Protein Turnover in Lactating Mink (Mustela vison) Is Not Affected by Dietary Protein Supply

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EXPANDED ABSTRACT

KEY WORDS: • $^{15}$N-glycine endpoint • synthesis • degradation • flux • carnivore

The mink is a strict carnivore and may therefore serve as a model for the cat. Current recommendations for protein supply for lactating mink are based on production experiments with preweaning kit growth as a measure of dietary adequacy (1,2). Recently, nitrogen balance and substrate oxidation have been used to obtain a more detailed knowledge of the protein utilization of the lactating mink (3–5). These results have shown that lactating mink dams are able to regulate protein oxidation rate and that milk yield, during the first 4 wk postpartum, was improved, and dam weight loss reduced, when protein supply was reduced below current recommendations and replaced with readily digestible carbohydrates (5,6). The effect of reduced protein supply on protein turnover is, however, still unknown. Tracer methodology with $^{15}$N-labeled amino acids has been used to measure the whole-body protein turnover in humans (7), growing pigs (8), and growing rats (9). In adult cats, both protein synthesis and breakdown were lower when feeding a low- than when feeding a high-protein diet [20 vs. 70% of metabolizable energy (ME) from protein] (10).

The objectives of this study were therefore to develop a $^{15}$N-glycine endproduct method for measurement of protein turnover in lactating mink dams and to evaluate if it was affected by different levels of dietary protein supply.

MATERIALS AND METHODS

Twelve mink dams with 7 kits each were transferred from our experimental farm to an intensive care unit ~2–3 days after parturition and placed in individual metabolism cages equipped with wood shavings-bedded nest boxes. The animals were kept under natural daylight conditions (55°N, 15°E, May). The dams were divided into 2 dietary treatment groups and fed ad libitum with diets either medium (M: 45% of ME) or low (L: 30% of ME) in protein content. The diets were based on fish and fish offal, animal byproducts, vegetable feedstuffs, vegetable oil, and animal fat. For chemical composition see Table 1. Balance experiments were performed in 3 separate 4-d periods in lactation wk 2 (d 7–11), 3 (d 14–18), and 4 (d 21–25) after parturition. Once in each balance period, protein turnover was determined by means of the $^{15}$N-glycine endproduct technique. The dams were given an oral dose (5 mg/kg body weight) of $^{15}$N-labeled glycine (99.8 atom % $^{15}$N, Campro Scientific). The $^{15}$N-glycine was added to 5 g of the diet and given to the dams at 0900 and consumed within 10 min. For measurement of the natural appearance of $^{15}$N, a urine sample was collected from each animal the day before administration of $^{15}$N. Urine was collected and weighed 6, 12, and 24 h after administration of the labeled amino acid and stored at −18°C pending analyses. Protein turnover was calculated according to a single-pool model. When the free amino acid pool is constant, the turnover rate (termed flux, Q) is given as $Q = S + E = B + I$, where S is synthesis of N, E is excretion, B is breakdown, and I is intake of N (digested nitrogen). The flux Q can be estimated as $Q = E \times 1/d$ where E is the rate of N excretion, d is the dose given, and 1 is the cumulated excretion of $^{15}$N in urine, which was calculated from the first 24-h collection after administration of the dose. This break-point time was based on results from an unpublished pilot study (A.-H. Tauson and J. Bujko, unpublished data), which suggested that label excreted later than 24 h represented recycled label. Net protein synthesis (NPS) was calculated as NPS = S – B (8), and the re-utilization rate of amino acids was calculated as $r = 100 \times S/Q$ (11).

The $^{15}$N content of urine was measured with emission spectroscopy using a NO17 (Fischer Analyser Instruments GMBH).

Statistical analyses were performed as repeated-measurements analyses by means of the procedure MIXED in SAS, version 8 (12) using a model comprising the fixed effects of diet and lactation week and their interaction effects. The autoregressive order 1 [AR(1)] covariance structure was fitted. Results are presented as least-squares means, the square root of residuals is used as the measure of variance, and differences are denoted significant if $P < 0.05$. Two observations

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5 Abbreviations used: B, breakdown; E, N excreted in urine; I, digestible N intake; L, low protein diet; M, medium protein diet; ME, metabolizable energy; NPS, net protein synthesis; Q, flux; R, re-utilization of amino acids; S, synthesis.
RESULTS AND DISCUSSION

The cumulated recovery of the label was, as expected, highest \( (P = 0.01) \) for dams on the M diet, but there was no effect of lactation week \( (\text{data not shown}) \). There was a tendency for a diet effect on flux \( (P = 0.10) \), values being higher for M than for L dams. Similarly, flux increased slightly but not significantly \( (P = 0.12) \) with progressing stage of lactation. For synthesis and breakdown, however, neither diet nor lactation week influenced the rates. Net synthesis was likewise not affected by diet, but it increased \( (P = 0.005) \) as lactation progressed. The re-utilization of amino acids was considerably more efficient \( (P = 0.005) \) among L than among M dams (Table 2). These results suggest that dietary protein had only a limited impact on protein turnover traits in lactating mink dams, contrary to findings in nonlactating adult cats \( (10) \). This discrepancy is most likely explained by the far greater difference in dietary protein supply between treatment groups in the cat study. Furthermore, kit live weight gain in this study did not differ between treatment groups \( (\text{data not shown}) \), suggesting that, unlike our previous findings \( (5,6) \), milk yield was not affected by the dietary protein:carbohydrate ratio. This suggested that a similar amount of milk protein was synthesized, and hence, it is unlikely that protein turnover would differ to any considerable extent between dietary treatments. The most evident impact of diet was on the rate of re-utilization of amino acids, which clearly showed that dams on the L diet were far more efficient in re-utilizing their limited supply of dietary amino acids. An estimate of protein output in milk was made based on data for milk intake per gram kit body gain and chemical composition of mink milk \( (13) \), and comparing those values with our results on net protein synthesis gave a fair agree-