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Creatine
muscle mass
protein-energy malnutrition

The Measurement of Muscle Mass in Children Using [^{15}N]Creatine

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Extract

In eight infants and children who had recovered from protein-energy malnutrition (PEM), muscle mass was estimated by measuring creatine turnover by an isotope dilution technique using [^{15}N]creatine, creatine concentration, and urinary creatinine output. Creatine turnover varied from 1.5 to 2.6% of the muscle creatine pool per day and muscle creatine concentration ranged from 1.7 to 3.9 μg/μg muscle DNA. Muscle mass was between 15% and 37% of total body weight. The results indicate that daily creatinine output is not a reliable indicator of muscle mass in children who have recently recovered from severe PEM.

Speculation

Short term fluctuations in the daily creatinine output in young children may be due to variations in muscle creatine concentration since creatine turnover, in the individual subject, remains remarkably constant. The wide variation in the proportion of body weight that is muscle may reflect significant body composition changes in fat, water, and muscle in children who have recovered from severe protein-energy malnutrition.

Skeletal muscle is the major component of lean body mass and is also the largest protein reservoir in the body. However, there is no accurate method for measuring muscle mass in man. A limited number of whole body dissection studies have been performed in relatively well nourished and in severely malnourished children (16, 18, 27). This direct method has limitations because it is difficult to ensure complete dissection of all muscle tissue, variable amounts of water and other fluids are lost during and after dissection, and studies are done at varying times after death. It is therefore difficult to extrapolate these findings to the dynamic situation in the living individual. Of the alternative and indirect methods of estimating muscle mass, that based on the 24-hr creatinine excretion is most widely used. The strong association between creatinine excretion and body weight led to the suggestion that muscle mass could be predicted from the daily output of urinary creatinine (24). The relationship has received continuing attention (7, 9, 21, 31) and constants (17, 31) or equations (8, 9) have been derived which seek to relate 24-hr urinary creatinine output to muscle mass.

The relationship is based on the observations that urinary creatinine is the sole breakdown product of creatine (3) and that more than 90% of body creatine is located in skeletal muscle (23, 35). Furthermore, the breakdown of creatine, or more accurately, creatine phosphate to creatinine, is a nonenzymic process (5) that occurs at a fixed rate. Under normal circumstances the rate of formation and excretion of urinary creatinine will therefore depend on the size and turnover rate of the creatine pool and the relationship between muscle mass and creatinine excretion will depend on the concentration of creatine in muscle.

Creatine catabolism has been measured with isotopically labeled creatine in normal individuals (13, 19, 21) and is remarkably constant although there is sufficient variation between individuals to affect the creatinine excretion per unit of body creatine. The concentration in muscle also shows individual variation (1). When the daily excretion of urinary creatinine is used to estimate muscle mass, it is important to recognize that both creatine turnover (13, 35) and creatine content of muscle (1) vary in the individual in differing clinical states. Other workers have pointed out the unreliability of using a single constant to relate muscle mass to creatinine excretion (21). In the present study we have measured creatine turnover and creatine concentration in muscle in estimating muscle mass in children.

THEORETICAL CONSIDERATIONS AND CALCULATIONS

Since more than 90% of body creatine is found in muscle, from a knowledge of the size of the creatine pool and the concentration of creatine in muscle, muscle mass can be estimated

$$\text{Muscle mass (kg)} = \frac{\text{creatine pool (g)}}{\text{creatine concentration in muscle } (\mu\text{g/mg wet weight of muscle})} \quad (1)$$

The size of the creatine pool may be estimated by an isotope dilution technique. A tracer dose of ^{15}N-labeled creatine is administered and after equilibration the size of the creatine pool can be calculated as follows: creatine pool = [(dose of [^{15}N]creatine remaining)/(degree of enrichment of muscle [^{15}N]creatine)]. This approach has a number of disadvantages. In order to calculate the dose of [^{15}N]creatine remaining after equilibration, the total loss of ^{15}N up to isotopic equilibrium must be measured accurately. It is technically difficult to ensure complete urine and stool collections over 72 hr, the period of equilibration, in infants and young children. Also, the degree of enrichment of urinary total nitrogen with ^{15}N towards the end of the equilibration period is so low that accurate measurements of isotope enrichment are not technically possible. It has also been shown in rabbits that after intravenous injection of [^{14}C]creatine, up to 25% of the dose given was secreted into the gastrointestinal tract where it was destroyed by micro-organisms (14). There is therefore a real possibility that after administering a dose of [^{15}N]creatine a significant proportion would neither mix with body creatine nor be accounted for in stool or urine. A final disadvantage is that muscle samples weighing several grams would be required to determine the enrichment of muscle creatine.

In order to avoid these difficulties another method to measure the size of the creatine pool was developed. After intravenous injection of [^{15}N]creatine and its equilibration with muscle creatine, the rate of loss of label as urinary [^{15}N]creatinine reflects the rate of breakdown of the muscle creatine pool. This is based on the observations that muscle creatine is not normally excreted as such, its sole nonreutilizable excretory end product is urinary creatinine (3), and the breakdown of creatine phosphate to creatinine is a nonenzymic process (5) and occurs at a fixed rate.

It follows that the size of the muscle creatine pool can be calculated as follows:

$$\text{Creatine pool (mg)} = \frac{\text{urinary creatinine excretion (,g/day)}}{\text{turnover of muscle creatine (\%/day)}} \times 100 \times 1.159 \quad (2)$$

where creatine turnover is defined as the percentage of the muscle creatine pool that is broken down and excreted per day and 1.159 is equal to the ratio of the molecular weight of creatine to that of creatinine. Turnover was calculated as the slope of the line obtained from the semilog plot of the daily urinary excretion of excess ^{15}N in creatinine as a function of time. The assumptions underlying this method are as follows. (*1*) Isotopic equilibration occurs in the creatinine precursor pool within 3 days after isotope administration. (*2*) Muscle creatine is the sole precursor of urinary creatinine. (*3*) The fractional rate at which creatinine is formed from creatine and excreted remains constant during the period of measurement. The validity of these assumptions is discussed under *Results*.

MATERIALS AND METHODS

Eight male Jamaican infants and children who had achieved full clinical recovery from protein-energy malnutrition were studied in a metabolic ward. Their ages ranged from 4 to 27 months (mean 14.8 months) and their weights, as a percentage of their expected weight for height, using the 50th percentile of the Boston standard, ranged from 90 to 107% with a mean of 98% (Table 1). All children were afebrile and free from overt infection during their studies. Body weight was measured daily and length once a week. The diet consisted of a proprietary milk mixture with added arachis oil and provided 135 kcal and 3.1 g protein/100 ml feeding (29). Full and informed parental consent was obtained for each study.

The children were given a single intravenous injection of sterile [^{15}N]creatine (30 atom %) in 0.9% NaCl at a dose of 1 mg ^{15}N/kg body weight. The synthesis of [^{15}N]creatine is described below. The time of injection was noted. On *day 3* and at intervals of 5–7 days for 3–4 weeks, urine was carefully collected on ice to minimize the interconversion of creatine and creatinine (32) for timed periods of approximately 24 hr and stored at −10° until analyzed. At 13.00 hr on *day 4* a sample of muscle was obtained either by percutaneous needle biopsy of the quadriceps femoris (25) or by open biopsy of the pectoralis major (34). The biopsy material was lightly blotted on filter paper to remove any adhering blood; any fat or connective tissue was removed by dissection with needles. The muscle sample was weighed at timed intervals on a torsion balance which allowed the original wet weight to be calculated (25). The sample was then frozen in liquid air and stored at −20° until analyzed.

Table 1. *Anthropometric measurements in eight male children after recovery from severe protein-energy malnutrition*

Subject	Age, mo	Wt, kg	Ht, cm	Expected wt for age, %	Expected wt for ht, %
RW	4	4.28	56.5	68	90
DA	12	9.36	72.5	94	100
HC	13	5.22	58	51	101
DJ	13	7.93	67	78	102
BJ	15	9.52	75	87	95
OJ	16	8.61	73	78	90
MT	18	10.85	80.5	94	97
CR	27	13.18	86	101	107
Mean	14.8			82	98
±SD	6.5			±15.3	±7.7

SYNTHESIS OF [^{15}N]CREATINE

[^{15}N]Glycine (95 atom % enriched) (34) was converted to [^{15}N]sarcosine (11). The acid solution of [^{15}N]sarcosine was applied to a 25-ml column of Dowex 50 ion exchange resin (H^+, 100–200 mesh) which was washed to neutrality with water and [^{15}N]sarcosine eluted with 7% ammonia solution in three 25-ml fractions. The crude sarcosine obtained on reducing these fractions to dryness was pooled and recrystalized from boiling 95% ethanol. Sarcosine was reacted with cyanamide (37) in the presence of ammonia to form creatine (4). Recrystallized [^{15}N]creatine was pure on the basis of melting point, infrared spectrum, and enzymic assay.

URINE ANALYSES

The Jaffe method was used to measure creatinine content of urine before and after chromatographic treatment (see below) and the values obtained were not significantly different. It was concluded that in these urine samples the contribution of nonspecific chromogen to the color developed in the Jaffe reaction was negligible and therefore the preliminary step of absorbing nonspecific chromogens with Lloyd's reagent was omitted. Creatinine was isolated from urine for mass spectrometry as follows. Urine containing 20 mg creatinine was adjusted to pH 2.5–3.0 with 6 M HCl and placed on a column (25 × 1 cm) containing Dowex 50 resin (Na^+, 100–200 mesh) equilibrated with water. One hundred milliliters of sodium citrate buffer (0.05 M Na^+ and pH 5.0) were allowed to run through the column and creatinine was eluted from the column by adding 120 ml 0.1 M sodium phosphate buffer, pH 6.60. Ten-milliliter fractions of eluate were collected and analyzed for creatinine content. Virtually all of the creatinine was accounted for in four fractions which also contained trace amounts of tyrosine and phenylalanine. Creatinine was separated from these amino acids by the addition of phosphotungstic acid (10% w/v) to form the highly insoluble precipitate, creatinine phosphotungstate. The precipitate was washed once with water, dried *in vacuo*, and submitted to Kjeldahl digestion for 24 hr. Nitrogen in the sample was liberated for mass spectrometry (30) and isotopic abundance determined on an AEI mass spectrometer, model MS3 (38), using the double collector technique. The purity of creatinine isolated from urine by this procedure was checked by comparing the observed and theoretical isotope abundance of [^{15}N]creatinine isolated from a urine sample to which was added a known amount of pure [^{15}N]creatinine. The observed atom per cent excess was between 98% and 102% of the predicted value.

Urinary creatine was isolated by a similar procedure. Urine containing 20 mg creatine was adjusted to pH 3.0 by the addition of 6 M HCl and placed on a column (25 × 1 cm) containing Dowex 50 resin (Na^+, 100–200 mesh). One hundred milliliters of sodium citrate buffer (0.2 M Na^+, pH 3.4) were allowed to run through the column and creatine was eluted with 50 ml sodium citrate buffer (0.05 M Na^+, pH 5.0). Samples containing creatine were prepared for mass spectrometry by the methods described above. Creatine content in urine was first estimated by an enzymic method (2) and later by a colorimetric method (12) which gave identical results and was therefore adopted.

MUSCLE ANALYSES

The muscle sample weighing 7–10 mg was homogenized in deionized water at 4° in a motor-driven all glass Duall tissue grinder (39) and divided into aliquots. Creatine was measured by an enzyme method (2), collagen and noncollagen protein were separated (34), and the protein in each fraction was determined by the Lowry method (22). DNA was assayed by the method of Kissane *et al.* (20), with minor modifications.

RESULTS

The total amount of excess of ^{15}N (40) excreted as urinary [^{15}N]creatinine per day was plotted on semilogarithmic paper as a

function of time and a typical result is shown in Figure 1. The lines obtained from a least squares fit represented a single log-linear process and regression analysis yielded correlation coefficients of 0.843–0.978 in eight subjects (Table 2). In one study (Fig. 1, *BJ*) when values for excess ^{15}N excreted on *days 1* and *2* were plotted, they fell on a steep slope that was different from the straight line obtained from the rest of the values. These results provide good evidence in support of the assumptions that isotopic equilibration occurs by *day 3* in the creatinine precursor pool and that muscle creatine turnover is constant over the period of measurement. The validity of the assumption that muscle creatine is the sole precursor of urinary creatinine was tested by comparing the enrichment of creatine isolated from muscle with that of urinary creatinine in one subject (*MT*).

Three days after the intravenous administration of [^{15}N]creatine a sample of muscle was obtained from the rectus abdominis muscle at an elective operation for repair of an umbilical hernia. Urine was collected during the 24 hr immediately preceding the operation. The atom per cent excess of muscle creatine was 0.085 as compared with 0.099 for urinary creatinine. This close agreement supports the assumptions that isotopic equilibration had been achieved in the muscle creatine pool and that the latter was the immediate precursor of urinary creatinine.

The creatine turnover rates in eight subjects are shown in Table 2. They ranged from 1.53–2.63%/24 hr and illustrate the wide variation between individuals.

The concentration of creatine in muscle also varied among individuals irrespective of whether the results were expressed per unit wet weight of muscle, per unit noncollagen protein, or per unit DNA (Table 3).

In three studies the amount of urinary creatine excreted per day and its degree of enrichment were measured and are shown in Table 4. From these data the turnover and the size of the nonmuscle creatine pool were calculated as for the muscle creatine pool. The degree of enrichment of urinary creatine was substantially less than that of urinary creatinine and the turnover rate of the nonmuscle creatine pool was 4–6 times faster than that of the muscle creatine pool. Table 5 shows the values of muscle mass calculated from muscle creatine turnover, creatinine excretion, and muscle mass equivalent to the excretion of 1 g creatinine in 24 hr. Muscle mass as a percentage of total body weight varied from 15 to 37 (mean 21.8 ± 7.8%) and the muscle mass equivalent to a daily excretion of 1 g creatinine ranged from 14 to 32 kg (mean 18.6 kg ± 6.60 kg).

DISCUSSION

Muscle mass has been estimated indirectly from the daily creatinine excretion, assuming that 1 g creatinine excreted/24 hr is equivalent to 20 kg muscle (6, 17). In deriving this factor of 20, muscle mass was estimated in normal children by assuming that muscle intracellular water was a constant percentage of total intracellular water. This assumption was based on measurements in the rat. Even if this assumption were valid in normal children, it is dubious that it, or the derived factor of 20, is applicable to the malnourished child in whom there are gross abnormalities and wide variations in body composition (16, 26, 33). Recent work in this unit has shown that intracellular water content of leukocytes was greater in malnourished children at the start of refeeding than in recovered children (28). Implicit in the use of a constant factor relating creatinine excretion to muscle mass are two concepts which were examined in this study. The first is that for a given muscle mass containing a given amount of creatine, a constant proportion of the latter is broken down and excreted as creatinine per day. However, the rates of creatine turnover found in this study varied from 1.5 to 2.6%/24 hr (Table 2). The other implicit concept is that the concentration of creatine in muscle is constant. This is not borne out by the data reported here which show that there was variation in the creatine content of muscle whether expressed on the basis of wet weight, noncollagen muscle protein, or muscle DNA (Table 3).

The basis of the present method of estimating muscle mass depends on measuring the size of the body or muscle creatine pool from the rate of loss of isotope following a single dose of labeled [^{15}N]creatine. A similar approach has been used recently (21). The pattern of excretion of label is consistent with a model of

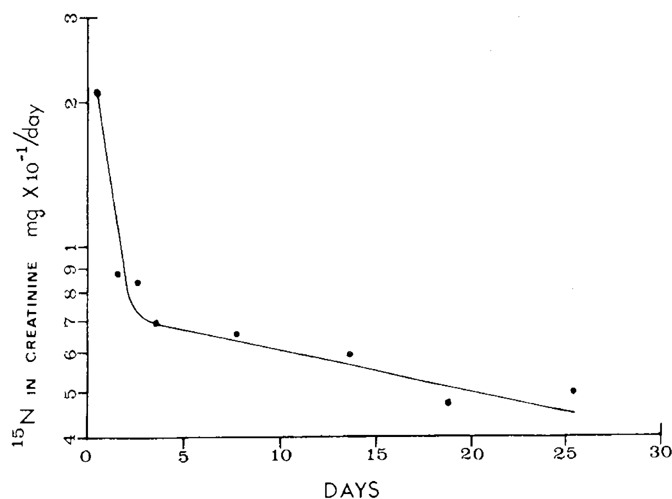

Fig. 1. Semi-log plot of ^{15}N excreted as urinary [^{15}N]creatinine per 24 hr as a function of time.

Table 2. *Creatine turnover in eight children who recovered*[1]

Subject	Creatine turnover, %/24 hr	*n*	*r*
RW	1.53	4	0.954
DA	2.63	4	0.978
HC	2.33	4	0.869
DJ	1.87	4	0.929
BJ	2.43	4	0.946
OJ	2.42	5	0.973
MT	2.38	5	0.938
CR	1.61	4	0.843
Mean	2.15		
±SD	0.42		

[1] Creatine turnover is expressed as the percentage of the creatine pool that is broken down per 24 hr. *n* denotes the number of observations in each study and the correlation coefficient *r* is calculated from linear regression analysis of the daily excretion of excess ^{15}N against time.

Table 3. *Muscle biopsy data: Concentration of creatine in muscle expressed relative to wet weight, noncollagen protein (NCP), and DNA in muscle*

	Muscle	Creatine	Concentration
Subject	μg/mg wet wt	μg/mg NCP	μg/μg DNA
RW	2.95	23.3	2.50
DA	2.92	21.4	1.69
HC	3.00	24.5	2.84
DJ	3.59	21.8	1.88
BJ	3.46	24.1	3.85
OJ	3.45	22.6	1.93
MT	3.32	35.0	2.61
CR	2.25	15.1	2.64
Mean	3.12	23.5	2.49
±SD	0.43	5.5	0.48

washout from a simple one-compartment system. However, a factor to be taken into account is the relatively large amounts of urinary creatine excreted by young children (10), an observation confirmed in this study. It was found that creatine excretion was unrelated to either age or body weight. The isotopic abundance of urinary creatine was 20–50% that of [^{15}N]creatinine in the same urine sample (Table 4). This implies that there are different precursor pools for urinary creatinine and creatine and that they are not in rapid equilibrium. Furthermore, the turnover rate of the precursor pool of urinary creatine appeared to be about 4–5 times as rapid as that of the precursor pool of creatinine. The size of the rapidly turning over creatine pool was calculated to be approximately 4–12% of the size of the creatinine precursor pool. It appears therefore that there are two pools of creatine, a small pool with a fast turnover because of excretion of creatine, and a much larger pool, the muscle creatine pool, which turns over at a slower rate through the formation and excretion of creatinine. Fitch *et al.* (13) have proposed a similar model to explain the creatinuria in patients and animals with muscular dystrophy. In the study reported here, the presence of a second creatine pool did not significantly affect the measurements of either turnover or pool size of body creatine because this secondary pool was of low isotopic abundance and small size and had a rapid turnover.

Muscle mass as a percentage of body weight varied widely and this may reflect individual differences in the proportions of fat, water, and muscle in the body. Although the mean value of 18.6 kg muscle mass/g creatinine excreted/24 hr is similar to that obtained by other workers (16), the individual values ranged from 13.8 to 31.9 kg muscle/g daily creatinine output and the standard deviation was ±35% of the mean value (Table 5). This large and unpredictable variation reduces the value of using a constant factor in predicting muscle mass on an individual basis from daily creatinine output. The data presented show that small but significant variations occur between individuals in both creatine turnover and muscle creatine concentration and that both of these parameters need to be measured in order to obtain a reasonably accurate estimate of muscle mass. The method described should have important applications in the study of body composition. One such application is the estimation of muscle mass in severe protein-energy malnutrition, a study which will be reported separately.

SUMMARY

A method to estimate muscle mass in children was developed. A tracer dose of [^{15}N]creatine was injected intravenously to label total body creatine, nearly all of which is in muscle. The loss of excess ^{15}N in urinary creatinine followed a monoexponential process from which the turnover rate in the muscle creatine pool was determined. From a knowledge of creatine turnover and creatinine output in urine, the size of the muscle creatine pool was calculated. Muscle mass was then estimated from the creatine pool

Table 4. *Comparison of isotopic enrichments of urinary creatine and creatinine and of turnover rates of muscle creatine (MC) and nonmuscle creatine (NMC) pools in three children*

	Urinary creatine		Urinary creatinine,	Turnover	Rate
	mg/24 hr	Atom % excess[1]	atom % excess[1]	% MC/24 hr	% NMC/24 hr
Subject BJ					
Day 3	51.3	0.1122	0.1974	2.43	8.0
Day 7	79.6	0.0477	0.1695		
Day 14	56.2	0.0338	0.1438		
Day 19	56.5	0.0315	0.1259		
Subject DJ					
Day 6	72.2	0.0869	0.1229	1.87	6.3
Day 14	185.5	0.0265	0.0862		
Day 21	112.0	0.0261	0.0683		
Subject MT					
Day 3	44.6	0.0993	0.0994	2.38	7.4
Day 9	114.1	0.0486	0.0684		
Day 16	113.2	0.0331	0.0577		
Day 23	79.2	0.0231	0.0528		
Day 31	91.7	0.0235	0.0417		

[1] Atom per cent of ^{15}N in excess of the natural abundance of ^{15}N.

Table 5. *Muscle mass and daily creatinine excretion and their relationship in eight children who recovered*

Subject	Body wt, kg	Creatinine excretion, mg/24 hr	Muscle mass kg	Muscle mass % body wt	Muscle mass equivalent to daily excretion of 1 g creatinine
RW	4.28	50.8	1.297	30.3	25.6
DA	9.36	105.0	1.586	16.9	15.1
HC	5.22	61.3	1.017	19.5	16.6
DJ	7.93	95.4	1.646	20.7	17.3
BJ	9.52	102.6	1.416	14.9	13.8
OJ	8.61	107.3	1.493	17.4	13.9
MT	10.85	130.0	1.911	17.6	14.7
CR	13.18	153.3	4.897	37.2	31.9
Mean				21.8	18.6
±SD				7.8	6.6

size after measurement of the concentration of creatine in a muscle sample obtained by biopsy. Data are presented to show that labeled creatine equilibrated in the creatinine precursor pool within 3 days, that muscle creatine was the sole precursor of urinary creatinine, and that the turnover rate in the creatine pool was constant during the period of measurement. There appears to be a secondary nonmuscle creatine pool which was much smaller and had a more rapid turnover rate than the muscle creatine pool. The results show that there was wide variation between individuals in creatine turnover and muscle creatine concentration. It is concluded that these variations seriously limit the usefulness of daily creatinine output as a predictor of muscle mass in the individual subject.

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40. Excess ^{15}N is the amount of ^{15}N in excess of that due to the natural abundance of ^{15}N.

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