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Effects of Streptozotocin-Diabetes and L-Thyroxine Treatment on Plasma Amino Acid Levels in Thyroidectomized Rats

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Summary

1) Thirty days after surgical thyroidectomy, one group of rats were made diabetic by treatment with streptozotocin and were studied for the next 14 days. These diabetic thyroidectomized animals were similar in body weight to their thyroidectomized controls but had higher plasma concentrations of most amino acids.

2) Treatment with 0.5, 1.0 or 2.0 μg of L-thyroxine/100 g body wt for 7 days prior to sacrifice produced no changes in either parameter in the diabetic thyroidectomized animals. On the contrary, in thyroidectomized controls, the L-thyroxine treatment was followed by a dose-dependent increment in body weight. In these animals, the administration of 0.5 μg of L-thyroxine per day was associated with a marked rise in the plasma level of most amino acids, while only basic amino acid levels increased with 1.0 μg, and levels decreased with 2.0 μg.

3) In the diabetic thyroidectomized rats treated with insulin for the last 7 days before sacrifice, body weight gain and the biphasic change of plasma amino acid levels were restored.

4) It is proposed that treatment of thyroidectomized controls with small doses of L-thyroxine accelerate protein breakdown accompanied by minor changes in amino acid utilization, while this latter effect increases with higher doses of the hormone.

5) Present results demonstrate that a certain amount of circulating insulin is required to obtain the response to exogenous thyroxine in diabetic thyroidectomized animals. Results are discussed in terms of the role of interhormone synergism as it affects normal sensitivity of the different hormones.

Key-Words: *Diabetes – Hypothyroidism – Plasma Amino Acids – Hormone Sensitivitiy – Thyroxine*

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Introduction

It is well known that thyroid hormones affect protein metabolism by enhancing the incorporation of amino acids into proteins (*Macho, Strbák* and *Hromadová* 1972; *Sokoloff* and *Kaufman* 1961; *Sokoloff, Kaufman, Campbell, Francis* and *Galboin* 1963) through the stimulation of ribosomal and nuclear RNA synthesis (*Degroot, Rue, Robertson, Bernal* and *Scherberg* 1977; *Tata* and *Widnell* 1966; *Zoncheddu, Viarengo, Accomando, Fugassa* and *Orunesu* 1977). It has also been proposed that they affect protein catabolism (*Goldberg* 1980; *Goldberg, Tischler, DeMartino* and *Griffin* 1980) and the use of amino acids for gluconeogenesis (*Singh* and *Snyder* 1978). The concurrence of these actions is probably responsible for the changes in

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plasma concentration of amino acids observed in hypo- and hyperthyroidism (*Goldberg* et al. 1980; *Ness, Takahashi* and *Lee* 1969; *Remesar, Arola, Palou, Herrera* and *Alemany* 1981). These parameters of protein metabolism are also markedly affected by insulin (*Adibi, Morse* and *Amin* 1975; *Ingle, Prestrud* and *Nezamis* 1947; *Luck, Morrison* and *Wilbur* 1928; *Manchester* 1970; *Manchester* and *Young* 1958; *Pozefsky, Felig, Tobin, Soeldner* and *Cahill* 1969; *Röthing, Stiller, Dahlmann* and *Reinauer* (1978) and it is known that the glucose/insulin relationships are also altered in hypo- and hyperthyroidism (*Aranda, Montoya* and *Herrera* 1972; *Malaisse, Malaisse-Lagae* and *McGraw* 1967 *Martinez, Llobera, Cornella* and *Herrera* 1977). Thus the changes in endogenous insulin levels may be involved in the thyroid-dependent changes in amino acid metabolism. These interactions could be important in diabetic rats since they are known to respond differently to thyroid hormones in other parameters (*González, Montoya* and *Jolín* 1980; *Paricas* and *Jolín* 1977). The purpose of the present study was to investigate the changes of circulating amino acids in normal and diabetic thyroidectomized rats and their responses to both thyroxine and insulin administration.

Materials and Methods

Animals. Male Wistar rats weighing 98.4 ± 7.8 g, kept in collective cages in a temperature (22 ± 1°C) and light (from 7:00 to 19:00 h) controlled room and fed a medium-residue, low-iodine diet (*Escobar del Rey, Morreale de Escobar, Jolín* and *López-Quijada* 1968) were surgically thyroidectomized.

Thirty days after the operation, food was withheld for 24 h and half of the animals received 4 mg/100 g body wt i.p. of streptozotocin (Upjohn Co., Kalamazoo, Mich.) freshly dissolved in citrate buffer pH 4.5, while the other half were injected with the buffer and used as thyrodectomized controls. Food was then restored and for the next 7 days, tail blood glucose concentration was 352 ± 27 mg/100 ml in the streptozotocin treated animals, considered diabetics, while it was 109 ± 12 mg/100 ml in the thyroidectomized controls ($p < 0.001$). At this time, the diabetic and control thyroidectomized rats were injected daily with 0, 0.5, 1.0 or 2.0 μg i.p. of L-thyroxine/100 g body wt.* Half of the diabetic thyroidectomized rats receiving each L-thyroxine replacement dose or saline were also injected s.c. with bovine insulin (0.5 IU/100 g body wt). These treatments with L-thyroxine and insulin were performed daily at 8:00 h for seven days. One hour after the last injections, the rats were killed by decapitation and without anesthesia. Age and sex matched intact normal animals were injected with saline and studied in parallel with the thyroidectomized groups. Blood was collected into heparinized chilled tubes and plasma samples were stored at -20°C until processing.

Amino acid evaluation. Aliquots of plasma were deproteinized with cold acetone (*Arola, Herrera* and *Alemany* 1977) and the supernatants were used for determination of individual amino acids (*Arola, Palou, Herrera* and *Alemany* 1976) by means of their combination with ^{14}C-dansyl-chloride (Radiochemical Center, Amersham), by thin layer chromatography separation and by evaluation of the radioactivity present in individual spots (*Arola* et al. 1976).

For a clearer understanding of their changes, the different amino acids were grouped according to their physiological and structural characteristics as follows: gluconeogenic amino acids (alanine, glutamic acid, glutamine, aspartic acid, asparagine, glycine, serine and threonine): imino acids (proline and hydroxyproline); basic amino acids (lysine, arginine, histidine, ornithine and citruline); sulphur amino acids (taurine, cysteine+cystine, cysteate and methionine); branched chain amino acids (valine and leucine+isoleucine); and aromatic amino acids (tyrosine, phenylalanine and tryptophan).

Statistical analysis of the data. Statistical comparison between groups was made with the student's "t" test, while comparison between several groups was performed with Tukey's comparison test as modified by *Snedecor* (1956). Individual values of each group were always expressed as mean ± standard error of the mean.

Results

Thirty days after thyroidectomy, the rats' body weight was 114 ± 1.1 g, while that of sex and age matched intact normal controls was 202 ± 2.1 g ($p < 0.001$). Streptozotocin treated thyroidectomized rats showed a slight weight loss (106 ± 0.9 g) after 7 days' administration, while the body weight of the thyroidectomized controls remained almost unchanged (113 ± 3.5 g). Daily thyroxine injections in the thyroidectomized diabetic animals, in doses up to 2.0 μg/100 g body wt, did not modify their weight during the 7 days of treatment (Fig. 1), while in the thyroidectomized controls, 0.5 μg of thyroxine were sufficient to produce a significant increment in weight which was greater with higher doses of the hormone (Fig. 1). Insulin treated thyroidectomized diabetic rats responded to the thyroxine

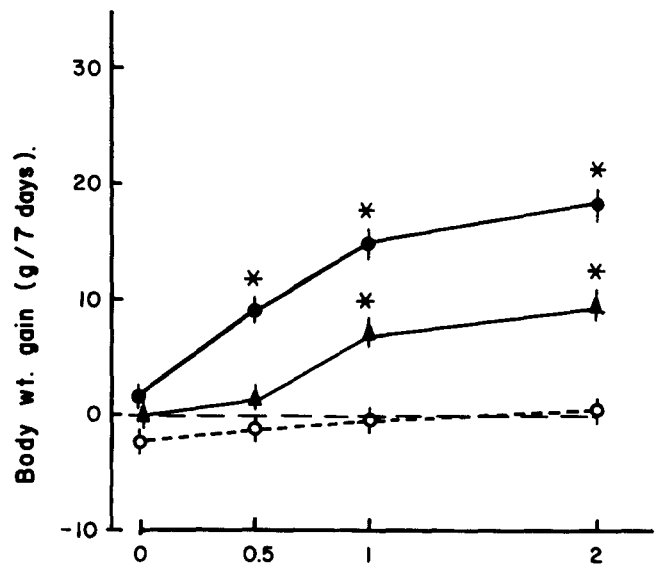

Fig. 1 Effect of treatment with L-thyroxine on the body weight gain of control (•), diabetic (o) and diabetic treated with insulin (▲) thyroidectomized rats.
Thirty days after thyroidectomy, one group of rats was made diabetic by treatment with streptozotocin and the rest remained as controls. Seven days thereafter, both groups received a daily i.p. injection of 0, 0.5, 1.0 or 2.0 μg of L-thyroxine/100 g body wt and half of the diabetic animals were also treated with a daily subcutaneous injection of 0.5 IU of bovine insulin/100 g body wt. These treatments with L-thyroxine and insulin lasted for 7 days and the values correspond to the net weight gain in each group during this period. Means ± S.E.M. Statistical comparisons of each group of animals treated with L-thyroxine *versus* the same group not receiving this treatment (0 doses) are denoted by asterisks: * = $p < 0.05$ or less. n = 8 rats/group.

* The L-thyroxine doses used were selected on the basis of determinations of RIA-TSH plasma levels (*González, Montoya* and *Jolín* 1980) in thyroidectomized controls treated with L-thyroxine and values in normal intact controls. Values (expressed as μg TSH/ml plasma) for thyroidectomized controls injected with saline were 6.19 ± 0.46; for those injected with 0.5 μg L-thyroxine/100 g body wt, values were 6.25 ± 0.38; for those injected with 1.0 μg, values were 5.20 ± 0.73, and for those injected with 2.0 μg values were 1.01 ± 0.25, while in normal intact age-matched animals, the values were 0.79 ± 0.28. Thus the 2.0 μg dose of L-thyroxine given to thyroidectomized controls produced plasma RIA-TSH levels that do not differ from those in intact animals ($p > 0.05$).

treatment with a net weight gain, although doses of 1.0 μg instead of 0.5 μg had to be used to produce a significant increment (Fig. 1).

Effects of thyroidectomy and L-thyroxine treatment on plasma amino acid levels. Table 1 summarizes the plasma concentration of individual amino acids identified in intact normal animals and thyroidectomized rats. In the thyroidectomized animals not treated with thyroxine, glycine and arginine levels were significantly augmented while levels of cystine + cysteine, glutamic acid, tyrosine, hydroxyproline, tryptophan, phenylalanine and valine decreased as compared with those in the normals. These differences were not very notable and they disappeared when the compiled values of total nonessential and essential amino acids and the values of total amino acids were compared. Doses of 0.5 or 1.0 μg of thyroxine produced significant increases in the levels of most amino acids in the plasma of thyroidectomized rats, making them higher than in intact controls. The thyroxine effect in those rats was reversed with doses of 2.0 μg which caused a return to levels observed in intact controls or even to below normal mean values. This biphasic effect of thyroxine on plasma amino acids in thyroidectomized animals is seen in Fig. 2 where compiled values are given for the amino acids, grouped according to their physiological affinity, showing that the effect was most pronounced in the gluconeogenic and sulphur amino acids. With the exception of the basic amino acids group, which required 1.0 μg doses of thyroxine to attain the highest level, the other amino acid groups showed the greatest increment with the 0.5 μg doses and decreased with higher doses (Fig. 2).

L-thyroxine response in diabetic thyroidectomized rats. As shown in Table 2, when the thyroidectomized animals were made diabetic by treatment with streptozotocin, the plasma levels of most amino acids appeared significantly enhanced as compared with controls. The compiled essential and nonessential amino acids and the total amino acid concentrations were significantly higher in the diabetic thyroidectomized rats than in their controls. The response to thyroxine treatment was also different in each group. Thus instead of the increase observed in most amino acids with low thyroxine doses in the thyroidectomized controls (Table 1), the response in diabetic thyroidectomized animals was minimal, and aspartic acid, hydroxyproline, alanine and proline were the only amino acids which increased with administration of 0.5 μg of thyroxine, and only alanine increased with doses of 1.0 μg. The other amino acids and the compiled essential and non-essential amino acids as well as the total ones were unchanged or even decreased with this treatment.

With 2.0 μg of thyroxine, most amino acid levels in diabetic thyroidectomized rats decreased significantly, as seen in Fig. 2. In contrast with the thyroidectomized controls, in no instance was the biphasic response to thyroxine treatment observed in diabetic thyroidectomized rats, but a progressive decrease in plasma amino acid groups was evident with increased doses.

Effect of insulin treatment in thyroidectomized diabetics during thyroxine treatment. Administration of insulin (0.5 IU/100 g body wt) to the thyroidectomized diabetic rats appeared to restore the biphasic response to thyroxine (Table 2). In these animals, as in the thyroidectomized controls, small doses of thyroxine (0.5 μg) produced a significant increment in most amino acids when compared with the values in both diabetic thyroidectomized rats treated with 0.5 μg of thyroxine and in non-treated subjects, while higher doses (1.0 or 2.0 μg) induced a return to basal or even lower levels in most amino acids.

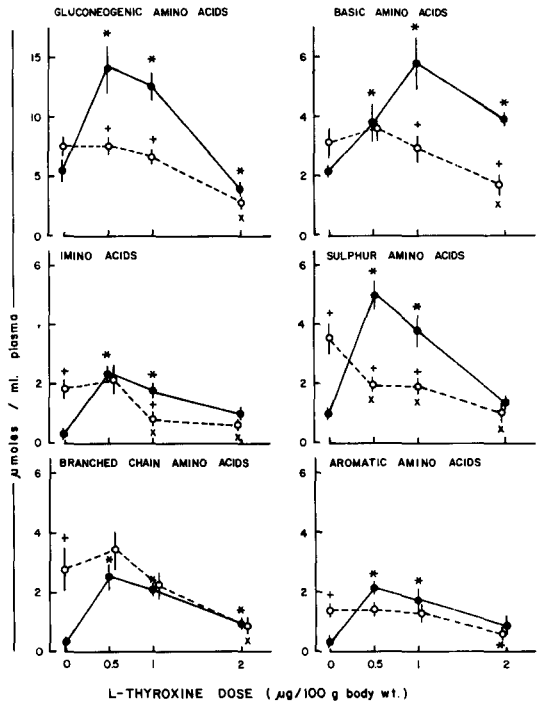

Fig. 2 Effect of treatment with L-thyroxine on the plasma level of amino acids grouped according to their physiological and structural characteristics in control (•) and diabetic (o) thyroidectomized rats.
Thirty days after thyroidectomy, half of the rats were made diabetic by treatment with streptozotocin. Seven days thereafter the animals of both groups received a daily i.p. injection of 0, 0.5, 1.0 or 2.0 μg of L-thyroxine/100 g body wt. All rats were then sacrificed by decapitation and individual amino acids were evaluated (*Arola* et al. 1976) in plasma aliquots deproteinized with cold acetone (*Arola* et al. 1977). Amino acids were grouped according to their physiological affinity (see text). Values are means ± S.E.M. of 8 to 14 rats per group. Statistical comparisons between each value of thyroxine treated animals and that of the same group not receiving thyroxine (0) are shown by asterisks and those between diabetic and control animals by crosses: * or + = $p < 0.05$ or less.

Discussion

Plasma levels of individual amino acids found in normal control rats were similar to those previously reported using similar methodology (*Arola, Herrera* and *Alemany* 1977; *Palou, Arola* and *Alemany* 1977; *Remesar* et al. 1981), whereas they differed from levels reported for humans. Comparative studies have already emphasized such differences (*Martín del Rio, Comprodón, Fabregat, Ramon* and *Herrera*, 1981), and our findings in rats should not be extrapolated to humans.

Table 1 Effects of L-T_4 on plasma amino acids (μmol/ml) in thyroidectomized (Thx) rats

	SALINE		L-T_4 treatment		
	Normal	Thx	Thx + 0.5	Thx + 1.0	Thx + 2.0
Essential Aminoacids					
Arginine	0.14±0.03	0.47±0.06*	0.64±0.28*	0.96±0.31*	0.28±0.14
Histidine	0.72±0.06	0.69±0.20	1.10±0.33	1.65±0.12*●	0.59±0.27
Threonine	0.24±0.03	0.22±0.03	1.18±0.16*●	1.37±0.24*●	0.46±0.07*●
Valine	0.26±0.03	0.15±0.03*	1.80±0.26*●	1.39±0.12*●	0.54±0.03*●
Methionine	0.39±0.12	0.20±0.06	0.80±0.21●	0.36±0.08	0.28±0.08
Phenylalanine	0.17±0.04	0.08±0.01*	0.92±0.08*●	0.83±0.10*●	0.18±0.01●
Leucine + Isoleucine	0.34±0.09	0.23±0.03	0.68±0.10*●	0.51±0.11●	0.46±0.09●
Tryptophan	0.53±0.13	0.14±0.04*	0.73±0.13●	0.30±0.04●	0.36±0.09●
Lysine	0.45±0.09	0.31±0.03	0.74±0.07*●	0.74±0.17●	0.14±0.03*●
Non-essential Amino acids					
Cystine + cysteine	0.26±0.04	0.12±0.03*	0.71±0.23*●	0.20±0.06	0.21±0.04●
Taurine	0.65±0.15	0.76±0.11	0.32±0.30	2.77±0.22*●	1.03±0.08*
Glutamic acid	0.25±0.03	0.17±0.01*	0.89±0.12*●	1.02±0.10*●	0.19±0.05
Aspactic acid	0.11±0.03	0.07±0.01	0.32±0.01*●	0.61±0.14*●	0.07±0.01
Ornitine	0.12±0.02	0.19±0.03	0.12±0.03	0.11±0.01	0.17±0.02
Serine	0.39±0.06	0.27±0.11	1.59±0.23*●	1.19±0.12*●	0.42±0.05●
Glutamine	1.63±0.47	2.73±1.15	4.56±0.73*●	3.60±0.84*●	1.09±0.23●
Asparagine	0.71±0.10	0.38±0.28	0.11±0.06*	0.09±0.05*	0.80±0.22●
Citrulline	0.74±0.20	0.38±0.28	0.79±0.20	2.19±0.80●	1.44±0.41●
Hydroxiproline	0.20±0.05	0.05±0.01*	0.37±0.08●	0.34±0.09●	0.14±0.02●
Glycine	0.38±0.07	0.60±0.06*	1.71±0.25*●	1.80±0.07*●	0.37±0.03●
Alanine	0.70±0.13	0.43±0.04	1.62±0.30*●	2.62±0.39*●	0.74±0.08●
Proline	0.37±0.12	0.27±0.01	1.89±0.18*●	1.98±0.17*●	0.69±0.09*●
Tyrosine	0.40±0.08	0.15±0.03*	0.59±0.12●	0.39±0.05●	0.37±0.08●
Total Amino Acids	10.15±1.13	9.06±1.22	24.18±3.41*●	27.02±2.71*●	11.02±0.82
Total Essential Amino Acids	3.24±0.31	2.49±0.33	8.59±2.21*●	8.11±0.79*●	3.29±0.21
Total Non-essential Amino Acids	6.91±0.76	6.57±0.87	15.59±2.16*●	18.91±1.74*●	7.73±0.55

Thirty seven days after surgical thyroidectomy (Thx) groups of 5 or 6 rats were i.p. injected with saline (0), 0.5, 1.0 or 2.0 μg of L-thyroxine (L-T_4)/100 g body wt. for 7 days. Results are expressed as means ± S.E.M. Asterisks and closed circles indicate statistical significance ($p \leq 0.05$ or less) between Thx and normal control rats also injected with saline, and between Thx on L-T_4 treatment and those of Thx on saline respectively.

The present study shows that thyroidectomized animals, which are markedly hypothyroid as seen by their retarded growth, have slight alterations in their circulating amino acid pattern in comparison with intact, normal rats. This finding is in agreement with previous reports (*Ness, Takahashi* and *Lee* 1969; *Remesar* et al. 1980) and indicates that in the fed condition, the hypothyroid rat has an equilibrium balanced between decreased protein synthesis (*Goldberg, DeMartino* and *Chang* 1978; *Yatvin, Wannemacher* and *Banks* 1964) and breakdown (*Goldberg* 1980; *Goldberg, DeMartino* and *Chang* 1978; *Goldberg* et al. 1980). Protein turnover has actually been found slower in thyroidectomized rats (*Goldberg* 1980; *Goldberg, DeMartino* and *Chang* 1978; *Rajwade, Katyare, Fatterpaker* and *Sreenivasan* 1975). Gluconeogenesis could also influence the peripheral amino acid concentration, but it has recently been reported that this pathway is unaffected in fed thyroidectomized rats (*Llobera* and *Herrera* 1980) and it was seen here that their gluconeogenic amino acid levels were not markedly altered. Thus, as already proposed for other metabolic parameters (*Aranda, Montoya* and *Herrera* 1972), a new equilibrium is apparently established in hypothyroidism which permits an almost normal steady-state concentration of most metabolites.

Our results indicate that this equilibrium for plasma amino acid levels was broken down by administration of small doses of exogenous thyroxine (0.5 μg/100 g body wt) which were not enough to restore animal growth rate to normal, although they caused a plasma amino acid level increase to values even greater than in intact normal animals. While this effect was evident in almost all amino acids, it was most pronounced in the gluconeogenic ones, due either to an enhanced release to the blood or a reduced renewal. There are no published data to resolve this question, but it is unlikely that thyroxine administration reduces amino acid renewal since it has been widely reported (although with higher doses and other experimental conditions) to enhance most pathways of amino acid utilization (*Macho, Strbák* and *Hromadová* 1972; *Singh* and *Snyder* 1978; *Sokoloff* and

Table 2 Effects of diabetes and insulin treatment on plasma amino acids (μmol/ml) in thyroidectomized rats treated with L-T_4).

Groups	Saline		L-T_4 treatment			L-T_4 + Insulin treatment		
	Thx	D.Thx	D.Thx + 0.5	D.Thx + 1.0	D.Thx + 2.0	D.Thx + 0.5	D.Thx + 1.0	D.Thx + 2.0
Essential Amino Acids								
Arginine	0.47±0.06	0.49±0.10	0.53±0.13	0.29±0.08	0.43±0.04	1.08±0.06*●	0.32±0.05*	0.35±0.11
Histidine	0.69±0.20	0.78±0.27	0.74±0.02	0.85±0.18	0.08±0.02*	2.30±0.67*●	5.29±0.11*●	0.32±0.08*●
Threonine	0.22±0.03°	0.85±0.13	0.82±0.09	0.74±0.08	0.30±0.08*	1.61±0.10*●	0.35±0.07*●	0.38±0.02*
Valine	0.15±0.03°	1.98±0.34	1.90±0.20	1.03±0.09*	0.36±0.03*	2.75±0.34*●	0.49±0.06*●	0.62±0.05*●
Methionine	0.20±0.06°	0.75±0.08	0.40±0.04*	0.22±0.03*	0.26±0.06*	0.36±0.03*	0.16±0.04*	0.17±0.04*
Phenylalanine	0.08±0.01°	0.65±0.20	0.80±0.11	0.68±0.12	0.22±0.02*	1.15±0.25*●	0.59±0.11	0.89±0.02●
Leucine+isoleucine	0.23±0.03°	0.78±0.11	0.28±0.05*	0.99±0.13	0.36±0.04*	1.80±0.12*●	0.84±0.12●	0.82±0.11●
Tryptophan	0.14±0.04°	0.26±0.04	0.25±0.04	0.14±0.01*	0.14±0.04*	0.26±0.13	0.34±0.06	0.17±0.03
Lysine	0.31±0.03	0.34±0.05	0.31±0.03	0.33±0.04	0.12±0.02*	0.28±0.04	0.29±0.04	0.26±0.03●
Nonessential Amino Acids								
Cystine+Cysteine	0.12±0.03°	0.27±0.05	0.14±0.02*	0.14±0.03*	0.09±0.02*	0.56±0.16●	0.15±0.03*	0.15±0.02*●
Taurine	0.76±0.11°	2.73±0.41	2.08±0.21	1.41±0.11*	0.61±0.07*	2.94±0.84	0.45±0.12*●	0.66±0.07*
Glutamic Acid	0.17±0.01°	0.59±0.08	0.69±0.07	0.55±0.04	0.27±0.04*	0.88±0.10*	0.15±0.02*●	0.40±0.05*●
Aspartic Acid	0.07±0.01°	0.15±0.03	0.31±0.04*	0.20±0.04	0.12±0.02	0.29±0.04*	0.09±0.02●	0.12±0.02
Ornitine	0.19±0.03°	1.55±0.01	0.10±0.09*	0.11±0.01*	0.15±0.04*	0.16±0.01*	0.03±0.01*	0.28±0.03*●
Serine	0.27±0.11°	0.94±0.08	1.03±0.10	0.98±0.11	0.35±0.05*	1.85±0.22*	0.27±0.05*●	0.58±0.11*●
Glutamine	2.73±1.15	2.35±0.52	2.48±0.31	2.00±0.22	0.69±0.13*	3.10±0.50	0.98±0.19*●	1.54±0.16●
Asparagine	0.38±0.28°	0.03±0.02	0.02±0.00	0.01±0.00	0.69±0.03*	0.03±0.01	0.07±0.03	0.41±0.09*●
Citrulline	0.38±0.28°	1.24±0.35	1.10±0.15	1.23±0.30	0.45±0.14*	5.29±1.56*●	0.24±0.06*●	0.94±0.15●
Hydroxyproline	0.05±0.01°	0.19±0.03	0.31±0.06*	0.11±0.02*	0.07±0.00*	0.21±0.05	0.08±0.03*	0.09±0.01*
Glycine	0.60±0.06°	1.36±0.15	1.15±0.12	0.96±0.09*	0.39±0.06*	2.02±0.35●	0.41±0.03*●	0.49±0.04*
Alanine	0.43±0.04°	1.18±0.07	1.55±0.19*	1.58±0.09*	0.67±0.06*	2.79±0.08*●	2.04±0.26*	0.98±0.06*●
Proline	0.27±0.01°	1.36±0.34	1.89±0.23*	0.70±0.08*	0.65±0.11*	3.10±0.30*●	1.37±0.22●	1.52±0.16●
Tyrosine	0.15±0.03	0.25±0.09	0.25±0.04	0.42±0.03	0.22±0.04	0.41±0.09	0.45±0.06*	0.33±0.06
Total Amino Acids	10.15 1.13°	21.07±1.58	19.14±1.53	15.69±1.05*	7.69±1.10*	35.22±2.64*●	15.35±1.34*	12.48±0.83*●
Total Essential Amino Acids	3.24±0.31°	6.88±0.56	6.04±0.53	5.27±0.34*	2.27±0.29*	11.59±0.27*●	8.58±0.73*●	3.99±0.24*●
Total Nonessential Amino Acids	6.91±0.76°	14.19±1.13	13.10±1.04	10.40±0.67*	5.42±0.77*	23.63±1.66*●	6.78±0.56*●	8.49±0.57*●

Thirty days after surgical thyroidectomy the rats were made diabetic by the injection of streptozotocin (D. Thx). Seven days thereafter groups of D.Thx rats were i.p. injected with saline or 0.5, 1.0 or 2.0 μg of L-T_4/100 g day for 7 days. Other D.Thx rats on L-T_4 were also daily injected with insulin for 7 days (0.5 IU/100 g BW/day). Results are expressed as means ± S.E.M. of 5 or 6 data/group. Asterisks and close circles indicate statistical significance ($p < 0.05$ or less) between D.Thx on L-T_4 vs. D. Thx on saline, and between D. Thx on L-T_4 + insulin vs. D. Thx on L-T_4, respectively. For comparison values of thyroidectomized control animals (Thx) on saline are also shown, their statistical significance vs. D.Thx on saline is shown by open circles ($p < 0.05$ or less).

Kaufman 1961; *Sokoloff* et al. 1963). Thus, in these conditions, protein breakdown may be accelerated by thyroxine doses which also produce minor modifications of amino acid utilization. This latter effect seems to appear with higher hormone doses, causing most circulating amino acids to return to basal levels. These results may be compared with findings of *Ness, Takahashi* and *Lee* (1969) after injecting L-triiodothyronine into thyroidectomized animals and again indicates the different sensitivity of the pathways in which the thyroid hormones are involved to their available amount. It should be noted that some of the observed thyroxine effects on amino acid concentrations in thyroidectomized animals may be secondary to the restoration of other endocrine functions such as the production of growth hormone by the pituitary (*Montes, Hervás* and *Jolín* 1977) or the release of insulin by the pancreas (*Malaisse, Malaisse-Lagae* and *McGraw* 1967) which are known to affect amino acid metabolism directly.

Insulin deficiency must be specially intense in the thyroidectomized rats made diabetic with streptozotocin. The plasma amino acid levels of these animals were augmented as compared with those in thyroidectomized controls and intact normal animals. Thus thyroid hormone deficiency in these animals apparently did not modify the augmented catabolism of the diabetic state. On the contrary, the insulin deficiency of these animals impeded the body weight gain and circulating amino acid responses to thyroxine administration. Insulin restored these effects, demonstrating that certain amounts of circulating insulin must be present to permit a proper thyroxine response.

The insulin effect in thyroidectomized diabetic rats treated with 0.5 μg of thyroxine deserves special attention. Insulin raised the plasma amino acid levels of these animals to values higher than in any of the other groups studied. This result contrasts with the well known hypoaminoacidemic effect of insulin (*Adibi, Morse* and *Amin* 1975; *Aoki, Brennan, Müller, Moore* and *Cahill* 1972). By this criterium, the thyroidectomized diabetic animals are treated with 0.5 μg of thyroxine and injected with insulin were more diabetic than those not injected with insulin. Minor amounts of insulin are evidently required in order for the small doses of thyroxine to exert their net proteolytic effect which was not seen in intensely diabetic animals. This is not the only instance in which the presence of minor amounts of a hormone is required in order for another hormone to exert some of its contrary effects, because minor amounts of circulating thyroid hormones are needed for a maximal release of pituitary thyrotropin in hypothyroid animals (*Purves* and *Adams* 1960) in spite of the negative feed-back regulation between thyroid hormones and thyrotropin secretion (*Wilber* and *Utiger* 1967).

Thyroidectomized rats received thyroxine and/or insulin for 7 days and were sacrificed just one hour after the last hormone injection. This experimental protocol did not permit determination of the duration of hormonal effects but gave an index of the actual acute response to treatments in the different experimental groups. Present data indicates that the direct effects of thyroid hormones on "in vivo" amino acid metabolism should not be evaluated without taking into account the effect of these hormones not only on other glands but also on the sensitivity of other hormones that, by themselves, affect amino acid metabolism. These interactions may determine the different responses of amino acids and protein metabolism to thyroid hormone administration according to the animals' age (*Macho, Strbák* and *Hromadová* 1972) and to the different experimental procols used, as well as making it difficult to produce "in vitro" responses in these parameters with these hormones.

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