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Metabolic determinants of body weight after cats were fed a low-carbohydrate, high-protein or a high-carbohydrate, low-protein diet *ad libitum* for 8 weeks

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Abstract

Overweight and obese conditions are common in cats, and are associated with the development of a number of diseases. Knowledge of metabolic determinants and predictors of weight gain may enable better preventative strategies for obesity in cats. Lean, healthy cats were fed either a low-carbohydrate, high-protein (*n* 16), or a high-carbohydrate, low-protein (*n* 16) diet *ad libitum* for 8 wk. Potential determinants and predictors of final body weight assessed were body fat and lean masses, energy required for maintenance, energy requirements above maintenance for each kg of weight gain, insulin sensitivity index, fasting, mean 24-h and peak plasma glucose, insulin and leptin concentrations, and fasting and mean 24-h serum adiponectin concentrations. In cats fed the low-carbohydrate, high-protein diet, after adjusting for initial body weight, those with higher energy requirements for weight gain and higher fasting glucose concentration had higher final body weights ($P \leq 0.01$). Predicted final body weights using initial body weight, fasting glucose and mean 24-h insulin concentrations (partial R^2 37.3%) were imprecise. An equation using just initial body weight and fasting glucose concentration would be of more practical value, but was

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marginally less precise. In cats fed the high-carbohydrate, low-protein diet, those with lower fasting leptin concentration initially had higher final body weights ($P = 0.01$). Predicted final body weights using initial body weight, energy requirements for maintenance, total body fat percentage and fasting leptin concentration (partial R^2 39.2%) were reasonably precise. Further studies are warranted to confirm these findings and to improve the precision of predicted final body weights.

Keywords: Fat mass; weight gain; energy requirements; glucose; adipocytokines; cats.

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1 Introduction

Overweight and obese conditions are commonly recognised in pet cats around the world, and are associated with the development of a number of diseases, including diabetes mellitus [1]. Although the prevalence of overweight and obese cats varies according to the population studied and methods used to determine body condition, the overall incidence is high in developed countries, varying from 17% to 63% [2-5].

As in humans [6], genetic and environmental factors are believed to predispose cats to weight gain. Environmental factors such as indoor housing [7], *ad libitum* feeding [8], neutering [4; 7; 8], and the underestimation of body condition status by owners [3] have been identified as risk factors for obesity in cats. Genetic factors determine the magnitude of weight gain in presence of excess food [9].

In adult humans, recognised predictors of weight gain are low metabolic rate, low levels of physical activity, low rates of fat oxidation, low sympathetic nervous system activity, and low fasting plasma leptin concentrations [10]. There is controversy in relation to the predictive value of insulin sensitivity in relation to weight gain in humans; in some populations increased insulin sensitivity is associated with weight gain, in others there is no association [10; 11]. Similarly, decreased fasting insulin concentration has been reported to be associated with subsequent weight gain in some studies [12; 13], whereas no association has been found in others [11].

There are no published studies investigating metabolic determinants of weight gain in cats. It is known that most of the excess weight in adult overweight and obese cats is from body fat [14; 15] and increased adipose tissue mass is associated with reduced insulin sensitivity [16; 17], increased circulating leptin [18-20] and decreased total adiponectin concentrations in cats [18]. These parameters might be useful markers for the prediction of weight gain in cats.

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Better knowledge of metabolic factors associated with weight gain may help explain why some cats gain weight more easily than others, and may enable more effective preventative strategies for obesity in cats. The aims of this study were to identify metabolic determinants of final body weight after 8 wk of *ad libitum* feeding in clinically healthy cats, and to identify predictive equations that could be used prospectively to identify cats that are likely to gain the most weight when fed *ad libitum*.

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2 Materials and Methods

2.1 Study overview

A retrospective single cohort study was conducted using data from a controlled trial. Cats were fed either a low-carbohydrate, high-protein diet or a high-carbohydrate, low-protein diet *ad libitum* for 8 wk. Potential determinants and predictors of final body weight were assessed using linear regression. Variables assessed were initial body fat and lean masses, initial maintenance energy requirements, energy requirements above maintenance for each kg of body weight gain, insulin sensitivity index, and fasting, mean 24-h and peak plasma glucose, insulin and leptin concentrations, and fasting and mean 24-h serum adiponectin concentrations. Initial body weight was fitted in all models.

Testing protocols, cat signalment, body condition variables and insulin and glucose concentrations have been previously reported as part of a study to assess effects of weight gain and diet on glucose and insulin concentrations [15], also on leptin and adiponectin concentrations [20], and in a study to assess the effect of dietary carbohydrate intake on adiponectin profiles [21]. Leptin and adiponectin concentrations have been reported in Coradini *et al*, 2013 [20], and part of the adiponectin results have been reported in Tan *et al*, 2011 [21]. Thirty-two neutered, lean, mixed breed and clinically healthy cats (sixteen males, sixteen females) of approximately 2 to 4 yr of age, were used in the study. Mean body weight was 3.31 kg (range 2.42 to 4.64 kg), and mean body condition score was 4.9 (range 4 to 5) on a nine-point body condition system [22]. Full description of the study protocol and dietary analyses have been reported [15].

The study consisted of three phases: baseline, stable-weight, and weight-gain. In the baseline phase, all cats were fed a baseline diet, moderate in carbohydrate, fat and protein (Table 1) [15], to maintain their body weight within 95 to 105% of their initial weight, for 3 wk and tests were

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conducted in the fourth week. Cats were paired based on sex, insulin sensitivity, and body weight, and were then randomly allocated to one of two diets, a low-carbohydrate, moderate-fat, high-protein diet and a high-carbohydrate, moderate-fat, low-protein diet (Table 1) [15].

In the stable-weight phase, cats were fed either a low-carbohydrate, high-protein or a high-carbohydrate, low-protein diet to maintain their body weight within 95 to 105% of their initial weight for the following 4 wk (study weeks 5 to 8), with testing in the eighth week of the study. In the weight-gain phase, cats were fed their respective test diets *ad libitum* for the subsequent 8 wk (weeks 9 to 16), and were tested in the 17th week of the study. During test weeks, cats were fed their maintenance energy requirements to allow comparison of results between the stable-weight and weight-gain phases and to determine the effects of 8 weeks of *ad libitum feeding* on the parameters tested [15].

All diets used were commercially available extruded dry feline products, made to comply with the Association of American Feed Control Officials standards. The study protocol, care and handling of the animals was approved by the University of Queensland's Animal Ethical Review Committee (approval number SVS/328/06/ARC), and by the WALTHAM Ethical Review Committee. During each test week, cats had a jugular catheter placed on day 1. On day 3, a dual-energy X-ray absorptiometry scan (Lunar prodigy, GE Lunar Incorporation, Madison, WI, USA) was performed, when lean body mass, total and abdominal body fat masses were measured. This was followed by a frequently-sampled intravenous glucose tolerance test on day 5, and a 24-h meal-feeding test on day 7 [15].

The insulin sensitivity index was determined by the computer program Minimal Model Millennium (MinMod Millennium, Version 6.02, MINMOD Incorporation, 2001, Los Angeles,

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CA, USA) [23], based on values obtained from plasma glucose and insulin concentrations during an insulin-modified frequently-sampled intravenous glucose tolerance test [15].

The meal-feeding test consisted of two fasting blood samples collected 30 and 5 min before a meal of 167 kJ/kg body weight, fed after a 24-h fasting period. At least 90% of the meal had to be consumed within 30 min for the test to proceed. Eleven samples were collected postprandially, over 24 h.[15] Blood samples for glucose, insulin and leptin analysis were placed into sterile tubes containing EDTA and the proteinase inhibitor, aprotinin (Trasylol, Bayer Ltd, Sydney, NSW, Australia) at 0.05 mL per mL of blood. Blood samples for adiponectin analysis were placed into sterile serum tubes. Samples were centrifuged for 8 min at 1500g. After centrifugation, plasma and serum were removed and stored in vials at -70°C until analysis. Erythrocyte autotransfusion was performed during this test to maintain red blood cell mass, as previously described [24]. In summary, after plasma was collected, the erythrocytes remaining in the EDTA tubes were washed with 0.9% saline, resuspended in saline to the initial volume of blood taken, and then autotransfused.

2.2 Sample analyses

Sample analyses have been described previously [15; 20]. Briefly, plasma glucose was determined using an enzymatic method (Hexokinase enzymatic UV; Olympus Diagnostic Systems Division, Melville, NY, USA) . Plasma insulin concentrations were determined by a commercially available RIA kit (Porcine Insulin RIA Kit; Linco Research Incorporation, St Charles, MO, USA). The assay has 100% specificity for human insulin, and was validated for the detection of feline insulin [15]. Plasma leptin concentration was measured using a commercially available radioimmunoassay kit (Multispecies Leptin RIA Kit, Linco Research Incorporation, St Charles, MO, USA), which has been validated for the detection of feline leptin [20; 25]. Serum total adiponectin concentration was determined by a commercially available murine/rat adiponectin

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ELISA kit (B-Bridge international, Otsuka, Tokyo, Japan), that has been validated for the detection of feline adiponectin [20; 26].

2.3 Calculations

For the 24-h meal-feeding test, fasting concentrations of blood glucose, insulin, leptin and adiponectin were estimated as the average of concentrations at -30 and -5 min. Peak glucose, insulin and leptin concentrations were defined as the highest concentrations observed after feeding, and were defined only for cats whose blood concentrations exceeded fasting as described previously [15; 20]. Mean analyte concentrations were calculated as areas under the curve for 24 h using the trapezoidal method [27] and divided by 24. Maintenance energy requirements were calculated for each cat based on the average metabolisable energy intake and body weight in the 3rd week of the stable-weight phase, according to the formula:

Energy intake (kJ/kg body weight/d) x body weight (kg)/ $(\text{body weight (kg)})^{0.40}$

The exponent of 0.40 was used because energy requirements for maintenance (kJ//kg/d) decline as a function of body weight raised to the power of 0.4 [28] so this equation accounted for differences in body weight.

Energy required above maintenance for each kg of body weight gain (energy required for weight gain), was defined as the average energy cost of each kg of body weight gain based on estimated metabolisable energy partitioned to weight gain during the 8 wk of *ad libitum* feeding; this was calculated for each cat as:

(Total energy intake during 8 wk of *ad libitum* feeding – sum of estimated daily energy requirements for maintenance during 8 wk of *ad libitum* feeding)/body weight gained.

This variable was calculated only for those cats that gained greater than 10% body weight (*n* 28), as absolute errors in measurement of either initial and/or final body weight would have had large impacts on this measure at smaller weight gains.

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2.4 Exclusions

In the stable-weight phase, one cat was excluded from the study in the second test week (week 8) because a catheter could not be placed in its jugular vein. Therefore, data from the stable-weight phase were analysed for 15 of the 16 cats enrolled in the low-carbohydrate, high-protein group, and all 16 cats enrolled in the high-carbohydrate, low-protein group. During the weight-gain phase, one cat in the low-carbohydrate, high-protein group was not sampled because a jugular catheter could not be inserted, and two cats enrolled in the high-carbohydrate, low-protein group were removed from the study at weeks 13 and 16, due to dietary intolerance. Therefore, during the third test week (week 17), dual-energy X-ray absorptiometry scans were performed in 15 of the 16 cats in the low-carbohydrate, high-protein group and in 14 of the 16 cats in the high-carbohydrate, low-protein group, and blood samples were collected from 14 cats in each dietary group.

2.5 Statistical analyses

Data were analysed using Stata versions 9.2 and 12.1 (StataCorp LP, College Station, TX, USA). Linear regression was used to identify determinants of final body weight (that is, at the end of the *ad libitum* feeding period). Initial body weight was fitted as a covariate in all models. This approach was preferable to modelling weight change directly, as the latter approach does not control for confounding due to differences in initial body weight [29] and does not maximise statistical power [30]. Initially, each possible determinant was fitted separately with initial body weight. Separate analyses were performed for each diet. Energy intake during the *ad libitum* feeding period was not assessed as this may have been an intervening variable, that is, part of the causal mechanism for other determinants of final body weight. Within each dietary group, all variables with bivariable p-values (that is, after adjustment for initial body weight) that were < 0.25 were then selected and a final multivariable model was developed using a backwards elimination process. Where variables were closely correlated, one variable was selected. For example, among

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fasting, mean 24-h and peak leptin concentrations, we selected fasting leptin concentration. All selected variables were fitted and the variables with the highest p-value sequentially removed and the model refitted with the remaining variables, until only variables significant at the 0.05 level remained. Once removed, variables were not eligible for reinclusion. Normality and homoscedasticity of residuals from final models were checked using histograms of residual and plots of residuals against fitted values, respectively.

To identify predictive equations that could be used prospectively to identify cats that are likely to gain the most weight when fed *ad libitum*, we used those variables that were used for the multivariable modelling process to identify determinants of final body weight other than energy required above maintenance for each kg of weight gain. This variable was not considered as it could not be measured prior to *ad libitum* feeding and so could not be used prospectively to identify cats that are likely to gain the most weight. The aim was to predict final weight (and hence weight gain, given that initial weight is known) for any particular cat as precisely as possible. Accordingly, variables were selected based on changes in the root mean square error for the model (that is, the square root of the residual mean sums of squares). This described the approximate average of the differences between predicted and actual final weight for the study cats [31]. Prediction equations for the calculation of final body weight were developed firstly using the model with the lowest root mean square error. Separate models were developed for each diet. Using a backwards elimination approach, the effect of each variable on the model's root mean square error was assessed by removing then replacing each. The model with the lowest root mean square error was selected, and the reduced set of explanatory variables fitted. This process was continued until no further reductions in root mean square error occurred on removal of further variables. Further equations were developed by fitting only subsets of variables from these predictive equations that were most readily measured by practitioners. Initial body weight was fitted in all models.

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Proportions of variability in final body weight accounted for by initial body weight were calculated from the univariable models with only initial body weight fitted as sum of squares due to initial body weight/total sum of squares. To assess the contribution of each additional variable in bivariable models, the partial R^2 value (the proportion of variability in final body weight not accounted for by initial body weight that was explained by the other variable) was calculated as sum of squares due to additional variable/(sum of squares due to additional variable + residual sum of squares). For multivariable models, proportions of variability in final body weight accounted for by the model were calculated as model sum of squares/total sum of squares. To assess the contributions of additional variables in multivariable models over and above the contribution of initial body weight, proportions of variability in final body weight not accounted for by initial body weight that were explained by each additional variable were calculated as sum of squares due to an additional variable/(sum of sums of squares due to each additional variable + residual sum of squares). These were summed to obtain the collective partial R^2 value (the proportions of variability in final body weight not accounted for by initial body weight that were explained by the additional variables collectively). Sums of squares were obtained using ANOVA. Root mean square errors were also reported for the models with just initial body weight fitted, and with the additional variables fitted to identify predictive equations.

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3 Results

3.1 Body weight, fat and lean masses

Body weight in the low-carbohydrate, high-protein diet group increased by 37% (1.22 ± 0.37 kg (mean ± SD)) and mean body condition score was 6.3/9, and in the high-carbohydrate, low-protein diet group body weight increased by 17% (0.5 ± 0.28 kg) and mean body condition score was 5.8/9 after 8 wk of *ad libitum* feeding, with proportional increases in body fat mass [15]. Lean mass increased with weight gain, however in smaller proportion relative to the increase in fat mass [15].

3.2 Determinants of final body weight after 8 wk of ad libitum feeding in cats fed the low-carbohydrate, high-protein diet

On univariable analysis, for each extra kg of initial body weight, final body weight increased by 1.10 kg (95% CI 0.81 to 1.40; $P < 0.01$; Table 2). This means that using the simplest equation provided in Table 3, for a cat that weighs 3 kg initially and eats excessively, so that final body weight after eating a low-carbohydrate, high-protein diet for 8 wk *ad libitum* is 4.16 kg, a cat that initially weighs 4 kg with the same propensity to gain weight, final body weight will be 5.26 kg. The latter cat is predicted to be 1.10 kg heavier than the first cat after weight gain, if all other factors are equal. The proportion of variability in final body weight accounted for by initial body weight was 83.2%.

After adjustment for initial body weight, energy requirements above maintenance for each kg of body weight gain and initial fasting glucose concentration were positively associated with final body weight (partial R^2 49.7 and 31.3% respectively; $P \leq 0.04$; Table 2). There was a negative

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association between initial mean 24-h insulin concentration and final body weight (partial R^2 29.1%; $P = 0.05$; Table 2).

Potential explanatory variables used in the multivariable modelling process were initial body weight, energy required for weight gain, initial body fat percentage, and fasting glucose, mean 24-h insulin and fasting adiponectin concentrations (Table 2). The final multivariable model consisted of initial body weight, energy required for weight gain, and initial fasting glucose concentration. After accounting for initial body weight and energy required for weight gain, for each extra mmol/L of fasting plasma glucose concentration, final body weight was 0.57 kg higher (95% CI 0.14 to 1.00; $P = 0.01$). After accounting for initial body weight and fasting glucose concentration, for each extra 10,000 kJ that the cat required for each kg of body weight gain, final body weight was 0.44 kg higher (95%CI 0.19 to 0.68; $P < 0.01$). The relationship between energy required for weight gain and daily energy intake in week 2 of *ad libitum* feeding is shown in Figure 1 (Pearson's correlation coefficient (r) = 0.80; 95% CI based on Fisher's transformation 0.49 to 0.93). After accounting for energy required for weight gain and fasting glucose concentration, for each extra kg of initial body weight, final body weight was 1.11 kg higher (95% CI 0.93 to 1.28; $P < 0.01$), the same relationship that was evident on univariable analysis.

The final multivariable model collectively explained 95.2% of the variability in final body weight. Of the variability in final body weight that was not accounted for by initial body weight, initial fasting glucose concentration accounted for 24.1% of the variability, and energy required for weight gain accounted for 44.5%.

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3.3 Determinants of the final body weight after 8 wk of ad libitum feeding in cats fed the high-carbohydrate, low-protein diet

On univariable analysis, for each extra kg of initial body weight, final body weight increased by 0.89 kg (95% CI 0.69 to 1.09; $P < 0.01$; Table 2). After adjustment for initial body weight, initial lean mass was positively associated with final body weight (partial R^2 47.8%; $P < 0.01$; Table 2). There was a negative association between final body weight and initial total and abdominal body fat mass percentages (partial R^2 47.3 and 40.3%, respectively; $P \leq 0.01$; Table 2), as well as initial fasting, mean 24-h and peak leptin concentrations (partial R^2 47.3, 48.8 and 45.1%, respectively; $P \leq 0.02$; Table 2).

Potential explanatory variables used in the multivariable modelling process were initial body weight, energy requirements for maintenance, initial body fat percentage, and initial mean 24-h glucose, mean 24-h insulin and fasting leptin concentrations (Table 2). The final multivariable model consisted of initial body weight, and initial fasting leptin concentration. After accounting for initial body weight, for each extra ng/mL of fasting plasma leptin concentration, final body weight was 0.34 kg lower (95% CI -0.55 to -0.12; $P = 0.01$). However, after accounting for fasting plasma leptin concentration, for each extra kg of initial body weight, final body weight was 0.86 kg higher (95% CI 0.71 to 1.01; $P < 0.01$).

The final multivariable model collectively explained 93.0% of the variability in final body weight. The proportion of variability in final body weight not accounted for by initial body weight that was explained by initial fasting leptin concentration was 47.3%.

3.4 Predictive equations

Predictive equations are shown in Table 3. For the low-carbohydrate, high-protein dietary group, the average predictive error, that is, the average of the differences between predicted and

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actual final weight for the study cats, was reduced from 0.37 kg based on initial body weight alone to 0.30 kg by also including fasting glucose concentration and mean 24-h insulin concentration. The proportion of variability in final body weight not associated with initial body weight that was collectively explained by these additional variables was only 37.3% (19.9% by fasting glucose concentration and 17.4% by mean 24-h insulin concentration). This reduced to 31.3% if the only additional variable was fasting glucose concentration.

For the high-carbohydrate, low-protein dietary group, the average predictive error was reduced from 0.27 kg based on initial body weight alone to 0.18 kg by also including energy requirements for maintenance, initial body fat percentage, and fasting leptin concentration. The proportion of variability in final body weight not associated with initial body weight that was collectively explained by these additional variables was 39.2% (12.4% by energy requirements for maintenance, 9.2% by initial body fat percentage, and 17.6% by fasting leptin concentration). This reduced to 23.9% if the only additional variable was energy requirements for maintenance, and the average predictive error (0.25 kg) was then similar to that when just initial body weight was used.

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4 Discussion

To our knowledge, this is the first study that has investigated metabolic determinants and predictors of final body weight, and hence weight gain, in cats and we believe the results could serve as a basis for future research in the prevention of obesity in cats. Firstly, in cats fed the low-carbohydrate, high-protein diet, the higher the energy requirements for weight gain, the higher the final body weight. Cats that had higher energy requirements for weight gain ate more when fed *ad libitum*, and therefore gained more weight. As demonstrated in Figure 1, this unexpected positive association appeared to have been, at least in part, because cats requiring more energy for each kg of weight gain ate more compared with cats with lower energy requirements to gain weight. This might have occurred as a compensatory mechanism for their lower efficiency to gain weight, and is similar to findings in other species [32]. This association was not observed in the cats fed the high-carbohydrate, low-protein diet, and this could have occurred because cats are reported to limit their carbohydrate intake [33]; this 'ceiling' effect might have limited food intake in the cats fed the high-carbohydrate, low-protein diet. High-protein diets (providing > 40% ME) are recommended to induce weight loss in cats and prevent loss of muscle mass that can occur with energy restriction. However, clients must be instructed to feed measured amounts of food based on the individual cat's daily energy requirements to achieve and maintain an ideal body condition. As demonstrated in this work and related publications by our group [15; 20], *ad libitum* feeding of these diets will promote weight gain, and therefore is not recommended.

Another finding was the positive relationship between fasting glucose concentration and final body weight. This might be explained because, in clinically normal individuals, the higher the amount of glucose in the bloodstream, the more glucose will be stored as glycogen in the muscles and liver, and also converted to fatty acids and stored as triglycerides in the process of lipogenesis

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[34]. There have been no reports of fasting glucose concentration as a determinant of weight gain in humans.

Initial body weight was a strong positive determinant of final body weight in the present study, regardless of the diet fed. This is in agreement with findings from human studies [11], and indicates that heavier cats at the beginning of the study were also heavier at the end. Adjusted regression coefficients for the association between initial and final body weight were near 1.00 (1.11 and 0.86 for the low- and high-carbohydrate diets, respectively), indicating that, within both diets, the absolute amount of weight gained was, on average, approximately similar in initially lighter and initially heavier cats. Initial body weight was fitted in all statistical models, as explained in the materials and methods section.

The finding that the higher the mean 24-h insulin concentration, the lower the final weight in cats fed the low-carbohydrate, high-protein diet might be associated with the appetite suppressant effect of insulin in the central nervous system [35; 36]. Insulin signaling in the brain causes a catabolic response that counteracts its anabolic effects in peripheral tissues, and involves regulation of genes that control feeding behaviour, which then subsequently reduce food intake [36]. The association between mean 24-h insulin and final body weight was much weaker and non-significant after adjustment for energy requirements to gain weight (results not shown), possibly because this latter variable indirectly accounted for some of the effects of insulin on energy intake. With the low-carbohydrate, high-protein diet, cats requiring more energy for each kg of weight gain ate more.

In the group fed the high-carbohydrate, low-protein diet, cats with lower fasting leptin concentration gained more weight. Leptin concentration increases in proportion to fat mass in different species, including cats [18; 20; 25], and is associated with decreased food intake and

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increased energy expenditure [37; 38]. Therefore, the lower the leptin concentration, the more the cats are likely to eat and gain weight, in agreement with reports from human studies [10]. Furthermore, initial fat mass (expressed as a percentage of body weight) was negatively associated with final body weight in cats of this group. That is, cats with higher fat mass at the start gained less weight. We therefore hypothesised that these cats with higher initial total body fat percentages did not eat as much as the cats with lower total body fat percentage during *ad libitum* feeding because cats with higher fat mass had higher leptin concentration. That leptin was involved in reducing food intake and weight gain in these cats with greater initial fat mass is supported by the observation that body fat mass expressed as percentage of body weight was not a significant determinant of final body weight in cats fed the high-carbohydrate, low-protein diet when fasting plasma leptin concentration was fitted in the model.

Consistent with the influence of initial body weight, lean mass had a positive association with final body weight in cats fed the high-carbohydrate, low-protein diet. This association is likely to be because the largest cats when lean (at the end of the stable-weight phase) were also those cats with more muscle mass. These large cats maintained their relatively larger muscle mass during the weight-gain phase compared with the smaller cats, although the increase in lean mass occurred in a smaller proportion relative to the increase in fat mass after weight gain [15].

The second part of the study was to determine predictive equations that could be used to quantify the amount of weight cats would gain after 8 wk of *ad libitum* feeding. These may be important preventative tools that veterinarians can use to identify those cats likely to gain most weight if fed *ad libitum* for a short period of time, and to advise owners accordingly. *Ad libitum* feeding is the most common feeding method employed by owners of pet cats [39], and studies have shown that it induces weight gain [15]. For the low-carbohydrate, high-protein diet group, the equation using just initial body weight and fasting glucose concentration would be more practical to

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use. However, fasting glucose concentration should be measured at home or after overnight hospitalisation, to minimise stress hyperglycaemia associated with travel to the veterinary clinic. This equation had similar predictive precision (mean predictive error of 0.32 kg) to the equation involving initial body weight, fasting glucose concentration and mean 24-h insulin concentration over 24 h (predictive error 0.30 kg). This latter equation would most likely only be feasible for use in research studies, since the measurement of plasma insulin concentration at thirteen time points over 24 h is time consuming and expensive, however predictions from both equations were imprecise.

In cats fed a high-carbohydrate, low-protein diet, the most precise equation included maintenance energy requirements, initial body fat mass and fasting leptin concentration. The equation involving these measures had reasonable precision (mean predictive error of 0.18 kg). However its use would most likely be limited to a research setting due to practical requirements to perform these measurements. In this group, there was little advantage in using the equation that involved the most accessible measures, maintenance energy requirements and initial body weight, over using the equation including only initial body weight, because their predictive precisions were similar (mean predictive error 0.25 kg and 0.27 kg, respectively). For both diets, precision of predicted final body weights by including metabolic determinants was only modestly improved over that achieved when just initial body weight was used.

We used two groups of cats that were fed diets of different composition. We initially considered pooling all cats across both diets and including diet as a covariate. However, it became evident that relationships differed markedly between diets necessitating fitting a large number of interaction terms, so we instead opted for simpler models. Therefore, other diets will need to be investigated because there were multiple differences between diets. Determinants and predictors of final body weight may differ between short-term periods of *ad libitum* feeding (8 wk in the present

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study) and excessive feeding over longer periods. However, in planning this study, it was considered unacceptable from a welfare perspective, to allow cats to eat to the point of obesity, because of the known increases in disease incidence in obese cats. Therefore, the study was designed to assess more moderate weight increases, to body condition scores common in the general pet cat population [3; 4; 8].

In conclusion, metabolic determinants and predictors of final body weight, and hence weight gain, after 8 wk of *ad libitum* feeding in cats differed according to the diet fed. In cats fed a low-carbohydrate, high-protein diet, typical of premium quality foods, including some indicated for weight management programs, cats with higher energy requirements for each kg of weight gain and higher fasting plasma glucose concentrations, had higher final weights. Although a predictive equation using fasting glucose concentration and initial body weight could be practical to inform clients of their cat's propensity to gain weight, predictions from this equation were diet dependant and imprecise; precision was improved only marginally by including mean 24-h insulin concentration in the equation. In cats fed a high-carbohydrate, low-protein diet, typical of the low-priced dry cat foods available in supermarkets in Australia, those with initially lower leptin concentration gained more weight. In these cats, energy requirements for maintenance, initial body fat percentage and fasting leptin concentration, in conjunction with initial body weight, predicted final weight with reasonable precision during *ad libitum* feeding. However the use of this equation would most likely be limited to a research setting because of difficulties in measuring these variables in cats in a veterinary practice setting. The results in this manuscript are applicable to young adult, neutered, lean, mixed breed and clinically healthy cats and so further research is required to validate our predictive equations in different populations of cats. These studies should include other diets and larger groups of cats that are fed *ad libitum*, ideally for a longer period of time, although obesity would likely occur in some cats. Other equations should also be

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investigated, using the same covariates that we used, with newly generated coefficients, and also different sets of covariates.

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Table 1. Macronutrient distributions and energy densities of the baseline, low-carbohydrate, high-protein and high-carbohydrate, low-protein diets. Composition values are expressed as percentage contribution to total metabolisable energy.

	Diet		
Approximate energy (ME)	Baseline[a]	Low-carbohydrate[b]	High-carbohydrate[c]
Energy density kJ/100g[d]	1518.0	1552.0	1550.0
Energy density kJ/100g[e]	1427.0	1434.0	1478.0
Protein (%)[e]	29.4	47.0	21.3
Fat (%)[e]	27.4	29.8	28.2
Carbohydrate (%)[e]	43.2	23.3	50.5

ME, metabolisable energy.

[a] Whiskas Adult with Vita-Bites, Mars Petcare, Raglan NSW Australia.

[b] Royal Canin Diabetic Feline, Royal Canin, Aimargues, France.

[c] Kitekat Krunch, Mars Petcare, Raglan, NSW, Australia.

[d] Metabolisable energy calculated using the equation proposed by the NRC, 2006 [40].

[e] Metabolisable energy calculated using the modified Atwater factors, NRC, 1985 [41].

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Table 2. Univariable and bivariable associations between final body weight at the end of the weight-gain phase, after 8 wk of *ad libitum* feeding either a low-carbohydrate, high-protein, or a high-carbohydrate, low-protein diet, and initial body weight, energy requirements for maintenance and above maintenance for each kg of body weight gain, initial fat and lean masses, and initial blood glucose, insulin, leptin and adiponectin concentrations.

Variables[a]	Low-carbohydrate, high-protein diet (*n* 15)				High-carbohydrate, low-protein diet (*n* 16)			
	Mean ± SD	Partial R^2 [b] (%)	Regression coefficient[c] (95% CI)	P-value[c]	Mean ± SD	Partial R^2 [b] (%)	Regression coefficient[c] (95% CI)	P-value[d]
Initial body weight	3.44 ± 0.73		1.10 (0.81 to 1.40)	<0.01	3.18 ± 0.76		0.89 (0.69 to 1.09)	<0.01
Energy requirements for maintenance (kJ/kg/d)[e]	368.2 ± 55.4	2.7	0.1[f] (-0.4 to 0.6)	0.57	438.2 ± 62.3	23.9	0.2[f] (-0.0 to 0.5)	0.06
Energy requirements per kg of body weight gain[g]	24353.9 ± 5280.9	49.7	0.5[h] (0.2 to 0.8)	<0.01	56392.8 ± 20724.4	5.6	-0.03[h] (-0.10 to 0.05)	0.46
Initial insulin sensitivity index [(mU/l)^-1.min^-1]	2.42 ± 1.35	6.3	0.07 (-0.10 to 0.25)	0.39	3.04 ± 1.38	7.5	0.05 (-0.06 to 0.17)	0.32
Initial total fat mass (%[i])	17.8 ± 5.4	12.0	-0.02 (-0.07, 0.02)	0.21	19.5 ± 5.1	47.3	-0.04 (-0.06 to -0.01)	<0.01
Initial abdominal fat mass (%[i])	3.9 ± 1.6	10.9	-0.08 (-0.22 to 0.06)	0.25	4.1 ± 1.3	40.3	-0.14 (-0.23 to -0.04)	0.01
Initial lean mass (%[i])	78.9 ± 5.1	13.8	0.03 (-0.02 to 0.07)	0.19	77.3 ± 4.9	47.8	0.04 (0.01 to 0.06)	<0.01
Initial fasting glucose (mmol/L)	5.0 ± 0.3	31.3	0.67 (0.05 to 1.30)	0.04	5.2 ± 0.7	0.0	-0.002 (-0.254 to 0.250)	0.99
Initial mean 24-h glucose (mmol/L)	5.4 ± 0.3	3.5	0.30 (-0.68 to 1.27)	0.52	6.4 ± 1.0	10.4	-0.08 (-0.23 to 0.06)	0.24
Initial peak glucose (mmol/L)	6.2 ± 0.7	3.3	0.09 (-0.26 to 0.45)	0.57	7.6 ± 1.6	6.4	-0.04 (-0.14 to 0.06)	0.36
Initial fasting insulin (pmol/L)	35.3 ± 14.2	8.2	-0.007 (-0.023 to 0.008)	0.32	45.9 ± 35.2	1.3	-0.001 (-0.006 to 0.004)	0.68
Initial mean 24-h insulin (pmol/L)	70.9 ± 21.2	29.1	-0.01 (-0.02 to -0.00)	0.05	106.0 ± 56.0	16.4	-0.002 (-0.005 to 0.001)	0.13
Initial peak insulin (pmol/L)	105.2 ± 42.5	20.4	-0.004 (-0.010 to 0.001)	0.12	190.6 ± 87.7	0.9	-0.0003 (-0.0022 to 0.0016)	0.74
Initial fasting leptin (ng/mL)	2.64 ± 0.75	3.7	-0.09 (-0.40 to 0.21)	0.51	2.60 ± 0.54	47.3	-0.34 (-0.55 to -0.12)	0.01
Initial mean 24-h leptin (ng/mL)	2.66 ± 0.73	3.8	-0.1 (-0.41 to 0.21)	0.50	2.71 ± 0.75	48.8	-0.25 (-0.41 to 0.21)	<0.01
Initial peak leptin (ng/mL)	3.09 ± 0.49	0.6	0.07 (-0.82 to 0.96)	0.85	3.36 ± 1.09	45.1	-0.19 (-0.34 to -0.04)	0.02
Initial fasting adiponectin (μg/mL)	4.88 ± 3.53	20.0	0.06 (-0.01 to 0.13)	0.11	6.73 ± 3.82	2.8	-0.01 (-0.06 to 0.03)	0.55
Initial mean 24-h adiponectin (μg/mL)	4.23 ± 3.02	13.5	0.05 (-0.03 to 0.14)	0.20	5.78 ± 3.05	1.7	-0.01 (-0.07 to 0.04)	0.65

[a] Initial values were obtained during the stable-weight phase test week (week 8 of the study), immediately before the commencement of the weight-gain phase.

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[b] Proportion of variability (sums of squares) in final body weight not accounted for by initial body weight that was explained by the variable. Partial correlation coefficients can be calculated from the square root of the partial R^2.

[c] Change in final body weight (kg) per unit increase in exposure variable. Initial body weight (that is, body weight immediately before the commencement of the weight-gain phase) was fitted in all models.

[d] P-value for the regression coefficient.

[e] Calculated from values obtained in the third week of the stable-weight phase. All cats fed the low-carbohydrate, high-protein (*n* 16), and high-carbohydrate, low-protein (*n* 16) diets were included.

[f] Change in final body weight (kg) per 100 units increase in maintenance energy requirements (described as (kJ/kg/d) x (body weight$^{(0.4)}$)).

[g] Calculated as: (total energy intake during 8 wk of *ad libitum* feeding – sum of estimated daily energy requirements for maintenance during 8 wk *ad libitum* feeding)/body weight gained. Excluding cats (*n* 3) fed the high-carbohydrate, low-protein diet for having gained less than 10% body weight by the end of the weight-gain phase.

[h] Change in final body weight (kg) per 10,000 kJ/kg increase in energy requirement for each kg of body weight gain.

[i] Mass expressed as percentage of body weight.

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Table 3. Equations for predicting final body weight (kg) after cats had been fed a low-carbohydrate, high-protein or a high-carbohydrate, low-protein diet *ad libitum* for 8 wk.

Equation to predict final body weight (kg)	Partial R^2 (%)[a]	Root mean square error for the model[b]
Low-carbohydrate, high-protein diet		
Initial body weight (kg) x 1.10 + 0.86		0.37
(Initial body weight (kg) x 1.20) + (initial fasting glucose concentration (mmol/L) x 0.52) – (initial mean 24-h insulin concentration (pmol/L) x 0.007) - 1.56	37.3	0.30
(Initial body weight (kg) x 1.14) + (initial fasting glucose concentration (mmol/L) x 0.67) –2.62	31.3	0.32
High-carbohydrate, low-protein diet		
(Initial body weight (kg) x 0.89) + 0.84		0.27
(Initial body weight (kg) x 0.80) + (energy requirements for maintenance x 0.13)[c] – (initial body fat percentage[d] x 0.02) – (initial fasting leptin concentration (ng/mL) x 0.21) + 1.42	39.2	0.18
(Initial body weight (kg) x 0.83) + (energy requirements for maintenance x 0.22)[c] + 0.07	23.9	0.25

[a] Proportion of variability (sums of squares) in final body weight not accounted for by initial body weight that was explained collectively by other variables in equation.

[b] The square root of the mean residual sums of squares. This describes the approximate average of the differences between predicted and actual final weight for the study cats.

[c] Daily maintenance energy requirements expressed in units of 100 kJ/(kg body weight)$^{0.4}$, calculated in the third week of the stable-weight phase, when cats were fed their respective test diets maintaining their lean body weight.

[d] Body fat mass expressed as a percentage of body weight

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Figure captions

Fig. 1. Association between metabolisable energy requirements above maintenance for each kg of body weight gain and amount eaten in the second week of *ad libitum* feeding in cats fed the low-carbohydrate, high-protein diet. The second week of *ad libitum* feeding was chosen for this evaluation because, in this week, the cats would have adapted to eating *ad libitum* but those with higher energy intakes would not have gained much weight. Later these cats would have been heavier, and possibly eating more to meet their increased maintenance requirements. Evaluation in week 2 allowed assessment of the relationship between energy required above maintenance for each kg of body weight gain and daily energy intake relatively unconfounded by differences in maintenance requirements.

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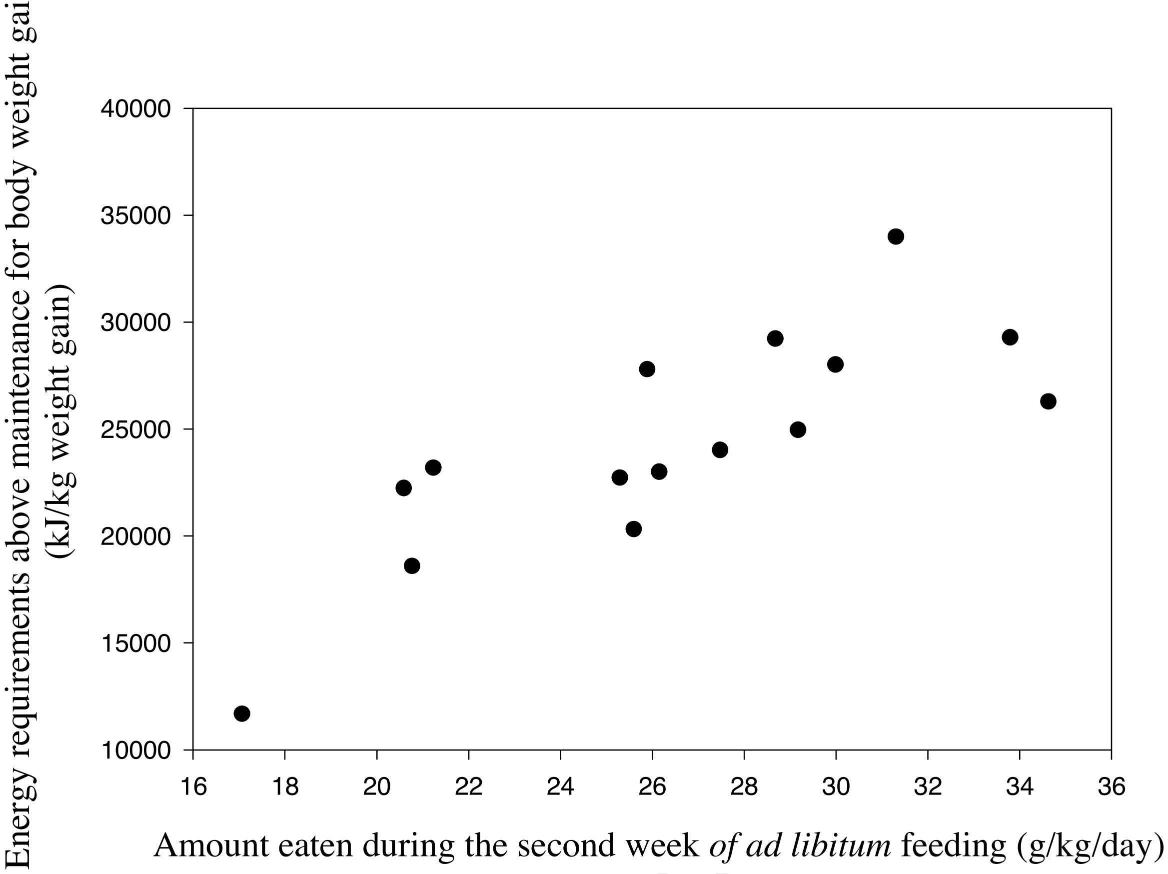

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