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Creatine
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total body water

Muscle Mass and Composition in Malnourished Infants and Children and Changes Seen after Recovery

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Summary

A method for measuring muscle mass in children with [^{15}N]creatine has been used to study changes in muscle after recovery from protein-energy malnutrition. Creatine pool size, muscle mass, total muscle cell number, muscle cell size, and total body water have been measured in seven malnourished and eight recovered children. After recovery there was a significant reduction in the muscle concentration (micrograms mg^{-1} wet wt muscle) of creatine (4.21 to 3.12), and a trend towards reduction in noncollagen protein (155 to 136) and DNA (2.13 to 1.34). The fractional turnover rate of creatine did not change but the creatine pool size increased significantly (4.2 to 5.6 g). Average muscle mass almost doubled (1.00 to 1.91 kg) and made up a greater percentage of body weight (16 to 22%). When muscle mass was expressed as a percentage of the expected muscle mass for a normal child of the same height the increase with recovery was from 49% to 92%. Total muscle noncollagen protein (NCP) increased after recovery (153 to 265 g) and accounted for a greater percentage of total body solids (6.6 to 8.5%). The average total muscle DNA was 2.049 g in the malnourished and 2.380 g in the recovered children and the ratio of NCP:DNA increased from 92 to 110 on recovery. Total body water as a percentage of body weight was not significantly different after recovery. Values for muscle mass in recovered children were similar to those reported for normal children of the same weight, height, and age.

Speculation

In children who have recovered from malnutrition by reaching their expected weight for height, muscle mass repletion is achieved by a combination of cellular hypertrophy and hyperplasia.

Muscle tissue provides the largest store of potentially available protein in the body (29) and is considerably reduced in the children with severe protein-energy malnutrition (PEM). From cadaver studies of children dying of severe PEM, the deficit in muscle was estimated to be about 70% based on either wet weight (18) or total muscle protein (15, 29). Muscle biopsy data showed a 45% reduction in total protein in malnourished as compared to recovered children (31). Both the size and the total number of muscle fibers were considerably smaller in the sartorius muscle, obtained at postmortem, of malnourished as compared to well nourished children (23). During recovery from PEM there is an accelerated rate of weight gain (2), and the daily excretion of creatinine has been used as an indirect index of muscle mass in malnourished and recovered children (1, 7, 13, 27). However, the validity of creatinine excretion as a reliable measure of muscle mass in the malnourished child remains to be established. This is important since changes in muscle cell size and total cell number during recovery from PEM have been based on this method of estimating muscle mass (7).

Recently we reported a more direct method of measuring muscle mass in children (25). This method has been used in the present study to determine quantitative changes in muscle mass, total cell number, and functional cell size in malnourished and recovered children. These results and those of total body water measurements are reported in this paper.

MATERIALS AND METHODS

The method for measuring total muscle mass *in vivo* with [^{15}N]creatine has been described in detail (25). A tracer dose of [^{15}N]creatine was injected intravenously to label total body creatine nearly all of which is in muscle (22, 32). The loss of excess ^{15}N (34) in urinary creatinine followed a monoexponential process from which the turnover rate of the muscle creatine pool was determined. From a knowledge of this and of daily urinary creatinine output, the size of the muscle creatine pool was calculated. Muscle mass was then estimated from the creatine pool size after measurement of the concentration of creatine in a muscle sample obtained by biopsy.

Urinary creatinine was measured by the Jaffe method. Isolation and purification of urinary creatinine and its analysis for ^{15}N abundance by mass spectrometry have been previously described (25). Creatine was measured enzymically (3). Muscle DNA was measured by the method of Kissane and Robins (20) with minor modifications. Total protein and noncollagen protein were assayed by a modification (31) of the Lowry method (21). The creatine, DNA, and protein were measured on muscle samples, obtained at biopsy, which weighed between 5 and 16 mg wet wt. All measurements were carried out in duplicate against a known standard. In preliminary studies on rat tissue values comparable to those in the literature were obtained for all the measurements. No studies have been performed on normal human biopsies.

Muscle "cell number" was calculated by dividing the total muscle DNA by 6.2 pg (11), and muscle "cell size" by dividing total muscle noncollagen protein (in grams) by total muscle DNA (8).

Total body water was measured by the technique of isotope dilution. A tracer dose of tritiated water was administered intravenously and its dilution was determined from the specific activity of plasma water from a blood sample obtained 3 hr after the injection of tracer. The dose was 0.2% of the recommended safe whole body dose which yielded counts 50–100% above background. The biologic half-life of the tracer was 30 hr.

PATIENTS

Seven malnourished children (malnourished group) were studied in a metabolic unit. Their ages, diagnoses, and anthro-

Table 1. *Clinical state of malnourished and recovered subjects*

Subject	Age, mo	Wt,[1] kg	Wt[2] for age, %	Ht, cm	Ht for age, %	Wt for ht, %	Day of study	Diagnosis[3]
Malnourished group								
KG	8	3.970	46	62	89	64	1	Marasmus
DA	9	5.280	58	69	97	63	3	Marasmus
DJ	11	4.438	46	61.5	83	73	4	Marasmus
GF	12	6.166	61	71	94	69	1	Undernourished
MT	15	7.722	72	78	99	73	1	Kwashiorkor
ED	17	5.950	53	73	90	63	1	Marasmus-kwashiorkor
CR	26	10.224	79	84.75	95	85	3	Kwashiorkor
Mean	14.0	6.250	59	71.32	92	70		
SD	5.92	2.14	12.6	8.33	5.5	7.9		
Recovered group								
RW	4	4.280	68	56.5	91	90		
DA	12	9.360	94	72.5	97	100		
HC	13	5.219	51	58	76	101		
DJ	13	7.934	77	67	88	102		
BJ	15	9.524	89	75	96	95		
OJ	16	8.605	78	73	92	90		
MT	18	10.845	95	80.5	98	97		
CR	27	13.180	101	86	96	107		
Mean	14.75	8.618	82	71.06	92	98		
SD	6.5	2.878	16.6	10.23	7.2	6.0		

[1] Lowest weight after admission.
[2] Boston standard 50th centile.
[3] Based on the Wellcome classification (33).

Table 2. *Total body water and body solids in malnourished and recovered subjects*

	Total body water		Total body solids	
Subject	Liter	%Wt	Kg	%Wt
Malnourished				
KG	2.695	59	1.849	41
DA	3.548	66	1.837	34
DJ	2.849	64	1.570	36
GF	4.057	66	2.090	34
MT	6.398	68	3.002	32
ED	4.307	66	2.268	34
CR	6.131	58	4.367	42
Mean	4.284	64	2.426	36
SD	1.475	3.8	.970	3.8
Recovered				
RW	2.829	66	1.451	34
DA	6.186	66	3.174	34
HC	N.A.[1]	N.A.	N.A.	N.A.
DJ	5.340	67	2.594	33
BJ	5.942	62	3.582	38
OJ	4.827	56	3.778	44
MT	7.042	65	3.803	35
CR	8.152	62	5.028	38
Mean	5.760	63	3.344	37
SD	1.693	3.8	1.033	3.8

[1] Not available.

Table 3. *Creatinine output, creatine turnover, and pool size and creatinine height index in malnourished and recovered subjects*

		Creatine		
Subject	Urinary creatinine, mg kg^{-1} day^{-1}	Fractional turnover, % day^{-1}	Pool size, g	Creatinine ht index
Malnourished				
KG	13.07	2.38	2.890	0.81
DA	11.85	2.14	3.449	0.68
DJ	11.00	1.59	3.554	0.70
GF	12.42	2.46	3.601	0.76
MT	8.74	2.42	3.933	0.63
ED	10.75	1.93	4.252	0.65
CR	10.06	1.61	7.591	0.64
Mean	11.13	2.08	4.181	0.69
SD	1.47	0.373	1.561	0.06
Recovered				
RW	11.86	1.53	3.853	0.94
DA	11.22	2.63	4.635	0.98
HC	11.75	2.33	3.051	1.04
DJ	12.02	1.87	5.906	1.10
BJ	10.77	2.43	4.902	0.87
OJ	12.47	2.42	5.146	0.98
MT	11.99	2.38	6.338	0.92
CR	11.63	1.61	11.042	0.89
Mean	11.71	2.15	5.609	0.97
SD	0.522	0.418	2.432	0.08

pometric data are shown in Table 1. All seven studies were done within 4 days of admission when the children were receiving a milk-based formula on which their body weight was maintained. After the initial study, children were given a high energy, milk-based diet on which they gained weight rapidly (19). Eight children were studied after they had recovered from PEM. They included four children who were also studied when they were malnourished. The ages and anthropometric data of children in the recovered group are shown in Table 1. Full and informed parental consent was obtained for each study performed.

RESULTS

Where applicable statistical analyses were carried out. Differences between the malnourished and recovered groups were tested for statistical significance by Student's *t*-test. Where groups' variance were significantly different, the Behring Fisher test was used (26).

The mean age and height of the malnourished group (14.0 months and 71.3 cm) were similar to the recovered group (14.8 months and 71.1 cm). The mean percentage weight for height

was significantly greater after recovery ($P < 0.001$) than in the malnourished state (Table 1).

Table 2 shows that in the malnourished children the average total body water (4.3 liters) and total body solids (2.4 kg) were reduced as compared to the recovered children (5.8 liters and 3.3 kg). In the paired studies this difference was significant ($P < 0.05$). However, when expressed as a percentage of total body weight, mean total body water (TBW%) and total body solids (65% and 35%, respectively) in the malnourished group did not differ from the values found after recovery (63% and 37%). The average urinary creatinine was not significantly greater in the recovered (11.7 mg kg^{-1} day^{-1}) than the malnourished (11.1 mg kg^{-1} day^{-1}) children (Table 3). There was a significant increase ($P < 0.005$) in the mean creatinine height index after recovery (0.68 to 0.97). The fractional turnover rate of creatine in the malnourished (mean 2.05% day^{-1}) and the recovered children (2.15% day^{-1}) did not differ significantly, although the creatine pool size increased significantly in the paired studies. The muscle biopsy data are shown in Table 4. There was a small but significant reduction in the average muscle concentration of creatine (4.21 to 3.12 μg mg^{-1} wet wt muscle, $P < 0.05$) on recovery. The increase in the concentration of noncollagen protein and DNA after recovery did not reach significance. The average muscle mass after recovery (Table 5) was almost twice that found in the malnourished group (1.91 versus 1.00 kg wet wt, respectively). In the paired studies the average increase in muscle mass was 130% and this accounted for a greater percentage of body weight (mean 56% range 1 to 131%). The muscle mass expressed as a percentage of the normal for a child of the same height increased on average from 49% in the malnourished to 92% on recovery. The average increase in muscle noncollagen protein with recovery was from 153 g to 265 g, an increase of 116% in the paired studies. The noncollagen protein accounted for a greater percentage of total body solids in the recovered (8.5%) as compared to the malnourished group (6.6%), a paired increase of 56%. The average muscle DNA in the malnourished (2.05 g) was lower than the recovered (2.38 g) and there was a paired increase of 55% with recovery. Similarly there was an increase in the ratio of noncollagen protein to DNA from 92 to 110, a 34% increase for the paired values.

Table 4. *Muscle biopsy data in malnourished and recovered subjects*

Subject	Creatine, μg mg^{-1} wet wt muscle	NCP, μg mg^{-1} wet wt muscle	DNA, μg mg^{-1} wet wt muscle	Creatine /NCP, %	Creatine /DNA, g/g
Malnourished					
KG	4.436	192.2	1.166	2.31	3.80
DA	4.440	161.4	2.078	2.75	2.14
DJ	3.941	133.6	2.095	2.95	1.88
GF	3.966	129.7	1.790	3.06	2.22
MT	4.88	156.4	4.100	3.12	1.19
ED	3.18	171.6	2.835	1.85	1.12
CR	4.607	138.7	0.824	3.32	5.59
Mean	4.207	154.8	2.127	2.77	2.66
SD	0.563	22.6	1.090	0.52	1.15
Recovered					
RW	2.970	127.6	1.189	2.33	2.50
DA	2.922	136.8	1.730	2.14	1.69
HC	3.001	122.5	1.056	2.45	2.84
DJ	3.587	164.6	1.912	2.18	1.88
BJ	3.463	143.5	0.900	2.41	3.85
OJ	3.448	152.3	1.787	2.26	1.93
MT	3.316	95.0	1.269	3.50	2.61
CR	2.247	148.6	0.850	1.51	2.64
Mean	3.119	136.4	1.337	2.35	2.49
SD	0.434	21.5	0.418	0.55	0.69

DISCUSSION

The value obtained for muscle mass is that at a single point in time when the muscle biopsy is taken. However, a single study extended over an average period of 4 weeks, because weekly samples of urine were needed in order to determine the kinetics of creatine catabolism. During this time a marked improvement in the nutritional status of the malnourished children had occurred. Yet the creatine turnover in any one individual remained remarkably constant. This is shown in the malnourished group where there was a high average correlation coefficient ($r = -0.937$) of the slope of the line expressing the monoexponential loss of isotope. Furthermore, there was no significant difference in the average creatine turnover rates between the malnourished and recovered groups. We conclude from these findings that creatine turnover is unaffected by

Table 5. *Muscle mass (MM) data in malnourished and recovered subjects*

Subject	MM, kg	MM/wt, %	MM expected for ht, %	NCP, g	NCP/total body solids %	DNA, g	Muscle nuclei, $\times 10^{12}$	NCP:DNA, g/g
Malnourished								
KG	0.652	14	46	125	6.8	0.760	0.123	165
DA	0.777	14	41	125	6.8	1.615	0.260	78
DJ	0.902	21	66	120	7.6	1.889	0.305	64
GF	0.908	15	44	118	5.6	1.625	0.262	72
MT	0.806	9	32	126	4.2	3.305	0.533	38
ED	1.337	20	61	229	10.1	3.791	0.611	61
CR	1.648	16	54	229	5.2	1.358	0.323	168
Mean	1.004	16	49	153	6.61	2.049	0.345	92.3
SD	0.356	4	11.9	52	1.9	1.050	0.169	52
Recovered								
RW	1.297	30	130	166	11.4	1.543	0.249	107
DA	1.586	17	73	217	6.8	2.744	0.443	79
HC	1.017	20	92	124	N.A.	1.074	0.173	116
DJ	1.646	21	93	271	10.4	3.148	0.508	86
BJ	1.416	15	60	203	5.7	1.274	0.206	159
OJ	1.493	17	67	227	6.0	2.668	0.430	82
MT	1.911	18	69	182	4.8	2.426	0.391	75
CR	4.897	37	155	728	14.5	4.162	0.671	175
Mean	1.907	22	92.4	265	8.5	2.380	0.384	110
SD	1.236	8	33.7	192	3.6	1.043	0.168	38

nutritional state. It follows from this that turnover is also not influenced by the size of the creatine pool, which increased by an average of 52% in the paired studies after recovery from malnutrition. Waterlow *et al.* (32) reported that in the rat, creatine turnover appeared to be little affected by age, sex, level of protein intake, and rate of weight gain or loss. There appears to be a remarkably narrow range of values for creatine turnover in the mouse, rat, dog, and man (32).

Muscle creatine concentration (micrograms mg^{-1} wet wt muscle) varied appreciably between individuals in both the malnourished and recovered groups (coefficient of variance, 13% and 14%, respectively). This may well be a reflection of the variation in the rate of creatine synthesis in man (10). Creatinine output, which has been used to estimate muscle mass (14), depends, among other things, on the muscle creatine concentration and turnover, both of which showed significant variation between individuals in the malnourished and recovered groups. The dietary intake of creatine and creatinine are known to influence the urinary excretion of creatinine (12). It has been suggested that creatinine excretion merely reflects the dietary intake of creatine (4). In a recent report the addition of creatine to the diet led to an increase in the daily creatinine excretion (9) and the subsequent feeding of a creatine-free diet resulted in a decrease in creatine pool size and urinary creatinine excretion (10). These authors have concluded that creatinine output can change independently of lean body mass. The data presented in this and other studies (9, 10, 25) support the view that there may be considerable error in predicting muscle mass from the daily excretion of urinary creatinine.

Of necessity the recovered studies were carried out some 2 months after the malnourished studies. In consequence any difference that is demonstrated between the groups tends to be the superimposition of two processes: the normal changes in growth and development that would have taken place over that period, plus the additional changes due to the repletion of tissues during recovery from malnutrition. We can try to differentiate the two processes either by looking at relative differences in the changes taking place within the study group itself, or, more ideally, we would like to be able to compare the changes we have observed with a suitable reference population. Data from the paired studies (Table 6) show that the increase in muscle mass (130%) and muscle noncollagen protein (116%) after recovery was greater than the increase in either body weight (56%) or total body solids (45%). These results confirm the suggestion that body weight deficit underestimates the extent of loss of muscle mass (30). One indicator of the abnormal body composition in PEM is the lower value for muscle mass as a percentage of body weight in the malnourished as compared to the recovered group.

Cheek *et al.* (7) calculated the muscle mass in malnourished and recovered children from the measured 24-hr excretion of creatinine in the urine, as described by Graystone (14). The ages of the children in their series were not significantly different from the ages of the children described here. When the two sets of data are compared on a group basis there is no significant difference in the mean muscle mass between the respective malnourished groups, nor is there any difference between the two groups after recovery. A comparison was made with the muscle mass of normal children of a similar age (6). The value of 22% for the recovered children is the same as that reported for normal male infants 0.2–2 years of age. The muscle mass of the malnourished children was significantly lower than normal. If the muscle mass was related to height rather than to age for the same children, then the increase with recovery brings the muscle mass to 92% of the expected value for their height. This suggests that the recovered children had repleted their muscle mass in relation to their weight and height.

The average total muscle DNA was not significantly different in the malnourished and recovered groups. In the paired studies total muscle DNA increased considerably after recovery (mean

Table 6. *Body composition changes in four subjects before and after recovery from protein-energy malnutrition*

Subject	Body wt, kg	Body solids, kg	Creatine pool, g	MM,[1] kg	MM/wt, %	NCP, g	NCP/TBS,[2] %	DNA, g	Muscle nuclei, $\times 10^{12}$	NCP: DNA, g/g	MM/ expected MM for ht, %
DA											
M	5.280	1.837	3.449	0.777	14.7	125	6.80	1.615	0.260	78	41
R	9.360	3.174	4.635	1.586	16.9	217	6.84	2.744	0.443	79	73
Δ %	+77	+73	+34	+104	+15	+74	+0.6	+70	+70	+1	+78
DJ											
M	4.438	1.570	3.554	0.902	20.3	120	7.6	1.889	0.305	64	66
R	7.934	2.594	5.906	1.646	20.7	271	10.4	3.148	0.508	86	93
Δ %	+79	+65	+66	+82	+2	+126	+37	+67	+67	+34	+41
MT											
M	7.722[2]	3.002	3.933	0.806	10.4	126	4.2	3.305	0.533	38	32
R	10.845	3.803	6.338	1.911	17.6	182	4.8	2.426	0.391	75	69
Δ %	+40	+27	+61	+137	+69	+44	+14	−27	−27	+97	+116
CR											
M	10.224[2]	4.367	7.591	1.648	16.1	229	5.2	1.358	0.323	168	54
R	13.180	5.028	11.042	4.897	37.1	728	14.5	4.162	0.671	175	155
Δ %	+29	+15	+46	+197	+131	+218	+179	+108	+108	+4	+187
Mean ± SEM											
M	6.916 (1.3)	2.694 (0.638)	4.632 (0.992)	1.033 (0.207)	15.4 (2.04)	150 (26.4)	5.95 (0.77)	2.042 (0.435)	0.355 (0.061)	87 (28.2)	48 (7.4)
R	10.330 (1.1)	3.650 (0.522)	6.980 (1.401)	2.51 (0.799)	23.1 (4.75)	350 (127.5)	9.14 (2.13)	3.12 (0.377)	0.503 (0.061)	104 (23.8)	97.5 (19.9)
Δ %	+56 (12.8)	+45 (14.2)	+52 (7.3)	+130 (25)	54 (29)	+116 (38)	+58 (41)	+54 (29)	+54 (29)	+34 (22)	106 (31.1)

[1] MM: muscle mass; TBS: total body solids; M: malnourished; R: recovered; Δ %: percentage of change.
[2] Minimum weight after loss of edema.

+55%), although in one study were was a decrease (−27%). It is not possible to equate total muscle DNA with total myofibrillar nuclei. Cheek *et al.* (8) have pointed out that during normal growth 25% of nuclei in human muscle may be outside the myofiber. Histologic studies of muscle biopsies from malnourished and recovered children in this unit have revealed that approximately two-thirds of the total nuclei count was accounted for by muscle nuclei in both the malnourished and recovered groups. Of these nuclei, those classified as myogenic, including presumptive myoblasts, myoblasts, and myotubes, made up a significantly greater percentage in the recovered than in the malnourished children (16). One child showed a fall in DNA with recovery. His initial biopsy, obtained when he was malnourished, showed an excess of nonmyofibrillar nuclei, and was considered to be technically unsatisfactory and probably unrepresentative of the muscle mass.

It is not possible to measure fiber size in a biochemical analysis. However the concept of "cell size," as the ratio of noncollagen protein to DNA, has been found useful as a functional expression of the active protoplasmic mass per nucleus or physiologic muscle cell (8). Because of the uncertain contribution of nonmyofibrillar nuclei to the total muscle DNA, the absolute value for the ratio of NCP:DNA for the muscle cell is uncertain. However, total muscle NCP per total muscle DNA may be considered to be a minimum estimate of average "cell size" as any contribution from nonmuscle nuclei would tend to lower the ratio. The increase in the ratio of NCP:DNA during recovery (34%) shows that cellular hypertrophy plays a role in the repletion of muscle mass. The finding that total DNA increases by 55% suggests that hyperplasia is also of importance, and that during recovery, muscle mass is repleted by a process of both hypertrophy and hyperplasia.

When changes are expressed on the basis of group means, it is possible that important individual variations be masked. It is difficult to make definite statements on the basis of a small series; however, the data do indicate a trend. If the initial deficits of weights and heights are ranked and compared with the ranked values for changes in muscle, NCP, and weight there is the suggestion that the least stunted children are more wasted and during repletion lay down relatively more muscle tissue. If this is so then the suggestion that during rehabilitation from malnutrition children make "balanced tissue" will have to be reexamined. We would suggest that this is not necessarily so. There is not a single metabolic stress of malnutrition, and the spectrum of marasmus to kwashiorkor is indicative of the differences. During rehabilitation the balance of tissues being repleted may vary depending upon the relative degree of stunting and wasting (17).

The changes in creatinine height index were similar to those reported previously (28). The creatinine excretion was highly correlated with body weight throughout recovery, irrespective of nutritional state ($r = 0.795$, $n = 126$, $P < 0.001$) provided that a correction was made in body weight for the presence of edema. This reflects the use of this index as a measurement of weight deficit in relation to height. The close relationship between daily creatinine excretion and body weight in normal infants has been ascribed to the muscle mass making up a fixed percentage of body weight (25%) during the first year of life (5). Our finding that this relationship holds in a variety of nutritional states when body composition is not necessarily normal suggests that muscle mass itself may not be the sole determinant of creatinine excretion.

An unexpected finding was that TBW% was the same in the malnourished and recovered groups. Similar results were obtained when paired data from four subjects were analyzed. All but one of the seven malnourished children were nonedematous, and none were dehydrated at the time of study. We have subsequently shown that TBW% in marasmic children on admission did not differ significantly from values obtained after recovery (24).

CONCLUSION

This study has demonstrated that during recovery from malnutrition there is a significant increase in muscle mass. This increase is proportionately greater than the increase in body weight, and may differ according to the initial degree of stunting or wasting. The evidence suggests that both hypertrophy and hyperplasia may take place, but the extent to which each process contributes to the muscle repletion has not been clarified and further work is required to elucidate this point.

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34. ^{15}N in excess of that due to the natural abundance in the compound is referred to as excess ^{15}N.
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