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Growth of muscle fibres during recovery from severe malnutrition in Jamaican Infants

BY F. M. HANSEN-SMITH*

Department of Anatomy, University of Michigan, Ann Arbor, Michigan 48109, *USA*

AND D. PICOU AND M. H. GOLDEN†

Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston 7
Jamaica, West Indies

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1. The growth of muscle fibres was analysed by light microscopy in biopsies from subjects when malnourished, during nutritional rehabilitation, and after clinical recovery.

2. Muscle fibres from malnourished subjects were extremely atrophic (cross-sectional area, 110 μm^2). The fibres doubled in size during the early period of rehabilitation. Growth of muscle fibres during later periods of rehabilitation occurred at a slower rate.

3. The absolute rates of change in fibre sizes differed considerably between subjects, but the rates of change relative to the rate of gain of total body-weight (expressed as % recovery or % expected weight-for height (Nelson, 1975)) were similar between subjects after the initial growth spurt. The pattern of recovery appeared to differ between older and younger subjects.

4. Fibre sizes correlated with body-weight but not with age in the malnourished subjects. A significant correlation between fibre areas and either weight or age was observed during rehabilitation and after clinical recovery.

5. Fibre sizes of clinically-recovered subjects (mean age, 13·8 months; weight, 8·7 kg) were only approximately 60 % of that for a well-nourished 6-month-old control subject (6.4 kg). These results suggest that a longer period of time is required for fibres to reach their expected size. Therefore, when the child has regained body-weight to that of a normal child of the same height, his muscles have not yet recovered and his body composition is abnormal.

Severe muscle wasting is one of the most obvious visible features of malnutrition in infants. Morphological examinations of muscle from malnourished infants and children show that individual muscle fibres become extremely atrophic (Vincent & Rademaker, 1959; Van Bogaert *et al.* 1961; Montgomery, 1962; Lessepos, 1963; Krishnamurthy *et al.* 1971; Nassar *et al.* 1974). The muscle fibres may even be thinner than those of a 31-36-week foetus (Montgomery, 1962; Krishnamurthy *et al.* 1971). Thus, the marked reduction in muscle mass is probably mediated largely by the diminution of fibre size, although a loss of individual fibres has also been noted (Montgomery, 1962).

In the past decade, studies of malnourished infants and children admitted to hospital have led to the development of a successful rehabilitation programme. Malnourished Jamaican children rehabilitated on a high-energy feeding regimen rapidly recover to the weight of a normal child of the same height (Ashworth *et al.* 1968). Biochemical measurements in the Jamaican children have shown that muscle mass approximately doubles by the period of time the patients have clinically recovered (Reeds *et al.* 1978). However, little is known about the manner by which this growth is achieved. Cheek *et al.* (1970) and Waterlow & Mendes (1957) showed that after rehabilitation protein : DNA increased in muscle from Peruvian and Jamaican children. These results indirectly suggest that muscle fibre sizes

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* Present address: University of Detroit, School of Dentistry, 2985 East Jefferson Avenue, Detroit, Michigan 48207 USA.

† On leave of absence from: London School of Hygiene and Tropical Medicine.

increase as the patients recover. From their measurements of myofibrillar and collagen-nitrogen, Nichols *et al.* (1972) postulated that changes in fibre size in muscle from Guatamalan children occurred late in the recovery process. Krishnamurty *et al.* (1971) were unable to observe a qualitative improvement in muscle histology of Indian children rehabilitated on a high-protein diet, but fibre sizes were not measured. The aim of this study, therefore, was to document quantitatively the severe atrophy of muscle fibres in malnourished children and to follow the changes in their size as a result of nutritional rehabilitation with an energy-rich diet.

METHODS

The twenty-five subjects in this study were infants and children who were admitted to the Tropical Metabolism Research Unit in Jamaica for treatment of severe protein-energy malnutrition. The nutritional rehabilitation regimen has been described elsewhere (Ashworth *et al.* 1968). The patients were studied at one or more of the following clinical stages: (*a*) malnourished, 1–7 d after admission, before weight gain was evident (restricted diet, 0·6 g protein+400 KJ/kg per d); (*b*) early recovery, 8–14 d after admission, after rapid weight gain became evident (recovery diet *ad lib.*); (*c*) mid-recovery, 15 or more d after admission, rapid weight gain in progress (recovery diet *ad lib.*); (*d*) clinically recovered, attainment of expected weight-for-height (Nelson, 1975); before discharge. Eleven patients were studied at two different stages and two were studied at three different stages. Anthropometric information relevant to the patients is shown in Table 1.

Samples of the vastus lateralis muscle of malnourished, recovering and clinically-recovered subjects were obtained by percutaneous needle biopsy (Nichols *et al.* 1968). Muscle samples from well-nourished control subjects were obtained incidentally to surgical procedures (Table 2). The muscle samples were fixed in phosphate-buffered glutaraldehyde (20 ml/l), post-fixed in OsO_4 (10 g/l), dehydrated, and then embedded in plastic blocks. Survey sections (1 μm) were cut on a microtome and stained by the method of Humphrey & Pittman (1974), which clearly distinguishes muscle fibres from collagen.

The diameters of 100 longitudinally sectioned fibres for each sample were measured by light microscopy (magnification ×400), using an ocular micrometer (Olympus). Areas of the samples which showed evidence of mechanical trauma, excessive stretching, or contracture, were excluded from the study. The biopsies did not yield enough suitable samples for direct measurement of the cross-sectional areas of the fibres. Therefore, the cross-sectional areas were calculated from the diameters assuming the fibres to be essentially circular. This assumption was supported by observations of cross-sectioned fibres. The statistical methods used in comparison of the results included Student's *t* test and the correlation coefficient (Sokal & Rohlf, 1969).

Ethical considerations. Full and informed parental consent was obtained for the biopsy of each child, and approval for the study was obtained from the local Ethics Committees. The biopsy procedure was well-tolerated by all children. They moved the limb freely immediately after the procedure, and there were no complications.

RESULTS

The frequency distribution of fibre cross-sectional areas was unimodal in every biopsy. Mean fibre areas for each clinical stage are shown in Table 3. No significant differences in fibre area were found between the groups of malnourished subjects when patients with or without oedema were compared (i.e. kwashiorkor+marasmic kwashiorkor *v.* marasmic+undernourished) or when subjects of different height deficits were compared (i.e. marasmic+marasmic kwashiorkor *v.* kwashiorkor+undernourished). However, fibre areas

Table 1. *Anthropometric features of malnourished, recovering and recovered Jamaican infants*

(Mean values with their standard errors)

Group* ...	Malnourished		Early recovery		Mid-recovery		Clinically recovered	
n Total	9		7		10		15	
♂	8		7		6		11	
♀	1		0		4		4	
Diagnosis:†								
Marasmus	13		3		3		6	
Marasmic-kwashiorkor	3		1		2		4	
Undernourished	1		2		0		0	
Kwashiorkor	2		1		5		5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
At admission:								
Age (months)	15·3	1·9	17·1	2·5	16·0	1·1	13·8	1·2
Body-wt (kg)	6·24	0·8	6·96	0·6	6·59	0·46	6·22	0·48
At biopsy:								
Body-wt (kg)	6·21	0·98	7·77	0·65	7·71	0·37	8·71	0·48
Percentage expected weight-for-height‡	71	2	82	2	81	2	97	1
Period after admission (d)	3·7	0·7	11·0	1·2	29·5	5·0	55·1	7·2
Recovery time (d):	50·1	7·9	41·9	5·8	64·4	9·8	55·1	7·2

* For definition, see p. 276.
† Classification of infantile nutrition, *Lancet* (1970).
‡ Nelson (1975).

Table 2. *Cross-sectional fibre areas in Jamaican infants (control subjects)**

Subject	Sex	Age (months)	Wt (kg)	Diagnosis	Muscle	Fibre area (μm^2)
1	♀	6	6·4	Congenital hip dislocation	Rectus femorus	417·8
2	♀	19	10·0	Abdominal hernia	Rectus abdominus	642·2
3	♂	23	16·9	Congenital hip dislocation	Rectus femorus	673.9

* For details, see p. 276.

from the marasmic groups (< 60% expected weight-for-height) tended to be smaller. During the early period of nutritional rehabilitation the mean areas of the fibres almost doubled when compared to their size in the malnourished subjects. Thereafter, there was a modest (not significant) steady increment in mean fibre area until clinical recovery was achieved. After clinical recovery, fibre areas were much smaller than those of even the youngest well-nourished control (Table 2).

The sharp increase in cross-sectional fibre areas during the early period of rehabilitation was particularly striking when serial biopsies were examined for each subject (Fig. 1). The fibre areas for individual biopsies were found to increase significantly between the early or mid-recovery stage and clinical recovery in all except four of the subjects in which serial biopsies were taken. Subjects whose fibre areas failed to increase during this period were significantly older (18·8 ± 1·3 months) than those in which a significant increase in fibre area occurred (12·8 ± 1·4 μm^2). No other differences between the two groups of recovering subjects were apparent from examination of the patients' records.

Because the recovery time of the subjects varied the measurements were also compared with respect to the recovery time. Fibre areas for each biopsy were related to the time interval that had elapsed after admission, expressed as a percentage of the total period of time taken for recovery (Fig. 2). Expression of the values in this way revealed that after

Table 3. *Cross-sectional fibre areas in malnourished recovering, and recovered Jamaican infants*†

Clinical stage	n	Fibre area µm² Mean	SE	
Malnourished:‡	9	110·2	15·7*	
Kwashiorkor + undernourished	3	144·2	32·0	NS
Marasmic + marasmic-kwashiorkor	6	93·2	14·7	
Kwashiorkor + marasmic-kwashiorkor	5	104·3	23·2	NS
Undernourished + marasmic	4	117·6	23·5	
Early Recovery§	7	203·6	33·9	
Mid-recovery§	10	222·6	19·3	
Clinically recovered§	15	244·3	16·0	

NS not significant.

* Significantly different from any other clinical stage ($P < 0·05$).

† For details, see p. 276 and Table 1.

‡ Classification of infantile nutrition, Lancet (1970).

§ For definition, see p. 276.

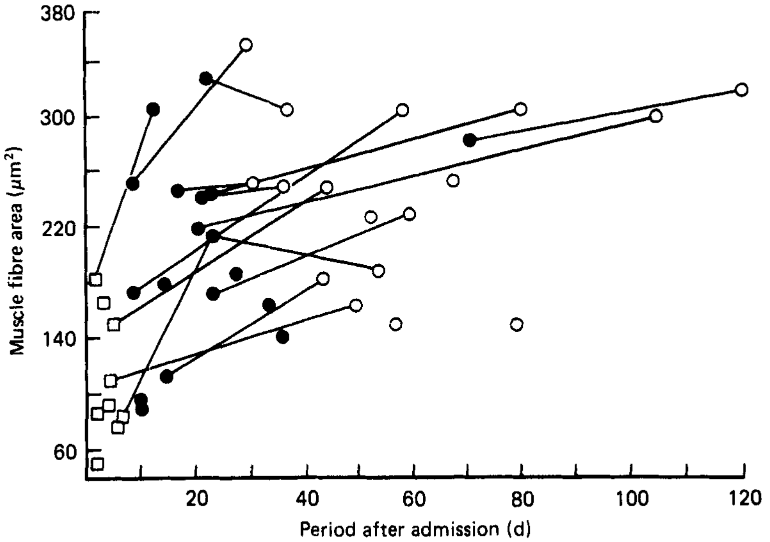

Fig. 1. Changes in muscle fibre areas (µm²) during recovery in malnourished Jamaican infants (for details, see p. 000 and Table 1.). (□), malnourished; (●), recovering; (○), clinically recovered (for definition of clinical stage, see p. 276).

the initial rapid growth of the fibres the majority of muscles biopsied increased in fibre area at a similar rate relative to total recovery time, even though the absolute rates of change were quite different. A comparable pattern of recovery was observed when fibre areas were related to the percentage of expected weight-for-height (Nelson, 1975) of the subject.

The relationships between muscle fibre areas and the age or weight of the subjects were also examined (Figs. 3 and 4). There was no significant correlation between age and fibre areas in the malnourished subjects. However, there was a significant correlation between these variables during recovery (r 0·709; $P < 0·01$) and at clinical recovery (r 0·773; $P < 0·01$). In contrast, a significant correlation was found between the fibre area and the weight of the subjects at all stages: malnourished (r 0·666; $P < 0·05$); recovering (r 0·744; $P < 0·01$); and clinical recovery (r 0·837; $P < 0·01$).

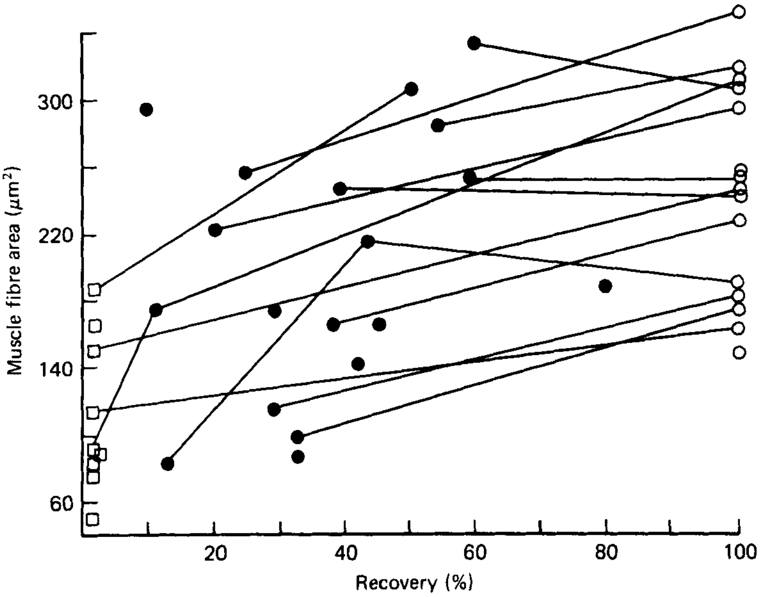

Fig. 2. Changes in muscle fibre areas (μm^2) relative to percentage of the total time taken for recovery after admission of malnourished Jamaican infants to hospital (for details, see p. 276 and Table 1. (□), malnourished; (●), recovering; (○), clinically recovered (for definition of clinical stages, see p. 276).

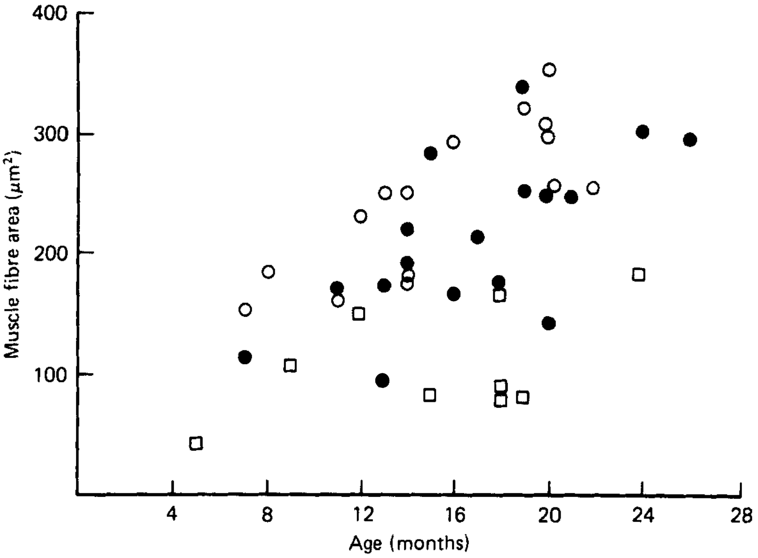

Fig. 3. Muscle fibre areas (μm^2) in relation to the age (months) of malnourished Jamaican infants. (□), malnourished; (●), recovering; (○), clinically recovered (for details, see p. 276).

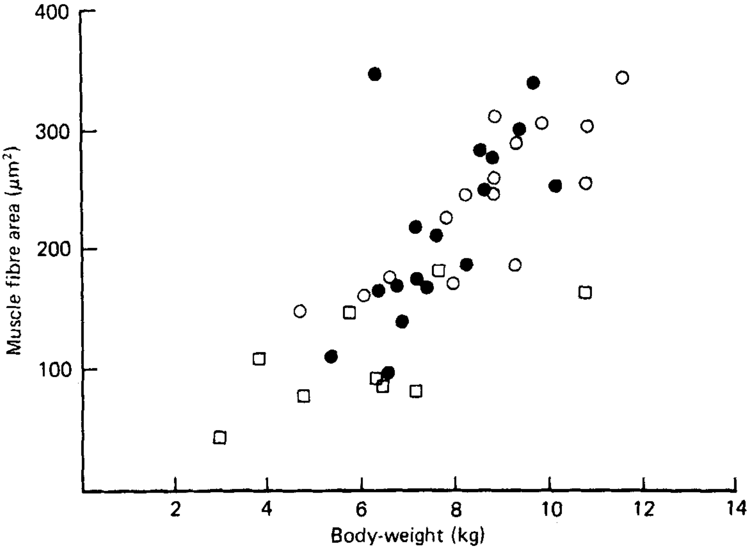

Fig. 4. Muscle fibre areas (μm^2) in relation to body-weight (kg) of the malnourished Jamaican infants. (□), malnourished; (●), recovering; (○), clinically recovered (for details, see p. 276).

DISCUSSION

The severe atrophy of muscle fibres in malnourished children has been documented (Vincent & Rademecker, 1959; Van Bogaert *et al.* 1961; Montgomery, 1962; Lessepos, 1963; Krishnamurthy *et al.* 1971; Nassar *et al.* 1974). However, there have been no previous reports dealing with the ability of nutritional rehabilitation programmes to reverse the severe atrophy. The results of the present study show that muscle fibres respond rapidly to high-energy feeding. Recovery growth of the muscle fibres occurred initially by a rapid expansion of the fibre, followed by a more gradual increase in fibre size. Although the major increment in fibre cross-sectional area appeared to occur within a short period of time after the initiation of the rehabilitation regimen, the rapid increase in size was not immediately paralleled by an increase in myofibrillar protein. Ultrastructural examination of the biopsies shows that myofibrils are often widely separated by sarcoplasmic spaces during early recovery. In some cases the area occupied by sarcoplasm exceeds that occupied by myofibrils. As recovery progresses the myofibrils become more densely packed within the fibre (Hansen-Smith, unpublished results). Measurements of myofibrillar N have also suggested a similar pattern of change in recovering Guatemalan children, but the results were interpreted as a delay in the growth of muscle fibre size (Nichols *et al.* 1972).

Muscle fibres approximately doubled in cross-sectional area during the process of recovery. These results compare favourably with the biochemical evidence for a twofold increase in muscle mass during this period (Reeds *et al.* 1978). It seems likely that the major increase in muscle mass may be attributed to the increased area of the fibres. Only slight changes in the height of the subjects occurred during recovery, so little increase in muscle length would be anticipated. The question of fibre number remains unresolved, but to date there is no strong qualitative morphological evidence for a substantial addition of new fibres during recovery (Hansen-Smith, unpublished results).

The absolute rates of increase in fibre areas varied widely between subjects, just as the individual recovery times varied. However, when the recovery patterns were standardized between subjects, either by percentage recovery time or by percentage of expected weight-for-height, the rates of increase in fibre areas appeared to be comparable between subjects after the initial growth spurt. This suggests that muscle fibre areas increase as a constant proportion of body-weight during rehabilitation. It also supports the hypothesis that recovery growth of muscle fibres is largely hypertrophic rather than hyperplastic.

In contrast to the over-all trend, four subjects failed to show a significant increase in fibre area between early or mid-recovery and clinical recovery. In one of the four subjects an extremely rapid growth spurt occurred in the fibres immediately after rehabilitation, and it is possible that a similar growth spurt occurred in muscle fibres from the other subjects. These subjects were significantly older than the subjects whose fibre areas increased gradually during the later stages of recovery. Cheek *et al.* (1970) have also noted a difference between older and younger subjects with regard to the biochemical parameters of muscle after rehabilitation. They found that muscle cell size (protein:DNA) was closer to normal in the older subjects. In the present study the age-differences were reflected primarily in the rate and pattern of increase in fibre areas, however.

The lack of correlation between fibre cross-sectional areas and age of the subjects demonstrated that the severity of malnutrition opposed and predominated over the normal growth of muscle fibres with age. However, as might be expected, a correlation between body-weight and fibre cross-sectional area was evident even in the malnourished subjects. As recovery progressed, muscle fibre areas were significantly correlated with both age and weight. Nevertheless, the fibre areas in the recovered subjects were only slightly more than half that of the youngest control subject, which suggests that a longer time interval may be required for the fibres to reach their expected size. Since the subjects were of normal weight for their height when 'recovered', the smaller amount of muscle must have been balanced by an excess of another tissue. Wheeler (1975) has shown increased body fat at the time of recovery. The 'recovered' subjects cannot be regarded as normal when weight-for-height is used as the measure of recovery; some measure of body composition should augment the criteria for recovery.

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