Hormone Res. 5: 129–140 (1974)

Effect of Thyroid Status on Glycerol Metabolism in Adipose Tissue of Fasted Male Rats[1]

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Abstract. We have studied the *in vitro* metabolism of glycerol by epididymal fat pads from thyroidectomized rats daily injected with either 0, 0.1, or 25 μg of thyroxine and intact controls after a previous fast of 48 h. The tissues coming from the thyroidectomized rats receiving 25 μg of thyroxine produce maximal amounts of glycerol while this parameter does not differ among the other groups. The same is true for the formation of $^{14}CO_2$ from 1-^{14}C glycerol while the uptake of this and its conversion to ^{14}C-labelled total lipids is maximal in the thyroidectomized rats no receiving thyroxine, it decreases in those treated with 0.1 μg and it is minimal in those receiving 25 μg and the controls. When proper corrections for the changes in the specific activity of the tracer are carried out it is seen that the rates of lipolysis and those of glycerol uptake and its conversion to lipids are augmented in the thyroidectomized rats receiving 0 or 25 μg of thyroxine, while that of the oxidation of glycerol to CO_2 is only elevated in the second group. The augmented lipolysis in the fasted hypothyroid animals found here is different from studies which omit to take account of the reutilization of glycerol by adipose tissue and can be used to explain the survival of these animals when food is withheld. In some way this could compensate the intense hypoglycemia found in the fasted hypothyroid animals which by itself could be contributing to the adipokinetic effect.

Key Words
Hypothyroidism
Hyperthyroidism
Adipose tissue metabolism
Glycerol utilization
Lipolysis

Introduction

By indirect studies on the effects of thyroid status on liver metabolism and circulating fuels, we have recently suggested that in the fasted state the hypothyroid rats mobilize more fat from peripheral depots than the euthy-

[1] Part of this study was carried out at the Departamento de Endocrinología Experimental, Instituto G. Marañón, CSIC, Madrid.

roid controls [1]. As this suggestion disagrees with the current belief that hypothyroidism is associated with unaltered or reduced adipose tissue lipolysis [2, 8, 12, 21], we decided to study this parameter by *in vitro* direct measurements on epididymal fat-pad from starved thyroidectomized rats (T) treated with different doses of exogenous thyroxine. We felt that the problem deserved special attention as we have shown that glycerol utilization by adipose tissue is considerably higher than previously thought and affects calculations of lipolysis and esterification which fail to take this factor into account [14, 15]. Thus, in the present study we have determined the rates of glycerol production and utilization by the tissues in order to make the proper corrections.

Materials and Methods

Animals. Male Wistar rats since the end of the weaning were fed on a medium residue, low-iodine diet (0.04–0.09 μg of iodine/g) [6] and surgically thyroidectomized. Two days after the thyroidectomy they were injected with 50 μCi of carrier free ^{131}I-sodium iodine to eliminate any remaining thyroid tissue and after two more days they were daily injected intraperitoneally with either 0, 0.1, or 25 μg of L-thyroxine/100 g body weight (T+0, T+0.1, or T+25, repectively) for 50–60 days until the time of killing which was done after a previous fast of 48 h. They were compared with age-matched intact male controls (C) under the same diet supplemented with 1.7 μg of KIO_3/g and injected daily with 0.9% NaCl during the same period. The size of the rats was determined by measuring the length from the snout to the beginning of the tail.

In vitro *incubations.* Animals were killed by decapitation without anesthesia and 35–43 mg pieces of epididymal fat-pad were placed in rubber sealed 20 ml vials each containing 0.5 ml of Krebs-Ringer bicarbonate buffer, pH 7.4 [20] supplemented with bovine albumin (20 mg/ml) purified by the method of CHEN [4]. At zero time 0.5 ml of the same buffer (without albumin) containing 0.5 μCi of (1-^{14}C) glycerol (15.3 mCi/mmol) was pipetted into each of the vials, after which they were rapidly sealed and gassed for 5 min with agitation (100 cpm) at 37 °C. The incubation was stopped by injecting 250 μl of hyamine 10× hydroxide into small polyethylene cups suspended from the cover of the vial and 0.5 ml of 10% (w/v) $HClO_4$ into the medium. The $^{14}CO_2$ evolved was trapped in the hyamine by gentle shaking at room temperature for 60 min. The medium was processed as previously described [14, 15] for the enzymatic determination of glycerol [11] and for the isolation of labelled glycerol. Lipids were extracted from the incubated tissue with chloroform-methanol [9] and the extracts were washed once with saline and twice with 1 M glycerol to remove any labelled glycerol bound to the tissue.

Radioactive assay. Samples were counted as already described [14]. The purity of the (1-^{14}C) glycerol used was determined as previously [14, 15]. It was found that more than 99.6% of the labelled product was in the form of pure glycerol.

Mathematical analysis of the data. Radioactive measurements were expressed as percentage of the total (1-^{14}C) glycerol added to each vessel related to the initial wet weight of the tissue and calculated as μmoles as a function of the specific activity of the appropriate counting standard. Regressions were determined following the standard methods described in the manuals of statistics [19]. Statistical comparisons between two groups of data were performed by the t-test of Student. Rates of lipolysis and glycerol utilization as functions of the production of glycerol and corrected for the renewal of (1-^{14}C) glycerol from the media were calculated as previously described [14]. Calculations were carried out in an Ataio-Compucorp 445 (Statistician) and comprobation of all the regressions was performed in an IBM 7090 computer.

Results

Body Weight and Size

The thyroid status of the different groups of rats was determined by the body weight and the size of the animals (table I). Although before the thyroidectomy there were no differences in the body weight of the rats in the different groups, at the time of killing, both the body weight and the size of T+0 rats were very much lower than that of the C animals, demonstrating the severe hypothyroidism in the former group. T+0.1 rats show a significant restoration of the growth capability, although not attaining the body weight nor the size of C (table I). T+25 rats show a reduction in the body weight that it is not caused by a smaller growth of the animals as their size does not differ from that in C.

Table I. Effect of thyroidectomy (T) and treatment with L-thyroxine on body weight and size in the rat (mean ± SEM)

Group	Body weight, g	Body size, cm
C[1]	238±7 (14)[2]	20.3±0.2 (8)
T+0[3]	88±5 (6)	14.1±0.4 (6)
p[4]	<0.001	<0.001
T+0.1	159±10 (8)	18.4±0.0 (6)
p	<0.001	<0.001
T+25	204±7 (18)	21.0±0.2 (15)
p	<0.01	NS

[1] C = Intact controls.
[2] ( ) = Number of rats/group.
[3] Doses of L-thyroxine (μg/100 g body weight/day).
[4] p = Statstical comparisons versus C (NS, not significant; i.e. p >0.05).

Glycerol Formation by Epididymal Fat-Pad

Pieces of epididymal fat-pad from 48 h fasted rats under the different thyroidal situations were incubated with (1-^{14}C) glycerol. At different times the incubation was stopped by the addition of $HClO_4$, which released the free glycerol of the tissue into the medium, and the amount of glycerol was determined. The results are summarized in table II. The only group which differs from C in the formation of glycerol at all the times studied was T+25, being higher than in the former group. In both T+0 and T+0.1 groups, the formation of glycerol was lower than in C practically all during incubation but due to the dispersion of the data, after 120 min of incubation was the only time when the difference between either group and C is stastistically significant. From the experimental data it is possible to adjust highly significant linear regressions of the formation of glycerol, g (t), as a function of time (t) of the type: g (t) = a+bt. The mean lines have been graphically represented in figure 1a. The slope of the lines obtained with the data from the T+25 animals was significantly higher than that of the C group, while the slope of the values from neither T+0 nor T+0.1 groups differs from that of C. As differences in the formation of glycerol by the tissues might partially be caused by differences in the reutilization of this metabolite, the amount of (1-^{14}C) glycerol taken up by the tissue was estimated.

Table II. Effect of thyroidectomy (T) and treatment with L-thyroxine on the *in vitro* glycerol formation by epididymal fat-pads from rats (μmol/100 mg, mean ± SEM of 6–9 rats/group)

Group	Minutes of incubation					
	0	30	60	90	120	180
C[1]	0.066±0.005	0.056±0.010	0.063±0.009	0.067±0.013	0.104±0.007	0.099±0.006
T+0[2]	0.056±0.012	0.041±0.008	0.039±0.008	0.041±0.008	0.057±0.013	0.097±0.011
p[3]	NS	NS	NS	NS	<0.01	NS
T+0.1	0.050±0.007	0.034±0.005	0.057±0.007	0.068±0.014	0.067±0.007	0.085±0.008
p	NS	NS	NS	NS	<0.05	NS
T+25	0.130±0.014	0.189±0.058	0.220±0.047	0.216±0.047	0.276±0.038	0.308±0.040
p	<0.01	<0.05	<0.01	<0.05	<0.001	<0.001

[1] C = Intact controls.

[2] Doses of L-thyroxine (μg/100 g body weight/day).

[3] p = Statistical comparisons versus C (NS, not significant; i.e. $p > 0.05$).

Utilization of (1-^{14}C) Glycerol

The amount of (1-^{14}C) glycerol taken up by the epididymal fat-pad from the different groups increases with the time of incubation (table III). At all the times of incubation studied the uptake of labelled glycerol is higher in the tissues from both T+0 and T+0.1 rats than from C, while in T+25 there is no difference with the values from the latter group. In all cases the data adjust well to first order regression plots (fig. 1b) but the highest slope is found in the values from the T+0 rats, followed by that from the T+0.1 animals, and in either case this parameter is significantly different when related with that in the C group. On the contrary, the slope of the values from the T+25 group does not differ statistically from that in C.

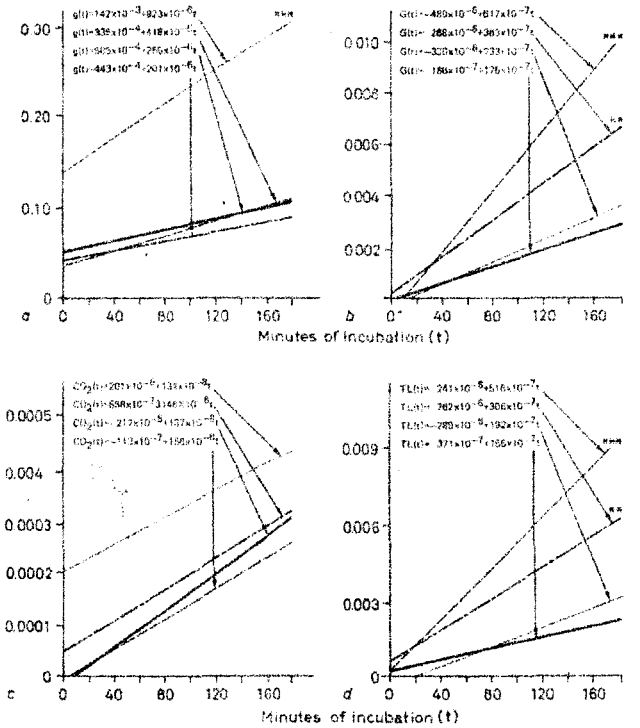

Fig. 1. In vitro handling of glycerol by epididyme fat-pad from rats under different thyroidal status. ——— = Intact controls; – – – = T+0; ——— = T+0.1; - - - = T+25. Significance of the differences between the slope of each line and that of the controls is shown by asterisks. *** = $p<0.001$; ** = $p<0.01$; no asterisk = $p<0.0.5$ *a* Formation of glycerol (g). *b* Uptake of (1-^{14}C) glycerol (G). *c* Formation of $^{14}CO_2$. *d* Formation of ^{14}C-total lipids (TL).

Table III. Effect of thyroidectomy (T) and treatment with L-thyroxine on the *in vitro* utilization of (1-C^{14}) glycerol by epididymal fat-pads from rats (μmol/100 mg $\times 10^5$, mean ± SEM of 6–9 rats/group)

Group	Minutes of incubation				
	30	60	90	120	180
Uptake of labelled glycerol					
C[1]	47 ± 8	99 ± 19	173 ± 19	226 ± 35	308 ± 47
T+0[2]	189 ± 18	306 ± 45	328 ± 39	805 ± 93	1,058 ± 185
p[3]	< 0.001	< 0.01	< 0.01	< 0.001	< 0.001
T+0.1	147 ± 25	330 ± 74	321 ± 43	716 ± 178	723 ± 93
p	< 0.01	< 0.01	< 0.01	< 0.01	< 0.001
T+25	46 ± 8	98 ± 24	168 ± 26	263 ± 25	383 ± 23
p	NS	NS	NS	NS	NS
Formation of $^{14}CO_2$					
C	2.8 ± 0.3	7.5 ± 2.1	21.8 ± 4.2	15.9 ± 4.1	29.5 ± 5.2
T+0	4.6 ± 1.0	10.9 ± 3.8	10.4 ± 2.1	10.6 ± 2.5	27.4 ± 6.4
p	NS	NS	< 0.05	NS	NS
T+0.1	3.7 ± 0.8	15.5 ± 3.1	15.5 ± 4.2	34.1 ± 10.4	27.7 ± 4.4
p	NS	NS	NS	NS	NS
T+25	4.6 ± 0.3	29.5 ± 10.1	27.9 ± 5.2	28.7 ± 4.2	91.3 ± 25.3
p	NS	< 0.05	NS	NS	< 0.01
Formation of ^{14}C-total lipids					
C	40 ± 6	91 ± 18	151 ± 18	210 ± 34	284 ± 54
T+0	183 ± 18	310 ± 49	325 ± 37	692 ± 52	941 ± 57
p	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001
T+0.1	141 ± 23	320 ± 72	294 ± 44	554 ± 164	609 ± 75
p	< 0.01	< 0.01	< 0.05	< 0.05	< 0.001
T+25	39 ± 7	81 ± 19	141 ± 22	224 ± 23	313 ± 16
p	NS	NS	NS	NS	NS

[1] C = Intact controls.
[2] Doses of L-thyroxine (μg/100 g body weight/day).
[3] p = Statistical comparisons versus C (NS, not significant; i.e., $p > 0.05$).

Most of the (1-^{14}C) glycerol taken up by the tissue is converted to ^{14}C-labelled CO_2 and total lipids (table III) as on other occasions [14, 15]. While the formation of $^{14}CO_2$ is slightly augmented in the tissues from the T+25 animals and the difference with the values of C was statistically different at 60 and 180 min of incubation, this parameter was not altered in the T+0

and T+0.1 rats. In all the groups the formation of $^{14}CO_2$ increases linearly with the time of incubation (fig. 1c) and no statistical differences were found between the slopes of the values from the T animals and those from the C rats.

Different from the $^{14}CO_2$ data, the amount of (1-^{14}C) glycerol converted to ^{14}C-labelled total lipids was higher in the T+0 and T+0.1 rats than in the C animals (table III). This parameter does not differ, however, in C and T+25 rats at any time of incubation. As shown in figure 1d, the values of ^{14}C-total lipid formation in adipose tissue from all the groups also adjust well to first order regressions. The slopes of the lines corresponding to T+0

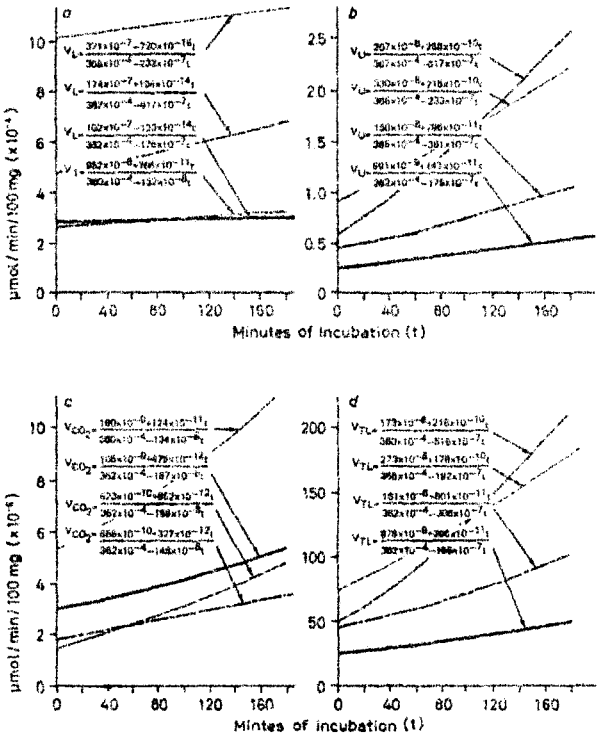

Fig. 2. Effect of thyroid status on the rates of glycerol release (lipolysis) and utilization by epididyme fat-pad from fasted rats. ——— = Intact controls; — — — = T+0; ——— = T+0.1; - - - = T+25. Details are given in the text. *a* Rate of glycerol release (V_L). *b* Rate of glycerol uptake (V_U). *c* Rate of CO_2 synthesis (V_{CO_2}). *d* Rate of total lipids synthesis (V_{TL}).

and T+0.1 are significantly higher than that in C ($p < 0.001$ and $p < 0.01$, respectively), while that of the T+25 does not differ from the value of the C group.

Rates of Adipose Tissue Lipolysis

As the reutilization of glycerol by adipose tissue differs among the groups depending on their thyroidal status, to calculate the rate of lipolysis we must correct for the amount of glycerol that is taken up by the adipose tissue. When the proper corrections are carried out [14] using the data described above, we obtain the rates of lipolysis that are schematically represented in figure 2a. When the values are extrapolated to zero time of incubation which theoretically should approximate the *in vivo* situation, the rate of lipolysis in the T+25 group is 260% above that in the C animals and in the T+0 animals it is 68% above the latter while in the T+0.1 rats the values obtained are practically identical to the C ones. Thus, the T+0 animals seem to be able to mobilize stored lipids when exogenous food is lacking. On the other hand, the data shows a biphasic effect of the administration of thyroxine as small doses (0.1 μg) reduce the rate of lipolysis of T+0 animals, while higher doses (25 μg) produce a tremendous elevation of this parameter.

The change of the rate of lipolysis with the time of incubation is hyperbolic in all the groups, but different from what we have observed previously [14], the rate of lipolysis increase as the incubation proceeds, while before, there was found a progressive fall. This difference is due to the fact that in the present study the values of the glycerol formation by the tissues have been adjusted to first order regressions ($g(t) = a + bt$), while previously it was found that the data fitted better to second order ones ($g(t) = a + bt + ct^2$). We are preparing for its publication a more specific analysis of this point.

Rates of Utilization of Glycerol

The augmented adipose tissue lipolysis in T+0 versus the C animals, despite the similar amount of glycerol formed during the time of incubation, is simply due to the fact that a greater proportion of the glycerol, that is coming out into the medium is being reutilized by the tissue of the T+0 animals, as suggested by the augmented uptake of (1-^{14}C) glycerol during the time of incubation (fig. 1b). The actual rate of glycerol being taken up by the tissue during the incubation period would depend not only on the percentage of ^{14}C-labelled glycerol captured by the tissue but also on the specific activity of the tracer, which at the same time would change according to the concentration of glycerol in the medium. When these points are taken

into account and the proper calculations are carried out [14], the observed rates of glycerol uptake by the epididymal fat-pads from the different groups are as shown in figure 2b. It can be seen that during the whole time of incubation this parameter is higher in the T animals under the different treatments than in the C ones, being maximal in the T+0 and T+25 animals.

As most of the glycerol taken up by the tissue is converted to both CO_2 and total lipids, the actual rates of formation of these compounds from glycerol were also estimated (fig. 2C, d). As it can be seen in figure 2d, the rate of formation of total lipids parallels quite well that of the uptake, being lowest in the C group and highest in both T+0 and T+25, while in the T+0.1 remains in the middle. The rate of formation of CO_2 is, however, very different, figure 2c, as it is maximal in the T+25 animals while in the T+0 and T+0.1 it is lower than in C during all the period of incubation.

Discussion

In the present work we have studied adipose tissue metabolism in fasted, thyroidectomized rats treated with different doses of exogenous thyroxine. We have previously shown that while in the fed state both hypo- and hyperthyroid animals obtain a balanced equilibrium which allows them to maintain a normal steady-state of several liver metabolites related with lipid metabolism, this equilibrium is broken down when food is withheld [1]. In some way this response to fasting might be related to the ability to mobilize lipids from adipose tissue depots.

Adipose Tissue Metabolism in Fasted Thyroidectomized Rats

We have seen here that the rates of both adipose tissue lipolysis and glycerol utilization for the synthesis of lipids are augmented in the thyroidectomized animals not receiving any exogenous thyroxine. These animals are markadly hypothyroid as shown by their great reduction in both body weight and size. These data would seen to be a surprise as up to now it was believed that hypothyroidism was associated with a decreased lipid mobilizing capability [2, 8, 12, 21].

Although no author has so far reported an augmented adipose tissue lipolysis in hypothyroid animals, on the basis of glycerol or free fatty acids formation, many have manifested their difficulties in obtaining a net decrease in these parameters when studied at basal conditions [2, 8, 12, 21]. Herein we have also seen that the net formation of glycerol by adipose tissue from

T+0 rats is not different or slightly reduced to that in the intact animals, but as the rate of reutilization of glycerol from the medium is tremendously augmented, we must come to the conclusion that the net rate of lipolysis is augmented in these animals. By indirect study we have come to the same conclusion [1]. In the fasted thyroidectomized rats there is an increase in liver acetyl-CoA and fatty acids and blood ketone bodies and these rats maintain the same levels of liver citrate as when fed [1], all of which would suggest an augmented arrival of fatty acids to the liver from peripheral depots. We do not know how this elevated turnover of lipids in the adipose tissue of the thyroidectomized rats might be driven *in vivo*, but actually in these animals there is a parallel reduction in the lipolytic hormones such as those secreted by the adenohypophysis [6, 7, 13, 18] and in the antilipolytic hormones such as insulin [1, 3, 16]. In any event, the ability to mobilize fat stores in the hypothyroid animals, even to a faster rate than in the normals, might play an important role in the hypothyroids metabolic adaptation to fasting which allows them to survive despite the low blood glucose levels that they attain in this situation [1]. This hypoglycemia by itself could be the primary factor in inducing the adipokinetic effect.

Effect of the Administration of Very Small Doses of Exogenous Thyroxine to Thyroidectomized Rats on Their Adipose Tissue Metabolism

As we have seen here 0.1 μg of thyroxine is enough to partially restore the growth capability of the thyroidectomized animals and to completely normalize the rate of adipose tissue lipolysis. The rate of whole glycerol utilization and specifically that for the synthesis of lipids is augmented in the T+0.1 animals. These doses of thyroxine are about one twentieth of those needed to decrease high rates of pituitary release of thyroid-stimulating hormone (TSH) to normal values [17] and actually we have measured the plasma levels of TSH in these animals and found that they remain as high as those from T+0 (unpubl. observations). We do not know the physiological implications of these findings, but the fact that not all the parameters are normalized in the thyroidectomized rats injected with 0.1 μg of thyroxine would suggest once more a different sensitivity of the pathways in which they are involved to the amount of thyroid hormone available.

Adipose Tissue Metabolism in Hyperthyroidism

We have seen here that the whole turnover of lipids is maximal in the thyroidectomized rats treated with 25 μg of thyroxine. Both lipoysis and glycerol utilization for either CO_2 or lipid synthesis are augmented in the

adipose tissue of these animals. These mixed effects might be induced by the high circulating levels of thyroid hormone through the activation of the lipolytic action of cathecolamines, concomitant with the elevation of circulating insulin [1], a hormone which we have found that by itself enhances the *in vitro* uptake and utilization of glycerol by adipose tissue [DOMINGUEZ and HERRERA, unpubl. observations].

Very probably, in the *in vivo* situation FFA and glycerol, the end products of this enhanced lipid mobilization by adipose tissue, are carried by the blood stream to other tissues, mainly to the liver where they might be used as substrates for other pathways such as ketogenesis and gluconeogenesis. In any event although it is evident from the data shown here that the whole enzymatic capacity for handling the lipids in adipose tissue is elevated in the hyperthyroid animals, this does not necessarily mean that the total amount of lipids that are liberated from adipose stores are very much elevated in these animals. We have seen here that although the size of the hyperthyroid animals does not differ from that of the intact controls, their body weight is lower and actually it has been shown that in hyperthyroidism, catabolism prevails over anabolism even in the fed state[10] which would unable these animals to mobilize enough lipids in the fasted state due to a lack of substrate and not to any fault in the lipolytic machinery. This interpretation is in agreement with our previous study on the steady-state concentration of metabolites in blood and liver in hyperthyroid animals kept under similar conditions [1], which demonstrates that the arrival of lipids to the liver is small in these animals when food is withheld.

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