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Effect of Maternal Dietary Restriction and Rehabilitation on the Muscle Protein Breakdown of Young Rats

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어미쥐의 식이제한과 식이회복이 새끼쥐의 근육단백질 분해에 미치는 영향

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Sprague Dawley 종의 어미쥐에게 수유기에 양적인 식이 제한을 한 후, 이유이후 새끼쥐에게 4 주일간 식이 회복을 시켰다. 각 새끼쥐에 있어서, 임의로 먹인 대조군에 비하여 식이 제한군의 체중, 근육 단백질, 근육 3-methylhistidine과 혈청 단백질 함량의 변화에 대하여 분석하였다. 식이 제한군의 체중은 대조군보다 매우 낮았으며, 식이 회복에 의하여 거의 정상으로 회복 되었다. 또한 식이 제한군의 근육 단백질, 3-methylhistidine 함량과 혈청 단백질 함량도 어미쥐의 식이 제한에 의하여 대조군에 비하여 매우 낮았으나, 4 주일간의 식이 회복에 의하여 빨리 증가하여, 대조군과의 유의 차가 없었다. 이로써 영양불량의 쥐에서는 3-methylhistidine의 대사가 장애를 받아 저하되나, 이유 이후의 식이 회복에 의해 거의 정상으로 회복됨을 알 수 있다.

INTRODUCTION

There has been a growing interest in recent years in the study of protein catabolism during protein-energy malnutrition. Skeletal muscle represents a large reserve of protein that can be made available during periods of dietary stress and is known to be affected in protein-energy malnutrition[1]. Studies with rats have shown that the rates of muscle protein synthesis in vivo and in vitro are decreased by feeding

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with low protein diets and protein-free diets[2)3)].

Asatoor and Armstrong[4)] have suggested that 3-methylhistidine is a suitable marker amino acid for myofibrillar degradation. Methylhistidine is found in actin and myosin[4)] and available evidence indicates that the methyl group is attached to free histidine after the formation of histidyl-tRNA[5)6)7)]. Young et al.[8)] studied the binding in vitro of various labelled amino acids to muscle tRNA and were unable to demonstrate a 3-methylhistidine-tRNA. This finding was later confirmed in studies in vivo[9)10)]. These data confirm that 3-methylhistidine is not incorporated into protein and that its release from myofibrillar protein will reflect the rate of degradation.

Haverberg et al.[11)] showed that skeletal muscle is likely to be the major source of urinary 3-methylhistidine and the latter is, in consequence, a reflection of myofibrillar protein breakdown in skeletal muscle. Thus many investigators studied the effect of diet on urinary 3-methylhistidine excretion. There has been a good correlation between the nutritive quality of the dietary protein and total urine 3-methylhistidine, the higher the protein quality, the greater being the excretion of total 3-methylhistidine[12)]. And children suffering from protein-energy malnutrition have reduced excretion of 3-methylhistidine[1)]. Assuming that tissues other than muscle are not significant source of urinary 3-methylhistidine[11)], reduced output of urinary 3-methylhistidine is due to an adaptive decrease in the rate of catabolism of muscle proteins. Studies in the rat have also demonstrated that total 3-methylhistidine excretion is reduced in response to a low protein diet[13)].

Muscle protein content is regulated at the level of protein degradation as well as at control points associated with protein sunthesis[13)14)]. When repleting the malnourished rats with an adequate diet brought about a rapid return to a normal growth rate, an increase in muscle mass[13)] and a rapid restoration of muscle protein[15)] Similarly, Narasinga Rao and Nagabhushan[1)] noted a marked increase in 3-methylhistidine output in malnourished children after 4 weeks of nutritional rehabilitation.

The effect of diet upon the content of 3-methylhistidine in muscle in rat has not been extensively studied. One report[13)] has shown that the 3-methylhistidine concentration in actin was 5% lower in protein-deficient rats compared with well-fed controls. Similarly, Fisher et al.[16)] also have shown that there was a reduction in the 3-methylhistidine content of chicken muscle with protein depletion.

Accordingly in this experiment, changes in the muscle protein breakdown in response to maternal ditary restriction and rehabilitation were performed. As a part of this investigation, determinations were made on the content of 3-methylhistidine and protein in the skeletal muscle, and the level of total protein in serum of offsprings.

EXPERIMENT

Virgin female Sprague Dawley rats weighing 200-230 g, supplied by Animal Breeding Laboratory of Seoul National University, were used in this experiment.

Two females were housed with one normal male per cage. Daily vaginal lavages were taken and the sperm-positive females were assigned randomly to individual cages. Twenty pregnant rats were randomly divided into two groups of ten rats each. The scheme of experimental design is shown in Fig. 1.

Control group : balanced dommercial diet Table 1. ad libitum during gestation and lactation.

Deficient group : quantitative restriction of diet from birth to 21 days of postnatal age. Ten pregnant rats were fed a balanced commercial diet ad libitum during gestation, and fed 30 g per day (60% of the normal average daily intake of a lactating rat) during lactation.

After weaning at 21 days, all offsprings were fed the same diet ad libitum for 4 weeks for rehabilitation.

At the age of birth, 1, 2, 3, 5, 7 week, body weight of all offsprings were weighed. At the same intervals, offsprings were randomly decapitated from each group, and bolld and hind leg muscles were removed. Serum and muscle were stored frozen in a screw-capped bottle until used. All tissues were weighed immediately prior to assay.

Total muscle protein was extracted according to

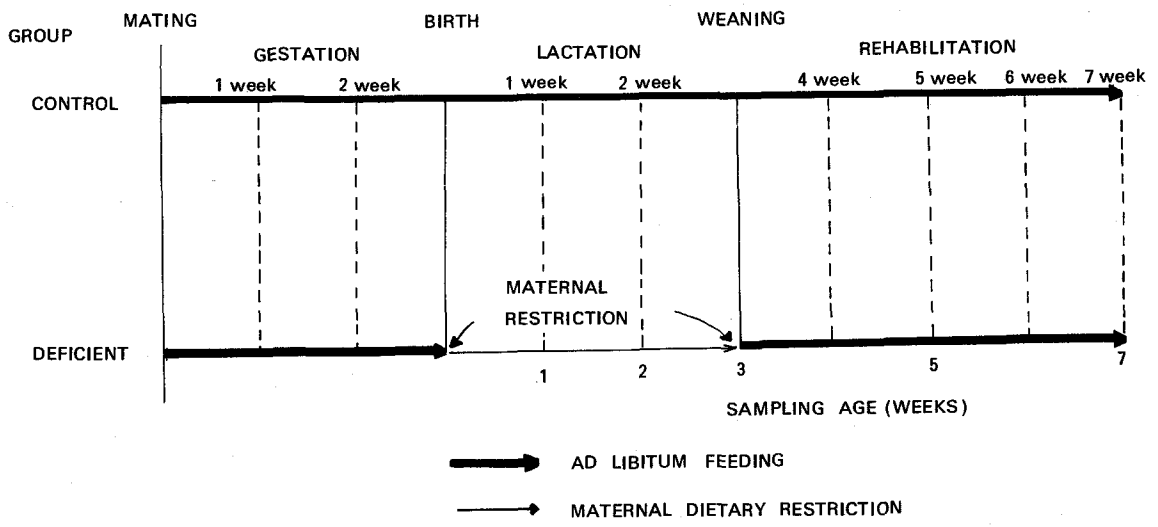

Fig. 1. Scheme of experimental design.

Table 1. Composition of Diet

Ingradients		%
Grain		55
Bran		11
Flour		20
Fish Meal		10
Misc		3
Content		**%**
Crude Protein	above	19.0
Crude Fat	above	3.0
Crude Fiber	Below	6.0
Crude Ash	Below	9.0
Ca	above	0.6
P	above	0.4
DCP (digestable crude protein)	above	16.5
TDN (total digestable nutrient)	above	73.0

(Calf chow, pellet form)

The above composition of diet was obtained from animal breeding laboratory of Seoul National University, to which it was submitted by Jeil Fodder Co. as a guaranteed Table of composition.

the method of Munro and Downie[17] and continued to 3-methylhistidine analysis by the method of Rhada and Bessman[18]. To dried hydrolysate of muscle[17] 1 ml water was added and mixed well in the vortex mixer.

About 1 g of Dowex 50 cation exchange resin (activated with 1 N HCl and washed with distilled water) in a test tube was equilibrated with of few ml of citrate buffer (pH 5) and mixed well in the vortex mixer and supernatant decanted. Repeated 3-4 times. Then hydrolysate solution was added into the resin which was well equilibrated.

To this, 5 ml of phosphate buffer (pH 7.8) was added and mixed well in the vortex mixer for 3-5 min., and centrifuged the resin to settle.

To 1 ml of this supernatant, the pH of the solution was adjusted to 5. And 0.5 ml of ninhydrin-OPT reagent was added and incubated for 10 min. at 45°C. Then added 0.5 ml of 2 N NaOH and mixed very well. Brought to room temperature, read the absorbance at 490 nm.

The yellow color that developed with ninhydrin-OPT under alkaline condition turned brown. This test is specific for 3-methylhistidine.

The muscle tissues were assayed for protein[19]. Serum protein level[20] was also determined.

Student's t-test was used to compare significance between the control and the deficient group.

RESULTS

1) Body weight

Depression in the rate of growth of the deficient group and marked increased growth after rehabilitation was seen in relation to that of the controls (Table 2).

Body weights of offsprings from deficient group were significantly lower (p 0.01) than were those of the control offsprings from 1 week of age, and these reduction in body weight of the deficient group continued to rehabilitate. At weaning, body weight of deficient group was about 60% of the control. Rehabilitation at weaning brought about a rapid growth response, thus the significant weight gain accurred in the deficient group. At the end of experimental period, there were no significant differences in body weight between the groups.

2) Muscle 3-methylhistidine content

Changes in 3-methylhistidine content in muscle during experimental period are presented in Table 3 and Fig. 2.

There were no significant differences between the groups in 3-methylhistidine content at 1 week of age. But deficient group exhibited a marked decrease compared to the control group at 2 and 3 week of age.

Table 2. Effect of Maternal Dietary Restriction and Rehabilitation on body weight of offsprings (G)

Age, weeks/group	Control	Deficient	
0	6.54 ± 0.22 (117)	6.54 ± 0.22 (117)	
1	14.08 ± 0.51 (42)	12.05 ± 0.71 (78)**	Restriction
2	27.86 ± 3.22 (30)	17.45 ± 1.95 (55)**	
3.	46.45 ± 4.52 (24)	27.45 ± 3.62 (42)**	
5.	109.10 ± 12.11 (19)	95.38 ± 13.74 (16)*	Rehabilitation
7	191.57 ± 20.58 (11)	170.38 ± 30.39 (8)	

* $p<0.05$ significantly different from control group

** $p<0.01$ significantly different from control group

( ) Number of animals used for calculation

(mean ± S.D.)

Table 3. Effect of Maternal Dietary Restriction and Rehabilitation on Muscle 3-methylhistidine content of offsprings (mg/g of muscle)

Age, weeks/group	Control	Deficient	
1	0.235 ± 0.012	0.221 ± 0.025	
2	0.405 ± 0.027	0.336 ± 0.022**	Restriction
3	0.357 ± 0.015	0.316 ± 0.023**	
5	0.340 ± 0.068	0.343 ± 0.096	Rehabilitation
7	0.358 ± 0.051	0.356 ± 0.068	

** $p<0.01$ significantly different from control group

(mean ± S.D.)

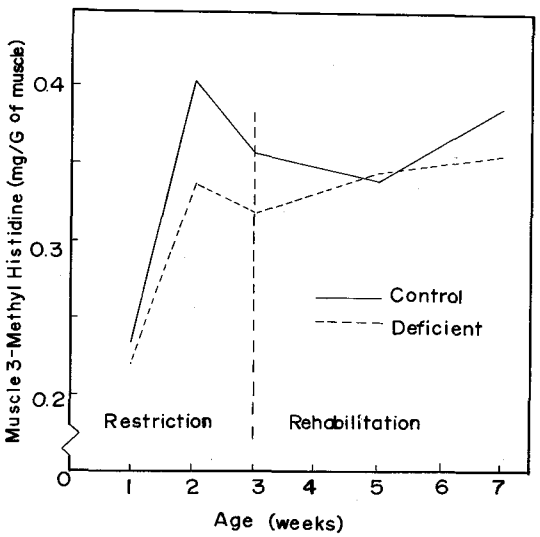

Fig. 2. Effect of Maternal Dietary Restriction and Rehabilitation on Muscle 3-methylhistidine content of offsprings.

The greatest decrease of 17% were observed at 2nd week. These changes also were confirmed by Haverberg et al.[13] and Fisher et al.[16] previously. Rehabilitation at weaning brought a response, 3-methylhistidine content were not significantly different at 5 and 7 week of age.

3) Muscle protein content

Table 4 shows muscle protein content. The protein levels in muscle before weaning were significantly lower in the deficient group than the control. In particular, muscle protein content of deficient group was about 16% lower than that of the control at 2nd week. Marked increase in muscle protein content was obtained during the early phase of dietary rehabilitation in deficient group. They showed a similar pattern in muscle protein content of the two groups after rehabilitation.

4) Total protein level in serum

Serum protein levels of offsprings are shown in Table 5. Serum protein levels of deficient group were significantly lower than those of the control during the first 3 weeks of maternal dietary restriction and then increased as the rehabilitation phase continued. At weaning, serum protein level of deficient group decreased 30% compared to that of the control.

DISCUSSIONS

In the present investigation, it was demonstrated that maternal dietary restriction during lactation affects body weight, muscle protein, muscle 3-methylhistidine content, and serum protein level of offsprings. These were nearly recovered by dietary rehabilitation after weaning.

Reductions in dietary intake were associated with decreases in rate of growth. Winick and Noble[21] proposed that the earlier a nutritional stress is imposed, the poorer the recovery, due probably to interference

Table 4. Effect of Maternal Dietary Restriction and Rehabilitation on Muscle protein of offsprings (mg/g of muscle)

Age, weeks/group	Control	Deficient	
1	117.5 ± 1.70	96.5 ± 1.55**	
2	126.9 ± 2.36	107.3 ± 3.24**	Restriction
3	122.5 ± 1.51	114.1 ± 1.42**	
5	136.4 ± 2.68	130.6 ± 4.30	Rehabilitation
7	121.6 ± 3.95	116.7 ± 5.23	

** $p<0.01$ significantly different from control group
(mean ± S.D)

Table 5. Effect of Maternal Dietary Restriction and Rehabilitation on Serum Total Protein level of Offsprings (G/100 ml serum)

Age, weeks/group	Control	Deficient	
1	4.09 ± 0.149	2.55 ± 0.076**	
2	4.47 ± 0.238	3.51 ± 0.061**	Restriction
3	4.93 ± 0.123	3.43 ± 0.092**	
5	6.42 ± 0.153	6.16 ± 0.174	Rehabilitation
7	6.73 ± 0.372	6.94 ± 0.242	

** $p<0.01$ significantly different form control group
(mean ± S.D.)

with cell replication. Maternal diets restricted in both protein and energy intake were also shown[22] to produce reduced liver and brain weight, and total organ DNA, RNA, and protein in pups at weaning. In this experiment, during maternal dietary restriction, weight gain of offsprings of deficient group were lower than those of the control. However, after 4 weeks of rehabilitation there were no significant differences between the two groups. Therefore, this observation leads that body weight reduction in offsprings due to maternal dietary restriction during lactation is conpletely recovered by dietary rehabilitation after weaning.

In present experiment, maternal dietary restriction caused a decrease in the 3-methylhistidine and protein content in muscle, and total protein level in serum. And dietary rehabilitation after weaning induced a marked increase. Haverberg et al.[13] showed that a diet deficient in protein causes the young rat to have a reduced output of 3-methylhistidine (reduced rate of muscle protein breakdown) which increases again during repletion on an adequate diet. Similarly, Garlick et al.[23] reported an immediate and progressive decrease in muscle protein breakdown when rats were given a reduced intake of food. Nagabhushan and Narasinga Rao[24] also observed that daily urinary excretion of 3-methylhistidine was markedly reduced in children suffering from clinical protein-energy malnutrition and moderately reduced in undernourished children, an muscle mass was also severely depleted in such cases. These observations suggest that breakdown of muscle protein is sensitive to malnutrition, such as is imposed by cessation of growth due to inadequate energy intake. Young and Munro[25] suggested that the fall in the rate of muscle protein breakdown with adaptation to a undernourishment is presumably due, at least in part, to the increased blood ketone body concentration that occurs under this circumstance.

An increase after rehabilitation in 3-methylhistidine content of undernourished rats implies an increased rate of muscle protein turnover. Hence, in malnutrition, the rate of muscle protein breakdown is reduced and nutritional rehabilitation is accompanied by an increased rate of muscle protein turnover. Young et al.[15] showed that refeeding depleted rats with an adequate diet brought about a rapid return to normal growth rate, and the rate of muscle protein synthesis was increased within 2 days of refeeding the malnourished rats with the adequate diet. Because muscle protein synthesis is reduced protein-depleted animals[23] and therefore, presumably in undernourished offsprings, the lowered rate of muscle protein breakdown in the offsprings of malnourished dams may be taken to reflect an adaptation in muscle protein metabolism, favoring the maintenance of balance between rates of protein synthesis and breakdown in muscle. Restoration of muscle protein, despite increase in breakdown rate during rehabilitation of deficient group, and be considered to be due to the prompt enhancement of the synthesis rate.

These results suggest that there is a quantitative

change in 3-methylhistidine metabolism in undernourished rats and these reductions can be nearly recovered by 4 weeks of dietary rehabilitation after weaning.

SUMMARY

A quantitative restriction of maternal diet was given to the Sprague Dawley rats during the lactation. The control group were fed a commercial diet ad libitum throughout the experimental period. Dietary restriction started from birth to weaning in deficient group. After weaning at 21 days, all offsprings were fed the same diet ad libitum for 4 weeks of rehabilitation. They were analyzed for body weight, muscle protein, muscle 3-methylhistidine and serum protein level of offsprings at 0, 1, 2, 3, 5, 7 weeks.

Body weight of offsprings of deficient group were significantly lower than the control group, but after rehabilitation there were no significant differences between two groups. Maternal dietary restriction caused a decrease in the 3-methylhistidine, protein content in muscle and total protein level in serum, and rehabilitation after weaning induced a marked increase.

These results suggest that there is a quantitative reduction in 3-methylhistidine metabolism in the undernourished rats and these reductions can be nearly recovered by 4 weeks of dietary rehabilitation after weaning.

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