Nutrient Requirements and Interactions

Chronic Low Protein Intake Reduces Tissue Protein Synthesis in a Pig Model of Protein Malnutrition1,2

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ABSTRACT To determine the effect of severe chronic protein deficiency on protein synthesis in different tissues and total protein in plasma, and on plasma biochemical constituents involved in amino acid metabolism, we fed diets containing either 20 or 3% protein to two groups of four age-matched piglets. After consuming the diets for 8 wk, the pigs received a primed-constant infusion of $^3$H-leucine for 8 h to measure the fractional synthesis rates (FSR) of tissue protein and total protein in plasma. Plasma urea and amino acid concentrations, particularly indispensable amino acids, were significantly lower in protein-deficient pigs. Fractional protein synthesis rates were lower in skin by 66% ($P < 0.01$), in jejunal mucosa by 50% ($P < 0.05$), in masseter muscle by 40% ($P < 0.05$), and in liver by 25% ($P < 0.02$); the fractional synthesis rate of the longissimus muscle was not different than controls. Although plasma protein concentration was significantly ($P < 0.01$) lower in protein-deficient pigs, the fractional synthesis rate of the total intravascular plasma protein pool was not different. We conclude that adaptation to a low protein diet involves a reduction in the rate of protein synthesis in most body tissues, with the most marked changes occurring in skin and intestine, two tissues which frequently exhibit severe functional impairment in protein malnutrition. J. Nutr. 126: 1481–1488, 1996.

INDEXING KEY WORDS:
• protein deficiency  • tissue protein synthesis  • stable isotope  • pigs

In severely malnourished patients, there is a marked reduction in whole-body protein turnover [Golden et al. 1977], a reduction in urea excretion and an increase in urea salvaging [Jackson et al. 1990, Picou and Phillips 1972] as the body adapts to a chronic reduction in protein intake by increasing the efficiency of nitrogen utilization to minimize loss. The extent to which protein turnover of the individual tissues contributes toward this adaptation to chronic protein deficiency in humans is not known, because it is difficult to conduct such studies. Early studies conducted in rats fed a low protein diet showed that there was either an increase or no change in the rate of synthesis of liver protein, whereas the rate of synthesis of muscle protein was decreased [Garlick et al. 1975, Waterlow and Stephen 1968]. In addition, rats fed a protein-free diet had an increased rate of synthesis of gut mucosal protein [Hirschfield and Kern 1969]. The findings of these studies suggest that the utilization of dietary amino acids by muscle is reduced during protein deficiency, thereby enabling dietary amino acids to be directed towards the maintenance of splanchnic protein synthesis. On the other hand, McNurlan and co-workers [McNurlan and Garlick 1981, McNurlan et al. 1982] have reported de-

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creased rates of protein synthesis in all major tissues in rats fed a protein-free diet, which suggests that all of the organ systems and muscles were involved in the adaptation to a chronic reduction in protein intake. To resolve these different findings, the present study was designed to determine the responses of protein synthesis rates of different tissues to chronic protein deficiency in weanling pigs.

In earlier studies, weanling pigs have been used (Edmonds and Baker 1987, Jahoor et al. 1995, Heard et al. 1958, Knowles 1957, Pond et al. 1965 and 1992) to develop a kwashiorkor-like model of malnutrition to study the deleterious biochemical and pathophysiological effects of chronic protein deficiency. However, none of the previous studies focused on the kinetic responses of protein in individual tissues. In the present study, we determined the effect of a deficient dietary intake of protein on several plasma constituents related to nitrogen metabolism and on the rates of synthesis of proteins in skeletal muscle, skin, liver and jejunal mucosa, and of total protein in plasma in weanling pigs fed a 3% protein diet for 8 wk.

**MATERIALS AND METHODS**

Experimental protocol. This study was approved by the Animal Protocol Review Committee of Baylor College of Medicine. Eight 2-wk-old crossbred piglets of either sex were obtained from the Department of Animal Science swine herd at Texas A & M University (College Station, TX). They were housed in individual pens with free access to water and a standard diet (Soweena, Merrick, Madison, WI) during a 5-d adaptation period. Pigs were then placed in two equal groups to receive either a control diet, containing 20% protein, or a 3% protein diet.

The pigs were weighed weekly when blood samples were taken by subclavian puncture for analysis of plasma biochemical constituents. After the pigs had consumed the diets for 8 wk, catheters were surgically inserted into the jugular vein and carotid artery. Three days later, a stable isotope infusion study with serial blood sampling was conducted to determine the effect of chronic protein deficiency on the synthesis of tissue and total protein in plasma. The pigs were killed with an intravenous injection of 0.33 mL/kg of Beuthanasia-D (129 mg sodium pentobarbital, 16.5 mg sodium phenoxytoin), obtained from Schering-Plough Animal Health, Kenilworth, NJ. Tissue samples were taken immediately.

Diet. Both diets (Table 1) were based on solvent-extracted soybean meal and corn meal, were low in fat, and were supplemented adequately with minerals and vitamins. A protein content of 3% was chosen for the low protein diet based on our earlier work (Jahoor et al. 1995, Lowrey et al. 1962, Pond et al. 1965 and 1992) and that of others (Edmonds and Baker 1987) showing the development of biochemical and pathophysiological changes similar to those seen in protein energy malnutrition. Dietary protein content was reduced without changing the amino acid profile by proportionate removal of soybean meal and corn meal, and replacement with cornstarch. The vitamin and mineral content of the low protein diet was increased to compensate for removal of the vitamin and mineral content of the protein sources, and to avoid deficiencies of nutrients other than protein. The control diet supplied energy as 20% protein, 67% carbohydrate and 12% fat. The low protein diet supplied energy as 3% protein, 85% carbohydrate, and 12% fat. Metabolizable energy content of both diets was 16 MJ/kg. The pigs had free access to feed and water.

Surgery. After being deprived of food overnight, pigs were anesthetized with 5% isoflurane (Aerrane, Ana- quest, Liberty Corner, NJ). Anesthesia was maintained with 2% isoflurane during aseptic insertion of 20-gauge polyethylene catheters [intramedic, Parsippany, NJ] into the jugular vein and carotid artery. Catheters were tunneled subcutaneously and exteriorized dorsally between the shoulders. Catheters were flushed with heparinized saline and sealed, before being secured to the skin in a gauze pouch with an elastic bandage. The incision was covered with Providone-iodine ointment (Professional Disposables, Orangeburg, NY). An antibiotic [0.1 mL/kg Baytril [enrofloxacin] Miles, Shawnee

| TABLE 1 |
| Composition of the control (20% protein) and the low protein (3% protein) diets |

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>Low protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn meal</td>
<td>200</td>
<td>33</td>
</tr>
<tr>
<td>Defatted soy flour</td>
<td>400</td>
<td>61</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>318</td>
<td>815</td>
</tr>
<tr>
<td>Corn oil</td>
<td>40</td>
<td>48</td>
</tr>
<tr>
<td>Mineral mixture¹</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Vitamin mixture²</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

¹ Vitamin premix supplied the following (units/kg of the control and low protein diets, respectively): retinol, 1.6 and 2.0 mg; cholecalciferol, 17.6 and 22.0 µg; RRR-α-tocopheryl acetate, 47 and 59 mg; menadione, 3.5 and 4.4 mg; vitamin B-12, 26 and 33 µg; riboflavin, 5.3 and 6.6 mg; D-pantothenic acid, 21 and 26 mg; niacin, 28 and 35 mg; biotin, 220 and 270 µg; folate, 980 and 1100 µg; thiamin, 2.2 and 2.7 mg.

² Mineral premix supplies the following (units/kg of the control and low protein diets, respectively): copper [as copper sulfate], 10 and 12.5 mg; iron [as ferrous sulfate heptahydrate], 160 and 200 mg; manganese [as manganese oxide], 100 and 125 mg; zinc [as zinc oxide], 100 and 125 mg; calcium [as calcium carbonate], 600 and 750 mg; and selenium [as sodium selenite], 100 and 125 µg.
Mission, KS] was administered intramuscularly. The pigs were monitored closely for about 5 h after recovery from anesthesia before free access to food and water was restored. The return of food intake to presurgery levels, and a normal body temperature and activity level were used as indices of recovery from surgery.

**Isotope infusion.** A sterile solution of L-[3,3,3-3H]-leucine (98.5% enriched, Cambridge Isotope Laboratories, Woburn, MA) was prepared in 4.5 g/L saline and infused through the jugular catheter. The control pigs were given a priming dose of 30 μmol/kg, followed by a constant infusion of 30 μmol/(kg·h) for 8 h. The protein-deficient pigs received a 45 μmol/kg priming dose and constant infusion of the isotope at 45 μmol/(kg·h) for 8 h. Because we anticipated that fractional synthesis rates (FSR) would be lower during protein deficiency, pigs receiving the low protein diet also received a higher tracer infusion rate to ensure reliable detection of tracer incorporation into protein pools which were turning over more slowly, particularly in muscle.

Studies were conducted in the fed state with pigs consuming about one twelfth of their daily food intake during each hour of the infusion. A 5-mL sample of arterial blood was taken before the infusion, and hourly thereafter. Blood was drawn into prechilled tubes containing 50 μL of a solution of 100 g/L Na2EDTA, 2 g/L sodium azide, 1 g/L mercaptoethanol, and 2 g/L soybean trypsin inhibitor. Blood samples were centrifuged 1,200 × g for 15 min immediately at 4°C. Plasma was removed and stored at −70°C for later analysis. Several tissues were sampled immediately after the animals were killed: liver, jejunum, longissimus dorsi muscle, masseter muscle and skin. The tissues were frozen immediately at −70°C.

**Sample analyses.** To monitor protein status, concentrations in plasma of albumin (using the bromocresol green dye technique), total protein (using the Biuret method), and urea (using urease) were determined weekly (Ciba-Corning 550 Express, Ciba-Corning Diagnostics, Gilford Systems, Oberlin, OH). Plasma amino acid concentrations were determined after the pigs had consumed the diets for 8 wk, by reverse-phase high-performance liquid chromatography using the phenylisothiocyanate derivative (Pico-Tag, Waters, Milipore, Milford, MA).

Amino acids in tissue and plasma were isolated and prepared for gas chromatography mass spectrometry (GCMS) as follows. Frozen tissue samples (about 200 mg) were homogenized in 10 mL of ice-cold 0.6 mol/L trichloroacetic acid and centrifuged 1,200 × g for 15 min. The protein-free supernatant containing tissue free amino acids was removed, and dried. After several washings, the protein pellet was hydrolyzed in 6 mol/L HCl at 110°C for 24 h, then dried. Both free and protein bound amino acids were resuspended in 1 mol/L acetic acid. Total plasma proteins were isolated in a similar manner. For analysis of free amino acids, plasma samples (200 μL) were acidified with 1 mL of 0.1 mol/L acetic acid.

Amino acids in all preparations were isolated by cation exchange chromatography and derivatized to their n-propyl ester heptafluorobutyramide derivatives as previously described (Reeds et al. 1992). Isotopic enrichment of leucine was determined by negative chemical ionization GCMS by monitoring ions at m/e 349 to 352.

Plasma alpha-ketoisocaproic acid (α-KICA) was used as an index of intracellular leucine enrichment. It was converted to its pentafluorobenzyl derivative as previously described (Reeds et al. 1992). Isotopic enrichment was measured by negative chemical ionization GCMS by monitoring ions at m/e 129 to 132.

**Calculations and statistics.** Isotopic equilibrium of plasma leucine and α-KICA was determined by demonstrating by linear regression that the slope of isotope enrichment against time was not different than zero. This criterion was made stringent by selecting the level of significance at P < 0.25.

Total leucine flux (Q) was calculated as follows:

\[ Q [\mu mol/(kg \cdot h)] = i [(E_f/E_{\alpha-KICA}) - 1] \]

where i is the tracer infusion rate [μmol/(kg·h)], E_r is tracer:tracer ratio of the tracer, E_f-KICA is the tracer:tracer ratio of plasma α-KICA at plateau.

Fractional synthesis rate (FSR) of total protein in plasma was calculated according to the precursor/product relationship.

\[ FSR (\% /d) = \frac{(E_f - E_t)}{E_{free} \times (t_f - t_t)} \times \frac{24 \times 100}{x} \]

where \( E_f - E_t \) is the increase in tracer:tracer ratio of leucine in plasma proteins over the period \( t_f \) to \( t_t \) (the last 4 h of the infusion), and \( E_{free} \) is the tracer:tracer ratio of intrahepatic free leucine.

The absolute synthesis rate (ASR) of the total plasma protein pool was determined by multiplying FSR by total intravascular protein pool size, where intravascular pool size is the product of plasma protein concentration and plasma volume. Plasma volume was calculated using measured hematocrits, assuming an average blood volume of 70 mL/kg for the control pigs and 75 mL/kg for the protein-deficient pigs (Ramirez et al. 1963).

The fractional synthesis rates of constitutive proteins in tissues were calculated from tracer:tracer ratios in samples taken at the end of the 8-h infusion.

\[ FSR (\% /d) = \frac{E_{bound} \times 24 \ h/d \times 100}{E_{free} \times 8 \ h} \]

The tracer:tracer ratio of free leucine in each tissue \( E_{free} \) was assumed to reflect the steady-state tracer:tracer ratio of the leucine pool from which pro-

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4 Abbreviations: ASR, absolute synthesis rate; α-KICA, alpha-ketoisocaproic acid; FSR, fractional synthesis rate; GCMS, gas chromatography mass spectrometry.
proteins in that tissue were synthesized. The tracer:tracer ratio of free leucine in each tissue at base line was assumed to be the same as plasma leucine. Isotopic steady state was achieved in plasma leucine and α-KICA within 1 h of starting the infusion; therefore the tracer:tracer ratio of intracellular leucine was assumed to be constant over the last 7 h of the infusion, and rapidly rising for the first hour. Because only one tissue sample was obtained after the 8-h tracer infusion period, only an estimate of FSR could be obtained. Because the rate of incorporation of leucine into protein was measured from time 0, the initial rate (during the first hour) when the precursor pool enrichment is still rising to plateau will be slower than the final rate of incorporation achieved when the precursor pool has reached isotopic steady state (from 1 to 8 h). Hence, it will underestimate the true rate of incorporation of labeled leucine into tissue proteins. Therefore, the calculated value for FSR in this study slightly underestimates the actual rate.

All results are presented as means ± SEM. The effect of diet over time on growth, albumin and total plasma protein concentration was determined by two-way ANOVA with repeated measures using BMDP Statistical Software (University of California, Berkeley, CA). The effect of diet on protein synthesis was determined by Student’s t test using equal or unequal variances as appropriate. P values of less than 0.05 were considered statistically significant.

RESULTS

The pattern of weight gain is shown in Figure 1. The average initial body weights of the two groups were not different (control: 7.6 ± 0.2 kg vs. protein-deficient: 7.5 ± 0.5 kg). Growth retardation in the pigs fed the low-protein diet was evident after 1 wk of consuming the diet [P < 0.05], and continued throughout the study. Feed consumption ranged from 90 to 60 g/(kg · d) over the final 4 wk of the study. After 8 wk, pigs fed the low protein diet weighed 11.3 ± 0.5 kg, an increase of only 50% of initial body weight. In contrast, pigs fed the control diet weighed 34.4 ± 0.5 kg, a 3.5-fold increase over their initial weight. In addition to the impaired growth, the pigs fed the low protein diet manifested other features typical of the kwashiorkor syndrome of malnutrition (Waterlow 1992) including dry scaly skin, coarse mottled hair, and towards the end of the study period, swelling in the neck area and lower legs indicating edema.

Consumption of the low protein diet also had an immediate negative effect on concentrations of albumin and total protein in plasma (Fig. 2). In the protein-deficient pigs, the concentration of total protein in plasma was significantly reduced [P < 0.05] after 1 wk of feeding, and declined by 30% during the study. In contrast, concentration of total protein in plasma increased by 12% in the control pigs. Changes in plasma albumin concentration followed a similar pattern. After 1 wk of feeding, albumin concentration declined significantly [P < 0.001] in the protein-deficient pigs and continued decreasing to 48% of the pre-diет value after 6 wk. In contrast, plasma albumin in the control pigs increased by 21% [P < 0.001] after 6 wk of feeding.

The plasma concentrations of most amino acids also were lower in the low protein group (Table 2). The exceptions were methionine, cystine, glycine, serine and alanine. Plasma urea concentration, over the final 4 wk, was also lower in the protein-deficient pigs (2.25 ± 0.36 mmol/L) compared with the control pigs (3.93 ± 0.33 mmol/L, P < 0.05).

The tracer:tracer ratios of plasma leucine and α-KICA reached steady states after 1 h of isotope infusion (Fig. 3). The different tracer doses among the groups resulted in different isotopic enrichments, however,
the relationships were similar as is typical of leucine tracer studies [Rennie et al. 1994]. In both groups, plasma KICA averaged 67% of plasma leucine enrichment. Tissue free leucine averaged 60% of plasma KICA enrichment. Based on plasma α-KICA isotopic enrichment, leucine flux was lower in protein-deficient pigs [109 ± 7 μmol/[kg·h]] compared with the well-nourished group [307 ± 34 μmol/[kg·h], P < 0.001]. Based on 8% leucine content of body protein, these data suggest a similarly lower rate of whole-body protein turnover in the protein-deficient pigs [4.6 ± 0.2 g/[kg·d)] vs. 12.3 ± 1.3 g/[kg·d], P < 0.001].

FIGURE 5 Concentration, fractional synthesis and absolute synthesis rates of total protein in plasma of pigs consuming the control [20% protein] or protein-deficient [3% protein] diet for 8 wk. Values are means ± sem, n = 4 pigs. *Significantly different (P < 0.05) than controls. **Significantly different (P < 0.01) than controls.
DISCUSSION

The growth potential of the pig is much more rapid than that of the human. Therefore, we reasoned that in this pig model of kwashiorkor-like malnutrition, induction of more severe growth retardation than is described in the Wellcome Trust classification for humans [Wellcome Trust Working Party 1970] would be necessary to produce the deleterious biochemical and pathophysiological changes characteristic of kwashiorkor. Previously, we have used a similar diet to investigate changes in serum biochemical constituents [Pond et al. 1992] and body composition [Pond et al. 1965], as well as impairment of glutathione homeostasis [Jaamoo et al. 1995]. In this study we observed features typical of kwashiorkor, including edema, and changes in hair and skin, and we investigated changes in protein synthesis in the whole-body, tissues and total protein in plasma.

The severe growth retardation of the protein-malnourished pigs suggests that when a young weaned animal is fed a protein-deficient diet, there is an overall reduction in the growth and development of all of the major tissues of the body. This response to the chronic deficiency of dietary protein is associated with a reduction in whole-body protein turnover, as indicated by the slower leucine flux of the protein-deficient group of pigs. Furthermore, this overall reduction in protein turnover includes most of the major tissues of the body, as evidenced by the slower rates of synthesis of protein in muscle, skin, liver, jejunal mucosa and in plasma. Thus, a reduction in protein synthesis rate is a major mechanism through which the growth failure of young animals weaned onto a protein-deficient diet is mediated.

The slower rate of whole-body protein turnover in the protein malnourished pigs was expected. Similar findings were reported in rats fed a low protein diet for 6 wk [Waterlow and Stephen 1968] and in protein-malnourished young children [Golden et al. 1977]. The study by Golden and co-workers showed that after recovery from malnutrition well-nourished children lost weight on a diet supplying adequate energy but only maintenance quantities of protein, whereas severely malnourished young children (who had lower protein synthesis and breakdown rates) were able to maintain their body weights and had a more positive nitrogen balance, suggesting a higher efficiency of utilization of dietary protein [Golden et al. 1977]. In the present study, the 50% increase in body weight during 8 wk of dietary treatment suggests that the protein-deficient pigs were able to maintain a positive nitrogen balance on the low protein diet. Thus, the reduction in protein turnover rate seems to be a necessary part of the metabolic adaptation to chronic protein insufficiency that ensures minimal nitrogen excretion and maximum efficiency of utilization of dietary protein, thereby permitting the organism to maintain nitrogen balance and to survive.

Our present results clearly indicate that protein synthesis in skin is markedly reduced during chronic protein deficiency in the young pig. These results are in agreement with several experiments in rats fed low protein or protein-free diets [Garlick et al. 1975, McNurlan and Garlick 1981, McNurlan et al. 1982, Waterlow and Stephen 1968]. The tissue proportionately most affected by chronic protein deficiency was skin. The fractional synthesis rate of protein in skin was decreased by two thirds in the protein-deficient pigs. This finding is in agreement with those of Waterlow and Stephen [1966], who reported that rats fed a low protein diet for 5–8 wk had a decrease in skin protein synthesis, and the skin lost more nitrogen proportionally than muscle. Similarly, when rats were starved for 2 d, protein synthesis in skin was reduced by 26% compared with fed controls [Preedy et al. 1983]. Together with our present results, these findings suggest that skin protein synthesis is highly sensitive to dietary protein intake, hence maintenance of skin integrity is especially vulnerable to a reduction in dietary protein. Nevertheless, the marked reduction in synthesis of skin proteins may be necessary during protein deprivation to ensure that most of the limited supply of dietary amino acids is available for synthesis of proteins in other organs that are more critical for survival. This reduction in protein synthesis in skin may underlie the formation of skin lesions observed in the kwashiorkor syndrome of protein-energy malnutrition. Furthermore, because the pig's skin contains about 10% of its total body protein [Bailey and Zobrisky 1968], this reduction in protein synthesis rate represents a substantial contribution toward the overall decrease in whole-body protein turnover in response to chronic protein insufficiency.

The rate of synthesis of jejunal mucosa protein was also severely affected by the protein-deficient diet: 50% lower in the protein-malnourished pigs compared with the well-nourished pigs. This is not surprising, because gut mucosa is very sensitive to changes in food intake and atrophies when there is a shortage of energy and protein [James 1971, Lowrey et al. 1962]. The present finding agrees with those of McNurlan and co-workers [McNurlan and Garlick 1981, McNurlan et al. 1982] who reported reductions in jejunal mucosa protein synthesis rate in rats either starved for 2 d or fed a protein-free diet for 9 d. Together, these findings suggest that gut mucosa protein synthesis rate decreases immediately in response to a shortage in dietary protein and that this response is sustained throughout the period of dietary protein deficiency. Our present findings and those of McNurlan and co-workers [McNurlan and Garlick 1981, McNurlan et al. 1982], however, dispute the conclusion of Hirschfield and Kern [1969] that atrophy of intestinal mucosa protein is delayed during protein deprivation because mucosal protein synthesis is
maintained by amino acids released from digestion of endogenous proteins. This conclusion was based on the increased incorporation of orally administered 3H-leucine into the jejunal mucosa protein in rats fed a protein-free diet for 23 d compared with rats fed a control diet. Because the intracellular free leucine (i.e., the precursor pool) specific activity was not measured by Hirschfield and Kern (1969), it is not possible to determine whether the more rapid incorporation of labeled leucine translated into an increased rate of synthesis of mucosal protein. Our findings suggest that protein-deficient animals have smaller amino acid pools, based on 1) reduced amino acid concentrations in plasma, and 2) the relatively highly enriched intracellular free leucine pool despite the higher tracer infusion rate. Therefore, it is likely that the intracellular specific activity of jejunal mucosa in the study of Hirschfield and Kern was much higher in the protein-malnourished rats, hence the higher incorporation of label may not necessarily reflect a faster rate of protein synthesis.

The rate of hepatic protein synthesis was 25% slower in the protein-deficient pigs relative to the control pigs. There is some disagreement with respect to the response of hepatic protein synthesis rate to dietary protein deprivation. Some workers have reported that protein deprivation causes a decrease in the rate of hepatic protein synthesis [McNurlan and Garlick 1981, McNurlan et al. 1982; Seve et al. 1986], whereas others have reported a higher liver protein synthesis rate despite significant loss of liver protein [Garlick et al. 1975, Haider and Tarver 1969, Waterlow and Stephen 1968]. Our present finding is in agreement with the former group of studies, suggesting that the reduction in liver protein content in response to chronic protein deficiency is mediated through a decrease in the rate of protein synthesis.

The fractional rate of synthesis of total protein in plasma was not different between the protein-deficient and control pigs, suggesting that the reduction in plasma protein concentration in response to dietary protein deficiency was probably due to a relatively higher rate of plasma protein breakdown compared with synthesis during the initial adaptation to the low protein diet. Although the rate at which the plasma protein pool was turning over was the same in both groups of pigs at 8 wk, the protein-deficient pigs were synthesizing only 60% as much plasma protein per kilogram of body weight. These findings are in agreement with that of Waterlow and Stephen (1966) who also reported no change in the fractional rate of synthesis of mixed serum proteins in rats fed a low protein diet for 10 d.

The fractional rate of synthesis of skeletal muscle proteins in both well- and protein-malnourished pigs was the slowest of all tissues. In the control pigs, the FSR of the masseter muscle tended to be higher than for the longissimus muscle. This difference is consistent with the higher proportion of Type I fibers, which have a higher FSR than Type II fibers (El Haj et al. 1986), in the longissimus muscle [van den Helm-Grooten et al. 1995]. Moreover, several other studies in rats in which the gastrocnemius or mixed muscles, composed predominantly of Type II fibers, were studied, protein deprivation was associated with a lower muscle fractional synthesis rate [Garlick et al. 1975, McNurlan et al. 1982, Seve et al. 1986, Waterlow and Stephen 1968]. The absence of a difference in the fractional rate of synthesis of protein in longissimus, however, is consistent with the findings of certain other studies (El Haj et al. 1986, Millward et al. 1975). The relevant difference between those studies that did find a difference and those that did not is the length of the dietary deprivation period. A comparison of these studies clearly illustrates that a reduction in FSR occurs acutely when protein intake is reduced, but with chronic low intakes, as in our study, this difference between malnourished and age-matched, well-nourished controls is no longer apparent. It is probable that FSR of the longissimus was significantly lower in pigs fed the low protein diet at an earlier stage, with this early reduction accounting for a lower rate of muscle accretion. The lower FSR of the masseter muscle, on the other hand, may reflect a delayed response to chronic undernutrition, those metabolic characteristics that render it more resilient to the effects of protein malnutrition being overwhelmed under conditions of chronic deprivation.

The present study and those of others (Garlick et al. 1975, Lowrey et al. 1962, Pond et al. 1965, Seve et al. 1986) have shown that when a young animal is weaned onto a protein-deficient diet, the metabolic adaptation results in an overall reduction in the growth and development of all major tissues of the body. The reduction in plasma of most amino acids and of urea suggests that the animals adapted to the deficient protein intake by increasing the efficiency of amino acid utilization for anabolic purposes, and by decreasing amino acid oxidation, thereby reducing net nitrogen loss. These findings are in agreement with the data of Edmonds and Baker (1987) which show decreased urinary urea excretion and activities of urea cycle enzymes in pigs fed 3% vs. 30% protein diets. In rats fed low protein diets, the activities of the urea cycle enzymes, and the three enzymes (glutamate dehydrogenase and alanine and aspartate aminotransferases) which facilitate the transfer of amino groups into the urea cycle in liver, are decreased [Das and Waterlow 1974]. Together, these data suggest that adaptation to dietary protein deficiency involves a reduction in the transfer of amino nitrogen to the urea cycle for urea synthesis. We conclude that despite the improved efficiency of protein utilization, chronic consumption of the 3% protein diet produced a marked shortage in protein which affected not only those stores of protein with slow turnover rates such as muscle tissues, but also protein pools which have faster turnover rates such as gut mucosa and plasma proteins.
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LITERATURE CITED


