Mechanisms for Defects in Muscle Protein Metabolism in Rats with Chronic Uremia

Influence of Metabolic Acidosis

R. C. May, R. A. Kelly, and W. E. Mitch
With the technical assistance of **G. Karapanos**
Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115

Abstract

Chronic renal failure (CRF) is associated with metabolic acidosis and abnormal muscle protein metabolism. As we have shown that acidosis by itself stimulates muscle protein degradation by a glucocorticoid-dependent mechanism, we assessed the contribution of acidosis to changes in muscle protein turnover in CRF. A stable model of uremia was achieved in partially nephrectomized rats (plasma urea nitrogen, 100–120 mg/dl, blood bicarbonate < 21 meq/liter). CRF rats excreted 22% more nitrogen than pair-fed controls ($P < 0.005$), so muscle protein synthesis and degradation were measured in perfused hindquarters. CRF rats had a 90% increase in net protein degradation ($P < 0.001$); this was corrected by dietary bicarbonate. Correction of acidosis did not reduce the elevated corticosterone excretion rate of CRF rats, nor did it improve a second defect in muscle protein turnover, a 34% lower rate of insulin-stimulated protein synthesis. Thus, abnormal nitrogen production in CRF is due to accelerated muscle proteolysis caused by acidosis and an acidosis-independent inhibition of insulin-stimulated muscle protein synthesis.

Introduction

Chronic renal failure (CRF)[1] is frequently characterized by loss of lean body mass, which suggests that CRF stimulates increased catabolism of muscle protein (1). Due to the metabolic complexities of CRF, the mechanisms responsible for this catabolic effect have not been elucidated. In studies of rats with experimental CRF, protein intake, starvation, and the degree of uremia appear to affect muscle protein metabolism (2–4). Besides these factors, the metabolic acidosis associated with CRF also might change muscle protein turnover. This possibility is raised because we have found that chronic metabolic acidosis alone, induced by feeding NH_4Cl or HCl to normal rats, increased their urinary nitrogen excretion, stunted their growth, and increased the rate of skeletal muscle protein breakdown (5). In these studies, the metabolic acidosis was severe (aortic blood pH 7.15); it is unknown whether the milder degree of acidosis associated with CRF would also increase catabolism of muscle protein. To address this question, pair-fed rats with stable chronic uremia were compared to sham-operated (SO) controls, and protein turnover in skeletal muscle was measured using the isolated perfused hindquarter technique. The results indicate that both muscle protein degradation and synthesis were abnormal in CRF rats. In investigating the mechanisms for these changes, we found that metabolic acidosis accounted for increased muscle proteolysis in CRF, but that there was an additional defect in insulin-stimulated protein synthesis that was independent of metabolic acidosis.

Methods

Male Sprague-Dawley rats weighing 125–150 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.) before surgery. They underwent right nephrectomy, which was followed 1 wk later by ligation of branches of the left renal artery to infarct five-sixths of the left kidney. SO rats underwent laparotomy without kidney damage. Rats recovered for at least 1 wk while being fed chow containing 14% protein (RMH 1000; Agway Country Foods, Syracuse, NY) ad lib. and were housed in individual metabolic cages in a temperature-controlled room with a 12-h light/dark cycle. SO rats drank water ad lib. while CRF rats were given 0.0375 M NaCl to drink. After recovery, all rats were switched to a diet similar to that used by Harter et al. which yielded normal growth in CRF rats (2). This diet, which contained 46% protein, was composed of casein, RMH 3000 protein chow (Agway Country Foods), and dextrin (40, 35, 25; wt/wt/wt). For 10 d, the rats were allowed free access to the diet to ensure a stable degree of uremia. After this period, rats were weighed and tail vein blood was obtained to measure plasma urea nitrogen (PUN) to establish bases for pairing.

Three separate pair-feeding comparisons were made; the food intake of each CRF rat was used to determine the intake of its paired rat. In study 1, CRF rats were paired by weight with SO rats and both groups were fed the diet alone. In study 2, CRF rats were paired by weight and PUN before being randomized to receive the diet with or without sodium bicarbonate mixed with the food (1.7% by weight). In study 3, CRF rats were paired by weight with SO rats and both groups received the diet mixed with 1.7% sodium bicarbonate. In each study rats were pair fed for 2 wk. In studies 1 and 3 the urine of six rats in each group was collected for 48 h after the rats had been pair fed for at least a week. At the beginning and end of the 48-h collection period, tail vein blood was obtained. Plasma and urine were analyzed for urea nitrogen to measure the urea appearance rate (6). Urinary total nitrogen and free corticosterone were measured as described previously (5).

The hindquarter perfusion technique was similar to that reported previously (6, 7) and is modified from the technique of Ruderman et al. (8). After the preparative surgery and just before initiation of the perfusion, aortic blood was obtained and P_{CO_2} and pH were measured (5). The calculated bicarbonate was used to estimate each rat's acid-base status to eliminate the influence of varying degrees of Co_2 retention during anesthesia and preparation for perfusion. The perfusate consisted of Krebs-Henseleit bicarbonate buffer, 3.5 g/dl bovine serum albumin (fraction V; Miles Laboratories, Elkhart, IN) that had been dialyzed for

Address reprint requests to Dr. May, Division of Clinical Pharmacology, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115.

Received for publication 19 June 1986 and in revised form 10 December 1986.

1. *Abbreviations used in this paper:* CRF, chronic renal failure; PUN, plasma urea nitrogen; SO, sham-operated.

J. Clin. Invest.

0021-9738/87/04/1099/05 $1.00
Volume 79, April 1987, 1099–1103

48 h at 4°C against Krebs-Henseleit bicarbonate buffer, aged, washed human erythrocytes (hematocrit 25%), 10 mM glucose, and amino acids in concentrations found in rat plasma (9). In some experiments in studies 1 and 3, 1 mU/ml of regular pork insulin (Eli Lilly & Co., Indianapolis, IN) was added and the glucose concentration in the perfusate was increased to 20 mM because preliminary studies showed that glucose fell to very low levels if the initial glucose concentration was 10 mM. The perfusate was gassed with 95%O_2/5%Co_2, equilibrated to 37°C, and the pH was adjusted to 7.4 immediately before each perfusion. The perfusate volume was 150 ml; the initial 30 ml was discarded and the remainder was continuously recirculated for a 30-min equilibration period followed by a 60-min experimental period while being gassed with 95%O_2/5%Co_2. At the conclusion of the equilibration and experimental periods, the perfusate was sampled to measure tyrosine and phenylalanine concentrations. In all studies, both animals of each pair were perfused simultaneously.

Protein turnover. Erythrocytes were separated and the "plasma" fraction of the perfusate sample was deproteinized using 10% trichloroacetic acid (TCA) (1:4 vol/vol). The rate of release of tyrosine from the hindquarter was calculated as the product of the perfusate volume and the change in tyrosine concentration during the experimental period. The release of tyrosine was used to estimate the rate of net protein degradation, since tyrosine is neither synthesized nor degraded by skeletal muscle (10–12). To exclude the possibility that changes in tyrosine transport could account for any differences in tyrosine release rates between CRF and SO rats, intracellular tyrosine levels were measured from anterior tibialis muscles. Intracellular tyrosine after the equilibration period (223±15 CRF vs. 218±11 μM SO; P = NS) did not change during the experimental period (216±12 CRF vs. 216±6 μM, SO; P = NS). Since net protein degradation is the difference between total proteolysis and protein synthesis, we also measured total protein degradation as tyrosine released when 0.5 mM cycloheximide was added to the perfusate (in study 2). This concentration inhibits muscle protein synthesis by > 95% (12). Tyrosine release was expressed per gram of skeletal muscle using relationships between body weight and the weight of perfused muscle established previously (6).

To measure protein synthesis, the perfusate was supplemented with L-U-^{14}C-phenylalanine (0.05 μCi/ml) and 0.5 mM phenylalanine (7, 11). Using this phenylalanine concentration, we have found that the extracellular phenylalanine specific radioactivity in the gastrocnemius muscle reaches a value equal to that in perfusate by the end of the 30-min equilibration period, and the specific radioactivity remains constant during the experimental period (7, 12). In the present study, protein synthesis rates were measured using the anterior tibialis muscle because it is composed of both red and white fibers (13) and is more easily biopsied than the medial gastrocnemius muscle we had studied previously. One anterior tibialis muscle was biopsied after the equilibration period and the contralateral muscle was sampled at the end of the experimental period. The samples were weighed and immediately homogenized in 1.5 ml of 10% TCA. The protein precipitate was washed once with 3 ml 10% TCA and then twice with 5 ml of ethanol/ether (1:1 vol/vol) before the precipitate was solubilized by incubating overnight at 37°C in 0.5 ml Soluene 100 (United Technologies, Packard Instruments Co., Downers Grove, IL). The radioactivity was determined by liquid scintillation counting with correction for quenching using an external standard and a quench curve generated using skeletal muscle protein. Scintillation counting was performed within 48 h of the perfusion. The phenylalanine intracellular specific radioactivity in the anterior tibialis muscle was measured as described previously (7, 12). It was not different statistically from the media and it remained constant (137±4 at 30 min, 133±3 dpm/nmol at 90 min) during the experimental period. Protein synthesis was calculated from the increase in radioactivity incorporated into muscle protein during the experimental period divided by the average of the intracellular specific radioactivities.

Tyrosine, phenylalanine, and urea were determined fluorometrically (14–17). Urinary nitrogen was determined by the Kjeldahl method and urinary free corticosterone was measured as described previously (5) using a radioimmunoassay (Cambridge Medical Diagnostics, Billerica, MA).

Statistics. Results were presented as means±SEM and were compared using analysis of variance and Student's t test. Results were considered significant at $P < 0.05$.

Results

The PUN of the CRF rats was significantly higher ($P < 0.001$) than that of SO rats, and was stable during the pair-feeding period (Table I). The presence of acidosis was defined as a blood bicarbonate < 21 meq/liter. Most (77%) of the CRF rats had a metabolic acidosis; the average blood bicarbonate of acidotic CRF rats was 16.0±0.5 meq/liter. Only two of the CRF rats receiving $NaHCO_3$ in studies 2 and 3 were acidotic; the average blood bicarbonate of CRF-HCO_3 rats did not differ from that of SO rats. The blood bicarbonate of SO rats receiving $NaHCO_3$ supplement was not changed (Table I).

The rates of urea appearance and urinary nitrogen excretion were significantly higher in the CRF rats compared with pair-fed SO rats (Fig. 1). These differences were reflected in a higher average daily weight gain (Table I) by SO rats (4.0±0.3 g/d SO vs. 3.0±0.4 g/d CRF; P = 0.052 by paired analysis). Differences in dietary protein could not account for the higher nitrogen excretion as the rats were pair fed. In study 2, the average daily weight gain of CRF-HCO_3 (3.3±0.3 g/d) was higher than the pair-fed CRF rats (2.2±0.4 g/d; $P < 0.01$). However, some of this difference could be due to the higher dietary sodium of the CRF-HCO_3 rats. In study 3, CRF and SO rats were pair fed the bicarbonate-supplemented diet. In this experiment, rats ate more of the diet per 100 g body weight than rats had eaten in study 1, and there was no difference in weight gain. However, in contrast to study 1 (Fig. 1), CRF did not increase total nitrogen excretion (604±65 CRF-HCO_3 vs. 598±25 mg nitrogen/100 g per 48 h SO-HCO_3; P = NS) or urea appearance rates (478±47 CRF-HCO_3 vs. 463±31 mg nitrogen/100 g per 48 h SO-HCO_3; P = NS). Presumably, the higher nitrogen excretion rates in this study compared with SO rats of study 1 were due to the greater protein intake.

Table I. PUN Values during the Period of Pair Feeding

Study	PUN		Blood bicarbonate	Weight	
	Initial	Final		Initial	Final
	mg/dl	*mg/dl*	*mM*	*g*	*g*
Study 1					
CRF	116±5	110±7*	16±1	237±5	293±8
SO	—	22±1	24±1	255±7	332±13
Study 2					
CRF	106±6	110±9	16±1	224±6	257±11
CRF-HCO_3	97±4	94±5	24±1	217±7	265±10
Study 3					
CRF-HCO_3	126±6	105±7*	24±1	213±6	265±8
SO-HCO_3	—	20±1	25±1	218±4	276±10

Values are means±SEM from at least 18 animals in each group. The PUN was determined at the time of the initial pairing and 2 wk later at the time of the experiment. Blood bicarbonate was determined at the time of the experiment. SO, pair-fed control; CRF-HCO_3 and SO-HCO_3, CRF and SO rats fed a diet supplemented to 1.7% sodium bicarbonate.
* $P < 0.001$ vs. SO.

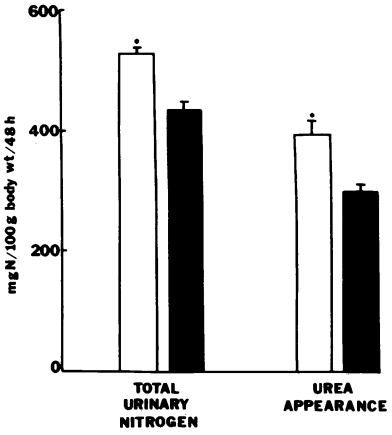

Figure 1. Total urinary nitrogen (N) and urea appearance rates of pair-fed chronically uremic (*open columns*) and SO (*solid columns*) control rats (study 1). Values are means±SEM from at least five rats in each group. *, $P < 0.005$ vs. SO.

Previously, we found that chronic metabolic acidosis increases corticosterone production. Similarly, urinary corticosterone excretion was elevated in rats with CRF compared with SO controls, although there was some overlap (15.97±2.83 CRF vs. 6.95±0.48 μg/kg per d SO; $P < 0.01$). However, the average values of corticosterone excretion did not differ between CRF rats and CRF-HCO_3 rats. By analysis of variance, the presence of CRF accounted for the increased urinary corticosterone excretion, and there was no independent effect of acidosis.

To determine the effects of chronic uremia on protein turnover, CRF rats were compared with pair-fed SO rats in study 1. In isolated hindquarters perfused without insulin, net protein degradation was significantly increased by CRF (Fig. 2). In a separate study, we measured rates of protein synthesis in anterior tibialis and lateral gastrocnemius muscles of CRF and SO rats. In the absence of insulin, CRF depressed protein synthesis in anterior tibialis (13.2±2.1 CRF vs. 20.1±2.9 nmol phenylalanine/g per h SO; $P < 0.05$), but this difference was quite small compared with the increase in net protein degradation measured in hindquarters of CRF rats (Fig. 2). Moreover, in study 2, the rate of protein synthesis was higher (23.2±4.5 nmol phenylalanine/g per h) in CRF, but not statistically different from the rate measured in study 1; the combined rate of protein synthesis of all CRF rats was 19.6±2.2 nmol phenylalanine/g per h. Thus, it seems doubtful that CRF causes a major defect in insulin-independent protein synthesis. The protein synthesis rate in lateral gastrocnemius (19.7±6.1 CRF vs. 28.4±4.2 nmol phenylalanine/g per h SO) was ~ 40% higher than that measured simultaneously in anterior tibialis muscles of the same rat (13.2±2.1 CRF vs. 20.1±2.9 nmol phenylalanine/g per h SO). Thus, differences in protein synthesis, though numerically different, were reflected in both muscles. Again, in lateral gastrocnemius, the difference in protein synthesis rates was small compared with the increase in net protein degradation caused by CRF (Fig. 2).

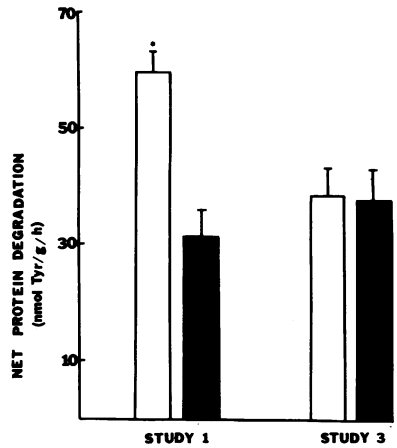

Figure 2. Net protein degradation in muscle of chronically uremic rats compared with that of pair-fed, SO control rats (*solid columns*). Values are means±SEM for hindquarters of at least eight rats in each group perfused without insulin. In study 3, rats were pair fed the diet supplemented with 1.7% $NaHCO_3$. *, $P < 0.001$ compared with control.

When CRF rats were given sodium bicarbonate, their plasma bicarbonate was corrected as was the increased net protein degradation (Fig. 2). To define how metabolic acidosis in CRF affects components of muscle turnover, CRF rats were pair fed with CRF-HCO_3 and compared in two series of experiments in study 2. In the first series, net protein degradation and protein synthesis were measured, and as shown in Table II, net protein degradation in muscles of acidotic CRF rats was significantly decreased by correcting their metabolic acidosis. In contrast, protein synthesis was unaffected by feeding bicarbonate. This suggests that muscle proteolysis was responsible for the acidosis-induced increase in net protein degradation. To address this possibility, cycloheximide was added to inhibit protein synthesis in a second set of experiments and total protein degradation was measured. Bicarbonate supplementation substantially decreased total protein degradation (140.4±7.5 CRF vs. 78.6±4.0 nmol tyrosine/g per h CRF-HCO_3; $P < 0.005$).

The influence of metabolic acidosis in CRF on insulin-stimulated muscle protein turnover was assessed by perfusing the hindquarters of rats in studies 1 and 3 with 1 mU/ml insulin and measuring both net protein degradation and protein synthesis. The hindquarters of fed SO rats perfused with insulin, glucose, and amino acids were apparently in neutral protein balance, since there was no net tyrosine release during the perfusion (Table III). Although insulin reduced the high rate of net protein degradation of CRF (Fig. 2, Table III), protein balance remained negative as indicated by the net release of tyrosine. Importantly, net protein degradation was significantly higher in hindquarters of acidotic CRF rats compared with SO rats, with CRF-HCO_3 rats, or with SO-HCO_3 rats. Moreover, net protein degradation in hindquarters of CRF-HCO_3 rats perfused with insulin was higher than that of pair-fed SO-HCO_3 rats (Table III). This contrasts with the results obtained without insulin since there was no difference in net protein degradation between these two groups (Fig. 2). The higher rate of net protein degradation in CRF-HCO_3 compared with SO-HCO_3 rats appeared to be caused by impairment of insulin-stimulated protein synthesis (Table III). Unlike protein degradation, the presence or absence of metabolic acidosis in CRF did not influence muscle protein synthesis; insulin-stimulated protein synthesis in hindquarters of CRF rats was only 60% of that measured in SO rats (Table III).

Table II. Comparison of the Effects of Acidosis on Muscle Protein Turnover in Chronic Uremia

	Net protein degradation	Protein synthesis
	nmol Tyr/g per h	*nmol Phe/g per h*
CRF	55.3±8.5*	23.2±4.5
CRF-HCO_3	36.4±5.6	20.1±3.1

Values are means±SEM from at least eight animals in each group perfused without insulin. For 2 wk, CRF rats were pair fed diets differing only in 1.7% $NaHCO_3$ (CRF-HCO_3).
* $P < 0.02$, compared with CRF-HCO_3.

Table III. Effects of Acidosis in Chronic Uremia on Muscle Protein Turnover Measured in the Presence of Insulin

	Net protein degradation	Protein synthesis
Study 1		
CRF	28.9±6.0	35.0±5.1
SO	−12.4±5.1	53.2±8.2
Study 3		
CRF-HCO_3	8.9±5.0	37.7±10.7
SO-HCO_3	−7.2±2.9	78.4±13.0

Values are means±SEM from at least five animals in each group perfused with insulin. For 2 wk, rats were pair fed; in study 3, the diet contained 1.7% $NaHCO_3$ (CRF-HCO_3, SO-HCO_3). By analysis of variance, there was an overall influence of chronic uremia on net protein degradation ($P < 0.001$) and protein synthesis ($P < 0.01$). Acidosis exerted an independent ($P < 0.001$) influence on net protein degradation, but no influence on protein synthesis was detected.

Discussion

In this report, we describe at least two abnormalities in skeletal muscle protein metabolism caused by chronic uremia that are reflected in an increase in urea appearance and urinary nitrogen excretion (Fig. 1). Firstly, in the absence of insulin, the net rate of protein degradation in hindquarters of uremic rats was nearly twice that of pair-fed, SO control rats. The mechanism for this abnormality depends on metabolic acidosis because, without changing the PUN, protein degradation could be normalized by simply adding sufficient dietary $NaHCO_3$ to correct the acidosis (Fig. 2). It should be emphasized that the degree of metabolic acidosis causing this defect was not severe (blood bicarbonate < 21 meq/liter). Presumably, the proteolytic abnormality induced by acidosis also required glucocorticoids, since we have shown previously that an increase in glucocorticoids was necessary for the accelerated protein degradation occurring in muscles of normal rats fed HCl or NH_4Cl to induce metabolic acidosis (5). CRF rats in this study exhibited a significant increase in corticosterone excretion, but increased glucocorticoid production alone could not have caused the proteolytic response because corticosterone excretion by CRF and CRF-HCO_3 rats (study 2) did not differ; yet, only the former group displayed an increase in muscle protein degradation. The second abnormality in muscle protein turnover attributable to CRF is suppression of insulin-stimulated protein synthesis. This occurred independently of metabolic acidosis since it was not corrected by bicarbonate (Table III).

In previous experimental studies of muscle protein turnover in CRF, the separate influence of metabolic acidosis has not been evaluated. Garber reported that alanine and glutamine release from muscle of fed CRF rats was increased and that radiolabeled leucine incorporation into muscle protein was decreased, which suggested that both protein degradation and synthesis were abnormal in CRF (18). Harter et al. compared net protein degradation rates in epitrochlearis muscles from uremic and control rats fed different levels of protein (2). At each level of dietary protein, net protein degradation was higher in CRF rats; the difference was greatest when 10% protein was fed even though the degree of azotemia was least in this group of rats. It is possible that the adverse effect of the 10% protein diet was the result of an inadequate protein intake, since at most 10 g of food was allowed each day and the average weight of both CRF and control rats decreased (2, 9). When CRF rats were given more protein, which lead to more severe azotemia, it is possible that these rats were acidotic, and that this contributed to the significant increase in net protein degradation compared with that in muscles of control rats. It should be reemphasized that we found no effect of uremia on basal muscle protein degradation and only a small difference in the presence of insulin if acidosis were prevented by bicarbonate (Fig. 2, Table III). In contrast to the studies of Garber (18) and Harter et al. (2), Li and Wassner (4) were unable to document any abnormalities in muscle protein turnover in the perfused hindquarters of fed CRF rats. With fasting, however, this and other groups have found that muscle protein synthesis is decreased and protein degradation is stimulated by CRF (3, 19). It is unclear why these reports differ from our findings and those discussed (2, 18). One possibility is that the fed CRF rats studied by Li and Wassner were not acidotic, since the degree of azotemia was mild (4, 19). It is unknown whether short-term fasting (10), like metabolic acidosis (5), stimulates muscle proteolysis directly, or whether in mild CRF fasting would cause acidosis and indirectly stimulate proteolysis.

Several factors in acute and chronic uremia have been implicated as causes of accelerated protein turnover, including abnormal glucose (7, 20) and vitamin D metabolism (21). Increased plasma levels of immunoreactive glucagon/proglucagon are frequently present in CRF (22), but at nonpharmacologic concentrations glucagon does not appear to change skeletal muscle metabolism (17). Although parathyroid hormone may affect skeletal muscle metabolism in normal rats, muscle of uremic rats appears to be resistant to its catabolic effects (23). It seems unlikely that feeding bicarbonate would affect these metabolic and hormonal factors, yet the present study indicates that correction of metabolic acidosis returns the abnormal muscle protein degradation and increased nitrogen excretion of CRF towards normal. These findings indicate that metabolic acidosis acts as a signal causing accelerated muscle proteolysis in CRF as well as in normal rats. Our previous studies have shown that NH_4Cl and HCl feeding to normal rats stunts growth, augments urinary nitrogen excretion, and increases muscle proteolysis, but does not change insulin-stimulated protein synthesis (5).

It is noteworthy that this catabolic effect of acidosis in normal rats required glucocorticoids. Several studies have shown that CRF is associated with abnormalities of the pituitary–adrenal axis that result in elevated plasma cortisol levels and falsely positive dexamethasone suppression tests (24, 25). The present results extend these reports by showing that CRF increases glucocorticoid excretion independently of metabolic acidosis. We have used 48-h corticosterone excretion rates as the estimate of glucocorticoid production because serum glucocorticoid levels in rats can be dramatically affected by handling or anesthetizing the animals before obtaining blood samples (26, 27).

In the presence of insulin, net protein degradation was higher both in CRF compared with pair-fed SO rats and in CRF-HCO_3 compared with pair-fed SO-HCO_3 rats (Table III). Whereas hindquarters of SO rats were in neutral protein balance when perfused with insulin, glucose, and amino acids, hindquarters of CRF-HCO_3 rats remained in negative protein balance. Since the rate of net protein degradation in muscle of SO and CRF-HCO_3 rats measured in the absence of insulin did not differ (Fig. 2), it is likely that the abnormality in muscle protein synthesis in CRF is quantitatively important only when high levels of insulin are present. In agreement with this conclusion is the observation that nitrogen excretion rates of fed CRF-HCO_3 and

SO-HCO_3 rats were not different. The mechanism(s) underlying the abnormality in insulin-stimulated muscle protein synthesis in CRF is unknown, but it seems unlikely that defective amino acid transport is responsible, since insulin stimulation of amino acid transport in muscle is preserved in acute uremia (28, 29). Zern et al. reported that albumin synthesis is reduced by CRF because of increased hepatic messenger RNA degradation (30). Whether a similar mechanism occurs in muscle or whether circulating inhibitor(s) of skeletal muscle protein synthesis (31) account for the depressed insulin-stimulated protein synthesis we observed (Table III) is unknown. The higher corticosterone production rate of CRF may have played a role since the major effect of glucocorticoids on muscle metabolism is to suppress protein synthesis (32).

These results highlight the deleterious effects of metabolic acidosis on skeletal muscle protein turnover. It is important to emphasize that the spontaneous acidosis of CRF, resulting from dietary protein, impairs both conservation of body nitrogen and muscle protein metabolism. The clinical importance of these findings is suggested by the report that $NaHCO_3$ can improve the nitrogen balance of patients with CRF (33). Correction of acidosis either by alkali supplementation or by restricting dietary protein should be a goal of therapy.

Acknowledgments

This work was supported by grants AM-37175 and AM-07131 from National Institutes of Health, and by a Clinician-Scientist Award (to Dr. Kelly) from the American Heart Association.

References

1. Coles, G. A. Body composition in chronic renal failure. 1972. *Quart. J. Med.* 41:25–47.

2. Harter, H. R., I. E. Karl, S. Klahr, and D. M. Kipnis. 1979. Effects of reduced renal mass and dietary protein intake on amino acid release and glucose uptake by rat muscle in vitro. *J. Clin. Invest.* 64:513–523.

3. Holliday, M. A., C. Chantler, R. MacDonnell, and J. Keitges. 1977. Effect of uremia on nutritionally induced variations in protein metabolism. *Kidney Int.* 11:236–245.

4. Li, J. B., and S. J. Wassner. 1986. Protein synthesis and degradation in skeletal muscle of chronically uremic rats. *Kidney Int.* 29:1136–1143.

5. May, R. C., R. A. Kelly, and W. E. Mitch. 1986. Metabolic acidosis stimulates protein degradation in rat muscle by a glucocorticoid-dependent mechanism. *J. Clin. Invest.* 77:614–621.

6. Mitch, W. E. 1981. Amino acid release from the hindquarter and urea appearance in acute uremia. *Am. J. Physiol.* 241:E415–E419.

7. Clark, A. S., and W. E. Mitch. 1983. Muscle protein turnover and glucose uptake in acutely uremic rats. *J. Clin. Invest.* 72:836–845.

8. Ruderman, N. B., C. R. S. Houghton, and R. Hems. 1971. Evaluation of the isolated perfused rat hindquarter for the study of muscle metabolism. *Biochem. J.* 124:639–651.

9. Wang, M., I. Vyhmeister, J. D. Kopple, and J. D. Swendseid. 1976. Effect of protein intake on weight gain and plasma amino acid levels in uremic rats. *Am. J. Physiol.* 230:1455–1459.

10. Goodman, M. N., M. A. McElaney, and N. B. Ruderman. 1981. Adaptation to prolonged starvation in the rat: curtailment of skeletal muscle proteolysis. *Am. J. Physiol.* 241:E321–E327.

11. Jefferson, L. S., J. B. Li, and S. R. Rannels. 1977. Regulation by insulin of amino acid release and protein turnover in the perfused rat hemicorpus. *J. Biol. Chem.* 252:1476–1483.

12. Clark, A. S., and W. E. Mitch. 1983. Comparison of protein synthesis and degradation in incubated and perfused muscle. *Biochem. J.* 212:649–653.

13. Ariano, M. A., R. B. Armstrong, and V. R. Edgerton. 1973. Hindlimb muscle fiber populations of five mammals. *J. Histochem. Cytochem.* 21:51–55.

14. Waalkes, T. P., and S. A. Udenfried. 1957. A fluorometric method for the estimation of tyrosine in plasma and tissues. *J. Lab. Clin. Med.* 50:733–736.

15. Andrews, T. M., R. Goldthorp, and R. E. Watts. 1973. Fluorometric measurements of the phenylalanine content of human granulocytes. *Clin. Chem. Acta.* 43:379–387.

16. Roman, R. J., J. V. Bonventre, and C. P. Lechene. 1979. Fluorometric assay for urea in urine, plasma and tubular fluid. *Anal. Biochem.* 98:136–141.

17. Clark, A. S., R. A. Kelly, and W. E. Mitch. 1984. Systemic response to thermal injury in rats. *J. Clin. Invest.* 74:888–897.

18. Garber, A. J. 1978. Skeletal muscle protein and amino acid metabolism in experimental chronic uremia in the rat. *J. Clin. Invest.* 62: 623–632.

19. Li, J. B., and S. J. Wassner. 1981. Muscle degradation in uremia: 3-methylhistidine release in fed and fasted rats. *Kidney Int.* 20:321–325.

20. May, R. C., A. S. Clark, M. A. Goheer, and W. E. Mitch. 1985. Specific defects in insulin-mediated muscle metabolism in acute uremia. *Kidney Int.* 28:490–497.

21. Harter, H. R., S. J. Birge, K. J. Martin, S. Klahr, and J. E. Karl. 1983. Effects of vitamin D metabolites on protein catabolism of muscle from uremic rats. *Kidney Int.* 23:465–472.

22. Bilbrey, G. L., G. R. Faloona, M. G. White, and J. P. Knochel, 1974. Hyperglucagonemia of renal failure. *J. Clin. Invest.* 53:841–847.

23. Garber, A. J. 1983. Effects of parathyroid hormone on skeletal muscle protein and amino acid metabolism in the rat. *J. Clin. Invest.* 71:1806–1821.

24. Mishkin, M. S., T. H. Hsu, W. G. Walker, and T. Bledsoe. 1972. Studies on the episodic secretion of cortisol in uremic patients on hemodialysis. *Johns Hopkins Med. J.* 131:160–164.

25. Wallace, E. Z., P. Rosman, N. Tosher, A. Sacerdote, and A. Balthazar. 1980. Pituitary-adrenocortical function in chronic renal failure: studies of episodic secretion of cortisol and dexamethasone suppressibility. *J. Clin. Endocrinol. Metab.* 50:46–51.

26. Kley, H. K., W. Elsasser, H. Dehnen, and H. L. Kruskemper. 1978. Evaluation of adrenal function in rats by the measurement of urinary free corticosterone, free aldosterone, and free 11-deoxycorticosterone. *Steroids.* 32:223–232.

27. Carney, J. A., and B. L. Walker. 1973. Mode of killing and plasma corticosterone concentrations in the rat. *Lab. Anim. Sci.* 23:675–676.

28. Maroni, B. J., G. Karapanos, and W. E. Mitch. 1986. System A amino acid transport in incubated muscle: effects of insulin and acute uremia. *Am. J. Physiol.* 251:F74–F80.

29. Maroni, B. J., G. Karapanos, and W. E. Mitch. 1986. System ASC and sodium-independent neutral amino acid transport in muscle of uremic rats. *Am. J. Physiol.* 251:F81–F86.

30. Zern, M. A., S. H. Yap, R. K. Strair, G. A. Kaysen, and D. A. Shafritz. 1984. Effects of chronic renal failure on protein synthesis and albumin messenger ribonucleic acid in rat liver. *J. Clin. Invest.* 73:1167–1174.

31. Cernacek, P., V. Spusiova, and R. Dzurik. 1982. Inhibitor(s) of protein synthesis in uremic serum and urine: partial purification and relationship to amino acid transport. *Biochem. Med.* 27:305–316.

32. Rannels, S. R., and L. S. Jefferson. 1980. Effects of glucocorticoids on muscle protein turnover in perfused rat hemicorpus. *Am. J. Physiol.* 238:E564–E572.

33. Papadoyannakis, N. J., C. J. Stefandis, and M. McGeown. 1984. The effect of the correction of metabolic acidosis on nitrogen and potassium balance of patients with chronic renal failure. *Am. J. Clin. Nutr.* 40:623–627.