Effects of Reduced Renal Mass and Dietary Protein Intake on Amino Acid Release and Glucose Uptake by Rat Muscle In Vitro

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ABSTRACT Epitrochlearis muscles obtained from normal male Holtzman rats used as controls (C) and rats with reduced renal mass (Nx) fed isocaloric diets of varying protein content were incubated in Krebs-Ringer buffer containing 5 mM glucose for 1 or 3 h with or without insulin.

Alanine (ALA) release rates from muscles of Nx rats were increased 40% above C values after 1 h of incubation regardless of protein intake. Addition of insulin decreased the ALA release from muscles of Nx rats to C values in animals fed 10 and 20% casein and chow but did not in rats fed 40% casein. After 3 h of incubation, all ALA release rates decreased by ≅40%. The ALA release from muscles of Nx rats fed 10% casein was comparable to C values and decreased further with the addition of insulin. On the other hand, ALA release from muscles of Nx rats fed 20 and 40% casein as well as chow remained significantly elevated above C values, but responded to the addition of insulin with a reduction in release rates to C values, except from the muscles of Nx animals fed 40% casein.

Tyrosine (TYR) and phenylalanine (PHE) release rates also were increased in muscles from Nx rats compared with C after 1 h of incubation. Release rates were highest in the Nx group fed 10% casein and decreased with increasing protein intake. Addition of insulin decreased the release rates of Nx rats to C values in each group. After 3 h of incubation, release rates of TYR and PHE in muscles from Nx rats remained significantly above C values for all groups, but responded to the addition of insulin with a decrease to C values. Glutamine and glutamate release were not significantly affected by reduction in renal mass.

Base-line glucose uptake by all groups of muscles from Nx rats was significantly greater than corresponding C values, but maximal insulin-stimulated glucose uptake was comparable in all groups. Tissue pool sizes for glycogen, ATP, phosphocreatine, ALA, glutamate, and glutamine were unaffected by reduction in renal mass.

The results indicate that Nx is associated with accelerated ALA, TYR, and PHE release from muscle. ALA release rose with increasing protein intake and decreased to values observed from C muscles after addition of insulin except in Nx animals fed 40% casein. TYR and PHE release decreased with increasing protein intake and also decreased to C values with the addition of insulin. The data also suggest that ALA release is not dependent upon glucose uptake in muscles from either C or Nx rats.

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INTRODUCTION

Uremia is associated with a number of metabolic derangements, including carbohydrate intolerance (1, 2), abnormal lipid metabolism (3), and elevated plasma levels of several hormones, such as growth hormone (4), glucagon (5), parathyroid hormone (6), and insulin (1, 4). Uremia may also be accompanied by a catabolic state characterized by muscle wasting (7, 8), decreased production of albumin (9, 10), and abnormalities in plasma amino acids, primarily determined by the dietary protein intake (7, 11). Wang et al. (7) have demonstrated that uremic rats use protein less efficiently and have impaired growth despite adequate protein and caloric intake. The incorporation of L-[^{14}C]leucine into muscle protein was shown to be reduced in uremia, whereas L-[^{14}C]leucine incorporation in liver was increased (11). Furthermore, the conversion of phenylalanine to tyrosine may be impaired, possibly because of the inhibition of the enzyme phenylalanine hydroxy-

lase by the uremic state (12). Previous studies have also demonstrated that the hepatic levels of alanine aminotransferase are increased by uremia and are responsive to changes in dietary protein intake (13). Furthermore, Garber (14) has demonstrated increased release of glutamine, glutamate, and alanine from uremic muscles studied in vitro without an effect on alanine utilization. The augmented release of these amino acids was felt to be the result of a decreased responsiveness to epinephrine reflected by decreased tissue cyclic AMP production in response to this hormone (15). Thus many derangements in protein metabolism have been demonstrated in uremia, but no systematic evaluation of the effects of uremia and diet on muscle protein metabolism exists.

Previous studies from this laboratory have demonstrated that the epitrochlearis muscle from rats can be rapidly removed and incubated in vitro for up to 6 h with stable metabolic parameters. Intracellular constituents during incubation approximate those of freeze-clamped muscles in vitro (16). Inasmuch as alanine, glutamine, and glutamate account for >70% of the total amino acids released from muscle, and because their release rate most likely reflects their formation from other amino acids (17), studying these amino acids provides an insight into protein metabolism in muscle. Tyrosine and phenylalanine release rates were also measured. The epitrochlearis muscle was therefore used to evaluate the effects of diet and reduced renal mass on glucose uptake, and pyruvate, lactate, and amino acid release in vitro.

METHODS

Animal studies. Male Holtzman rats with normal or decreased renal mass weighing 130–140 g were housed in individual metabolic cages under constant humidity and temperature. The animals were fed either Purina chow (Ralston Purina Co., St. Louis, Mo.) or the diets described below, and were given one-quarter normal saline to drink (18).

Surgical procedures. Renal mass was reduced (Nx)[1] by five-sixths nephrectomy as previously described (19) and summarized briefly here. Using diabutol anesthesia, 35 mg/kg body wt, the rats underwent a 70–75% left renal infarction by ligating most of the terminal branches of the renal artery. 1–2 d later, a right nephrectomy was performed. The sham-operated pair-fed control animals (C) were also anesthetized and the abdominal cavity entered, but no other surgical procedure was performed. After surgery, all animals were placed in individual metabolic cages and fed their respective diets, and were studied 10 d after surgery.

Diets. Rats were pair-fed either standard rat chow in powdered form (Ralston Purina Co.) containing 23% protein from both animal and vegetable sources or isocaloric diets containing 10, 20, or 40% casein. The animals were pair-fed by initially giving both C and Nx animals 10 g of their respective diets. Thereafter, the quantity of food eaten by the Nx rat was given to the C animal the following day. Because the Nx animals ate at a slower rate, the quantity of food eaten by each Nx animal was fed to the C animal in two divided feedings, morning and afternoon. The composition of the 20% casein diet used in this study has been previously described (20), and the other diets were prepared from the 20% casein diet by exchanging protein for sucrose to maintain an isocaloric intake. The casein and vitamin mixtures were obtained from ICN Nutritional Biochemicals Div., Cleveland, Ohio. To ensure stable salt and trace metal intake, salt mixtures for the diet were divided into two parts. Salt mixture 1 contained $CaCO_3$, $MgSO_4$, $FeSO_4$, $CuSO_4$, KF, $MnSO_4$, $CdCl_2$, and NaI. Salt mixture 2 contained NaH_2PO_4, Na_2HPO_4, NaCl, K_2HPO_4, and KH_2PO_4. Each salt mixture was added to the synthetic diet in amounts appropriate to allow for the needed daily requirements of salt and trace minerals as defined by Ralston Purina Laboratories (21).

Experimental procedure. All animals were studied in the fed state and had free access to one-fourth normal saline as their drinking solution. Blood samples were obtained from each animal for the determination of glucose, urea nitrogen, phosphate and creatinine, glucagon, and insulin. The animals were killed by a blow to the head and to the lumbar vertebral column to prevent muscle spasm. The epitrochlearis muscles, weighing 15–30 mg, were then removed rapidly and washed initially in a saline Krebs-Ringer bicarbonate buffer at pH 7.4. They were blotted and placed in 500 μl of Krebs-Ringer bicarbonate containing 5 mM glucose, 5 mM Hepes buffer, and, where specified, 0.01 U/ml of purified glucagon-free porcine insulin, Lilly lot 615-D63-10 (Eli Lilly and Company, Indianapolis, Ind.). The calcium concentration of the Krebs-Ringer buffer was reduced to 1.2 mM as albumin was not added to the incubation media. One muscle from each animal was incubated with insulin, the other without insulin. Muscles were randomly assigned to each group. For determination of zero time tissue pool sizes, the muscles were rapidly removed, washed, blotted, frozen in liquid nitrogen, and stored at −80°C until assays were performed.

The muscles were incubated for either 1 or 3 h using continuous 95% O_2, 5% CO_2 bubbling in a metabolic shaker (88 oscillations/min) at 37°C. Solutions were prepared fresh on the day of the study and assayed for sodium, potassium, phosphate, calcium, glucose, and pH before use to ensure identical composition in all studies. The pH of the incubation medium was also determined randomly at the end of the incubation. After incubation, the muscles were quickly removed, blotted, and frozen in liquid nitrogen. Incubation media were then placed in ice and subsequently heated for 2 min at 90°C to inactivate enzymes released from the muscles (16). Media and muscles were stored at −80°C until the assays were performed.

Release rates for alanine, glutamine, glutamate, tyrosine, phenylalanine, pyruvate, and lactate as well as glucose uptake were determined for each muscle. All uptake and release rates are expressed as nanomoles per gram wet weight per minute; intracellular levels are expressed as nanomoles per milligram wet weight. Each value represents the mean±SEM. Significance was determined by Student's *t* analysis for unpaired samples.

Assays. Plasma glucose, urea, phosphate, and creatinine were determined by techniques described previously (22). Enzymatic fluorometric techniques were adapted for determination of glucose (23), pyruvate (24), alanine (24), lactate (23), glutamine, and glutamate (16). Tyrosine and phenylalanine were assayed by using a transfer-RNA method as described by Rubin et al. (25). Insulin (26) and glucagon

[1] *Abbreviations used in this paper*: BUN, blood urea nitrogen; C, sham-operated pair-fed controls; Nx, reduced renal mass; PTH, parathyroid hormone.

TABLE I

Plasma Levels of Glucose, Phosphate, BUN, Creatinine, Insulin, and Glucagon in C and Nx Rats

Diet	Glucose	Phosphate	BUN	Creatinine	Insulin	Glucagon
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*μU/ml*	*pg/ml*
10% Casein						
C(22)	130±2	8.2±0.3	11.2±1.2	0.35±0.02	12.8±3.8	167±21
Nx(22)	118±3*	8.2±0.2	29.4±6.5‡	0.60±0.03*	8.2±2.2	575±49‡
20% Casein						
C(40)	132±4	8.4±0.2	13.3±0.9	0.37±0.02	50.0±7.7‡	167±12
Nx(37)	123±4	7.8±0.3	36.5±2‡	0.86±0.06‡	16.8±3.3	496±20.5‡
Chow						
C(36)	120±2	8.9±0.3	11.8±0.5	0.39±0.01	49.4±3.4*	163±10
Nx(28)	125±3	8.6±0.3	47.2±2.9‡	1.00±0.05‡	34.1±3.6	527±59‡
40% Casein						
C(24)	130±2	9.0±0.2	37.2±1.7	0.24±0.02	52.2±8.0‡	157±27
Nx(18)	134±3	9.6±0.6	106.8±2.8‡	0.76±0.06‡	29.4±2.5	632±73‡

Number of animals studied appear in parentheses.
* $P < 0.05$ compared with corresponding C.
‡ $P < 0.001$ compared with corresponding C.

(27) were assayed using techniques previously described from this center. The preparation of tissues and the enzymatic reactions used to determine tissue levels of ATP, phosphocreatinine, alanine, glutamine, and glutamate have been described previously (16). Tissue glycogen content was determined by a modification of the method of Huijing (28).

Reagents. Adenylate kinase (EC 2.7.4.3), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glutamate dehydrogenase (EC 1.4.1.3), glutaminase (EC 3.5.1.2), hexokinase (EC 2.7.1.1), lactate dehydrogenase (EC 1.1.1.27), NAD^+, NADH, and $NADP^+$ were obtained from the Sigma Chemical Co. (St. Louis, Mo.).

RESULTS

Rats fed the 10% casein diet did not gain weight over the 10-d period. Nx rats lost 0.6±0.8 g and C rats 1.1 ±0.9 g during the course of the study on an average daily intake of 8.5±0.3 g. Nx animals fed 20% casein gained 21.3±2.2 g and C animals 27.5±2.3 g ($P < 0.05$) on an average daily intake of 10.4±0.2 g. The 40% casein-fed Nx rats gained 29.7±2.9 g and the C rats 30.1±2.8 g ($P > 0.1$) on an average daily intake of 13.0 ±0.4 g. Chow-fed Nx rats gained 21.6±4.8 g, whereas the C rats gained 24.7±2.2 g ($P < 0.1$) on an average daily intake of 11.8±0.3 g.

Table I summarizes the mean values for plasma glucose, phosphate, blood urea nitrogen (BUN), creatinine, insulin, and glucagon in the different groups of rats. There was no significant difference in plasma glucose among the various groups studied except for the Nx animals fed 10% casein. BUN levels rose in all Nx groups as well as with increases in dietary protein. C rats fed 40% casein had a significant increase in BUN levels when compared with C rats in the other dietary groups ($P < 0.01$) and had values comparable to Nx animals fed 10 or 20% casein. Creatinine levels were significantly increased in all four groups of Nx rats. Plasma insulin rose in all groups as protein intake increased and was significantly higher in the C group compared with Nx animals except in the group fed 10% casein. Glucagon levels, on the other hand, were significantly higher in the Nx groups compared with C groups.

Alanine release. Alanine release rates during 1 h of incubation from muscles of C or Nx rats fed the different diets are shown in Fig. 1. Alanine release rates were significantly greater from Nx muscles when compared with C muscles when the animals were fed 10, 20, or 40% casein or chow ($P < 0.005$ compared with

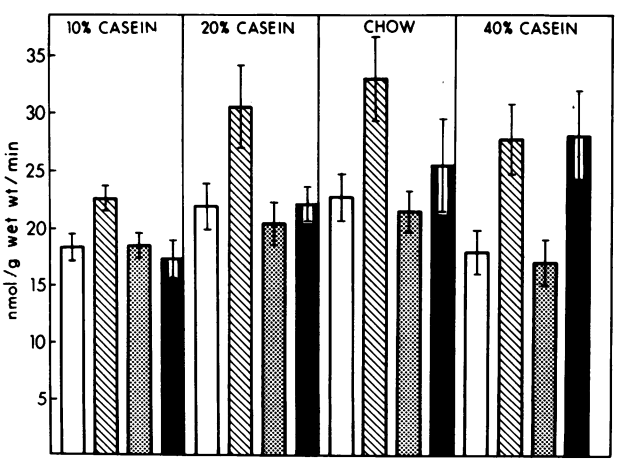

FIGURE 1 Effects of varying protein intakes on alanine release from C and Nx muscles after 1 h of incubation. □, muscles from C rats (n = 18); ▨, muscles from Nx rats (n = 18); ▩, muscles from C rats incubated with insulin (n = 18); ■, muscles from Nx rats incubated with insulin (n = 18).

corresponding C). Muscles from Nx animals fed 10% casein released significantly less alanine than did Nx muscles from other groups ($P < 0.01$). When insulin was added to the incubating media, no effect was seen in C muscles, but muscle alanine release decreased to control levels in the Nx groups fed 10 or 20% casein as well as chow. No insulin effect was seen in either C or Nx groups fed 40% casein.

Alanine release rates after 3 h of incubation are shown in Fig. 2. All values were ≅40% lower when compared with the corresponding 1-h values. Both C and Nx muscles from animals fed 10% casein released alanine at a comparable rate and further decreased the release with the addition of insulin ($P < 0.001$ compared with noninsulin-treated muscle). On the other hand, muscles from Nx animals fed 20% casein or chow released significantly more alanine than their corresponding controls ($P < 0.001$), and the addition of insulin caused an enhanced suppression of alanine release compared with the effects seen in C muscles. It is of interest to note that whereas the C muscles from the group fed 40% casein significantly reduced their alanine release after 3 h of incubation and further responded to the addition of insulin, the muscles from Nx animals continued to release alanine at an accelerated rate ($P < 0.001$ compared to C). The addition of insulin to the media caused an enhanced suppression of alanine release from Nx muscles although the release did not return to C values.

Tyrosine and phenylalanine release. Tyrosine release rates after 1 h of incubation (Fig. 3) were highest in the 10% casein-fed Nx group ($P < 0.01$ compared with other Nx groups) and were significantly greater in all Nx groups compared with C values ($P < 0.01$). Although insulin did not affect tyrosine release rates from C muscles, the addition of insulin did decrease the release rate from Nx muscles to C values in all groups studied. Tyrosine release rates remained constant over 3 h for all Nx and C groups not incubated with insulin as depicted in Fig. 4. In each group studied, release rates from Nx muscles were significantly greater than corresponding C values ($P < 0.001$). With the addition of insulin to the incubating media, tyrosine release was significantly reduced from C muscles of all dietary groups ($P < 0.01$ compared with noninsulin-treated C), and the insulin effect was greatest in the chow- and 40% casein-fed groups. Insulin reduced tyrosine release to C values in all Nx groups except the animals fed 20% casein ($P < 0.05$).

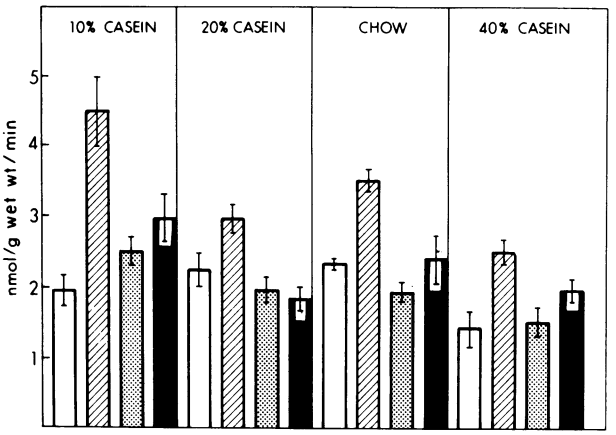

FIGURE 3 Effects of varying protein intakes on tyrosine release from C and Nx muscles after 1 h of incubation. □, muscles from C rats (n = 10); ▨, muscles from Nx rats (n = 10); ▩, muscles from C rats incubated with insulin (n = 10); ■, muscles from Nx rats incubated with insulin (n = 10).

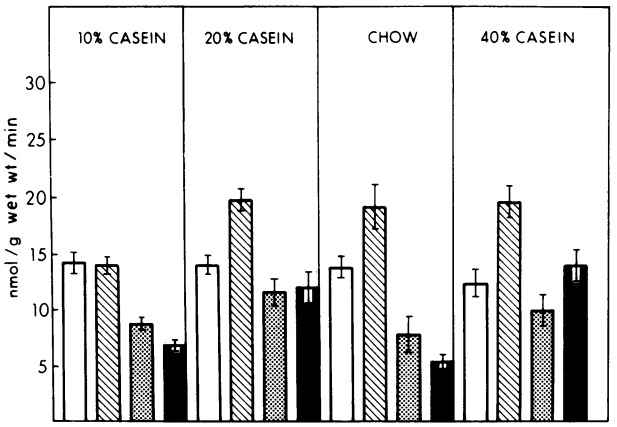

FIGURE 2 Effects of varying protein intakes on alanine release from C and Nx muscles after 3 h of incubation. □, muscles from C rats (n = 18); ▨, muscles from Nx rats (n = 18); ▩, muscles from C rats incubated with insulin (n = 16); ■, muscles from Nx rats incubated with insulin (n = 16).

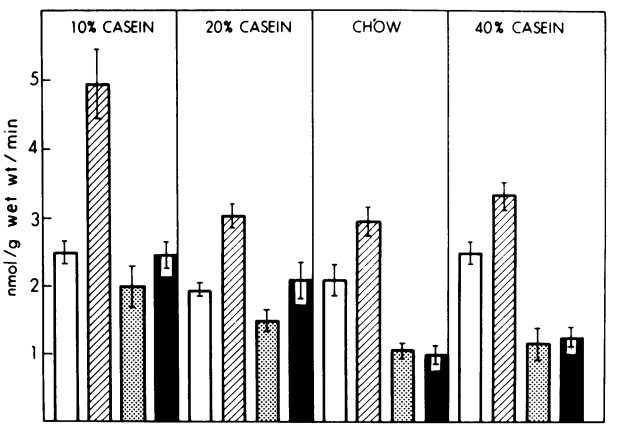

FIGURE 4 Effects of varying protein intakes on tyrosine release from C and Nx muscles after 3 h of incubation. □, muscles from C rats (n = 10); ▨, muscles from Nx rats (n × 10); ▩, muscles from C rats incubated with insulin (n = 10); ■, muscles from Nx rats incubated with insulin (n = 10).

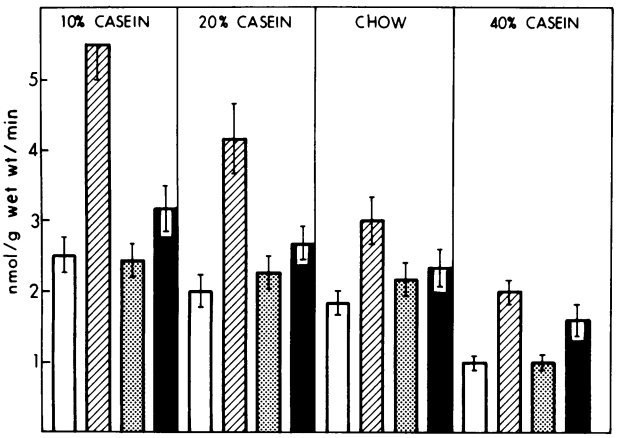

FIGURE 5 Effects of varying protein intakes on phenylalanine release from C and Nx muscles after 1 h of incubation. □, muscles from C rats (n = 10); ▨, muscles from Nx rats (n = 10); ▩, muscles from C rats incubated with insulin (n = 10); ■, muscles from Nx rats incubated with insulin (n = 10).

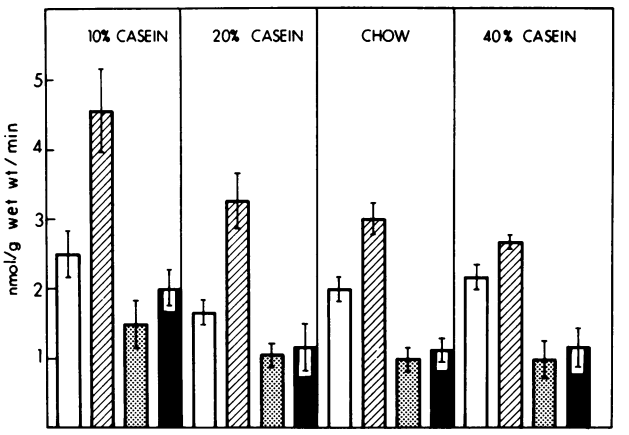

FIGURE 6 Effects of varying protein intakes on phenylalanine release from C and Nx muscles after 3 h of incubation. □, muscles from C rats (n = 10); ▨, muscles from Nx rats (n = 10); ▩, muscles from C rats incubated with insulin (n = 10); ■, muscles from Nx rats incubated with insulin (n = 10).

After 1 h of incubation (Fig. 5), the release rates for phenylalanine decreased in both C and Nx groups as dietary protein was increased and were significantly greater in the corresponding Nx group compared with C ($P < 0.001$). Insulin did not affect phenylalanine release from C muscles but reduced release in all Nx groups to C values ($P < 0.001$), although not completely in the 10 and 40% casein-fed groups. Release rates after 3 h of incubation (Fig. 6) remained constant in all C and Nx groups and were significantly greater in the corresponding Nx groups ($P < 0.01$). Insulin produced a significant reduction in phenylalanine release rates in all C groups ($P < 0.001$ compared with noninsulin-treated C), and all Nx groups responded to insulin with a reduction in release to their corresponding C values.

Glutamine release (Table II). Muscles from Nx rats had no increase in glutamine release after 1 h, and insulin had no effect on the release rates in either C or Nx rats. Muscles obtained from Nx rats fed 40% casein had a slight but not significant increase in gluta-

TABLE II
Glutamine and Glutamate Release Rates

	1 h of incubation				3 h of incubation			
	Glutamine		Glutamate		Glutamine		Glutamate	
Diet	−I*	+I‡	−I	+I	−I	+I	−I	+I
	nmol/g wet wt/min				*nmol/g wet wt/min*			
10% Casein								
C(12)	26.4±1.7	23.7±1.3	7.5±0.7	7.1±0.4	19.9±0.9	15.7±1.6	3.2±0.2	3.9±0.4
Nx(12)	24.8±0.9	24.7±2.0	7.5±0.5	7.2±0.4	18.8±0.8	14.1±0.9	3.9±0.3	3.5±0.2
20% Casein								
C(18)	32.2±1.7	30.8±1.5	7.8±0.9	8.6±0.7	24.7±1.1	20.4±1.1	3.2±0.4	3.6±0.5
Nx(18)	33.9±2.1	31.5±1.5	8.7±1.1	9.5±1.2	27.9±2.1	21.7±2.4	3.6±0.3	2.9±0.3
Chow								
C(16)	33.3±2.2	31.9±2.1	8.7±0.6	8.7±0.5	22.2±0.8	15.0±0.8	3.6±0.3	2.9±0.3
Nx(16)	34.8±1.9	26.5±2.8	9.7±0.9	9.6±1.0	20.1±0.2	13.1±0.7	2.8±0.2	3.0±0.3
40% Casein								
C(18)	29.2±2.0	27.8±1.8	10.4±0.9	9.3±0.7	20.0±0.8	16.7±0.7	5.3±0.6	5.6±0.7
Nx(18)	33.9±2.6	30.6±2.9	13.6±1.8	11.5±1.6	22.2±0.9	17.3±1.5	6.9±0.8	6.1±0.8

Number of muscles studied appear in parentheses.
* Without insulin.
‡ With insulin.

TABLE III
Lactate and Pyruvate Release Rates

Diet	1 h of incubation				3 h of incubation			
	Lactate		Pyruvate		Lactate		Pyruvate	
	−I*	+I‡	−I	+I	−I	+I	−I	+I
	nmol/g wet wt/min				*nmol/g wet wt/min*			
10% Casein								
C(12)	371±53	393±23	30±2.2	31±2.0	196±23	202±20	15±0.4	16±0.7
Nx(12)	328±35	419±36	29±1.9	30±1.2	213±14	238±22	19±1.0	18±0.7
20% Casein								
C(18)	329±34	399±27	25±1.4	25±1.7	353±22	324±61	18±1.2	17±3.5
Nx(18)	330±46	408±40	22±2.0	25±2.3	368±65	313±41	21±2.8	23±3.5
Chow								
C(16)	453±47	546±37	26±2.5	26±1.7	347±31	446±44	18±0.5	18±1.8
Nx(16)	454±63	574±55	27±2.3	28±1.0	406±53	418±56	20±2.0	20±2.0
40% Casein								
C(18)	546±59	576±41	29±2.6	31±2.0	316±30	346±30	18±1.3	23±2.3
Nx(18)	550±43	494±32	38±2.7	38±3.2	299±30	316±23	24±2.8	26±3.1

Number of muscles studied appear in parentheses.
* Without insulin.
‡ With insulin.

mine release. On the other hand, muscles of both C and Nx rats fed 10% casein had significantly lower glutamine release rates than the other groups ($P < 0.01$).

After 3 h of incubation, glutamine release fell significantly in all groups ($P < 0.001$ compared with 1-h values). No difference in release rates was noted between C and Nx rats. Insulin decreased release rates significantly in all groups ($P < 0.01$ compared with noninsulin-treated muscles).

Glutamate release (Table II). Glutamate release rose as protein intake was increased in both C and Nx rats and was most marked in Nx rats. The muscles from Nx animals fed 40% casein had a release rate significantly greater than the corresponding C muscles ($P < 0.05$). Release rates of C and Nx rats fed 40% casein and Nx rats fed chow were significantly greater than the release rates from muscles of animals fed 10% casein ($P < 0.01$). Insulin did not significantly affect these release rates.

After 3 h release rates fell by ≅50% in all groups and were unaffected by insulin. The release rates from animals fed 40% casein were significantly greater than any of the other groups ($P < 0.001$).

Lactate and pyruvate release (Table III). Lactate release rates after 1 h of incubation were comparable in C and Nx groups, although an increase in release with increased protein intake was noted. After 3 h of incubation, release rates fell by 10–30% but no differences were noted between C and Nx groups. Modest increases in lactate release were noted with the addition of insulin but were not statistically significant. Pyruvate release at 1 h was not affected by diet or insulin except that release rates from muscles of Nx rats fed 40% casein, with or without insulin, were significantly higher than the corresponding C ($P < 0.001$). After 3 h of incubation, pyruvate release fell by 40% as compared with 1-h values but were comparable in all C and Nx groups and were not affected by insulin.

Glucose uptake. After 1 h of incubation (Fig. 7), muscles from C rats fed 10% casein had an uptake of 35.7±9.3 which rose to 171.7±13.1 ($P < 0.001$) with insulin. Muscles from Nx rats had a significantly greater

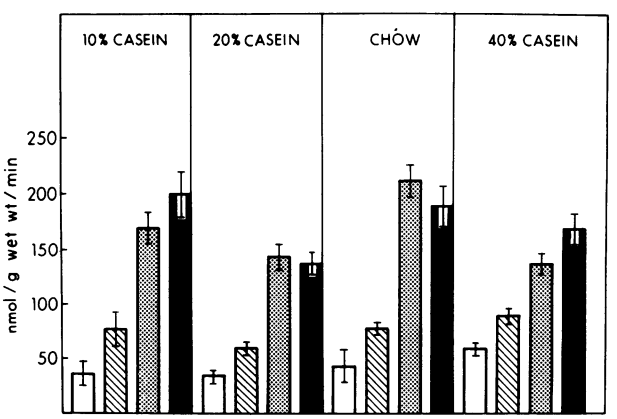

FIGURE 7 Effects of varying protein intakes on glucose uptakes from C and Nx muscles after 1 h of incubation. □, muscles from C rats ($n = 18$); ▨, muscles from Nx rats ($n = 18$); ▩, muscles from C rats incubated with insulin ($n = 16$); ■, muscles from Nx rats incubated with insulin ($n = 16$).

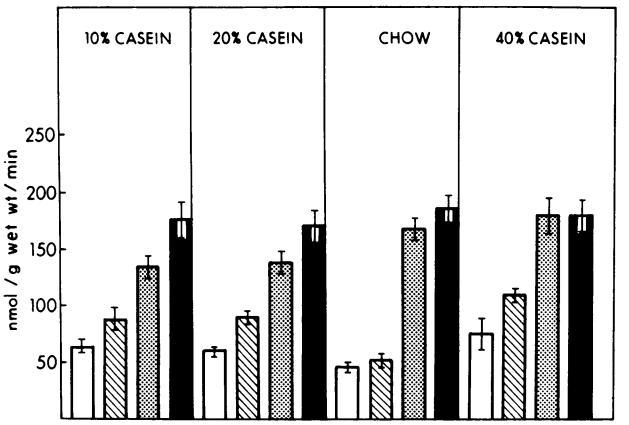

FIGURE 8 Effects of varying protein intakes on glucose uptakes from C and Nx muscles after 3 h of incubation. □, muscles from C rats (n = 18); ▨, muscles from Nx rats (n = 18); ▩, muscles from C rats incubated with insulin (n = 16); ■, muscles from Nx rats incubated with insulin (n = 16).

glucose uptake (77±17.8) than C ($P < 0.01$), but had a comparable increase in uptake when insulin was added (199.5±19.5).

C rats fed 20% casein had a basal glucose uptake of 33.0±5.4, which rose when insulin was added to 143.2±11.4 ($P < 0.01$). Muscles from Nx animals had significantly higher uptakes (59.0±5.4) than C muscles ($P < 0.01$). The maximal uptake in response to insulin was, however, similar to C values (137.8±9.7).

The data obtained with the chow diet were comparable to the 20% casein-fed group. Glucose uptake in C muscles was 43.8±14.5 and increased to 212±13.1 with insulin. Basal glucose uptake in Nx rats (77.6±6.8) was significantly greater than C values ($P < 0.01$), but the maximal uptake with insulin was similar (190.2 ±18.7).

Glucose uptake in C animals fed 40% casein was 59±5.4 without and 137.8±9.7 with insulin ($P < 0.001$). Basal glucose uptake for Nx muscles was 90.2±6.8 ($P < 0.001$ compared with C) and increased to 168.6 ±15.0 with insulin ($P < 0.05$ compared with C with insulin).

Glucose uptake rates after 3 h of incubation are shown in Fig. 8. Glucose uptake remained constant in all groups studied. Basal uptake in Nx rats was significantly greater than the corresponding C values ($P < 0.01$) except in the group fed chow (C, 46.2±5.5 and Nx, 52.8±7.3). Addition of insulin produced comparable increases in glucose uptake except in the animals fed 10% casein (C, 133.3±10.2 and Nx, 176.2 ±17.0 [$P < 0.01$]).

Tissue substrate levels. Tissue levels of glycogen, ATP, phosphocreatine, alanine, glutamine, and glutamate were determined in a representative number of muscles from each group studied (Table IV). All data are expressed as nanomoles per milligram wet weight ±SEM. No significant differences in tissue substrate levels were noted between fast-frozen muscles and those incubated for 1 h except for a decrease in ATP levels. A comparable fall in ATP was noted between C and Nx muscles.

Neither the addition of insulin to the incubating media nor diet affected the pool sizes at 1 or 3 h for the substances measured. For this reason the data were combined. Reduction of renal mass did not affect the pool size of any substance measured. After 3 h of incubation, tissue levels of glycogen fell ($P < 0.01$ compared with 1 h values), but no significant difference was noted between the C and Nx muscles. Tissue alanine and glutamine levels also decreased significantly in both C and Nx muscles ($P < 0.01$ compared with 1-h

TABLE IV
Tissue Pool Sizes

		Glycogen	ATP	PCR*	ALA‡	GLN§	GLU‖
				nmol/mg wet wt			
Fast-frozen	C(10)	28.1±1.4	5.5±0.3¶	16.6±0.5	2.0±0.2	3.6±0.3	1.4±0.2
	Nx(10)	29.9±2.9	5.4±0.2¶	15.3±0.5	2.0±0.2	3.5±0.3	1.3±0.1
1 h	C(17)	27.9±2.2	4.8±0.3	14.0±1.0	2.0±0.2	3.9±0.4	1.2±0.1
	Nx(17)	24.7±2.0	4.8±0.3	14.1±0.8	2.1±0.2	3.3±0.5	1.3±0.2
3 h	C(17)	25.8±2.8	4.8±0.3	14.0±0.9	1.4±0.2	3.3±0.5	1.1±0.2
	Nx(17)	23.0±2.6	4.4±0.4	13.7±0.7	1.6±0.4	2.7±0.4	1.1±0.1

Number of muscles studied appear in parentheses.
* Phosphocreatine.
‡ Alanine.
§ Glutamine.
‖ Glutamate.
¶ $P < 0.05$ compared with corresponding group after 1 or 3 h of incubation.

value) but no difference was noted between C and Nx muscles.

DISCUSSION

The present study suggests that increased muscle catabolism exists when renal mass is reduced. Tyrosine and phenylalanine, both noncatabolizable amino acids at the level of muscle (29), demonstrated increased release from muscles of uremic rats when compared with corresponding controls. Of interest, the release of these amino acids decreased from muscles of uremic rats as protein intake increased, suggesting that muscle catabolism was reduced. The data are supported by the fact that growth of the uremic animals increased with increasing protein intake, and in animals fed 40% casein the growth of both control and uremic animals was comparable. The response of tyrosine and phenylalanine release to the addition of insulin decreased as protein intake increased.

The present study also demonstrates that increased alanine production and release occurs in muscles obtained from rats when renal mass is reduced. Alanine release rates from muscles of uremic rats after 1 h of incubation were significantly greater than values obtained from control animals. The uremic animals fed 10% casein had alanine release rates comparable to control values after 180 min of incubation, but as the protein intake was increased the release rates from uremic animals persisted significantly above control values without changes in pool size. Glutamate release was also increased above control, but only in uremic animals fed 40% casein was the increase significant.

The increased alanine release was not directly related to an increase in BUN, as control animals fed 40% casein had normal release rates and a BUN similar to that found in uremic animals fed 10 or 20% casein (Table I). Furthermore, the enhanced release of alanine did not reflect depletion of intracellular pools as no significant differences were noted between control and uremic groups at zero time or after 1 or 3 h of incubation. Despite the fact that a decrease in pool size for alanine was noted between the 1- and 3-h values, no differences in pool size between control and uremic groups were seen. Any differences, therefore, in release between the two groups of muscles may reflect a defect in alanine transport, increased proteolysis, decreased protein synthesis, increased synthesis of alanine, or combinations of these factors.

Because release of tyrosine and phenylalanine decreased with increasing protein intake, which was associated with improved growth of the uremic animals, net protein catabolism must have decreased. Whether this reflects decreased proteolysis or increased synthesis cannot be documented with certainty by these studies. Of interest, alanine release increased as protein intake increased, suggesting that the release of this amino acid may be dependent upon factors other than net protein catabolic rates, such as increased synthesis from other amino acids. The fact that alanine release continued at an accelerated rate from the uremic muscles of animals fed chow and 40% casein would suggest a decrease in the cell-to-medium gradient for alanine and a possible alteration in its transport system (30). Data are not available from this study to exclude this possibility.

Other possible mechanisms for the increased alanine release from muscles of Nx animals exist. It is possible that changes in hormone levels affect the release of alanine. It has been clearly demonstrated that plasma glucagon levels increase as uremia progresses (5). In the present study glucagon levels were significantly elevated in the uremic animals when compared with pair-fed C animals. The effects of glucagon on muscle in uremia are unknown. It is of interest that muscles from uremic animals fed 10 or 20% casein responded to insulin after 1 h of incubation, whereas those fed the higher protein diets required 3 h of incubation. Furthermore, all C muscles required 3 h of incubation before any insulin effect on depressing alanine release was noted. Recent data using the perfused rat hemicorpus (31) have demonstrated that alanine release, protein synthesis, and protein degradation are all affected by insulin but only after 2–3 h of perfusion. Our data are in agreement with these observations. The fact that muscles from uremic rats fed lower protein diets had increased alanine release rates after 1 h of incubation which responded to insulin may be the result of decreased insulin levels in these animals. On the other hand, insulin levels rose as protein intake increased, whereas alanine release remained elevated and was not affected by insulin until incubated for 3 h. Furthermore, the fact that muscles from uremic animals fed 40% casein had only a modest reduction in alanine release in the presence of insulin for 3 h would suggest decreased responsiveness in this group.

The results seen in muscles from uremic rats are similar to those obtained in muscles from diabetic (streptozotocin), thyroxine, or cortisone-treated rats (32). All three of these groups of animals had increased alanine release rates, whereas glutamine release was either normal or depressed. Because cortisol and thyroxine cause insulin antagonism, and diabetes mellitus is associated with an absolute deficit of this hormone, the increased release of alanine may be at least partially related to the cellular effects of insulin. The data from this study on muscle from uremic rats would imply that the counterregulatory effect of insulin on alanine release has been reduced because of a deficit in or decreased responsiveness to this hormone.

The increased release of alanine does not appear to be dependent on glucose metabolism. Despite continuing accelerated glucose uptake in the presence of insulin, alanine release actually decreased in all groups of animals studied after 180 min of incubation. In previous studies from this laboratory, increased glucose uptake produced by increasing glucose concentration in the incubating media was not associated with any change in the rate of release of alanine (16). This suggests that insulin plays some regulatory role in the release of alanine independent of its effect on glucose metabolism.

Starvation is also associated with increased catabolism and increased release rates of alanine from muscle (32). Starvation, however, cannot account for the observed changes, because as protein content in the diet was increased, weight gain was actually observed in uremic animals. Furthermore, the uremic animals fed 40% casein had a weight gain similar to pair-fed C animals, and yet had the most severe changes in alanine release (Figs. 1 and 2). On the other hand, the increased release of phenylalanine and tyrosine would imply accelerated muscle catabolism and may explain the poor growth rate of uremic animals fed low protein intakes (7). The present studies demonstrate that growth rates of Nx animals can be impaired significantly by reducing protein intake, data that are in agreement with previous studies (7, 13). By feeding high protein diets, weight gain in uremic animals was improved. Previous studies would suggest that weight gain in uremic animals cannot be corrected to C values even with high protein feeding (7). The differences between this study and previous studies, however, may reflect changes in experimental design, diet used, age of animals studied, or duration of uremia.

Recent data from Garber (15) have suggested that the increased release of alanine from uremic muscle may be caused by decreased responsiveness to epinephrine or serotonin. He has shown, in vitro, a decrease in alanine release from control muscles associated with augmented cyclic AMP production in response to epinephrine. This effect was blunted or absent in uremic muscles (15). What effect diet has on plasma epinephrine levels in uremia is unknown.

Parathyroid hormone (PTH) levels are significantly elevated in uremia and are responsive to the amount of phosphate ingested (6). Even though attempts were made in this study to partially control the phosphate intake, with increasing protein intake phosphate intake rose modestly, as reflected by an elevated plasma phosphate level in the animals fed 40% casein. It is possible that the animals fed the highest percentage of protein had the highest PTH levels. Garber (33) has shown that addition of synthetic PTH (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.) to the incubating media increases the release rates of alanine, glutamine, and glutamate in normal muscle, but that PTH has no effect on uremic muscle (33). We were unable, however, to confirm these observations with either synthetic 1-34 PTH (Beckman Instruments) or purified 1-84 bovine PTH. No change in cyclic AMP production by the muscle, glucose uptake, or amino acid release was documented (unpublished observations).

Plasma levels of glutamine, glutamate, and alanine were either normal or slightly decreased in these uremic rats (unpublished observation). This occurred in the presence of increased in vitro release rates of alanine from skeletal muscle, suggesting an increased uptake of this amino acid by other tissues. As the liver is the major organ responsible for alanine uptake and as alanine is a major gluconeogenic precursor (34), increased alanine and glucose turnover would be expected to occur. Recent turnover studies in uremic patients, obtained by Rubenfeld and Garber (35), have shown this to be true. Increased alanine-glucose turnover may explain part of the carbohydrate intolerance seen in patients with chronic renal failure.

Base-line glucose uptake was significantly increased (30–50%) in all groups of uremic muscles, and this increase persisted even after 180 min of incubation (Figs. 7 and 8), despite lower plasma insulin values in these animals. It is unlikely that the changes in glucose uptake were induced by diet, because all animals had pair-fed C, and base-line glucose uptakes by C muscles were comparable in each group. On the other hand, insulin-stimulated glucose uptake was comparable in both uremic and C muscles.

Uremia may be associated with decreased activity of sodium:potassium ATPase in different tissues, presumably due to nonspecific inhibitors (36). Furthermore, inhibition of sodium:potassium ATPase with ouabain in muscle has been shown to increase glucose uptake modestly (37). It is possible that in uremic muscle the activity of the sodium:potassium ATPase is at least partially inhibited, analogous to the ouabain effect, thus changing the intracellular concentrations of sodium and potassium, leading to the observed mild increase in glucose uptake seen in uremic muscle. Because the effect of ouabain on glucose uptake is absent when supramaximal concentrations of insulin are present (37), no difference in maximally stimulated glucose uptake would be expected to occur.

In summary, these studies demonstrate an effect of a reduction in renal mass on alanine, tyrosine, phenylalanine, glutamine, and glutamate release, and the contribution that increasing protein intake may have on net muscle catabolic rates. The net protein catabolic rate of muscles in uremia is insulin responsive and decreases as protein intake increases. On the other hand, the response of alanine, tyrosine, and phenyl-

alanine release from uremic muscle to insulin decreases with increasing protein intake, with no apparent further change in renal function. Furthermore, the release of alanine from both control and uremic muscles appears to be independent of glucose uptake. There is augmented base-line glucose uptake by muscles from uremic animals with normal maximal responses to insulin, implying that the suggested insulin resistance for glucose uptake in uremia may not occur at the level of muscle.

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