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The effects of increasing water content to reduce the energy density of the diet on body mass changes following caloric restriction in domestic cats.

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Summary

Caloric restriction induces body mass loss that is often regained when restriction ends. This study aimed to determine if dietary energy density modulates the extent of post-restriction body mass regain. Water (20% wt:wt) was added to a standard dry commercially available feline diet. Twenty-seven domestic short-haired cats underwent a 20% caloric restriction on this diet. Following restriction, cats were offered the same dry diet ad libitum either without additional water or with 40% added water, therefore maintaining macronutrient composition whilst manipulating energy density. Despite no significant difference in energy intake during ad libitum consumption, post-restriction body mass regain was greater on the high energy dense (0% hydrated), compared to the low energy dense (40% hydrated) diet. The same protocol was repeated with a separate cohort of 19 cats with additional measures of physical activity, gut transit time and energy digestibility. Activity levels on the low energy dense diet were significantly higher than in cats on the high energy dense diet ($P = 0.030$) and were similar to those recorded during caloric restriction. These results suggest that body mass gain following caloric restriction is ameliorated, and physical activity enhanced, by feeding a diet which is low in energy density due to the addition of 40% water.

Introduction

Obesity results from a chronic imbalance between energy intake and energy expenditure. The prevalence of human obesity has dramatically increased in recent decades and is also the most common nutritional disorder in companion animals (German 2006). Factors implicated in companion animal obesity include neutering, inactivity and feeding energy dense food (Scarlett, Donoghue et al. 1994; Lund, Armstrong et al. 2005; German 2006). Estimates of feline obesity in the United States vary from 25-35% (Scarlett, Donoghue et al. 1994; Lund, Armstrong et al. 2005). Caloric restriction is the most frequent self- and physician prescribed treatment to promote body mass loss for both humans and companion animals, but is rarely successful in the long-term and the lost mass is often regained. This trend has been widely observed in humans (Anderson, Vichitbandra et al. 1999), dogs (Laflamme and Kuhlman 1995), cats (Villaverde, Ramsey et al. 2008) and laboratory mice (Hambly, Mercer et al. 2007). Several dietary and behavioral strategies aimed at promoting maintenance of mass loss have been identified in human subjects, such as consumption of low fat foods and increased physical activity (Schoeller, Shay et al. 1997; Butryn, Phelan et al. 2007). In cats, body mass regain following restriction is often attributed to a lack of owner compliance to the diet (Kienzle and Berglert 2006), but there is also evidence suggesting that there is a decrease in mass-adjusted energy expenditure during caloric restriction that promotes regain (Villaverde, Ramsey et al. 2008).

A previous study has examined the effect of dietary energy density manipulation on feline body mass by alterations in the ratio of meat to gravy content (Morris, Calvert et al. 2006). Dry matter intake remained constant over a 10 wk period resulting in greater body mass gain in cats fed a higher energy dense diet. The diets used in this study however, were not matched for nutritional content and therefore it is impossible to determine whether the effects seen were purely due to manipulations in energy density or whether macronutrient differences also had an effect. Also, Vasconcellos et al (2009) examined the effects of manipulating protein content on weight loss and subsequent weight management. This study found that high protein diets enabled a greater energy intake, thus reducing the extent of the energy restriction and weight maintenance required more energy after weight loss. The aim of the following studies was to identify whether alterations in dietary energy density via manipulations of water content would modulate post-restriction body mass increases in domestic cats in the absence of macronutrient differences (fat, protein, carbohydrate).

Materials and Methods

Study 1

Study design: the study was of a randomized 2-way crossover design. Twenty-seven (16 female, 11 male) neutered domestic short-haired cats, born and housed at the WALTHAM Centre for Pet Nutrition participated in the study following approval by the WALTHAM Ethics Committee. Cats were housed in two different social rooms with free access to water but were individually fed for 45 min twice daily in individual feeding boxes without access to water. Habitual dietary intake of each cat on the dry (0% hydrated) study diet was monitored for 8 wk prior to commencement of the study. Cats were matched for social room, sex, age (range 2.8 to 9.9 y) and start body mass before being randomized within pairs to one of two groups. The test diets were based on a commercially available complete dry diet (Whiskas TOP^{TM} :120g/kg moisture (12% basal hydration), 42.3 g/kg protein, 14.5 g/kg fat, 1.6 g/kg crude fiber). The 3 diets of varying energy density were 0% hydrated, whereby the diets was not manipulated in any way, or created by adding 20% wt/wt tap water (20% hydrated), or 40% wt/wt tap water (40% hydrated). The water was always added to the diets immediately before feeding to minimize evaporation. It took around 10 minutes for the water to be absorbed in the 20% hydrated diet and twice as long for the 40% hydrated diet. Diets were mixed with the water continuously until there was no free water in the mixing bowl. The kibbles absorbed the water around the edges but maintained their structure. Evaporation from start of preparation to the end of the feeding time was < 2% of the total mass (measured in triplicate).

During the first period (phase 1), cats were calorically restricted by feeding the 20% hydrated diet at 80% of their individual habitual energy intake for 6 weeks. During phase 2, cats in group 1 were offered the 0% hydrated diet, and cats in group 2 were offered the 40% hydrated diet for 3 wk ad libitum for two, 45 min feeding periods daily. A further, 6-week period of caloric restriction (phase 3) followed, in which cats were fed the same number of calories as phase 1. Finally, during phase 4, group 1 cats received the 40% hydrated diet and group 2 received the 0% hydrated diet ad libitum for 3 wk for two, 45-minute feeding periods daily.

Measurements: Food intake (± 0.01g, Sartorius L2200P top-pan balance) and body mass (±0.1g, Sartorius FB 34 3DE P top-pan balance) were recorded daily and was recorded three times per wk. Body composition was assessed in fasted cats at the start and end of each phase by means of dual energy x-ray absorptiometry (DXA) (Lunar Hologic QDR-1000W, Waltham, MA, USA). Cats were sedated with Domitor® (50µg/kg) and Torbugesic (0.4mg/kg) and reversed with Antisedan® (125µg/kg) (all drugs from Pfizer UK Ltd., Kent, UK). This method has been previously validated in cats (Speakman, Booles et al. 2001).

Blood Measurements: A fasted blood sample (3.4 ml) was taken from the jugular vein, prior to sedation, at the start and end of each phase. Plasma insulin and leptin were analyzed by means of radioimmunoassay (insulin RIA kit, CAT no: PI-12K, multi-species leptin RIA kit, CAT no: XL-85K; Linco Research, Millipore, MA, USA).

Digestibility: Six cats (3 male, 3 female) were randomly selected at the end of the study. Each cat was offered each of the three diets in a randomized order; ad libitum for 7 d. Cats were individually housed in the same feeding boxes as study 1. Food intake was recorded daily (± 0.01g, Sartorius L2200P top-pan balance) and all fecal deposits were collected. Total fecal output for each cat was homogenized, freeze dried for 48 h (BOC Edwards, West Sussex, UK), ground into a powder and sieved to remove hair. Adiabatic bomb calorimetry was used to determine the gross energy (GE) of the feces (Gallenkamp, Loughborough, UK). Mean GE was calculated from a mean of two replicates within ±0.4 kJ/g. The apparent energy absorption efficiency (AEAE) and net energy assimilation (EA) were calculated accordingly:

$$\text{AEAE (\%)} = \frac{(\text{hydrated food intake (g) x GE food}) - (\text{dry fecal mass (g) x GE feces})}{(\text{hydrated food intake (g) x GE food})}$$

$$\text{EA (kJ/g)} = \text{energy consumed x AEAE}$$

Ancestry: An ancestral pedigree was constructed using breeding records. By tracing all the paths which connect two individuals through a nearest common ancestor, the relatedness for each pair of cats was calculated. The relatedness for an individual was calculated as half the sum of the genetic contribution from the individual’s sire and dam. For example, if two cats were siblings, relatedness would be (0.5+0.5)/2=0.5.

Study 2

Study design: Nineteen different neutered domestic shorthaired cats (12 male, 7 female) were used to assess the repeatability of the effect seen in study 1 and to investigate the mechanism by which altered energy density might affect the post-restriction changes in body mass. As previously, cats were sex, age (range 2.3 to 8.9 y) and start body mass matched across two groups. Feeding and diet preparation were as detailed in study 1. The two groups were housed separately in social rooms which did not differ with the exception of a slightly different layout. The study was of a parallel design with two phases. In phase 1, cats were calorically restricted for 5 wk on the 20% hydrated diet. Cats received an 18% caloric restriction (by mass) relative to each individual's daily energy requirements (determined prior to the study). In phase 2, group 1 cats were offered the 0% hydrated diet and group 2 were offered the 40% hydrated diet for 3 wk ad libitum.

Measurements: Measurements were as detailed above, with the exception of body mass that was recorded twice weekly. As previously, DXA was performed on fasted cats at the start and end of each phase (Lunar Prodigy Advance, GE Lunar, Waltham, MA, USA).

Gut transit and digestibility: Each cat was fed 16 plastic beads (2x1x1 mm tubes; Malte Haaning Plastic, Denmark) with a separate colored bead fed to each cat. Feces were then collected from each room for the following 7 d. Every fecal deposit was examined for the presence of beads. Gut transit time was determined by the appearance of one or more beads. All beads were removed from the feces in order to assess GE as described in study 1.

Estimation of Physical Activity: Activity monitors (Actical®, MiniMitter Company Inc., Bend, Oregon, USA) were attached to the collars of 15 cats (n = 8 and n = 7 from groups 1 and 2 respectively) during the final 6-12 d of each phase. As there were not enough monitors for all the cats, the cats with monitors were selected at random. An activity count was recorded by the monitor once a minute and all data were excluded from the first and last day and when cats were DXA scanned. Hard-drive video recorders (JVC, London, UK) also continually recorded two 24 h periods of each group, during each phase. The recordings were analyzed by noting the predominant activity level within each group every 60 min. Activity levels were categorized as: ‘low level’ (prolonged periods of little movement, such as sleeping), ‘medium level’ (grooming, slowly walking around), or ‘high level’ (interacting with other cats, running around).

Statistics

All statistical analyses were performed with MINITAB version 13.1. A general linear model (GLM) was used to determine the presence of order effects for every parameter measured. The data were pooled from each study treatment when order effects during the body mass regain phase were non-significant ($P < 0.05$). The statistical significance of differences in the mean values of measured parameters was assessed using paired Student’s t tests or a one-way analysis of variance (ANOVA) as appropriate. Repeated measures ANOVA was used for repeated measurements of body mass and food intake in the same animals. GLM was also used to find the significance of factors impacting on post-restriction body mass regain, including the relevant interactions. Correlation coefficients were assessed using linear least squares regression or reduced major axis regression where appropriate. Data are expressed as means ± SD unless otherwise stated. Differences were considered significant when $P < 0.05$.

Results

Study 1

Twenty five cats completed study 1. Two cats were removed from the study (one from each group) due to ill health at different time-points in the experiment, but all data from these animals were excluded. Mean body mass at the start of the study for group 1 was 4782 ± 765 g and 4715 ± 735 g for group 2 (range: 3745-6580 g) (P > 0.05). Mean age of group 1 was 6.4 ± 2.4 yr and 5.9 ± 1.8 yr for group 2 (P > 0.05). Complete DXA data were available for 25 cats and blood data for 19 cats. Samples were missing due to tolerance to the treatment and animal compliance.

Body mass and composition: Data were pooled for all phases for the 2 test groups as there were no residual dietary effects as a result of using the crossover design (P > 0.05). The washout was effective to separate the phases, such that body mass at the start and end of the restriction phases was not different between the groups (P > 0.05). The body mass loss during caloric restriction did not differ (phase 1 = 172.0 ± 96.5 and phase 3 = 176.5 ± 135.1 g) (**Fig. 1**) which was a mean overall loss of 3.5 ± 1.9% compared to the start of the phase (mean start mass: 4828 g, range: 3745-6580 g). Mean fat mass loss during restriction was 127.9 ± 104.5 g which was not different between the two phases or groups ($P > 0.05$).

Following ad libitum feeding, mean body mass regain was 330.2 ± 164.3 g on the 0% hydrated diet and 266.5 ± 134.9 g on the 40% hydrated diet ($P = 0.026$). These represented mean body mass gains, in comparison to starting body mass, of 6.9 ± 3.0% and 5.7± 2.8% respectively. Mean fat mass gain during the regain phase was 147.3 ± 118.5 g for the 0% hydrated diet and 137.4 ± 137.6 g for the 40% hydrated diet. Body mass regain was significantly correlated with fat mass gain ($P < 0.001$), with a significant effect of diet ($P = 0.044$).

Food Intake: There were no significant differences in mean dry matter intake between the 2 caloric restriction phases (phase 1, 51.5 ± 8.0 and phase 3, 51.3 ± 8.2 g/d). During the ad libitum phases, mean wet matter intake (g of diet) was significantly greater on the 40% (131.2 ± 27.2 g/d) than the 0% hydrated diet (86.7 ± 18.4 g/d) ($P < 0.001$). Body mass regain was significantly influenced by wet mass food intake ($P < 0.001$) and there was a highly significant diet-by-food intake interaction ($P = 0.001$). Dry matter intake was significantly lower on the 0% hydrated diet (86.7 ± 18.4 g/d (1381 ± 292 kJ/d) versus the 40% hydrated diet (93.7 ± 19.4 g/d (1493 ± 306 kJ/d) ($P < 0.001$). As expected, body mass gain during the regain phase was significantly influenced by energy intake ($P < 0.001$), with no significant effect of diet ($P = 0.189$), but a significant diet-by-energy intake interaction ($P = 0.026$). During the regain phase, for the same mean number of kilojoules consumed (1438 kJ), cats gained 125g more body mass on the 0% hydrated diet when compared to the 40% hydrated diet (**Fig. 2**).

Three factors were significantly associated with the body mass increase observed following restriction: body mass loss during restriction ($P < 0.001$), the mass of the cat at the start of the phase ($P = 0.042$), and energy intake ($P < 0.001$). The energy density of the diet did not directly influence body mass regain ($P = 0.513$), but had significant interacting effects with both energy intake ($P < 0.001$) and the starting mass of the cat ($P = 0.007$).

Blood Chemistry: Leptin and insulin concentrations significantly decreased during caloric restriction (leptin: $P < 0.001$, insulin: $P = 0.011$), and increased during regain (leptin: $P = 0.012$, insulin: $P < 0.001$). There was no significant effect of diet in either case. Leptin concentrations were not significantly correlated to changes in fat mass during either the restriction or regain phases, and there was no significant effect of diet. Insulin concentrations were significantly related to changes in fat mass during restriction only (*P* = 0.018), with a significant effect of diet (*P* = 0.028).

Digestive efficiency: In study 1, mean fecal GE was 14.79 ± 0.69, 14.43±0.38 and 14.88 ± 0.59 kJ/g on the 0, 20% and 40% hydrated diets respectively (*P* > 0.05). AEAE, energy intake and net energy assimilated were not significantly different between the diets (*P* > 0.05 for all parameters tested).

Ancestry: In the pedigree there were three sets of siblings, and one maternal association. All cats were related to at least one of two sires. The four factors significantly influencing body mass regain (mass loss during restriction, starting body mass, energy intake and diet) accounted for 75% of the variability in the mass increase. Some of the remaining 25% of the variance could have been due to genetic similarities between cats. The residual variance from the GLM was found for each cat and the differences between these variances were calculated for each pair of cats. This was plotted against the corresponding relatedness for each pair of cats. If genetics played a significant role in post-restriction body mass increase, it was predicted that responses would be more similar in closely related pairs of cats. If this were the case, a negative correlation between residual variance and relatedness was predicted. There was no evidence for a genetic effect on body mass regain within the population tested (**Fig. 3**).

Study 2

All 19 cats completed study 2. The mean body mass of cats at the start of the study was 5366 ± 674 g (range: 4260-6530 g).

Body mass and composition: Mean body mass at the start of the experiment for groups 1 and 2 respectively were 5215 ± 753 and 5407 ± 642 g. Mean body mass at the end of caloric restriction (start of ad libitum feeding) was 5085 ± 735 and 5318 ± 639 g respectively. Caloric restriction induced significantly greater mean body mass losses in group 1 (129.4 ± 83.0 g) compared to group 2 (88.7 ± 96.5g) ($P > 0.05$). Mean body mass loss during restriction was 2.4 ± 1.5% and 1.6 ± 1.9% of start body mass. Mean fat and lean mass losses were 73.6 ± 132.8 and 57.6 ± 199.9 g respectively ($P > 0.05$). Mean body mass regain in cats fed the 0% hydrated diet was 368.3 ± 120.7 g and cats fed the 40% hydrated diet was 312.8 ± 95.9g ($P = 0.280$). This was a mean body mass gain was 6.7 ± 1.8% and 5.6 ± 1.9% of start body mass for groups 1 and 2 respectively. Mean fat and lean mass gains during the regain phase were 27.8 ± 148.4 and 222.0 ± 154.8 g respectively and not significantly different between the diets.

Food intake: During restriction, mean wet mass food intake of the 20% hydrated diet was 65.8 ± 7.3 and 69.1 ± 7.3 g/d (dry mass: 53.9 ± 6.0 and 56.6 ± 5.9 g/d) in groups 1 and 2 respectively ($P = 0.162$). During the regain phase, mean wet mass food intake was significantly greater on the 40% hydrated diet (129.5 ± 18.0 g/d) when compared to the 0% hydrated diet (82.5 ± 14.8 g/d) ($P < 0.001$). Body mass regain showed a significant association with wet mass food intake ($P < 0.001$) with a significant effect of diet ($P < 0.001$). Dry matter intake during the regain phase was significantly greater (86.7 ± 18.4 g/d (1299 ± 232 kJ/d) on the 0% hydrated diet when compared to 40% hydrated diet (77.7 ± 10.8 g/d (1208 ± 172 kJ/d) ($P < 0.05$). Body mass regain was significantly related to energy intake ($P < 0.001$), with no significant effect of diet or diet-by-energy intake interaction ($P = 0.555$).

Gut transit and digestive efficiency: Mean fecal GE was 15.38, 14.60 and 15.51 kJ/g on the 0, 20 and 40% hydrated diets respectively (*P* = 0.379). Gut transit time was not different on the three diets and the majority of the beads emerged together (**Table 1**). There were no significant differences in AEAE, EA or EI between the three diets when the data were pooled across both experiments (**Table 2**).

Physical activity: Inter-day activity levels, as measured by the activity monitors, were consistent within each dietary phase. Activity levels were significantly higher during the restriction than the regain phase (*P* < 0.001) (**Fig. 4*A***). Analysis of the video footage showed the amount of time dedicated to moderate and high levels of activity was greater when the diet was restricted compared to when either diet was fed ad libitum. Activity was significantly affected by time of day (*P* < 0.001) showing large peaks when food was offered in the morning. In the regain phase, there was a significant effect of time of day (*P* < 0.001) and of diet group (*P* = 0.030) on activity levels (**Fig. 4*B***). Activity, as measured by the activity monitors, was significantly higher in the group on the 40% hydrated diet compared to the cats fed the 0% hydrated diet. This was also evident from the video footage. Cats fed the 0% hydrated diet spent the majority of the day sleeping, whilst cats fed the 40% hydrated diet were moderately-to-highly active, and behaviors resembled those observed during restriction.

Discussion

In humans, consumption of low energy dense foods has been shown to promote weight loss maintenance following caloric restriction (Greene, Malpede et al. 2006). However, the effects of nutrient intake are often not controlled (Morris, Calvert et al. 2006; Vasconcellos, Borges et al. 2009). The aim of this study therefore, was to investigate if changes to dietary energy density, whilst controlling for nutritional composition, could modulate post-restriction body mass regain in cats. There is evidence to suggest that body mass has a genetic background (Rankinen, Zuberi et al. 2006) and heredity has been shown to influence food intake and dietary energy density in humans (De Castro 2006). Although there was a wide range of relatedness between the cats; there was no evidence of a genetic contribution to post-restriction regain once the other factors had been taken into account. As all cats were related to one of 2 sires, the genetic variation may not have been great enough to demonstrate an effect.

In study 1, actual energy intake was less on the 0% than the 40% hydrated diet. During the regain phase, for the same mean number of kilojoules consumed, cats regained more body mass on the more energy dense 0% hydrated diet, than the 40% hydrated diet. The same trend, although not significant, was observed when the protocol was repeated in a separate group of cats in study 2. The lack of significance may have been due to lack of power in experiment 2 because of the absence of the crossover design. There are a number of possible mechanisms that could explain this phenomenon. The first was that the reduced body mass gain was a result of increased energy expenditure. Activity levels were significantly greater during restriction than ad libitum feeding. This result has also been reported in rodents (Holloszy 1997; Dixon, Ackert et al. 2003) and is hypothesized to be a food searching behavior, although paradoxically this behavior drives animals further into energy deficit (Hambly and Speakman 2005). Activity levels between the two groups were not significantly different during restriction despite the significant difference in body mass loss between the 2 groups. There were, however, significant differences in post-restriction activity as quantitatively assessed by the use of activity monitors and subjectively assessed by the use of video recordings and behavioral classification. The types of activity observed in cats consuming the low energy dense (40% hydrated) diet were similar to when they were restricted, in that they were more active in comparison to when consuming the 0% hydrated diet. To our knowledge, this is the first time reduced energy density diets have been associated with an increase in physical activity levels. The mechanism responsible for this observation requires further investigation.

It may be possible that cats were in a perceived state of energy restriction on the low energy dense diet, and were actively searching for more food. However, this seems contrary to expectations because low energy diets are usually associated with greater stomach fill for the same caloric intake (De Castro 2006; Vasconcellos, Borges et al. 2009) leading to satiation (De Graaf, Blom et al. 2004). These previous data refer to low energy dense diets high in fiber, and there may be differences in satiety between methods of altering energy density (fiber vs. water). The difference in hydration levels of the cats may also have influenced subsequent activity, for example on the 40% hydrated diet cats may have been more hydrated and therefore more active. On the other hand, cats that are dehydrated on the 0% diet may spend time seeking water and may be more active. The impact of hydration status on activity levels requires more work. Carbannel *et al* (1994) has previously shown that altering the energy density of human meals by adding water did not alter satiety, and further investigation in cats is required.

Alternatively, the increased activity levels observed may indicate a learned behavior such as a response to appetite regulation hormones that would override an initial satiety effect of increased stomach fill. In study 1, there was no significant effect of diet on serum concentrations of either leptin or insulin indicating that they were not responsible for the increased activity levels. There are however many other hormones involved in appetite regulation (Mercer and Speakman 2001; Field, Wren et al. 2008) which were not measured and may also have had important effects. Further studies are required to examine the association, if any, of these hormones with spontaneous physical activity levels in cats.

Another potential mechanism to explain the reduced body mass gain in the 40% hydrated group was reduced gut transit time and a subsequent reduction in energy digestibility (Slavin 2005). However, our data suggest that adding water did not impact on digestive efficiency, as measured by gross fecal energy. This is similar to data reported in humans (Carbannel, Lémann et al. 1994). Dry diets have been linked to slow rates of gastric emptying in cats (Goggin, Schryver et al. 1993). However, gut transit time has also been reported to be between 24 and 37 h in cats consuming wet foods (Peachey, Dawson et al. 2000; Kinga, Angella et al. 2006). There were also no differences in gut transit time as measured by the use of the emergence of plastic beads, suggesting that varying the hydration levels of food by up to 40% did not affect gut transit time in these cats. However, the method of measurement of transit time has not been validated in cats and could have been improved, for example by using radio-opaque markers (Peachey, Dawson et al. 2000). The method of administration of the water to the diet may also be important and it has been suggested that the water must be incorporated into the food rather than consumed alongside, at least in humans (Stubbs, Ferres et al. 2000). The 20% and 40% diets had completely absorbed all of the water by thorough mixing during diet preparation, so it was not the case that water emptied from the stomach faster than the diet.

In conclusion, body mass regain following caloric restriction was dependent on dietary energy density when manipulated by water content, whereby cats fed a low energy dense diet (higher water content) regained less body mass without significantly increased energy intake. Our data suggest that this phenomenon can, at least in part, be explained by increased physical activity levels in cats feeding on low energy dense (high water content) diets. We suggest that modulation of energy density alone via water content in the post-restriction phase may be a valuable strategy to aid maintenance of body mass following caloric restriction in cats.

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FIGURE LEGENDS

Fig. 1 Mean body mass during study 1. Each phase is separated by a dashed line. Values are mean ± SEM, n = 27 cats.

Fig. 2 Mean body mass change in relation to energy intake of the 0% and 40% hydrated diets in the regain phase for each cat in study 1. Dashed vertical lines were added at mean daily energy intake (1437.59 kJ) and horizontal lines were added to illustrate a difference of approximately 125 g in body mass on the two diets. n = 27 cats.

Fig. 3 The differences in residual variance between pairs of cats, plotted against the corresponding relatedness between each pair of cats. Variance was evenly distributed at all levels of relatedness, suggesting there were no genetic effects on post-restriction body mass. n = 25 cats.

Fig. 4*A* Physical activity during the caloric restriction and regain phase for all cats in study 2. Activity was significantly higher when cats were calorically restricted than when consuming either diet ad libitum. ***B*** Activity during the regain phase for cats consuming 0% hydrated food was significantly lower than cats consuming 40% hydrated food. Values are mean ± SEM, n = 19 cats.

TABLE 1 Gut transit time (h) on the three study diets (0, 20 and 40% hydrated), determined by the time it took for one or more inert plastic beads (out of 16 consumed) to emerge in the feces.

	0% hydrated[2]	**20% hydrated**[1]	**40% hydrated**[3]
24 h	8	6	8
27 h	1	3	2
32 h	0	4	0
48 h	0	5	0
Bead never found	0	1	0

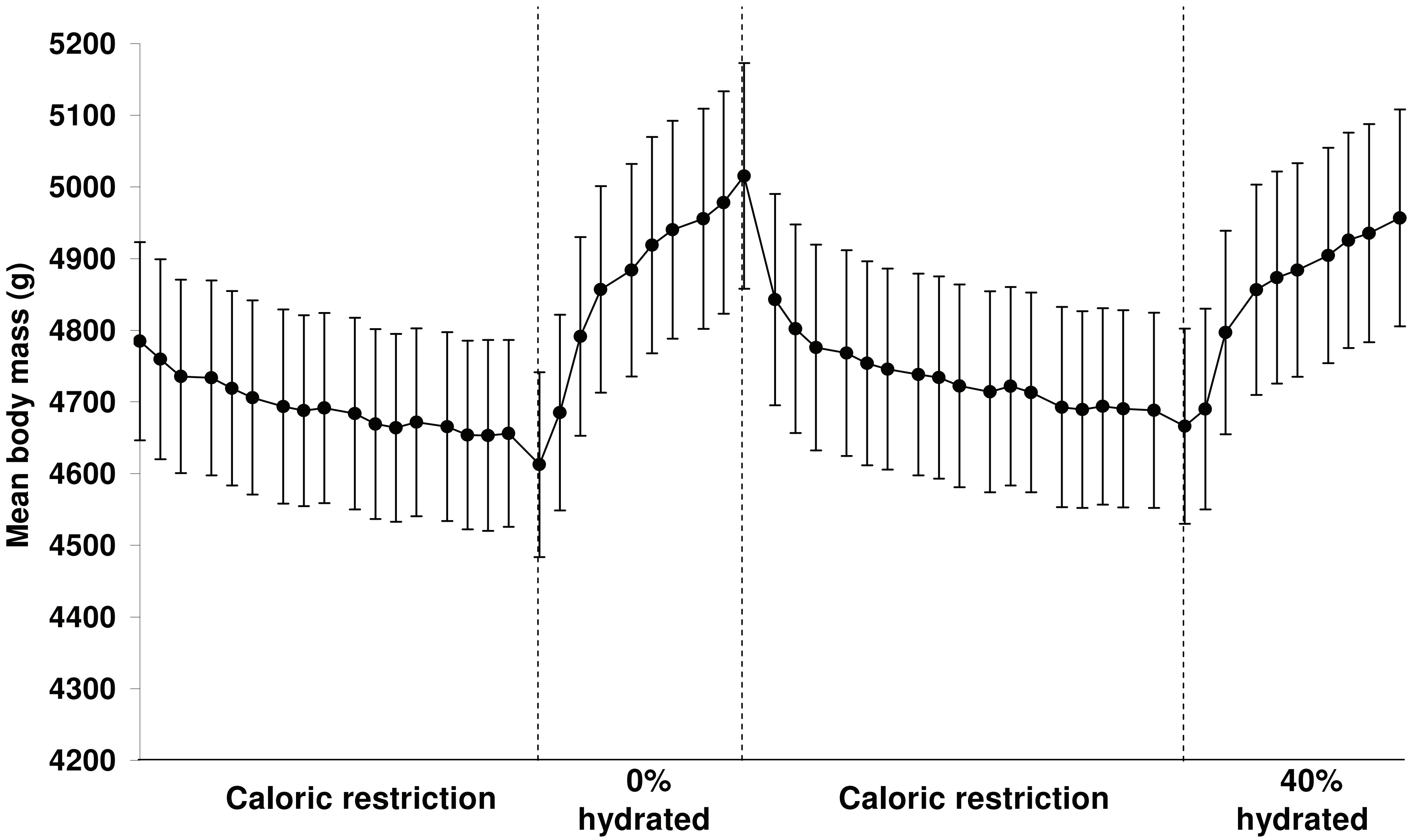
1. n=19
2. n=10
3. n=9

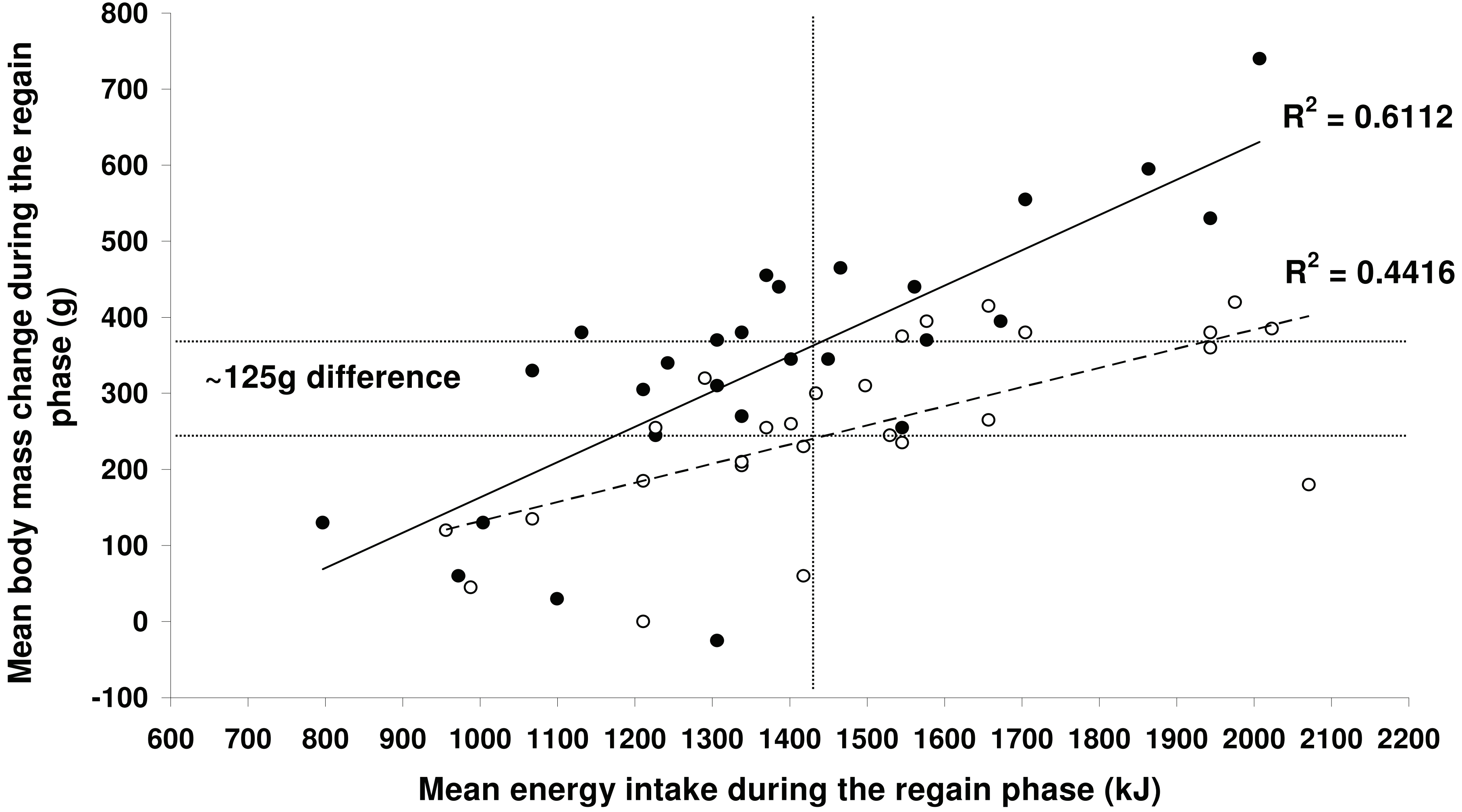
TABLE 2 Digestive efficiency data measured from on the three study diets. Data were pooled from all cats in studies 1 and 2 consuming 0, 20 and 40% hydrated diets[2].

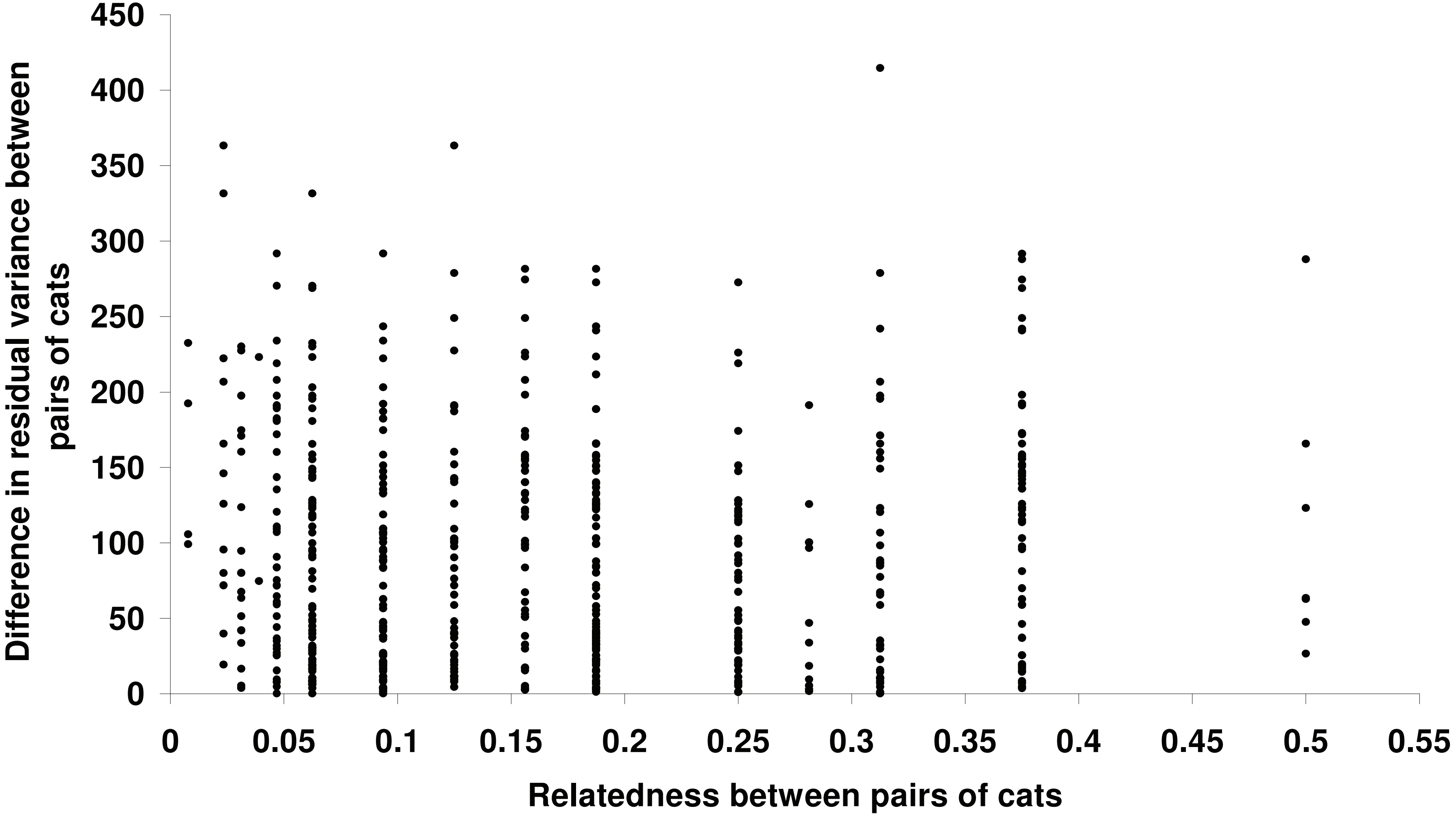
	0% hydrated	**20% hydrated**	**40% hydrated**
Fecal deposits (g/d)	14.04 ± 2.88	14.53 ± 2.86	14.35 ± 3.80
Food intake (g/d)	52.94 ± 7.65	62.59 ± 9.78	78.87 ± 11.60*
AEAE (%)[1]	87.65 ± 2.78	88.25 ± 3.33	84.87 ± 6.25
Energy intake (kJ/d)	1140.34 ± 237.93	1014.18 ± 135.67	1002.48 ± 260.69
Energy assimilation (kJ/d)	985.81 ± 215.76	890.56 ± 138.08	840.45 ± 165.03

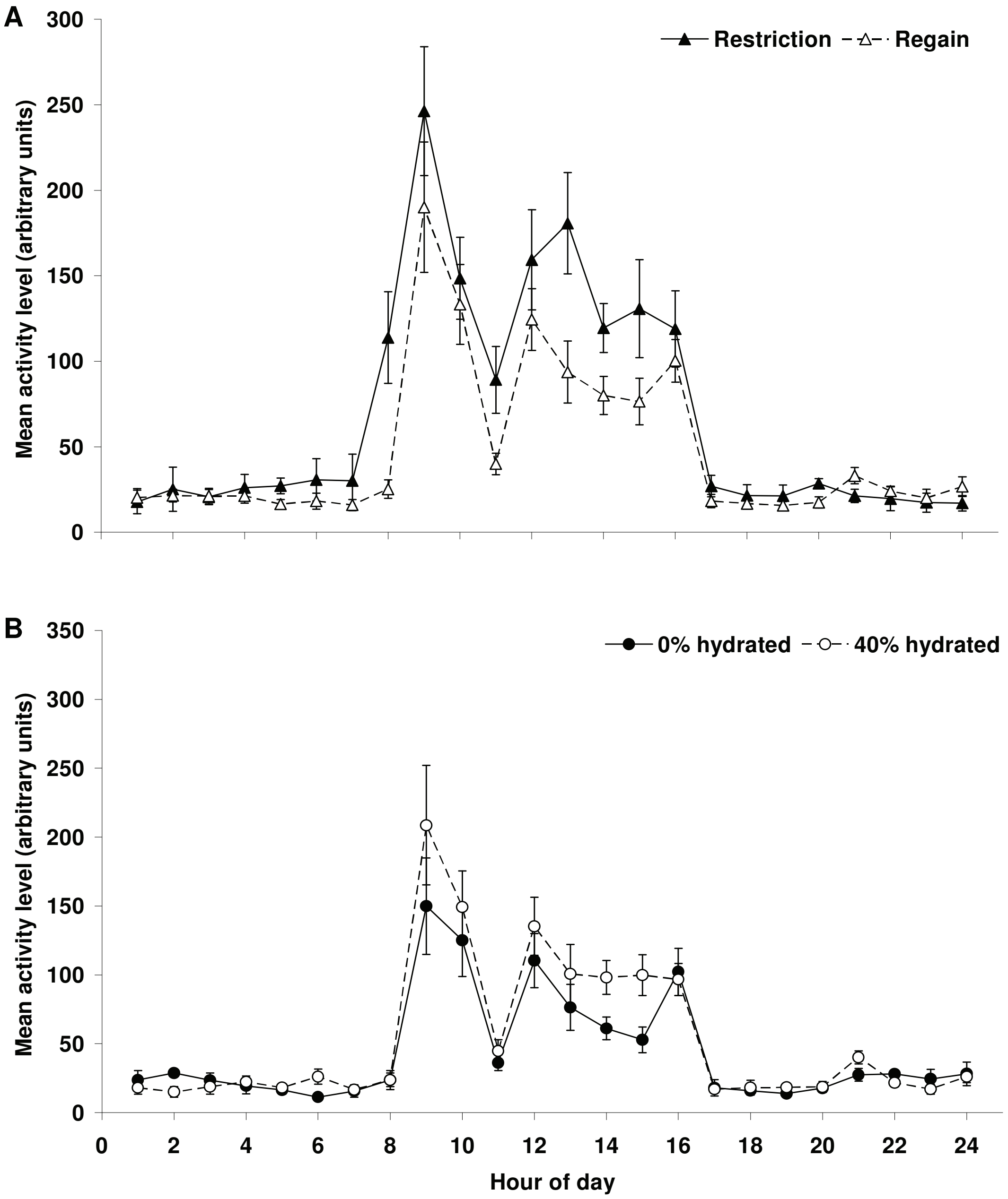
[1] AEAE, apparent energy assimilation efficiency.

[2] Values are means ± SD, n = 46. Differences between the diets significant when *$P \leq 0.001$


5200
5100
5000
4900
4800
4700
4600
4500
4400
4300
4200
Mean body mass (g)
Caloric restriction
0%
hydrated
Caloric restriction
40%
hydrated


Mean body mass change during the regain phase (g)
800
700
600
500
400
300
200
100
0
-100
Mean energy intake during the regain phase (kJ)
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
2200
$R^2 = 0.6112$
$R^2 = 0.4416$
~125g difference


Difference in residual variance between pairs of cats
0
50
100
150
200
250
300
350
400
450
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
Relatedness between pairs of cats


A
Restriction
Regain
Mean activity level (arbitrary units)
0
50
100
150
200
250
300
B
0% hydrated
40% hydrated
Mean activity level (arbitrary units)
0
50
100
150
200
250
300
350
0
2
4
6
8
10
12
14
16
18
20
22
24
Hour of day