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Leucine Catabolism and Incorporation into Tissue Proteins in Thyroparathyroidectomized Rats[1] (42633)

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Abstract. We investigated parameters of leucine metabolism in thyroparathyroidectomized (TPX) and pair-fed control rats using a technique of continuous infusion of [1-^{14}C]leucine. The rate of leucine turnover was significantly smaller in TPX than in control rats (42.5 ± 2.6 vs 35.1 ± 1.9 μmole/hr/100 g, mean ± SEM, six rats). There was no significant difference between rates of α-decarboxylation of leucine by the two groups of rats. The protein incorporation of leucine was significantly smaller in the muscle of TPX than control rats (39 ± 5 vs 24 ± 4 pmole/mg protein, mean ± SEM, six rats) but in liver it was not significantly different. Thyroparathyroidectomy also had no significant effect on concentration of either leucine or its ketoacid (α-ketoisocaproate) in plasma, liver, and muscle. We conclude that hypothyroidism does not alter catabolism of leucine but reduces its incorporation into muscle protein. © 1988 Society for Experimental Biology and Medicine.

Metabolism of leucine is limited to two pathways, (a) incorporation into body proteins and (b) oxidation. The oxidation is initiated by transamination of leucine to α-ketoisocaproate (KIC) followed by α-decarboxylation of KIC. This decarboxylation, since it is irreversible, is the first step in catabolism of leucine.

A variety of nutritional, metabolic, and hormonal alterations have been shown to affect catabolism of leucine (1); among these alterations is the change in thyroid gland function. Although a previous study showed enhancement of α-decarboxylation of leucine in thyrotoxic patients (2), the mechanism of this effect remains unclear. One possibility could be that there is a relationship between the state of energy metabolism and the leucine catabolism. If this is the case, then there should be a decrease in α-decarboxylation of leucine in hypothyroidism. To investigate this possibility, in the present experiment, we have determined the effect of thyroparathyroidectomy on α-decarboxylation of leucine *in vivo* in rats. Recently we described a rat model for such an investigation (3). The model also provides information on concentrations of leucine and KIC in plasma and tissues, whole body rate of leucine turnover, and incorporation of leucine into muscle and liver proteins (3).

Materials and Methods. *Treatment of animals.* Thyroparathyroidectomized (TPX) and sham-operated (control) Sprague–Dawley rats (all male) were obtained from Zivic–Miller Laboratories (Allison Park, PA). All rats were housed in individual cages in air-conditioned quarters (temperature approximately 24°C) with controlled 12-hr light and dark cycles. Rats had free access to Purina Laboratory Chow and drinking water containing calcium lactate (1%).

TPX rats were maintained for 5 weeks after the operation to deplete the circulating levels of thyroid hormones. The hormone depletion was verified by measuring the plasma concentrations of thyroxine (T_4) and triiodothyronine (T_3). The hormone concentrations were measured by radioimmunoassay using reagent kits from Clinical Assays (Cambridge, MA).

The mean daily food intake of TPX rats over the 5-week period was 20.0 ± 0.3 g/rat (mean ± SEM, six rats). Control rats were housed under similar conditions as described above for TPX rats. They were pair-fed to TPX rats and received drinking water without calcium lactate.

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Leucine catabolism and turnover. We investigated rates of leucine catabolism and turnover by using [1-^{14}C]leucine infusion as previously described (3). The priming dose of [1-^{14}C]leucine (50 mCi/mmole, Amersham, Arlington Heights, IL) was 10 μCi and the rate of constant infusion was approximately 0.0069 ml/min or 0.069 μCi/min. The duration of the infusion was 240 min. The concentrations and specific activities of leucine and KIC in plasma and tissues were determined as described previously (3).

CO_2 specific activity. CO_2 specific activity was determined as described previously (3). Briefly, expired air was collected in hydroxide of methylbenzethonium chloride (Hyamine-10×, Rohm & Haas, Philadelphia, PA)–ethanol (1:1, v/v), and radioactive CO_2 was measured by liquid scintillation spectrometry. Total CO_2 production was determined by trapping expired air in $Ba(OH)_2$. Specific activity of CO_2 was calculated by dividing the radioactive CO_2 by the total amount of CO_2.

The retention of $^{14}CO_2$ produced as a result of α-decarboxylation of [1-^{14}C]leucine was determined in TPX and pair-fed control rats by measuring rates of $^{14}CO_2$ recovery during infusion of $NaH^{14}CO_3$ (53 mCi/mmole, New England Nuclear, Boston, MA) as described previously (3).

Incorporation of leucine into protein. After 240 min of [1-^{14}C]leucine infusion, samples of liver and gastrocnemius muscle were obtained to determine the incorporation of leucine into protein. Incorporation of leucine into tissue proteins was determined as previously described (4). Incorporation was calculated based on the specific activity of leucine in tissues which was determined by a previously described method (3).

Calculations. The rates of leucine turnover and α-decarboxylation were calculated by the following formulas:

$$Q = \frac{I}{SA_{pl}}$$

Where Q = turnover rate of leucine (μmole/100 g/hr), I = infusion rate of [1-^{14}C]leucine (dpm/100 g/hr), and SA_{pl} = specific activity of leucine in plasma under steady-state conditions (dpm/μmole).

$$D = \frac{PR}{SA_{pl} \times FR}$$

Where D = decarboxylation rate of leucine (μmole/100 g/hr), PR = $^{14}CO_2$ production rate (dpm/100 g/hr), SA_{pl} = specific activity of leucine or KIC in plasma at steady state (dpm/μmole), and FR = fraction of infused $NaH^{14}CO_3$ recovered as $^{14}CO_2$ under steady state conditions.

Statistics. Students' t test was used for the statistical analysis of the data (5).

Results. *Body weight.* Five weeks after the operation, the plasma concentration of T_4 in TPX rats was reduced by 78% (57.4 ± 3.0 vs 12.6 ± 0.9 ng/ml, mean ± SEM, six rats, $P < 0.01$) and that of T_3 by 65% (0.71 ± 0.04 vs 0.25 ± 0.02 ng/ml, mean ± SEM, six rats, $P < 0.01$). Despite equal food intake, control rats had modestly greater (18%) body weight at sacrifice than TPX rats (276 ± 12 vs 233 ± 8 g, mean ± SEM of six rats, $P < 0.01$).

Concentrations and specific activities of leucine and KIC. Concentrations of leucine and KIC in plasma and tissues are summarized in Table I. There was no significant difference between concentrations of leucine or KIC in plasma, muscle, or liver of control and TPX rats. The plasma specific activity of leucine and KIC reached a near constant value after 180 min of infusion of [1-^{14}C]leucine in control and TPX rats (Fig. 1).

Leucine turnover. TPX rats had significantly ($P < 0.05$) reduced rates of leucine turnover. The rates (μmole/hr/100 g, mean ± SEM in six rats) of leucine turnover in

TABLE I. EFFECT OF THYROPARATHYROIDECTOMY ON CONCENTRATIONS OF LEUCINE AND α-KETOISOCAPROATE IN PLASMA AND TISSUES

	Control	TPX
Leucine		
Plasma (nmole/ml)	108 ± 6	105 ± 4
Muscle (nmole/g)	95 ± 9	82 ± 4
Liver (nmole/g)	165 ± 23	187 ± 14
α-Ketoisocaproate		
Plasma (nmole/ml)	22.6 ± 1.2	18.2 ± 2.6
Muscle (nmole/g)	7.6 ± 0.7	5.7 ± 1.0
Liver (nmole/g)	<1.0	<1.0

Note. Each value represents the mean ± SEM of five to six rats.

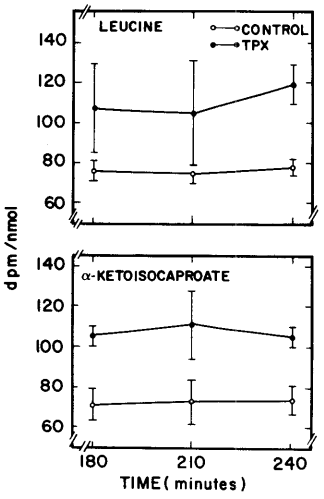

FIG. 1. Specific activities of leucine and α-ketoisocaproate in plasma. Rats were given a priming dose followed by an infusion of [1-^{14}C]leucine for 240 min. At 180, 210, and 240 min, blood samples were taken to determine specific activities of leucine and α-ketoisocaproate in plasma. Each value is the mean ± SEM of five to six rats.

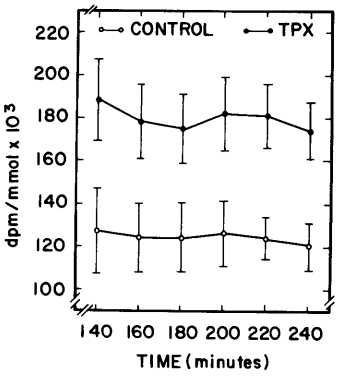

FIG. 2. Specific activity of CO_2 in expired air. Rats were given a priming dose followed by an infusion of [1-^{14}C]leucine for 240 min. Rats were placed in metabolic cages and expired air was collected. The specific activity of CO_2 in expired air was determined at the indicated time intervals. Each value is the mean ± SEM of five to six rats.

control and TPX rats were 42.5 ± 2.6 and 35.1 ± 1.9, respectively.

Leucine catabolism by whole body. Determination of the rate of α-decarboxylation of leucine by whole body required investigation of the extent of recovery of $^{14}CO_2$ produced as the result of this decarboxylation and demonstration that specific activity of CO_2 in expired air reaches a near constant value. The recovery of $^{14}CO_2$ was nearly 100% in control and TPX rats. The specific activity of CO_2 in expired air reached a near constant value after 140 min of infusion of [1-^{14}C]leucine in control and TPX rats (Fig. 2). The fact that specific activity of CO_2 in expired air and specific activity of leucine and KIC in plasma had all reached a plateau, allowed calculation of rates of leucine decarboxylation under steady state conditions. These calculations were based on plasma leucine as well as plasma KIC specific activity. Regardless of the specific activity used for calculation, there were no significant differences in rates of leucine decarboxylation between TPX and control rats (Fig. 3).

Leucine incorporation into tissue protein. To investigate whether a decrease in protein synthesis accounted for the decreased leucine turnover, we measured the incorporation of leucine into liver and muscle proteins in control and TPX rats (Table II). Leucine incorporation into muscle protein was significantly smaller in TPX than in control rats. There was no significant difference in leucine incorporation into liver proteins between the two groups of rats.

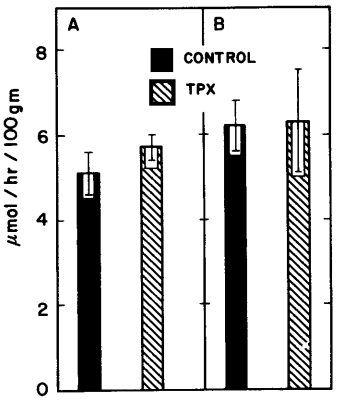

FIG. 3. Rates of α-decarboxylation of leucine by whole body. Rats were given a priming dose followed by an infusion of [1-^{14}C]leucine for 240 min. Rates of leucine decarboxylation were determined from the expired $^{14}CO_2$ collected under steady-state conditions. (A) Rate of leucine oxidation calculated based on plasma leucine specific activity. (B) Rate of leucine oxidation calculated based on plasma α-ketoisocaproate specific activity. Each value represents the mean ± SEM of five to six rats.

TABLE II. EFFECT OF THYROPARATHYROIDECTOMY ON LEUCINE INCORPORATION INTO TISSUE PROTEINS

Tissue	Leucine incorporation	
	Control	TPX
	(pmol/mg protein)	
Liver	328 ± 46	309 ± 36
Muscle	39 ± 5	24 ± 4*

Note. Each value represents the mean ± SEM of five to six rats.

* Significantly different from control at $P < 0.05$.

Discussion. The results of the present experiment show that thyroparathyroidectomy, which lowers plasma concentrations of T_4 and T_3 by 78 and 65%, respectively, does not alter leucine catabolism by whole body in rats. This lack of effect could be explained by the examination of the following factors involved in regulation of leucine catabolism.

Branched-chain keto acid dehydrogenase plays a key role in regulation of leucine catabolism (1). This mitochondrial enzyme exists in active and inactive forms. The activation and inactivation of branched-chain ketoacid dehydrogenase is brought about by dephosphorylation and phosphorylation of the protein moiety of this enzyme, respectively (1). A variety of factors appear to regulate the activity status of branched-chain keto acid dehydrogenase (1). Among these factors, concentrations of leucine and KIC seem to be physiologically most important (1). Therefore, absence of any significant change in plasma and tissue concentrations of leucine and KIC (Table I) may have been responsible for lack of alteration of leucine catabolism in TPX rats.

In addition to α-decarboxylation, the other pathway for leucine metabolism is incorporation into tissue proteins. There is evidence that thyroid hormone is involved in regulation of protein turnover in skeletal muscle (6–8). For example, in TPX rats, rates of protein synthesis are reduced in the gastrocnemius muscle (7). These rates are increased when thyroid hormone is administered to these rats (7). The result of the present experiment is consistent with the above observations by showing reduced leucine incorporation into muscle proteins in TPX rats (Table II). The present results further show that the effect of thyroid hormone is specific to muscle protein, since there was no significant difference in the incorporation of leucine into liver proteins between control and TPX rats (Table II).

It is pertinent to note that despite equal food intake, TPX rats gained less weight than control rats. This has been commonly observed previously (9, 10). Although we did not investigate the mechanism of stunted growth in TPX rats, others have suggested that it is secondary to growth hormone deficiency (11).

Finally, the results of the present experiment show that there is no relation between protein synthesis in skeletal muscle and leucine catabolism by whole body. For example, a decrease in protein synthesis in muscle of TPX rats was not accompanied by a significant change in leucine catabolism by whole body. Since the rate of leucine catabolism is increased in conditions with altered protein turnover, such as diabetes and starvation (3, 12, 13), the results of this study suggest that leucine catabolism is more directly related to stimulation of protein degradation than to inhibition of protein synthesis.

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