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Muscle Protein Breakdown in Young Rats Fed on an Energy-Depleted Diet

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The urinary excretion of 3-methylhistidine has been determined in rats fed an energy-depleted diet. Young male rats were fed over a 21-day period on either an adequate control diet (18 % lactalbumin), or an energy-depleted diet (containing half of the amount of carbohydrates of the control diet). Urinary urea-N, creatine, creatinine and 3-methylhistidine, as well as body weight changes were monitored throughout the experiment. At the end of the experiment, the levels of insulin and corticosterone, and the weights of livers and gastrocnemius, soleus, tibialis anterior and extensor digitorum longus muscles were determined.

A significant ($p < 0.05$) reduction in body and liver weight was found in the energy-depleted rats, but no weight differences were found in the four excised muscles. Urinary outputs of urea-N, creatine and creatinine were significantly ($p < 0.05$) increased in this latter group. Output of 3-methylhistidine showed an initial rise followed by a significant ($p < 0.05$) and progressive decline throughout the experiment in the rats fed the energy deficient diet. Insulin concentration was significantly ($p < 0.01$) reduced in those animals, but no differences were found in the serum levels of corticosterone.

It is assumed that lack of energy in the diet decreases the rate of myofibrillar protein breakdown in growing rats.

It has been widely shown that skeletal muscle represents a large reserve of protein that can be made available during periods of dietary stress (10, 16, 17). This fact has been demonstrated to occur both in man and small animals, such as the rat, although in these animals losses from the skeletal muscle are usually only observed as result of prolonged dietary restriction, due to the fact that skin and visceral organs also serve as energy stores (1).

Studies with rats have shown that the rates of muscle protein synthesis *in vivo* and *in vitro* are reduced by feeding these animals with low-protein diets and pro-

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tein-free diets (15, 29), and starvation increases breakdown in several muscles (7). HAVERBERG *et al.* (12) investigated the effect of protein and protein and energy depleted diets on the rate of muscle protein breakdown, as measured by the urinary excretion of the amino acid 3-methylhistidine: they found that these diets reduced the rate of muscle protein breakdown in the rat. OMSTEDT *et al.* (21), using a similar approach demonstrated that there is a relationship between the nutritive quality of the dietary protein and the urinary excretion of 3-methylhistidine in rats.

The aim of this study was to assess the effect of a single energy-depleted diet on the rate of myofibrillar protein breakdown by determining the urinary excretion of 3-methylhistidine. The rationale and validation of this approach have been discussed in previous studies from our laboratory (13, 18, 35): in short, the vast majority of 3-methylhistidine formed in the body is present in the skeletal muscle; methylation of histidine takes place after its incorporation into the growing peptide chains of actin and myosin; after breakdown of these proteins, the liberated 3-methylhistidine is not reutilized, and about 98 % of it is excreted in the urine. The total 3-methylhistidine excreted in the urine has therefore been proposed as an index of muscle protein catabolism rates.

Since serum levels of insulin and corticosterone are closely related to the nutritional status (32), the concentrations of these hormones in the experimental animals and their changes by the dietary restrictions have been investigated in this study. It was found that feeding growing rats on an energy-deficient diet brings about an initial increase of 3-methylhistidine output, followed by a persistent decrease in the excretion of this metabolite, indicating parallel changes in the rate of myofibrillar protein breakdown.

Materials and Methods

Intact young male rats, 30 days old and weighing about 100 g (Charles River Breeding Laboratories, Wilmington, Mass.) were housed in non-metabolic cages. For three days they were allowed to eat *ad libitum* a purified control diet containing 18 % lactalbumin (12), so that they could adapt to this new diet and sorrounding. Then, they were housed in metabolic cages, and randomly divided into two groups of six animals each. One of them was fed *ad libitum* on the adequate diet mentioned above, and the other was fed on an energy-restricted diet, containing 36 % lactalbumin; this later diet was offer to the rats at half the amount of diet consumed by rats fed on the adequate diet, in order that the protein intake in both groups remained the same.

The composition of the diets is shown in table I. Food intake and body weight changes were recorded daily; 24 h urine excretion was collected in each rat, and pooled within each group; 0.1 ml of toluene was used as preservative in each urine-flask. Samples of urine were centrifuged for 10 m at 2000 r.p.m. and the clean urine was frozen at —20° C for further urea-N, creatine, creatinine and 3-methylhistidine determination.

On the last day of the experimental period all rats were killed by decapitation, and a sample of blood was taken for insulin and corticosterone determinations. Immediately, liver, as well as gastrocnemius, soleus, tibialis anterior and extensor digitorum longus muscles from both rear legs, were excised by careful dissection and weighed (9).

Urinary analysis. In three-day pooled samples, urinary urea-N was determined by the method of FOSTER and HOCHOLZER (6), creatine and creatinine were assayed by the picrate alkaline procedure as described by OWEN *et al.* (22), and 3-methylhistidine was evaluated by the meth-

od of BILMAZES *et al.* (4) and HAVERBERG *et al.* (13). In short, the urine was hydrolyzed with 2 N HCl in a boiling water bath for 2 h. After that, the sample was desalted using a cation exchange resin (Dowex AG50 X8). The fraction containing the acidic and neutral amino acids was eluted with 12 ml of 2.0-2.5 N HCl. Finally, the fraction containing the basic amino acids (and 3-methylhistidine among them), was eluted with 18 ml of 4.0-4.5 N HCl. This eluate was dried in a rotatory evaporator, reconstituted with 2-4 ml of citrate buffer (pH 2.2) and injected into an automatic amino acid analyzer Beckman 121.

Blood analysis. Blood was obtained on the last day of the experiment by bleeding the rats from the decapitation wound, it was allowed to clot at room temperature, and then centrifuged for 10 minutes at 2000 r.p.m. The separated serum was stored in small aliquots at —20° C. Insulin and corticosterone concentrations were evaluated by radioimmunoassay method, using kits purchased from Bio Rad (Louisville, Ky.) and Inter Science Institute (Los Angeles, Calif.), respectively (30).

Statistical evaluations. Statistical calculations were carried out by conventional one- and two-way analysis of the variance (5).

Results

Effect of energy-restriction on body weight, organ weights and serum insulin and corticosterone concentrations. Body weight gain, expressed in g/day throughout the experimental period was significantly reduced ($p < 0.01$) in the animals fed the low-energy diet, compared to the well-nourished rats. The weight of the liver was significantly reduced ($p < 0.05$) in the rats fed the low-energy diet, compared to the control ones. The weight of the four muscles dissected, representing predominantly red (soleus), white (extensor digitorum longus) and mixed (gastrocnemius and tibialis anterior) fibers, showed a similar pattern, in the sense that no significant differences were noted between the two dietary groups. Serum insulin concentration was significantly ($p < 0.01$) lower in the energy-restricted animals as compared to the control rats. Serum corticosterone levels were not significantly different between the two dietary experimental groups.

Table I. *Diet constituents (g/100 g dry diet).* The energy-deficient diet was offered to rats at half the amount of diet consumed by rats fed on the adequate one. The mineral mix was purchased from General Biochemicals, Chagrin Fall, Ohio. Composition of mineral and vitamin mixtures was as described by Rogers and Harper (25). Choline was added to the diet as an aqueous solution containing 1 g of choline hydrochloride/5 ml. The corn oil was obtained from Wesson Oil Sales, Fullerton, Calif.

Component	Adequate diet	Energy-deficient diet
Dextrine	44.2	28.4
Sucrose	22.1	14.2
Lactalbumin	18.0	36.0
Mineral mix	5.0	10.0
Vitamin mix	0.5	1.0
Choline	0.2	0.4
Corn oil	10.0	10.0
Agar	4.0	4.0
Water	100	100.0

Urinary excretion of urea-N, creatine, creatinine and 3-methylhistidine. In the rats fed the low-energy diet, urinary urea-N output underwent a sharp and significant ($p < 0.01$) increase during the first 10-12 days of the experiment, but from then on values tended to match those of the well-nourished rats. Creatine and creatinine excretions did not show any change during the three first days of the experiment, but from that time on, a

Table II. *Body weight, liver and muscle weights and serum insulin and corticosterone concentrations of young male rats fed for 21 days on either an adequate control diet (18 % lactalbumin) or energy-deficient diet (36 % lactalbumin, and half the caloric content of the control diet).*

The adequate diet was offered to the rats *ad libitum*, and the energy-deficient diet was offered at one half the intake of adequate diet consumed by the rats fed on this diet. Entries are mean ± S.E.M. from six rats. EDL = extensor digitorum longus muscle. Muscles were excised from both rear legs. Blood was obtained directly from the decapitation wound. For 1 h it was allowed to clot at room temperature, and then it was centrifuged for 10 min at 2000 r.p.m. and stored at —20 °C until assay.

Diet	Body weight gain g/day	Liver g/100 g b. wt.	Gastrocnemius g/100 g b. wt.	Soleus mg/100 g b. wt.	Tibialis mg/100 g b. wt.	EDL mg/100 g b. wt.	Serum concentration	
							insulin μlU/ml	corticosterone μg/100 ml
Adequate	7.14 ± 1.65	5.1 ± 0.7	1.12 ± 0.10	77 ± 6	346 ± 16	93 ± 14	85.59 ± 2.52	35.50 ± 2.23
Energy-deficient	3.81 ± 0.74 *	3.8 ± 0.4 *	1.13 ± 0.11	75 ± 16	365 ± 24	92 ± 10	26.25 ± 6.88 *	38.23 ± 2.10

* $p < 0.05$, compared to values of the rats fed on the adequate diet.

Table III. *Urinary urea-N, creatine, creatinine (mg/100 g body weight) and 3-methylhistidine (A: μmol/100 g body weight; B: μmol/mg creatinine) excretion in young male rats fed on either an adequate control diet (18 % lactalbumin) or an energy-deficient diet (Low-E: 36 % lactalbumin, and half the caloric content of the control diet) for 21 days.*

The control diet was given to the rats *ad libitum* and the low-energy diet was given to the rats at half the amount of the control diet consumed by the rats fed this diet. Entries are values from six-rats-pooled samples in each dietary group taken each three days. LSD, least significant difference for $p < 0.05$ between values from rats fed the control and low-energy diets.

Day	Urea-N		Creatine		Creatinine		3-methylhistidine			
							(A)		(B)	
	Adequate	Low-E	Adequate	Low-E	Adequate	Low-E	Adequate	Low-E	Adequate	Low-E
3	56.8	507 *	0.28	0.29	1.9	1.8	0.96	1.05 *	0.50	0.58 *
6	21.8	660 *	0.31	0.40 *	1.9	1.2 *	1.00	1.06 *	0.53	0.88 *
9	19.6	564 *	0.23	0.37 *	1.3	1.9 *	0.91	0.91	0.65	0.48 *
12	25.2	193	0.20	0.37 *	1.0	1.9 *	0.92	0.89 *	0.92	0.46 *
15	24.6	76.4	0.25	0.34 *	1.3	2.0 *	0.95	0.81 *	0.73	0.41 *
18	15.8	26.6	0.22	0.27 *	1.2	1.8 *	0,89	0.68 *	0.68	0.38 *
21	23.5	30.6	0.23	0.30 *	1.5	2.5 *	0.85	0.72 *	0.72	0.29 *
LSD	251.1		0.05		0.06		0.01		0.03	

* $p < 0.05$, compared to values of the rats fed on the adequate diet.

significant ($p < 0.05$) increase was noted in the output of creatine. Creatinine excretion was reduced between days 4th and 8th, but after that a significant ($p < 0.05$) increase was monitored in the output of this metabolite. Per unit of body weight, the excretion of 3-methylhistidine was significantly increased ($p < 0.05$) in the rats fed the low-energy diet during the first six days of the experiment; then, for 3-4

days, the output was similar to that of the control rats, and from the 10th-11th days onward, 3-methylhistidine output was significantly reduced ($p < 0.05$) in the energy-depleted rats compared to the well-nourished ones. A similar pattern was displayed when 3-methylhistidine output was expressed per mg of creatinine (an index of total muscle mass); however, in this case, after the rise exhibited during the first six days, values did not become similar, but significantly less ($p < 0.05$) than those of the control animals throughout the experiment.

Discussion

This study clearly shows that the excretion of 3-methylhistidine is affected by the energy content of the diet provided to young growing rats. Energy deficiency caused an immediate, but short (5 days) rise in 3-methylhistidine output (expressed both per unit of body weight and per mg of creatinine output, used as a measure of muscle mass), indicating that the rate of muscle protein breakdown increased when the rats were fed diets lacking carbohydrates. These results are similar to those reported in the reviews by YOUNG and MUNRO (35) and MUNRO and YOUNG (18). This response to the energy supply is also evident in the data of LI and GOLDBERG (14). Our results also showed that once the elevation of 3-methylhistidine output occurred a progressive and clear decrease in the excretion of this metabolite was displayed throughout the experiment. This response agrees with that reported by several investigators studying the effect of protein-deficient diet (28), protein-free diet (19) and starvation (20) on the rate of muscle protein breakdown in rats.

Although rats fed the energy-depleted diet grew significantly less than the well-nourished ones, they did not lose weight throughout the experiment. This response is different from that shown by HAVERBERG *et al.* (12) after feeding rats on a both energy and protein depleted diet; they found evidence of a net loss of body weight and increased 3-methylhistidine excretion, and therefore, increased myofibrillar protein degradation for the first 11 days of their experiment (in contrast with the 5-6 days of increased 3-methylhistidine exhibited in this experiment). It appears logical that since rats fed the energy-deficient diet can use the normal protein content of it as a caloric and amino acid source, their metabolic need of using their own bodily proteins is less than if they were fed a diet lacking both protein and carbohydrates (20). This would explain the dramatic rise in urea-N excretion monitored during the first days of the experiment, in agreement with the data reported by other investigators (2). The subsequent decrease in breakdown noted after the 5-6 days of elevated breakdown may represent adaptation to utilization of body fat as a principal source of energy, such as it has been reported in studies with humans (24, 33); this observation also implies a metabolic adaptation in order to conserve muscle protein from losses through breakdown (12, 13).

It should be pointed out that the net reduction in liver mass detected in the energy-depleted animals might compensate for the lack of energy provided by the diet by breaking down the hepatic content in carbohydrates and proteins, sparing thus the muscle protein. This result correlates with the investigations reported by VITERI and ARROYAVE (27) in their studies on protein-calory malnutrition in humans. Although a total reduction in muscle weights was shown in the individual rats fed the energy-depleted diet, no differences were found when muscle weights were expressed per unit of body weight, compared to the rats fed the control diet; this means that no specific effect on muscle size was caused by carbohydrate deprivation in the diet, and

that the reduction in muscle size was parallel to the reduction in body weight (8, 11).

In growing-well-fed animals, output of creatinine relative to unit of body weight remains basicaly constant, which in this experiment was evident from days 5-6 onward. Generally, output of creatinine and creatine are considered as an index of total muscle mass (31). It appears that energy deficiency in the diet increases the output of creatine and creatinine per unit of body weight indicating a rise in the degradation of muscle protein other than myofibrillar; therefore in this experiment the measurement of creatinine may not be an accurate index of muscle mass. However, creatinine determination allows for the examination of the 3-methylhistidine/creatinine ratio as an indication of the rate of muscle protein degradation relative to muscle mass.

Finally, it has been shown that the metabolic adaptation in both calorie and protein malnutrition occurs by hormonal interaction, by servomechanisms at the cellular level and by yet poorly understood generalized body reactions (27). In this sense, the reduction in serum insulin concentrations detected in the energy-depleted rats agrees with previously reported data in the literature, both in humans (34) and rats (3) suffering from different types of malnutrition. These insulin changes compensate for the dietary restrictions by increasing fat mobilization from adipose tissue. Corticosterone changes are detected after prolonged malnutrition or severe stress, as reported by several workers (23, 26). From the data of this study, it can be concluded that restriction of energy in the diet provided to young male growing rats causes a slight initial increase in the rate of myofibrillar protein breakdown, followed by a subsequent decrease, which persisted throughout the rest of the experimental time.

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Resumen

Se ha determinado la excreción urinaria de 3-metilhistidina en ratas alimentadas con una dieta baja en energía. Ratas macho jóvenes se alimentaron durante 21 días con una dieta control adecuada (18 % lactalbúmina), o con una dieta deficiente en energía (conteniendo la mitad de la concentración de hidratos de carbono en relación con la dieta control). Se determinan, durante el experimento, la excreción urinaria de urea, creatina, creatinina y 3-metilhistidina, y las variaciones del peso de los animales y al final la concentración de insulina y corticosterona en el suero, así como el peso del hígado y de los músculos gastrocnemius, tibialis anterior, soleus y extensor digitorum longus.

Las ratas sometidas a la dieta baja en energía mostraron una significativa reducción de peso corporal y peso de hígado ($p < 0,01$), pero no se encontraron diferencias en los pesos de los cuatro músculos extraídos. La excreción urinaria de urea, creatina y creatinina fue significativamente ($p < 0,05$) más elevada en los animales de este grupo. La excreción de 3-metilhistidina experimentó un aumento inicial, seguido por una reducción significativa ($p < 0,05$) durante el resto del tiempo del experimento. La concentración sérica de insulina resultó significativamente ($p < 0,01$) más baja en las ratas alimentadas con dieta baja en energía que en las controles; no se encontraron diferencias significativas en los niveles séricos de corticosterona.

Se concluye que la falta de energía en la dieta suministrada a ratas en crecimiento origina una reducción en el nivel de degradación de las proteínas miofibrilares del tejido muscular.

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