High Protein Intake Affects Lean Body Mass but Not Energy Expenditure in Nonobese Neutered Cats¹,²

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EXPANDED ABSTRACT

Dietary composition affects body composition; the role of fat and carbohydrates is well documented (1,2). Proteins are more satiating than carbohydrates, may produce different effects on glycemia (2), and are more thermogenic (1). Indeed the efficiency of cellular conversion of energy to high-energy yielding phosphate bonds from fatty acids is 90%, from carbohydrates 75%, but only 55% for amino acids (3).

High-protein diets produced greater weight loss in some studies of obese humans (4,5) but not others (6). Other human studies found that although the high-protein diet did not promote weight loss in excess of control diets, it did preserve lean tissue mass (7) and promoted the loss of fat tissue (8).

Companion animals also suffer from obesity (9), and a high-protein, energy-restricted diet was successful in reducing the body weight and body fat of overweight dogs (10) and cats (11), and in conserving lean body mass of dogs (12). However, there have been few studies of the effects of protein intake on the body composition in normal-weight individuals of any species.

The current study aimed to determine the effect of a limited energy intake of a high-protein diet on the body composition and least observed metabolism (LOM)⁵ of adult neutered cats.

MATERIALS AND METHODS

Sixteen normal-weight cats (11 female and 5 male, aged 4.5 ± 0.1 y, all Domestic Shorthair, neutered) participated in the study. For the 6 mo before the study all cats had free access to a commercially available extruded food (340 g/kg crude protein and 14.64 MJ ME/kg as fed).

The study was conducted as a crossover, with two consecutive 6-mo test periods. Mean initial body weight (BW) was 4.83 ± 0.13 kg, and body condition score was 3.4 ± 0.1. Cats were allocated to one of two groups, and were fed one of two test diets for 6 mo, before being transferred (without washout period) to the second diet for 6 mo. The extruded diets were either high protein (HP) (containing 528 g/kg crude protein) or moderate protein (MP) (containing 297 g/kg crude protein) (Table 1).

Cats were group housed, but fed individually. Each cat was offered throughout the study a mean energy allowance of 209 kJ ME/kg BW/d (according to BW at the beginning of each period). This energy allowance is considered sufficient to maintain optimal weight under relatively inactive conditions (13). The actual value for each cat was calculated at the end of each study period according to the mean BW during that period. All cats had access to fresh drinking water at all times.

All experimental protocols were approved by the Animal Use and Care Advisory Committee of the Nantes Veterinary School, and adhered to European Union guidelines.

Body composition and energy expenditure were measured before, and at the end of, each 6-mo period.

Body composition

Body composition was determined from isotopic dilution of 2H₂O at the beginning and end of each period. On the day of the study, 12-h food-deprived cats were placed in individual cages for water restriction and body water equilibration from 2 h before to 3 h after tracer injection. Cats were injected subcutaneously (0.50 g/kg BW) with physiological saline 2H₂O (99.9% ²H/H, Euriso-top, Gif-sur-Yvette, France). Venous blood samples were obtained before, and 2.5 h subsequent to, injection of the isotope, and plasma was stored at −20°C in sealed vials. Plasma deuterium was determined by Fourier-transform infrared spectroscopy as described previously (14). Total body water (TBW) was determined from the dilution space of the isotope. Fat-free mass (FFM) was calculated as TBW/hydration rate of FFM, using a feline-specific hydration rate of 0.769 (15).

Table 1

Energy expenditure was calculated using the abbreviated formula:

\[ \text{calculated} = \text{minute-by-minute after a 20-min equilibration period.} \]

In all cases, Berkeley, CA). In all cases were expressed in L/d (17).

Statistics

Energy expenditure

Least-observed metabolism (LOM) (16) was determined by indirect calorimetry. Cats were food-deprived overnight, then confined to a metabolism chamber connected to a breathe gas-exchange monitor (GEM) (Deltatrac II metabolic monitor, Datex, Helsinki, Finland) for 14 h. The GEM was calibrated once hourly, using standard gas mixtures. The open-circuit system was ventilated by atmospheric air, pumped through the metabolism chamber at a flow rate of 4 L/min. The rate of \( \text{CO}_2 \) production (rCO\(_2\)) and \( \text{O}_2 \) consumption (rO\(_2\)) were calculated minute-by-minute after a 20-min equilibration period. Energy expenditure was calculated using the abbreviated formula:

\[ EE = (1.11 \times r\text{CO}_2 + 3.94 \times r\text{O}_2) \times 4.184, \]

where \( r\text{CO}_2 \) and \( r\text{O}_2 \) were expressed in L/d (17).

Because LOM is, in principle, the lowest amount of energy expended by the unfed individual at rest, the lowest value for each rolling 30-min period was used. This was calculated from the 15 min before and the 15 min after each reading. Reported LOM is the lowest observed 30-min value.

Statistics

The data are presented as mean ± SE. The effect of diet was investigated using repeated measure ANOVA and the sequence effect was tested as a between factor (SuperANOVA V1.11, Abacus Concepts, Berkeley, CA). In all cases \( P < 0.05 \) was considered significant.

RESULTS

Energy intake

Both diet groups were offered, and consumed similar amounts of food; energy intake of cats when fed the MP diet (204 ± 5 kJ/kg/d) was not significantly different from when fed the HP diet (207 ± 5 kJ/kg/d).

Body composition

Most cats lost BW during the study, with the exception of six animals when fed the HP diet and one when fed the MP diet. Cats fed the MP diet lost \(-8.7 ± 1.7\% \) of BW (\( P < 0.001 \)) (amounting to \(-0.42 ± 0.09 \) kg), whereas cats fed the HP diet lost \(-2.8 ± 1.8\% \) (\( P > 0.05 \)) (amounting to \(-0.15 ± 0.08 \) kg). There was no effect of sequence for either group.

Adipsity decreased over each 6-mo feeding period, but there was no significant effect of diet. Relative change in body fat content over the 6-mo feeding periods was \(-5.4 ± 1.6\% \) and \(-4.5 ± 1.6\% \) for MP and HP diets, respectively.

The effect of diet on changes in FFM was significant. FFM increased by \(4.2 ± 1.7\% \) amounting to \(0.114 ± 0.048 \) kg (\( P = 0.016 \)) when cats were fed the HP diet whereas FFM did not change when fed the MP diet \((-1.2 ± 1.0\% \) amounting to \(0.039 ± 0.029 \) kg), and there was neither effect of sequence nor diet interaction.

Energy expenditure

There was no significant difference (\( P > 0.05 \)) in LOM of cats at the start of each 6-mo period, either when corrected for BW (163 ± 5 kJ/kg) or FFM (255 ± 5 kJ/kg). LOM measured at the end of the study was not significantly different (\( P > 0.05 \)) from the starting values, for either diet, either when corrected for BW (165 ± 3 kJ/kg) or FFM (240 ± 4 kJ/kg).

There was no significant effect (\( P > 0.05 \)) of diet on the change in LOM, either when corrected for BW (\(-0.6 ± 3.4 \) and \(1.9 ± 2.7\% \), for MP and HP diets, respectively) or FFM (\(-6.7 ± 3.2 \) and \(-4.0 ± 2.5\% \), for MP and HP diets, respectively).

DISCUSSION

These results confirm that, as in other species, dietary protein content influences the body composition of neutered cats. When fed the MP diet, cats lost body weight with no change in FFM, whereas, when fed the HP diet, cats gained FFM without change in body weight.

When fed the MP diet the BW loss appeared to be comprised mainly of fat tissue because there was no change in absolute FFM. This is in contrast to the HP diet, when cats gained FFM without any change in BW. However, there was a similar loss of fat tissue on each diet; the simultaneous gain in FFM in HP-fed cats would be reflected in the absence of net BW variation. These findings suggest that a high-protein diet is useful for improving the body composition of neutered cats and preventing and/or treating obesity.

A reduction of energy intake results in BW loss that is usually accompanied by a lean tissue loss. The moderate-protein level in the MP diet supported, however, FFM despite the reduction in energy intake. The finding that when cats were fed the HP diet they gained FFM without BW variation is interesting. Gain of lean tissue in adult animals is unusual in itself, in the absence of prior malnutrition or bodybuilding, although there have been few studies conducted in healthy normal-weight adults.

The measure of FFM in the current study includes all nonfat tissue. The observed increase in FFM could therefore be attributed to several sources, all of which may potentially respond to protein intake, at least in the short term. Skeletal muscle probably does not respond to increase in protein intake, because there was no change in muscle mass in human weight trainers fed a high-protein diet for 1 mo (18). The protein content and size of the splanchnic organs may be expected to reflect protein intake due to variation in the functional demand. However, although changes in hepatic size are often significant in hepatic size are reported in immature animals of several species, including pigs (19) and kittens (20), there is a lack of equivalent studies in adults. In adult rats, changes in hepatic size may be minimal (21), although unquantified changes in kidney size are reported (22).

A trend toward an increase in nitrogen balance when nitrogen intake is increased is reported (23) although most of the protein intake in excess of requirement is excreted. This tends to support the findings above. However, when nitrogen balance studies are conducted there is an apparent retention of...
nitrogen that progressively increases as the protein content of the diet is increased (24), a phenomenon that is also apparent in the cat (25). Whether this apparent retention is real, or an artifact of the measurement technique, remains the subject of debate (26). It has been suggested that it may reflect changes to a "labile protein store" (27), which may primarily reside in the splanchic tissues (28). It would be of interest to revisit this issue, and assess nitrogen balance and body composition at high protein intakes, using a sensitive isotopic technique for measuring lean body mass.

The increase in FFM of cats fed the HP diet may be the result of the increased availability of amino acids for protein synthesis. Cat has a high protein requirement (13), but the MP diet contained approximately twice, and the HP diet four times this amount of protein. The extra protein intake from the HP diet appears to have been utilized, supporting an increased lean tissue mass. Whole-body protein turnover is known to be related to intake in this species (29), as in others, and the high turnover measured at high protein intakes will reflect any increases in lean tissue mass as well as an increased rate (per unit tissue).

The FFM increase in the current study should also be considered in relation to physiological relevance and the methodological error margin. The methodology is validated and is accurate in dogs (14). Nevertheless the FFM change is determined by the difference of two measurements of a large variable (TBW), which is further converted to FFM, and conclusions must therefore be stated with caution. The timing of the increase is also critical. If the increased FFM occurred very quickly after changing to the HP diet, then remained stable, this could reflect a short-term adaptation to diet. However, if the increase in FFM was gradual and progressive over the 6-mo feeding period, this has implications for long-term effects of the HP diet. Increases in FFM cannot continue indefinitely.

There was no effect of protein intake on LOM in the current study. LOM is close to basal metabolism but is likely to be a little less because LOM occurs during sleep whereas basal metabolism must be measured shortly after waking (16). This lack of effect is supported by studies in a variety of obese human subjects (6,30). It is possible that a small increase in thermogenesis as a result of increased protein intake would be masked by the inherent variability in energy expenditure. As we measured energy expenditure in unfasted cats, we can also hypothesize that metabolic rate rapidly switches back to the basal value independent of the diet commonly fed.

Whatever the cause, our results show that the least-observed metabolism was strongly related to the FFM and amounted to ~250 kJ (60 kcal)/kg FFM with a very low variability.

In summary, a high-protein diet may promote an increased lean tissue mass in cats that may be helpful in preventing or treating obesity. The fact that the extra protein is useful to the cat and not simply excreted is interesting and warrants further study.

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LITERATURE CITED