THE RESPONSE OF MUSCLE CELLS DURING COMPENSATORY GROWTH IN RATS

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OPSOMMING: DIE REAKSIE VAN SPIERSELLE TYDENS KOMPENSERENDE GROEI IN ROTTE

In hierdie studie is die spierselgroei van rotte tydens die vroeë stadium van herstel na ondervoeding bestudeer. Groepe diere is voor- of nadat hulle gespeen is ondervoed en is daarna op beperkte of onbeperkte voedingspeile geplaas. Die toename in liggaamsmassa was die vinnigste by die twee groepe diere wat ondervoed was voordat hulle gespeen is. Laasgenoemde groep diere wat onbeperkte voeding ontvang het se spier-selle het teen die hoogste tempo vermenigvuldig, maar die toename in spierselgrootte was laag. Die tendense in die toename van die verskillende spierkomponente van rotte wat beperkte voeding na die periode van ondervoeding ontvang het, het 'n verwantskap getoon tot die verskille in groeipotensiaal tussen die verskillende groepe diere.

SUMMARY

This study was concerned with the cellular growth of muscle tissue in rats during the early stages of recovery from undernutrition. Groups of animals were undernourished either pre- or post-weaning and realimentated on either unrestricted intakes or on a restricted intake regime. Rates of increase in bodymass were highest on both dietary treatments in animals undernourished after weaning. On unrestricted intakes these animals also had the most rapid multiplication of muscle "cells" but their rate of increase in muscle "cell size" was low. Patterns of increase in muscle components during realimentation on the restricted intake regime were related to differences in potential for growth between groups.

An animal whose growth has been retarded exhibits, when the restriction has been removed, a rate of growth greater than that which is normal in animals of the same chronological age (Wilson & Osbourn, 1960). This phenomenon is known as compensatory growth and it is taken account of in the management of livestock in areas which experience periods of seasonal food shortage.

Today much is known of the interplay of the factors which determine rate and degree of recovery from undernutrition. Again, a wealth of information is available on changes in form and chemical composition of animals during realimentation. However, present knowledge has been derived largely from empirical observations and there is, as yet, little understanding of the mechanisms operative during recovery. Further clarification of the nature of compensatory growth may be obtained from studies on the influence of nutrition on the cellular growth of animals. A knowledge of changes in cell number and cell size during the periods of undernutrition and realimentation could be of considerable value in the elucidation of mechanisms involved in recovery and in the integration of diverse observations relating to the phenomenon of compensatory growth.

The question arises as to which cells should be studied in order to provide the most useful information on the cellular events which occur during compensatory growth. The logical choice is muscle cells as this tissue constitutes the bulk of the cellular phase of the body. In addition, muscle tissue is readily accessible for study in farm livestock, as biopsy samples. This paper reports observations on the influence of nutrition on muscle cell growth in rats and discusses the possible significance of measurement of cellular parameters in farm livestock.

Procedure

In experiment 1, male Sprague-Dawley rats were reared from birth to weaning in litters of 3 (group A), 8 (Group (B) and 16 (Group C). A creep feed was made available to Group A from day 16 and to the other groups from day 21. All animals were weaned at 28 days and were subsequently fed *ad libitum*, on a diet which promoted rapid growth for 16 weeks. Individual bodymasses were recorded by-weekly.

In experiments 2 and 3 animals were reared along different growth patterns to 50 g livemass. Three groups grew directly to this mass in litters of 3 (Group A), 8 (Group B) and 16 (Group C). A fourth Group (D) was reared in litters of three to approximately 75 g livemass and subsequently food intakes were reduced to between 1,5 and 2,0 g per day so that bodymass returned slowly to approximately 50 g. Only animals whose growth pattern conformed to that of their group prior to the attainment of 50 g were taken for experiment.

Some animals from each treatment group were killed at 50 g for measurement of muscle parameters. Others were realimentated from this mass on unrestricted intakes of a diet which promoted a high rate of bodymass gain to 200 g livemass (Experiment 2).

In addition, some animals from Groups B, C and D were placed on a restricted intake regime once they had reached 50 g by the selected growth patterns (Experiment 3). For the first week they received 6 g of food per day, for the second 8 g and for the third week 10 g. This latter experiment was designed to measure the response of muscle cells in animals which received the same amount of food from a common "starting mass".
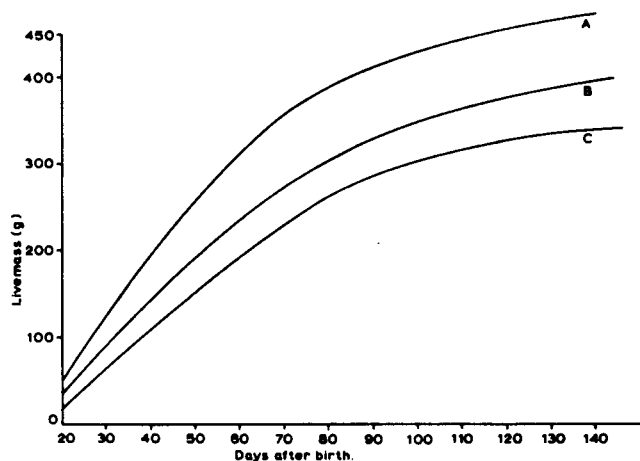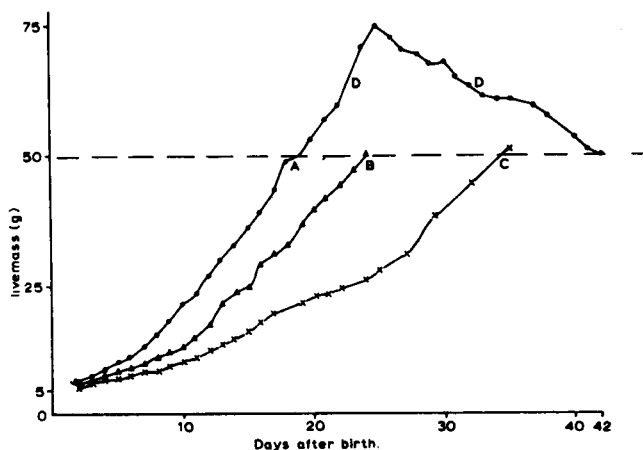
Fig. 1 *Pattern of livemass gain after weaning*

Protein and desoxyribonucleic acid (DNA) determinations were carried out on two hind limb muscles, the *anterior tibialis* and the *vastus lateralis*. The pattern of cellular response was similar for the two muscles and is reported here for the *anterior tibialis* only. The muscles were dissected from the limbs of animals in each treatment group at 50 g and 200 g livemass and at the end of the period of restricted feeding. During dissection the tendons of insertion were lifted and the deep surface of the muscles cleared as far as the tendon of origin. DNA in muscle from the left limbs was extracted by the method of Schneider (1945) and measured by a diphenylamine method (Burton, 1956). Nitrogen contents of muscles from right limbs were measured by a semi-micro Kjeldahl method with a mercury catalyst. The factor 6,25 was used for the calculation of protein from nitrogen contents.

Results

Pre and post-weaning of growth

Growth rates from birth differed markedly in animals reared in litters of different size. By 21 days after birth the bodymasses of Group A animals were approximately two and a half times heavier than those of Group C animals (Fig. 1). Absolute increases in livemass were similar in all groups during the first few weeks after weaning. Growth rates from approximately 200 g live mass were higher in Group A than in Group C animals.

Growth to 50 g bodymass

The growth curves of animals reared to 50 g bodymass along different patterns are shown in Fig. 2. Despite the severe nutritional restriction experienced by Group D, the animals in this group remained healthy as adjudged by their frenetic activity at feeding times and subsequent rapid recovery.

Chemical components of muscle at 50 g bodymass

Mass, protein content and protein concentration were significantly lower ($p < 0,01$) in Group A than in the other groups (Table 1). Protein content was highest in Groups C and D.

DNA contents in Group C were lower ($p < 0,01$) than in other groups. Total DNA was highest in Groups B and D and concentration of DNA was highest in Groups B and A. The ratio of protein to DNA, a measure of cell size, was lowest in Group A, intermediate in Groups B and D and highest in Group C.

Chemical components of muscle at 200 g livemass

Masses and protein contents in Groups A and D were similar and significantly lower ($p < 0,01$) than in Groups B and C (Table 2). DNA content and concentrations were lowest in Group C and highest in Group A. Protein/DNA ratio was lowest in Groups A and D, intermediate in Group B and highest in Group C.

Fig. 2 *Representative growth curves of four groups of animals reared along different patterns to 50 g livemass*

Table 1

Mass, protein and DNA contents and concentrations, and ratio of protein to DNA in anterior tibialis muscles in groups of rats reared to approximately 50 g bodymass by different growth patterns

	Group*				LSD		
	B	A	C	D	p=0,05	p=0,01	D₁ **
Empty bodymass (g)	50,2	51,7	49,7	50,7	1,8	2,5	71,0
Mass (mg)	86,8	66,5	90,5	94,8	5,8	7,9	117,8
Protein (mg)	15,6	8,7	18,5	17,6	1,6	2,2	20,7
Protein conc. (mg/g)	179,7	130,8	204,4	185,7	13,5	18,4	175,7
DNA (ug)	233,4	183,3	158,6	230,5	15,1	20,6	242,0
DNA conc. (ug/mg)	2,69	2,77	1,78	2,43	0,22	0,30	2,05
Ratio protein/DNA	67,0	51,6	113,1	76,4	7,5	10,0	85,5

* 5 animals per group

** Measurements on Group D animals, prior to period of mass loss

Table 2

Mass, protein and DNA contents and concentrations and ratio of protein to DNA in anterior tibialis muscles in groups of animals reared by different growth patterns to approximately 50 g livemass and subsequently fed ad libitum to approximately 200 g livemass

	Group *				LSD	
	B	A	C	D	p=0,05	p=0,01
Empty bodymass (g)	191,2	187,6	186,2	185,6	4,2	5,7
Time from 50 g bodymass (days)	29,4	27,2	30,0	21,4	–	–
Mass (mg)	360,8	320,2	358,2	312,6	14,9	20,6
Protein (mg)	75,2	63,4	75,5	61,8	3,2	4,4
DNA (ug)	352,0	398,5	326,0	371,0	14,5	20,0
DNA conc. (ug/mg)	0,96	1,20	0,90	1,19	0,05	0,07
Ratio protein/DNA	213,6	159,1	231,6	166,6	13,0	18,0

* 5 animals per group

Table 3

Mass, protein and DNA contents and concentrations and ratios of protein to DNA in anterior tibialis muscles in groups of animals reared by different growth patterns to approximately 50 g livemass and subsequently placed on the same restricted intake regime for three weeks

	Group *			LSD	
	B	C	D	p=0,05	p= 0,01
Empty bodymass (g)	125,4	123,2	144,1	3,3	4,6
Mass (mg)	224,5	243,3	268,2	15,3	21,4
Protein (mg)	44,0	44,8	53,5	3,7	5,1
Protein conc. (mg/g)	195,9	184,1	199,8	11,3	16,3
DNA (ug)	267,5	278,0	257,6	26,8	37,2
DNA conc. (ug/mg)	1,19	1,14	0,97	0,10	0,14
Ratio protein/DNA	164,5	161,2	207,7	15,6	21,9

* 4 animals per group

Chemical components of muscle at end of realimentation on restricted intakes

Analysis of the data presented in Table 3 revealed that mass and protein contents were higher in Group D than in the other groups. Protein concentration was similar in Groups B and D and was lower in Group C. DNA contents were similar in all groups but concentration was lowest in Group D. Protein/DNA ratio was highest in the latter animals.

Discussion

The most significant finding from Experiment 1 was the differences in rate of growth after the animals had reached 200 g livemass. As a result of this finding, 200 g was chosen as the "final mass" for subsequent experiments. The rationale behind this decision was that the measurement of selected parameters might provide pointers to the reasons for the differences in potential for growth. In turn, these pointers could provide guidelines for studies on larger animals.

In Experiments 2 and 3 comparisons were made initially on animals of similar "starting" mass. Treatment groups included animals which had been severely undernourished after weaning. As noted by Winick and Noble (1966), undernutrition from birth resulted in a reduction of DNA content (Group C, Table 1). Cell number and cell size were maintained during the period of mass loss in Group D animals.

The results on muscle parameters at 200 g livemass are confounded by differences in the time taken to reach 200 g by animals on the different treatments (Table 2). Calculations of increases per day in the respective components in each group between 50 g and 200 g livemass revealed that increases in mass were highest in Group D. Daily increases in protein contents were similar in all groups, while increments in DNA were highest in Groups A and D. Of particular note was the finding that a fast rate of compensatory growth in the latter animals was associated with a relatively low rate of increase in muscle "cell size".

These findings support those of Waterlow & Mendes (1957) and of Howarth & Baldwin (1970). The former workers noted a lag period in the synthesis of protein in the muscles of rats which had been fed a protein deficient diet and which were realimentated on a stock diet containing 18 protein. Howarth & Baldwin (1970) also observed that a compensatory acceleration in body growth was not accompanied by an enhanced rate of synthesis of muscle protein. On the other hand, rate of synthesis of DNA during recovery was greater than normal.

With regard to the results of the experiment on restricted intakes (Experiment 3), the inference may be drawn that, even though Group D animals had the highest bodymass gains, these animals suffered the greatest degree of nutritional stress. This inference is based on inspection of protein/DNA ratios in the muscles at the end of the period of restricted feeding and of increases in muscle protein and DNA contents during this period. Of particular note were the differences in the parameters between Groups C and D. The latter group had higher protein/DNA ratios, higher increases in protein contents and lower increases in DNA contents. Elliott & Cheek (1968) and Hill *et al.* (1970) found that restriction of energy intake resulted in a greater reduction of DNA than protein accumulation, so that cell size continued to increase. Thus the above observations indicated that Group D animals had been subjected to a relatively more severe nutritional deprivation than had Group C.

From *a priori* considerations it would be anticipated that, when animals of similar livemass are fed the same limited amount of food, those with the greatest potential for growth would be stressed to the greatest extent. As adjudged from patterns of growth during the initial phase of realimentation from 50 g livemass on *ad libitum* intakes, Group D animals had the greatest potential for growth. Thus it is evident that observations on patterns of growth on unrestricted intakes and the measurement of changes in muscle parameters on restricted intakes provide separate indices of differences in potential for growth.

The findings of this study have shown that the response of muscle cells during realimentation in the rat is dependent on previous nutritional history and on plane of nutrition. The question arises as to what extent similar response might be expected in animals such as cattle and sheep.

In areas which experience uneven seasonal distribution of rainfall, grazing animals encounter periods of undernutrition which result from both quantitative and qualitative deficiencies in the diet. At the onset of the rains, when liberal supplies of food become available, the animals frequently exhibit compensatory growth. The rate and degree of recovery are determined by a number of factors (Wilson & Osbourn, 1960) which include the stage of growth at which the restriction is imposed. For example, Mentz (1968) found that calves which were severely undernourished in the early postnatal period did not show compensatory growth during rehabilitation. Again, Allden (1968) observed that growth and herbage consumption during rehabilitation were similar to those of the control group in lambs subjected to growth restrictions during the first six months of life. By contrast, sheep which suffered severe nutritional deprivation later in life exhibited rapid compensatory growth.

The results of the present study provide pointers to the explanation of the differences in response described above. It can be hypothesized that the recovery from undernutrition of the last-mentioned sheep is, like that of rats undernourished after weaning, associated initially with a relatively small protein/DNA ratio in the cells of muscle tissue. Experiments are planned to test this hypothesis.

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