

## Creatine Metabolism in Men: Creatine Pool Size and Turnover in Relation to Creatine Intake<sup>1</sup>

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**ABSTRACT** Creatine metabolism was studied in relation to creatine intake and creatinine excretion. Young men were fed 0.23 g creatine/day for 9 days and then 10 g/day for 10 days consecutively. Thereafter, the diet fed was creatine-free. From day 81 through 90, isonitrogenous amounts (4 g N/day) of either an equimolar mixture of the creatine precursors arginine and glycine or of alanine were added to the diet. As reported in a previous paper, creatinine excretion increased during creatine feeding, continued to remain elevated immediately afterwards and then decreased gradually during the period of feeding the creatine-free diet, whereas two subjects not fed creatine showed no significant changes in creatinine output throughout the experiment. The present paper describes studies in which di-<sup>15</sup>N-creatine was injected into the same subjects on two occasions during the creatine-free period, near the beginning of this period and 43 days later. By isotope dilution, the creatine pool sizes were calculated and the rate of conversion of this pool to creatinine was computed. The pool of body creatine diminished during the creatine-free period in parallel with the daily output of creatinine, that is, the fractional rate of conversion of creatine to creatinine was very similar for all subjects ( $0.0169 \pm 0.0006 \text{ day}^{-1}$ ,  $n = 13$ ). In contrast to the marked constancy of the rate of conversion of creatine to creatinine, apparent fractional creatine synthesis rates were much more variable between subjects ( $0.011$  to  $0.016 \text{ day}^{-1}$ ). Administration of the creatine precursors arginine and glycine significantly increased apparent creatine synthesis, whereas administration of alanine depressed synthesis. From these data on di-<sup>15</sup>N-creatine metabolism, it can be concluded that (a) the size of the body pool of creatine can be influenced by dietary creatine, (b) administration of precursor amino acids can increase the rate of synthesis of creatine, (c) creatinine output is a constant fraction of the body creatine pool and can change independently of lean body mass. *J. Nutr.* 106: 371-381, 1976.

**INDEXING KEY WORDS** creatine pool · creatine turnover · dietary creatine · creatinine

Only a few tracer studies of creatine metabolism in humans have been published (1-6). Hoberman and his associates (1, 2) studied the metabolism of isotopic creatine in two normal males receiving a diet free of meat and meat products, but adequate in protein and energy. They reported that creatine synthesis was decreased when creatine was fed and was increased by administering methyltestosterone. No informa-

tion on changes in gross body composition was presented. These and other investi-

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gators calculated fractional turnover rates and/or half-times of creatine in humans (3-6).

All of the reported rates of conversion of creatine to creatinine in normal humans are of the same order of magnitude, but there is almost a two-fold difference between the extremes of the range, suggesting that the rate of conversion of creatine to creatinine may not be constant. Borsook and Dubnoff (7) found the *in vitro* rate constants for conversion of creatine and creatine phosphate to creatinine were equivalent to  $0.0106 \text{ day}^{-1}$  and  $0.0264 \text{ day}^{-1}$ , respectively. These values are in the range of the reported rates of creatinine formation in humans, dogs, guinea pigs, pigs, rabbits and rats. No conclusive evidence of enzyme mediation of these reactions has been reported (8, 9). Thus, the variation in the reported rates of *in vivo* conversion of creatine to creatinine may reflect changes in the proportion of creatine to creatine phosphate in the body. However, another explanation of the variations in conversion rate is possible. Most investigators have assumed that a steady state exists such that creatine synthesis is equal to (and in consequence becomes a measure of) the rate of creatinine formation. In fact, creatine synthesis rate may be rather variable over short periods of time. If this is so, the calculated rate of conversion of creatine to creatinine would also show variability.

We have previously reported the creatine and creatinine excretions of men fed a creatine-free diet to which creatine was added during the first 19 days of a 90-day experiment (10). The present paper reports the results of a concurrent study of di- $^{15}\text{N}$ -creatine metabolism. Creatine pool sizes and the rates of conversion of creatine to creatinine were measured. The results of the present study indicate that the steady state assumption may be invalid for the body creatine pool in man, particularly when dietary creatine content varies.

#### METHODS

*Subjects.* A 90-day study was conducted in the human metabolic unit of the Department of Nutritional Sciences at the University of California at Berkeley. Most of the details of the experiment have been described in a previous paper (10). Eight

healthy young men, 20-28 years old, participated in the study. Four men (S1, S2, S3, S4) completed the entire period of 90 days; two (S7 and S8) stayed from day 1 to day 29 only, and two others (S5 and S6) were admitted on day 33 and stayed to the end of the experiment.

*Diet.* The basic diet consisted of a creatine-free formula (containing 75 g protein from egg albumen, a variety of carbohydrates and fats and sufficient quantities of macrominerals to provide the recommended daily allowances), additional low-protein foods to provide an adequate energy intake, and vitamin, choline and trace mineral supplements in capsule form.

The experiment was divided into four consecutive metabolic periods (MP) of 9, 10, 61, and 10 days duration (MP 1, MP 2, MP 3 and MP 4, respectively). MP 1 was a standardization and training period during which 0.23 g creatine was added to the noon meal of the daily formula. In MP 2, 2.5 g of creatine was added to each of the four daily meals. In MP 3 and MP 4, the diet was free of creatine. The two subjects (S5 and S6) admitted late to the experiment (day 33) were not fed creatine. Throughout MP 4, all subjects were fed an additional 4 g of nitrogen in the form of amino acid supplements. Four men (S1, S2, S3 and S6) received equimolar amounts of L-arginine HCl and glycine, precursors in the creatine synthetic pathway, and the other two men (S4 and S5) received L-alanine as a nitrogen control.

*Creatine pool size and turnover determinations.* At the beginning (day 23) and near the end of MP 3 (day 65), creatine labelled with  $^{15}\text{N}$  was administered to each subject for the determination of creatine pool size by isotope dilution. In addition, isotopic creatine was administered to S5 shortly after his admission to the experiment (day 38).

Creatine hydrate, 94% labeled with  $^{15}\text{N}$  in both nitrogens of the amidine group,<sup>4</sup> was dried to a constant weight *in vacuo* over  $\text{P}_2\text{O}_5$  at  $56^\circ$ . On the day prior to administration, the dried creatine (di- $^{15}\text{N}$ -creatine) was added quantitatively to a sterile, pyrogen-free 5% solution of glu-

<sup>4</sup> Purchased from Isomet Corporation, Palisades Park, N.J.

cose<sup>5</sup> to make a final known concentration of about 1% creatine. This solution was passed through a sterile filter unit containing a 0.22 micron filter.<sup>6</sup> A sample was taken for bacterial cultures and the remaining solution was refrigerated.

On the day of isotope administration, for each subject, a measured weight of the sterile, 1% creatine solution was transferred aseptically to a preweighed one liter bottle of sterile, pyrogen-free 5% glucose (providing 10-14 mg creatine/kg body weight). This dilute creatine solution was administered intravenously over an 8-hour period. The subjects were ambulatory during this interval and consumed meals as usual. Treadmill exercise was suspended on the day of the infusion. Each creatine infusion was followed by the infusion of 50-100 ml of sterile normal saline, so that no labeled creatine remained in the intravenous tubing. The bottles containing creatine solution were weighed before and after the infusions as well as in their clean dry state to permit accurate measurement of the amount of di-<sup>15</sup>N-creatinine administered to each subject.

Urinary creatinine was isolated as the ZnCl salt by the picrate precipitation method of Benedict (11) daily for at least two weeks following isotope administration. Each creatinine-ZnCl sample was recrystallized from acetic acid by the method of Edgar (12) and reconverted to creatinine by Benedict's (11) method of solution in warm concentrated ammonia. Some difficulties were encountered recovering creatinine from the urine samples in the first few days. Subsequently this was corrected by first concentrating each sample by rotary evaporation and then proceeding with the isolation procedure.

The atom percent excess of di-<sup>15</sup>N-creatinine in selected samples was measured by a computer-assisted high resolution mass spectroscopy method<sup>7</sup> (13), and by gas mass spectroscopy.<sup>8</sup> For the latter determination, 18 selected recrystallized creatinine-ZnCl samples were digested in H<sub>2</sub>SO<sub>4</sub> for 24 hours (microkjeldahl procedure using selenized granules as the catalyst and H<sub>2</sub>O<sub>2</sub> for the final oxidation). The ammonia was liberated with NaOH, distilled into boric acid and titrated with HCl. These solutions were then oxidized

with hypobromite to release N<sub>2</sub> gas for analysis of isotope concentration. Eight of the 18 samples of isolated creatinine were analyzed for creatinine and nitrogen concentration. These samples contained 23.3 ± 0.9% (SD) nitrogen and 64.7 ± 2.0% creatinine. Creatinine nitrogen thus accounted for 103 ± 1.6% of the nitrogen measured. These results indicate that the samples had no significant contamination with nitrogen-containing compounds other than creatinine.

Creatine pool size was calculated by isotope dilution on the basis of the atom percent excess of the urinary creatinine on the third day after injection, to allow time for equilibration with the muscle creatine pool. Isotope excreted prior to the time of the pool-size determination was subtracted from the injected dose. Where data for a given subject were not complete as to the urinary loss of isotope prior to the time of calculation of pool size, the average percentage of injected dose excreted by the other subjects was assumed (the assumed losses are shown in parenthesis in table 1). The amount of doubly-labeled creatine administered minus the equivalent isotope lost in the urine prior to the pool-size determination (creatinine retained on day 3) was divided by the atom percent excess of urinary creatinine on that day to estimate total body creatine by isotope dilution.

The second time the subjects were given isotope, the value for the residual atom percent excess on day 67 due to the preceding injection was taken into account by using the slope of the dilution of isotope from days 67 to 79 for each subject (shown in table 2) and the atom percent excess determined just prior to the second administration of the isotope (day 63) to predict by linear regression the residual isotope concentration of the body on day 67. The estimated residual isotope concentration on day 67 was then subtracted from the observed atom percent excess in urinary creatinine to determine the isotope concentration due to the second injection. This

<sup>5</sup> Cutter Laboratories, Berkeley, Calif.

<sup>6</sup> Filter unit XX11047-00 and filter 6SWP-047-50, Millipore Corporation, Bedford, Mass.

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<sup>8</sup> We are grateful to C. C. Delwiche, University of California, Davis, Calif. for measuring the isotope concentration of these samples.

TABLE 1  
Creatine pool size and turnover

Sub- ject	Day <sup>1</sup>	di- <sup>15</sup> N creatine injected	di- <sup>15</sup> N creatine <sup>2</sup> excreted	di- <sup>15</sup> N creatine <sup>3</sup> retained	Atom <sup>4</sup> % excess	Residual <sup>5</sup> atom % excess	Corrected <sup>6</sup> atom % excess	Creatine <sup>7</sup> pool	Creatinine <sup>8</sup> excretion	Creatine pool excreted as creatinine per day
		g	g	g	%	%	%	g	g/day	%
MP 3, Immediately after creatine feeding										
1	25	1.093	0.068	1.024	0.634			160.6	2.29	1.67
2	25	0.776	(0.051)	0.725	0.567			127.2	1.88	1.72
3	25	0.868	(0.060)	0.808	0.550			146.1	2.09	1.65
4	25	0.955	0.062	0.893	0.527			168.6	2.41	1.66
7	25	0.864	(0.057)	0.808	0.565			142.2	1.96	1.59
8	25	0.623	(0.040)	0.583	0.482			120.3	1.64	1.59
								150.6 ± 18.2 <sup>10</sup>	2.17 ± 0.23	1.68 ± 0.03
MP 3, 47 days after creatine feeding										
1	67	0.778	0.029	0.749	0.945	0.378	0.567	131.2	1.97	1.76
2	67	0.574	0.021	0.553	0.829	0.338	0.490	112.2	1.65	1.73
3	67	0.646	0.023	0.623	0.805	0.310 <sup>11</sup>	0.495	125.8	1.87	1.72
4	67	0.690	0.025	0.664	0.787	0.320	0.468	141.5	2.06	1.70
								127.7 ± 12.2	1.89 ± 0.18	1.73 ± 0.02
MP 3, subjects not fed creatine										
5	40	0.930	0.038	0.892	0.693			127.9	1.85	1.70
5	67	0.704	(0.024)	0.680	1.001	0.475	0.525	128.7	1.85	1.66
6	67	0.971	(0.034)	0.936	0.745			124.3	1.86	1.76

<sup>1</sup> Day pool size was calculated (on the third day after injection of di-<sup>15</sup>N-creatine). <sup>2</sup> Atom percent excess multiplied by grams creatine (urinary creatinine × 1.159) excreted and summed for the 2 days prior to pool size determination. Numbers in parentheses represent values not directly determined on both days 1 and 2 which were estimated on the basis of average directly determined values for each time of injection (4.67 and 1.80% for the first and second days of the first injection, respectively, n = 3, and 1.92 and 1.71% for the second injection respectively, n = 4). In subjects receiving a second injection on day 65 the amount of di-<sup>15</sup>N-creatinine excreted due to the second injection has been corrected for di-<sup>15</sup>N-creatine still present in the pool from the previous injection (see Methods). <sup>3</sup> Injected minus excreted equals retained creatine on day of pool size calculation. <sup>4</sup> Estimate atom % di-<sup>15</sup>N-creatinine on the day of pool size calculation as measured by high resolution mass spectroscopy (13). <sup>5</sup> These subjects received two injections; the entry in the table is an estimate for day 67 of di-<sup>15</sup>N-creatine still present from the first injection and is based on the measured di-<sup>15</sup>N-creatinine atom % excess observed on day 63 (see Methods). <sup>6</sup> Corrected atom percent excess = atom percent excess minus residual atom percent excess, this number is the atom percent excess due to the second injection. <sup>7</sup> [(Grams di-<sup>15</sup>N-creatine retained/atom percent excess of di-<sup>15</sup>N-creatine due to injection) × 100] minus g di-<sup>15</sup>N-creatine retained. <sup>8</sup> Three-day mean around day of isotope pool measurement (taken from ref. 10). <sup>9</sup> [(Grams creatinine excreted/day) × 1.159/creatinine pool size] × 100, a measure of the fractional rate of conversion of creatine to creatinine. <sup>10</sup> Mean ± SD of subjects 1 to 4. <sup>11</sup> A correction factor was applied to the day 67 residual atoms percent excess of subject 3. See Results.

value for the isotope concentration after the second injection was then divided into the amount of isotope retained to obtain the size of the creatine pool by isotope dilution. In this case, retained creatine was estimated using the atom percent excess due to the second injection (calculated as above).

After each administration of di-<sup>15</sup>N-labeled creatine, the amount of retained isotopic creatine was subtracted from the calculated pool.

## RESULTS

*Creatine pool size and conversion to creatinine.* Figure 1 (page 376) displays semi-log plots of urinary di-<sup>15</sup>N-creatinine concentrations over time following the two administrations of labeled creatine. Subjects 1 and 3 were fed creatine during MP 2 (days 10 through 19) and the creatine

precursor amino acids glycine and arginine during MP 4 (days 81 through 90). Subject 5 received no creatine at any time and was given alanine during MP 4. From these data, tables 1 and 2 are derived. Differences between mean slopes of the lines were analyzed statistically for significance by the method of Crow et al. (18).

Creatine pool size was calculated by isotope dilution on the third day after injection as discussed in Methods. These data are presented in table 1. For these calculations it was assumed that creatinine was the only product of body creatine, that the isotopic creatine mixed with and was metabolized in the same way as unlabeled creatine, and that no significant loss of body creatine occurred other than by urinary excretion of creatinine. [These assumptions are warranted by the work of others (1, 3, 14-17)]. Thus, the concentra-

TABLE 2  
Effect of feeding amino acids on the rate of dilution of  $^{15}\text{N}$ -creatinine by newly synthesized creatinine<sup>1</sup>

Subject	M.P. <sup>2</sup>	Interval <sup>3</sup> expt. days	n <sup>4</sup>	Slope <sup>5</sup>	b <sup>6</sup>	r <sup>7</sup>
Basic diet, no amino acids added						
1	3	67-79	9	-0.0106 ± 0.0016	+0.655	-0.92
2	3	67-79	8	-0.0134 ± 0.0016	+0.711	-0.96
3	3	67-79	7	-0.0158 ± 0.0016	+0.841	-0.98
6	3	67-79	7	-0.0143 ± 0.0006	+0.665	-0.99
Mean slope				-0.0135 ± 0.0022 <sup>8</sup>		
Basic diet, plus glycine and arginine						
1	4	81-90	9	-0.0203 ± 0.0020	+1.439	-0.97
2	4	81-90	7	-0.0211 ± 0.0018	+1.329	-0.96
3	4	81-90	7	-0.0230 ± 0.0020	+1.446	-0.98
6	4	81-90	7	-0.0191 ± 0.0016	+1.042	-0.98
Mean slope				-0.0209 ± 0.0016 <sup>9</sup>		
Basic diet, no amino acids added						
4	3	67-79	8	-0.0139 ± 0.0017	+0.695	-0.96
5	3	67-79	8	-0.0162 ± 0.0024	+1.025	-0.94
Mean slope				-0.0151		
Basic diet, plus alanine						
4	4	81-90	6	-0.0116 ± 0.0019	+0.476	-0.95
5	4	81-90	7	-0.0117 ± 0.0029	+0.646	-0.87
Mean slope				-0.0116		

<sup>1</sup> The rate of reduction in  $^{15}\text{N}$  concentration with time was calculated by plotting log atom percent excess  $^{15}\text{N}$  in urinary creatinine against days following di- $^{15}\text{N}$ -creatinine administration. The table shows the components of the regression equation: atom percent excess = slope  $\times$  experimental days + b (intercept at time zero). <sup>2</sup> Metabolic period. <sup>3</sup> Inclusive interval of the experiment days from which the data in the table were derived. <sup>4</sup> Number of values (days) used to calculate the linear regression. <sup>5</sup> Slope of the calculated regression  $\pm$  the SE of the slope (18). <sup>6</sup> The y-intercept of the calculated regression. <sup>7</sup> Coefficient of correlation for the line. <sup>8</sup> Mean  $\pm$  SD. <sup>9</sup> Significantly different from the mean when no amino acids were added ( $P < 0.05$ ).

tion of the isotope in creatinine was assumed to reflect the isotope concentration in the body creatine pool. The body creatine pool is defined as the sum of creatine and creatine phosphate which are assumed to be in equilibrium. Thus, the fractional rate of conversion of creatine to creatinine is the weighted mean of the combined fractional conversion rates for creatine and creatine phosphate. Daily creatinine excretion, as a percentage of the creatine pool (mole/mole), was calculated as a measure of the fractional rate of creatine conversion to creatinine. For all of the above calculations, it was not necessary to assume that the creatine pools were in a steady state,

but rather only that no significant changes in the creatine pools or creatinine excretions of the subjects occurred during the one to two days around the day of pool determination (i.e., quasi-steady state).

As shown in table 1, the creatine pools of subjects S1, S2, S3 and S4 decreased from an average of 150.6 g early in MP 3 (day 25) to 127.7 g late in MP 3 (day 67). (A correction factor was applied to the day 67 residual atom percent excess  $^{15}\text{N}$  of subject 3. The rationale for this adjustment is discussed below). These subjects were all fed creatine during MP 2 and the urinary creatinine excretions of all decreased during MP 3 when creatine was no longer

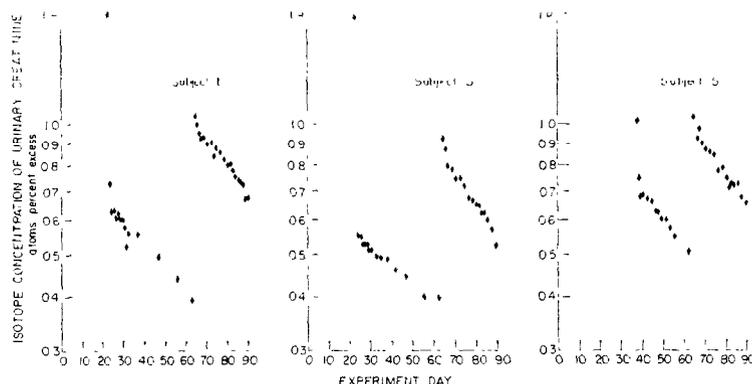


Fig. 1 Isotope concentration in creatinine isolated from the urines of three subjects at various times after dosing with di- $^{15}\text{N}$ -creatinine. Di- $^{15}\text{N}$ -creatinine was administered intravenously on days 23 and 65 to subjects 1 and 3, and on days 38 and 65 to subject 5. Subjects 1 and 3 were fed 0.23 g creatine/day on days 1 through 9 and 10 g creatine/day on days 10 through 19. Subject 5 was not fed creatine. The values for the other subjects were similar. Di- $^{15}\text{N}$ -creatinine was analyzed by high resolution mass spectroscopy (13).

fed. There was no significant change in the creatine pool size (127.9 g to 128.7 g) or urinary creatinine excretion of the control subject, S5, who was not fed creatine. Nevertheless, the calculated fractional rate of conversion of creatine to creatinine was very similar for all subjects irrespective of the sizes of their creatine pools. The average for all 13 determinations was  $0.0169 \pm 0.0006$  (mean  $\pm$  SD). These results, which directly measure the proportion of the pool excreted as creatinine, were much less variable than any of the previously discussed literature values, which were largely based on the assumption that creatine synthesis and creatinine formation are in equilibrium.

In order to confirm the analytical data on which these conclusions are based, a selection of samples of di- $^{15}\text{N}$ -creatinine isolated from 18 urines were analyzed by gas mass spectroscopy. The isotope concentration in these samples was  $88.5 \pm 4.8\%$  of that estimated to be present using high resolution mass spectroscopy. From this series of samples, an independent though rough estimate of creatine pools and rate of conversion to creatinine was calculated. The data are qualitatively similar to those presented in table 1 in so far as subjects S1 through S4 showed a decline in estimated creatine pool size during MP 3 ( $173.9 \pm 19.2$  g to  $135.2 \pm 13.2$  g,  $n = 4$ ), and the rates of conversion of creatine to

creatinine were similar for all subjects ( $1.52 \pm 0.09$ ,  $n = 13$ ). However, the absolute values were quite different due to the consistent difference between the two methods for measuring  $^{15}\text{N}$  concentration in the isolated creatinine. Comparison of the isotope concentrations obtained by high resolution mass spectroscopy and by gas mass spectroscopy showed that the sample used to determine residual isotope concentration for subject 3 on day 63 was more divergent than the other samples analyzed by both methods. The gas mass spectroscopy value was only 73% of the high resolution value, more than three standard deviations less than the mean. In addition, when the high resolution mass spectroscopy values were used to calculate the creatine pool size for subject 3 on day 67, there was no change during MP 3 (144.3 g creatine) despite a marked decline in creatinine output. It was concluded that the high resolution mass spectrographic value for this sample was in error and an estimate of the latter value was obtained by applying an average correction factor to the gas mass spectrometer value (based on observed differences in values of the other samples by the two methods following omission of this sample).

*Creatine synthesis rate.* The metabolic fate of the injected di- $^{15}\text{N}$ -creatinine was also used to compute apparent creatine synthesis rates. This was not applied to the

first isotope injection, since the progressive decline in creatinine excretion during this period was incompatible with a steady state. If the creatine pools are in a steady state or quasi-steady state (no significant changes over the period of the measurement) the rate of dilution would be equal to the average fractional rate of creatine synthesis over the measured interval. Based on the constancy of creatinine excretion discussed earlier for the same subjects (10), the subjects were close to a steady state with respect to their creatine pool at the time of the second isotope administration, late in MP 3 (day 65). The introduction of measurable amounts of isotopic creatine (0.5% of the pool) may have been sufficient to affect the steady state condition, but this should have had a transient effect of only 1 or 2 days duration.

The rate of change of isotope concentration in creatinine with time was obtained by linear regression analysis of the change in the logarithm of isotope concentration from days 67-79 inclusive (table 2). The calculated slopes for this interval (fractional creatine synthesis rates) vary from 0.011 to 0.016 day<sup>-1</sup>. This variation is similar to that reported by others (1-6) who equated this with creatinine formation assuming a steady state. Comparison of these synthesis rates with the rates of creatinine formation (table 1) shows that only S3 and S5 were close to a steady-state; the others had decreasing pools due to reduced synthesis compared with rate of conversion of creatine to creatinine. This finding agrees with the observation that the creatinine excretion of S2 and S4 continued to decline late in MP 3 at a slow rate (10). The rates of creatine synthesis of S1 and S6 seem to be low in spite of constant creatinine excretion rates as shown previously (10). A declining pool size at the time of measurement of the synthesis rate would make the estimated rates of synthesis slightly high. Similarly, if the creatine pool were increasing, the calculated rates of synthesis would be slightly lower than the actual rates of synthesis, depending on the rate of increase of the pool.

*Effect of feeding precursor amino acids.* Feeding of glycine and arginine resulted in a significant increase in the synthesis rate measured by dilution of the isotopic cre-

atine of each subject compared to his own rate immediately prior to the diet change. In contrast, the two subjects fed alanine had significantly decreased rates of creatine synthesis after amino acid administration. The new rates probably were due largely to changes in the rate of creatine synthesis rather than to changes in creatine pool size. That is, if creatine synthesis were constant, changes in the order of 30% to 50% in the creatine pools would be necessary to have produced these rate changes and the creatinine excretion rate would have to show proportional changes. However, a doubling of the creatine synthesis rate for 10 days would result in only a very small change in the creatine pool size, about 1 g/day, which would increase creatinine excretion by only 10-20 mg/day. This is well within the analytical error of the creatinine method ( $\pm 5\%$ , which is about 50-100 mg creatinine in a 24 hour urine). Similarly the decrease in rate of isotope dilution in the subjects fed alanine probably represents an inhibition of synthesis. A decrease in the urinary creatine excretions [reported previously (10)] of these subjects coincides with the conclusion that creatine synthesis was inhibited.

#### DISCUSSION

*Variations in creatine pool size.* The constant rate of creatinine formation, as measured in the present experiment, supports the concept that daily creatinine excretion is directly proportional to the size of the body creatine pool under our experimental conditions and thus that the conversion of creatine to creatinine in the body is indeed non-enzymatic. Thus the size of the creatine pool was increased by feeding creatine and decreased by the removal of exogenous creatine, yet the proportion of pool excreted daily as creatinine remained unchanged. If the increases and subsequent decreases in the creatine pools observed in our studies were distributed throughout the muscle mass of the subjects, a change in muscle creatine concentration of less than 0.1 g/100 g wet muscle occurred, which is within the reported range of human muscle creatine concentration, 0.3% to 0.5% (19, 20). The decreases in the size of the creatine pools of the subjects fed creatine-free diets in our experi-

ments occurred in spite of positive apparent nitrogen and potassium balances (10), and in the absence of any significant changes in body composition as measured by body density, body  $^{40}\text{K}$  and total body water which will be reported later.<sup>9</sup> The data from this study indicate that the creatine synthesis rate and the size of the creatine pool in man can vary and in a manner independent of lean body mass. Thus, the correlation of creatinine excretion with lean body mass appears to have little physiological validity and may simply reflect the similar dietary intake of creatine by the population, as has been suggested by Bleiler and Schedl (21).

Other data regarding the constancy of the creatine pool are in conflict. Hoberman, Sims and Peters (1) calculated creatine pool size by two methods. In the first method, which assumed a steady state of creatine synthesis and creatinine output, the slope of the log-linear curve of the decrease in isotope concentration with time was assumed to be equivalent to the fraction of the creatine pool which was synthesized and excreted per day. Daily creatinine excretion, expressed as creatine, divided by the slope gave the creatine pool size. In the second method, isotope concentration at zero time was estimated by extrapolating back to zero time from the log linear curve. The creatine pool was then calculated by dividing isotope concentration at zero time by the amount of isotope available for mixing with the body tissues and subtracting the grams creatine administered, and required no assumption of a steady state. Since both methods gave comparable results, Hoberman, Sims and Peters used the log-linear slope analysis method and applied it in their subsequent study (2). Bleiler and Schedl (21) challenged the steady state assumption of Hoberman's group on the basis that their own data showed a declining creatinine excretion on a creatine-free diet. Internal evidence also suggests this in the Hoberman, Sims and Peters study (1), in which the fraction of the creatine pool excreted as creatinine per day, calculated by the first method (slope), was  $0.0164 \text{ day}^{-1}$ . However, division of the amount of creatine excreted as creatinine per day (1.89 g) by the pool size calculated by the sec-

ond method (105 g) gives a higher value,  $0.018 \text{ day}^{-1}$  (our calculations). The difference between these rates suggests that the creatine pool was not in a steady state.

Fitch and associates (3, 4) report experiments in which  $^{14}\text{C}$ -creatine was injected into seven patients, five of whom had a muscular disease. In patients without significant creatinuria, comparison of the curves generated by the decline in creatinine specific activity with time showed that a log-linear decrease occurred, with slopes of  $0.013\text{--}0.017 \text{ day}^{-1}$ , whereas in patients with significant amounts of creatine in their urine, the curves were biphasic. Fitch and his associates assumed that the creatine pools of their subjects without creatinuria were all in a steady-state and thus that the rate of decline of the log of the isotopic creatinine concentration was equal to the fractional turnover rate of creatine to creatinine. This assumption seems unlikely since there was, in fact, considerable variation in their data. Fitch and associates also assumed that control of dietary creatine intake was unnecessary; that the total amounts of nonradioactive creatine in the various body compartments remained constant for the period of observation, 50–60 days; and that creatine synthesis was identical for all cases. The validity of these assumptions is questionable in light of the results reported in the present paper.

Kreisberg, Bowdoin and Meador (6) reported the creatine fractional turnover rates of 4 subjects as "determined from the time curve of the radioactivity remaining in the body plotted at daily intervals." If there were no significant creatinuria, this rate of loss of creatine from the body should be a valid measure of the rate of conversion of the body creatine to urinary creatinine. However, the range of reported rates,  $0.0138 \text{ to } 0.0188 \text{ day}^{-1}$ , are in contrast to the constant rates found in the present study and may represent errors inherent in the estimates. The isotope curves were not presented and creatine excretion was not measured. Comparison of their data with ours or that of other authors is thus difficult.

Bleiler and Schedl (21) felt that, in the presence of a declining creatine pool, the

<sup>9</sup> Unpublished results.

body would selectively preserve its supply of creatine phosphate which is converted more rapidly to creatinine (7). A comparison of the creatine pool excreted as creatinine on day 25 with that on day 67 shows no marked increase in the rate as would have had to occur if there had been an increase in the proportion of creatine phosphate in the pool (7). Thus, the decreases in creatine pools measured in the present experiment do not appear to be the result of marked selective loss of either creatine or creatine phosphate.

Assuming that creatinine excretion reflects total creatine pool size (as our data indicate), then from creatinine excretion data it can be said that significant increases in the creatine pool can result from feeding less than a gram of creatine per day for many weeks (22, 23), or 10–20 grams per day for 10 days (24). Similarly, creatine pools can decrease when exogenous creatine is removed from the diet (21, 22–25). No decline in creatinine excretion occurred in our two subjects not fed creatine throughout the experiment. These subjects exercised more vigorously than the subjects in the previous experiment where creatinine excretion fell (25) and more than those in Bleiler and Schedl's report (21). Control of creatine pool size may be subject to variations in physical fitness, amino acid requirements, the exogenous creatine intake prior to the experiment, or other unrecognized factors.

*Control of creatine synthesis.* The evidence from our studies indicates clearly that conversion of creatine to creatinine occurred at a constant fractional rate (table 1). In the absence of exogenous creatine, or significant creatinuria, regulation of the creatine pool must thus be controlled by creatine synthesis. The large range of apparent creatine synthesis rates, as estimated by the slope of isotopic dilution in the present study and as reported by others (1–6), is evidence that creatine synthesis is sufficiently variable to be hypothesized as the factor controlling creatine pool size. Changes in the creatine pool occur gradually, due to the slow rate of conversion of creatine to creatinine. Thus, short-term evaluation of urinary creatinine excretion is an insensitive means of determining whether the creatine pool

is in a steady state. As control of creatine pool size does not appear to be closely regulated in humans, the slow rate of conversion of creatine to creatinine may be a particularly important factor in maintaining creatine pool size.

Although body creatine was assumed to be one pool for the purpose of isotope analysis, in fact body creatine is distinctly compartmentalized (3, 4). This complicates any attempts to determine the factors controlling creatine pool size. However, it may be noted that the urinary excretion of di-<sup>15</sup>N-creatine injected into the subjects fed creatine (S1, S2, S3, S4; MP 2) was significantly higher on the day of the initial injection, ( $4.67 \pm 0.38\%$  of the dose,  $n = 3$ ), than on the day of the second injection, ( $1.92 \pm 0.06\%$  of the dose,  $n = 4$ ). The excretions were, however, similar on the second day after each injection ( $1.80 \pm 0.01\%$ ,  $n = 3$  and  $1.71 \pm 0.07\%$ ,  $n = 4$  on days 26 and 66 respectively). The proportionately greater loss of the injected creatine after the first injection indicates that there may be a greater resistance to exogenous creatine uptake when the creatine pool has been increased by creatine feeding. Thus some control of creatine pool size may be exerted by the excretion of excess in the urine. Our isolation method does not allow us to determine whether the excreted form was creatine or creatinine.

Creatine synthesis appears to be controlled by feedback inhibition of the transaminase enzyme by creatine (26–28). As transamidation is extramuscular (29), and the bulk of the creatine pool is located in skeletal muscle, then control of synthesis must depend on changes in the concentration of the relatively small amounts of circulating creatine and its precursors. Thus, factors affecting creatine entry and retention in muscle must be important secondary regulators of creatine synthesis, and the steady state size of the creatine pool.

Fitch et al. (30) reported *in vivo* and *in vitro* studies which indicate that creatine enters rat skeletal muscle by a transport site specifically adapted to interact with an amidine group. L-arginine, glycine, creatinine,  $\beta$ -alanine and glucose were among a group of compounds reported as having no effect on creatine transport into skeletal

muscle in vitro. An earlier study by Fitch and Shields (31) showed that creatine enters rat skeletal muscle by an energy-dependent saturable process at a sufficient rate to replace about 4% of the total amount of creatine in muscle each day. The data of Koszalka and Andrew (32) implicate insulin in mediation of creatine uptake in vivo by the skeletal muscle in the normal and X-irradiated rat. It has been hypothesized that defects in creatine transport and creatine trapping are the primary causes of reduced muscular creatine in humans (4).

The addition of glycine and arginine to the diet stimulated creatine synthesis in excess of that required to maintain a steady-state. This indicates that intake of methyl donor compounds was not a factor limiting creatine synthesis, and that an increase in the other precursor amino acids will stimulate creatine synthesis. This may indicate that (a) substrate limitation was controlling creatine synthesis or (b) transaminase enzyme inhibition by creatine was reversed by increased precursor concentrations, or (c) synthesis of the transaminase enzyme was increased by increased precursor concentrations (26-28, 33) or (d) that insulin concentrations in the plasma were changed by the arginine and alanine resulting in changes in the extracellular creatine pool due to the facilitation of creatine transport by insulin (32). Clearly, a complex variety of factors may be postulated as having effects on the control of creatine synthesis and its deposition in the muscle tissue.

The metabolic value of an increased creatine pool was not thoroughly investigated in the present experiment. Further controlled testing is necessary to determine whether or not accumulation of creatine is a significant factor in muscle metabolism.

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