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Release of free fatty acids by adipose tissue from rats treated with triiodothyronine or propylthiouracil

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SUMMARY

Adipose tissue from rats treated with propylthiouracil released less free fatty acids (FFA) into the medium and contained less FFA at the end of a 1-hr incubation period than did tissue from euthyroid animals. The elevation of the concentration of tissue FFA and enhancement of FFA release induced in normal tissues by addition of epinephrine to the medium was not observed in tissues from animals treated with propylthiouracil. Tissues from rats treated with triiodothyronine released more FFA into the medium and contained more FFA after 1 hr of incubation than did control tissues. The tissues from rats treated with triiodothyronine, however, also contained more FFA before incubation than did control tissues and, as with control fat pads, there was no net accumulation of FFA during the incubation period. From measurement of total accumulation of glycerol during the same time, it was concluded that the rate of lipolysis in tissues from rats treated with triiodothyronine was increased above the control level. The rate of esterification of fatty acid (glyceride synthesis) was apparently also elevated. The effect of epinephrine on the release of FFA and glycerol was greater in tissues from triiodothyronine-treated rats than in the control tissues.

Debons and Schwartz (1) found that when epididymal fat pads from propylthiouracil (PTU)-treated rats were incubated *in vitro*, release of free fatty acids (FFA) into the medium was strikingly low and was not increased by the addition of epinephrine. Conversely, in fat pads from rats pretreated with triiodothyronine (T_3), release of FFA was greater than that from normal tissues, and the effect of epinephrine on this process was markedly exaggerated. After incubation of adipose tissue *in vitro*, a large fraction (under some conditions, essentially all) of the FFA in the system is found in the tissue, not in the medium. In addition, it should be noted that FFA may be released even when there is no net increase in the amount of FFA in the system (2). For these reasons, tissue as well as medium FFA were measured in experiments similar to those of Debons and Schwartz. In addition, we undertook to determine if there was, in fact, net production of FFA during incubation of tissues from rats treated with the T_3.

Net changes in the amount of FFA are determined by the balance between the rate of production of FFA by lipolysis of triglycerides and the rate of esterification of FFA (glyceride synthesis). Thus, inferences about rates of lipolysis cannot be drawn from data on FFA alone. On the other hand, glycerol, which is also formed in the lipolysis of triglycerides, is metabolized little, if at all, by adipose tissue (3–7) and therefore serves as a measure of lipolysis. Comparison of glycerol production by tissues from T_3-treated rats with that by control tissues indicated an elevated rate of lipolysis in the former.

METHODS

Epididymal fat pads (150–500 mg each) were obtained from male Sprague-Dawley rats, which had been decapitated. Methods for incubation of tissues and for quantification of FFA and of glycerol in tissues and in medium are described in detail in a previous paper from

this laboratory (2). In all flasks, the molar ratio of FFA to albumin was less than 2 at the end of the incubation period.

Rats treated with PTU received, for a period of 18–21 days, ground Purina chow to which 1% PTU (w/w), was added. Controls were pair-fed with the treated rats. All other animals received Purina chow pellets *ad libitum*. For injection, L-triiodothyronine was dissolved in a small volume of 0.1 N NaOH and then diluted so that the concentration of NaOH in the injected solution was less than 0.002 N. Rats were given T_3 by three intraperitoneal injections: 50 μg, 21 hr before; 200 μg, 6 hr before; and 200 μg, 3 hr before the experiment. Control animals received intraperitoneal injections of the solvent alone.

RESULTS

As shown in Table 1, the tissues from rats treated with PTU released less FFA into the medium during 1 hr, and contained less FFA at the end of the hour than did their controls. Tissues from T_3-treated rats released more FFA and, at the end of the incubation, contained more FFA than did their controls. Effects of epinephrine on FFA were absent in tissues from PTU-treated animals and were significantly increased over those of the controls in tissues from T_3-treated rats.

Tissues from T_3-treated rats also released more glycerol into the medium and responded to epinephrine with a greater increase in glycerol release than did tissues from control rats (Table 2).

In the experiments summarized in Table 3, one tissue from each rat was analyzed for glycerol and FFA immediately after excision. The contralateral tissue was

TABLE 1. EFFECT OF EPINEPHRINE (E) ON RELEASE OF FFA AND TISSUE CONCENTRATION OF FFA*

Conditions	No. of Pairs	FFA in Medium		FFA in Tissue	
		Without E†	Increase Due to E‡	Without E†	Increase Due to E‡
		μEq/g tissue		*μEq/g tissue*	
Fasted 18 hours:					
Control	12	3.3 ± 0.36	3.0 ± 0.56	3.0 ± 0.23	4.1 ± 0.46
PTU-treated	12	1.0 ± 0.42	0.4 ± 0.28	2.0 ± 0.32	0.3 ± 0.27
Not Fasted:					
Control	3	0.5 ± 0.39	6.6 ± 2.08	3.1 ± 0.38	5.0 ± 0.17
PTU-treated	3	0.1 ± 0.02	0.1 ± 0.39	1.0 ± 0.36	0.1 ± 0.47
Not Fasted:					
Control	7	0.1 ± 0.06	1.2 ± 0.09	2.2 ± 0.22	1.5 ± 0.39
T_3-treated	8	2.4 ± 0.30	4.1 ± 0.31	4.8 ± 0.83	7.6 ± 0.68

* Pairs of tissues from normal, PTU-treated, and T_3-treated animals were incubated in 3 ml of Krebs' bicarbonate medium containing bovine serum albumin, 30 mg/ml; one of each pair with, and one without epinephrine, 0.1 μg/ml. FFA was measured in tissue and medium after 1 hr of incubation.

† Mean ± standard error of the mean.

‡ Mean of differences between paired tissues ± standard error of the mean.

TABLE 2. EFFECT OF EPINEPHRINE (E) ON GLYCEROL AND FFA IN TISSUES FROM RATS TREATED WITH TRIIODOTHYRONINE*

	Glycerol in Medium		FFA in Medium	
	Without E†	Increase Due to E‡	Without E†	Increase Due to E‡
	μmole/g tissue		*μEq/g tissue*	
Control	1.8 + 0.04	1.2 ± 0.26	0.1 ± 0.06	2.7 ± 0.09
T_3-Treated	2.6 ± 0.25	3.8 ± 1.06	1.3 ± 0.14	7.3 ± 1.63
p Value for control vs treated	<0.05	<0.025	<0.01	<0.025

* Conditions of incubation were as described in Table 1. Three rats were in each group.

† Mean ± standard error of the mean.

‡ Mean of differences between paired tissues ± standard error of the mean.

TABLE 3. NET CHANGES IN FFA AND GLYCEROL DURING INCUBATION OF FAT PADS FOR 1 HR*

	Glycerol					FFA				
	Tissue-0′†	Tissue-60′†	Medium-60′†	Δ60′‡	Lipolysis	Tissue-0′†	Tissue-60′†	Medium-60′†	Δ60′‡	Esterification
	μmoles/g tissue				*μEq FFA/g/hr*	*μEq/g tissue*				*μEq FFA/g/hr*
Normal	0.7 ±0.20	0.5 ±0.08	1.2 ±0.12	+1.0 ±0.12	3.0 ±0.36	1.1 ±0.17	1.0 ±0.08	0.2 ±0.13	+0.1 ±0.14	2.9. ±0.33
T_3-treated	0.8 ±0.06	0.8 ±0.09	1.9 ±0.04	+1.9 ±0.20	5.7 ±0.60	3.2 ±0.54	1.8 ±0.22	1.7 ±0.14	+0.3 ±0.37	5.4 ±0.19
p Value for normal vs treated		<0.05	<0.005	<0.005		<0.005	<0.005	<0.001		<0.001

* Six fed rats were in each group. One of each pair of tissues was analyzed, without incubation, for glycerol and FFA. The contralateral tissue was incubated for 1 hr in 3 ml Krebs' bicarbonate medium containing bovine serum albumin, 30 mg/ml, and the tissue and the medium then analyzed for glycerol and FFA.

† Mean ± standard error of the mean.

‡ Mean of values calculated for each pair of tissues ± standard error of the mean.

incubated for 1 hr and glycerol and FFA were measured in tissues and in medium. From these data, it is possible to calculate the net changes in glycerol and in FFA over the course of incubation. If it is assumed that the amount of glycerol that accumulates is a measure of lipolysis, that 3 moles of FFA were formed with each mole of glycerol, and that the only quantitatively significant pathways of fatty acid formation and removal are lipolysis and esterification (glyceride synthesis) respectively; then the average rate of lipolysis and of esterification over the hour of incubation can be calculated. The calculations and the assumptions upon which these depend have been discussed in detail elsewhere (2). As shown in Table 3, the greater release of glycerol by tissues from T_3-treated rats as compared with the control tissues reflects a greater total accumulation of glycerol in the system, presumably the result of a greater rate of lipolysis. On the other hand, the concentration of FFA in the tissues at zero time was significantly higher in tissues from T_3-treated rats, and the significantly greater accumulation of FFA in the medium of these tissues was entirely at the expense of the tissue FFA. There was no net change in the amount of FFA in the system during the incubation period with either group of tissues. The rates of both lipolysis and esterification were greater in the tissues from T_3-treated animals than in control tissues; but the rates of the two processes were balanced in both groups of tissues.

DISCUSSION

As observed by Debons and Schwartz (1), release of FFA from tissues of rats treated with T_3 was elevated. We found, in addition, that the tissues from T_3-treated rats analyzed after incubation contained more FFA than did the control tissues. Tissues from T_3-treated rats contained more FFA at zero time, however, and in neither control nor experimental group was there a net change in the total amount of FFA in the system during the period of incubation. Since comparison of net changes in glycerol during the same time indicated a greater rate of lipolysis in the tissues from T_3-treated rats, fatty acid esterification was presumably increased to an equivalent extent. The rates of the two processes remained balanced just as they were in the tissues from control animals, and the total amount of FFA in the system was maintained unchanged. Whether the elevated rate of esterification was secondary to increased lipolysis, or whether the processes of esterification are influenced in some other way in the tissues from T_3-treated rats, is not known.

The effect of epinephrine on glycerol release is exaggerated in tissues from rats treated with T_3, as is its effect on release of FFA, which indicates a greater than normal response of the lipolytic system to epinephrine in the experimental tissues. This could be due to an increased sensitivity of the tissue to epinephrine, resulting, for example, from a slower rate of inactivation of epinephrine in tissues from T_3-treated rats. If an hypothesis of this sort were to explain the effects of T_3-treatment on behavior of fat pads without epinephrine added *in vitro*, it would be necessary to assume that, even in the absence of added epinephrine, the lipolytic system is to some extent under the influence of catecholamines contained in the tissue. On the other hand, thyroid status may influence the lipolytic system itself in such a way that both its basal level of operation and the magnitude of its response to epinephrine is affected.

The concentration of FFA in tissues from T_3-treated rats was higher than that in the control tissues. During incubation, the concentration of FFA fell in the former tissues due to release of FFA into the medium whereas the concentration did not change appreciably in the control tissues. In neither group, as shown in Table 3, was there any net production of FFA during the incubation. These animals were not fasted and and it may be that in neither group was the adipose tissue releasing FFA into the blood at the time of sacrifice. The concentrations of FFA in the plasma at that time were not determined. Rich and co-workers (8) have reported higher than normal concentrations of FFA in the plasma of patients with induced or spontaneous hyperthyroidism in the fasted state. Perhaps, even when fatty acid mobilization is at a minimum in the hyperthyroid animal, there are higher than normal concentrations of FFA in plasma and adipose tissue and elevated but balanced rates of lipolysis and esterification in the latter tissue. This interpretation is quite compatible with the abnormally high rate of fatty acid release that apparently occurs in the fasted hyperthyroid animal. The equivalence of these rates of lipolysis and esterification observed *in vitro* in tissues from both normal and T_3-treated rats (not fasted) thus may reflect the situation that exists *in vivo* in the nonfasted animals. On the other hand, it is possible that, in one or both groups of tissues, this balance is a result of metabolic adjustments to the experimental conditions.

The data reported above confirm the observations of Debons and Schwartz (1) that tissues from PTU-treated rats release less FFA into the medium than do tissues from euthyroid rats and that the effect of epinephrine added *in vitro* is absent. We observed, in addition, parallel differences in the concentration of FFA in

tissues of the two groups after incubation. By analogy with the findings in tissues from T_3-treated rats, it seems quite possible that the low levels of FFA release and of FFA in the incubated tissues are a reflection of a lower than normal concentration of FFA in the tissues of PTU-treated rats at the time of excision. Net changes in FFA and glycerol were not determined in this group. The rates of lipolysis and esterification relative to those in control tissue cannot be estimated.

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