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Metabolic Response to Short Periods of Starvation in Hypo- and Hyper-Thyroid Male Rats

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Summary

1) Thyoridectomized rats were fed with a low iodine diet, injected daily with 0, 0.1, 1.8 or 25 μg of L-thyroxine/100 g body wt., and compared with intact controls. 2) Plasma protein-bound iodine was decreased in the rats given the 0 and 0.1 μg doses, unchanged in those given the 1.8 μg doses, unchanged in those given the 1.8 μg dose increased in those given the 25 μg one. 3) The liver content of DNA-P, phospholipid-P, proteins and fatty acids was decreased in the rats that did not receive thyroxine, practically recuperated in those receiving 0.1 μg and normal in those given 1.8 or 25 μg of thyroxine. 4) 3 h of starvation produced a reduction in the liver content of total fatty acids that disappeared after 24 h. 5) When fed, liver glycogen concentration was low in the rats given 25 μg of thyroxine. 6) With starvation, the fall in liver glycogen and blood glucose, and the rise in liver acetyl-CoA and citrate and blood glycerol concentrations were faster in the thyroidectomized rats that did not receive thyroxine than in the other groups. 7) The rise in plasma free fatty acid and blood ketone bodies concentrations were similar in all the groups, the greater level of the first parameter being observed after 6 h of starvation in the rats given 25 μg of thyroxine and in the second one after 24 h in the rats given either 0.1, 1.8 or 25 μg of thyroxine. 8) The rapid decrease in the availability of carbohydrate stores with starvation in the thyroidectomized rats could be responsible for their fast call for lipid utilization. The slower response to fasting in the hyperthyroid animals is probably a consequence of their reduced amount of endogenous substrates to be mobilized.

Key-Words: *Hypothyroidism – Hyperthyroidism – Liver Metabolism – Steady State – Starvation*

Introduction

It is well known that changes in the thyroid status are followed by intense alterations in intermediary metabolism (for recent reviews on this subject see *Freinkel and Metzger* 1971; *Metzger and Freinkel* 1971; *Hoch* 1974). Thyroidectomized rats fed *ad libitum* on a diet of low iodine content show normal concentration of acetyl-CoA, citrate and glycogen in liver and of glucose and ketone bodies in blood (*Aranda* et al. 1972). Thus althoug the turnover rates of metabolites may be altered, the hypothyroid animals have been able to establish a new equilibrium supported by parallel changes in anabolism and catabolism. This equilibrium is broken when food is withheld for 48 h, as both blood glucose and liver glycogen decline to minimal values (*Aranda* et al. 1972). In hyperthyroid animals there is a net catabolic state already in the fed condition (*Freinkel and Metzger* 1971). Very probably their difficulties to respond to 48 h of starvation (*Aranda* et al. 1972) are due to the low endogenous reserves available. This starvation period is the time of maximal mobilization of endogenous lipidic stores in the normal rat, as suggested by the peak in ketonemia (*Herrera and Freinkel* 1968; *McGarry* et al. 1973). There exists the possibility that the time of metabolic adaptation to starvation will be variable according to the thyroid status of the animals, as well as enzymatic capabilities. To estudy this point, which would allow us to obtain a better understanding of the carbohydrate-lipid interaction in hypo- and hyperthyroidism, in the present work we have studied the effect of short periods of starvation on the steady state concentration of key metabolites in thyroidectomized rats treated with different doses of exogenous thyroxine.

Material and Methods

Animals. Young male Wistar rats weighing 40–50 g were fed on a medium residue, low-iodine diet (0.05–0.09 μg of iodine/g) (*Escobar del Rey* et al. 1968), surgically thyroidectomized (*Zarrow* et al. 1964) and injected daily intraperitoneally thereafter with 0, 0.1, 1.8 or 25 μg of L-thyroxine/100 g body wt. for 40 days, after which they were killed. They were compared with age-matched intact controls under the same diet supplemented with 1.7 μg of KIO_3/g and injected daily with 0.9 % NaCl during the same period of time. For the whole experiment the animals were housed in full air-conditioned animal quarter maintained at 22–24 °C. Lights in the animals room were automatically controled under a 12 hours on 12 off cycle, beginning at 11.0 hours. The rats were killed at 11.0 by decapitation without anesthesia. Starvation was timed on the basis of the previous period of food deprivation by adequate removal of the food at present times. During the starvation period the animals had unrestricted access to distilled water. Blood was collected into heparinized chilled beakers and a piece of liver was clamped with liquid N_2 cooled aluminium thongs in less than 6s after decapitation. Samples were placed and kept in liquid N_2 until processing.

Blood components. Blood was deproteinized with $Ba(OH)_2$-$ZnSO_4$ (*Somogyi* 1945) and analyzed for glucose (*Hugget and Nixon* 1957) and total ketone bodies (*Bessman and Anderson* 1957). Plasma free fatty acids were measured by the method of *Falholt* et al (1973). Protein-bound iodine in plasma was determined by a modified Zak procedure (*Benotti and Benotti* 1963).

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Analysis of liver. Portions of the frozen liver were analyzed for protein by the procedure of *Lowry* et al. (1951) after alkali digestion, using bovine serum albumin as standard. Other portions of the frozen liver were digested with KOH for precipitation of glycogen with ethanol (*Good* et al. 1933) and further hydrolysis (*Aranda* et al. 1972) and for glucose assay (*Huggett & Nixon* 1957). Another portion of frozen liver was used for lipid extraction and purification (*Folch* et al. 1957). An aliquot of the lipid extract was used to determine lipid-phosphorus (*Fiske & Subbarow* 1925) after digestion with 72% (v/v) $HClO_4$, as described by *Freinkel* (1958). A second aliquot was dryed with a stream of nitrogen at 45 °C, saponified with 1 M KOH in 95% (v/v) ethanol for 2 h at 100 °C, and the fatty acids were extracted into heptane after acidification. Final heptane extracts were again dried and resuspended in chloroform for fatty acids determination by the method of *Duncombe* (1963). DNA was isolated from the residual pellet after the lipid extraction (*Schmidt and Thannhauser* 1945) and its phosphorus contend was determined (*Fiske and Subbarow* 1925) after digestion with 72% $HClO_4$

Another portion of frozen liver was grinded under liquid nitrogen and used for $HClO_4$ extraction (*Herrera and Freinkel* 1967) in order to determine fluorimetrically its acetyl-CoA (*Herrera and Freinkel* 1967) and citrate (*Moellering and Gruber* 1966) content.

Statistical analysis. Student's "t" test was used for the comparison of the mean values in each group with those of the intact controls.

Results

Circulating levels of protein-bound iodine, and body and liver weights in thyroidectomized rats treated with L-thyroxine. Plasma protein-bound iodine levels were measured in order to have an index of the circulating concentration of thyroid hormones in the different groups (Table 1). In the rat, thyroidectomy at the end of weaning and the use of a diet with low iodine content during 40 days, produced a significant decrease in the plasma protein-bound iodine concentration (Table 1). The daily injection of 0.1 μg of thyroxine/100 g body wt. tripled the plasma protein-bound iodine level in thyroidectomized rats, but anyway the values remained significantly lower than in the controls. The differences versus the controls disappeared completely in thyroidectomized rats treated with 1.8 μg of thyroxine. The level was twice the one in the controls when the daily injection of thyroxine was boosted to 25 μg. Starvation did not affect the qualitative relationships among the groups.

The changes in body wt. of the rats throughout the experiment are shown in Fig. 1. Before the thyroidectomy (day 0) there were no differences in the body weights of the rats of different groups. From the 6th day of the thyroidectomy the increase in body weight of the thyroidectomized animals was retarded as compared with that in the intact controls. The daily injection of 0.1 μg of thyroxine/100 g body wt. to the thyroidectomized rats was sufficient to restore partially their growth capability. The body weights became equal to those of the intact controls when the thyroidectomized animals received 1.8 μg of thyroxine daily. The thyroidectomized rats treated with 25 μg of thyroxine/100 g body wt. showed a diminution of body weight as compared with the intact controls from the 30th day after the thyroidectomy and treatment. This was probably due to the catabolic status of these animals, and not to a retardation in the growth rate, as their body size was equal to that of the intact controls at the time the animals were killed (19.2 ± 0.2 cm body length in the thyroidectomized rats treated with 25 μg of thyroxine and 19.7 ±0.3 cm in the intact controls; n = 15–18/group, difference not significant). The changes of body and liver weights with starvation in the different groups are shown in Table 1. The lower percentual decrease of body weight was observed in the thyroidectomized rats not treated with thyroxine and the higher in those injected with 25 μg of thyroxine. Liver weight was significantly lower in the thyroid-

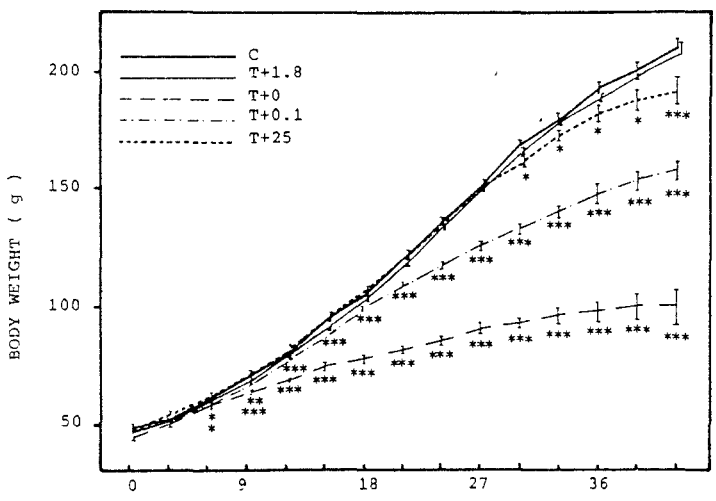

Fig. 1

Table 1 *Effect of starvation on plasma protein-bound iodine, liver weight and the change of body weight in thyroidectomized rats treated daily with L-thyroxine.* Rats were thyroidectomized after weaning and injected intraperitoneally with different dosis of L-thyroxine during 40 days. They were killed by decapitation. The results are given as means ± S.E.M. of 5 animals/group. P values refer to the differences between each group and the intact controls (N.S., not significant, i.e., $p > 0.05$). The statistical comparison of the starved groups versus the fed ones are shown by asterisks (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Other details are given in the Materials and Methods section.

Group (μg of L-thyroxine/ 100 g body wt.)	Dietary status	Plasma protein-bound iodine (μg/100 ml)	p'	Body wt. lost with fasting (g)	p'	Liver wt. (g)	p'
Intact controls (0)	Fed	4.59 ± 0.25		0.		10.64 ± 0.35	
	3h-starved	5.44 ± 0.57		6.83 ± 1.01		10.28 ± 0.50	
	6h-starved	5.26 ± 0.32		8.40 ± 0.92		9.45 ± 0.37*	
	24h-starved	2.81 ± 0.14***		16.16 ± 1.64**		7.45 ± 0.36***	
Thyroidectomized (0)	Fed	0.23 ± 0.36	< 0.001	0.	NS	4.64 ± 0.68	< 0.001
	3h-starved	0.36 ± 0.11	< 0.001	1.72 ± 0.40	< 0.001	3.82 ± 0.30	< 0.001
	6h-starved	0.44 ± 0.13	< 0.001	2.66 ± 0.84	< 0.01	3.35 ± 0.36	< 0.001
	24h-starved	0.53 ± 0.10	< 0.001	8.37 ± 1.43**	< 0.05	2.79 ± 0.23*	< 0.001
Thyroidectomized (0.1)	Fed	0.68 ± 0.28	< 0.001	0.	N.S.	6.50 ± 0.63	< 0.001
	3h-starved	0.97 ± 0.54	< 0.01	3.35 ± 0.71*	< 0.05	6.91 ± 0.32	< 0.001
	6h-starved	0.84 ± 0.29	< 0.001	3.50 ± 0.92*	< 0.01	5.10 ± 0.41	< 0.001
	24h-starved	1.14 ± 0.01	< 0.001	10.00 ± 2.00**	< 0.05	4.44 ± 0.18*	< 0.001
Thyroidectomized (1.8)	Fed	4.46 ± 0.28	N.S.	0.	N.S.	10.31 ± 0.41	N.S.
	3h-starved	4.69 ± 0.71	N.S.	3.85 ± 1.40**	N.S.	9.71 ± 0.56	N.S.
	6h-starved	4.96 ± 0.33	N.S.	4.40 ± 0.92	< 0.05	8.99 ± 0.37*	N.S.
	24h-starved	2.99 ± 0.65*	N.S.	15.00 ± 2.27*	N.S.	6.64 ± 0.49***	N.S.
Thyroidectomized (25)	Fed	9.55 ± 0.71	< 0.001	0.	N.S.	11.94 ± 0.77	N.S.
	3h-starved	10.80 ± 1.23	< 0.01	4.80 ± 0.51	N.S.	11.46 ± 0.56	N.S.
	6h-starved	10.03 ± 1.61	< 0.01	7.33 ± 0.84*	N.S.	9.74 ± 0.80	N.S.
	24h-starved	8.08 ± 1.07	< 0.001	22.83 ± 2.4***	< 0.05	9.46 ± 0.42*	< 0.01

ectomized rats treated with 0 or 0.1 μg of thyroxine than in the controls both fed and starved. Thyroidectomized rats treated with 1.8 μg of thyroxine showed the same liver weights than those of intact controls (Table 1), while the thyroidectomized rats treated with 25 μg of thyroxine had liver weights greater than those of the controls.

Liver composition in rats of different thyroidal situations after starvation. The results are summarized in Table 2. DNA-phosphorus was measured as an index of cellularity. The concentration of DNA-phosphorus per whole liver was significantly reduced in thyroidectomized rats as compared with the intact controls. The treatment with 0.1 μg of thyroxine partially restaured this parameter. Neither the DNA-phosphorus content per whole liver in thyroidectomized rats treated with 1.8 μg of thyroxine, nor those treated with 25 μg differed from that in the controls. The content of DNA-phosphorus in the whole liver did not change in any group with starvation, as had been found in other experiments (*Herrera and Freinkel* 1968; *Herrera* et al. 1969). The amount of proteins in the whole liver was significantly reduced in the thyroidectomized rats versus the intact controls (Table 2). This difference was maintained at all lengths of starvation studied. The treatment of thyroidectomized rats with 0.1 μg of thyroxine reduced the differences with the controls. In thyroidectomized rats treated with either 1.8 or 25 μg of thyroxine, the amount of proteins per whole liver was the same as in the intact controls. Starvation produces a no significant decrease in the amount of protein in the liver of all the animals. In the thyroidectomized rats, however, after 24 h of starvation, the decrease in this parameter is significant when related to the values observed in fed animals. The amount of phospholipid-phosphorus per whole liver was lower in the thyroidectomized rats treated with either 0 or 0.1 μg of thyroxine than in the intact controls, while the thyroidectomized rats treated with either 1.8 or 25 μg of thyroxine showed equal phospholipid-phosphorus concentrations than those of intact controls. 24 h of starvation did not produce a significant change in the concentration of phospholipid-phosphorus in the intact controls and in thyroidectomized rats treated with either 1.8 or 25 μg of thyroxine. However, in the thyroidectomized animals there was a significant decrease in the amount of phospholipid-phosphorus after both 6 and 24 h of starvation and in the thyroidectomized rats treated with 0.1 μg of thyroxine after 24 h of starvation (Table 2). The amount of total fatty acids in whole liver was low in the thyroidectomized rats treated with either 0 or 0.1 μg of thyroxine, while it was like that of the controls in the rats treated with either 1.8 or 25 μg of thyroxine. Three hours of starvation were enough to produce an intense and significant decrease in the concentration of total fatty acids in the liver of the thyroidectomized rats. This effect was smaller at 6 h of starvation than at 3 h and disappeared completely at 24 h. Thyroidectomized rats

Table 2 *Effect of starvation on liver composition in thyroidectomized rats treated with L-thyroxine.* Rats were thyroidectomized after weaning and injected intraperitoneally with different dosis of L-thyroxine during 40 days. They were killed by decapitation. The results are given as means ± S.E.M. of 5 animals/group. P values refer to the differences between each group and the intact controls (N.S., not significant, i.e. $p > 0.05$). The statistical comparison of the starved groups versus the fed ones are shown by asterisks (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Other details are given in the Materials and Methods section.

Group (μg of L-thyroxine/100 g body wt.)	Dietary status	DNA P whole liver (μg)	p'	Proteins whole liver (mg)	p'	Phospholipid P whole liver (mg)	p'	Fatty acids whole liver (μm)	p'	Glycogen (%)	p'
Intact controls (0)	Fed	1697±110		1463±69		13.57±1.04		1171±81		4.68±0.25	
	3h-starved	1871±174		1374±32		13.02±1.02		839±67		3.77±0.20*	
	6h-starved	1782±182		1126±91		11.86±0.86		907±52		2.59±0.18***	
	24h-starved	1932±184		1289±85		10.63±0.89		833±95		0.32±0.09***	
Thyroidectomized (0)	Fed	894±125	<0.001	939±82	<0.01	7.41±0.48	<0.001	782±130	<0.05	5.06±0.53	N.S.
	3h-starved	971±113	<0.001	688±98	<0.01	6.26±0.83	<0.001	306±43**	<0.001	2.84±0.21**	<0.01
	6h-starved	899±134	<0.001	622±86	<0.05	5.20±0.40**	<0.001	367±38*	<0.001	1.76±0.46**	N.S.
	24h-starved	792±141	<0.01	519±44**	<0.01	3.77±0.26***	<0.001	609±123	N.S.	0.23±0.08***	N.S.
Thyroidectomized (0.1)	Fed	1138±187	N.S.	1029±236	N.S.	8.24±0.98	<0.01	592±138	<0.01	5.47±0.59	N.S.
	3h-starved	1039±112	N.S.	1021±59	<0.01	9.07±0.31	<0.01	776±48	N.S.	3.90±0.54	N.S.
	6h-starved	1006±112	N.S.	771±110	N.S.	7.84±0.61	<0.01	565±83	<0.01	1.81±0.34***	N.S.
	24h-starved	1168±169	N.S.	624±76	<0.01	5.84±0.59*	<0.01	638±104	N.S.	0.16±0.06***	N.S.
Thyroidectomized (1.8)	Fed	1539±168	N.S.	1473±178	N.S.	13.85±1.61	N.S.	1190±122	N.S.	5.15±0.56	N.S.
	3h-starved	1609±105	N.S.	1350±162	N.S.	12.67±0.91	N.S.	1055±183	N.S.	3.30±0.52*	N.S.
	6h-starved	1509±70	N.S.	1176±139	N.S.	11.58±0.38	N.S.	875±17	N.S.	2.49±0.34***	N.S.
	24h-starved	1346±206	N.S.	941±179	N.S.	10.02±1.33	N.S.	754±142	N.S.	0.43±0.05***	N.S.
Thyroidectomized (25)	Fed	1704±156	N.S.	1575±157	N.S.	13.74±1.13	N.S.	1095±141	N.S.	1.31±0.16	<0.001
	3h-starved	1682±118	N.S.	1468±143	N.S.	14.03±1.87	N.S.	1206±142	<0.05	1.17±0.31	<0.001
	6h-starved	1799±237	N.S.	1332±153	N.S.	10.34±0.74*	N.S.	1039±190	N.S.	0.57±0.07*	<0.001
	24h-starved	1545±216	N.S.	1242±187	N.S.	11.38±1.10	N.S.	824±100	N.S.	0.11±0.03***	<0.05

treated with 0.1 μg of thyroxine also showed a lower total fatty acids concentration in liver than the intact controls but, as it happened in the other groups, starvation does not produce any significant change in this parameter. The percentage of liver glycogen was the same in thyroidectomized rats treated with 0, 0.1 or 1.8 μg of thyroxine and in intact controls when fed, while it was significantly lowered in the liver of the thyroidectomized animals treated with 25 μg of thyroxine (Table 2). 3 h of starvation produced a fall in the concentration of liver glycogen in all the groups, the effect being maximal in the thyroidectomized rats not treated with thyroxine, that showed values significantly lower than those of the controls, and minimal in the thyroidectomized rats treated with 25 μg of thyroxine, where this effect was not significant. A longer starvation period produced a progressive fall in liver glycogen in all the groups, being the thyroidectomized rats that received 25 μg of thyroxine the group that showed the lower values after 24 h of starvation.

Changes of liver acetyl-CoA and citrate with short periods of starvation in thyroidectomized rats treated with thyroxine.

As observed previously (*Aranda* et al. 1972), acetyl-CoA concentration in the liver of all the animals under different thyroidal status was the same in the fed condition (Table 3). 3 h of starvation produced a significant increase in the concentration of acetyl-CoA in the liver of the thyroidectomized rats not treated with thyroxine, while it did not change in the other groups. In all the animals studied, the concentration of acetyl-CoA in liver was higher after 6 h and 24 h of starvation than in the fed condition, and the values did not differ significantly among the groups. The concentration of liver citrate did not differ significantly between the thyroidectomized rats treated with either 0, 0.1, 1.8 or 25 μg of thyroxine and the controls. 3 h of starvation did not affect these relationships but 6 h of starvation produced an increase in the liver concentration of citrate in the intact controls and the thyroidectomized rats treated with 1.8 and 25 μg of thyroxine, while it did not change in those treated with 0 or 0.1 μg of thyroxine, making the difference of these groups with the values of the controls statistically significant. After 24 h of starvation it appeared a subsequent decrease in the citrate concentration in the liver from the intact controls and the thyroidectomized rats treated with either 1.8 or 25 μg of thyroxine, while there was a slight rise in those treated with 0 or 0.1 μg of thyroxine, supressing the difference observed after 6 h of starvation between these groups and the controls. At

Table 4 *Effect of starvation on blood components in thyroidectomized rats treated with L-thyroxine.* Rats were thyroidectomized after weaning and injected intraperitoneally with different dosis of L-thyroxine during 40 days. They were killed by decapitation. The results are given as means ± S.E.M. of 5 animals/group. P values refer to the differences between each group and the intact controls (N.S., not significant, i.e. $p > 0.05$). The statistical comparison of the starved groups versus the fed ones are shown by asterisks (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Other details are given in the Materials and Methods section.

Groups (μg of L-thyroxine/100 g body weight	Dietary status	Blood glucose (mg/100 ml)	p'	Plasma glycerol (μm/l)	p'	Plasma free-fatty acids (μm/ml)	p'	Blood ketone bodies (μm/l)	p'
Intact Controls (0)	Fed	126.75±2.92		68.90±6.35		0.14±0.03		291.7±43.2	
	3h-starved	119.41±2.62		71.49±5.88		0.25±0.04		265.0±63.4	
	6h-starved	111.87±7.38		118.38±22.43*		0.50±0.08**		426.3±90.0	
	24h-starved	89.92±4.52***		149.75±12.31***		0.61±0.06**		886.6±55.2***	
Thyroidectomized (0)	Fed	117.48±6.61	N.S.	75.72±8.94	N.S.	0.19±0.03	N.S.	273.2±18.6	N.S.
	3h-starved	92.53±7.50*	<0.01	105.26±12.46*	<0.01	0.30±0.05	N.S.	268.8±59.7	N.S.
	6h-starved	96.60±3.62	N.S.			0.28±0.06	N.S.	465.0±43.3**	N.S.
	24h-starved	87.88±3.46**	N.S.	191.89±10.03***	N.S.	0.65±0.09**	N.S.	907.1±53.9***	N.S.
Thyroidectomized (0.1)	Fed	106.15±5.28	<0.01	83.62±12.68	N.S.	0.15±0.03	N.S.	228.4±69.6	N.S.
	3h-starved	111.67±7.05	N.S.	76.72±6.96	N.S.	0.24±0.03	N.S.	306.9±55.1	N.S.
	6h-starved	92.03±4.87	N.S.	104.17±10.96	N.S.	0.38±0.06*	N.S.	347.5±42.4	N.S.
	24h-starved	85.07±3.42**	N.S.	189.88±26.42*	N.S.	0.68±0.09**	N.S.	1346.1±132.5***	<0.01
Thyroidectomized (1.8)	Fed	116.00±5.25	N.S.	69.68±7.62	N.S.	0.12±0.01	N.S.	229.2±28.2	N.S.
	3h-starved	109.91±6.82	N.S.	68.85±7.81	N.S.	0.25±0.03**	N.S.	296.9±27.6	N.S.
	6h-starved	101.66±5.23	N.S.	119.44±25.02	N.S.	0.49±0.06**	N.S.	294.7±27.0	N.S.
	24h-starved	87.51±6.18*	N.S.	146.59±20.41**	N.S.	0.69±0.10**	N.S.	1263.5±83.9***	<0.01
Thyroidectomized (25)	Fed	138.11±16.28	N.S.	97.53±17.61	N.S.	0.17±0.03	N.S.	445.7±71.7	N.S.
	3h-starved	125.87±6.73	N.S.	74.74±5.64	N.S.	0.21±0.01	N.S.	459.6±89.2	N.S.
	6h-starved	113.52±6.37	N.S.	166.55±11.33*	N.S.	0.97±0.07**	< 0.01	559.8±17.3	N.S.
	24h-starved	86.46±12.65*	N.S.	175.95±8.98***	N.S.	0.69±0.10**	N.S.	1303.9±163.4***	<0.05

vation produces a decrease in the liver content of glycogen, protein and phospholipid-P, but in the present study it has been shown that these changes occur with shorter periods of starvation in the thyroidectomized animals than in the intact controls. The content of liver total fatty acids decreased dramatically after 3 h of starvation in the thyroidectomized rats; as this change is not paralleled by a similar change in the liver content of phospholipids, it can be concluded that that reduction is due mainly to a fall in neutral lipids fatty acids content. At this time of starvation, liver acetyl-CoA and citrate and plasma glycerol concentrations are increased in the thyroidectomized rats. All these data allow us to suggest that with short periods of starvation the thyroidectomized animals shift to the utilization of lipids earlier than the intact controls.

The rapid fall in blood glucose and liver glycogen could be one of the main factors responsible for the rapid call for lipid utilization in the starved thyroidectomized animals, as it has been suggested by several groups of investigation that decreased quantities of available carbohydrate in the liver is an important factor contributing to an increase in the rate of fatty acids hepatic catabolism in insulin deficiency (*Mayes and Felts* 1967; *McGarry and Foster* 1971; *Topping and Mayes* 1972; *Wieland* 1968; *Wieland and Matchinsky* 1962; *Krebs* 1966; *Woodside and Heimberg* 1976). Actually, we have previously observed that thyroidectomized rats also are insulin deficient animals (*Aranda* et al. 1972).

The rapid decrease of carbohydrate storages in the thyroidectomized rats should be due to their difficulties in increasing the synthesis of glucose more than to a higher utilization of this substrate as it is well established that glucose consumption is diminished in both fed and fasted hypothyroid rats (*Scow and Cornfield* 1954; *Halmi* et al. 1959; *Elrick* et al. 1961; *Lamberg* 1965; *Andreani* et al. 1968; *Andreani* et al. 1970; *Edwards* et al. 1971; *Katsilambros* et al. 1972; *Renauld* et al. 1974; *Seino* et al. 1975), while gluconeogenesis is either normal or diminished (*Bargoni* et al. 1966; *Menahan and Wieland* 1969; *Bottger* et al. 1970; *Castro and Herrera* 1973). Actually, we have recently observed that *in vivo* gluconeogenesis does not increase with 3 h of starvation in the thyroidectomized rats, while it increases in the intact controls (*Llobera* 1977). Thus, the slower gluconeogenetic response to fasting in the thyroidectomized animals could be the responsible for the rapid decrease in the availability of carbohydrates which forces the early shift to the utilization of lipids.

Effect of small doses of thyroxine on the response to starvation in thyroidectomized rats. The daily administration of 0.1 μg of thyroxine to thyroidectomized rats keep them in a hypothyroid state as indicated by their low levels of plasma protein-bound iodine.

These small doses of thyroxine are however enough to normalize most of the metabolic changes observed in the thyoridectomized rats and to partially restore the growth rate of the animals. This, in the presence of the difficulties in observing *in vitro* effects of the thyroid hormones on metabolic parameters results in the need to recognize that an important proportion of the metabolic changes found in hypothyroidism are not directly due to the decrease in the levels of thyroid hormones themselves but to other endocrine alterations that follow the states of intense difficiency of these hormones. The metabolic adaptation to starvation and the growth rate of the animals are completely normalized when the thyroidectomized rats are treated daily with 1.8 μg of thyroxine. This treatment allows the maintenance of plasma protein-bound iodine concentrations normal in the thyroidectomized animals; thus, it can be suggested as an ideal experiment control in metabolic studies with thyroidectomized rats.

Response to starvation in hyperthyroid rats. Thyroidectomized rats treated with 25 μg of thyroxine are in an intense catabolically oriented situation when fed, as shown by their decreased body weight in the presence of normal body size. The catabolic situation is most pronounced in the mass of extrahepatic tissues, as liver weight is even augmented. These changes are mainly localized in the peripheral fat stores, of which these animals are practically depleted. Thus, despite the well known hyperactive adipose tissue metabolism in hyperthyroidism (*Goodman and Bray* 1966; *Fisher and Ball* 1967; *Zederman* et al. 1972; *Nikkila and Kekki* 1972; *Montoya and Herrera* 1974), the concentration of plasma free fatty acids, glycerol ad ketone bodies do not differ from that in the intact controls, probably due to a reduced amount of substrates to be mobilized. Although it would be expected a rapid response to fasting in these animals due to their adaptation to the net catabolic state, very probably for the depletion of fat stores the changes in same parameters occur even more slowly than in the normal controls. Actually, contrary to what happend in the controls and to what was observed in other conditions (*Herrera and Freinkel* 1968), 6 h of starvation are not enough to produce a significant increase in the steady state concentration of liver acetyl-CoA in the hyperthyroid rats. After 24 h of starvation, the liver concentration of citrate and the blood ketone-bodies levels are higher in the thyroidectomized rats treated with 25 μg of thyroxine than in the intact controls in despite of the normal concentration of acetyl-CoA in liver. This allows to suggest that the intrahepatic lipid breakdown is maximally enhanced in these animals by starvation when the liver glycogen reserves are completely exhausted. We have previously observed that this dramatic situation in the thyroidectomized rats treated with 25 μg of thyroxine is also maintained after 48 h of starvation (*Aranda* et al. 1972), producing a generalized weakness of metabolic origin that causes them to be of very low resistance to starvation, as observed by the higher death incidence in these animals during food deprivation.

It is known that starvation has major effects on the convertion of T_4 to T_3 (*Spaulding* et al. 1976; *Pamblab* et al. 1977) as well as in the level of other hormones not directly related with the thyroid. Thus, it can not be excluded the possibility that several of the metabolic changes observed in the different groups have been influenced by these endocrine changes that appear in the fasting state, which very probably are different depending on the thyroidal status of the animals.

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