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Effects of Hypo and Hyperthyroidism on the Response to Glucose Loading of Blood Glucose, Ketones and Insulin and Liver Glycogen as Studied in the Fasted Rat *

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After receiving an i.p. glucose load, 24 h fasted thyroidectomized rats showed a progressive increase in blood glucose and a slow decrease in blood ketone bodies. Both liver glycogen and plasma insulin levels showed no differences within 60 min of the glucose administration. It is suggested that the glucose intolerance in these animals is partly due to an insulin deficiency. Thyroidectomized rats treated daily with 25 μg of L-thyroxine/100 g body weight for 40 days responded to the glucose test with a supranormal and more persistent elevation of blood glucose but with a faster and a greater fall in blood ketone bodies, as compared to controls. Sixty min after the glucose loading, liver glucogen levels were lower and plasma insulin were slightly higher than controls. It is suggested that a diminished extraction of glucose during transhepatic passage can be responsible for the impaired glucose tolerance observed in the hyperthyroid animals.

Blood glucose levels in hypothyroidism are normal or slightly decreased (2, 3, 9, 17, 21, 22, 32, 34). However, the utilization of glucose appears to be reduced (9, 10, 17, 21, 22, 32-34). There are, however, conflicting data on this subject, specially those involving the determination of plasma insulin (1, 7, 8, 25, 31). Moreover, the type of relationship between diabetes and hypothyroidism is not well established, as reviewed by METZGER and FREINKEL (26). On the contrary, considerable clinical and experimental evidence indicates that hyperthyroidism, is diabeto-

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genic, although the mechanisms involved have not been fully understood [reviews by FREINKEL and METZGER (14) and by HOCH (18)] and the results obtained from glucose tolerance tests are also often conflicting. A comparison of the conclusions derived from all these studies is rather meaningless, because of the heterogeneity of the experimental situations involved eventually complicated by species differences.

Continuing our previous work on the variations of several parameters of intermediary metabolism resulting from changes in the thyroid status of the rat (2-4, 29), in the present study we have measured the response to glucose administration in hypo- and hyperthyroid thyroidectomized rats.

Materials and Methods

After weaning, male Wistar rats were fed a medium residue, low-iodine diet (11) and surgically thyroidectomized. During a period of 40 days, a group of animals were daily injected (i.p.) with 25 μg of L-thyroxine/100 g body weight and an other group with saline. The animals were compared with age and sex matched intact controls maintained under the same diet, supplemented with 1.7 μg of KIO_3/g of food for the same period of time and injected daily with saline. All the animals drank distilled water. After 24 h of fasting, several animals from each group were sacrificed by decapitation. Blood was collected from the neck into dry heparinized beakers for insulin evaluation in plasma by radioimmunoassay (16), and a piece of liver was immediately frozen in liquid nitrogen for glycogen extraction (15) and quantification (2). The other animals were injected i.p. with 0.2 g of glucose/100 g body weight. Blood was collected from the tip of the tail into heparinized dried porcelain plates just before the injection and 15 and 30 min thereafter. At 60 min the rats were sacrificed and blood collected from the neck as described above. After deproteinization (36), the supernatants of the blood samples were used for glucose, acetoacetate and β-hydroxybutyrate determination by enzymatic procedures (19, 38).

Results

The situation of excess or deficiency in thyroid hormones of the animals was assessed by the changes in their body weight (table I). Although the body weight of the three groups of rats at the time of the thyroidectomy was practically the same, at the time of sacrifice the thyroidectomized rats treated with saline

Table I. *Effect of thyroidectomy and chronic treatment with L-thyroxine on body weight and length in the rat.*

Mean ± S.E.M. n = 10-15 rats/group. Body size corresponds to the length from the snout to the beginning of the tail. p = statistical comparisons versus controls (N.S., not significant; i.e. $p > 0.05$).

	Before operation	40 days after operation	
	Body weight (g)	Body weight (g)	Body lenght (cm)
Intact controls	56.3 ± 2.2	208.0 ± 5.9	19.4 ± 0.2
Thyroidectomized	54.9 ± 1.5	114.9 ± 7.1	15.9 ± 0.6
p	N.S.	< 0.001	< 0.01
Thyroidectomized + 25 μg L-T_4/100 g b.w./day	58.6 ± 2.6	184.0 ± 4.1	19.6 ± 0.3
p	N.S.	< 0.05	N.S.

(T + 0) weighed almost half as much as the controls, and their body length was also significantly reduced (table I). The thyroidectomized rats injected daily with 25 μg of L-thyroxine (T + 25) show a significantly decreased body weigth with regard to the controls. However, this was due to a reduction in the lean mass rather than to a diminished growth: the body length was the same in both groups (ta-

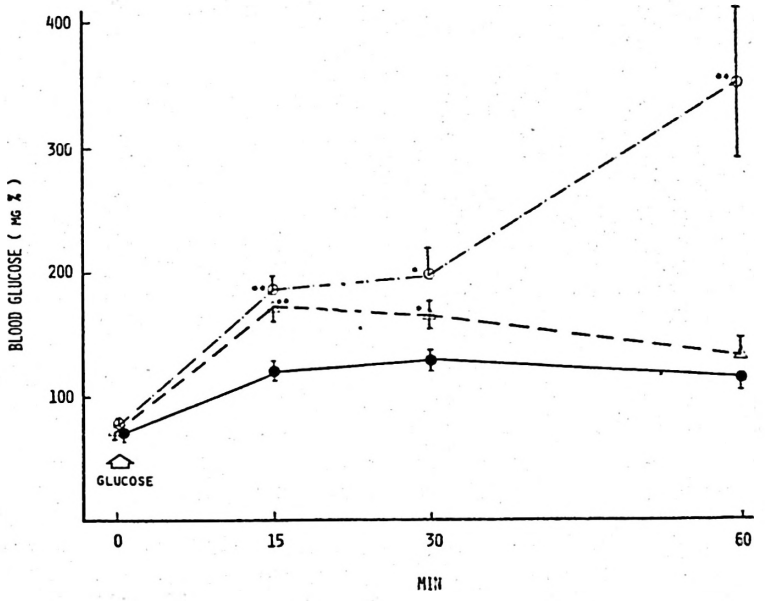

Fig. 1. *Blood glucose levels after i.p. injection of a glucose overload (0.2 g/100 g body weight) in 24 h fasted thyroidectomized rats daily injected with saline (T + 0)* O—·—·—O *or 25 μg of L-thyroxine/100 g body weight (T + 25)* △———△, *for 40 days, and intact controls* ●—●.
Values are means ± S.E.M. of 8-9 rats/group. Statistical comparisons versus controls are shown by asterisks: * $p < 0.05$, ** $p < 0.01$.

Table II. *Effect of thyroidectomy and chronic treatment with L-thyroxine on blood ketone bodies in 24 h fasted rats after i.p. administration of 0.2 g glucose/100 g body weight.* Mean ± S.E.M. n = 6-9 rats/group. p = statistical comparisons versus 0 time p′ = statistical comparisons versus controls. N.S. = not significant; i.e. $p > 0.05$.

Time after glucose administration (min)	Acetoacetate (mg/l) 0	15	30	60	β-hydroxy-butyrate (mg/l) 0	15	30	60
Intact controls	43.2± 3.9	21.9±3.9	13.8±2.9	31.8±5.6	120± 9	60± 7	31±5	42±5
p		<0.01	<0.001	N.S.		<0.001	<0.001	<0.001
Thyroidectomized	33.9± 4.7	25.5±5.9	16.1±3.9	21.9±3.6	73± 7	53±10	36±9	33±6
p		N.S.	<0.05	N.S.		N.S.	<0.01	<0.001
p′	N.S.	N.S.	N.S.	N.S.	<0.01	N.S.	N.S.	N.S.
Thyroidectomized + 25 μg L-T_4/100 g/day	81.4±19.1	25.4±7.0	7.7±3.0	26.7±4.2	170±27	79±21	32±7	48±8
p		<0.05	<0.01	<0.05		<0.05	<0.001	<0.001
p′	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

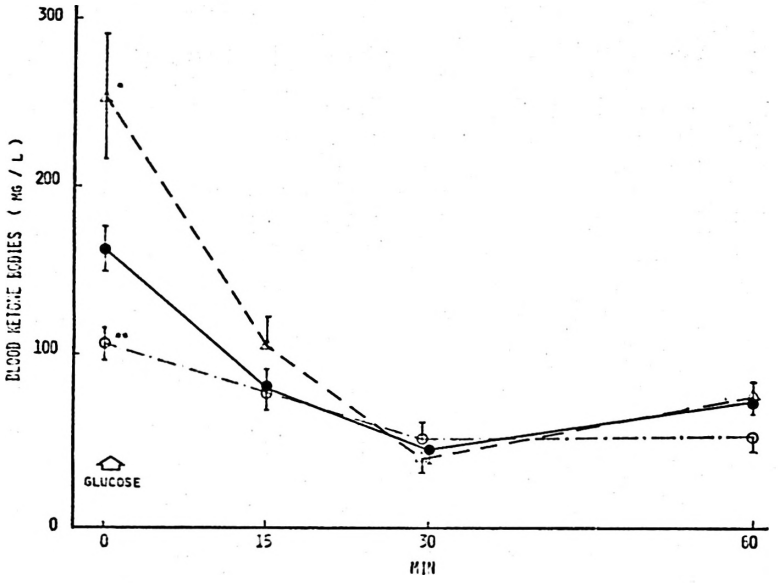

Fig. 2. *Blood total ketone bodies (β-hydroxy-butyrate + acetoacetate) levels after i.p. injection of a glucose overload (0.2 g/100 g body weight) in 24 h fasted thyroidectomized rats daily injected with saline (T + 0)* O—·—·—O *or 25 μg of L-thyroxine/100 g body weight (T + 25)* Δ——Δ *for 40 days, and intact controls* ●—●.
Values are means ± S.E.M. of 8-9 rats/group. Statistical comparisons versus controls are shown by asterisks: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

ble I). Blood glucose levels were the same for all groups studied just before onset of the test, and after a 24 h fast (fig. 1). In the T + 0 group there is a significantly impaired glucose tolerance as shown from the data at 15, 30 and 60 min after the loading. In the T + 25 rats this impairment is observed at 15 and 30 min, but no longer at 60 min. Blood acetoacetate and β-hydroxybutyrate levels in these animals, and the combined values of acetoacetate + β-hydroxybutyrate levels (total ketone bodies) are summarized in table II and figure 2 respectively. Before the glucose loading (time 0), the total ketone bodies levels (acetoacetate + β-hydroxybutyrate) were higher in T + 25 and lower in T + 0 than in the controls, these differences being statistically significant ($p < 0.05$). After the glucose load there is a fall in blood ketone bodies in the three groups studied, which was steeper in T + 25 and more flattened in T + 0, than in controls. These differences disappeared grossly from the 15 min onwards. From 30 to 60 min blood ketones started to increase towards normal values in the T + 25 animals and the controls, but no change was observed in the T + 0 ones (fig. 2). These changes in the blood ketone bodies are reflected more closely by the changes in blood β-hydroxybutyrate than by those in acetoacetate (table II). Liver glycogen concentrations before the administration of glucose were normal in the T + 0 group and decreased in the T + 25 one as related with the values observed in the controls (fig. 3). When the rats were sacrificed 60 min after glucose loading, liver glycogen increased significantly in the controls but did not change in the T + 0 and the T + 25

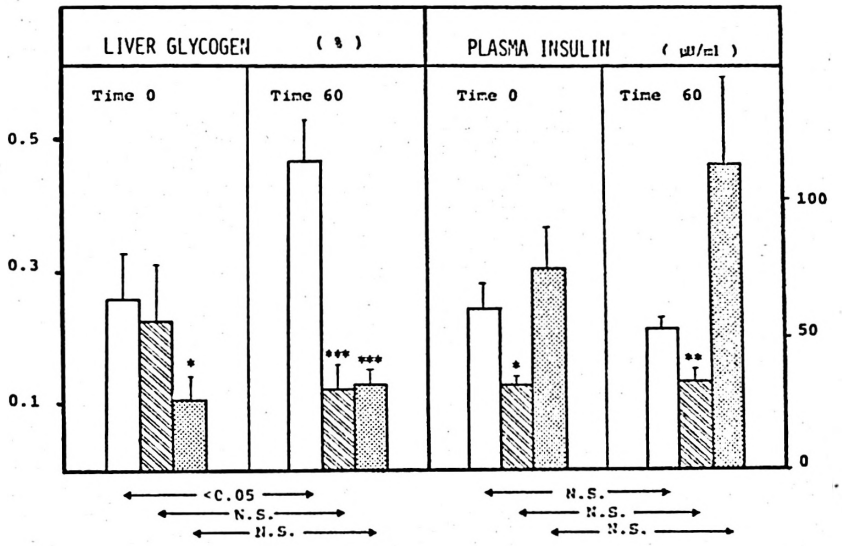

Fig. 3. *Effect of thyroidectomy (T) and chronic treatment with L-thyroxine on liver glycogen and plasma insulin levels in 24 h fasted rats before and 60 min after I.p. administration of a glucose overload (0.2 g/100 g body weight).*
Means ± S.E.M. n = 5-9 rats/group. ☐ controls; ▨ T + 0; ▩ T + 25. p vs. controls; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

animals, their differences with the controls being statistically significant (fig. 3). At this time, plasma insulin levels were significantly lower in the T + 0 treated animals than in the controls, while mean values were higher in the T + 25 ones, although not significantly.

Discussion

The present study shows that both hypo- and hyperthyroid animals have an impaired glucose tolerance; this agrees with the results of other authors (9, 17, 20, 21, 32-34). However, the mechanism underlying these diabetogenic responses seems to be quite different in each situation. In hypothyroid rats blood glucose levels keep rising up to 60 min after injecting the glucose load. This fact seems to be the result of an intense inability to metabolize glucose, as suggestted by the maintenance of low liver glycogen concentrations. It would also be suggested by the slower decrease in the blood ketone body levels in response to a glucose load observed in these animals: indeed as reported by FOSTER (13), insulin- induced hypoglycemia in the fasted rat and the decrease in blood ketones are concomitant, suggesting that both glucose and ketone body utilization are normally interrelated. The abnormal glucose tolerance in the hypothyroid rats might be due, at least partially, to insulin deficiency, as suggested by the low plasma insulin levels observed here and elsewhere in these animals (2). The low circulating insulin levels agree with the results of MALAISSE *et al.* (23) which reported a decreased *in vitro* secretion of insulin by pancreas obtained from thyroidectomized rats. The reduced growth of these animals represents an intense growth hormone deficiency (30, 35), secondary to the lack of thyroid hormone; this deficiency could

contribute to the decreased response of the pancreas of these animals to the glucose stimulus.

The glucose tolerance curves of the hyperthyroid animals must be interpreted in quite a different way, despite their closer resemblance to the curves of the hypothyroid rats than to those of the control ones during the first 30 min. Although there is some controversy, most authors agree with the existence of an increased glucose utilization rate by the extrahepatic tissues in hyperthyroidism (5, 10, 22, 24, 28), a normal insulin secretion by the pancreas (23, 24) and a normal or augmented blood glucose removal after *in vivo* insulin administration (10, 24). This idea would agree with the higher mean plasma insulin levels observed here and elsewhere (2, 10), and the fast decrease in blood ketone bodies observed in the present work after the administration of glucose. Diminished extraction of glucose during transhepatic passage could contribute to the supranormal and more persistent increase in blood glucose levels found here in the hyperthyroid rats. We have indeed seen in the present study that liver glycogen reaccumulation is less than normal when the fast is terminated by the i.p. glucose administration, a fact that was already observed as early as 1933 by COGGESHALL and GREENE (6). An augmented gluconeogenesis, supported by the heightened availability of glycerol, coming from the activated lipolysis (13, 37) and of amino acids, from activated protein catabolism (27) in hyperthyroidism could also contribute to the diabetogenic picture observed in the hyperthyroid rat but its contribution to the response to glucose loading in the fasted state remains yet to be established.

Resumen

Tras administrarles una inyección i.p. de glucosa, las ratas tiroidectomizadas sometidas a un ayuno de 24 horas, presentan un incremento progresivo en la glucemia, así como un leve descenso en los niveles de cuerpos cetónicos en sangre. No se observan variaciones en los niveles de glucógeno hepático, ni en los de insulina plasmática, durante los 60 minutos siguientes a la administración de la sobrecarga de glucosa. Estos resultados parecen sugerir que la intolerancia de estos animales a la glucosa se deba en parte a una deficiencia de insulina. Las ratas tiroidectomizadas tratadas durante 40 días con 25 μg de L-tiroxina/100 g de peso corporal y día, respondieron a la prueba de sobrecarga de glucosa con un incremento de la glucemia superior y más persistente que el de los controles, pero provocando un descenso en la cetonemia más rápido y acusado. A los 60 minutos de la administración de glucosa los niveles de glucógeno hepático observados en estos animales fueron menores que los de los controles y su insulinemia ligeramente aumentada. Estos datos sugieren que la alteración de la tolerancia a la glucosa encontrada en los animales hipertiroideos puede deberse a su menor extracción durante su paso por el hígado.

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