

Dependence of the lipolytic action of epinephrine *in vitro* upon thyroid hormone^{*†}

ALBERT F. DEBONS and IRVING L. SCHWARTZ

Medical Research Center,
Brookhaven National Laboratory,
Upton, Long Island, New York

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SUMMARY

The influence of thyroid hormone on the epinephrine-induced release of free fatty acids (FFA) from rat epididymal adipose tissue was studied *in vitro*. Untreated tissue from euthyroid animals released only small amounts of FFA. However, the tissues responded to treatment with epinephrine with a significant increase in the rate of release of FFA, confirming observations previously reported by others. In the case of fat pads removed from hypothyroid animals, no increase in the rate of release of FFA was observed after epinephrine; in the case of fat pads removed from hyperthyroid animals, the epinephrine-induced release of FFA was markedly exaggerated. This thyroidal enhancement of epinephrine action on the fat pad was not evident when tissues were removed from euthyroid animals 3 hours after intraperitoneal injection of triiodothyronine, but it was maximal in tissues removed after 15 hours from animals that had received repeated intraperitoneal injections of triiodothyronine. When triiodothyronine was added *in vitro* to fat pads from euthyroid rats, the basal release of FFA was not affected nor was the normal response to epinephrine altered. These studies show that the thyroid hormone is essential for the epinephrine-induced release of FFA from adipose tissue.

Accelerated mobilization of fat in hyperthyroid subjects is associated with an increase in the concentration of nonesterified, or free fatty acids (FFA), in plasma (1). The administration of epinephrine is also associated with an increase in the plasma FFA level *in vivo* (2, 3, 4) and, in addition, with lipolysis (5) and the release of FFA from adipose tissue *in vitro* (6, 7). Because the calorogenic effect (8, 9) and many circulatory effects (10) of epinephrine are modified by the thyroid status of the animal, we have investigated the relationship between the action of epinephrine and thyroid hormone on the release of FFA from adipose tissue.

In the present study it has been found that the response of isolated adipose tissue to epinephrine requires the mediation *in vivo* of thyroid hormone.

MATERIAL AND METHODS

Male Sprague-Dawley rats weighing approximately 275 to 325 g were used for all experiments. They were

maintained on Purina Rat Chow and drinking water *ad libitum*. Rats were arranged in three groups: (a) hypothyroid, treated with 0.05% propylthiouracil in their drinking water; (b) euthyroid; (c) hyperthyroid, treated with 0.2 μ g L-triiodothyronine per 100 g of body weight subcutaneously daily. The L-triiodothyronine was dissolved in 0.1 N NaOH and diluted with isotonic saline to give the desired dose in 0.1 ml solution (11); the final concentration of alkali was approximately 0.001 N. Groups (a) and (b) received control injections of the solvent with L-triiodothyronine omitted. After 11 to 15 days, without preliminary fasting, animals from each group were sacrificed by means of a sharp blow on the head and section of the spinal cord. The epididymal fat pads were excised and placed in ice-cold isotonic saline prior to treatment as described below under "In Vitro Conditions." The thyroid glands were dissected free of fascia, removed, and weighed on a Roller Smith Torsion Balance. Blood samples were also taken at this time, for measurement of the concentration of total cholesterol (12).

Another group of animals was given L-triiodothyronine by intraperitoneal injection at 3-hour intervals. These animals were prepared, and epididymal fat pads removed for *in vitro* study in the manner described

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above, at 3 hours or at 15 hours after the first intraperitoneal injection.

In Vitro Conditions. Portions of epididymal fat pads weighing approximately 200 mg were cut free hand, and incubated in 4 ml of a Krebs-Ringer Bicarbonate Buffer (13) containing 5% Albumin (Bovine, Fraction V, Armour Co.). This buffer-albumin mixture had been previously adjusted to $\text{pH } 7.40 \pm 0.02$. The flasks, containing tissue and medium, were exposed to a stream of 95% oxygen-5% carbon dioxide for 1 minute, stoppered, and placed in a shaking water bath at 37° . Zero time control flasks were allowed to equilibrate for 15 minutes; all other flasks were incubated for 4 hours following a similar initial equilibration period, that is, for a total period of 4.25 hours. Epinephrine¹ ($2.5 \mu\text{g}$, $0.0137 \mu\text{moles}$) was included per each ml of buffered medium of selected flasks at zero time; in these flasks, therefore, the initial concentration of epinephrine was $13.7 \mu\text{M}$. Duplicate aliquots were taken from the incubation medium of control flasks at the end of the 15-minute equilibration period and 4 hours later, that is, after 4.25 hours of total incubation time. The FFA concentration was determined by the method of Dole (2).

RESULTS

The relation between the amount of FFA released from the epididymal fat pad incubated under the conditions outlined above and the dose of epinephrine added to the incubation medium is shown in Figure 1. FFA release was roughly proportional to the initial concentration of epinephrine over a range from 0 to $12.5 \mu\text{M}$, but did not increase when greater amounts of epinephrine were added to the medium, nor when 1 to 3 μmoles of ascorbic acid was included in the medium to prevent oxidation of epinephrine. Therefore, the standard epinephrine challenge employed in this study, $2.5 \mu\text{g}$ ($0.0137 \mu\text{moles}$) per ml of incubation medium, was capable of producing a maximal release of FFA from fat pads taken from euthyroid animals.

Figure 2 shows a comparison among the three groups of animals studied (euthyroid, hypothyroid, and hyperthyroid) with respect to the release of FFA from the epididymal fat pad, both in the basal state and in response to epinephrine challenge. In the absence of epinephrine, the release of FFA from fat pads taken from euthyroid rats averaged $3.22 (\pm 1.36) \mu\text{moles}$

¹ This preparation of epinephrine has been recently shown to have the D-configuration, although it is levorotatory. Therefore, it should be referred to as D(-)-epinephrine, instead of L-epinephrine, as given in the United States Pharmacopeia (14).

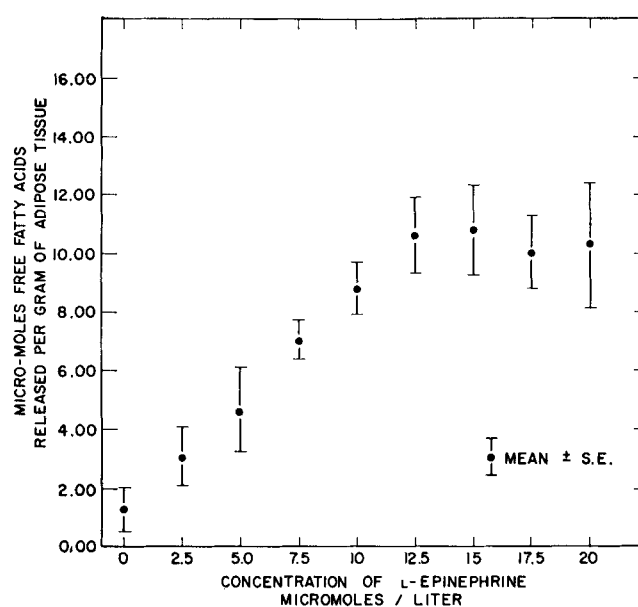


Fig. 1. Relation of the release of FFA from adipose tissue to the concentration of epinephrine in the medium.

per g of fat pad per 4-hour incubation period. The basal release of FFA from adipose tissue taken from hyperthyroid rats averaged $10.81 (\pm 3.56) \mu\text{moles}$ per g of fat pad per 4-hour incubation period, and was significantly greater ($p < 0.05$) than the basal FFA release from adipose tissue taken from the euthyroid animals. Conversely, the basal release of FFA from fat pads taken from the hypothyroid animals averaged $1.54 (\pm 1.62)$, and was less than the basal FFA release from the pads taken from the euthyroid group of rats; however, the difference was not significant ($p > 0.30$).

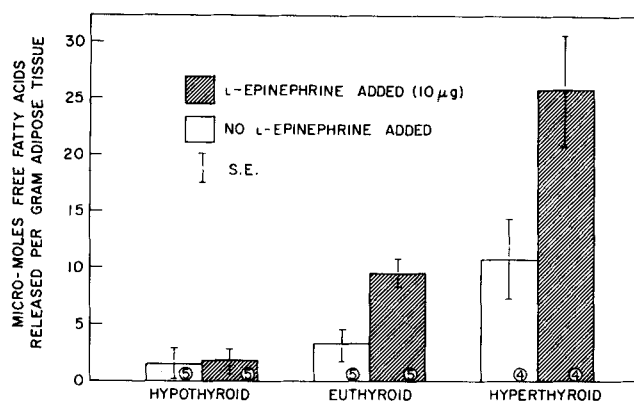


Fig. 2. Basal release and epinephrine-stimulated release of FFA from adipose tissue taken from hypothyroid, euthyroid, and hyperthyroid rats. The encircled numbers indicate the number of animals studied in each group.

TABLE 1. EFFECT OF *in Vivo* EXPOSURE TO L-TRIIODOTHYRONINE ON THE ACTION OF L-EPINEPHRINE ON ADIPOSE TISSUE *in Vitro*

| Group | Treatment | μ moles of FFA Released per G Adipose Tissue | |
|-----------|--|--|--------------------------------|
| | | No L-Epinephrine | 10 μ g L-Epinephrine |
| Euthyroid | None* | 2.10 \pm 0.30† | 6.90 \pm 0.31 |
| | L-Triiodothyronine (50 μ g)‡ (1 intraperitoneal injection only) | 3.84 \pm 0.64 (p < 0.05) | 7.86 \pm 0.74 (p > 0.05) |
| | None* | 1.44 \pm 0.12 | 6.97 \pm 1.88 |
| | L-Triiodothyronine (50 μ g)§ (every 3 hours for 12 hours) | 2.43 \pm 0.29 (p < 0.01) | 23.04 \pm 3.33 (p < 0.01) |

* Two groups of four animals were injected intraperitoneally with saline-NaOH solvent containing no hormone.

† Mean and standard error.

‡ Two groups of four animals were sacrificed 3 hours following the injection of L-triiodothyronine, epididymal fat pads excised and treated under *in vitro* conditions.

§ Two groups of six animals were sacrificed 3 hours following the last injection of L-triiodothyronine, epididymal fat pads excised and treated under *in vitro* conditions.

Following challenge with 0.0137 μ moles of epinephrine per ml of medium, FFA release averaged 9.45 (\pm 1.21) μ moles per g of fat pad per 4-hour incubation period in the case of the euthyroid animals. This response was significantly less (p < 0.01) than that observed when fat pads from hyperthyroid rats were treated in an identical manner. FFA release from the adipose tissue taken from the latter group averaged 25.7 (\pm 5.0) μ moles per g of fat pad per 4-hour period. This exaggerated lipolytic effect observed when fat pads from hyperthyroid rats were challenged with epinephrine contrasted sharply with the failure of the fat pads removed from the hypothyroid animals to exhibit any response to epinephrine. The release of FFA from "hypothyroid" adipose tissue following challenge with epinephrine averaged 1.72 (\pm 1.2) μ moles per g of fat pad per 4-hour period, and therefore did not differ significantly from the basal rate of FFA release from fat pads observed in the experiments in

which epinephrine was not included in the medium (Fig. 2).

The thyroidal enhancement of the lipolytic action of epinephrine on adipose tissue was not evident when tissues were removed from euthyroid animals 3 hours after the injection of 50 μ g of L-triiodothyronine intraperitoneally, but was maximal in tissues removed from animals that had received four such injections at 3-hour intervals (Table 1).

In a separate group of experiments, L-triiodothyronine was added to epididymal fat pads *in vitro*, both in the presence and absence of epinephrine; and, conversely, epinephrine was added to epididymal fat pads *in vitro*, both in the presence and absence of L-triiodothyronine.

The addition of L-triiodothyronine *in vitro* did not alter the basal release of FFA from adipose tissue of euthyroid animals and did not affect the normal response to 10 μ g (0.055 μ moles) of epinephrine (Table 2). Nor did the addition of epinephrine evoke any greater release of FFA from fat pads exposed to L-triiodothyronine *in vitro* than from fat pads that had not been exposed to L-triiodothyronine.

Additional evidence for the status of the euthyroid, hypothyroid, and hyperthyroid animals (Fig. 1) was obtained by determining the thyroid weights, calculated as mg of thyroid tissue per 100 g of body weight, which proved to be as follows: euthyroid 5.25 \pm 0.22, hypothyroid 17.13 \pm 1.61, hyperthyroid 3.47 \pm 0.28. The concentration of total cholesterol (expressed as mg/100 ml) in the serum of the three groups averaged as follows: euthyroid 81.8 \pm 8.6, hypothyroid 89.5 \pm 17.6, hyperthyroid 67.0 \pm 7.3.

DISCUSSION

The foregoing results show that thyroid hormone is

TABLE 2. ADDITION OF L-EPINEPHRINE AND L-TRIIODOTHYRONINE *in Vitro**

| L-Epinephrine (μ g) | 0 | 0 | 0 | 10 | 10 | 10 |
|--|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| L-Triiodothyronine (μ g) | 0 | 1 | 5 | 0 | 1 | 5 |
| μ moles of FFA released per g adipose tissue | 2.08 \pm 0.48† | 1.57 \pm 0.35 | 1.83 \pm 0.55 | 6.48 \pm 0.82 | 7.75 \pm 1.26 | 7.71 \pm 0.89 |
| p | | >0.05 | >0.05 | | >0.05 | >0.05 |

* Values represent the results of 24 experiments conducted with adipose tissue taken from four euthyroid animals.

† Mean \pm standard error.

required for the epinephrine-induced release of FFA from adipose tissue. This phenomenon depends on the exposure of the adipose tissue to a critical minimum level of active thyroid principle *in vivo* for a period of several (more than 3 but less than 15) hours prior to challenge with epinephrine *in vitro*. In other words, the enhancement of the lipolytic action of epinephrine on adipose tissue depends on adequate treatment of the whole animal with triiodothyronine prior to excision of the adipose tissue for *in vitro* study. In addition to the requirement of minimal amounts of thyroid hormone for even a minimal effect of epinephrine on the release of FFA from adipose tissue, it is clear that when the hormone is present in excessive amounts, the effect of epinephrine is strikingly exaggerated.

These findings are in accord with the report that the epinephrine-induced *in vivo* mobilization of FFA in rhesus monkeys is dependent upon optimal thyroid function (15). It is noteworthy that the increased mobilization of FFA from fat depots in response to fasting and growth hormone does not show a similar thyroid requirement (15, 16). Shafrir *et al.* (17) also failed to observe an elevation in plasma FFA after the administration of epinephrine to hypophysectomized dogs, confirming the findings of Goodman and Knobil (15). However, Shafrir and Steinberg (18) have reported subsequently that adrenalectomy alone abolished the epinephrine-induced increase in the plasma level of FFA in dogs *in vivo*, and treatment with cortisone restored this response, indicating a role for cortisone in the response of adipose tissue to epinephrine. They also noted that cortisone treatment alone restored the response of the plasma FFA fraction to epinephrine in hypophysectomized dogs. These authors pointed out, however, that the time interval between hypophysectomy and the execution of their studies was too short to exclude the persistence of thyroid function in their animals.

The mechanism by which the thyroid hormone modifies the capacity of epinephrine-stimulated adipose tissue to release FFA is not clear. It is possible that thyroidal inhibition of adipose tissue enzymes involved in degradation of epinephrine may be a significant factor in view of the fact that there is evidence of thyroidal inhibition of monoamine oxidase activity (19, 20, 21). Also, the fact that thyroidectomy increases the hypoglycemic response to insulin (22) suggests that a thyroid-insulin antagonism may be involved.

However, we consider it more likely that this thyroid effect is due largely or entirely to an increase in the content of an epinephrine-activated lipase of adipose tissue, or to an increase in the availability of a co-factor required for activation of this enzyme, or to an increase of both (23).

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