Starvation-Induced Decreased Sensitivity of Resting Metabolic Rate to Triiodothyronine

Abstract. *The decrease in resting oxygen consumption induced by starvation was found to occur not only in euthyroid rats but also in hypothyroid and even in hypothyroid animals treated with triiodothyronine. Furthermore, the effectiveness of triiodothyronine was decreased when given to starved hypothyroid animals.*

The activity of many hormones is not only dependent on the availability of the hormones, but can also be influenced by the sensitivity of target tissues. The latter aspect of hormone activity, with respect to thyroid hormones, was studied in the present work. As a measure of tissue sensitivity, we used the change in oxygen consumption per minute per 100 grams of body weight ($\dot{V}O_2$) during starvation, since O_2 consumption is known to decrease during caloric deprivation. Thyroid hormone metabolism also changes during starvation: in man triiodothyronine (T_3) production is reduced (*1*), and in rat serum T_3 and thyroxine (T_4) concentrations decrease, reflecting a decrease in T_4 production and metabolism (*2*). We therefore determined the extent to which the decrease in $\dot{V}O_2$ is related to decreased thyroid hormones and to changes in tissue sensitivity.

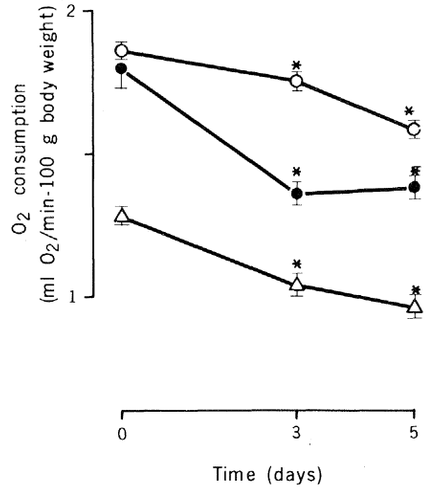

Fig. 1. The $\dot{V}O_2$ (expressed as milliliters of O_2 per minute per 100 g of body weight) was measured in male Wistar rats (300 to 350 g) when they were fed and after 3 and 5 days of starvation. Symbols: ○, intact euthyroid rats (N = 10); △, hypothyroid rats (N = 10); ●, hypothyroid rats injected daily for 10 days before and each day during the experiments with 365 ng of T_3 per 100 g of body weight (N = 5). Hypothyroidism was induced surgically at 6 to 8 weeks of age and was followed 7 days later by an injection of 150 μCi of ^{131}I. To allow growth, we gave these animals T_3 in their drinking water until 4 weeks prior to the experiments. The value for $\dot{V}O_2$ decreased significantly after 3 and 5 days of starvation. Asterisks indicate (P < .01 to < .001); vertical bars indicate standard errors of the mean.

These studies were performed in male Wistar rats. We measured $\dot{V}O_2$ in a closed-circuit calorimeter at thermoneutrality (29°C) (*3*). First we examined the relation between thyroid hormone production rates and $\dot{V}O_2$. In ten intact euthyroid animals fasted for 5 days we confirmed the decrease in basal $\dot{V}O_2$ (Fig. 1). However, in ten hypothyroid animals with unmeasurable serum T_3 and T_4 values and decreased $\dot{V}O_2$ in the fed state, starvation induced a further decrease in $\dot{V}O_2$. In intact euthyroid rats starved for 3 days, the percentage decrease of $\dot{V}O_2$ was 6.7 ± 1.8 percent (*4*); after 5 days of starvation the value was 14.7 ± 2.6 percent. In hypothyroid rats $\dot{V}O_2$ decreased by 18.1 ± 3.4 and 24 ± 4.3 percent after 3 and 5 days of starvation, respectively. There was no significant difference in the percentage decrease of $\dot{V}O_2$ between the two groups (P > .05 < .1). Another group of five hypothyroid rats received a daily injection of T_3 (365 ng per 100 g of body weight) starting 10 days before and continuing throughout the period of starvation. This dose normalized the $\dot{V}O_2$ and the concentration of thyroid-stimulating hormone present in the serum. In these T_3-substituted animals, $\dot{V}O_2$ decreased after 3 days of starvation by 29.0 ± 3.5 percent. This decrease was even greater than that in the intact euthyroid or hypothyroid rats (P < .001). This difference is not easy to explain; however, one could postulate that T_4 and T_3 together have an unknown regulatory effect that is not exhibited by T_3 alone.

Although our data excluded a direct control of $\dot{V}O_2$ by thyroid hormones, the rapid decline of $\dot{V}O_2$ in the presence of continued T_3 administration suggested an altered tissue response. In a second group of experiments we used hypothyroid animals because of their increased sensitivity to T_3 (*5*). First we studied the changes in $\dot{V}O_2$ for 72 hours in animals given a single injection of T_3 (365 ng per 100 g of body weight, injected intraperitoneally). In another experiment, 15 animals were divided into two groups. The rats in one group (N = 8) received a single injection of T_3 while being fed on their usual pelleted rat chow and after 2 weeks they were starved for 3 days and then they received a second dose of T_3. In the other group (N = 7) of animals we used the reverse procedure, that is, we first starved the animals for 3 days. The results (*5*) showed that only those animals in the fed state responded to their dose of T_3, the maximum response being at 12 hours after the injection. This response remained significantly increased for 48 hours.

We then constructed a dose-response curve for fed animals and animals starved for 3 days. Groups of eight animals received half, five, and 25 times the replacement dose of 365 ng of T_3 per 100 g of body weight (Fig. 2). In starved animals, an approximately five to ten times greater dose of T_3 was needed to demonstrate a significant increase in $\dot{V}O_2$ compared to the fed animals. With the highest dose, the $\dot{V}O_2$ of the starved animals increased further and reached the level of fed animals. Even with this dose the maximum response was still 12 hours after injection.

We conclude from these experiments that starvation altered thyroid hormone activity in at least two ways. Clearly, the effective serum concentrations of T_3 and T_4 were decreased by starvation. In addition, starvation decreased the response to a given dose of T_3. This effect seemed to be primarily related to a decreased

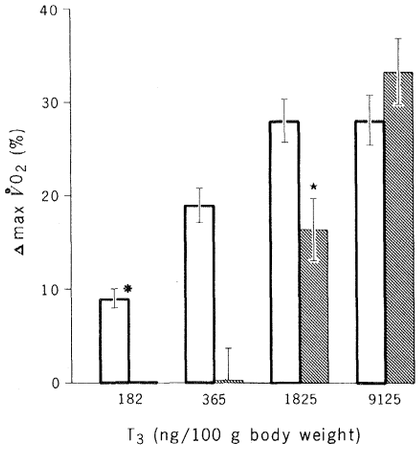

Fig. 2. The maximum increase of $\dot{V}O_2$ over baseline is shown for single injections of T_3 in hypothyroid rats (300 to 350 g). The open bars represent the results from fed animals; the hatched bars the results during starvation (groups of eight rats). In the fed state half of the replacement dose (182 ng/100 g) elicited a significant response. During starvation, five to ten times higher doses of T_3 (1825 ng/100 g) were necessary to elicit a significant response. This response however was still significantly lower than in the fed state. Asterisks indicate P < .05. With the highest dose, the response was the same in fed and fasted animals. Vertical bars indicate standard errors of the mean.

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sensitivity to T_3, particularly with replacement doses. The capacity to respond, however, seemed not to be altered because high doses of T_3 elicited the same response in starved and fed animals. The decrease of $\dot{V}O_2$ in starvation, in the presence of adequate replacement doses of T_3, favors the hypothesis that the sensitivity of $\dot{V}O_2$ to T_3 provides a finer and more efficient control on thyroid hormone activity than that brought about by alterations in thyroid hormone metabolism.

Three groups of investigators (*6*) have recently found a decrease in hepatic nuclear T_3 receptors in starved rats. Tata *et al.* (*7*) described in this situation a lack of stimulation of protein synthesis, even when supraphysiological doses of T_3 (50 μg/100 g) were given. More specifically, Tarentino *et al.* (*8*) and Dillmann *et al.* (*9*) reported that the induction of the cytosolic enzyme, malic dehydrogenase, known to be inducible by thyroid hormones, is blocked by starvation. The similarity between these subcellular changes and thermogenesis is striking. Even though thermogenesis is considered to be one of the major actions of thyroid hormones, it represents a late event and many unrelated factors could modify the interaction of these hormones and thermogenesis. Although this relationship may be complex, its physiological relevance can hardly be doubted and might serve as the basis for more analytical studies of the mechanisms involved in thyroid hormone action.

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4. Statistical analysis was done according to the method of L. Sachs, *Angewandte Statistik* (Springer-Verlag, Heidelberg, 1974), pp. 391–411. The data are expressed as means ± standard error; nonparametric tests were used (comparison of two groups: Mann-Whitney test; of multiple groups: analysis of variance and test by Scheffé).
5. The values for $\dot{V}O_2$ before and 6, 12, 48, and 72 hours after the injection were, respectively, as follows. Hypothyroid rats (when fed): 1.32 ± 0.01; 1.34 ± 0.03; 1.58 ± 0.03 ($P < .001$); 1.38 ± 0.02 ($P < .05$); and 1.28 ± 0.02 ml of O_2 per minute per 100 g of body weight. Hypothyroid rats (when starved): 1.16 ± 0.02; 1.15 ± 0.02; 1.17 ± 0.03; 1.07 ± 0.03, and 1.04 ± 0.03. Euthyroid rats (when fed): 1.66 ± 0.03; 1.69 ± 0.03; 1.74 ± 0.03; 1.68 ± 0.04, and 1.60 ± 0.04 (analysis of variance and multiple comparisons of means by Scheffé). Unless otherwise specified, the values were not significant in comparison to the baseline.
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Sister Chromatid Exchanges in Human Lymphocytes After Exposure to Diagnostic Ultrasound

Abstract. *The frequency of sister chromatid exchanges increased in freshly isolated human lymphocytes as well as in a continuously growing lymphoblast line by exposure to diagnostic levels of ultrasound for 30 minutes. The results confirm previous findings indicating that ultrasound of diagnostic intensities can affect the DNA of animal cells.*

The use of ultrasonic studies in medical diagnosis has greatly increased, particularly in obstetrics. It is estimated that by the mid-1980's virtually all infants in the United States will have been exposed to ultrasound in utero (*1*). Diagnostic levels of ultrasound are now regarded as innocuous to the developing fetus. Investigators have been unable to demonstrate deleterious effects of ultrasound on human chromosomes (*2–7*). However, as recently reported (*8, 9*), exposure to pulsed ultrasound generated by a commercial diagnostic instrument appeared to have deleterious effects on rapidly growing mammalian and insect tissues. We have detected disturbances in HeLa cell DNA and in the growth characteristics of C3H mouse cell cultures exposed to ultrasound in the diagnostic range (*10*). Here we report the effects of ultrasound on the incidence of sister chromatid exchange (SCE), a sensitive direct indicator of chromosome damage (*11, 12*). Exposure to diagnostic levels of ultrasound increased the frequency of SCE's in freshly isolated human lymphocytes as well as in a continuously growing human lymphoblast line (SKL-7).

Human blood containing heparin (10 U/ml) was allowed to stand for 60 minutes so that the leukocytes were separated by gravity from the erythrocytes. The supernatant serum containing lymphocytes was dispersed into growth medium at a concentration of approximately 2×10^5 white cells per milliliter of medium. The lymphocytes were grown in the dark in McCoy's 5A medium containing 15 percent fetal bovine serum, phytohemagglutinin-16 (PHA-16, 1 μg/ml; Burroughs Wellcome), and heparin (5 to 10 U/ml). Bromodeoxyuridine (BrdU), at a final concentration of 5 to 10 μg/ml (Sigma), was added approximately 22 hours after the culture was initiated. The lymphocytes were harvested 72 hours later, after completion of two rounds of DNA replication. Colcemid (0.2 μg/ml) was added for 2 hours to arrest cells in metaphase, and the cells were then resuspended in 0.075*M* KCl for 10 minutes and fixed in three changes of a 3:1 mixture of absolute alcohol and glacial acetic acid. The human SKL-7 lymphoblast line was grown under dark conditions in McCoy's 5A medium containing 20 percent fetal bovine serum in the presence of 10 μg of BrdU per milliliter for two division cycles (approximately 36 hours) before the cells were harvested, as in the experiments with peripheral blood lymphocytes.

Differential staining was obtained by a modification of the fluorescence plus Giemsa technique (*13*). Metaphase spreads were stained with Hoechst 33258 (1 μg/ml) for 10 minutes, exposed to an ultraviolet light source (General Electric, G8T5) for 15 minutes at a 5-cm distance, and then stained with 10 percent Giemsa solution in phosphate buffer at *p*H 7 for 5 minutes.

The cells were exposed to ultrasound between their first and second divisions (48 hours after addition of the mitogen in the fresh lymphocyte culture, and at 18 hours in the lymphoblast line). This was done because it has been shown that cells must pass through S-phase in order to express ultraviolet damage by SCE (*14, 15*). In a number of experiments, 0.1 μg of mitomycin per milliliter was added to portions of the lymphocytes in separate cultures to provide control preparations with high frequencies of SCE's.

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