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Malnutrition muscle myogenic cells needle biopsy

Quantitative Analysis of Nuclear Population in Muscle from Malnourished and Recovered Children

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Summary

Muscle tissue obtained by needle biopsy from the quadriceps femoris of eight malnourished and eight recovered children were examined histologically. In both groups about two-thirds of the total nuclear count was due to muscle nuclei. The remaining one-third was made up of vascular, nerve, and interstitial cells and isolated nuclei in similar proportions in the two groups. A significantly greater percentage of muscle nuclei consisted of myogenic cells in recovered children as compared to malnourished children (20.7 ± 6.4 *vs*. 2.6 ± 1.4%)

Speculation

The presence of a higher percentage of myogenic cells in muscle of children who have recovered from protein-energy malnutrition suggests that muscle repletion involves hyperplastic growth and that muscle maturation in apparently fully recovered children may not be complete.

The concept of a DNA unit, or "cell" (3) has been found useful in defining a subunit of control within the muscle mass, even though it is well recognized that mature skeletal muscle fibers are multinucleate, rather than discrete mononucleate cells. Precursors of the myofiber, however, exist as individual mononucleate cells before joining to form a myotube. DNA content or concentration is frequently used as a reference to express biochemical responses of other muscle constituents to protein-energy malnutrition (PEM) (2, 3, 10, 14) and to nutritional rehabilitation (2, 10). The use of DNA as a reference is based on the assumption "that no cells are present in the extracellular phase and that connective tissue cells are minimal" (2). This is not strictly valid since in animal muscle it has been shown that approximately one-third of the nuclei in the tissue are associated with nonmuscle cells rather than with the myofiber (5, 12). In severe PEM there are marked alterations in body composition, with severe muscle wasting (14). However, there is no quantitative information on the extent to which nonmuscle nuclei contribute to the DNA in muscle tissue in PEM. Histologic changes such as apparent loss of muscle fibers without a notable change in vascular cells (7) and infiltration of the tissue by white cells (7, 13) have been reported in muscle from malnourished children. These observations suggest that in PEM, contrary to Cheek's statement (2), a significant number of the total population of nuclei in muscle may be associated with interstitial cells. There is also little information on the distribution of nuclei in muscle of children who have recovered from PEM. Thus, interpretation of DNA measurements must be viewed with caution.

The purpose of this investigation was to determine the distribution of nuclei among the cell types in muscle of children before and after recovery from severe PEM.

MATERIALS AND METHODS

Muscle samples were obtained by percutaneous needle biopsy (7) of the quadriceps femoris. Biopsies from eight malnourished Jamaican children were obtained 2–7 days after their admission for severe PEM. The mean age (±SD) of these children on admission was 15.9 (±4.8) months. Biopsies were taken from eight other children who had recovered from severe PEM and had achieved, on average, 95% of their expected weight for height (50th percentile of the Boston standards). Their mean ages (±SD) on admission and after recovery were 16.0 (±3.2) and 18.5 (±3.2) months, respectively. The anthropometric measurements of the malnourished children who were biopsied and of the recovered group when they were admitted for PEM were similar (Tables 1 and 2). Full and informed parental consent was obtained for the biopsy of each child and approval for the study was obtained from the local Ethics Committee. The biopsy procedure was well tolerated by all children, who moved the limb freely immediately after the procedure, and there were no complications. The muscle specimens were immediately fixed in 2% phosphate-buffered glutaraldehyde. Subsequently they were postfixed in 2% OsO_4, dehydrated in alcohol, and embedded in a low viscosity plastic resin. Sections for light microscopy (1 mμ) were cut on an LKB ultramicrotome and stained (6). The total number of nuclei in each section was determined at a magnification of 400× on an Olympus microscope. Counts were made on sections from five different specimen blocks per subject. A mean (±SEM) of 1283 (±239) nuclei were counted per subject in the malnourished group and 1137 (±167) in the recovered group. Student's *t*-test was used for statistical analyses. Statistical significance was accepted at $P < 0.05$.

The nuclei were classified according to the following cell types (Fig. 1).

MUSCLE CELLS

These consisted of myofibers and myogenic cells. Associated with the former were subsarcolemmal nuclei of mature striated myofibers, as well as satellite cells, which were not always distinguishable from the true subsarcolemmal myonuclei because of compression in some parts of the tissue. Myogenic cells included presumptive myoblasts, myoblasts, and myotubes.

VASCULAR AND NERVE CELLS

These were structural cells of capillaries, small blood vessels and, rarely, nerve fibers.

Table 1. *Diagnosis and anthropometric data of eight malnourished infants and children*

Subject	Sex	Diagnosis[1]	Age, mo	Wt, kg	Ht, cm	Expected[2] wt for age, %	Expected[2] wt for ht, %	Expected[2] ht for age, %
RB	M	Kwashiorkor	15	6.94	73.0	65	73	93
AO	M	Kwashiorkor	10	5.38	65.5	58	73	91
GO	M	Marasmus	18	6.18	73.0	55	65	90
AW	M	Marasmus	11	5.86	69.5	60	69	95
DS	F	Masrasmus	18	4.86	63.0	43	74	77
AY	M	Masrasmic kwashiorkor	12	5.70	70.5	58	64	94
AD	M	DO	19	6.38	72.0	55	69	87
MM	M	Undernourished	24	8.20	76.5	66	80	88
Mean			15.9	6.19	70.4	57.5	71	89
±SD			4.8	1.03	4.4	7.2	5.2	5.7

[1] Based on Wellcome Classification (4)
[2] Boston standards, 50th percentile.

Table 2. *Diagnosis and anthropometric data of eight male children before and after recovery from protein-energy malnutrition*[1]

Subject	Diagnosis	Status	Age, mo	Wt, kg	Ht, cm	Wt for age, %	Wt for ht, %	Ht for age, %
BF	K	M	19	7.98	79.0	69	74	
		R	20	10.88	80.0	93	99	96
RS	K	M	13	6.99	75.0	69	71	96
		R	17	9.27	77.0	84	89	99
RD	Mar	M	19	4.48	66.0	39	60	96
		R	21	9.38	71.0	79	104	80
GC	Mar	M	17	5.10	67.5	46	64	84
		R	19	9.09	72.5	79	97	84
AD	M-K	M	19	6.38	72.0	55	69	88
		R	22	9.67	75.5	80	96	87
NH	M-K	M	17	6.19	73.0	56	65	88
		R	21	9.70	79.5	82	89	91
KS	M-K	M	11	5.65	65.5	59	77	94
		R	13	8.42	68.5	83	102	89
AJ	U	M	13	6.66	69.0	65	79	90
		R	15	7.46	69.5	70	87	91
								89
Mean		M	16.0	6.18	70.9	57	70	90
±SD			3.2	1.10	4.7	10.7	6.6	6.1
Mean		R	18.5	9.32	74.2	81	95	91
±SD			3.2	1.00	4.5	6.4	6.4	4.3

[1] K: kwashiorkor; Mar: marasmus; M-K: marasmic kwashiorkor; U: undernourished; M: malnourished; R: recovered.

INTERSTITIAL CELLS

These comprised fibroblasts, macrophages, and miscellaneous unidentified mononucleate cells in the interfiber space and in collagen bundles. Isolated nuclei with no apparent surrounding cytoplasm were found in the collagen deposits and were included in the counts.

RESULTS AND DISCUSSION

The results are shown in Table 3. Muscle nuclei accounted for approximately two-thirds of the total nuclei count. This proportion was not significantly different in malnourished and recovered children. A significantly greater percentage of muscle nuclei consisted of myogenic cells in the recovered group (20.7 ± 6.4%, mean ± SEM) as compared to the malnourished group (2.6 ± 1.4, mean ± SEM). Myogenic cells are thought to be derived from the satellite cells of the myofiber (1). Since it is believed that myogenic cells may be capable of mitosis (1) the results suggest that the recovery process in muscle may involve hyperplastic growth.

No significant differences between the malnourished and recovered groups were observed in the distribution of the nonmuscle nuclei which made up the remaining one-third of the total nuclear count. Although invasion by white cells has been reported in malnourished children (7, 13) the presence of interstitial phagocytic cells was not common in either group. Nor was there a suggestion of a disproportion between vascular cells and muscle cells when judged by the percentage of nuclei in each group.

DNA content in muscle biopsies has been used to estimate changes in muscle "cell" number (2, 3, 10) and size (15, 2, 3, 10). However, this study shows that approximately one-third of all nuclei in muscle tissue is accounted for by nonmuscle nuclei and this holds true for muscle obtained from both malnourished and recovered children. Thus, to reasonably estimate the number of muscle cells from the DNA content of muscle, it is necessary to correct the latter by a factor of one-third. When the total number of muscle cells from biopsy data and a value for total muscle mass are estimated the assumption is made that the composition of the sampled muscle is representative of the

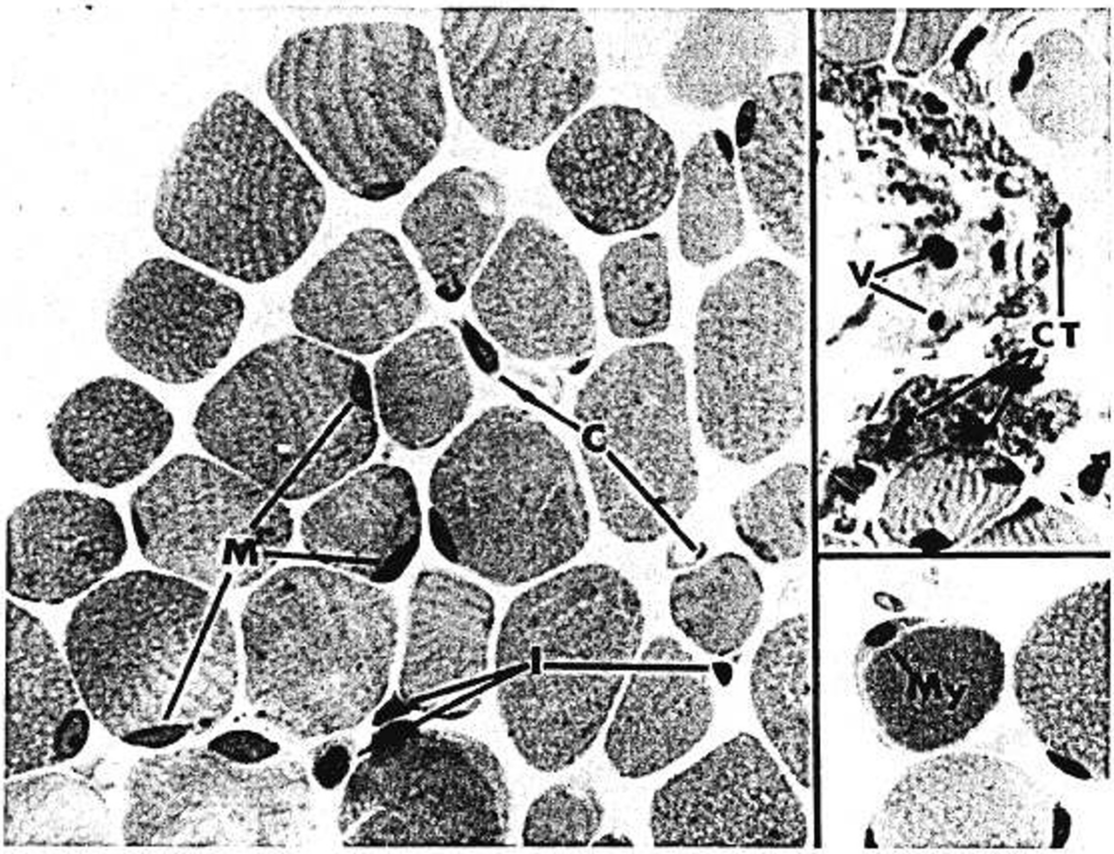

Fig. 1. Representative types of nuclei from a biopsy of a recovered subject: M: muscle fiber; C: capillary; I: interstitial cell; CT: connective tissue; V: small blood vessel; My: presumptive myoblast (1100×).

Table 3. *Proportion of nuclei in muscle from malnourished and recovered children*

Subject	Total muscle	Myofiber	Myogenic cells	Vascular and nerve	Interstitial cells
Malnourished					
GO	68.6	68.1	0.5	10.3	20.4
RB	74.0	67.0	7.0	14.8	11.0
AW	61.5	61.5	0.0	24.9	14.0
AO	60.6	58.2	2.4	26.9	12.0
MM	62.6	62.6	0.0	24.7	12.1
AY	63.5	63.5	0.0	20.5	14.0
AD	64.8	64.8	0.0	19.9	16.0
DS	80.3	76.1	4.2	8.7	11.0
Mean ± SE	67.0 ± 2.4	65.2 ± 1.9[1]	1.8 ± 0.9[1]	18.7 ± 2.5	14.1 ± 1.1
Recovered					
AD	58.9	54.2	4.7	26.1	15.3
NH	49.0	45.7	3.3	25.1	25.9
RD	54.1	48.1	6.0	26.7	19.7
BF	80.5	58.7	21.8	16.0	3.4
RS	63.9	59.0	4.9	14.0	22.1
AJ	72.1	45.4	26.7	10.9	17.0
GC	68.6	58.2	10.4	17.5	13.9
KS	62.5	54.3	8.3	26.3	12.5
Mean ± SE	63.7 ± 2.0	53.0 ± 2.0	10.8 ± 3.1	20.6 ± 2.4	16.2 ± 2.4

[1] $P < 0.05$ compared to recovered subjects.

muscle mass as a whole. No human data are available to test this assumption but studies in normal and malnourished rats suggest that changes in DNA content of the quadriceps femoris parallel those of other muscles (3, 11). The size or mass of a functional cell unit in muscle has been calculated from the ratio of muscle protein to muscle DNA (2, 3, 10, 15). An underlying assumption of this mathematic expression is that nonmuscle cells in the muscle sample contribute insignificantly to the protein and DNA measurements. The latter is certainly invalid (Table 3), although this study provides no information on the former. Furthermore, although this ratio is an informative measurement, the "cell" size so calculated is an average value and does not take into consideration the heterogeneity in size between the two muscle cell types, myofibers and myogenic cells. The latter appear visually to have a much smaller proportion of cytoplasm per nucleus than the former.

CONCLUSION

The concept of a DNA unit of muscle has been used as a reference to express biochemical responses. However, the usefulness of this reference is reduced if the proportion of nonmuscle nuclei in this muscle differs with nutritional states. Measurements have been made on eight malnourished and eight recovered children of the distribution of nuclei in muscle. It is concluded that muscle nuclei account for approximately two-thirds of the total nuclei count and that this proportion does not differ significantly in the malnourished child.

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