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THE TIME COURSE OF THE EFFECT OF THYROID HORMONES UPON BASAL OXYGEN CONSUMPTION AND PLASMA CONCENTRATION OF FREE FATTY ACID IN RATS

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SUMMARY

1. Basal O_2 consumption and plasma free fatty acid (FFA) concentration were measured in normal and hypothyroid rats at various intervals after the initiation of a course of treatment with thyroid hormones.

2. A significant rise in basal O_2 consumption was detectable in both groups of rats before there was any change in plasma FFA level.

3. It is concluded that the stimulatory effect of thyroid hormones on the basal O_2 consumption of the whole animal is not mediated entirely by a rise in plasma FFA level.

INTRODUCTION

Rich, Bierman & Schwartz (1959) showed that the plasma concentration of FFA is raised in thyrotoxicosis and after intravenous injections of triiodothyronine into normal men. The rise in plasma FFA concentration was later shown to be due to stimulation of the output of FFA from adipose tissue by thyroid hormone (Debons & Schwartz, 1961). Since it has also been shown that the rate of oxidation of FFA by skeletal muscle incubated *in vitro* is roughly proportional to the concentration of FFA in the medium (Fritz, Davis, Holtrop & Dundee, 1958; Eaton & Steinberg, 1961) it has been suggested that the stimulatory effect of thyroid hormones upon basal O_2 consumption of the whole animal may be mediated wholly or in part by a rise in plasma FFA concentration (Eaton & Steinberg, 1961). In an attempt to test this suggestion we have made serial measurements of basal O_2 consumption and plasma FFA concentration in rats given injections of thyroid hormones.

METHODS

The rats were males of the Wistar strain weighing 220–250 g. They were housed in a room maintained at a constant temperature of 22° C and were given M.R.C. Diet No. 41 (Bruce & Parkes, 1946) *ad libitum*. The thyroxine and triiodothyronine used for injection were dissolved in 0·5 ml. of dilute NaOH (pH 8·6). At each injection of hormone into a treated rat, a control animal was injected with the same volume of a similar solution of NaOH containing no hormone. The thyroids were destroyed by giving the rats 600 μc of ^{131}I intraperitoneally (Goldberg, Chaikoff, Lindsay & Feller, 1950). The basal O_2 consumption was measured by the closed circuit method of Maclagan & Sheahan (1950), with slight modifications. The rat was anaesthetized lightly with an intraperitoneal injection of 5 mg of Nembutal and was then placed in the desiccator (internal volume, 4 l.), which was immersed completely in a water-bath kept at constant temperature. Oxygen was flushed through the desiccator for 2 min, and the tap to air was closed. Readings of the pressure in the desiccator were taken at 5 min intervals for 25–30 min, after allowing 3–5 min for the temperature inside the desiccator to reach that of the water-bath. Measurements were made on four rats at a time, each rat being kept in a separate desiccator. The temperature of the water-bath was 28° C for the rats with intact thyroids and 33° C for the hypothyroid rats, since O_2 consumption in rats is minimal at these temperatures (Leidig & Gray, 1957). Plasma FFA concentrations were measured by the method of Dole (1956), modified by using hexane instead of heptane in the extraction mixture. Blood was taken from the heart with a heparinized syringe after lightly anaesthetizing the rat with an intraperitoneal injection of 5 mg of Nembutal, care being taken to avoid restraining the rat during the injection.

Time course of injections. In the experiments in which O_2 consumption was measured at 18, 21 and 36 hr after the beginning of a course of injections of hormone, the first injection was given at 10 p.m. In all other experiments, the first injection was given at 9.30 a.m.

Statistical analysis. All values are given as averages with S.E. of mean. Student's t test was used for testing for the significance of the difference between averages.

RESULTS

Effect of thyroxine in rats with intact thyroids. The basal O_2 consumption was measured in nine pairs of rats, matched for weight. As soon as the measurement was completed, one rat from each pair was given 50 μg of thyroxine by intraperitoneal injection, the other member of the pair being used as a control. The injection was repeated once every 12 hr until the animal was killed. O_2 consumption and plasma FFA concentration were measured in the treated and control animals at various intervals throughout the course of injections. In some rats measurements were made on two or more occasions, the whole group of eighteen animals providing thirty-eight estimates of O_2 consumption and eighteen of plasma FFA concentration, in addition to those made before the first injection.

Figure 1 shows all the values obtained from rats given courses of injection extending from 18 hr to 5 days. The average O_2 consumption measured in the eighteen rats before the initial injection was $0{\cdot}94 \pm 0{\cdot}02$ ml./hr/g body weight. At 18 hr after the first injection there was no increase in O_2 consumption, but the values obtained at 24 and 27 hr were significantly

higher in the treated animals ($1{\cdot}18 \pm 0{\cdot}05$ ml./hr/g) than in the controls ($0{\cdot}94 \pm 0{\cdot}03$) measured at the same time ($P < 0{\cdot}002$ for the four pairs of values at 24 and 27 hr). At all subsequent intervals the values from the treated animals were significantly higher than those from the controls, the values in the treated group rising to $1{\cdot}46 \pm 0{\cdot}07$ at 5 days.

The plasma FFA concentration 24 hr after the first injection was not significantly higher in the treated than in the control rats. At 3 days the

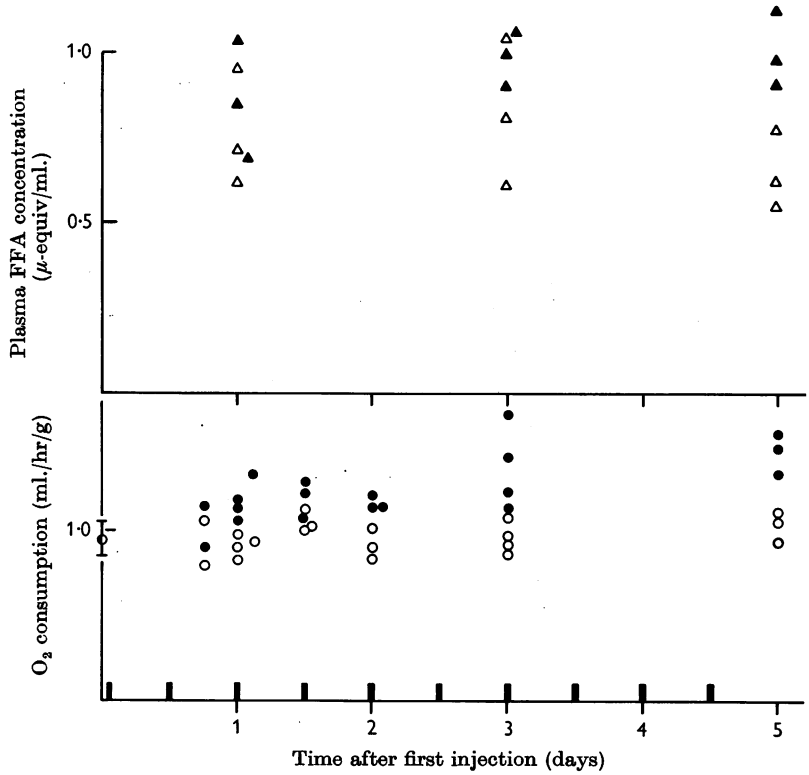

Fig. 1. Effect of injections every 12 hr of 50 μg of thyroxine on O_2 consumption (○, ●) and plasma free fatty acid level (△, ▲) in rats. Thyroxine injections shown by vertical bars; open symbols, control rats injected with dilute NaOH; filled symbols, treated rats.

average value from the treated rats ($0{\cdot}99 \pm 0{\cdot}06$ μ-equiv/ml.) was higher than that from the controls ($0{\cdot}85 \pm 0{\cdot}14$ μ-equiv/ml.), but the difference was not significant. At 5 days, the average value from the treated rats was significantly higher than that from the controls ($P < 0{\cdot}05$).

Effect of triiodothyronine in rats with intact thyroids. Basal O_2 consumption and plasma FFA concentration were measured in a total of twenty-one pairs of rats. One rat from each pair was then given 25 μg of triiodo-

thyronine by intraperitoneal injection and the injection was repeated once every 12 hr until the animal was killed. The other member of the pair was used as a control. O_2 consumption and plasma FFA concentration were measured at intervals ranging from 18 to 66 hr after the first injection. In eight of the pairs, measurements were made on more than one occasion, the whole group of forty-two rats providing fifty-seven measurements of

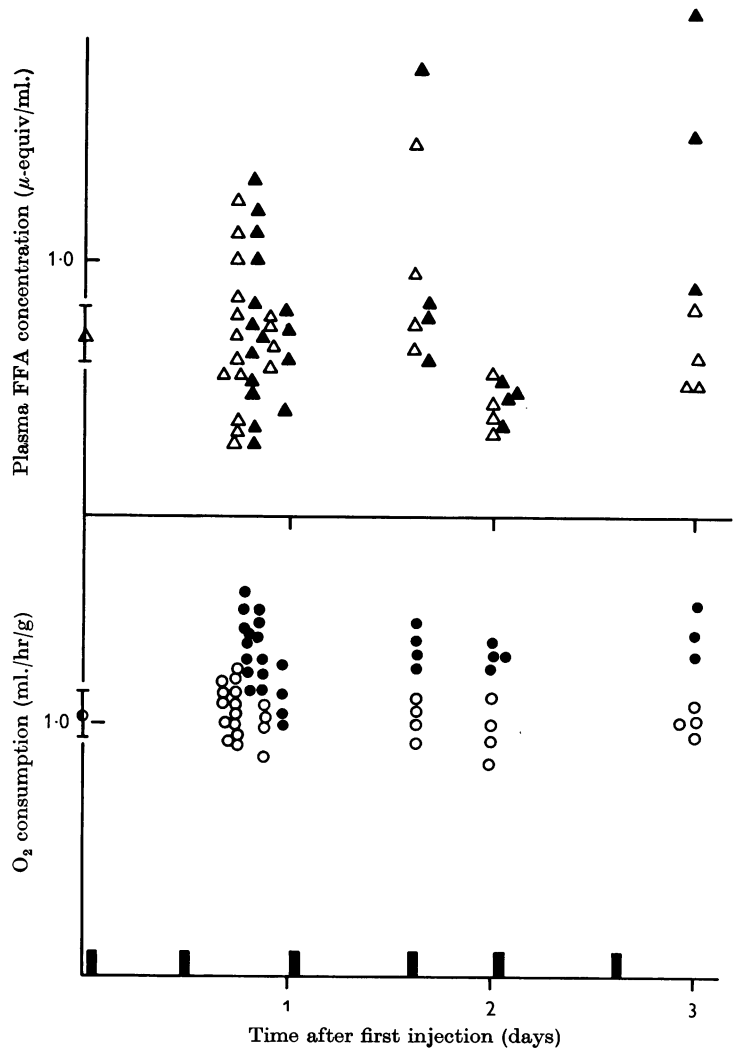

Fig. 2. Effect of injections every 12 hr of 25 μg of triiodothyronine on O_2 consumption (○, ●) and plasma free fatty acid level (△, ▲) in rats. Triiodothyronine injections shown by vertical bars; open symbols, control rats injected with dilute NaOH; filled symbols, treated rats.

O_2 consumption and fifty-five of plasma FFA concentration in addition to the initial measurements made before the first injection.

Figure 2 shows all the values obtained. In the whole group of rats tested before the first injection, the average O_2 consumption was $1{\cdot}03 \pm 0{\cdot}03$ ml./hr/g and the average plasma FFA concentration was $0{\cdot}74 \pm 0{\cdot}05$ μ-equiv/ml.

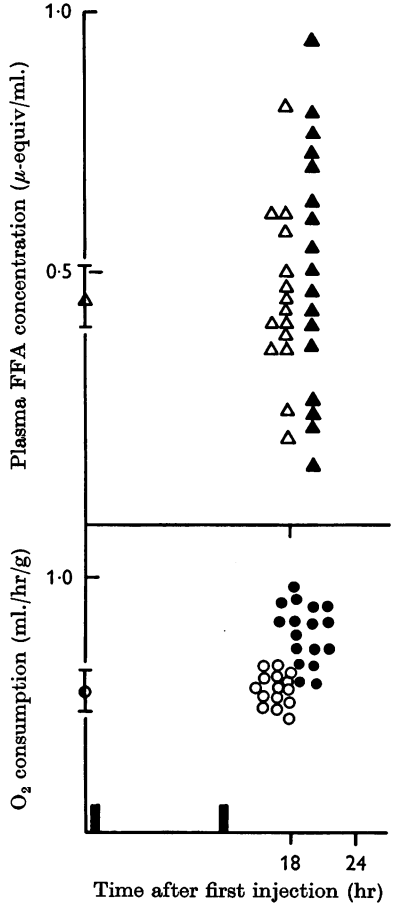

Fig. 3. Effect of injections every 12 hr of 25 μg of triiodothyronine on O_2 consumption (○, ●) and plasma free fatty acid level (△, ▲) in rats made hypothyroid by intraperitoneal ^{131}I. Triiodothyronine injections shown by vertical bars; open symbols, control rats; filled symbols, treated rats.

At 18 hr after the first injection the average O_2 consumption in the treated rats ($1{\cdot}25 \pm 0{\cdot}03$ ml./hr/g) was significantly higher than that in the controls ($1{\cdot}04 \pm 0{\cdot}03$) ($P < 0{\cdot}002$). At all subsequent intervals the average values in the treated rats were higher than those in the controls. At 18, 21, 40 and 48 hr after the first injection, the plasma FFA concen-

trations in the treated rats were not significantly different from those in the controls, but at 66 hr the average value in the treated rats ($1{\cdot}39 \pm 0{\cdot}30$ μ-equiv/ml.) was significantly higher than that in the controls ($0{\cdot}85 \pm 0{\cdot}14$) ($P < 0{\cdot}02$).

Effect of triiodothyronine in hypothyroid rats. Basal O_2 consumption and plasma FFA concentration were measured in a total of thirty-two hypothyroid rats 72 or more days after giving them ^{131}I. Seventeen of the rats were injected with 25 μg of triiodothyronine, followed by a second injection 12 hr later. The remaining fifteen hypothyroid rats were used as controls. Basal O_2 consumption and plasma FFA concentration were measured in the thirty-two rats 18 hr after each animal had had its first injection.

The results of this experiment are shown in Fig. 3. In the whole group of thirty-two rats, the average O_2 consumption before the first injection of triiodothyronine was $0{\cdot}59 \pm 0{\cdot}04$ ml./hr.g and the average plasma FFA concentration was $0{\cdot}44 \pm 0{\cdot}05$ μ-equiv/ml. At 18 hr after the first injection, the O_2 consumption in the treated rats ($0{\cdot}76 \pm 0{\cdot}02$ ml./hr/g) was significantly higher than that in the controls ($0{\cdot}57 \pm 0{\cdot}01$) ($P < 0{\cdot}002$). There was no significant difference between the values for plasma FFA concentration in the treated and control rats.

DISCUSSION

Our results show that serial injections of thyroid hormones may bring about a significant rise in basal O_2 consumption before there is any detectable change in plasma FFA concentration. A dissociation of the two effects is especially noticeable in the hypothyroid rats injected with triiodothyronine (Fig. 3).

In the present work, a significant increase in O_2 consumption was observed in the hypothyroid rats 18 hr after the first injection of triiodothyronine. In comparable experiments, Tata, Ernster, Lindberg, Arrhenius, Pedersen & Hedman (1963) observed a latent period of 20–30 hr after single subcutaneous injections of triiodothyronine. This difference in latency may be due to the fact that our rats received two injections separated by an interval of 12 hr. Rich *et al.* (1959) noted an increase in basal O_2 consumption and in plasma FFA level within 6 hr of an intravenous injection of triiodothyronine into normal men. The difference between their results and ours may be due to a difference between man and the rat in their sensitivity to thyroid hormones or, perhaps, to the difference in the route of administration of the hormone in the two experiments.

Although it has been clearly shown that the rate of oxidation of FFA by skeletal muscle *in vitro* increases as the FFA concentration in the

medium is raised (Fritz *et al.* 1958; Eaton & Steinberg, 1961), there is no unequivocal evidence for a similar relation between concentration and rate of oxidation of FFA in the whole animal. Nor is it certain that the increased rate of FFA oxidation observed at high FFA concentrations *in vitro* is necessarily accompanied by an increase in the over-all consumption of O_2 by skeletal muscle. Indeed, since FFA are known to inhibit glucose utilization by skeletal and heart muscle (Randle, Garland, Hales & Newsholme, 1963), it might be expected that the increased FFA oxidation brought about by raising the FFA concentration would tend to be offset by a fall in glucose oxidation. Eaton (1964) found no increase in the total O_2 consumption of skeletal muscle *in vitro* when the rate of oxidation of palmitate was increased by raising the FFA concentration in the medium. On the other hand, Challoner & Steinberg (1966) found an increase in the total O_2 consumption of perfused rat hearts when the concentration of palmitate in the perfusing fluid was raised. This finding provides some support for the hypothesis that the rise in plasma FFA concentration found in thyrotoxicosis or after injections of thyroid hormone contributes to the rise in basal O_2 consumption. Against this, however, Eaton, Steinberg & Thompson (1965) have shown that infusions of nicotinic acid or glucose, in amounts sufficient to lower the plasma FFA level by suppressing the output of FFA from adipose tissue, have no effect on the basal O_2 consumption of human subjects pretreated with triiodothyronine. Our own results, showing a dissociation between plasma FFA concentration and O_2 consumption in the early stages of a course of hormone treatment, are difficult to reconcile with the view that the whole of the effect of thyroid hormone upon basal O_2 consumption is mediated by a rise in plasma FFA concentration. But they do not exclude the possibility that the further increase in O_2 consumption that occurs when the treatment is prolonged for several days (Fig. 1) is due in part to a rise in the plasma FFA level.

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