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Effect of the Thyroid Status and Protein-Calorie Malnutrition of the Rate of Myofibrillar Protein Degradation in Mature Male Rats

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The urinary excretion of N^{τ}-methylhistidine (3-methylhistidine: 3-Mehis), an index of the rate of myofibrillar protein catabolism, was determined in intact and thyroidectomized mature male rats, receiving intraperitoneally either vehicle (0.9 % NaCl) or thyroxine (T_4) replacement (2 μg/100 g body weight/day) during 20 days. Rats were fed either an adequate control or a low-protein low-energy diet. In addition, body weight changes and food intake were recorded throughout the experiment. At the end of the 20-day period, livers and several muscles from hind limbs were excised and weighed. A sample of blood was then taken for serum insulin, triiodothyronine (T_3) and thyroid-stimulating hormone (TSH) determination.

As compared to the well-nourished animals, a significant ($p < 0.05$) reduction in the rate of growth, food intake, 3-Mehis and serum insulin and T_3 concentrations was observed in the rats fed the low-protein low-energy diet. In both dietary groups, thyroidectomy increased serum TSH levels and tended to reduce 3-Mehis output and liver and muscle sizes, although there was a different response according to the type of muscle excised. T_4 replacement improved growth and restored T_3 levels, especially in the well-fed animals, but it failed to restore either serum insulin concentrations or 3-Mehis output in either dietary group.

In conclusion, both thyroidectomy and protein-calorie malnutrition reduced the rate of myofibrillar protein breakdown in the mature rat and T_4 replacement had no effect in restoring the normal range of myofibrillar protein degradation.

The rate of muscle protein synthesis and degradation is deeply affected by both dietary and hormonal factors (16, 23). It has been reported that thyroid hormones increase the rate of growth and RNA concentration and muscle protein synthesis in the skeletal musculature in the rat (3, 9). Hypophysectomy or thyroidectomy brings about a significant re-

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duction in growth, muscle protein synthesis and muscle protein degradation (9, 14). On the other hand, both protein and energy deprivation lead to a reduced rate of muscle protein tunover in the young rat as measured either by the urinary excretion of 3-methylhistidine or through the method of the constant infusion of labelled amino acids (13, 16, 20). Furthermore, nutritional status is also affected by thyroid status. Triiodothyronine plasma levels are reduced during fasting (2) and increased in subjects overfed with carbohydrates (6).

The purpose of this study was to extend our previous investigations (1,19) by reporting the effect of thyroid status on the rate of myofibrillar protein catabolism in mature male rats fed a low-protein low-energy diet. The urinary excretion of 3-methylhistidine will be used as an *in vivo* index of the rate of myofibrillar protein breakdown in the rat. The rationale and validation of this approach has been discussed in previous publications from this laboratory and others (10, 17, 23). Briefly, N^{τ}-methylhistidine (3-methylhistidine), an amino acid present in myofibrillar protein is released on breakdown of the myofibrillar proteins myosin and actin, and excreted quantitatively chiefly via urine. Through the use of this parameter, the separation of synthesis and degradation effects is possible.

It was found that thyroidectomy brought about a significant reduction in the excretion of 3-methylhistidine both in the well-nourished rats and in those suffering protein-calorie malnutrition. Furthermore, this reponse was not affected by thyroxine replacement.

Materials and Methods

Intact and thyroidectomized male rats Sprague Dawley (Charles River Breeding Laboratories, Wilmington, Mass.), 29 days old and weighing about 100-110 g were housed in individual non-metabolic cages, and fed *ad libitum* on an adequate diet (18 % lactalbumin) for 67 days. Water was always supplied *ad libitum*. Then, the animals were housed in individual metabolic cages, and randomly assigned to two dietary-treated groups of fifteen animals each. One was fed the control diet mentioned above, and the other was fed a low-protein low-energy diet. Composition of the diets, as well as procedure of feeding were as described by HAVERBERG *et al.* (13). Within each of these groups, three hormone-treated groups of five animals each were made as follows: intact, receiving vehicle injection (0.9 % NaCl), thyroidectomized, receiving the same vehicle, and thyroidectomized receiving 2 μg of thyroxine (Sigma) per 100 g body weight daily. Injections of both vehicle and hormone were given intraperitoneally for a 20 days experimental period, between 9.00 and 10.00 h, after the animals were weighed. Body weight changes and food intake were daily recorded.

Daily urine excretion was collected in each rat; 0.1 ml of toluene was used as preservative. Individual excretions were pooled within each group and prepared for further analysis. Urinary output of 3-methylhistidine was assessed with the aid of an automatic amino acid analyzer Beckman 121, as previously described (10, 12, 18).

At the end of the experiment, all rats were killed by decapitation. Immediately, livers, as well as gastrocnemius, soleus, tibialis anterior and extensor digitorum longus muscles from hind limbs were carefully excised and weighed (11). A sample of blood was taken at the killing time from the decapitated body. From 1 h it was allowed to clot at room temperature. Then, it was centrifuged 10 min at 2,000 r.p.m. The separated serum was stored in small aliquots at —20° C until assay. Serum concentrations of insulin, triiodothyronine and thyroid-stimulating hormone were determined by radioimmuno-

assay method (22), using kits purchased from Bio Ria (Louisville, Ky.), Corning Medical Diagnostic (Medfield, Mass.) and New England Nuclear (Boston, Mass.) respectively.

Statistical evaluations were carried out by conventional one- and two-way analysis of the variance test (4). Least significant differences were calculated and are given in tables I and II and in figure 1.

Results and Discussion

Results of the experiment are summarized in tables I and II and in figure 1, which also contain the least significant differences between pairs of observations based on the residual error of each analysis of variance. As previously reported by us in young rats (1, 19) and others (5), thyroidectomy caused a significant reduction ($p < 0.05$) in the rate of growth and liver size. These effects were overcome by the administration of 2 μg of thyroxine per 100 g body weight. Similar results have been reported by EVANS *et al.* (8), in which it was shown that doses between 2 and 5 μg of thyroxine were able to restore both growth and energy metabolism. Thyroidectomy also caused an impairment in the rate of muscle protein catabolism as indicated by the significant reduction ($p < 0.01$) in the urinary excretion of 3-methylhistidine, expressed either as μmoles per unit of body weight (fig. 1) or as μmoles per 100 g of excised leg muscles. This fact correlates with widely reported data in the literature (2, 7, 9) and with recent observations from our laboratory in young growing rats (1, 19). However, it is interesting to point out that thyroxine replacement to thyroidectomized rats, although restored the rate of growth, did not affect the urinary excretion of 3-methylhistidine (and therefore the rate of myofibrillar protein breakdown), in contrast with the effects observed in the young thyroidectomized rat receiving the same thyroxine replacement (1). It might be possible that higher doses of the hormone would cause a more substantial effect. FLAIM *et al.* (9) found that doses of 5 μg of thyroxine were necessary to almost optimize not only the rate of growth, but also muscle protein synthesis and RNA content. In any case, under the circumstances of this experiment, it seems that the response of growth to thyroxine replacement occurred much earlier than it did myofibrillar protein breakdown. On the other hand, since serum triiodothyronine levels, virtually suppressed by thyroidectomy, were significantly brought up ($p < 0.05$) to the normal range, it seems that the response of muscle protein catabolism to thyroxine replacement is independent of the thyroid status, as observed in young animals (19).

Although thyroxine replacement did not restore significantly ($p < 0.05$) serum insulin levels, it almost doubled the concentration exhibited by the thyroidectomized rats receiving only vehicle injection. This fact correlated with the improved rate of growth displayed by the hormone treated animals, and agreed with our previously reported data (1).

The main purpose of this study was to investigate the effect of protein-calorie malnutrition and thyroid status on the rate of growth and myofibrillar protein catabolism in the mature rat. As compared to the well-fed animals, a significant reduction ($p < 0.05$) in both the rate of growth and liver size, together with a reduction in food intake, was displayed by all thyroidectomized rats, regardless of the hormone treatment. Nevertheless, thyroxine replacement slightly alleviated the impairment in the rate of growth caused by protein-calorie malnutrition. Furthermore, serum insulin and triiodothyronine levels were significantly reduced ($p < 0.05$), in correlation with the data reported by other investigators studying the effect of protein-calorie malnutrition in human subjects (21). As expected, thyroxine replacement to those animals

Table I. *Body weight gain, food intake, organ weights and urinary output of 3-methylhistidine (3-Mehis) of intact and thyroidectomized (T_x) mature male rats (117 days old) receiving either vehicle (0.9 % NaCl) or 2 μg of thyroxine (T_4)/100 g body weight/day, intraperitoneally during 20 days.*

Rats were fed on an adequate control diet or a low-protein low-energy diet. Entries are mean values ± S.E.M. from five rats in each experimental group. For 3-Mehis entries are mean values for the entire experiment. Rats were killed by decapitation 24 h after the last injection of either vehicle or hormone. Immediately livers and muscles from hind limbs were excised and weighed. EDL, extensor digitorum longus.

Diet	Group	Body weight gain g/day	Food intake g/100 g b. wt.	Organ weights/100 g body weight					3-Mehis μmoles/100 g leg muscle
				Liver (g)	Gastrocnemius (g)	Soleus (mg)	Tibialis (mg)	EDL (mg)	
Control	Intact	2.4±0.4	7±2	3.3±0.6	1.26±0.10	97± 3	376±26	106± 7	47±1
	T_x + veh.	1.4±0.5 [b]	7±2	3.0±0.4 [b]	1.15±0.13	70± 7 [b]	389±29	96± 8	35±2 [b]
	T_x + T_4	3.1±0.5	10±3 [b]	3.4±0.3	1.10±0.06 [b]	66± 1 [b]	370±26	93±11 [b]	30±2 [b]
Low-protein	Intact	—4.8±0.3 [a]	4±1 [a]	2.3±0.2 [a]	1.38±0.09	110±12 [a]	416±12 [a]	116± 8	39±1 [a]
Low-energy	T_x + veh.	—1.7±0.2 [ab]	3±1 [a]	2.0±0.2 [ab]	1.01±0.09 [ab]	83± 7 [ab]	370±15 [b]	97± 6 [b]	37±1
	T_x + T_4	—2.4±0.2 [ab]	3±1 [a]	2.2±0.1 [a]	1.17±0.05 [b]	83± 6 [ab]	418± 8 [ab]	108± 5 [a]	37±1 [a]
[1]LSD		1.0	1.5	0.3	0.13	10.0	29	11	4

[1] LSD, least significant differences for $p < 0.05$ (one- and two-way ANOVA).
[a] $p < 0.05$, as compared to the group fed on the control diet (dietary effect).
[b] $p < 0.05$, as compared to the intact group fed on the same diet (hormonal effect).

slightly improved the concentration in serum of these hormones, especially insulin. The reduction in growth showed by the rats fed the low-protein low-energy diet was accompanied by a overall reduction in the urinary output of 3-methylhistidine regardless of the thyroid status, in agreement with the observations of HAVERBERG *et al.* (13). However, as compared to the intact well-fed animals, there was an initial increase in the excretion of 3-methylhistidine (fig. 1) in the undernourished animals, indicating that the rate of myofibrillar protein breakdown initially increased when both energy and protein are restricted, in agreement with studies carried out in rats (13, 15) and humans (24).

Regarding to serum TSH levels it is noteworthy to point out that, as compared to the well-fed rats, there was a significant reduction ($p < 0.05$) in the rats fed the low-protein low-energy diet, to the point where they became undetectable (21). Nevertheless, in both dietary regimes, thyroidectomy caused a dramatic rise in TSH concentration, probably due to the suppression of the negative feedback inhibition by the thyroid hormone on the secretion of TSH by the hypophysis. Thyroxine replacement had no effect at all in restoring the physiological levels of TSH in either dietary group. This indicated that the response of the hypophysis relative to TSH secretion to a daily single dose injection of thyroxine is very different from that of the intact, well-regulated thyroid gland; similar response was observed in a recently reported study from our laboratory (1).

The response of the four muscles excised in the rats, which represent predominantly red (soleus), white (extensor digitorum longus) and mixed (gastrocnemius and tibialis anterior) fibers, was in general difficult to interpret and varied according to the type of muscle. Expressing their weights per unit of body weight, thyroidectomy brought about an overall reduction in the size of these muscles in the two dietary regimes. In most of the cases, this reduction was not restored by

Table II. *Serum insulin, triiodothyronine (T_3) and thyroid-stimulating hormone (TSH) of intact and thyroidectomized (T_x) mature male rats (117 days old), receiving either vehicle (0.9% NaCl) or 2 μg of thyroxine (T_4)/100 g body weight/day, intraperitoneally, for 20 days.* Rats were fed on an adequate control diet or a low-protein low-energy diet. Entries are mean values ± S.E.M. from five rats in each group. Rats were killed by decapitation 24 h after the last injection of either vehicle or hormone. Blood was then collected from the decapitation wound, and serum was separated and assayed for the hormones. UD, undetectable.

Diet	Group	Insulin μU/ml	T_3 ng/ml	TSH μIU/ml
Control	Intact + veh.	160 ± 18	1.03 ± 0.31	1.88 ± 0.51
	T_x + vehicle	50 ± 8 [b]	UD	7.25 ± 1.06 [b]
	T_x + T_4	98 ± 10 [b]	1.15 ± 0.09	0.06 ± 0.01 [b]
Low-protein Low-energy	Intact + veh.	36 ± 10 [a]	0.87 ± 0.09 [a]	UD
	T_x + vehicle	22 ± 6 [ab]	UD	5.20 ± 0.82 [a]
	T_x + T_4	40 ± 9 [a]	0.88 ± 0.09 [a]	UD
LSD [1]		8	0.16	0.83

[1] LSD, least significant difference for $p < 0.05$ (one-way ANOVA) for both dietary and hormone treated-groups.
[a] $p < 0.05$, compared to the group fed on the control diet (dietary effect).
[b] $p < 0.05$, compared to the intact group fed on the same diet (hormonal effect).

thyroxine replacement. Nevertheless, these weights provided an excellent means of expressing the urinary output of 3-methylhistidine, since this amino acid derivative mainly comes from the skeletal musculature.

In conclusion, the results of this study suggest that both thyroidectomy and protein-calorie malnutrition caused a reduction in the rates of growth and myofibrillar protein catabolism in the adult rat. Furthermore, a replacement dose of 2 μg of thyroxine per 100 g body weight, although improving the rate of growth in the rats fed either the adequate or the low-protein low-energy diet, had no effect on the rate of myofibrillar protein catabolism as measured by the urinary excretion of 3-methylhistidine.

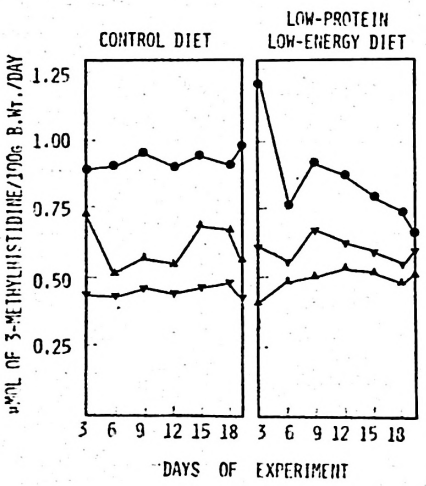

Fig. 1. *Urinary output of 3-methylhistidine in intact and thyroidectomized mature male rats receiving different hormonal and dietary treatments.*

Each point is a pooled sample from five rats in each of the following hormone-treated groups: intact, receiving vehicle injection (0.9% NaCl) (●—●), thyroidectomized, receiving the same vehicle injection (▲—▲), and thyroidectomized receiving 2 μg of thyroxine/100 g body weight/day during 20 days (▼—▼). Injections were given intraperitoneally. Animals were fed on either an adequate control diet or a low-protein low energy diet. Least significant differences for $p < 0.05$ (two-way ANOVA) for the groups fed the control and the low-protein low energy diets were 0.10 and 0.15 respectively, and for the entire experiment, 0.11.

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Resumen

Se ha determinado la excreción urinaria de N^{τ}-metilhistidina (3-metilhistidina: 3-Mehis), en ratas macho adultas intactas o tiroidectomizadas a las que se les inyectaba vehículo (ClNa 0,9 %) o tiroxina (T_4) (2 μg/100 g de peso corporal/día) durante 20 días. Las ratas se alimentaron con una dieta control o con una dieta deficiente en proteína y en energía. Se determinaron además las variaciones ponderales y el consumo de dieta a lo largo del experimento. Al final del período experimental se pesaron los hígados y diferentes músculos de las patas traseras, y se determinó en suero la concentración de insulina, triyodotironina (T_3) y hormona estimulante de la tiroides (TSH).

Comparados con los animales alimentados con la dieta control las ratas alimentadas con la dieta baja en proteína y en energía presentaron una reducción significativa ($p < 0,05$) en la velocidad de crecimiento, consumo de alimento, excreción de 3-Mehis y concentraciones séricas de insulina y T_3. En los dos grupos dietéticos, la tiroidectomía llevó consigo un aumento de los niveles séricos de TSH y reducción en la excreción de 3-Mehis, así como en el tamaño de los hígados y de los músculos. La administración de T_4 mejoró el crecimiento y restableció los niveles séricos de T_3, sobre todo en los animales alimentados con la dieta control, pero no consiguió restablecer los niveles de insulina ni aumentar la eliminación urinaria de 3-Mehis en ninguno de los dos grupos dietéticos.

Se concluye que tanto la tiroidectomía como la alimentación con dietas deficientes en proteína y energía reduce la degradación de las proteínas miofibrilares en la rata adulta, y que la administración de T_4 no restablece los valores de degradación de las proteínas miofibrilares al nivel de los animales control.

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