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Effect of Thyroidectomy on Circulating Components and Liver Metabolism in Fed and Fasted Rats

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Abstract. To study intermediary metabolism in hypothyroidism, thyroidectomized rats were compared with thyroidectomized rats daily injected with 1.5 μg of thyroxine and with intact controls. When fed, thyroidectomized animals show decreased plasma protein-bound iodine (PBI) and immunoreactive insulin (IRI) levels and body and liver weights, normal blood glucose and ketone bodies, liver DNA-P concentration and *in vitro* liver (^{14}C) glucose synthesis from (U-^{14}C) alanine, and elevated liver glycogen concentration and *in vitro* (U-^{14}C) alanine and (1-^{14}C) glucose uptakes and (^{14}C) lactate from both substrates. After 48 h of starvation, PBI and IRI remain lower in thyroidectomized rats than in the other groups, normal blood glucose and glycogen concentration fall more in this than in the other groups while the rise in ketone bodies does not differ among the groups. The uptake of (U-^{14}C) alanine by the liver slices remains elevated in fasted thyroidectomized rats while the other parameters, studied on *in vitro* liver metabolism, are equal to the other groups. Thus, different from what it was thought, liver metabolic activity is increased in thyroidectomized rats when corrected by their smaller liver and body weights, allowing them to maintain a normal homeostasis of glucose in the fed state although not when food is withheld.

Introduction

In hypothyroidism, carbohydrate metabolism is very much altered but an equilibrium is established between decreased anabolism and diminished catabolism [19] which allows to maintain normal concentrations of liver glycogen and blood glucose [2, 13, 17]. This equilibrium is broken after prolonged fasting where both parameters fall in hypothyroid animals below those in normal controls [2]. These alterations seem to be mainly localized in the liver where the activity of glycolytic and gluconeogenic enzymes is diminished in hypothyroid animals [3, 7, 18, 24]. Actually, the rate of liver gluconeogenesis has been reported to be decreased in these animals [18], but the greater production of urea by their perfused livers [18] and the normal steady-state concentration in the liver of regulatory metabolites for gluconeogenesis in them [2] would point to a different conclusion. To gain a better understanding of these alterations in hypothyroidism, we have investigated in the present work the *in vitro* utilization of alanine and glucose by livers from thyroidectomized rats. For a better characterization of these changes, the study was performed in animals both fed and starved for 48 h.

Materials and Methods

Female Wistar rats, weighing 75 ± 5 g, were fed a low-iodine diet (0.04–0.09 μg of iodine/g) [9], surgically thyroidectomized and injected daily intraperitoneally thereafter with either 0 or 1.5 μg of L-thyroxine/100 g body weight for 45–60 days. They were compared with age-matched intact female 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. Animals were killed by decapitation and without anaesthesia. Blood was collected into heparinized chilled beakers and a piece of liver was rapidly placed in liquid N_2. The rest of the liver was placed in fresh Krebs-Ringer bicarbonate buffer, pH 7.4 [22]. Deproteinized blood [21] was used to analyze glucose [16] and total ketone bodies [5]. Immunoreactive insulin was measured in the plasma by a double-antiboby technique [12] by using a radioactive insulin kit obtained from The Radiochemical Centre, Amersham, Bucks, UK. Rat insulin (kindly given by Novo Industries, Copenhagen, Denmark) was used as standard. Plasma was used for determination of the protein-bound iodine [4].

Portions of the frozen liver were digested with KOH for precipitation of glycogen with ethanol [11]. The purified precipitate was hydrolised (2.5 M H_2SO_4; 2 h; 100 °C) and analyzed enzymatically with glucose oxidase [16]. DNA-P was isolated from the residual pellet after lipid extraction with chloroform-methanol (2:1, v/v) of another portion of frozen liver [20]; inorganic phosphorus was determined [10] after digestion with 72% $HClO_4$.

The fresh liver was used for the preparation of slices and incubation during 90 min in Krebs-Ringer bicarbonate medium, pH 7.4, supplemented with 0.5 μCi/ml of either (U-^{14}C) alanine (10^{-3}M) or (1-^{14}C) glucose (1 mg/ml), as described previously [6, 8]. The incubations were stopped by injecting 0.1 ml of 1 N H_2SO_4 into the medium and after centrifugation, aliquots of the medium were used for the isolation of radioactive metabolites by paper ascending chromatography [6].

Results

Circulating components. After 45–60 days of being thyroidectomized and fed a low-iodine diet, the rats show a great reduction in the plasma protein-

Table I. Effect of thyroidectomy and starvation on blood components in the female rat. p denotes the significance of the differences between mean ± SEM values of thyroidectomized rats and its respective controls. The significance of the differences between the mean for fed animals and animals starved for 48 h is denoted by asteriks: * = $p<0.05$; ** = $p<0.02$; *** = $p<0.01$; **** = $p<0.001$; N.S. = not significant, i.e. $p>0.05$. The number of rats in each group is shown in parentheses

	Fed			Starved for 48 h		
	intact controls	thyroidectomized + 1.5 μg L-T$_4$	thyroidectomized	intact controls	thyroidectomized + 1.5 L-T$_4$	thyroidectomized
Plasma protein-bound iodine, μg/100 ml	5.7±0.3 (13)	4.0±0.5 (13)	0.28±0.02 (11)	4.0±0.4 (11) ***	4.7±0.4 (19) N.S.	0.7±0.1 (9) ****
p		<0.01	<0.001		N.S.	<0.001
Blood glucose mg/100 ml	123±6 (15)	97±5 (10)	116±3 (13)	89±4 (13) ****	78±4 (14) **	70±2 (8) ****
p		<0.01	N.S.		N.S.	<0.01
Plasma insulin μU/ml	47±4 (20)	53±3 (16)	28±2 (11)	22±2 (19) ****	23±2 (21) ****	16±2 (10) ****
p		N.S.	<0.01		N.S.	<0.05
Blood ketone bodies, μM/ml	579±127 (7)	502±77 (10)	419±77 (7)	2414±148 (13) ****	2489±198 (17) ****	1894±248 (9) ****
p		N.S.	N.S.		N.S.	N.S.

bound iodine concentration when compared with thyroidectomized animals daily injected with 1.5 μg of thyroxine/100 g body weight and with intact controls under the same diet (table I). After 48 h of fasting, the relative differences between the groups remained the same as in the fed state (table I). Blood glucose is the same in the thyroidectomized rats as in the intact controls while in the thyroidectomized animals treated with 1.5 μg of thyroxine, it was lower than in the other groups (table I). After 48 h of starvation, blood glucose concentration fell in all the groups but the greatest fall was observed in the thyroidectomized rats, the difference with the controls being statistically significant (table I). Plasma concentration of insulin in the fed state is slightly augmented in the thyroidectomized rats treated with 1.5 μg of thyroxine and, as in similar conditions [2], this could explain the fall in blood glucose in these animals. The concentration of plasma insulin is, however, significantly decreased in the thyroidectomized rats when compared to the other two groups (table I). After 48 h of fasting, the plasma insulin concentration fell in all groups when compared with fed rats, and it remains lower in the thyroidectomized animals than in the controls. Blood total ketone bodies concentration is similar in all the groups with the same dietary status, and starvation made this parameter increase in all the experimental conditions (table I).

Body and liver weights and liver DNA-P and glycogen. Although there were no differences in the weight of the rats of the different groups before thyroidectomy, the growth rate was slowing down in the thyroidectomized rats from the 19th day after the surgery, obtaining, at the time of the sacrifice, values of body weight significantly lower than when treated with 1.5 μg of thyroxine and than in the intact controls (table II). In all groups, there is a significant fall in the body weight with 48 h of starvation, and the decrease in percent does not differ among the groups. Parallel changes are found in the liver weight in such a way that the difference among the groups disappears when the liver weight is expressed per 100 g body weight (table II). This differs from that we have previously found in male animals maintained under similar conditions where liver weight/100 g of body weight is lower in the thyroidectomized rats than in the controls [2] and could be due to the different sex of the animals. Despite these differences in the absolute weight of the livers, the concentration of DNA-P in liver does not differ among the groups when fed (table II). After 48 h of starvation, the concentration of DNA-P rose in all groups (table II) and as on other occasions [2, 14, 15], the total amount of DNA-P in the whole liver was found to be the same in

Table II. Effect of thyroidectomy and starvation on body and liver weights and liver components in the female rat. Statistical comparisons among the groups are as described in table I.

	Fed			Starved for 48 h		
	intact controls	thyroidectomized + 1.5 μg L-T_4	thyroidectomized	intact controls	thyroidectomized + 1.5 μg L-T_4	thyroidectomized
Body weight, g	183 ± 5 (13)	181 ± 5 (19)	99 ± 3 (19)	163 ± 4 (13) *	164 ± 5 (16) *	89 ± 3 (20) *
p		N.S.	<0.001		N.S	<0.001
Liver weight, g	7.4 ± 0.3 (16)	7.1 ± 0.2 (13)	4.0 ± 0.2 (14)	5.2 ± 0.1 (19) ****	5.7 ± 0.2 (18) ****	2.7 ± 0.2 (17) ****
p		N.S.	<0.001		<0.02	<0.001
Liver weight g/100 g body weight	4.12 ± 0.2 (16)	4.30 ± 0.15 (13)	4.10 ± 0.10 (14)	3.33 ± 0.07 (19) ****	3.37 ± 0.06 (18) ****	3.16 ± 0.12 (17) ****
p		N.S.	N.S.		N.S.	N.S.
Liver DNA-P μg/g	232 ± 18 (17)	251 ± 9 (12)	212 ± 8 (4)	318 ± 18 (6) **	324 ± 7 (12) ****	328 ± 18 (8) **
p		N.S.	N.S.		N.S.	N.S.
Liver glycogen, %	4.0 ± 0.5 (15)	2.8 ± 0.9 (18)	6.1 ± 0.8 (9)	0.17 ± 0.04 (16) ****	0.12 ± 0.04 (25) ****	0.10 ± 0.02 (11) ****
p		N.S.	<0.05		N.S.	N.S.

Table III. Effect of thyroidectomy and starvation on the disposal of (U-^{14}C) alanine (10^{-3}M) and (1-^{14}C) glucose (1 mg/ml) *in vitro* by liver slices from female rats. The disposal of labelled substrates to the different metabolites during the 90 min of incubation has been expressed as a function of the total counts initially present within each flask. Statistical comparisons among the groups are as described in table I

	Fed			Starved for 48 h		
	intact controls	thyroidectomized + 1.5 μg L-T_4	thyroidectomized	intact controls	thyroidectomized + 1.5 μg L-T_4	thyroidectomized
Disposal of (U-^{14}C) alanine						
Uptake	35.6±1.9 (8)	34.4±2.8 (6)	45.3±1.4 (7)	22.3±2.9 (9) ****	21.5±1.4 (8) ****	31.2±3.2 (7) *
p		N.S.	<0.01		N.S.	<0.05
(^{14}C) Glucose	3.9±0.8 (7)	4.6±1.1 (6)	4.5±0.6 (7)	5.5±1.1 (11) N.S.	5.7±1.2 (7) N.S.	8.8±1.4 (4) ***
p		N.S.	N.S.		N.S.	N.S.
(^{14}C) Lactate	14.7±2.1 (8)	14.3±2.2 (5)	28.7±1.2 (7)	7.4±0.6 (9) ***	7.7±1.1 (6) **	9.7±1.0 (6) ****
p		N.S.	<0.001		N.S.	N.S.
Disposal of (1-^{14}C) glucose						
Uptake	4.0±0.6 (8)	4.9±0.6 (5)	5.5±0.3 (8)	5.0±0.6 (6) N.S.	3.8±0.2 (7) N.S.	3.4±0.4 (5) ***
p		N.S.	<0.05		N.S.	N.S.
(^{14}C) Lactate	1.51±0.19 (6)	1.47±0.09 (6)	2.1±0.2 (8)	1.37±0.13 (7) N.S.	1.32±0.13 (6) N.S.	1.3±0.2 (6) **
p		N.S.	<0.05		N.S.	N.S.

starved as in fed animals. These data suggest that the number of cells in the liver of the thyroidectomized rats is reduced, although the size of the hepatocyte is the same as in the controls and that fasting does not produce a change in the total number of cells in neither group. The concentration of glycogen is higher in the liver of the fed thyroidectomized rats than in those treated with 1.5 μg of thyroxine and than in the intact controls (table II). This fact is also different from that of male thyroidectomized animals where the liver glycogen concentration is the same as in the intact controls [2]. Fasting produces a fall in the glycogen concentration of the liver from all the groups, the differences disappearing among them (table II) which means that the fall is maximal in the thyroidectomized animals as they started from a higher level when fed.

Alanine and glucose utilization by liver slices. The uptake of (U-^{14}C) alanine by liver slices incubated *in vitro* is higher in the thyroidectomized rats than in their controls (table III). Fasting makes this parameter decrease in all the groups, and the tissues from the thyroidectomized animals remain taking up more ^{14}C-labelled alanine than those from the other two groups. The percentage of initial radioactivity converted to (^{14}C) lactate is also augmented in the thyroidectomized animals when fed although after fasting, this parameter falls more in these animals, the differences disappearing among the groups (table III). The formation of (^{14}C) glucose from (U-^{14}C) alanine is not different in the livers from thyroidectomized rats injected with either 0 or 1.5 μg of thyroxine and in the intact controls in either fed or fasted state, suggesting a preservation of liver gluconeogenesis in the former group, even when food is withheld.

The uptake of (1-^{14}C) glucose and the formation of ^{14}C-labelled lactate from this substrate is also augmented in the liver from fed thyroidectomized rats when compared with those receiving 1.5 μg of thyroxine and with those of intact controls, the difference is disappearing when the animals are under fasting 48 h before the sacrifice (table III).

Discussion

In the present study, we have seen that the growth rate is very much decreased in thyroidectomized animals fed on a low iodine diet, and this is accompanied by a parallel decrease in the liver weight and in the plasmatic immunoreactive insulin concentration. These changes are primarily induced by the decrease in the supply of thyroid hormones to the tissues as thyroidectomized rats fed on the same diet, but daily injected with substitutive doses of exogenous thyroxine do not show such alterations. Despite these changes that demonstrate an intense alteration in the whole endocrine system in the thyroidectomized rats, these animals are able to maintain normal levels of circulating glucose and ketone bodies and even to accumulate in their liver a greater percentage of glycogen than their controls. The liver should be playing a very important role in the maintainance of this balanced equilibrium of the carbohydrate metabolism in the hypothyroid animals. Actually, we have seen here that the hepatic metabolism of these animals is quite active, as shown by the increased uptake of labelled alanine and glucose by liver slices incubated *in vitro* and the increased conversion of these two substrates to ^{14}C-labelled lactate. The conversion of (U-^{14}C) alanine to glucose by liver slices from thyroidectomized animals is the same as that in the intact controls which agrees with the normal steady-state concentration in the liver, from animals under similar conditions, of regulatory metabolites for gluconeogenesis, as previously reported [2]. The low concentration of circulating insulin might contribute to the maintainance of liver gluconeogenesis in the hypothyroid animals. The preferential conversion of alanine and glucose to lactate in the thyroidectomized animals agrees with the elevated lactate/pyruvate ratio found in the liver of hypothyroid rats [1] and would suggest a reduced cytoplasmic potential that would facilitate the reduction of 1,3-diphosphoglycerate for its conversion to glucose [23]. It might be inferred that the whole liver gluconeogenic capacity in these rats is lower in the thyroidectomized rats than in the controls due to the smaller livers of the former group, but this difference disappears when it is taken into account of the smaller body weight of these animals.

After 48 h of starvation, the peripheral lipids are mobilized in the thyroidectomized animals at least as much as in the controls, as suggested by previous findings [2] and by the normal rise of blood ketone bodies found here. Despite this lipid availability, the glycaemia is no longer preserved indicating that the utilization of glucose is greater than its synthesis. We have seen, however, that the uptake of (U-^{14}C) alanine is higher, and its conversion to (^{14}C) glucose and (^{14}C) lactate is the same in the fasted thyroidectomized rats than in their controls, and the uptake of (1-^{14}C) glucose and its conversion to (^{14}C) lactate do not differ between both groups. The preservation of liver gluconeogenesis in the fasted thyroidectomized animals would agree with the increased production of urea by the perfused liver of rats under comparable conditions [18] and with the normal liver steady-state concentration of regulatory metabolites for gluconeogenesis in hypothyroid animals [2]. It has been shown, however, that the activity of key gluconeogenic enzymes is reduced in the liver of the fasted hypothyroid animals [3, 7, 18, 24], but they seem to be compensated by a parallel reduction in the activity of some glycolytic enzymes [3, 7, 24].

Although, as pointed out above, the primary defect of the thyroidectomized animals is the reduction in the supply of thyroid hormones to the peripheral tissues, we must emphasize that most of the metabolic changes observed in these animals are probably secondary to the other endocrine alterations that accompany this situation of extreme hypothyroidism. In agreement with this point are our previous results showing that when the thyroid hormone deficiency is not high enough to alter the growth rate and the fed and fasted plasma insulin levels, as indexes of an unimpaired endocrine system, none of the changes here observed in the thyroidectomized animals are present [8].

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