimates can result in overfeeding and weight gain. As well, the shift in RQ toward carbohydrate usage may be associated with glucose intolerance and insulin resistance. Finally, stress can impair behaviors related to food intake, which may also negatively impact EE and RQ.[5]

To our knowledge, no previous studies have compared EE and RQ between cats that have been partially or fully acclimated to the temporary space restriction necessary to carry out indirect calorimetry. Therefore, the aim of this study was to build on observations made by Gooding et al[3] regarding acclimation to indirect calorimetry chambers by investigating energy and macronutrient metabolism changes throughout the acclimation procedure. Respiration rate (RR) was also observed as an added behavioral marker of cat stress. The study objectives were to validate a behavioral acclimation procedure for cats to calorimetry chambers and to measure EE and RQ alongside observed indicators of stress. We hypothesized that all cats would be successfully acclimated to the calorimetry chambers based on CSS, NOT, and RR data returning to baseline levels and that EE and RQ would be lower at the end of temporary space restriction than at the beginning, indicating that stress increases EE and alters RQ.

Materials and Methods

All procedures were reviewed and approved by the University of Guelph Animal Care Committee (animal use protocol No. 3999) and were carried out in accordance with the Animals for Research Act and Canadian Council on Animal Care. All cats were deemed healthy on entering and throughout the study according to standard veterinarian evaluations of health.

Animals, diet, and housing

A total of 8 (4 spayed females, and 4 neutered males) domestic shorthair and longhair cats (mean ± SEM age, 2.5 ± 1.5 years; mean ± SEM body weight [BW], 4.8 ± 1.8 kg) were used. Cats were fed a control diet (I19 Ideal Solution Support Skin, Coat and Stomach; Nutram Pet Products) individually in cat condos (91.5 X 60 X 128 cm) once per day at 08:00 hours. Cats were fed to individual maintenance requirements as determined on the basis of historical feeding records. Each cat was permitted 1 hour to eat, and orts (food refusals) were recorded daily. Measurements of BW as well as visual and physical assessment of body condition score (9-point system) were done weekly and monthly, respectively.

For the duration of the study, all cats resided in the Animal Biosciences Cat Colony at the University of Guelph and were housed in an indoor free-living environment, except for during acclimation, indirect calorimetry measurements, and morning feedings. The room was furnished with environmental enrichment including beds, toys, scratching posts, and climbing apparatuses. All cats had daily social interaction with previously familiarized humans for a maximum of 2 hours each day including petting, grooming, and playing with restricted-access toys. A 12-hour light cycle that started at 07:00 hours was implemented, and the room temperature and relative humidity were maintained at 22 ± 1.5 °C and 40% to 70%, respectively. Fresh distilled water was provided daily, and cats had ad libitum access.

Acclimation procedure

The acclimation procedure was adapted from Gooding et al.[3] This procedure was extended from the 11 weeks used by Gooding et al[3] to 13 weeks in the present study to account for older cats potentially requiring a longer acclimation time. Cats were acclimated to the primary researchers prior to the study and had exposure to the sounds associated with the indirect calorimetry equipment (eg, large rotary vein pumps). Once normal behavior was observed and cats had become acclimated to the primary researchers, baseline (week 0) behavioral observations were recorded. The acclimation procedure for weeks 1 to 3 (chamber placed in permanent housing room) and weeks 4 to 6 (cats introduced to study room) were identical to that of Gooding et al,[3] except that only 1 chamber (with the door open) was accessible in the housing room during weeks 1 to 3 and that, for weeks 4 to 6, the cats were exposed to the study room 3 times a week, not 5. The remainder of the acclimation period was based on the final stage of the protocol reported by Gooding et al[3] but was separated into weeks 7 to 8 and 9 to 13.

For weeks 7 to 8, cats were restricted in their individual cages during morning feeding for increasing lengths of time to prepare for increased time restriction within indirect calorimetry chambers. Cats also continued to receive weekly access to the study room throughout these weeks. During week 7, cats were restricted in their cages for 2 hours during feeding at 08:00 hours. During week 8, cats were restricted in their cages for 1 hour prior to and 2 hours following feeding for a total of 3 hours beginning at 07:00 hours. As the cats remained in their home environment, CSS, NOT, and RR were not recorded.

During week 9, the cats were restricted in the chambers in the study room 2 times each on subsequent days for 1 hour per restriction period following morning feeding at 08:00 hours. Following restriction, cats were allowed free access to the study room and received positive reinforcement from the primary researchers as described previously. For weeks 10 to 13, the cats were restricted for 5, 10, 15, and 20 hours, respectively, all beginning at 06:00 hours. The cats all received positive reinforcement upon exiting the chambers, and when restriction coincided, food was offered to the cats during their regularly scheduled feeding time at 08:00 hours.

Behavioral observations

The CSS system was used to determine the cats' stress level based on postural and behavioral indicators and has been described at length by Kessler and Turner.[6] Stress level was evaluated with CSS in accordance with the protocol utilized by Gooding et al,[3] with the exception being that at baseline and during weeks 1 to 3, cats were assessed 3 times per week rather than 5 times per week. Videos of at least 10 seconds were taken for each CSS observation period and were later scored independently by 2 researchers, and the average score was used. The researchers remained the same throughout the study, had previously become familiar with and practiced scoring, and did not have access to each other's score until both were submitted. Interobserver reliability on the CSSs was conducted by means of calculating Kappa statistics with commercially available statistical software (SAS version 9.4; SAS Institute Inc).

An NOT was utilized following the protocol defined by Gooding et al.[3] During weeks 1 to 6 the novel object was placed in the center of the room, and during weeks 9 to 13 the object was placed in the front right corner of the chamber.

Respiratory rate was measured visually over a 30-second period. All measurements were taken by the same 2 primary researchers in duplicate, 2 minutes apart, and the mean value was used. Before the start of acclimation, baseline RRs were determined for each cat from 3 values collected over a 5-day period. All measurements were taken at the 85% time point. After each daily feeding, any remaining food was weighed and recorded, and daily food intake was calculated for each cat.

Indirect calorimetry

Indirect calorimetry chambers (53.3 X 53.3 X 76.2 cm) made of Plexiglass were used to determine EE and RQ quantified by means of respiratory gas exchange. The chambers were outfitted with a food and water bowl, litter box, toy, fleece bed, and elevated level. The chamber allowed for separation of feeding, sleeping, and elimination areas. Chambers, bowls, litter boxes, toys, and fleece beds were cleaned and disinfected after every use.

Chambers had an open-circuit, flow-through design, with room air drawn into chambers at a rate of 5 to 8 L/min. Chamber air was dried by columns of a commercially available desiccant (Drierite; W. A. Hammond Drierite Co Ltd) and similarly again prior to gas analyzers. Calorimetry data were collected using calorimetry software (C950-MCGES; Qubit Systems Inc). Data collection occurred in 5-minute periods every 25 minutes, and the last 3 minutes of each data period were averaged and used. During weeks 10 to 13, cats were fasted overnight and placed into chambers the following morning at 06:00 hours. Approximately 2 fasted respiratory gas measurements were taken following a 30-minute gas equilibration period to determine fasted volumes of CO_2 produced (VCO_2) and O_2 consumed (VO_2). Cats were fed their entire daily ration following the fasted measurements, and indirect calorimetry continued as per the weekly acclimation timeline. Recalibration of the CO_2 and O_2 analyzers was conducted approximately every 8 hours or when a drift of > 5% occurred for the reference gases or atmospheric pressure. The abbreviated Weir equation[7] was used to calculate EE. Fasted and fed values of EE and RQ were calculated and compared; however, fed values were selected to evaluate differences for acclimation purposes.

Statistical analysis

Data were analyzed by means of a repeated-measures mixed model with commercially available statistical software (SAS version 9.4; SAS Institute Inc). The independent variable was the week of acclimation, and dependent variables included CSS, NOT, RR, food intake, EE, and RQ. Cat was treated as a random effect, and week as a fixed effect. If the main fixed effect was significant, means were compared with the Tukey multiple comparison test. Pearson correlation coefficients were used to test for correlation between CSS, NOT, and RR. Results were considered statistically significant at $P \leq 0.05$. Values were reported as mean ± SEM.

Results

It should be noted that for weeks 7 and 8, cats were confined for increased durations of time but within a familiar environment (their own individual cages). Since the cats were normally fed in these cages, they were observed to be very excitable in anticipation of food while confined during these 2 weeks. This made it difficult to collect accurate behavioral data, and these data were ultimately removed from the analysis.

Cat stress scores

On week 0, the mean ± SEM baseline CSS was established (2.5 ± 0.25), and only mean CSS values on weeks 4, 9, and 10 differed from baseline **(Figure 1)**. Cat stress scores were greater during week 4, when cats were first introduced to the study room, than all other weeks aside from weeks 9 and 10. The CSS on week 9, when cats were first confined to the calorimetry chambers, was greater than all other weeks except week 10, and CSS returned to baseline levels by week 11.

During weeks 9 to 12, the CSS at the 10% time point was greater than at the 80% time point (data not shown), and during week 13, the CSS at the 10% time point was greater than the 50% and 80% time points (data not shown). When pooled across all weeks, the CSS at the 10% time point was significantly greater than the 50% and 80% time points, but the 50% and 80% time points did not differ from each other (data not shown).

Within each week, there was no difference between CSS values on days 0, 1, and 4 (data not shown); however, when pooled across all weeks, mean CSS on day 0 was greater than on day 4 ($P \leq 0.05$), but neither day 0 nor 4 differed from day 1.

Novel object test

Latency to approach the novel object was greater on weeks 9 and 10 (first 2 weeks of chamber restriction) than all other weeks, aside from weeks 1 and 4 **(Figure 2)**, coinciding with the initial introduction of the chamber to the cats' home room and the cats' initial introduction to the study room, respectively. There was no correlation between latency to approach the novel object and CSS or RR (data not shown).

Respiration rate

One cat displayed higher-than-normal RRs throughout the trial, and those data were removed from analysis. Respiration rate was higher on weeks 4, 5, 6, and 9 than on all other weeks aside from week 10 **(Figure 3)**. From week 10 and onward, RRs were either not different from or less than baseline levels. Within-week differences were only observed on weeks 4 and 5, with RRs on day 4 being less than on days 0 and 1 (data not shown); however, when pooled across weeks, there was an overall decrease in RR from day 0 to days 1 and 4 (data not shown). There was a positive correlation between RR and CSS ($r = 0.22$; data not shown).

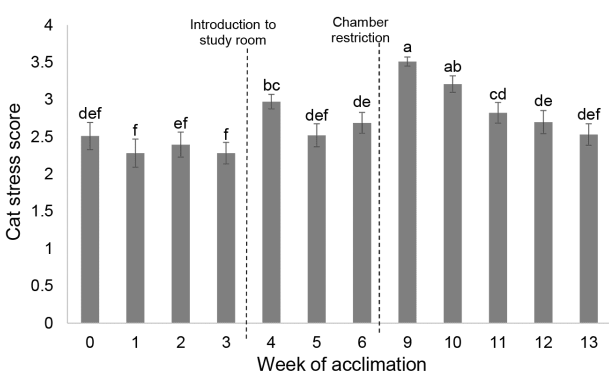

Figure 1—Mean ± SEM cat stress scores over time for 8 cats undergoing acclimation to indirect calorimetry equipment and the associated environment. [a–f]Means across all weeks with no common superscript letter differ significantly ($P \leq 0.05$).

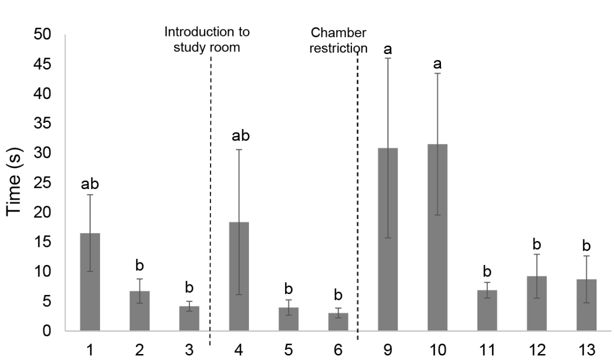

Figure 2—Mean ± SEM latency to approach a novel object within a 5-cm radius over time for the cats of Figure 1. ***See*** Figure 1 for key.

Food intake

There were no differences in food intake, as represented by orts, between baseline and weeks 1 to 8 and 13 (data not shown). Food intake was significantly less (orts were significantly greater) on weeks 9 to 12 (first 4 weeks of chamber restriction) than on weeks 0 to 3 and 5 to 8 (data not shown) but did not differ from week 4, when cats were first introduced to the study room, or week 13 (data not shown).

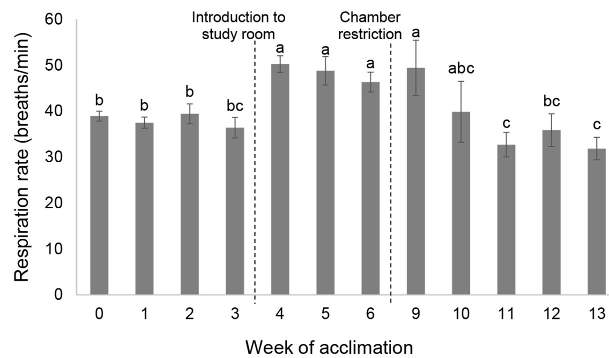

Figure 3—Mean ± SEM respiration rate over time for 7 of the cats of Figure 1. ***See*** Figure 1 for key.

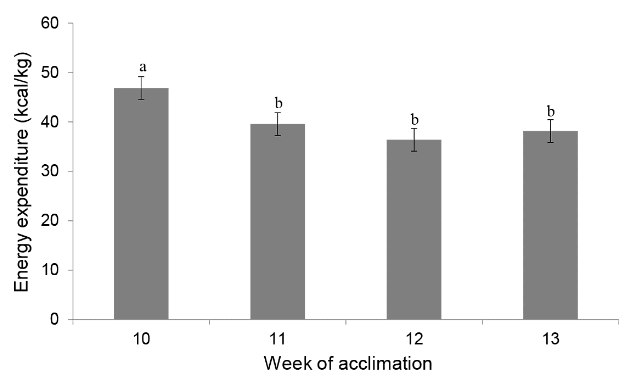

Figure 4—Mean ± SEM postprandial energy expenditure over time for the cats of Figure 1. ***See*** Figure 1 for key.

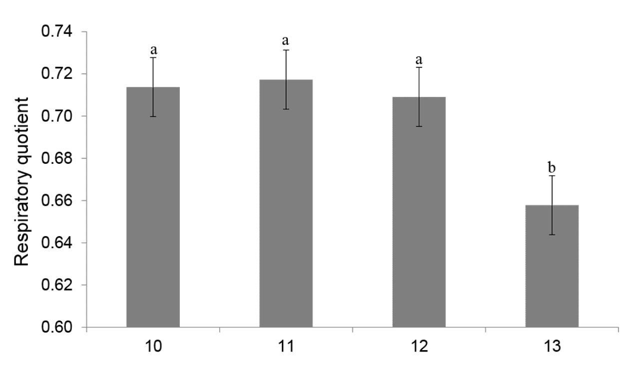

Figure 5—Mean ± SEM postprandial respiratory quotient over time for the cats of Figure 1. ***See*** Figure 1 for key.

Energy expenditure and respiratory quotient

Fasted EE and RQ data could not be consistently collected due to varying times of temporary space restriction, and postprandial EE and RQ data could not be collected during week 9 as it only consisted of 1 hour of space restriction. Therefore, those data were not included in the analysis. Postprandial EE was greater on week 10 than on weeks 11 to 13 **(Figure 4)**, and mean EE did not differ between week 11, 12, or 13. There were no differences in postprandial RQ between week 10, 11, or 12 (mean of 0.71); however, postprandial RQ during weeks 10 to 12 was greater than during week 13 (mean of 0.65) **(Figure 5)**.

Discussion

To our knowledge, this study was the first to report changes in EE and RQ as outcomes of an incremental acclimation procedure of cats to respiration chambers used for indirect calorimetry. These data were reported alongside changes in RR as well as observed CSS and responses to NOT during the acclimation in an attempt to quantify stress associated with temporary restriction. Based on the combined data presented herein, all cats were considered to have been successfully acclimated to the calorimetry chambers by the end of the 13-week acclimation period. The current observations regarding CSS and NOT returning to baseline values by week 11 were similar to those reported for younger cats in the study by Gooding et al,[3] and behavioral acclimation was supported further with RR similarly returning to baseline values by week 11. However, Gooding et al[3] did not measure respiratory gases, and these data lend support to an extended acclimation protocol (from 11 to 13 weeks) to ensure that energy and macronutrient metabolism are observed by fully acclimated cats and not affected by stress.

Based on the EE data presented herein, cats may be considered acclimated to this temporary restriction by week 11 of the acclimation protocol. This notion was supported by CSS and NOT latency data, as these values had returned to baseline levels by week 11. The mean ± SEM EE of the cats at the end of the present study was 37.47 ± 2.30 kcal/kg BW*day, which was reduced by approximately 25% from week 10, when the mean EE was greatest (49.13 ± 2.54 kcal/kg BW*day). This elevated EE at week 10 coincided with the greatest observed CSS and NOT latency values, indicating the cats may have been experiencing a stress response that increased EE, supporting our hypothesis that collection of data from cats experiencing stress may lead to overestimation of energy requirements. Additionally, the mean EE at week 10 fell within the range of EE reported in previous studies[8–12] that failed to acclimate cats to respiration chambers (44.0 to 67.5 kcal/kg BW*day). While this wide range suggests that there are likely considerable differences in individual cat stress responses, the magnitude of these EE values illustrate the importance of acclimating cats to the temporary restriction within indirect calorimetry chambers with regard to collecting reliable and accurate respiratory gas data. Furthermore, previous studies[1,13–15] in which acclimation included < 1 week of exposure to the chambers revealed a similar range in EE as those without acclimation, indicating that these short-term acclimation procedures may still be insufficient.

Unlike EE, RQ values did not decrease until week 13, suggesting that the effects of temporary restriction and the associated stress on RQ may be longer lasting than on EE in young adult cats. These RQ data suggest that an extended acclimation protocol may be necessary for older cats as opposed to younger cats utilized in the study by Gooding et al.[3] However, it should be noted that in that previous study,[3] RQ data were not collected, so it is possible that young cats also may require an extended adaptation period if RQ data are considered alongside that of CSS and NOT. Future research investigating the acclimation of younger cats (eg, < 1 year old) to the temporary restriction in indirect calorimetry chambers while measuring changes in EE and RQ is warranted to elucidate whether they too require an extended adaptation period.

Like EE, mean RQ was also greatest during week 10 (0.71), yet these mean values dropped considerably by week 13 (0.65). In brief, an RQ of approximately 0.8 indicates the oxidation of a mixed diet, while 0.6 to 0.7 indicates fat metabolism and 0.9 to 1.0 indicates carbohydrate metabolism.[16] As such, mean RQ values obtained throughout all 4 weeks of extended space restriction in the present study indicated primarily fat oxidation rather than a shift in macronutrient metabolism toward carbohydrate oxidation that has previously been associated with acute stress.[4] A variety of factors may have contributed to this, including that the cats were only fed once per day, allowing only a single insulin response. Furthermore, cats are evolutionarily adapted for fat oxidation due to their carnivorous nature and may maintain RQ that trends toward fat oxidation, even when fed increased levels of carbohydrates.[17] Studies[1,10,14,15] in which little to no acclimation of indirect calorimetry respiration chambers was provided with cats of similar age, BW, and macronutrient intake revealed RQ values ranging from 0.8 to 0.85. However, food intake in these studies was either described as ad libitum or multiple meals, which may result in greater RQ values. Nonetheless, mean RQ in the present study was still statistically lower by the end of acclimation compared to week 10, indicating that an acclimation procedure of at least 13 weeks is necessary to accurately measure macronutrient utilization in cats by use of indirect calorimetry chambers.

This study yielded results for CSS similar to those of a previous study[3] of younger cats; however, the latency to approach a novel object was much greater in the present study (> 30 seconds) than in that study (< 7 seconds). This difference in latency to approach may be attributed to age (eg, differences in play or curiosity between kittens and young adults) or to the cats' previous experience, as all cats in the present study were from shelters; as such, their experience with and reaction to novel stimuli may vary greatly. Kessler and Turner[6] reported that the age of cattery-kept cats did not influence stress level; however, the cats used in that study were a research cohort of purpose-bred cats and they were likely raised in a different environment than the cats in the present study.

Based on NOT responses, stressful events for the cats of the present study included the first week of chamber exposure (week 1), first week of study room exposure (week 4), and first 2 weeks of confinement (weeks 9 and 10). However, largely due to the degree of variation, only weeks 9 and 10 were statistically greater than at the end of the 13 weeks when the cats were deemed fully acclimated. The large degree of variation in these NOT data was likely due to the fact that cats respond to stimuli on an individual level and can be classified as responders and nonresponders.[18,19] For example, an animal may respond to familiar and unfamiliar events in a similar manner, a phenomenon that is indicative of their coping mechanisms and may vary from animal to animal.[20] As such, if a population has any responders, the greater latency to approach in these cats will inflate the mean despite the fact that most cats in that population are nonresponders and thus would approach the object relatively quickly. Overall, the behavioral variability seen between cats can be attributed to factors such as differences in genetics, maternal care, and previous experience.[20–22]

In conclusion, the 13-week acclimation procedure in the study reported here led to successful acclimation of young adult cats (mean ± SEM age, 2.5 ± 1.5 years) to respiration chambers and the associated study environment. This procedure may be used in the future to train cats for indirect calorimetry studies and is anticipated to have a high success rate. Based on CSS, NOT, and RR data, older and random-sourced adult cats do not appear to require more time to acclimate to calorimetry chambers than young cats (mean age, 10 ± 2 months). Nevertheless, the RQ data presented herein indicated that it may be beneficial to extend the length of acclimation (from 11 to 13 weeks) for cats. Overall, acclimation of cats to temporary restriction in indirect calorimetry chambers is necessary to minimize cat stress, optimize welfare, and obtain accurate data related to feline macronutrient metabolism that can be readily applied in both clinical and research settings.

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