Dietary Protein Level Affects Protein Metabolism during the Postabsorptive State in Dogs\textsuperscript{1,2}

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EXPANDED ABSTRACT

KEY WORDS: • dietary protein level • protein metabolism • \textsuperscript{13}C-leucine method • dogs

The effects of dietary protein level on whole-body protein metabolism have usually been assessed in dogs by nitrogen balance studies based on the difference between nitrogen intake and excretion from the body (1,2). Although this method has been widely accepted for evaluation of nutritional status and determination of dietary protein requirement (3), it fails to distinguish between the effects of individual changes in protein synthesis, oxidation and breakdown and the overall response of the body to altered nutritional intake. \textsuperscript{13}C-Leucine infusion is now regarded as the reference method (4) for determination of whole-body protein metabolism. Theoretically, the oxidation of any essential amino acid in steady-state conditions is accompanied by the catabolism of all amino acids in the proteins of origin and should thus provide information equivalent to that of total nitrogen excretion. In a previous study using \textsuperscript{13}C-leucine infusion, our group estimated that the equivalent to that of total nitrogen excretion. In a previous study using \textsuperscript{13}C-leucine infusion, our group estimated that the minimal nitrogen requirement in dogs is between 0.41 and 0.55 g N/kg metabolic body weight (BW\textsuperscript{0.75})\textsuperscript{4} per d (5).

The kinetic parameters of protein metabolism in adult dogs can be modulated notably in the short term by food (energy) intake and in the long term by variations in whole-body protein (6), which is also the case for humans (7). However, very little is known about the effects of graded dietary protein level on whole-body protein kinetics in normally fed dogs. It is likely that modifications of whole-body protein kinetics occur once dogs adapt to graded dietary protein levels and that these changes are partly attributed to enhancement of the oxidative pathway by excess protein. In humans in the fasting state, graded dietary protein level has no effect on protein synthesis, but increases protein breakdown (8). In dogs, the level of dietary protein intake is higher than that in humans, and very little is known about the effects of high protein level on whole-body protein kinetics during the postabsorptive phase. In the present study, the \textsuperscript{13}C-leucine method was used to estimate the effects of graded dietary protein levels on protein synthesis, breakdown and oxidation in dogs during the post-absorptive phase.

MATERIALS AND METHODS

Animals

The eight adult beagle dogs used in the study were housed according to the regulations for animal welfare of the French Ministry of Agriculture and Fisheries. The experimental protocols adhered to European Union guidelines and were approved by the Animal Use and Care Advisory Committee of the University of Nantes. Only healthy animals were enrolled: hematocrit > 38%, leukocyte count < 18,000/mm\textsuperscript{3}, good appetite, no medications, normal stools and body temperature (38.5–39.5°C).

Protocol design

The eight dogs [body weight (BW) = 14.2 ± 0.8 kg; mean ± se] were studied after a 2-wk adaptation period to each of the following diets: 1) low protein (P1); 430 kcal ME/100 g; 32 g CP/Mcal ME (g of crude protein per Mcal ME); 2) medium protein (P2); 380 kcal ME/100 g; 63 g CP/Mcal ME); 3) generous protein (P3; 375 kcal ME/100 g; 100 g CP/Mcal ME); and 4) very high protein (P4; 380 kcal ME/100 g; 148 g CP/Mcal ME) (only seven of the dogs received this last diet). Commer-
TABLE 1

Leucine fluxes in adult beagle dogs fed either a low-protein (P1, n = 8), a medium-protein (P2, n = 8), a generous-protein (P3, n = 8) or a very high protein diet (P4, n = 7)!

<table>
<thead>
<tr>
<th></th>
<th>RaLeu</th>
<th>OxLeu</th>
<th>NOLD</th>
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<tbody>
<tr>
<td>P1</td>
<td>263 ± 14#</td>
<td>42 ± 4</td>
<td>221 ± 11#</td>
</tr>
<tr>
<td>P2</td>
<td>298 ± 17</td>
<td>51 ± 4</td>
<td>248 ± 13</td>
</tr>
<tr>
<td>P3</td>
<td>250 ± 10##</td>
<td>54 ± 5</td>
<td>195 ± 7###</td>
</tr>
<tr>
<td>P4</td>
<td>191 ± 15##</td>
<td>43 ± 6</td>
<td>148 ± 11###</td>
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</table>

1 Plasma leucine appearance rate (RaLeu), leucine oxidation (OxLeu) and nonoxidative leucine disposal (NOLD) are expressed as mean ± SE in μmol/(kg h). P-values with ANOVA were <0.05 for RaLeu and NOLD and were not significant for OxLeu.

*, **, ***: significant difference vs. P2, P < 0.05, P < 0.01 and P < 0.001, respectively.

#:##, significant difference vs. P4, P < 0.05 and P < 0.01, respectively.

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The body weights of the dogs were not significantly affected by the diets.

Leucine flux values are shown in Table 1. Protein breakdown, synthesis and oxidation were calculated from leucine fluxes. P1 provided a slight (not statistically significant) decrease in protein breakdown (−11%) and synthesis (−10%) as compared to P2 (Fig. 1). P3 decreased protein breakdown (−15%) and synthesis (−20%) significantly as compared to P2 (Fig. 1). P4 inhibited protein breakdown (−32%) and synthesis (−37%) significantly as compared to P2. Compared to P3, P4 induced a 20% decrease in protein breakdown (P < 0.05) and a 21% drop in protein synthesis (P < 0.05) (Fig. 1). Whole-body postabsorptive protein oxidation remained unaltered regardless of dietary protein level. However, a rising trend in protein oxidation level was observed when dietary protein increased from P1 to P3 (Fig. 1).

DISCUSSION

Continuous infusion of stable isotopes was used in this study to investigate the effects of graded dietary protein levels on whole-body protein kinetics in healthy adult dogs. Moderate protein restriction did not significantly affect protein turnover, but an increase in dietary protein above a high threshold decreased whole-body protein turnover. Protein breakdown and synthesis were reduced, without significant change in protein oxidation.

The 13C-leucine method was used to assess leucine kinetics in dogs (Table 1), and leucine flux values were extrapolated to protein fluxes on the basis of commonly accepted assumptions (11). For dogs fed a medium-protein diet (63 g CP/Mcal ME), leucine oxidation, plasma appearance rate and nonoxidative disposal were 51 ± 4, 298 ± 17 and 248 ± 13 μmol/(kg h), respectively. These values are consistent with those previously reported elsewhere (5). Briefl y protein intake was 0.75 kg (−20%) and 0.85 kg (−37%) as compared to P2. Compared to P3, P4 decreased protein breakdown (−15%) and synthesis (−20%) significantly as compared to P2 (Fig. 1).

13C-leucine infusion protocol

The protocols used for each infusion were previously described elsewhere (5). Briefly, 13C-leucine infusion, performed to assess leucine appearance rate (RaLeu) and leucine oxidation (OxLeu), was immediately followed by infusion of 13C-bicarbonate to assess total CO2 production. RaLeu was calculated by measuring plasma 13C-enrichment in α-ketoisocaproic acid (KIC) by GCMS (9–11). Total CO2 production and OxLeu were calculated by measuring 13CO2 enrichment in breath by GC-IRMS. Nonoxidative leucine disposal (NOLD) was calculated from OxLeu and RaLeu. Leucine fluxes, RaLeu, OxLeu and NOLD were extrapolated nitrogen fluxes, expressed in g N/(kg BW0.75 d−1). Assuming that 1 g of protein contains 590 μmol of leucine and that 6.25 g of protein contain 1 g of nitrogen.

Statistical analysis

Data (presented as means ± SE) were compared between treatments using repeated analysis of variance (ANOVA) measurements and an unpaired Student’s t-test with Bonferroni correction. Significance was established at P < 0.05.

RESULTS

A steady state was observed over the last 60 min of each isotope infusion in 13C-enrichments of plasma KIC and breath CO2.

### FIGURE 1

Effects of low-protein (P1), medium-protein (P2), generous-protein (P3) and very high protein (P4) diets on protein breakdown (B), oxidation (Ox) and synthesis (S) [all values expressed in g N/(kg BW0.75 d−1)] in adult beagles. Each column represents the mean ± SE of the values for 8 dogs for P1, P2 and P3, and for 7 dogs for P4. P-values with ANOVA were <0.05 for RaLeu and NOLD and not significant for OxLeu. *, **, ***: significant difference vs. P2, P < 0.05, P < 0.01 and P < 0.001, respectively. #, ##: significant difference vs. P4, P < 0.05 and P < 0.01, respectively.

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5 Diet P1 contained: wheat, corn, animal fat, fish meal, milk protein extract, potato protein extract, soybean protein extract, sucrose, minerals, vitamins; Diet P2 contained: grains, meat, meat by-products, vegetable by-products, animal fat, vegetable oils, fish and fish by-products, minerals, dried yeast, vitamins; Diet P3 contained: chicken and turkey meals and by-products, corn meal, corn fiber, poultry fat, poultry liver, Scandinavian fish meal, brewer’s yeast, beet pulp, soybean oil, egg powder, L-methionine, minerals, vitamins; Diet P4 contained: poultry by-product meal, corn gluten meal, cellulose, rice gluten meal, beet pulp, barley, poultry oil, poultry liver digest, psyllium, brewer’s yeast, fructooligosaccharides, L-carnitine, minerals, vitamins.

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as assessed by the 13C-leucine method. In the puppy, the activities of enzymes involved in transamination and oxidation of amino acids were correlated with dietary protein level (3). In the current study, no effects of dietary protein level on protein oxidation were observed.

Pacy et al. (8) found that a higher dietary protein level decreased protein breakdown and increased protein synthesis in the fed state in humans. Our study showed that a low-protein diet, as in human subjects (8), did not significantly affect protein breakdown or synthesis in the food-deprived dog, whereas an increase in dietary protein level dramatically reduced protein breakdown and synthesis. However, protein breakdown was enhanced and protein synthesis remained unaltered when food-deprived humans were adapted to an increasing dietary protein level (8). This discrepancy could be the result, in part, of the difference in the range of dietary protein level tested in our study and that of Pacy et al. The higher protein level in the human study (44 g CP/Mcal ME) (8) was in between the low- and medium-protein diets tested in our study. In these conditions, the generous and very high protein-rich diets may have been depleted in other nutrients, such as carbohydrates and lipids. In fact, glucose has been shown to be an effective regulator of whole-body protein metabolism in humans (13). Thus, it is likely that the protein/carbohydrate ratio in the generous and very high protein-rich diets was not adequate to increase protein turnover in response to increased protein intake, an effect observed in humans receiving more carbohydrate-rich diets than those tested in our study.

These data also suggest that the timing of response of protein metabolism to a protein meal could differ between species. The protein breakdown or synthesis assessed by us in the postabsorptive state could reflect a remnant effect of the dogs' adaptation to the diet that was not observed in humans.

Pannemans et al. (14) also found that high protein intake (21 vs. 12% of energy) resulted in increased protein turnover, but questioned the notion that a protein turnover threshold exists in humans, beyond which a further increase in protein intake would not lead to greater protein turnover. The adaptation to dietary protein level could differ, depending on whether this level is lower or higher than the requirement. In the first case, prior diet could prime the body to conserve dietary amino acids, notably by reducing protein breakdown. In the case of surfeit protein consumption, amino acids, which are toxic in excess and cannot be reduced, protein oxidation remains unclear. Leucine oxidation has been shown to be correlated with plasma leucine concentration in humans (15) but not in dogs (16). With respect to the regulation of protein turnover, specific amino acids could play a role by modulating protein synthesis. Indeed, enteral glutamine was shown to stimulate nonoxidative leucine disposal in healthy humans (17). It is also possible that glutamine administered by the intragastric route limits amino acid oxidation and has a sparing effect on whole-body amino acids in hypercatabolic dogs in the fed state (Humbert et al. unpublished data). However, this anabolic effect of glutamine was not found in hypercatabolic food-deprived dogs when amino acid was provided intravenously (16).

In conclusion, assessment of whole-body protein metabolism 24 h after the last meal is not indicative of all the mechanisms that may occur in response to modulation of dietary protein level. However, the current study suggests that protein oxidation and turnover could be regulated by different mechanisms in dogs and that dietary protein level has a more enduring effect on protein turnover than on protein oxidation.

acknowledgment

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literature cited