Early Treatment of Obese (ob/ob) Mice with Triiodothyronine Increases Oxygen Consumption and Temperature and Decreases Body Fat Content (43814)

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Abstract. **An early abnormality in the ob/ob mouse is a low circulating level of the thyroid hormone, triiodothyronine (T_3). The possibility was explored that early T_3 treatment of ob/ob mice will increase oxidative metabolism and lower body fat content. Doses of T_3, ranging from 0.0 to 25.0 μg/100 g body wt were injected, ip, into ob/ob and non-ob/ob mice daily from 3 weeks until 6 weeks of age. Food intake was equalized across all groups to that consumed by non-ob/ob saline-treated group. At 6 weeks of age, body weight, serum concentrations of thyroxine (T_4), T_3, insulin and glucose, oxygen consumption, colonic temperature, and body composition were analyzed. T_3 treatment decreased body weight, increased body oxygen consumption, increased colonic temperature, and decreased body fat without a significant change in body protein in ob/ob mice. T_3 treatment also increased serum T_3, and decreased serum T_4, insulin, and glucose concentrations in ob/ob mice. Because total body protein did not change as a result of T_3 treatment, the increased oxidative metabolism due to T_3 treatment was probably via the change of metabolic activity of the total lean body mass or the specific metabolically active tissues in the ob/ob mice, such as brown adipose tissue, liver, or muscle.** [P.S.E.B.M. 1994, Vol 207]

The genetically obese hyperglycemic (ob/ob) mouse has several metabolic and endocrine disorders as early as 2 weeks of age and is a useful model of some types of human obesity. These mice exhibit depressed oxygen consumption (1–3), low body temperature (2, 3), and higher body fat content (1). Thyroid hormones are also low during thermal stress (4, 5). Ohtake *et al.* (6), however, reported that thyroid-pituitary axis of ob/ob mice was normal and that hypothermia was not due to hypothyroidism. Also, these mice maintain elevated levels of food intake over much of their adult life (7). Dubuc (8) reported that these mice, regardless of feeding regimen, maintained absolutely higher carcass fat levels than their lean littermates.

Thyroid hormone is required for normal growth and tissue development and is an important regulator of energy metabolism. Triiodothyronine (T_3) is the metabolically active form of thyroid hormone and known to be 3 to 8 times more potent than thyroxine (T_4). T_3 also exerts negative feedback inhibition of thyroid-stimulating hormone (TSH) secretion by the pituitary. Serum T_3 plays a more important direct role than serum T_4 in the feedback regulation of serum TSH in euthyroid or mildly hypothyroid rats (9). Varied results (either lower, higher, or the same as in non-ob/ob mice) in concentrations of thyroid hormones in serum of ob/ob mice have been reported (10–13).

Hypothyroidism is a very early detectable symptom in ob/ob mice, possibly because of abnormal regulation of the hypothalamic-pituitary-thyroid at early ages (4). Insensitivity to the effect of circulating hormones by nonthyroid tissues might be present at later ages (12, 13). We reported that T_3 treatment increased animal oxygen consumption (14). In that report, insulin-stimulated and non-insulin-stimulated glucose utilization in diaphragm of ob/ob mice was also increased by T_3 treatment. The ob/ob mice required higher doses of T_3 than did non-ob/ob mice to increase diaphragm

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glucose utilization. This finding was consistent with the concept of T_3 resistance by the tissues. Van der Tuig *et al.* (15) reported that thyroxine treatment increased heat production and decreased body weight in ob/ob mice. Possible changes in body composition were not examined. In studies with obese humans, T_3 treatment increased the metabolic rate (16), decreased body weight, and increased serum T_3 and TSH (17). Wolman *et al.* (18) reported that body weight loss after T_3 treatment in obese patients was from lean body mass rather than body fat.

Past experiments used pharmacological doses of T_3 for a short period of time. The experiments reported here in pair-fed ob/ob mice explore the possibility that early physiological levels of T_3 treatment of ob/ob mice will increase oxidative metabolism and diminish the accumulation of body fat.

Materials and Methods

Animals and Diets. Male obese (C57BL/6J-ob/ob) mice and their nonobese (non-ob/ob) littermates were obtained at 2 weeks of age from the animal colony of the Food Science and Human Nutrition department of Iowa State University. At 2 days of age, litter size was adjusted to 7–9 mice per litter. Genetically ob/ob and non-ob/ob mice were identified 16 days of age on the basis of their individual oxygen consumption (3). At 3 weeks of age, all animals were housed individually in solid-bottom, plastic cages with aspen wood shavings for bedding in an animal room at 23° ± 1°C with a dark cycle from 0600 to 1800 hr and a light cycle from 1800 to 0600 hr. All animals were allowed free access to tap water. Food intake was equalized daily across all groups to the average consumed by the non-ob/ob saline-treated group on the preceding day. The mice were fed a high-carbohydrate diet during the dark period from 1000 to 1600 hr so that meal patterns would be identical in all groups as an adjustment for possible meal-feeding effects in the restricted groups. In our experience, the mice adapt to the feeding program within 3–4 days. The diet consisted of the following: casein, 20.0 g; DL-methionine, 0.3 g; AIN mineral mix 76,[2] 3.5 g; AIN vitamin mix 76,[3] 1.0 g; choline chloride, 0.2 g; corn oil, 5.0 g; fiber, 5.0 g; dextrose, 60.0 g; dextrin, 5.0 g per 100 g diet. Diet ingredients were purchased from ICN Biomedicals (Costa Mesa, CA). The diet was pelleted to facilitate the measurement of food consumption. Food consumption and body weight were measured daily until 6 weeks of age.

Treatments. Obese (ob/ob) and nonobese mice were divided into seven groups. Nontreated ob/ob and non-ob/ob mice were injected with 9 g/l saline, pH 9.1. Six levels of L-3, 3′, 5-triiodothyronine (T_3) (Calbiochem-Behring Co., La Jolla, CA) (2.5, 5.0, 7.5, 10.0, 12.5, and 25.0 μg/100 g body weight) dissolved in 0.9% saline (final pH 9.1) were injected ip daily, between 0900 and 0945 hr from 3 to 6 weeks of age. Body weight was measured at the time of the treatment. Oxygen (O_2) consumption, colonic temperature, and final body weight were measured at 6 weeks of age.

Oxygen Consumption. All measurements of O_2 consumption were conducted in the animal room. O_2 consumption was measured before treatment and at the end of treatment period by differential constant pressure respirometry, as reported by Kaplan and Leveille (3), with the apparatus submerged in a water bath at 25°C. Duplicate measurements were made for each mouse. O_2 consumption was corrected to standard temperature and pressure.

Colonic Temperature. Colonic temperature was measured immediately after removal of the mouse from the chamber in which O_2 consumption was measured by using a thermister with a round tip applicator (YSI model 43TA single-channel telethermometer; Yellow Springs Instrument Co., Yellow Springs, OH). Each mouse was held in a mouse restrainer, and the thermister was inserted about 1 cm into the colon and held 15 sec until the needle of the meter was stabilized.

Body Composition. The contents of the gastrointestinal tracts were removed from mice, and the carcasses were stored at −20°C for future analysis. Each frozen carcass was chopped into small pieces and homogenized using a 1.14-l Waring blender with addition of sufficient (approximately 100 ml) water so that the blender could operate freely. The entire homogenate was transferred into a preweighed dry flask, frozen at −20°C, and was subsequently lyophilized. Lyophilized samples were weighed and stored dessicated at 4°C until analyzed for protein and fat. For the protein determination, weighed samples were digested in 36 *N* H_2SO_4, 3 g/l SeO_2 using the digestion system (Tecator ab, Hoganas, Sweden) at 420°C for 1 hr or until heated samples were clear. The resultant $(NH_4)_2SO_4$ was determined colorimetrically (19). Lipid was extracted from weighed samples by chloroform-methanol (2:1, v/v) and determined gravimetrically (20).

Serum Analysis. At 6 weeks of age, all mice were killed by decapitation between 0930 and 1000 hr,

[2] AIN mineral mix 76, ICN Biomedicals, Costa Mesa, CA. Composition of nutrients per kg mineral mixture: calcium phosphate dibasic, 500 g; sodium chloride, 74 g; potassium citrate monohydrate, 220 g; potassium sulfate, 52 g; magnesium oxide, 24 g; manganous carbonate (43%–48% Mn), 3.5 g; ferric citrate (16%–17% Fe), 6 g; zinc carbonate (70% ZnO), 1.6 g; cupric carbonate (53%–55% Cu), 0.3 g; potassium iodate, 0.01 g; sodium selenite, 0.01 g; chromium potassium sulfate, 0.55 g; sucrose, finely powdered, 118 g.

[3] AIN vitamin mix 76, ICN Biomedicals, Cost Mesa, CA. Composition of nutrients per kg vitamin mixture: thiamine HCl, 600 mg; riboflavin, 600 mg; pyridoxine HCl, 700 mg; nicotinic acid, 3.0 g; D-calcium pantothenate, 1.6 g; folic acid, 200 mg; D-biotin, 20 mg; cyanocobalamine, 1.0 mg; retinyl palmitate (250,000 IU/g), 1.6 g; DL-α-tocopherol acetate (250 IU/g), 20 g; cholecalciferol (400,000 IU/g), 250 mg; menaquinone, 5 mg; sucrose, finely powdered, 972.9 g.

which was prior to the start of the feeding period. Blood was collected in microcentrifuge tubes, allowed to clot, and centrifuged. Serum was collected and saved frozen at −20°C until analysis for T_3, T_4, insulin, and glucose. Because a small volume of serum was collected from each mouse, sera from three mice were pooled to create one sample for the assays in serum. The serum samples were collected from several sets of experiments to create enough sample numbers in a treatment group. Serum T_3, T_4, and insulin were assayed using the radioimmunoassay (RIA) procedures as described in the RIA kits with human sera standards; Amerlex-M T_4, Amerlex-M T_3, and insulin RIA kit, respectively (Amersham, Arlington, IL). Serum glucose was assayed using glucose hexokinase reagent kit (Sigma Chemical Co., St. Louis, MO) in which glucose was assayed using the coupled enzymatic reactions catalyzed by hexokinase and glucose-6-phosphate dehydrogenase (21).

Statistics. Overall significance was determined by two-way analysis of variance (22, 23). Main effects include T_3 treatment and phenotype. Significant main effects and interactions were identified, and means within these main effects were tested with the protected *t* test which uses the mean square of the error term from the analysis of variance (ANOVA). Probability of 0.05 or less was taken to be statistically significant.

Results

Body weight and Food Consumption. At 6 weeks of age, no significant differences in body weight between ob/ob and non-ob/ob mice in saline-treated groups were observed, mainly because of equalized food intake (Fig. 1). T_3 treatment decreased body weight in both phenotypes but decreased more in ob/ob mice. Total food consumption during the experimental period was similar in both phenotypes because food was equalized across all ob/ob and non-ob/ob groups throughout the experimental period (Table I). The average weekly food consumption per mouse in all groups were 8.3 g (4 weeks), 17.1 g (5 weeks), and 21.7 g (6 weeks).

Serum Concentrations of Insulin and Glucose. No significant differences in serum insulin and glucose were observed between saline-treated ob/ob and non-ob/ob mice (Fig. 2). T_3 treatment decreased both serum insulin and glucose in both phenotypes. Serum glucose was significantly decreased at all doses of T_3.

Serum Concentrations of T_4 and T_3. Serum T_4 was lower in saline-treated ob/ob mice (Fig. 3). All doses of T_3 significantly decreased serum T_4 in both phenotypes. No differences in serum T_4 levels were observed in T_3-treated ob/ob and non-ob/ob mice. Serum T_3 was lower in saline-treated ob/ob mice (Fig. 3). T_3 treatment increased serum T_3 significantly in ob/ob

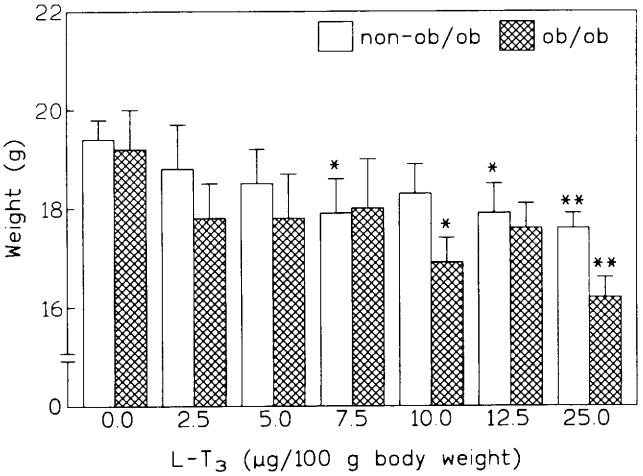

Figure 1. Body weight of ob/ob and non-ob/ob mice at 6 weeks of age. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. Values are the means ± SEM for eight to nine mice per group. Mean square error is 3.97. Significant differences between means were determined by using the protected *t* test; *significantly different from 0.0 µg T_3 at $P < 0.05$; **significantly different from 0.0 µg T_3 at $P < 0.001$.

Table I. Total Food Consumption per Mouse of ob/ob and Non-ob/ob Mice During the T_3 Treatment Period[a]

Treatment (µg T_3/100 g body wt)	Non-ob/ob	ob/ob
	(g/3 weeks)	
0.0	48.9 ± 0.9	47.9 ± 0.7
2.5	44.1 ± 1.1	46.6 ± 1.4
5.0	46.7 ± 0.9	46.0 ± 1.9
7.5	47.7 ± 1.1	47.4 ± 1.1
10.0	46.9 ± 0.8	47.5 ± 1.1
12.5	48.3 ± 1.0	47.7 ± 1.1
25.0	46.1 ± 0.8	47.4 ± 0.7

[a] Values are the means ± SEM for 7–9 mice per group. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods.

mice even at the 2.5 µg T_3/100 g body wt dose. Only the highest dose of T_3 (25.0 µg/100 g body wt) increased serum T_3 values in non-ob/ob mice. The levels of serum T_3 attained by exogenous injection of T_3 were all within or near the physiological range.

Body Oxygen Consumption and Colonic Temperature. Because the mode of expression of oxygen consumption has been debated over the years, three manners of expressing animal oxygen consumption were calculated: Microliters of O_2 consumed per hour per (i) whole animal, (ii) $g^{0.75}$ body weight, or (iii) $g^{0.75}$ body protein. Values of whole body oxygen consumption per animal in saline-treated ob/ob mice were approximately 67% of the values observed in non-ob/ob mice (Fig 4). T_3 treatment significantly increased O_2 consumption in ob/ob mice, while values in the non-ob/ob did not change with T_3 treatment. Obese mice treated at the 25.0-µg T_3 level had O_2 consumption

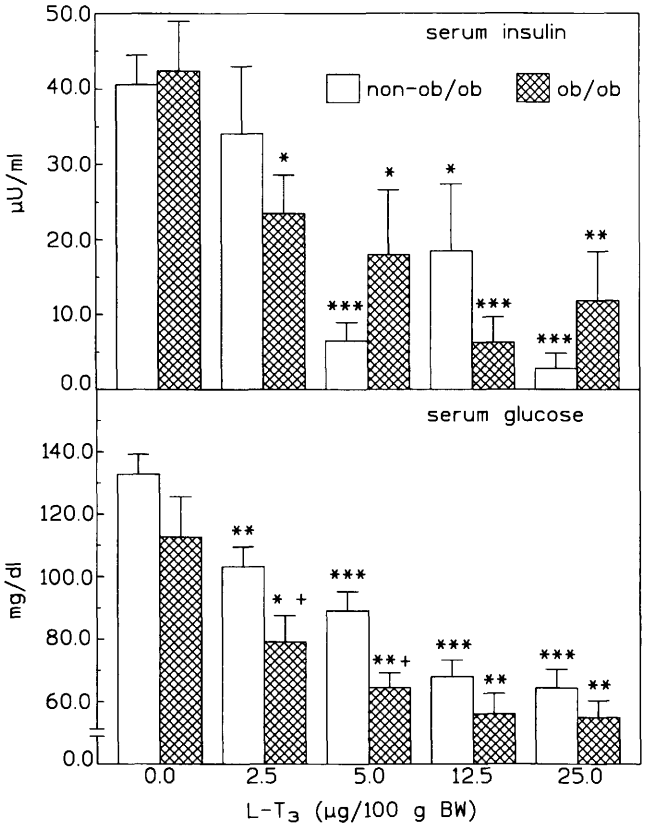

Figure 2. Serum insulin and glucose concentrations of ob/ob and non-ob/ob mice. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. Values are the means ± SEM of 8–12 samples. Sera from three individual mice were pooled to create each sample. For insulin, the ANOVA showed a significant T_3 effect at $P < 0.0001$. For glucose, the ANOVA showed significant T_3 effect at $P < 0.0001$ and phenotype effect at $P < 0.0001$. Mean square errors for insulin and glucose are 311.04 and 461.61, respectively. Significant differences between means were determined by using the protected *t* test: *significantly different from 0.0 μg T_3 at $P < 0.05$; **significantly different from 0.0 μg T_3 at $P < 0.005$; ***significantly different from 0.0 μg T_3 at $P < 0.001$; +significantly different from non-ob/ob at $P < 0.05$.

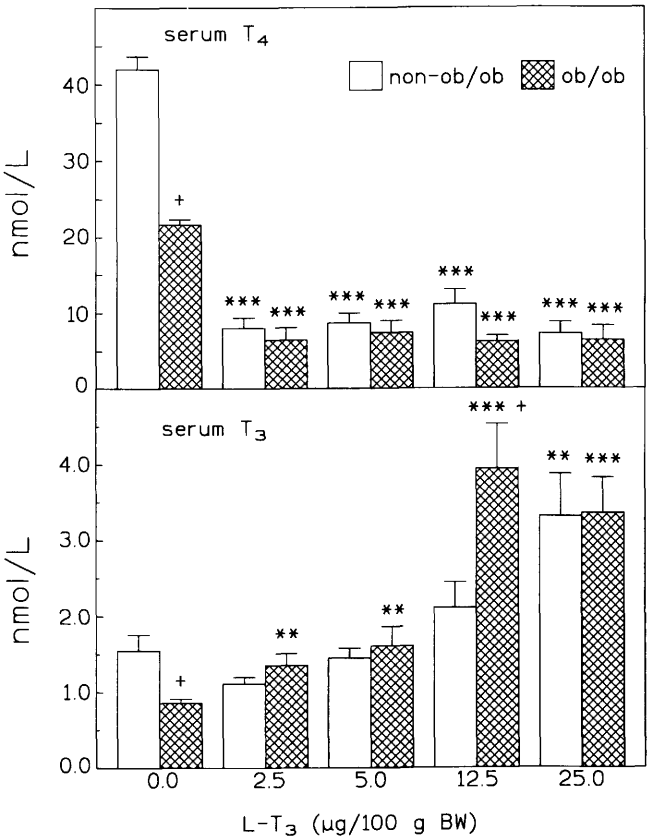

Figure 3. Serum concentrations of T_4 and T_3 of ob/ob and non-ob/ob mice. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. Values are the means ± SEM for 8–12 samples. Sera from three individual mice were pooled to create each sample. For serum T_4, the ANOVA showed significant T_3 effect at $P < 0.0001$, phenotype effect at $P < 0.0001$, and T_3 × phenotype effect at $P < 0.0001$. For serum T_3, the ANOVA showed significant T_3 effect at $P < 0.0001$ and T_3 × phenotype effect at $P < 0.0148$. Mean square errors for T_4 and T_3 are 24.02 and 1.25, respectively. Significant differences between means were determined by using the protected *t* test: *significantly different from 0.0 μg T_3 at $P < 0.05$; **significantly different from 0.0 μg T_3 at $P < 0.025$; ***significantly different from 0.0 μg T_3 at $P < 0.001$; +significantly different from non-ob/ob at $p < 0.001$.

values that were approximately the same as observed in saline-treated non-ob/ob mice.

Oxygen consumption per $g^{0.75}$ of body weight was lower in saline-treated ob/ob than in saline-treated non-ob/ob mice (Fig. 5). T_3 treatment significantly increased O_2 consumption of ob/ob mice, even at the lowest dose (Fig. 5). At 7.5 μg/100 g body wt treatment level, differences between ob/ob and non-ob/ob were no longer observed. The same data are presented per $g^{0.75}$ of body protein in Figure 6. On a per protein basis, the saline-treated ob/ob had a lower O_2 consumption than the saline-treated non-ob/ob mice, although these differences were not statistically significant. In the T_3-treated ob/ob and non-ob/ob mice, no differences in O_2 consumption on a whole body protein basis were found. T_3 treatment significantly increased O_2 consumption only in ob/ob mice at the highest levels of T_3.

Colonic temperature was lower in saline-treated ob/ob than in saline-treated non-ob/ob mice (Fig. 7). T_3 treatment increased the colonic temperature in ob/ob mice. At the 10.0 μg T_3/100 g body wt and higher doses, colonic temperature were similar between ob/ob and non-ob/ob mice.

Body Composition. Total body protein in ob/ob mice was less than that in non-ob/ob mice in both saline-treated and T_3-treated groups (Fig. 8). T_3 treatment did not significantly offset protein content. Percent body protein per unit of dry carcass weight in ob/ob mice was lower than that in non-ob/ob mice (Fig. 9). However, T_3 treatment significantly increased the percent protein in ob/ob mice at 5.0 μg T_3/100 g body wt and higher levels. Total body lipid in ob/ob mice was higher than that in non-ob/ob mice in all treatment groups (Fig. 8). At 10.0 μg T_3/100 g body wt

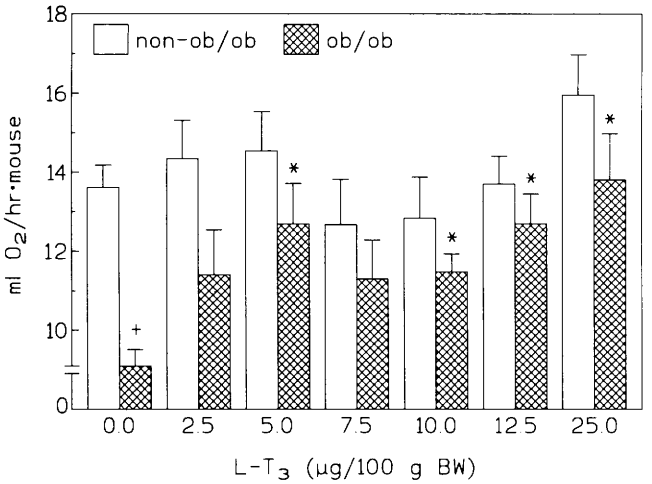

Figure 4. Oxygen consumption per animal in ob/ob and non-ob/ob mice. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. Values are the means ± SEM for nine mice per group. The ANOVA showed significant T_3 effect at $P < 0.0015$ and phenotype effect at $P < 0.0001$. Mean square error is 8.10. Significant differences between means were determined by using the protected *t* test: *significantly different from 0.0 μg T_3 at $P < 0.005$; +significantly different from non-ob/ob at $P < 0.001$.

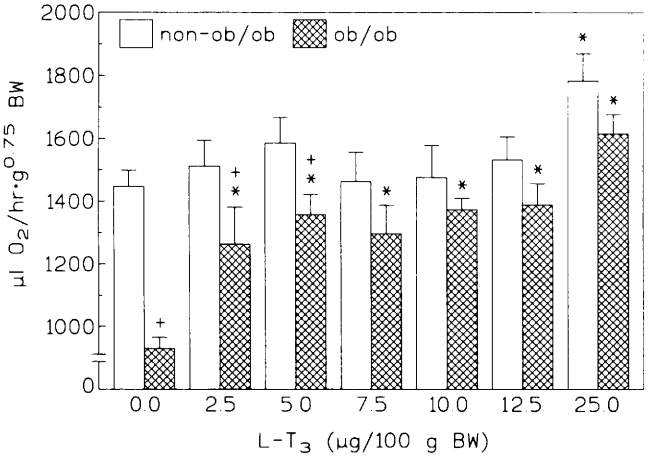

Figure 5. Oxygen consumption per unit of body weight in ob/ob and non-ob/ob mice. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. Values are the means ± SEM for nine mice per group. The ANOVA showed significant T_3 effect at $P < 0.0001$, phenotype effect at $P < 0.0001$, and T_3 × phenotype effect at $P < 0.0300$. Mean square error is 60963.84. Significant differences between means were determined by using the protected *t* test: *significantly different from 0.0 μg T_3 at $P < 0.025$; +significantly different from non-ob/ob at $P < 0.05$.

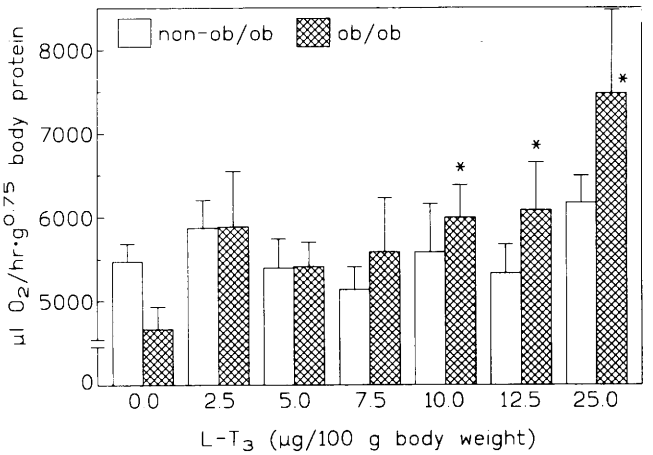

Figure 6. Oxygen consumption per unit of body protein in ob/ob and non-ob/ob mice. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. Values are the means + SEM for nine mice per group. The ANOVA showed a significant T_3 effect at $P < 0.0029$. Mean square error is 1585590.12. Significant differences between means were determined by using the protected *t* test: *significantly different from 0.0 μg T_3 at $P < 0.05$.

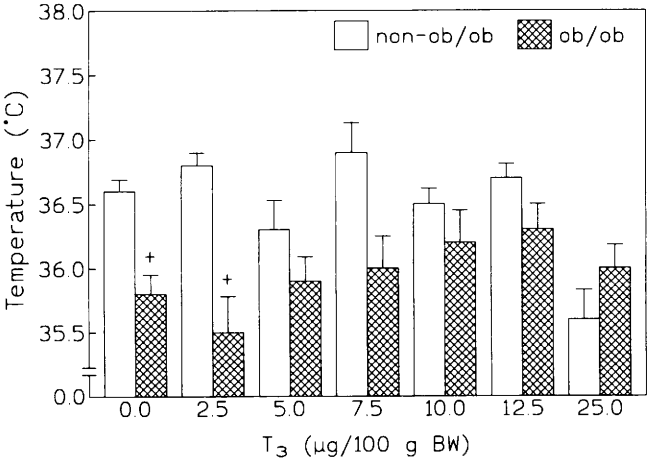

Figure 7. Colonic temperature of ob/ob and non-ob/ob mice. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. Values are the means ± SEM for 10 mice per group. ANOVA showed significant T_3 effect at $P < 0.0025$, phenotype effect at $P < 0.0001$, and T_3 × phenotype effect at $P < 0.0002$. Mean square error is 0.39. Significant differences between means were determined by using the protected *t* test: +significantly different from non-ob/ob at $P < 0.01$.

and higher doses, T_3 treatment significantly decreased body fat in ob/ob mice. Percent lipid per unit of dry carcass weight in ob/ob mice was higher than that in non-ob/ob mice in all groups (Fig. 9). Only at the highest dose of T_3 (25.0 μg T_3/100 g body wt) did T_3 treatment significantly decrease the percent lipid in ob/ob mice. Total body water in ob/ob mice was lower than in non-ob/ob mice in all groups (Fig. 8). T_3 decreased body water only in non-ob/ob mice at 7.5 μg T_3/100 g body wt and higher levels.

Discussion

The saline-treated ob/ob mice in these experiments clearly showed lower oxygen consumption, body temperature, and concentrations of thyroid hormones, T_4 and T_3, and higher body fat at 6 weeks of age. These findings are consistent with past reports (2, 5, 8). A low metabolic rate (3) in preweaning ob/ob mice may be the result of a reduction in circulating thyroid hormones (12).

T_3 treatment increased circulating T_3 levels in the ob/ob mice to within the normal range (1.0–3.0 nmol/l) and decreased serum T_4 in both phenotypes as ex-

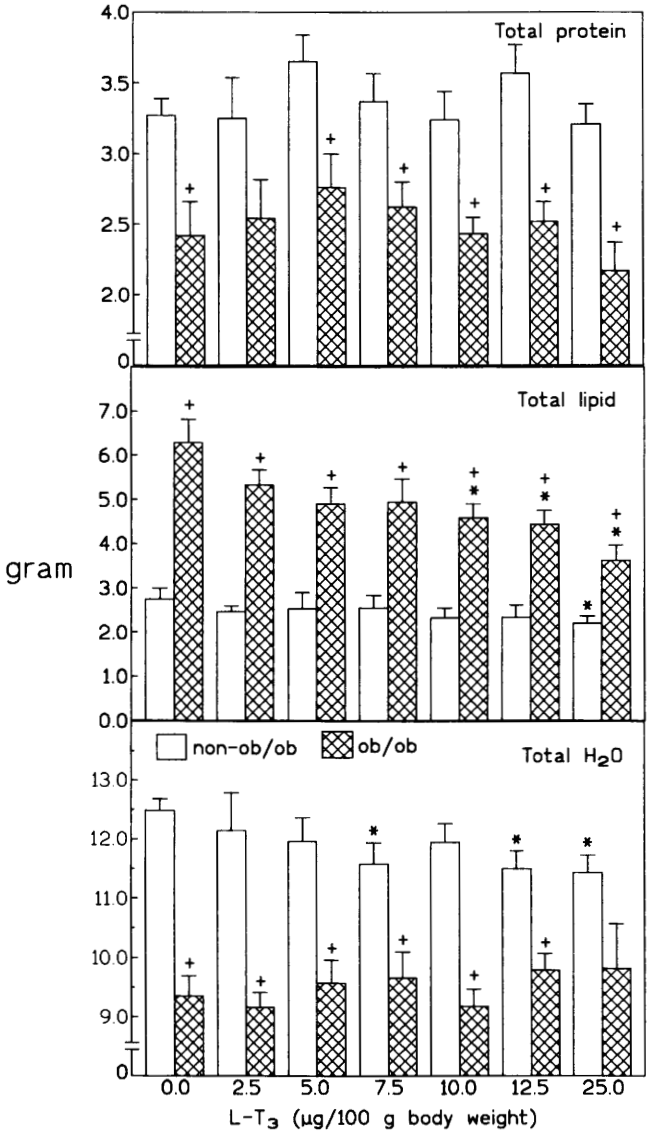

Figure 8. Total body protein, lipid, and water of ob/ob and non-ob/ob mice. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. Values are the means ± SEM for eight mice per group. For total protein, the ANOVA showed a significant phenotype effect at $P < 0.0001$. For total lipid, the ANOVA showed significant phenotype effect at $P < 0.0001$ and $T_3 \times$ phenotype effect at $P < 0.0157$. For total water, the ANOVA showed a significant phenotype effect at $P < 0.0001$. Mean square errors for protein, lipid, and water are 0.35, 0.97, and 1.07, respectively. Significant differences between means were determined by using the protected *t* test: *significantly different from 0.0 μg T_3 at $P < 0.05$; +significantly different from non-ob/ob at $P < 0.001$.

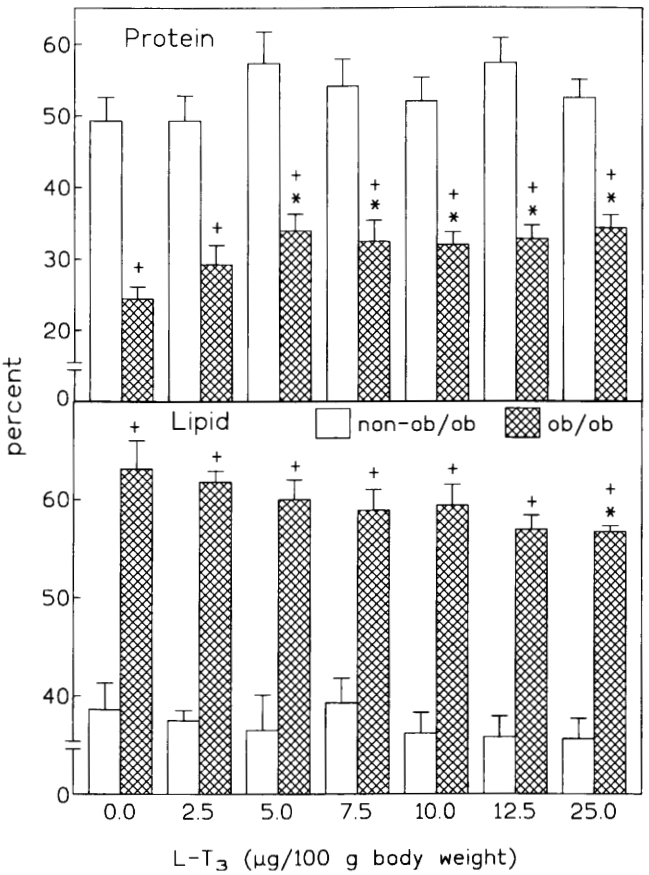

Figure 9. Percent protein and lipid on a dry weight basis in ob/ob and non-ob/ob mice. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. Values are the means ± SEM for eight mice per group. For percent protein, the ANOVA showed a significant phenotype effect at $P < 0.0001$. For percent lipid, the ANOVA showed a significant phenotype effect at $P < 0.0001$. Mean square errors for percentage protein and lipid are 97.70 and 55.63, respectively. Significant differences were determined by using the protected *t* test: *significantly different from 0.0 μg T_3 at $P < 0.01$; +significantly different from non-ob/ob at $P < 0.001$.

pected. In the results reported here, T_3 treatment significantly increased animal oxygen consumption and body temperature, and decreased body fat of young ob/ob mice without a change in total body protein.

No differences were found in the body weight of ob/ob mice and non-ob/ob mice in all groups mainly because of equalized food consumption to prevent gross obesity in ob/ob mice during experimental periods (Fig. 1). These results are different from our earlier paper (14), which reported that the body weight of saline-treated ob/ob mice was higher than that of saline-treated non-ob/ob mice when the ob/ob were allowed to be hyperphagic. T_3 treatment decreased the accretion of body weight in both phenotypes. Prevention of excessive weight gain in ob/ob mice after T_3 treatment was previously reported by us (14) as well as others (13, 24). Body fat reduction was also reported in ob/ob mice fed *ad libitum* with thyroid powders added to the diet (24). The serum T_4 and T_3 levels attained by this treatment were not reported.

Serum insulin and glucose concentrations in saline-treated ob/ob mice and non-ob/ob mice were similar. In hyperphagic ob/ob mice glucose and insulin levels are higher than in non-ob/ob mice (4, 8) which shows that hyperphagia *per se* can probably account for many of the phenotypic differences. T_3 treatment significantly decreased serum insulin and glucose in both phenotypes, but to a greater degree in ob/ob mice. Serum glucose was significantly decreased in

both phenotypes in a dose-dependent manner. Thenen and Carr (24) reported that T_4 treatment lowered blood glucose in the ob/ob mice, but the hyperinsulinemia persisted. The differences in our results were probably due to the feeding regimen, which was restricted.

Oxygen consumption in saline-treated ob/ob mice was lower than saline-treated non-ob/ob mice regardless of the different modes of expression (Fig. 4 and 5). Whole animal oxygen consumption expressed on a body protein basis was lower in saline-treated ob/ob than in non-ob/ob mice (Fig. 6), but these differences were not statistically significant. T_3 treatment increased O_2 consumption in ob/ob mice regardless of which mode of expression was used. Wimpfheimer *et al.* (26) reported that hypothyroid rats had enhanced sensitivity to T_3 by increasing O_2 consumption. Also, the metabolic rate was increased by T_3 treatment in obese men (16). Both of these findings are consistent with the results reported here.

The body temperature of ob/ob mice increased after T_3 treatment (Fig. 7). The increase of body temperature is matched with the increase in body oxygen consumption and a decreased accretion of body fat.

Body composition may influence the whole body metabolism. Thyroid hormone is known to increase animal metabolism. However, it is not clear whether thyroid hormone can increase the metabolism by changing body composition in young animals. The saline-treated ob/ob mice showed higher total fat and lower total protein than non-ob/ob mice (Fig. 8). Because tissues with high protein content, such as muscle, are metabolically more active than tissues with high fat content, such as white adipose tissue, this abnormally low ratio of protein to adipose mass can partially explain the lower O_2 consumption in the ob/ob mice. T_3 treatment decreased total fat content in a dose-dependent manner but did not significantly change total protein, even though it showed a tendency to increase. The increase in percentage body protein (Fig. 9) without significant change in total protein content reflects the significant decrease in total fat content. The increase in body O_2 consumption without the increase in total body protein suggests that the lean body mass or high protein-containing tissues increased oxidative activity in response to T_3 treatment. Alternatively, T_3 may have induced thermogenesis in tissues other than muscle, such as brown adipose tissue.

Contradictory reports exist about serum concentrations of T_4 and T_3 in ob/ob mice and whether the defects in metabolism reside in malfunction of pituitary-thyroid-axis or insensitivity of extrathyroidal tissues to circulating hormones. The experiments reported here showed that ob/ob mice exhibit decreased serum T_4 and T_3 concentrations. The lower levels of T_3 treatment in the early stage of obesity increased the serum T_3 levels of the ob/ob mice to match those observed in non-ob/ob mice. Concomitantly, serum T_4 levels of both phenotypes were suppressed so as to be equal. These findings suggest that low concentrations of serum T_3 and T_4 in nontreated ob/ob mice are from insufficient production of these hormones in the thyroid gland. T_3 treatment increased body temperature and body oxygen consumption to the range exhibited by untreated non-ob/ob mice. Also, T_3 treatment decreased total body fat in the ob/ob mice but did not normalize body composition. Because total body protein was not significantly changed after T_3 treatment, the increase in whole body metabolism was probably due to increased oxidative activity in tissues that comprise the lean body mass or the most oxidative tissues such as brown adipose tissue, liver, or muscle.

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