

Mechanisms Contributing to Muscle Wasting in Acute Uremia: Activation of Amino Acid Catabolism

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Abstract. Acute uremia (ARF) causes metabolic defects in glucose and protein metabolism that contribute to muscle wasting. To examine whether there are also defects in the metabolism of essential amino acids in ARF, we measured the activity of the rate-limiting enzyme for branched-chain amino acid catabolism, branched-chain ketoacid dehydrogenase (BCKAD), in rat muscles. Because chronic acidosis activates muscle BCKAD, we also evaluated the influence of acidosis by studying ARF rats given either NaCl (ARF-NaCl) or NaHCO₃ (ARF-HCO₃) to prevent acidosis, and sham-operated, control rats given NaHCO₃. ARF-NaCl rats became progressively acidemic (serum [HCO₃]⁻ = 21.3 ± 0.7 mM within 18 h and 14.7 ± 0.8 mM after 44 h; mean ± SEM), but this was corrected with NaHCO₃. Plasma valine was low in ARF-NaCl

and ARF-HCO₃ rats. Plasma isoleucine, but not leucine, was low in ARF-NaCl rats, and isoleucine tended to be lower in ARF-HCO₃ rats. Basal BCKAD activity (a measure of active BCKAD in muscle) was increased more than 17-fold ($P < 0.01$) in ARF-NaCl rat muscles, and this response was partially suppressed by NaHCO₃. Maximal BCKAD activity (an estimate of BCKAD content), subunit mRNA levels, and BCKAD protein content were not different in ARF and control rat muscles. Thus, ARF increases branched-chain amino acid catabolism by activating BCKAD by a mechanism that includes acidosis. Moreover, in a muscle-wasting condition such as ARF, there is a coordinated increase in protein and essential amino acid catabolism. (J Am Soc Nephrol 9: 439–443, 1998)

When dietary protein is diminished, both humans and rats activate metabolic mechanisms to reduce the oxidative degradation of essential amino acids (1,2). This adaptive response conserves branched-chain amino acids (BCAA; leucine, isoleucine, valine) for protein synthesis. It could also influence protein turnover, because leucine and its ketoacid, α -ketoisocaproate, suppress protein degradation in muscles (3). Consequently, any stimulus that prevents or overrides the adaptive suppression of BCAA degradation could increase essential amino acid requirements and contribute to muscle atrophy. In chronic renal failure, the stimulus for increased BCAA oxidation is metabolic acidosis (4). Valine and leucine decarboxylation in incubated muscles of chronically uremic rats is higher than in muscles of similar rats given NaHCO₃ or pair-fed, sham-operated control rats (4). BCAA oxidation also is increased in muscles of normal rats fed a diet containing NH₄Cl because there is increased activity of the rate-limiting enzyme for BCAA degradation in muscle, branched-chain ketoacid dehydrogenase (BCKAD) (5,6).

BCKAD is present in two forms: an active, dephosphorylated form and an inactive, phosphorylated form produced by a kinase that is tightly associated with the BCKAD enzyme complex. The ratio of the active:inactive BCKAD forms (*i.e.*, the activation state) varies considerably among tissues and with nutritional status. In normal rats, less than 5% of the total BCKAD enzyme is typically active in muscle (7). In muscles of acidotic rats, the amount of dephosphorylated, active BCKAD is greater than in control rats, and the maximal amount of BCKAD activity (an estimate of BCKAD content) is also increased (6).

In experimental acute renal failure (ARF), there are abnormalities in glucose and protein metabolism, along with increased release of amino acids from muscle (8–11). In rats, ARF causes anorexia, which should trigger a reduction in BCAA catabolism (1,8). Because ARF rats exhibit muscle wasting despite being anorexic, we examined whether ARF overrides this adaptive metabolic response by measuring BCKAD activity in muscle. We also evaluated whether the acidosis associated with ARF contributes to this condition.

Materials and Methods

ARF Model

All studies were conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague Dawley rats (Charles River Laboratories) weighing approximately 225 g were fed a 14% protein diet *ad libitum* for 7 d before inducing ARF by bilateral ureteral ligation (8), whereas control rats underwent a laparotomy with closure. Because ARF rats eat and drink

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minimal amounts, food and water were removed after surgery to avoid the confounding influence of erratic eating and drinking, which could influence amino acid metabolism (8). After 40 h, rats were anesthetized, arterial blood was taken to measure blood gas and plasma amino acids, and gastrocnemius muscles were immediately frozen in liquid nitrogen. The bicarbonate concentration in arterial blood was used to evaluate the acid-base status *in vivo* (12). Previously, we found that varying degrees of CO₂ retention caused by anesthesia make blood pH values difficult to interpret (12).

To evaluate the influence of acidosis on BCKAD activity, some ARF rats were given 1.25 mmol NaHCO₃/100 g body wt per d by gavage (ARF-HCO₃) to prevent acidemia. To ensure that any influence of NaHCO₃ was related only to correction of the acidosis, another group of ARF rats was given an equimolar amount of NaCl (1.25 mmol/100 g body wt per d [ARF-NaCl]). Sham-operated control rats (SO-HCO₃) were given an equimolar amount of NaHCO₃. In preliminary experiments, ARF-NaCl and ARF-HCO₃ rats were studied at 6, 18, 27, or 44 h after surgery to demonstrate that acidemia was prevented (Figure 1).

BCKAD Activity

Basal and total BCKAD activities were measured for each gastrocnemius muscle as described (6). The percentage of active BCKAD was calculated for each muscle by dividing the basal activity by the respective total activity.

BCKAD Protein Content

Immunoreactive BCKAD protein content in mitochondria of gastrocnemius muscles of ARF and control, sham-operated rats was compared by Western blot analysis as described (6,13). A mitochondrial protein fraction prepared according to Greenawalt was studied (14). Equal amounts of protein (15 µg) were separated by electrophoresis in a 12% sodium dodecyl sulfate-polyacrylamide gel and were transferred to a Hybond-enhanced chemiluminescence membrane (Amersham Life Science, Arlington Heights, IL). The E1α, E1β, and E2 subunits of the BCKAD complex were detected with a polyclonal BCKAD complex antisera, using the Amersham enhanced

chemiluminescence detection system (6,15). To ensure that the corresponding band intensities for BCKAD subunits were within the linear detection range, the optimal protein:antisera ratio was determined using serial dilutions of mitochondrial protein and antisera (6,13). This protein:antisera ratio was then used to measure BCKAD protein in experimental samples. Additionally, each membrane was exposed for different times to confirm that band intensities did not exceed the linear detection range of the film. Band intensities of immunoreactive proteins were measured with a Bio-Rad densitometer (Hercules, CA).

BCKAD Subunit mRNA Analysis

Total RNA was extracted from gastrocnemius muscles of rats as described (16). RNA (15 µg) was separated by formaldehyde agarose gel electrophoresis and transferred to a Bio-Rad ZetaProbe GT membrane for hybridization (6,17). The cDNA probes used for hybridization were derived from mouse E2 and E1β subunits (gifts of Dr. Jeffrey M. Chinsky, Johns Hopkins University, Baltimore, MD), human BCKAD E1α subunit (a gift from Dr. Dean Danner, Emory University), and rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Autoradiographic band densities were quantified with the Bio-Rad densitometer. Relative amounts of BCKAD subunit mRNA were compared after correcting autoradiographic values for minor variations in gel loading, using the respective GAPDH values. GAPDH mRNA levels were not different between ARF and control rats (Table 4).

Statistical Analyses

Values are reported as means ± SEM. Comparisons between two treatment groups were performed using an unpaired *t* test, whereas three or more treatment groups were compared by one-way ANOVA followed by *post hoc*, pairwise comparisons of experimental groups using the Bonferroni test. *P* < 0.05 was considered significant.

Results

ARF rats given 1.25 mmol NaHCO₃/100 g body wt per d or an equimolar amount of NaCl lost less body weight compared with the sham-operated control rats given NaHCO₃ (Table 1). ARF-NaCl rats became acidemic within 18 h, and the severity of the acidemia increased with time (Table 1, Figure 1). Giving ARF rats oral NaHCO₃ prevented acidemia (Table 1, Figure 1).

Plasma levels of valine and isoleucine in ARF-NaCl rats were significantly diminished compared with values in SO-HCO₃ rats (Table 2). Valine, but not isoleucine, was also

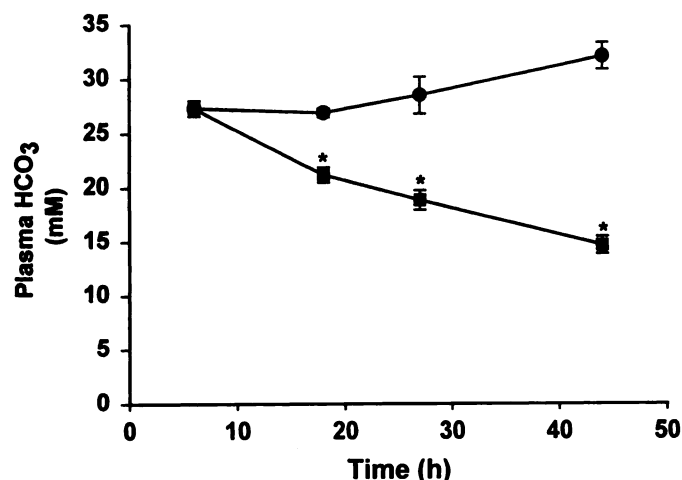


Figure 1. Bicarbonate administration prevents acute renal failure (ARF) rats from developing acidosis. ARF rats were given 1.25 mmol of NaHCO₃ or NaCl/100 g body wt per d. At the indicated times, arterial blood was drawn for blood gas analysis. Circles represent ARF rats given NaHCO₃, and squares represent ARF rats given NaCl. * *P* < 0.05 versus ARF-NaHCO₃ rats.

Table 1. Weight and acid-base status of ARF rats^a

Category	SO-HCO ₃	ARF-NaCl	ARF-HCO ₃
Initial weight	224 ± 2	223 ± 3	224 ± 5
Final weight	193 ± 2	208 ± 2 ^b	209 ± 5 ^b
Arterial [HCO ₃]	25.6 ± 0.8 ^c	13.3 ± 0.6 ^c	29.1 ± 0.5 ^c

^a Rats were randomly divided into three groups: sham-operated given NaHCO₃ (SO-HCO₃); ARF rats given HCO₃ (ARF-HCO₃); and ARF rats given NaCl (ARF-NaCl). Results are expressed as the mean ± SEM for six to nine rats per group at the time of surgery or 40 h after surgery. ARF, acute renal failure.

^b By ANOVA, *P* < 0.05 versus SO-HCO₃ rats.

^c *P* < 0.05 for all groups.

Table 2. The influence of ARF on plasma branched-chain amino acid concentrations^a

Group	Valine	Isoleucine	Leucine
SO-HCO ₃ (9)	307 ± 9	144 ± 6	240 ± 11
ARF-NaCl (9)	268 ± 11 ^b	111 ± 10 ^b	248 ± 22
ARF-HCO ₃ (5)	233 ± 11 ^b	128 ± 9	228 ± 16

^a Plasma branched-chain amino acid concentrations (μM) were measured in arterial blood of SO-HCO₃, ARF-HCO₃, and ARF-NaCl rats 40 h after surgery. Results are expressed as mean ± SEM, and the number of rats is indicated in parentheses. Abbreviations as in Table 1.

^b By ANOVA, *P* < 0.05 versus sham-HCO₃ rats.

statistically lower in ARF-HCO₃ rats (Table 2). The levels of leucine were not statistically different in ARF-HCO₃, ARF-NaCl, and SO-HCO₃ rats.

The basal level of BCKAD activity was increased more than 17-fold (*P* < 0.01) in muscles of ARF-NaCl rats compared with SO-HCO₃ rats (Table 3). In ARF rats given NaHCO₃, basal BCKAD activity in muscle was approximately 3 times higher than the value measured in SO-HCO₃ rat muscles, but this change was not statistically significant. The maximal level of BCKAD activity, a measure of the amount of BCKAD complex in muscle, was not different between ARF-HCO₃, ARF-NaCl, and SO-HCO₃ rat muscles. When the activation state of BCKAD was calculated, ARF complicated by acidemia resulted in an increase in BCKAD activation by more than 18-fold above SO-HCO₃. Although giving ARF rats NaHCO₃ reduced this activation of BCKAD, it remained threefold higher than in muscles of SO-HCO₃ rats (Table 3).

Because chronic metabolic acidosis in normal rats increases the levels of mRNA for BCKAD subunits in muscle (6), we compared these mRNA levels in muscles of ARF-HCO₃, ARF-NaCl, and SO-HCO₃ rats. The levels of E1α, E1β, and E2 mRNA were not different in muscles of acidotic and nonacidotic ARF and sham-operated rats (Table 4). We also compared the relative amounts of BCKAD E1α, E1β, and E2 subunit proteins in mitochondrial proteins isolated from muscle, using Western blot techniques and polyclonal antibodies to

Table 3. BCKAD activity in muscles of ARF and control rats^a

Group	BCKAD Activity (nmol/min per g protein)		Activation State
	Basal	Total	
Sham-HCO ₃ (13)	31 ± 5	2045 ± 124	1.5 ± 0.3
ARF-NaCl (9)	536 ± 101 ^b	2006 ± 78	27.7 ± 5.3 ^b
ARF-HCO ₃ (9)	95 ± 25	1869 ± 234	4.8 ± 1.0

^a Results are expressed as mean ± SEM, and the number of rats is indicated in parentheses. Activation state is the basal activity divided by the total activity × 100. BCKAD, branched-chain ketoacid dehydrogenase. Other abbreviations as in Table 1.

^b By ANOVA, *P* < 0.01 versus SO-HCO₃ and ARF-HCO₃ rats.

the BCKAD complex. No differences in the amounts of BCKAD subunit proteins were detected (Figure 2).

Discussion

Mortality remains high in ARF patients despite different nutritional support strategies; nitrogen balance is negative and urea production is increased, indicating that muscle protein catabolism is accelerated (18). Accelerated muscle protein degradation and depressed protein synthesis can be demonstrated in rats with experimental ARF (8,10,11,19,20), but the impact of ARF on the irreversible degradation of BCAA has not been studied. On the basis of the accelerated proteolysis and depressed protein synthesis in muscles of rats with ARF (8,9,11), we expected that the plasma levels of BCAA would be increased, but the levels of these essential amino acids were comparable to or lower than those in control rats. Because there were no differences in dietary amino acid intake between ARF and sham-operated control rats and because amino acid uptake by muscle is depressed (21,22), BCAA oxidation must be increased. This was confirmed by finding higher BCKAD activity in ARF rat muscle. These results provide evidence for integrated regulation of BCAA and protein degradation in muscle of ARF rats, similar to the catabolic processes occurring in muscles of rats with chronic uremia, sepsis, cancer, and thermal injury (23). The questions that remain unanswered are: What is the stimulus activating BCKAD? and How is the change in BCKAD achieved?

In chronic uremia, the principal stimulus causing muscle proteolysis and BCAA oxidation is metabolic acidosis, because correcting the associated acidemia resulted in a normalization of the accelerated rate of protein degradation and BCAA oxidation in muscles of chronically uremic rats (4,12,24). Likewise, in normal rats given NH₄Cl to produce acidosis without azotemia, there is increased muscle proteolysis and BCKAD activity in muscle (5,6). The higher BCKAD activity results from an increase in both the activation state of BCKAD and maximal enzyme activity (6). ARF rats also progressively develop acidemia. When the acidosis of ARF is prevented with oral NaHCO₃, the statistically significant increase in BCKAD activity is blocked, but the basal activity of BCKAD in muscles of ARF-HCO₃ rats was still three times higher than the value in muscles of control rats. This suggests there may be a stimulus of BCKAD activity in ARF that is independent of acidosis (and apparently not present in rats with chronic uremia). In fact, stimuli other than acidosis have been found to independently influence BCKAD activity and BCKAD protein content (13).

In nonacidemic ARF rats, the plasma levels of valine were below those in control rats and there also was a downward trend in the levels of isoleucine. The mechanism for this result was not identified, but Bergstrom *et al.* reported similar findings when they measured the plasma and free amino acids in muscle of hemodialysis patients (25). One potential explanation involves BCKAD. It is notable that valine is the preferred BCAA substrate for purified BCKAD (26). Although basal activity of BCKAD and its activation state were not statistically higher in muscles of ARF-HCO₃ rats compared with

Table 4. BCKAD subunit mRNA levels in ARF and sham rat muscles^a

Group	GAPDH	BCKAD Subunit		
		E1 α	E1 β	E2
SO-HCO ₃	2.409 \pm 0.23	2.56 \pm 0.18	2.69 \pm 0.23	1.17 \pm 0.11
ARF-NaCl	2.398 \pm 0.26	2.97 \pm 0.20	2.915 \pm 0.23	1.45 \pm 0.07
ARF-HCO ₃	2.745 \pm 0.23	3.03 \pm 0.13	2.845 \pm 0.15	1.40 \pm 0.08

^a Total RNA was extracted from gastrocnemius muscles of SO-HCO₃, ARF-NaCl, and ARF-HCO₃ rats and analyzed by Northern blot hybridizations with cDNA probes to human BCKAD E1 α , mouse BCKAD E1 β , or E2 subunits or rat GAPDH. Autoradiographs were subjected to densitometric scanning. Results are expressed in arbitrary units and represent the mean \pm SEM of the ratios of each BCKAD subunit value divided by the respective GAPDH value ($n = 6$ for each group). By ANOVA, there were no statistically significant ($P < 0.05$) differences between experimental groups for GAPDH or each BCKAD subunit. Abbreviations as in Tables 1 and 3.

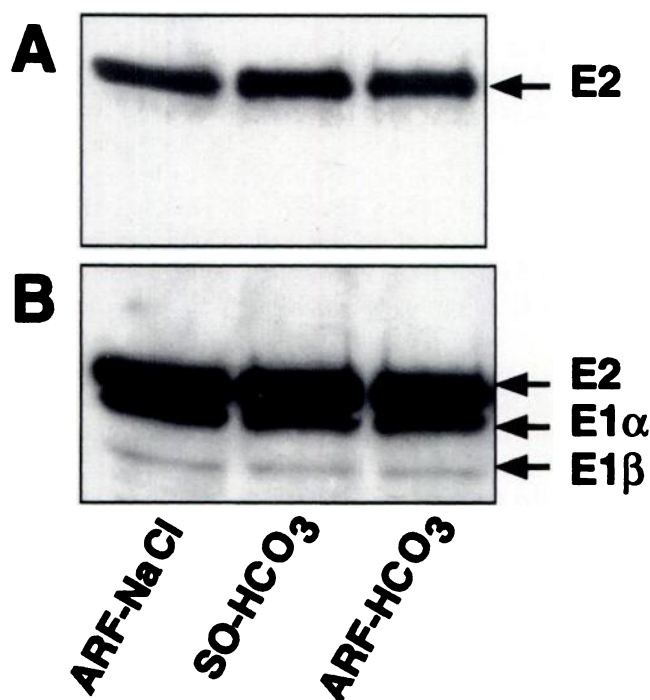


Figure 2. ARF does not alter the levels of branched-chain ketoacid dehydrogenase (BCKAD) subunit proteins in muscle. Mitochondrial fractions were prepared from muscles of SO-HCO₃, ARF-NaCl, and ARF-HCO₃ rats. A polyclonal antisera against the BCKAD complex was used to detect the E1 α , E1 β , and E2 subunits in equal amounts of mitochondrial protein. Representative results are shown. (A) The blot was exposed to film for 2 min to avoid overexposure of the E2 subunit band. (B) The same blot was exposed to film for 10 min to detect the E1 α , E1 β , and E2 subunits.

control rats, the activities were almost three times greater than in muscles of control rats given NaHCO₃. Alternatively, feeding NaHCO₃ to acutely uremic rats or patients may suppress protein degradation and amino acid release below the levels in control rats fed NaHCO₃.

In NH₄Cl-fed normal rats, the increase in muscle BCKAD activity involved both an activation of the enzyme (*i.e.*, dephosphorylation of BCKAD) and an increase in BCKAD production. Consistent with increased maximal BCKAD activity, the levels of mRNA encoding the E1 α , E1 β , and E2 BCKAD

subunits in muscle were found to be higher in acidotic rats. Because acidemia in normal rats increases the maximal activity of BCKAD, it is not clear why acidosis did not increase maximal BCKAD activity in muscles of ARF rats. This difference could be due to the relatively brief duration of the catabolic state (approximately 44 h in ARF compared with more than 2 wk of acidosis in chronic renal failure or 5 d of NH₄Cl-induced acidosis in normal rats). Alternatively, acute uremia may interfere with transcription of BCKAD subunit genes, thus blocking the mechanism for increased maximal BCKAD activity.

In summary, ARF increases BCKAD activity in muscle. As with chronic uremia, metabolic acidosis is an important stimulus for this catabolic process that impairs the ability of the organism to adapt to dietary protein restriction. We believe that these findings provide evidence for an integrated catabolic response to pathologic conditions, including degradation of muscle protein and irreversible oxidation of BCAA in a manner that maintains the available amino acid pools.

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