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Effects of Thyroxine, Epinephrine and Cold Exposure on Lipolysis in Genetically Obese (ob/ob) Mice[1] (40295)

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Mayer and Barrnett (1) observed that genetically obese (ob/ob) mice were unable to withstand exposure to a cold environment and that the administration of thyroid hormone before cold exposure slightly prolonged their survival. More recent studies provided evidence that ob/ob mice were hypothyroid (2, 3) and that thyroid hormone administration corrected the observed hypothermia during cold exposure (4). Experimentally produced hypothyroidism in rats prevents normal epinephrine-stimulated lipolysis (5), and *in vitro* studies using adipose tissue from ob/ob mice have demonstrated a similar reduction in epinephrine-stimulated free fatty acid (FFA) release (6–8). These observations support the hypothesis that hypothyroidism in ob/ob mice results in defective lipolysis, thus limiting FFA as a substrate for thermogenesis during cold exposure. However, *in vivo* studies in ob/ob mice at ambient temperature failed to show defective lipolysis either in response to catecholamines (9) or during fasting (10).

In order to examine this apparent discrepancy between the *in vivo* and *in vitro* data in the literature and to study the metabolic effects of cold stress in ob/ob mice more precisely, an experiment was designed to investigate the hormonal influences on lipolysis and the relevant parameters of carbohydrate metabolism during cold exposure in these animals. Specifically, this study measured the effects of pharmacological doses of thyroxine (T_4) on both *in vivo* and *in vitro* FFA release in cold-exposed ob/ob mice in comparison to the effects in non-obese mice. In addition, the effect of T_4 treatment on epinephrine-stimulated FFA release from adipocytes was assessed.

Materials and methods. Male weanling mice of the obese strain, C57BL/6J-ob were purchased from Jackson Laboratories, Bar Harbor, ME. The obese (genotype, ob/ob) and non-obese (genotypes, +/ob and +/+) mice were fed an experimental diet containing 20% casein, 32% glucose, 32% sucrose, 10% corn oil, 5% salts (11), 0.5% vitamin mix (12) and 0.2% choline chloride. At 18 weeks of age and prior to T_4 treatment and cold exposure, nonfasting blood samples were taken from the retro-orbital sinus in heparinized tubes for basal glucose and insulin determinations. At 24 hr before cold exposure, half of the obese and nonobese mice were injected ip with 100 μg L-thyroxine (Sigma Chemical Co., St. Louis, MO) in 0.25 ml of 0.9% NaCl adjusted to pH 12 with NaOH, and the remaining half were injected with alkaline NaCl alone. These injections were repeated immediately before cold exposure. After 90 min at 4°, animals were killed by decapitation and blood collected in heparinized tubes for determinations of plasma glucose (13), insulin (14), and FFA (15).

In vitro lipolysis was measured in preparations of adipocytes isolated from 1 g portions of epididymal adipose tissue by the method of Rodbell (16). The washed fat cells were suspended in 9 ml of Krebs-Ringer bicarbonate buffer containing 3% fatty acid-free albumin. Three ml of this suspension was used to determine DNA (17). For determination of FFA release, 0.9 ml samples of fat cell suspension were incubated in duplicate vials for two hours at 37° in 2.1 ml Krebs-Ringer bicarbonate buffer containing 3% fatty acid-free albumin with and without 1.1×10^{-5} *M* epinephrine (Fisher Scientific Co., Fairlawn, NJ). At the end of the 2-hr incubation period, these test samples containing fat cells and incubation medium were cooled to 4°, homogenized and extracted for lipid (18). Zero time samples were prepared in duplicate by adding 0.9 ml portions of fat cell

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suspension to 2.1 ml of buffer at 4°. They were homogenized without incubation and the lipid was immediately extracted (18). The FFA content of lipid extracts from zero time and test samples was determined (15) and the total FFA content of the homogenates of fat cells plus incubation medium was calculated. The FFA in zero time samples was measured to provide an index of intracellular levels of FFA after cold exposure, as well as for calculation of FFA release from triacylglycerol during the incubation period, since the FFA content of the zero time samples was subtracted from that of the incubated test samples. FFA release was expressed as μeq FFA/μg DNA/hr. Statistical comparisons were made by Student's *t* test (19).

Results. The effects of T_4 treatment on plasma glucose, insulin and FFA after cold exposure for 90 min are presented in Table I. T_4 treatment had no statistically significant effect on any of these parameters in either obese or nonobese mice. However, there was a tendency toward higher plasma FFA values in T_4-treated mice, particularly for the obese.

Under non-fasting conditions at ambient temperature, obese mice had plasma glucose values of 228 ± 20 (mean ± SE) mg/dl and insulin values of 96 ± 8 μU/ml, while nonobese mice had glucose values of 187 ± 19 mg/dl and insulin values of 24 ± 1 μU/ml. In comparison to these basal values, the elevated glucose and depressed insulin values shown in Table I indicate the response to the stress of cold exposure in both obese and nonobese mice. The higher plasma glucose and insulin values of the obese in comparison to nonobese mice under basal conditions are characteristic of this genotype. These same differences were observed in cold-exposed obese and nonobese mice.

The intracellular concentrations of FFA in isolated adipocytes are also shown in Table I. Adipocytes from obese mice treated with T_4 had a significantly higher concentration of FFA than those from untreated obese mice. In contrast, fat cells from nonobese mice had similar FFA concentrations regardless of treatment, and these were not significantly different from the mean value for untreated obese mice. This elevated zero time FFA concentration only in T_4-treated obese mice suggests an increased *in vivo* lipolytic response to T_4 in these animals.

Figure 1 illustrates the results of the meas-

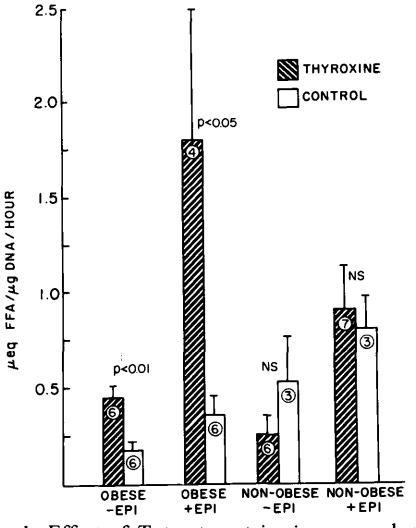

FIG. 1. Effect of T_4 treatment *in vivo* on production of FFA by hydrolysis in fat cells isolated from epididymal adipose tissue of cold-exposed mice and incubated with (+) and without (−) 1.1×10^{-5} *M* epinephrine (EPI). Number of animals are indicated on each column, bars represent SEM, and P values compare differences between paired column means.

TABLE I. EFFECT OF THYROXINE TREATMENT ON OBESE AND NONOBESE MICE EXPOSED TO THE COLD (4°) FOR 90 MIN.

Group	Treatment	Plasma glucose (mg/100 ml)	Plasma insulin (μU/ml)	Plasma FFA (μeq/liter)	Adipocyte FFA (μeq/μg DNA)
Obese	Saline	379 ± 54 (6)[a]	24 ± 5 (6)	722 ± 142 (6)	0.25 ± 0.08 (6)
	Thyroxine	375 ± 64 (6)	23 ± 3 (6)	855 ± 208 (6)	1.33 ± 0.43 (6)
		NS	NS	NS	$P < 0.05$
Non-obese	Saline	256 ± 58 (6)	17 ± 1 (6)	697 ± 75 (4)	0.37 ± 0.09 (3)
	Thyroxine	237 ± 11 (7)	18 ± 1 (7)	716 ± 65 (5)	0.41 ± 0.05 (7)
		NS	NS	NS	NS

[a] Values are mean ± SE. Number of mice sampled in parentheses.

urements of *in vitro* lipolysis after cold exposure for 90 min. Lipolysis in isolated adipocytes is presented as μeq FFA released per μg DNA per hr of incubation in order to express the results in terms related to cell number rather than cell mass. The data from the untreated control mice show that adipocytes from cold-exposed obese mice had reduced lipolytic activity in comparison to those from nonobese mice. The addition of epinephrine to the incubation medium of fat cells from both obese and nonobese control mice increased FFA release, but the values were not significantly different from those under non-stimulated conditions. The epinephrine-stimulated release of FFA from the fat cells of untreated obese mice remained significantly ($P < 0.05$) lower than the release from fat cells of untreated nonobese mice.

T_4 treatment of obese mice before cold exposure had a striking effect on *in vitro* lipolysis in contrast to the small but not significant effect in nonobese mice (Fig. 1). Adipocytes from T_4-treated obese mice released significantly more FFA both in the presence ($P < 0.05$) and in the absence ($P < 0.01$) of epinephrine in comparison to adipocytes from corresponding untreated obese mice. The response to epinephrine of adipocytes from T_4-treated obese mice was more than three times greater than that of fat cells from untreated control obese mice. However, adipocytes from nonobese mice showed no increase in FFA release when treated with T_4 prior to cold exposure, although there was a significant ($P < 0.05$) rise in FFA release in response to epinephrine in T_4-treated nonobese mice.

Discussion. From the data presented it is apparent that the failure of ob/ob mice to survive during cold exposure was not attributable to insufficient circulating FFA since plasma values in obese mice were comparable to those in nonobese mice after cold exposure for 90 min at 4°. Other studies in this laboratory (20) showed that a more prolonged cold exposure (up to 4 hr) also resulted in similar plasma FFA values in obese and nonobese mice. The plasma FFA values obtained in the present experiment were similar to those found by Abraham *et al.* (9) for obese and nonobese mice after norepinephrine administration and after a 24-hr fast. Their study and our study during cold stress showed normal *in vivo* lipolysis for ob/ob mice. Thyroid hormone treatment, which is known to alleviate the hypothermia in ob/ob mice (4) also did not significantly alter FFA values *in vivo*.

The *in vitro* results indicated an inhibition of basal and epinephrine-stimulated lipolysis in adipocytes from ob/ob mice after cold stress, a condition in which lipolysis should be maximally stimulated. This was similar to the inhibition of FFA release from adipose tissue of ob/ob mice found at ambient temperature by other investigators (6–8), except that Marshall and Engel (6) did not find inhibition under basal conditions (without epinephrine). In addition, Herberg *et al.* (21) reported increased release of FFA from epididymal adipose tissue under both basal and epinephrine-stimulated conditions. However, these latter investigators pre-incubated adipose tissue in Krebs-Ringer bicarbonate buffer with albumin and glucose (no insulin) before measuring lipolysis. It is possible that insulin was "washed out" by this preincubation and no longer exerted its known inhibitory effect on lipolysis (22). Otto *et al.* (3) found elevated lipolysis as measured by glycerol release from adipose tissue of ob/ob mice under basal conditions, but reduced sensitivity to epinephrine and thyroid hormone administration. Although FFA release should parallel glycerol release during lipolysis, this may not occur under these conditions differing from ours, in which adipose tissue in contrast to adipocytes were incubated in the presence of glucose.

The coexistence of our *in vivo* results showing similar plasma FFA after cold exposure in both T_4-treated and untreated obese and nonobese mice and the *in vitro* results showing variable FFA release from fat cells is possible for several reasons. First, while fat cells from untreated obese mice released less FFA on a cell number basis, the increased number of fat cells in these obese mice (23) could be sufficient to maintain plasma FFA at similar concentrations to those in nonobese mice. Also, *in vitro* conditions are not analogous to those *in vivo*. For example, it is possible that a more rapid turnover of circulating FFA or an inhibition of FFA release into the blood occurs *in vivo*. FFA determinations

were also made on homogenates of fat cells and incubation medium and, therefore, represented fatty acid release from triacylglycerol, but not necessarily release from fat cells. Cushman *et al.* (24) have shown that under some circumstances intracellular FFA concentrations increased without increasing FFA release from the cell. Since the intracellular FFA concentration in the T_4-treated obese mice was significantly higher than in any other group, as determined from the zero time samples, it is possible that all of the FFA released during lipolysis *in vivo* were not released into the circulation.

The significant increase in FFA release from lipid stores in adipocytes in response to epinephrine in both nonobese and obese mice treated with T_4 is in agreement with observations in other rodents in which T_4 potentiated the action of epinephrine (25) and in which the lipolytic response of adipocytes *in vitro* was affected by the *in vivo* thyroid status of the animal (5, 26). However, the accentuated epinephrine-stimulated lipolysis in ob/ob mice treated with T_4 as compared to the lesser effect in nonobese mice suggests an increased sensitivity to epinephrine in obese mice once the hypothyroid status is corrected.

Although this study did not directly test the hypothesis that decreased thermogenesis in ob/ob mice during cold exposure was a result of decreased FFA availability, the evidence of reduced lipolysis by adipocytes which was reversed by T_4 treatment supports this hypothesis. Although circulating FFA concentrations were not significantly affected by T_4 treatment, it is possible that T_4 potentiated the rate of FFA release from adipose tissue *in vivo* as well. A similarly increased rate of FFA uptake and oxidation could allow for increased thermogenesis, while maintaining plasma FFA concentrations constant.

Summary. Treatment of ob/ob mice with T_4 24 hr prior to cold exposure did not alter plasma concentrations of glucose, insulin and FFA during cold exposure although ob/ob mice remained hyperglycemic and hyperinsulinemic when compared to nonobese mice. FFA content of and FFA release from isolated adipocytes were significantly elevated in T_4-treated obese mice after cold stress as compared to untreated obese mice. T_4 treatment also produced a marked increase in epinephrine-stimulated FFA release from fat cells of obese mice *in vitro*.

These results indicate that correction of the hypothyroid status of ob/ob mice with pharmacological doses of T_4 improved the *in vitro* lipolytic response of fat cells, but did not alter the circulating concentrations of important energy sources for thermogenesis *in vivo*.

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