Effects of Dietary Protein Intake on Muscle Protein Synthesis and Degradation in Rats with Gentamicin-Induced Acute Renal Failure

Radhakrishna Baliga, M.D., and Sudhir V. Shah, M.D.

ABSTRACT
In the study presented here, the muscle protein synthesis and degradation in gentamicin-induced acute renal failure were examined in rats fed a low (7%), normal (22%), and high (35%)-isocaloric protein diet. Male Sprague-Dawley rats were fed equivalent amounts of these diets for 10 days and then received daily subcutaneous injections of either 1 mL of sterile isotonic saline or 100 mg/kg of gentamicin for 7 consecutive days. The rats were sacrificed the following day, and epitrochlearis muscles were obtained for measurement of protein turnover. The serum creatinine in each of the gentamicin-treated groups was significantly higher than that in the saline-treated controls but were no different from each other. Muscle protein synthesis (calculated from the incorporation of radiolabeled [U-14C] phenylalanine) was slightly but not significantly decreased in gentamicin-treated rats as compared with that of the corresponding saline controls in each of the dietary groups. Net protein degradation (the rate of tyrosine release into media) in the 7 and 22% gentamicin-treated groups was similar to that in the corresponding saline controls. In contrast, net protein degradation was significantly greater in the 35% gentamicin group of rats when compared with the 7 and 22% gentamicin groups and its own control. In the 7 and 22% saline- and gentamicin-treated protein groups, there was a reduction in net protein degradation in response to insulin. In contrast, the net protein degradation continued to remain significantly elevated in the 35% gentamicin-treated group, despite addition of insulin, when compared with that in the 7 and 22% gentamicin groups and its own control. The results of these studies indicate that there are no marked alterations in the protein turnover in rats with gentamicin-induced acute renal failure fed a normal protein diet. In contrast, rats on a high-protein diet have marked weight loss and a marked increase in muscle protein catabolism which is unresponsive to insulin.

Key Words: Gentamicin-induced acute renal failure, protein synthesis, degradation

It is generally accepted that severe reduction in renal function is accompanied by evidence of abnormal protein metabolism, including negative nitrogen balance and loss of lean body mass (1, 2). This has been attributed to nutritional deficiencies, resistance to the compensatory mechanisms that regulate protein metabolism, and stimulation of catabolism by metabolic abnormalities that result from decreased renal function.

Previous studies of protein turnover in acute renal failure have been largely carried out in surgically induced acute renal failure rats (3–5). In these studies, it has been shown that there is an early marked increase in protein degradation followed later by a depression in protein synthesis (6). Because the majority of the studies were carried out within 48 h from the onset of surgically induced acute renal failure, the acceleration of the normal catabolic response from surgical stress, starvation, and inadequate food intake may have contributed to the changes in the protein turnover observed in these studies. Thus, the changes in protein turnover in postsurgical acute renal failure may not be applicable to other models of acute renal failure. Indeed, Goodship et al. have recently reported that patients with chronic renal insufficiency have nitrogen balance and adaptation to variation in dietary protein similar to those of control subjects, suggesting that renal insufficiency per se may not be responsible for alterations in pro-
tein metabolism (7). There have been no previous studies of protein turnover in other clinically relevant forms of acute renal failure.

Aminoglycoside antibiotics including gentamicin have been widely used in the treatment of gram-negative infections. A major complication of the use of this drug has been nephrotoxicity. About 10 to 26% of patients taking aminoglycosides may develop renal toxicity. It has been a common practice to alter protein intake in acute renal failure. Harter et al. (8) have shown in a surgical model of acute renal failure an improved weight gain with a high-protein diet associated with a decrease in protein degradation. The effects of varying dietary protein on muscle protein synthesis and degradation in a nonsurgical model of acute renal failure have not been previously tested. We therefore examined the effect of a low (7%)-, normal (22%)-, and high (35%)-isocaloric protein diet on protein turnover in a gentamicin-induced acute renal failure model.

METHODS

The experimental protocol we used is described in Figure 1. Male Sprague-Dawley rats weighing 210 ± 4 g were maintained in individual cages for 48 h on a 12-h light/12-h dark cycle for 2 days with free access to food and water. They were then divided equally by weight into three groups and paired 7, 22, or 35% protein diets for 10 days (Table 1). The diets were isocaloric with identical calcium, phosphorus, mineral, and vitamin content. The 7% protein diet group of rats ate 10.0 g/day, and the same amount was fed to the 22 and 35% protein group of rats. On day 11, the animals in each group were randomly assigned to receive daily s.c. injections between 0900 and 1100 h of either 100 mg/kg of gentamicin or 1 ml of sterile isotonic saline for 7 consecutive days. On day 18 and at 24 h after the last injection, the rats were anesthetized with pentobarbital at 5 mg/100 g body wt. Blood and epitrochlearis muscle were obtained for analysis, followed by exsanguination of the animal while sedated.

Epitrochlearis Muscle Incubation

Epitrochlearis muscles from anesthetized rats were quickly dissected free, rinsed with iced saline, blotted, weighed, and incubated in flasks containing 3 mL of Krebs-Henseleit bicarbonate buffer, 10 mM glucose, and 0.5 mM phenylalanine (with or without 0.05 μCi of [U-14C]phenylalanine per mL). The flasks were then stoppered, gassed for 3 min with 95% O2-5% CO2, and placed in a rotating (60 cycles/min) bath maintained at 37°C. After a 30-min preincubation, the muscles were removed with a blunt forceps, blotted on cotton gauze, transferred to flasks containing 3 mL of the same media, with or without 1 μM/mL of insulin, and incubated for an additional 2-h period.

At the end of the incubation period, the flasks were placed on ice and the muscles were quickly rinsed with ice-cold saline and were then plunged into 1.5 mL of 10% perchloric acid, followed promptly by homogenization with a dounce-type rotating homogenizer. A sample of 2.5 mL of the incubation media was reserved for determination of protein degradation. The resulting homogenate was transferred to large Sarstedt test tubes and centrifuged at 4°C at 5,000 × g for 10 min. The supernatant was saved to measure intracellular specific radioactivity. The protein pellet was washed once with 3 mL of iced 10% perchloric acid and twice with 5 mL of iced ethanol/ether (1:1, vol/vol). Each washing consisted of vigorous vortexing for 10 s, followed by centrifugation at 4°C at 5,000 × g for 10 min. After the final wash,
the pellet was resuspended in 0.5 mL of tissue solubilizer and incubated overnight at 37°C. A 4.5-ml sample of scintillation cocktail was added to each tube 15 h later; the solution was then vortexed and transferred to vials to be counted in a liquid scintillation counter.

Protein synthesis was measured as the rate of incorporation of [U-14C]phenylalanine into muscle protein divided by the extracellular specific radioactivity of phenylalanine.

**Protein Degradation**

To 2.5 mL of the incubation media from each vial, 0.5 mL of 50% trichloroacetic acid was added. The resulting solution was centrifuged at 1,000 rpm for 3 min, and the supernatant was saved for tyrosine determination. Muscle protein degradation was calculated as the rate of release of tyrosine into the incubation media by the fluorometric method of Waalkes and Udenfriend (9). The procedure was similar to that used previously to study muscle protein synthesis and degradation in incubated muscles (10,11).

Plasma urea nitrogen and creatinine were measured using a Beckman BUN Analyzer 2 and a Beckman Creatinine Analyzer (Beckman Instruments, Inc., Fullerton, CA). The data are expressed as mean ± SE. The results were analyzed statistically by analysis of variance and Fisher’s test for post-ad hoc analysis. The changes induced by insulin in protein degradation and protein efficiency ratios were compared by a paired t test.

**RESULTS**

On being fed identical amounts of respective protein diets before gentamicin administration, the 7% protein group lost significant weight compared with the 22 and 35% protein groups. The 35% protein group gained weight compared with the rest of the groups (Table 2). All three gentamicin-treated groups of rats before sacrifice lost weight. However, it was most pronounced in the 35% protein group and was significant when compared with its respective control (Table 3). Weight change during this phase in the 35% gentamicin group correlated significantly with the muscle protein degradation estimated at the end of the phase (r = 0.74; P = 0.01). None of the animals had diarrhea to account for the weight loss. The food intake was similar in each of the three dietary groups (Table 3). The protein efficiency ratios calculated as grams of weight gained or lost per gram of protein consumed were markedly decreased in the gentamicin-treated group of rats compared with those of the respective saline controls (Table 3).

**TABLE 2. Effect of varying protein diets on weights before gentamicin administration**

<table>
<thead>
<tr>
<th>N</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Change in Weight (g)</th>
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<tbody>
<tr>
<td>7%</td>
<td>12</td>
<td>210 ± 3</td>
<td>194 ± 4</td>
</tr>
<tr>
<td>22%</td>
<td>12</td>
<td>213 ± 3</td>
<td>212 ± 4</td>
</tr>
<tr>
<td>35%</td>
<td>10</td>
<td>207 ± 4</td>
<td>215 ± 5</td>
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</tbody>
</table>

* Values are mean ± SE.
* P < 0.01 compared with 22 and 35% protein diets.

**TABLE 3. Effects of varying protein diets on weights in the gentamicin- and saline-treated groups before sacrifice**

<table>
<thead>
<tr>
<th>N</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
<th>Change in Weight (g)</th>
<th>Food Eaten (g/day)</th>
<th>Protein Efficiency Rates</th>
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7% Protein
Saline 6 191 ± 5 188 ± 5 -3 ± 4.5 10 ± 0.8 -0.16 ± 0.42
Gentamicin 6 197 ± 6 179 ± 7 -18 ± 4.8 10 ± 0.8 -1.4 ± 0.35p

22% Protein
Saline 6 206 ± 6 210 ± 5 4 ± 6.3 11 ± 0.5 0.15 ± 0.31
Gentamicin 6 218 ± 6 211 ± 10 -7 ± 6.9 10 ± 0.8 -0.52 ± 0.46p

35% Protein
Saline 5 210 ± 8 214 ± 4 4 ± 8 11 ± 0.5 0.09 ± 0.21
Gentamicin 5 220 ± 5 198 ± 4 -22 ± 2.5p 10 ± 0.8 -0.82 ± 0.14p

* Values are mean ± SE.
* P < 0.05 compared with respective saline group.
The serum urea nitrogen was higher in the gentamicin-treated group of rats and, as expected, varied with the level of protein intake. The serum creatinine in each of the gentamicin-treated groups were significantly higher than that in the saline-treated controls but no different from each other (Table 4).

The protein synthesis in the saline-treated groups were not significantly different from each other, and the values were similar to those previously reported (5). Muscle protein synthesis was slightly but not significantly decreased in gentamicin-treated rats as compared with that in the corresponding saline controls in each of the dietary groups (Table 5). The net protein degradation in the 7 and 22% gentamicin-treated groups was similar to that in the corresponding saline controls. In contrast, net protein degradation was significantly greater in the 35% gentamicin group of rats when compared with that in the 7 and 22% gentamicin groups and its own control (Figure 2).

We examined the effects of insulin, which is known to decrease protein degradation (12). In the 7, 22, and 35% saline- and gentamicin-treated group of rats (Figure 2). In the 7 and 22% saline-treated dietary groups, addition of insulin caused reduction in net protein degradation. No effect of insulin was observed in the 35% saline-treated control group. In the 7 and 22% gentamicin-treated protein groups, there was reduction in net protein degradation in response to insulin. In contrast, net protein degradation continued to remain significantly elevated in the 35% gentamicin-treated group, despite addition of insulin, when compared with that in the 7 and 22% gentamicin groups and its own control.

<table>
<thead>
<tr>
<th>TABLE 4. Renal function in the gentamicin- and saline-treated groups receiving varying protein diets*</th>
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<tbody>
<tr>
<td>N</td>
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<tr>
<td>7% Protein</td>
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<tr>
<td>Saline</td>
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<tr>
<td>Gentamicin</td>
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<td>22% Protein</td>
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<td>Gentamicin</td>
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<td>35% Protein</td>
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<td>Saline</td>
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<td>Gentamicin</td>
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* Values are mean ± SE.

* Values are mean ± SE.

* P < 0.01 when compared with respective saline group.

* P < 0.01 when compared with 7 and 22% gentamicin groups.

<table>
<thead>
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<th>TABLE 5. Protein synthesis in gentamicin- and saline-treated rats receiving varying protein diets</th>
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<tr>
<td>Protein</td>
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<tr>
<td>7% Protein</td>
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<td>Gentamicin</td>
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<td>22% Protein</td>
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<td>Saline</td>
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<td>Gentamicin</td>
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* Values are mean ± SE.

Figure 2. Effect on insulin on net protein degradation in the gentamicin- and saline-treated rats.

DISCUSSION

The acceleration of the normal catabolic response from surgical stress may have contributed to the changes in protein turnover observed in surgically induced acute renal failure rats (3–5). We employed the present rat model to reduce the catabolic effects resulting from the trauma of surgery.

In the study presented here, we have examined the muscle protein synthesis and degradation in gentamicin-induced acute renal failure in rats fed a low (7%), normal (22%), and high (35%)-isocaloric protein diet. There were no significant differences in renal function as measured by serum creatinine between the rats on various diets, although recently it
has been suggested that level of prior protein intake may alter the susceptibility of the kidney to injury (13, 14). In addition, the rats on various diets had a similar amount of food intake. Thus, any observed differences cannot be attributed to differences in food intake or to the degree of renal failure.

The gentamicin-treated group of rats in our study had muscle protein synthesis lower but not significantly different than that in the saline-treated controls. This finding is similar to those of previous reports of surgically induced acute renal failure (15). Studies performed with both incubated and perfused muscle preparations have indicated that rats with surgically induced acute renal failure have accelerated muscle protein wasting and that this wasting was due to enhanced protein degradation (4, 15, 16). The results of our study show that the weight loss and the protein degradation in the gentamicin-treated group of rats fed the normal protein diet (22%) was not significantly different from those in the saline-treated controls, despite a marked reduction in renal function and a decrease in the protein efficiency ratio. Although the failure to detect differences may be related to the limitations of short-term muscle incubations, these findings are nonetheless in contrast to those of reports of increased protein catabolism in surgically induced renal failure and suggest that factors other than the renal failure may contribute importantly to increased protein catabolism.

The major metabolic change that occurs in response to feeding a low-protein diet has been a decrease in amino acid oxidation and protein degradation (17). Goodship et al. (7) have shown that metabolic adaptation to a low-protein diet is unimpaired in patients with moderate chronic renal failure. Rats fed a 7% protein diet had significant weight loss before the administration of gentamicin. Yet, at the termination of the study, their protein turnover was not significantly different from the rats fed 22% protein diets. It is possible that these rats might have undergone some degree of adaptation resulting in a decrease in protein breakdown. The amino acids liberated by intracellular breakdown of protein along with those derived from protein intake may replenish the diminished free amino acids pool which is the source of protein synthesis.

Insulin has been a major potent regulator of the net protein balance (11, 12). In uremia, there has been an increased resistance to insulin-stimulated glucose uptake and amino acid transport in the muscles, causing a shift in the normal protein metabolism (3, 5, 18). Addition of insulin decreased the tyrosine release from muscles of gentamicin-treated rats fed 12% and 22% isocaloric protein diets to a similar degree. The above findings are in contrast to observations of increased insulin resistance in surgically induced acute renal failure but similar to that observed by Harter et al. (8) in an acute renal failure model studied 10 days after surgery.

The major finding in our study has been the detrimental effect of a high-protein diet on muscle protein catabolism in the gentamicin-treated group of rats. Rats fed a high-protein diet (35%) had marked increase in weight loss as well as muscle protein degradation with a decrease in protein efficiency ratio. Moreover, the weight change noted during this phase correlated significantly with the protein degradation of the end of the study. The net tyrosine release despite addition of insulin continued to remain significantly elevated in this group compared with that in the 7% and 22% gentamicin-treated rats and its control. This detrimental effect of a high-protein diet is in contrast to that reported by Harter et al. (8) where the release rates of tyrosine were maximum in the 10% protein-fed rats and decreased with increasing protein intake. Our results, however, are similar to those reported by May et al. (19) in which they found that rats fed a 46% protein diet had a 90% increase in net protein degradation. Although we did not examine the mechanisms responsible for the detrimental effect of a high-protein diet, May et al. (19) have shown that the increased protein degradation in response to a high-protein diet is corrected by dietary bicarbonate. It is possible that the 35% gentamicin-treated rats had metabolic acidosis secondary to severe azotemia from increased dietary protein intake that might have contributed to the significant increase in net protein degradation. In addition, the lack of response to insulin in the 35% gentamicin-treated group may be related either to the metabolic acidosis (19) and/or to an increase in the cytosolic free calcium (20).

The results of our studies indicate that there are no marked alterations in the protein turnover in rats with gentamicin-induced acute renal failure fed a normal protein diet. In a surgically induced acute renal failure model, Harter et al. (8) have shown improved weight gain with a high-protein diet associated with a decrease in net protein degradation. In contrast, in gentamicin-induced acute renal failure, high-protein intake was associated with a markedly increased catabolism, as reflected in both the increased muscle degradation unresponsive to insulin and increased weight loss.

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REFERENCES


