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The effect of L-triiodothyronine on oxidative metabolism of muscle, adipose tissue, and liver in genetically obese mice

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Oh, Soomee Sohn, Ph.D.

Iowa State University, 1993

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The effect of L-triiodothyronine on oxidative metabolism of muscle, adipose tissue, and liver in genetically obese mice

by

Soomee Sohn Oh

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Food Science and Human Nutrition
Major: Nutrition

Approved:

Signature was redacted for privacy.
In Charge of Major Work

Signature was redacted for privacy.
For the Major Department

Signature was redacted for privacy.
For the Graduate College

Members of the Committee

Signature was redacted for privacy.

Iowa State University
Ames, Iowa

1993

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INTRODUCTION

Obesity has been cited as the known health hazard of our time and the dangers of being overweight have come to be increasingly recognized particularly during the last forty years. There is a linear relationship between the degree of obesity and excess mortality and morbidity (Lew and Garfinkel 1979, Waaler 1983). However, it is not the obesity itself which provokes a high degree of morbidity and mortality, but the large range of metabolic disorders to which many obese people are susceptible. A 12-year follow-up study by the American Cancer Society shows that persons who are greater than 40% overweight suffer increased mortality from diabetes, coronary heart disease, and some cancer (Lew and Garfinkel 1979). The importance of less severe obesity has remained controversial in that moderate obesity is not associated with increased mortality (Andres 1980, Harrison 1985, Keys 1980).

Obesity is defined as that physiological state in which there is excessive accumulation of body fat in both the absolute and the relative sense (McBride 1964). This state results from the excessive intake of energy relative to energy expenditure caused by genetic, metabolic, endocrinological, environmental, or behavioral factors or hypothalamic injury

(Bray and York 1979). The endocrine aspects of obesity have assumed a prominent place in consideration of the pathogenesis, diagnosis, prognosis, and treatment of obesity (Glass 1989). Several metabolic changes accompany obesity such as hyperinsulinemia (Beard et al. 1987, Cohen et al. 1986, Genuth 1973), insulin resistance (Cohen et al. 1986, Kolterman et al. 1980, Zuniga-Guajardo et al. 1986), reduced growth hormone level (Meistas et al. 1982, Slavnov and Epstein 1977) and blunted growth hormone responsiveness to stimulation (Davies et al. 1985, Kopelman and Noonan 1986), increased cortisol turnover with normal serum levels (Abou-Samra et al. 1985, Kopelman et al. 1988, Kurtz et al. 1987, Strain et al. 1982), elevated parathyroid hormone level (Anderson et al. 1986, Bell et al. 1985), increased serum epinephrine (Schwartz et al. 1980), and decreased (Burman et al. 1980, Chomard et al. 1985, Kventy 1985) or normal (Bray et al. 1973, Strata et al. 1978) thyroid hormone metabolism. The failure of these abnormalities to be normalized after weight reduction raises the possibility of an underlying disorder leading to both endocrine dysfunction and obesity, rather than to the endocrine dysfunction being a consequence of the obesity.

Thyroid hormone is required for normal growth and tissue development and is an important regulator of energy metabolism. A number of disease states is associated with altered thyroid hormone metabolism and action. Decreased

concentrations of thyroid hormones in plasma are found in a high percentage of diabetic (Schlienger et al. 1982) and obese patients (Bray et al. 1976). Hypothyroidism, which is clearly associated with some types of obesity in humans and in animal models, precedes the onset of hyperinsulinemia, insulin resistance, hyperphagia, and obesity (York et al. 1978).

The genetically obese (ob/ob) mouse is an excellent animal model in which to study the effect of thyroid hormone. These mice have a number of metabolic and endocrine disorders as early as 2 weeks of age, among which are depressed oxygen consumption (Kaplan and Leveille 1974), low body temperature (Joosten and Van der Kroon 1974), decreased thermogenic activity of brown adipose tissue (Goodbody and Trayhurn 1982), higher body fat content (Boissonneault et al. 1978), and hypothyroidism (Mobley and Dubuc 1979). These disorders persist throughout adulthood in obese mice. I reported that T_3 treatment increased whole body O_2 consumption in ob/ob mice (Oh and Kaplan 1989). However which metabolically important tissues are responsible for increased O_2 consumption and what effects early T_3 treatment has on concentrations of thyroid hormones, insulin, and glucose in serum and on possible changes in body composition are not yet known.

This dissertation explores the role of thyroid hormone (T_3, triiodothyronine) administration on oxidative metabolism in

ob/ob mice early in the development of obesity, before gross abnormalities of obesity are apparent.

Explanation of dissertation format

This dissertation contains an abstract, general introduction, literature review, two papers submitted for publication, general discussion and summary, and literature cited in general introduction, literature review, and general discussion and summary.

LITERATURE REVIEW

The genetically obese hyperglycemic (ob/ob) mouse is extensively used as a model for the study of early onset obesity and mature onset diabetes in human. These ob/ob mice have a number of metabolic and endocrine disorders that can be detected as early as 2 weeks of age before the obesity is phenotypically expressed. These mice exhibit depressed oxygen consumption (Boissonneault et al. 1978, Kaplan 1981, Kaplan and Leveille 1974), low body temperature (Kaplan 1981, Kaplan and Leveille 1974), and higher body fat content (Boissonneault et al. 1978). Also, thyroid hormones are low during thermal stress in ob/ob mice (Bray and York 1971, Joosten and Van der Kroon 1974). Ohtake et al. (1977), however, reported that the thyroid-pituitary axis of ob/ob mice was normal and that hypothermia was not due to hypothyroidism. These mice showed decreased thermogenic activity of brown adipose tissue as measured by GDP binding to mitochondria, which was also detectable early, when obesity was not apparent (Goodbody and Trayhurn 1982, Himms-Hagen and Desautels 1978). Oxygen consumption of brown adipose tissue was lower in ob/ob than in non-ob/ob mice (Thurlby and Trayhurn 1980). Generally thyroid hormones potentiate the norepinephrine-stimulated GDP binding to brown adipose tissue mitochondria. This thermogenic activity is reduced in the brown adipose tissue of ob/ob mice

(Himms-Hagen 1983a, Hogan and Himms-Hagen 1981). Also these mice maintain elevated levels of food intake over much of their adult life (Chlouverakis and White 1969). Dubuc (1976) reported that these mice, regardless of feeding regimen, maintained absolutely higher carcass fat levels than did 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 on TSH (thyroid stimulating hormone) secretion by the pituitary. Serum T_3 plays a more important, direct role than does serum T_4 in the feedback regulation of serum TSH in the euthyroid or mildly hypothyroid rats (Emerson et al. 1989). Varied results in serum concentrations of thyroid hormones in ob/ob mice are reported (Ikeda et al. 1988, Kaplan et al. 1985, Mobley and Dubuc 1979, York et al. 1978), which were either lower or higher or the same as in non-ob/ob mice.

Hypothyroidism was a very early lesion in ob/ob mice, possibly because of abnormal regulation of the hypothalamic-pituitary-thyroid at early ages (Bray and York 1971). Insensitivity to the effect of circulating hormones by non-thyroid tissues might be present at later ages (Mobley and Dubuc 1979, York et al. 1978). I reported that T_3 treatment

increased animal oxygen consumption (Oh and Kaplan 1989). 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 glucose utilization. This was consistent with the concept of T_3 resistance by the tissues. In studies with obese humans, T_3 treatment increased the metabolic rate (Abraham et al. 1985), decreased body weight, and increased serum T_3 and TSH (Rozen et al. 1986). Wolman et al. (1985) reported that body weight loss after T_3 treatment was from lean body mass rather than body fat.

Thyroid hormones are low in the ob/ob mice during thermal stress of cold temperature (Bray and York 1971, Joosten and Van der Kroon 1974). The defects in thermogenesis in ob/ob mice may be attributed to impaired thyroid hormone action. Supportive of this concept are reports that the thyroid hormone-sensitive Na^+-K^+-ATPase is lower in the liver and skeletal muscle of ob/ob mice than in same tissues of non-ob/ob mice (Lin et al. 1979, York et al. 1978a). Recently, Hillgartner and Romsos (1988) reported that the ob/ob mice had lower hepatic thyroid hormone uptake than non-ob/ob mice. This may account for the impaired thyroid hormone action and T_3 production in the liver, which is the major site for T_3 production from T_4.

Thyroid hormones are known to increase metabolic rate. Previously, I reported that T_3 treatment increased animal oxygen consumption and both non-insulin-stimulated and insulin-stimulated glucose utilization in ob/ob mice and eliminated the insulin resistance by diaphragm muscles (Oh and Kaplan 1989). Others have shown that T_3 treatment also increases anaerobic glycolysis in muscle (Nicole and Johnston 1981). Skeletal muscle plays a major role in glucose utilization (DeFronzo et al. 1981, Katz et al. 1983). Significant amounts of energy are also used by the skeletal musculature (Martin and Fuhrman 1955). Because O_2 consumption is lower in some muscles of ob/ob mice (Conway and Kaplan 1977, Kaplan and Oh 1991), the role of T_3 on muscle O_2 consumption was further explored.

Thyroid hormones are known to increase fatty acid mobilization from adipose tissue in rat (Fain and Rosenthal 1971). In ob/ob mice, the impaired norepinephrine-stimulated lipolysis was improved by exogenous thyroid hormone (Otto et al. 1976, Thenen and Carr 1978), but York et al. (1978b) reported that adipose tissue lipolysis of ob/ob mice was less sensitive to thyroid hormone than that of non-ob/ob mice. In preadipocyte cell lines from both ob/ob and non-ob/ob mice, T_3 increased both fatty acid synthesis and lipolysis (Pou and Torresani 1989). Thyroid hormones stimulate hepatic fatty acid synthesis and esterification in the rat (Diamant et al.

1972, Mariash et al. 1980). Also T_3 treatment increased O_2 consumption in isolated perfused liver (Horst et al. 1989, Müller and Seitz 1980) and in isolated hepatocytes (Gregory and Berry 1991). Liver utilizes 30-40 % of the resting oxygen consumption (Martin and Fuhrman 1955) and therefore should be explored as a possible site for the increased total O_2 consumption in response to T_3.

Past experiments used pharmacological doses of T_3 for a short period of time. The experiments reported here in ob/ob mice explore the possibility that increasing the serum T_3 levels early in the development of obesity to physiological and pharmacological levels over a 3 week period, at a critical developmental period, will alter energy metabolism and reduce body fat. Food consumption was equalized across all groups to remove the role of hyperphagia per se on the parameters studied. The metabolic responses to thyroid hormones are different in many tissues (Barker and Klitgaard 1952, Schwartz 1983). T_3 treatment increased whole body oxygen consumption in ob/ob mice (Oh and Kaplan 1989, Oh and Kaplan 1993). Major metabolically important tissues should account for the change in whole body metabolism. Although a controversy exists about the role of brown adipose tissue in human obesity, in which brown adipose tissue is almost nonexistent, it is a thermogenically important tissue in ob/ob mice (Hogan and Himms-Hagen 1981). White adipose tissue is important in

energy balance and should also be explored in terms of oxidative metabolism. The present report explores the possibility that early T_3 treatment of ob/ob mice over a 3-week period will alter the energy expenditure of important metabolically active tissues.

PAPER I. EARLY TREATMENT OF OBESE (OB/OB) MICE WITH TRIIODOTHYRONINE INCREASES OXYGEN CONSUMPTION AND TEMPERATURE AND DECREASES BODY FAT CONTENT

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 weight (BW) were injected intraperitoneally 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 the non-ob/ob 0.154M NaCl (saline)-treated group. At 6 weeks of age, the following parameters were analyzed: body weight, concentrations of T_4, T_3, TSH, insulin, and glucose in serum, oxygen consumption, colonic temperature, and body composition. 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, decreased serum T_4, TSH, 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.

Keywords obesity, triiodothyronine, oxidative metabolism, body composition, temperature

INTRODUCTION

Genetically obese hyperglycemic (ob/ob) mice have a number of metabolic and endocrine disorders as early as 2 weeks of age and are a useful model of some types of human obesity. These mice exhibit depressed oxygen consumption (Boissonneault et al. 1978, Kaplan 1981, Kaplan and Leveille 1974), low body temperature (Kaplan 1981, Kaplan and Leveille 1974), and higher body fat content (Boissonneault et al. 1978). Thyroid hormones are also low during thermal stress of cold temperature (Bray and York 1971, Joosten and Van der Kroon 1974). Ohtake et al. (1977), 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 (Chlouverakis and White 1969). Dubuc (1976) reported that these mice, regardless of feeding regimen (ad lib. or restricted), maintained absolutely higher carcass fat levels than did their lean littermate.

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 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 does serum T_4 in the feedback regulation of serum TSH in the euthyroid or mildly hypothyroid rats (Emerson et al. 1989). Varied results in concentrations of thyroid hormones in serum of ob/ob mice are reported (Ikeda et al. 1988, Kaplan et al. 1985, Mobley and Dubuc 1979, York et al. 1978), which were either lower or higher or the same as in non-ob/ob mice.

Hypothyroidism is a very early detectable symptom in ob/ob mice, possibly because of abnormal regulation of the hypothalamic-pituitary-thyroid at early ages (Bray and York 1971). Insensitivity to the effect of circulating hormones by non-thyroid tissues might be present at later ages (Mobley and Dubuc 1979, York et al. 1978). I reported that T_3 treatment increased animal oxygen consumption (Oh and Kaplan 1989). 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 glucose utilization. This finding was consistent with the concept of T_3 resistance by the tissues. In studies with obese humans, T_3 treatment increased the metabolic rate (Abraham et al. 1985), decreased body weight, and increased serum T_3 and TSH (Rozen et al. 1986). Wolman et al. (1985) reported that body weight loss after T_3 treatment 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 ob/ob mice explore the possibility that increasing the serum T_3 levels early in the development of obesity to both physiological and pharmacological levels over a 3-week period between 3 and 6 weeks of age, which is a critical developmental period, will alter energy metabolism and reduce body fat. Food consumption was equalized across all groups to remove the effect of hyperphagia per se on the parameters studied. Hyperphagia can possibly change sympathetic nervous system activity, hormonal status of insulin, epinephrine, and glucocorticoids, increase body fat tissues (Bray 1991).

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 to allow pups to even access to nurturing female mouse. Oxygen consumption was measured at 16 days of age on the basis of the low oxygen consumption exhibited by future obese mice (Kaplan and Leveille 1974). In this study, 2000 μl O_2/hr·g body weight at STP was used as the value below which 2-week-old mouse pups are identified as obese with 99.9% reliability (the average value of O_2 consumption is 2322.0 for non-ob/ob and 1439.9 μl/hr·g BW for ob/ob mice). All mice were housed individually in solid bottom plastic cages with aspen wood shavings for bedding in an animal room at 23 ± 1°C with a 12-hour dark-light cycle (dark cycle 0600-1800 hr, light cycle 1800-0600 hr). All mice were fed a high carbohydrate diet during the 6-hour feeding period from 1000 to 1600 hr and allowed free access to tap water. The diet consisted of: casein, 20.0 g; DL-methionine, 0.3 g; AIN mineral mix 76[1], 3.5 g; AIN vitamin mix 76[2], 1.0 g; choline chloride, 0.2 g; corn oil, 5.0 g; fiber, 5.0 g; dextrose, 60.0 g; and dextrin, 5.0 g per 100 g diet. The diet was pelleted to facilitate the

measurement of food consumption. Food intake was equalized daily across all groups to the average consumed by non-ob/ob saline-treated group on the previous day. Food consumption and body weight were measured daily until 6 weeks of age. Mice were treated with L-triiodothyronine(T_3) as indicated below.

Treatments. Obese and nonobese mice were divided into 7 groups. Six levels of 2.5, 5.0, 7.5, 10.0, 12.5, and 25.0 μg L-T_3/100 g BW, dissolved in 0.154M NaCl (saline) (final pH 9.1 to keep the T_3 soluble), were injected intraperitoneally daily, between 0900 and 1000 hr from 3 to 6 weeks of age. Untreated ob/ob and non-ob/ob mice were injected with saline, pH 9.1. Body weight was measured daily at the time of the treatment. Oxygen 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 6 weeks of age by differential constant pressure respirometry as reported (Kaplan and Leveille 1974) with the apparatus submerged in a water bath at 25°C. Duplicate measurements were made consecutively for each mouse. Oxygen 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 thermistor with a round tip applicator (YSI model 43TA single-channel tele-thermometer, Yellow Springs Instrument Co., Yellow Springs, OH). Each mouse was held in a mouse restrainer, and the applicator was inserted about 1 cm into the rectum and held 15 seconds until the needle of the meter was stabilized.

Body Composition. The contents inside the gastrointestinal tracts were removed as much as possible from mice, and the carcasses were stored at -20°C for future analysis. Each frozen carcass was chopped into small pieces and homogenized by using a one quart size Waring blender with addition of sufficient (approximately 100 ml) water so that the blender could operate freely. The entire homogenate was transferred into a pre-weighed dry flask, frozen at -20°C, and subsequently lyophilized. Lyophilized samples were weighed and stored desiccated at 4°C until analysis for protein and fat. For the protein determination, weighed samples were digested in 36N H_2SO_4, 0.3% SeO_2 by using the digestion system (Tecator ab, Höganas, Sweden) at 420°C for 1 hr or until heated samples were clear. The resultant $(NH_4)_2SO_4$ was determined colorimetrically (Chaney and Marbach 1962), and the protein factor of 6.25 was used in calculation. Lipid was extracted from weighed samples by chloroform-methanol (2:1, v/v) and determined gravimetrically (Griminger and Gamarsh 1972).

Serum Analysis. At 6 weeks of age, all mice were killed by decapitation between 0930 and 1000 hr, 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 triiodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH), insulin, and glucose. Because very small volume of serum was collected from one mouse, sera from three mice were pooled to create each sample in the assay. Also, serum assay was performed last after a few sets of experiments to create enough sample numbers in a group. Serum T_3, T_4, TSH, and insulin were assayed by using radioimmunoassay (RIA) procedures as described in the RIA kits: Amerlex-M T_4, Amerlex-M T_3, TSH coated tube assay, and insulin RIA kit, respectively (Amersham, Arlington, IL). Serum glucose was assayed by using glucose hexokinase reagent kit (Sigma Chemical Co., St. Louis, MO) in which glucose was assayed by using the coupled enzymatic reactions catalyzed by hexokinase and glucose-6-phosphate dehydrogenase (Bondar and Mead 1974).

Chemicals. L-3,3',5-triiodothyronine was purchased from Calbiochem-Behring Co. (La Jolla, CA). All RIA kits were purchased from Amersham (Arlington, IL). The glucose hexokinase reagent kit was purchased from Sigma Co. (St. Louis, MO). All other reagents were purchased from Fisher

Scientific Co. (Fair Lawn, NJ). Diet ingredients were purchased from ICN Biomedicals (Costa Mesa, CA).

Statistics. Overall statistical significance was determined by analysis of variance (Ray 1982, Snedecor and Cochran 1980). 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 ANOVA.

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 (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 1). The average weekly food consumption 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 serum insulin and glucose in both phenotypes. Serum glucose was significantly decreased at all doses of T_3.

Serum concentrations of TSH, T_4, and T_3. Serum TSH was higher in saline-treated ob/ob mice than saline-treated non-ob/ob mice (Fig. 3). T_3 treatment significantly decreased serum TSH at 2.5 μg T_3/100 g BW to the level observed in non-ob/ob mice. The highest dose of T_3 (25.0 μg/100 g BW) reduced the ob/ob TSH values even further. T_3 did not affect serum TSH in non-ob/ob mice. Serum T_4 was lower in saline-treated

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 8-9 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 $P < 0.001$.

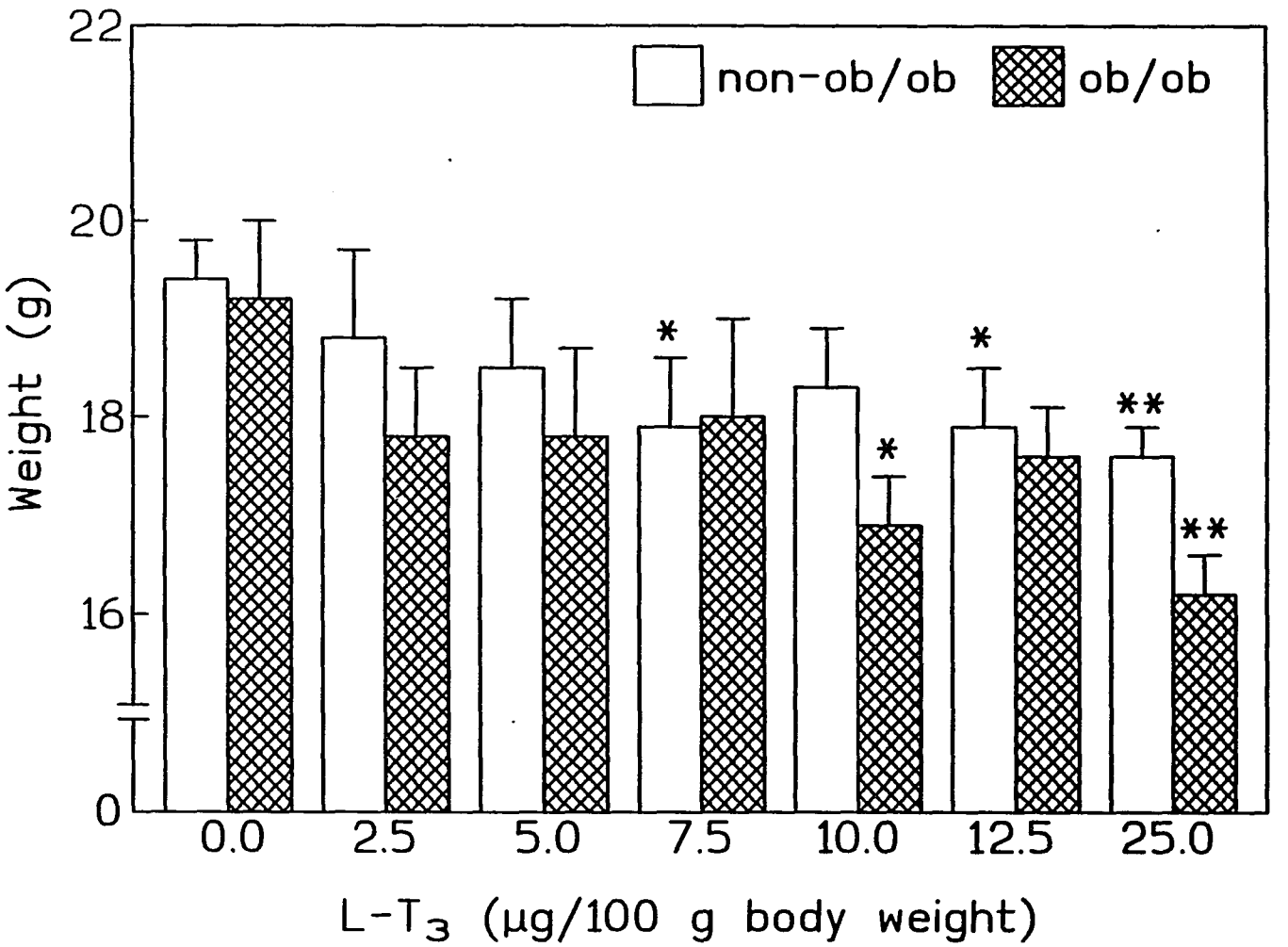

non-ob/ob
ob/ob
Weight (g)
22
20
18
16
0
0.0
2.5
5.0
7.5
10.0
12.5
25.0
L-T3 (µg/100 g body weight)
*
*
*
**
**

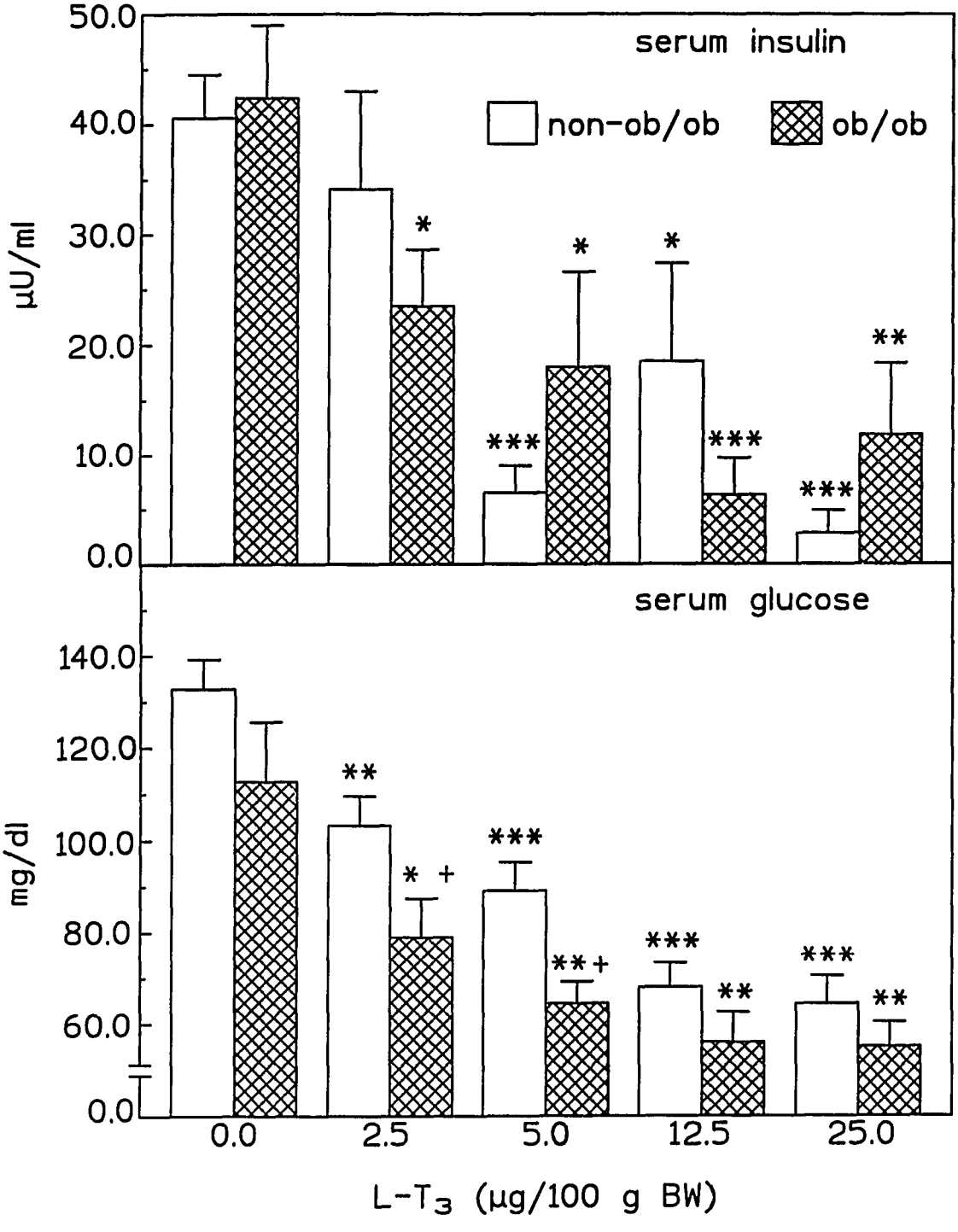
TABLE 1

Total food intake of ob/ob and non-ob/ob mice during the T_3 treatment period[1]

Treatment	non-ob/ob	ob/ob
μg T_3/100 g BW	*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

[1]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.

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 3 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 $P < 0.05$.


serum insulin
non-ob/ob
ob/ob
μU/ml
50.0
40.0
30.0
20.0
10.0
0.0
serum glucose
mg/dl
140.0
120.0
100.0
80.0
60.0
0.0
0.0
2.5
5.0
12.5
25.0
L-T3 (μg/100 g BW)

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 mice even at the 2.5 μg T_3/100 g BW dose. Only the highest dose of T_3 (25.0 μg/100 g BW) 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. Serum T_3 levels of ob/ob mice were more sensitive to the exogenous source of T_3 than were the non-ob/ob mice.

Body oxygen consumption and colonic temperature. Because the mode of expression of oxygen consumption has been debated over the years, more than one manner of expressing animal oxygen consumption was calculated. These were μl of O_2 consumed per hour in the basis of whole animal or $g^{0.75}$ body weight or $g^{0.75}$ 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 values that were approximately the same as observed in saline-treated non-ob/ob mice.

FIGURE 3 Serum concentrations of TSH, 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 3 individual mice were pooled to create each sample. For TSH, the ANOVA showed significant T_3 effect at $P < 0.0001$ and T_3 x phenotype effect at $P < 0.0069$. For serum T_4, the ANOVA showed significant T_3 effect at $P < 0.0001$, phenotype effect at $P < 0.0001$, and T_3 x phenotype effect at $P < 0.0001$. For serum T_3, the ANOVA showed significant T_3 effect at $P < 0.0001$ and T_3 x phenotype effect at $P < 0.0148$. Mean square errors for TSH, T_4, and T_3 are 0.0003855, 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 $P < 0.001$.

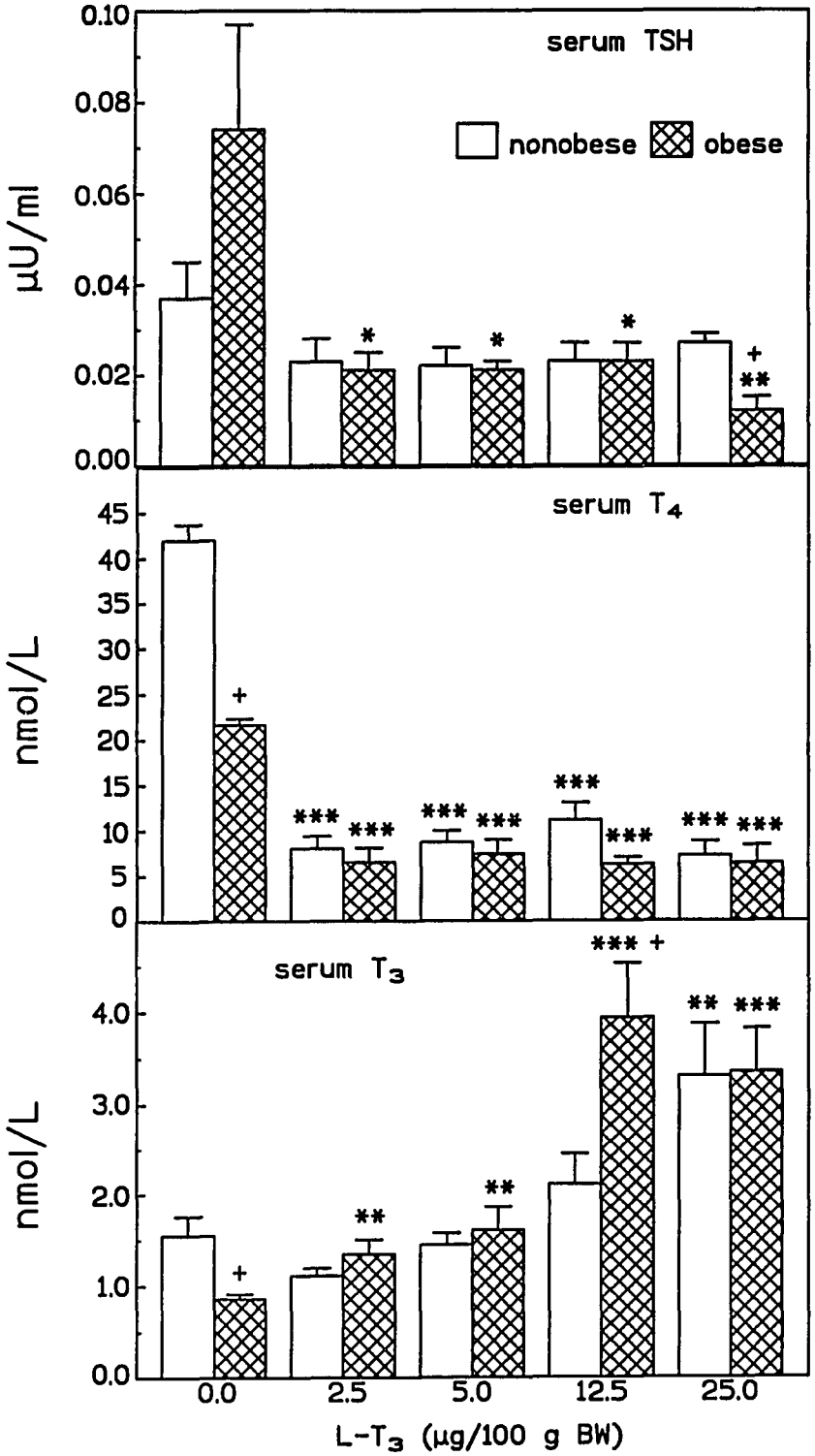
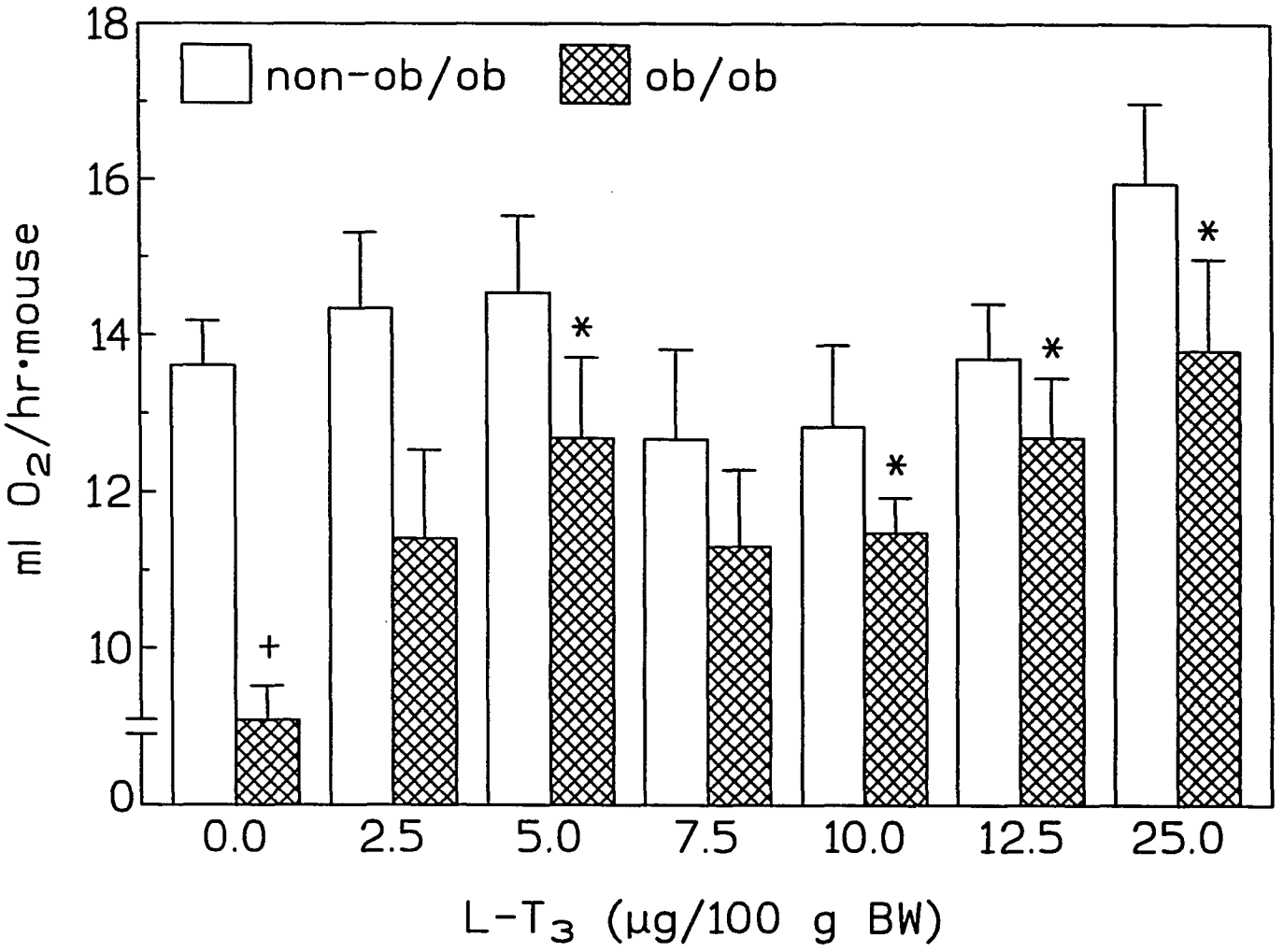

serum TSH
nonobese
obese
μU/ml
0.10
0.08
0.06
0.04
0.02
0.00
serum T4
nmol/L
45
40
35
30
25
20
15
10
5
0
serum T3
nmol/L
4.0
3.0
2.0
1.0
0.0
0.0
2.5
5.0
12.5
25.0
L-T3 (μg/100 g BW)

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 9 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 $P < 0.001$.


non-ob/ob
ob/ob
ml O_2/hr•mouse
18
16
14
12
10
0
+
*
0.0
2.5
5.0
7.5
10.0
12.5
25.0
L-T_3 (μg/100 g BW)

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. At 7.5 μg/100 g BW treatment level, differences between ob/ob and non-ob/ob were no longer observed. The same data are presented in Figure 6 per $g^{0.75}$ of body protein. 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 BW 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). Total body lipid in ob/ob mice was higher than that in non-ob/ob mice in all treatment groups. T_3 treatment did not significantly offset body protein content. At 10.0 μg T_3/100 g BW and higher doses, T_3

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 9 mice per group. The ANOVA showed significant T_3 effect at $P < 0.0001$, phenotype effect at $P < 0.0001$, and T_3 x 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 $P < 0.05$.

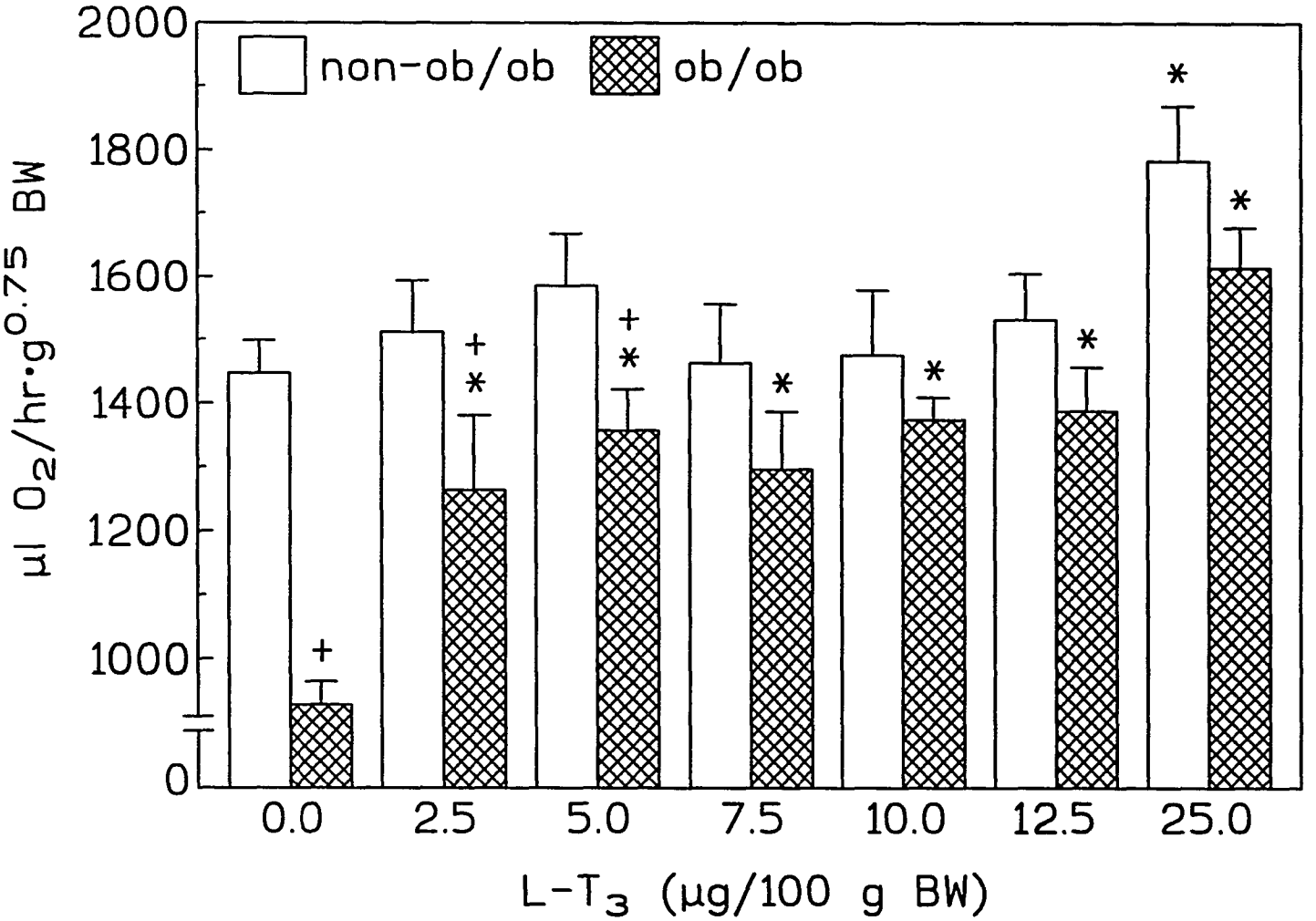

non-ob/ob
ob/ob
2000
1800
1600
1400
1200
1000
0
μl O_2/hr•$g^{0.75}$ BW
0.0
2.5
5.0
7.5
10.0
12.5
25.0
L-T_3 (μg/100 g BW)

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 9 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 $P < 0.05$.

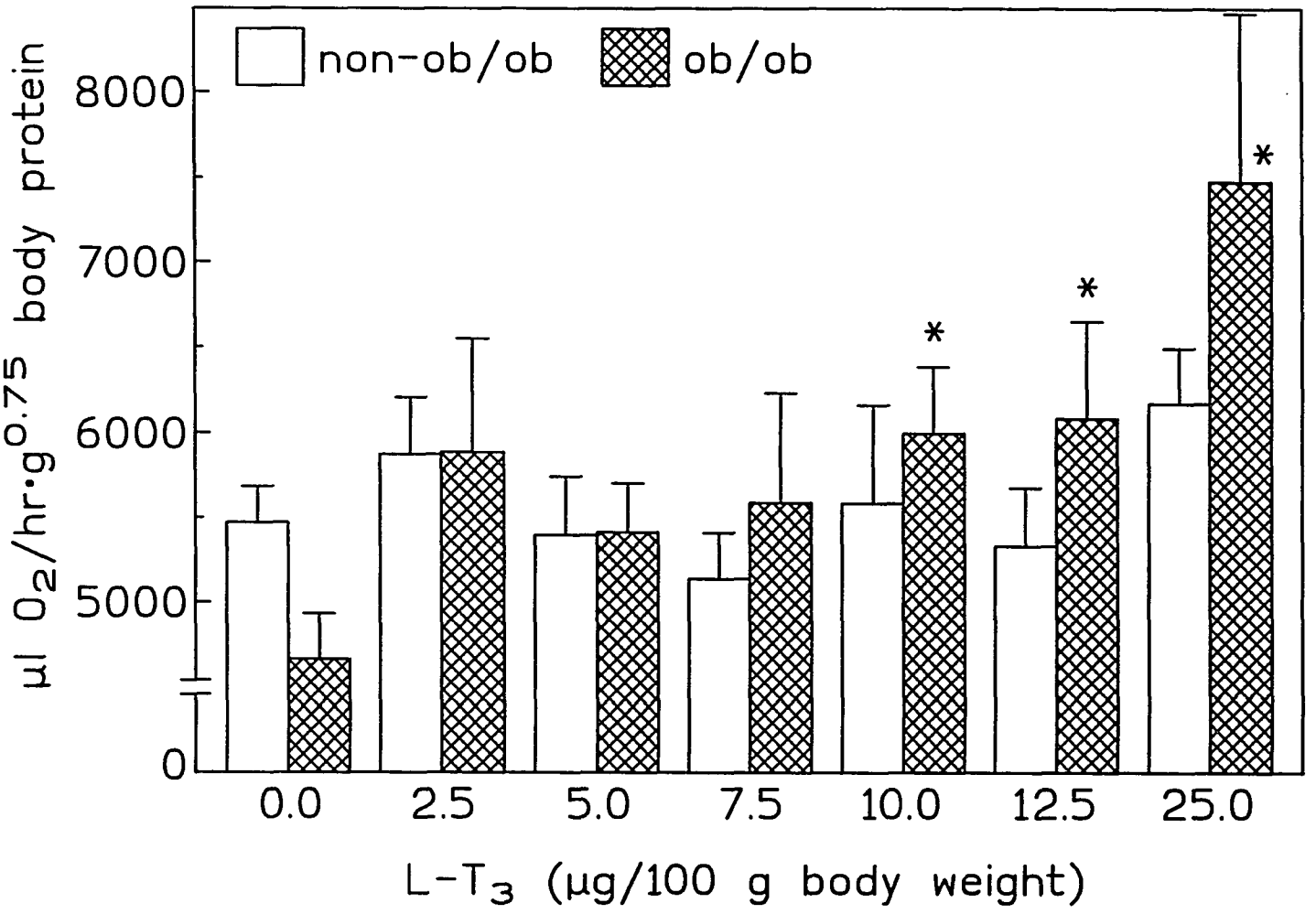

non-ob/ob
ob/ob
8000
7000
6000
5000
0
μl O_2/hr•$g^{0.75}$ body protein
*
*
*
0.0
2.5
5.0
7.5
10.0
12.5
25.0
L-T_3 (μg/100 g body weight)

FIGURE 7 Colonic temperature of ob/ob nad 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 x 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 $P < 0.01$.

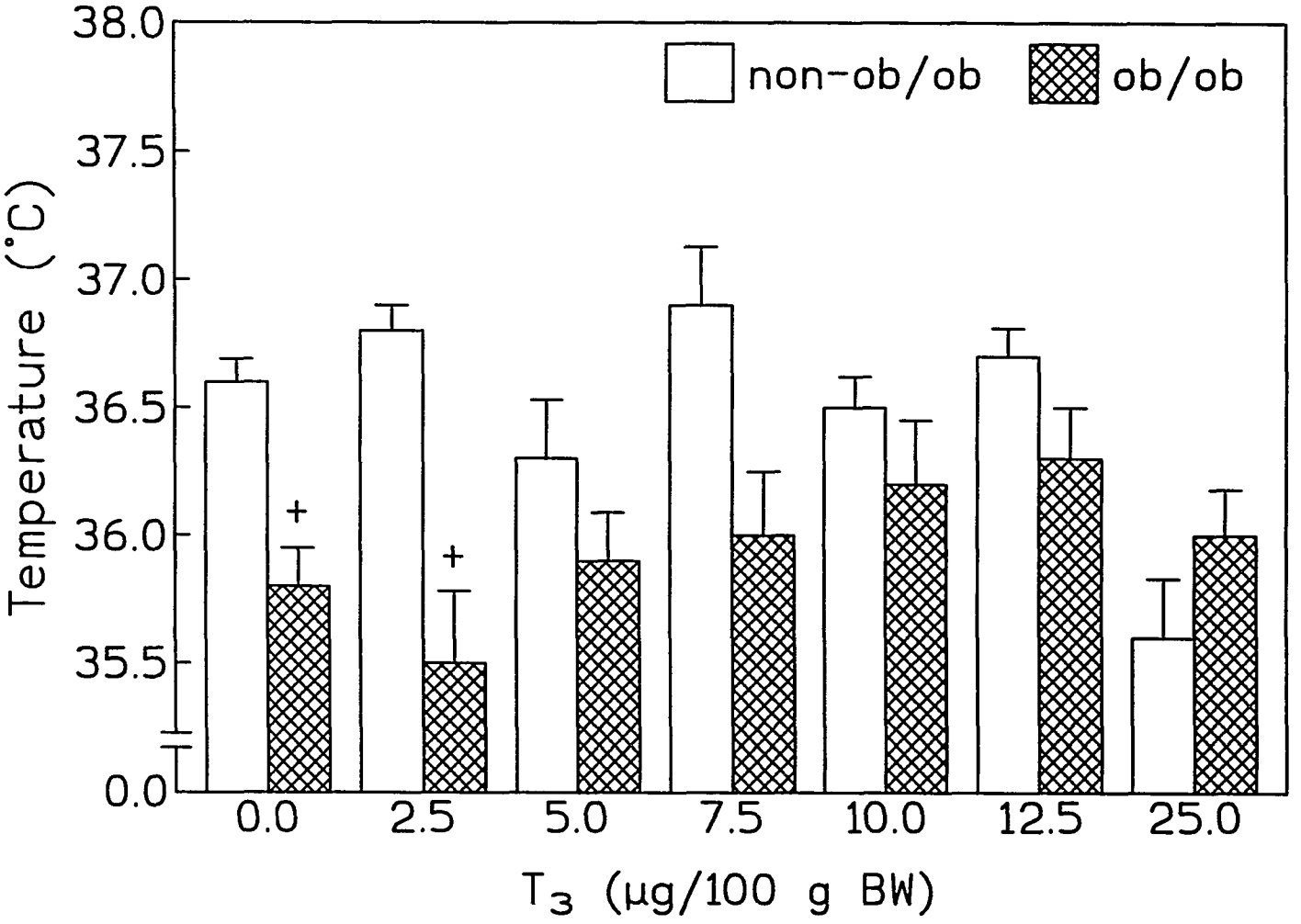
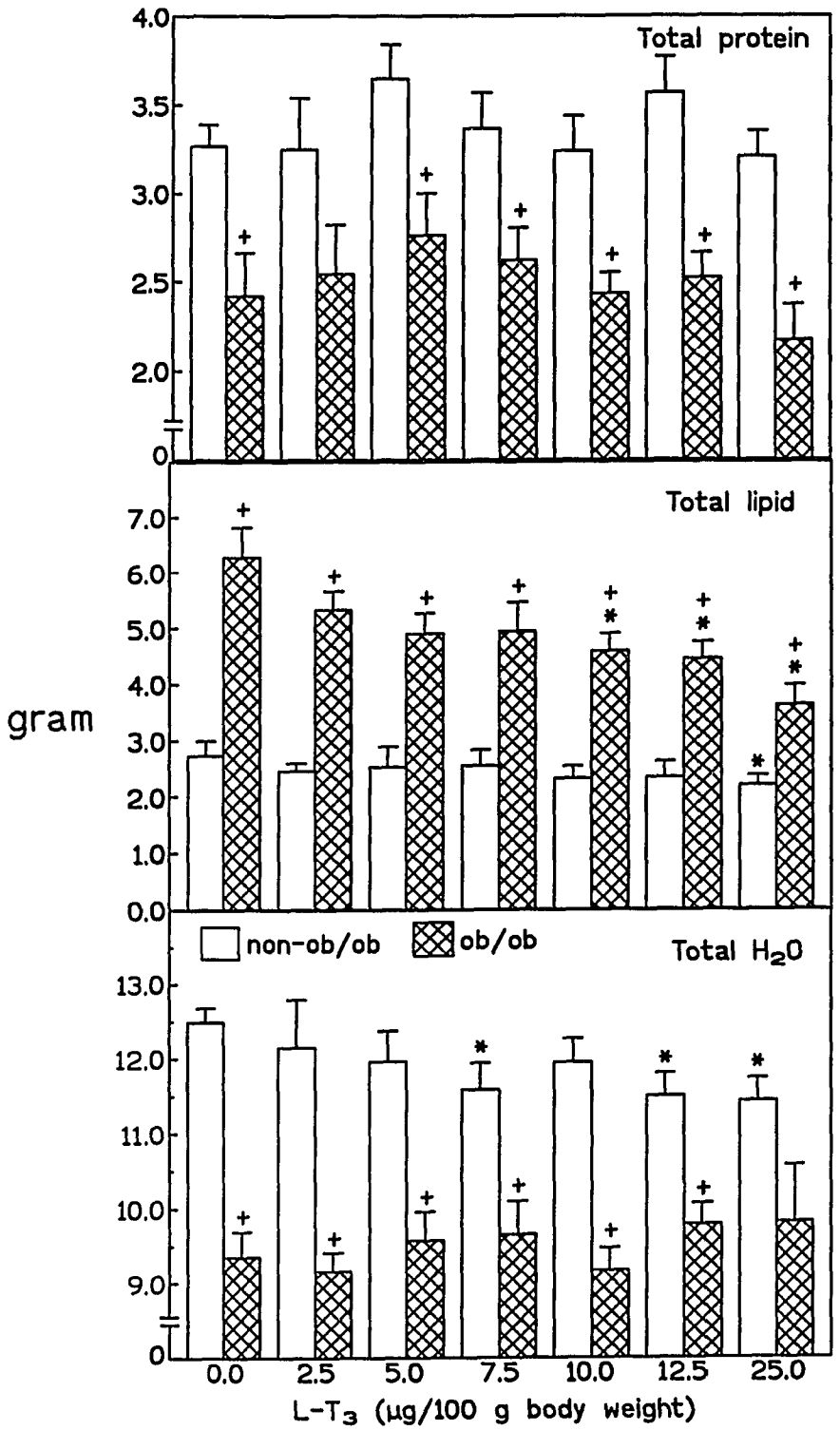
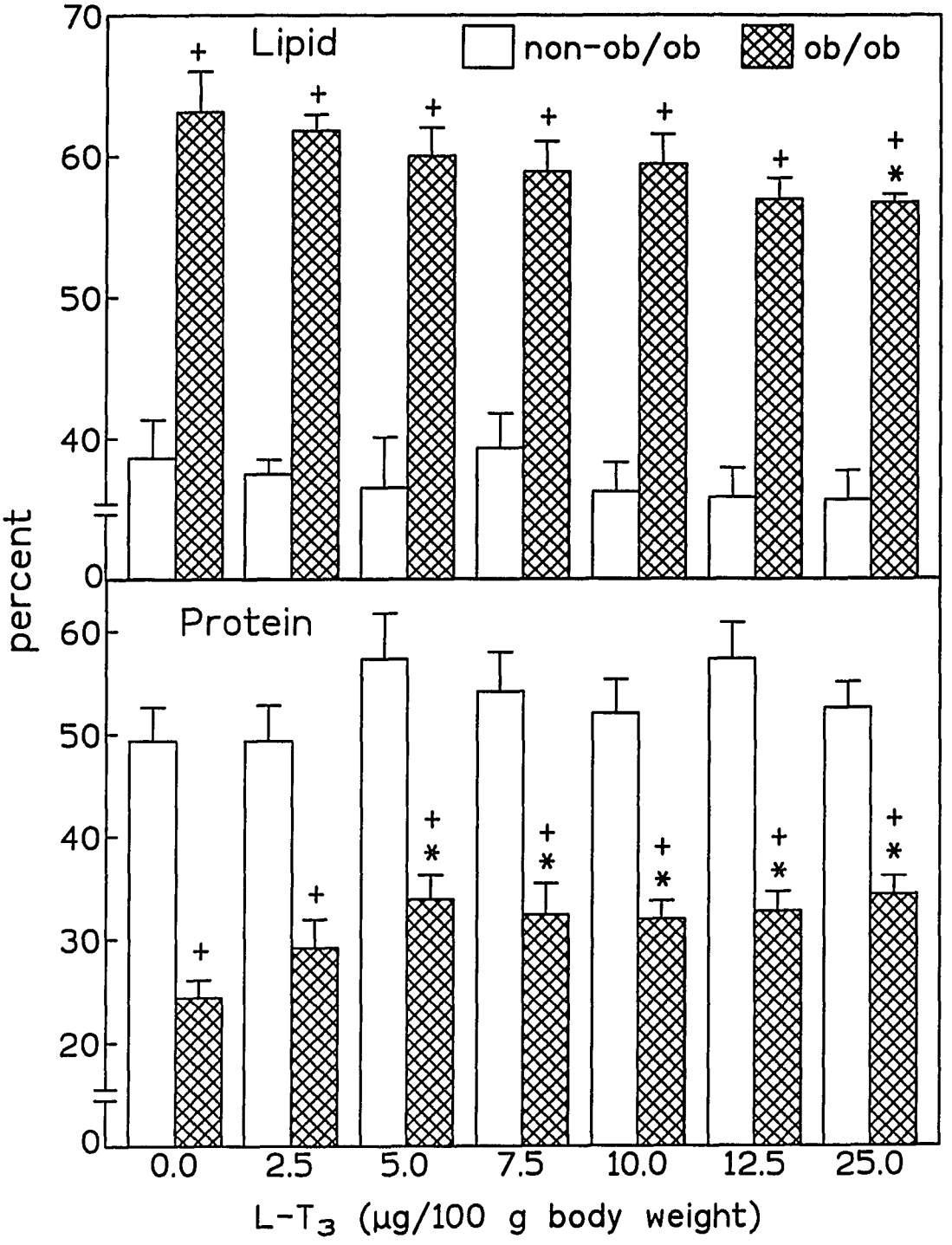

38.0
37.5
37.0
36.5
36.0
35.5
0.0
Temperature (°C)
non-ob/ob
ob/ob
+
+
0.0
2.5
5.0
7.5
10.0
12.5
25.0
T3 (µg/100 g BW)

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 8 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 x 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 $P < 0.001$.


Total protein
Total lipid
Total H_2O
gram
non-ob/ob
ob/ob
4.0
3.5
3.0
2.5
2.0
0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
13.0
12.0
11.0
10.0
9.0
0
0.0
2.5
5.0
7.5
10.0
12.5
25.0
L-T_3 (µg/100 g body weight)

treatment significantly decreased body fat 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 BW and higher levels. 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 BW) did T_3 treatment significantly decrease the percent lipid in ob/ob mice. 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 BW and higher levels.

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 8 mice per group. For percent lipid, the ANOVA showed a significant phenotype effect at $P < 0.0001$. For percent protein, the ANOVA showed a significant phenotype effect at $P < 0.0001$. Mean square errors for % lipid and % protein are 55.63 and 97.70 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 $P < 0.001$.


Lipid
non-ob/ob
ob/ob
Protein
percent
70
60
50
40
0
60
50
40
30
20
0
0.0
2.5
5.0
7.5
10.0
12.5
25.0
L-T3 (µg/100 g body weight)

DISCUSSION

In genetically obese hyperglycemic (ob/ob) mice, the early periods of life, prior to gross deposition of fat, are probably critical to the development of the obese syndrome. Genetic differences in some aspects of metabolism probably are present early and cause abnormal physiological function and subsequent expression of the phenotype. I propose that this critical period might be between 3 weeks of age, immediately after weaning, and 6 weeks of age when the obese phenotype is easily detectable by visual inspection. The saline-treated ob/ob mice in these experiments clearly showed lower oxygen consumption, lower body temperature, lower 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 (Dubuc 1976, Joosten and Van der Kroon 1974, Kaplan 1981). A low metabolic rate (Kaplan 1974) in preweaning ob/ob mice may be the result of a reduction in circulating thyroid hormones (Mobley and Dubuc 1979).

T_3 treatment increased circulating T_3 levels in the ob/ob mice to within the normal range (1.0-3.0 nmol/L). In the results reported here, T_3 treatment significantly increased animal oxygen consumption, increased 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). T_3 treatment decreased body weight in both phenotypes. Higher T_3 doses had a greater effect on preventing weight gain in the ob/ob than in non-ob/ob mice. A weight loss in ob/ob mice after T_3 treatment was previously reported by us (Oh and Kaplan 1989) as well as by others (Thenen and Carr 1980, York et al. 1978).

Serum insulin and glucose concentrations in saline-treated ob/ob mice and non-ob/ob mice were similar (Fig. 2). Values reported here are different from the reported hyperglycemia and hyperinsulinemia in ob/ob mice (Bray and York 1971). In this experiment, food consumption was equalized across all groups which may account for differences from other reports in which the ob/ob mice were hyperphagic. Dubuc (1976) reported higher serum glucose and insulin in ob/ob mice, which were allowed free access to food and were hyperphagic, than non-ob/ob mice. T_3 treatment significantly decreased serum insulin and glucose in both phenotypes, but greater degree in ob/ob mice. Serum glucose was significantly decreased in both phenotypes in a dose-dependent manner. Thenen and Carr (1980) reported that T_4 treatment lowered blood glucose in the ob/ob mice, but the hyperinsulinemia persisted. The differences in my results were probably due to the feeding regimen, which was

restricted. The food restriction itself in the ob/ob mice might cause a decrease in serum insulin in the saline-treated ob/ob mice.

Serum T_3 and T_4 were lower and serum TSH was higher in saline-treated ob/ob mice (Fig. 3). The high TSH levels of the ob/ob mice were probably related to the low T_4 and T_3 levels. Pituitary secretion of TSH is regulated by negative feedback control by T_4 and T_3 (Larsen 1982). The output of TSH by the pituitary is the principal factor modifying thyroid activity. T_3 treatment significantly decreased serum TSH at 2.5 μg T_3/100 g BW in ob/ob mice to the level of saline-treated non-ob/ob mice. However, T_3 treatment did not change serum TSH in non-ob/ob mice. This result suggests that exogenous T_3 causes negative feedback regulation of TSH secretion from the pituitary in ob/ob mice. Although serum TSH in saline-treated ob/ob mice was higher, it did not appear to trigger thyroid gland production of more T_3 or T_4, which suggests a defect in the pituitary-thyroid axis in ob/ob mice.

T_3 treatment significantly decreased serum T_4 in both phenotypes at all doses of T_3, which was expected, because T_3 imposes negative feedback regulation on the pituitary-thyroid axis, probably at the level of the thyroid gland. This finding is consistent with the reported suppression of serum T_4 by injected T_3 (Bray and Ohtake 1976).

Other investigators report that food restriction decreased serum T_3 in ob/ob mice (Himms-Hagen 1985) and in obese men (Katzeff et al. 1990, Rozen et al. 1986). York et al. (1978) reported that ob/ob mice had lower T_4 but normal T_3. The low T_3 found in this experiment may be due to food restriction.

Oxygen consumption in saline-treated ob/ob mice was lower than that in saline-treated non-ob/ob mice regardless of the different modes of expression (Fig. 4, 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. Kaplan (1981) reported that the ob/ob mice showed a lower O_2 consumption on a protein basis than non-ob/ob mice when animals were fed with free access to food. T_3 treatment increased O_2 consumption in ob/ob mice. Wimpfheimer et al. (1979) 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 (Abraham et al. 1985) which are all consistent with the results reported here.

Obese mice showed a low body temperature at an early age, even before weaning. This symptom persists after weaning through all developmental stages of obesity. At 6 weeks of age, saline-treated ob/ob mice had a lower colonic temperature than did non-ob/ob mice. This difference disappeared after T_3

treatment (Fig. 7). The increase in body temperature is matched with the increase in body oxygen consumption.

The change in body composition may influence the whole body metabolism. The saline-treated ob/ob mice showed higher total fat and lower total protein than non-ob/ob mice (Fig. 8). Because muscle tissues are metabolically more active than adipose tissues, this abnormally low ratio of protein to adipose mass can 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 percent 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.

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 and that T_3 treatment in the early stage of obesity corrected these

variables. These findings suggests that low serum T_3 and T_4 are from insufficient production of these hormones in thyroid glands. Also, thyroid gland did not respond to increased serum TSH in ob/ob mice to produce more T_4 and T_3. Because serum T_4 was much below the normal range (60-160 nmol/L) than was serum T_3 (1.0-3.0 nmol/L) (Fig. 3), I believe that the deiodination of T_4 to T_3 in ob/ob mice is not totally defected. Also, T_3 treatment increased body temperature, body oxygen consumption and decreased total body fat in the ob/ob mice. Because total body protein was not significantly changed, the increase in whole body metabolism after T_3 treatment is probably due to increased activity of the protein fractions or the most metabolically active tissues.

ACKNOWLEDGMENTS

The authors thank Ruth Koschorreck and her staff for care of the mice and Mark Troendle for assistance in the analysis of body composition.

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Footnotes

[1]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.

[2]AIN vitamin mix 76, ICN Biomedicals, Costa 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-alpha-tocopherol acetate (250 IU/g), 20 g; cholecalciferol (400,000 IU/g), 250 mg; menaquinone, 5 mg; sucrose, finely powdered, 972.9 g.

PAPER II. EARLY TREATMENT OF OBESE (OB/OB) MICE WITH TRIIODOTHYRONINE INCREASES OXIDATIVE METABOLISM IN MUSCLE BUT NOT IN BROWN ADIPOSE AND LIVER

ABSTRACT

Early defects in the ob/ob mouse are low body oxidative metabolism and low levels of circulating thyroid hormones. I explored the possibility that early T_3 treatment may improve oxidative metabolism in metabolically important tissues such as muscle, white and brown adipose tissue, and liver in the ob/ob mice. T_3, ranging from 0.0 to 25.0 μg/100 g BW, was injected intraperitoneally 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 the non-ob/ob saline-treated group to remove the effects of hyperphagia. At 6 weeks of age, the O_2 consumption of the above tissues was measured. The saline-treated ob/ob mice showed lower muscle weights, higher fat pad and liver weights, and larger fat cell sizes than saline-treated non-ob/ob mice. In ob/ob mice, tissue O_2 consumption was lower in muscle and brown and white adipose tissue but higher in liver as compared with that in same tissue of saline-treated non-ob/ob mice. Early T_3 treatment in ob/ob mice during growth resulted in significantly lower values for body weight, liver weight, hepatocyte number, liver protein, epididymal fat pad weight, and white adipocyte number and size than those values in saline-treated ob/ob mice. T_3 treatment increased muscle,

liver and brown adipose tissue O_2 consumption in non-ob/ob mice. In ob/ob mice, T_3 only increased muscle O_2 consumption and required higher doses than in non-ob/ob mice to achieve a change. These data are consistent with the concept of tissue T_3 resistance in ob/ob mice. Low T_3 levels and tissue resistance to T_3 seem to be important early defects in this obesity syndrome.

Key words triiodothyronine, oxidative metabolism, muscle, liver, white adipose tissue, brown adipose tissue

INTRODUCTION

The genetically hyperglycemic obese (ob/ob) mouse is extensively used for the study of human obesity. Major characteristics of ob/ob mouse are low oxygen consumption and low body temperature, which can be detected at 2 weeks of age and before the obesity is phenotypically expressed (Kaplan and Leveille 1974). These mice show decreased thermogenic activity of brown adipose tissue as measured by GDP binding to mitochondria, which was also detectable early and when obesity was not apparent (Goodbody and Trayhurn 1982, Himms-Hagen and Desautels 1978). Oxygen consumption of brown adipose tissue was lower in ob/ob than in non-ob/ob mice (Thurlby and Trayhurn 1980). Thyroid hormones potentiate the norepinephrine-stimulated GDP binding to brown adipose tissue mitochondria. This thermogenic activity is reduced in the brown adipose tissue of ob/ob mice (Himms-Hagen 1983a, Hogan and Himms-Hagen 1981).

Thyroid hormones are lower in ob/ob mice than in non-ob/ob mice during thermal stress (Bray and York 1971, Joosten and Van der Kroon 1974). The defects in thermogenesis in ob/ob mice may be attributed to impaired thyroid hormone action. Supportive of this concept are reports that the thyroid hormone-sensitive Na^+-K^+-ATPase is lower in the liver and skeletal muscle of ob/ob mice than of non-ob/ob mice (Lin et

al. 1979, York et al. 1978a). Recently, Hillgartner and Romsos (1988) reported that the ob/ob mice had lower hepatic thyroid hormone uptake than did non-ob/ob mice. This may account for the impaired thyroid hormone action and T_3 production in the liver, which is the major site for T_3 production from T_4.

Thyroid hormones are known to increase metabolic rate. Previously, I reported that T_3 treatment increased oxygen consumption and both non-insulin-stimulated and insulin-stimulated glucose utilization in ob/ob mice, and eliminated the insulin resistance by skeletal muscle (Oh and Kaplan 1989). Others have also shown that T_3 treatment increased anaerobic glycolysis in muscle (Nicole and Johnston 1981). Skeletal muscle plays a major role in glucose utilization (DeFronzo et al. 1981, Katz et al. 1983). Significant amounts of energy are also used by the skeletal musculature (Martin and Fuhrman 1955). Because O_2 consumption is lower in some muscles of ob/ob mice (Conway and Kaplan 1977, Kaplan and Oh 1991), the role of T_3 treatment of ob/ob mice on muscle O_2 consumption was further explored.

Thyroid hormones are also known to increase fatty acid mobilization from adipose tissue in rat (Fain and Rosenthal 1971). In ob/ob mice, the impaired norepinephrine-stimulated lipolysis was increased by exogenous thyroid hormone (Otto et al. 1976, Thenen and Carr 1978), but York et al. (1978b)

reported that adipose tissue lipolysis of ob/ob mice was less sensitive to thyroid hormone than that of non-ob/ob mice. In preadipocyte cell lines from both ob/ob and non-ob/ob mice, T_3 increased both fatty acid synthesis and lipolysis (Pou and Torresani 1989). Thyroid hormones stimulated hepatic fatty acid synthesis and esterification in the rat (Diamant et al. 1972, Mariash et al. 1980). Also, T_3 treatment increased O_2 consumption in isolated perfused liver (Horst et al. 1989, Müller and Seitz 1980) and in isolated hepatocytes (Gregory and Berry 1991). Liver utilized 30-40 % of the resting oxygen consumption (Martin and Fuhrman 1955) and therefore should be explored as a possible site for the increased total O_2 consumption in response to T_3 treatment.

The metabolic responses to thyroid hormones are different in many tissues (Barker and Klitgaard 1952, Schwartz 1983). T_3 treatment increased whole body oxygen consumption in ob/ob mice (Oh and Kaplan 1989). Major metabolically important tissues should account for the change in whole body metabolism. Although a controversy exists about the role of brown adipose tissue in human obesity, it is a thermogenically important tissue in ob/ob mice (Hogan and Himms-Hagen 1981). White adipose tissue is important in energy balance and should also be explored in terms of oxidative metabolism. The present report explores the possibility that early T_3

treatment of ob/ob mice over a 3-week period will alter the energy expenditure of major metabolically active tissues.

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 to allow pups to even access to nurturing female mouse. Oxygen consumption was measured at 16 days of age on the basis of the low oxygen consumption exhibited by future obese mice (Kaplan and Leveille 1974). In this study, 2000 μl O_2/hr·g body weight at STP was used as the value below which 2-week-old mouse pups are identified as obese with 99.9% reliability (the average value of O_2 consumption is 2322.0 for non-ob/ob and 1439.9 μl/hr·g BW for ob/ob mice). All mice were housed individually in solid bottom plastic cages with aspen wood shavings for bedding in an animal room at 23 ± 1°C with a 12-hour dark-light cycle (dark cycle 0600-1800 hr, light cycle 1800-0600 hr). All mice were fed a high carbohydrate diet during the 6-hour feeding period from 1000 to 1600 hr and allowed free access to tap water. The diet consisted of: casein, 20.0 g; DL-methionine, 0.3 g; AIN mineral mix 76[1], 3.5 g; AIN vitamin mix 76[2], 1.0 g; choline chloride, 0.2 g; corn oil, 5.0 g; fiber, 5.0 g; dextrose, 60.0 g; and dextrin, 5.0 g per 100 g diet. The diet was pelleted to facilitate the

measurement of food consumption. Food intake was equalized daily across all groups to the average consumed by non-ob/ob saline-treated group on the previous day. Food consumption and body weight were measured daily until 6 weeks of age. Mice were treated with L-triiodothyronine(T_3) as indicated below.

Treatments. Obese and nonobese mice were divided into 7 groups. Six levels of 2.5, 5.0, 7.5, 10.0, 12.5, and 25.0 μg L-T_3/100 g BW, dissolved in 0.154M NaCl (saline) (final pH 9.1 to keep the T_3 soluble), were injected intraperitoneally daily, between 0900 and 1000 hr from 3 to 6 weeks of age. Untreated ob/ob and non-ob/ob mice were injected with saline, pH 9.1. Body weight was measured daily at the time of the treatment. At 6 weeks of age, mice were killed by decapitation and skeletal muscle, adipose, or liver tissues were dissected and processed as described below.

Muscle oxygen consumption. The soleus muscles were dissected and stretched on pre-weighed horse-shoe shaped stainless steel wire clips. The muscles were then weighed and placed into 25 ml polycarbonate incubation flasks that contained 3 ml of incubation media, consisting of 5 mM glucose, 0 or 1000 μU/ml insulin in Krebs-Ringer bicarbonate (KRB) buffer (DeLuca 1964), pH 7.4, with one half the recommended calcium level to bring calcium to a physiological level (1.22% $CaCl_2 \cdot 2H_2O$), and gassed with 95% O_2, 5% CO_2 at

37°C in a gyrotory metabolic shaker. The diaphragm muscles were also dissected with ribs attached. After adipose tissue and other unwanted muscles and blood vessels were trimmed, the diaphragm was cut in half and rinsed in 0.9% saline. The hemidiaphragms were placed into incubation flasks with the same conditions as the soleus muscles. After 30 min of incubation, the diaphragms were dissected from the ribs. Then, soleus and diaphragm muscles were transferred to the O_2 electrode chamber, which contained the same media, and the chamber was gassed to saturation with 95% O_2, 5% CO_2. The chambers were sealed, bubbles were carefully removed, and the injection ports were sealed with mineral oil to minimize the leakage of O_2. Oxygen content of the media was determined by the method of Robinson and Cooper (1970). Oxygen consumed by muscle tissue in the chamber was recorded by YSI multi-channel biological oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, OH) for 10 min.

Muscle protein assay. Each muscle sample was homogenized in 1 ml of H_2O. The homogenates were analyzed for protein content by the biuret method (Layne, 1957) with bovine serum albumin (BSA) as the protein standard.

Adipocytes isolation. Epididymal adipose tissue and interscapular brown adipose tissue were dissected and weighed. Fat pads were rinsed in 0.9% saline and snips of both white and brown fat pads were fixed with 3% OsO_4 in 50 mM collidine

(2,4,6-trimethylpyridine) buffer, pH 7.4 (Hirsch and Gallian 1968) for later determination of cell number and size. The white adipocytes were isolated by the method of Rodbell (1964). For epididymal fat pads, the remnants of the fat pads were placed into 25 ml polypropylene flask which contained 3 ml of KRB, 3% BSA, 0.1% type IV collagenase, pH 7.4 at 37°C. The tissues then minced with scissors into smaller pieces. The flasks were placed in a gyrotory metabolic shaker at 37°C and gassed with 95% O_2, 5% CO_2 for one hour. After the incubation, the digestion mixture was filtered through a 250-μm nylon screen (Tetko Co.) and rinsed with KRB-3% BSA twice. Then, the filtrate was resuspended in KRB-3% BSA, pH 7.4, centrifuged at 150 x g for 1 min and rinsed twice with KRB, 3% BSA. After final centrifugation, the white adipocytes were suspended in KRB, 1% BSA, pH 7.4. Rough estimation of cell count was determined in a Fuchs-Rosenthal counting chamber (0.2 mm deep) microscopically by suspension in 0.3% trypan blue, 0.9% NaCl. Cells were generally 90% viable as determined by exclusion of the dye. A 0.2 ml of each cell suspension was fixed in 3% OsO_4 in 50 mM collidine buffer, pH 7.4, to obtain a precise cell count at a later date.

For brown adipocytes, cells were isolated in the same manner except that concentration of type IV collagenase was increased to 0.2% in KRB as recommended by Fain (1975). During incubation, the flasks with minced brown fat pads were

removed from the shaker and shaken by hand every 10 min for 10-15 sec to accelerate cell isolation. Viability of brown adipocytes was determined as in white adipocytes except that brown adipocytes were counted in Lange-Levy chamber (0.1 mm deep).

Adipocytes oxygen consumption. The volume of isolated cell suspension was adjusted to have approximately 0.5 x 10^6 cells/ml. Each 1 ml of diluted fresh cell suspension was added to tubes which contained 2 ml of one of the following: no substrate, 5 mM glucose, 2 mM pyruvate, 1 mM palmitate, or 2 mM acetate in KRB buffer with 1% BSA, pH 7.4. These tubes with cells and substrates were incubated at 37°C for 15 min in a gyrotory metabolic shaker and gassed with 95% O_2, 5% CO_2. After incubation, 2 ml of each cell suspension with substrate were placed into O_2 electrode chamber and gassed to saturation with 95% O_2, 5% CO_2. The chambers were sealed, and mineral oil was injected into the injection ports. Oxygen consumption of adipocytes was recorded for 10 min with a YSI oxygen monitor. Oxygen content of each medium was determined by the method of Robinson and Cooper (1970).

Adipose tissue and cell fixation for determination of cell number and size. Fixed cells were counted (Hirsch and Gallian 1968) with a Coulter counter, model Z_B, (Coulter Electronics Inc., Hialeah, FL) and sized in 10 categories of cell diameter as previously described (Kaplan et al. 1980). The average

cell size was also calculated for each sample as a grouped mean from the frequency distribution.

Hepatocyte isolation. The liver was dissected with the portal vein intact, rinsed in cold 0.9% saline, blotted, and weighed. A snip of liver was saved and frozen for later determination of protein. Hepatocytes were isolated by a modification of the method of Howard et al. (1973). The liver was perfused at 10 ml/min via the portal vein with cold calcium-free KRB buffer, pH 7.4, which contained 20 mM HEPES, 5 mM glucose, 1 mM pyruvate, 0.05% type II collagenase, 0.1% hyaluronidase, 95% O_2, 5% CO_2, for 5 min. The perfusate was not recirculated, and the reservoir of perfusate was continuously gassed with 95% O_2, 5% CO_2. Removal of blood from the liver was noted by blanching of all the lobes within 2 min. The liver was minced with a scalpel and placed into a 25 ml polycarbonate flask which contained 5 ml of the same media as the perfusate and incubated at 37°C in a metabolic shaker for 30 min, and under 95% O_2, 5% CO_2. After 30 min, 0.05 ml of 1.86% $CaCl_2 \cdot 2H_2O$ was added, and incubation was continued for another 30 min. The filtrate with cells were collected by filtering through a 100 μm nylon screen and rinsed with complete KRB (KRB with calcium), 20 mM HEPES, 1% BSA, 95% O_2, 5% CO_2, pH 7.4. The cell suspension was centrifuged at 120 x g for 1 minute. The hepatocytes were resuspended in complete KRB, 20 mM HEPES, 1% BSA, pH 7.4, and

centrifuged at 50 x g for 2 min. This resuspension-centrifugation repeated twice to rinse out enzymes. The final pellet was suspended in the same media. The cells were counted in a Lange-Levy hemocytometer chamber (0.1 mm deep) and the viability was determined by exclusion of 0.6% trypan blue. One ml of cell suspension was saved for later protein determination.

Hepatocyte oxygen consumption. Oxygen consumption of isolated hepatocytes was measured with a Clark-type oxygen electrode as described for the adipocyte O_2 consumption method.

Hepatocyte protein assay. The protein contents in the liver snip and an aliquot of the cell suspension were determined by the method of Lowry et al. (1951). I assumed the liver cells accounted for almost all protein in the liver.

Chemicals. L-3,3',5-Triiodothyronine was purchased from Calbiochem-Behring Co. (La Jolla, CA). Dextrose (anhydrous, granular) was purchased from Mallinckrodt Chemical Works (New York, NY). Porcine insulin and bovine serum albumin (RIA grade) were purchased from Sigma Chemical Co. (St. Louis, MO). A gyrotory water bath shaker (model 76) was purchased from New Brunswick Scientific (Edison, NJ). Collidine (2,4,6-trimethylpyridine) was purchased from Aldrich (Milwaukee, WI). Osmium tetroxide (OsO_4) was purchased from Electron Microscopy Sciences (Fort Washington, PA). Collagenases (type II and

type IV) and hyaluronidase were purchased from Worthington Biochemical Co. (Freehold, NJ). Nylon screens were purchased from Tetko Co. (Elmsford, NY). Folin-Ciocalteau reagent was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Diet ingredients were purchased from ICN Biomedicals (Costa Mesa, CA).

Statistics. Overall statistical significance was determined by analysis of variance (Ray 1982, Snedecor and Cochran, 1980). 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 ANOVA. Adipocyte size distribution was analyzed by multivariate analysis of variance (MANOVA), and using proportions weighted for the number of cells counted in each sample. The Wilks' Lambda test after conducting the ANOVA (GLM) was used to determine the discriminatory power of the model (SAS Institute Inc. 1985).

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 in three different sets of experiments (Table 1, 2, 3). T_3 treatment significantly decreased body weight in both phenotypes. 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 this period. The average total food intake during the T_3 treatment period for all groups was 47 g/mouse·3 weeks. The average weekly food consumption in all groups in the first set of experiment was 8.3 g (4 weeks), 17.1 g (5 weeks), and 21.7 g (6 weeks). The second and third sets of experiments followed the same trend. Because food consumption was equalized across all groups, no significant differences were found in total food consumed by all groups in these series of experiments.

Muscle weight and muscle protein. In saline-treated ob/ob mice, both soleus and diaphragm muscle weights were significantly lower than those of saline-treated non-ob/ob mice (Table 1). Overall, T_3 treatment did not change soleus muscle weight in either phenotype. In diaphragm muscles, T_3 treatment decreased the dissectable muscle weight in non-ob/ob but not in ob/ob mice. No differences in total soleus muscle

TABLE 1

Muscle weight and total muscle protein in T_3 treated ob/ob and non-ob/ob mice[1]

T_3 (μg/100 g BW)		Body wt. (g)	Soleus muscle wt.(mg)	Soleus muscle protein (mg)	Hemidiaphragm muscle wt.(mg)	Hemidiaphragm muscle protein (mg)
0.0	non-ob/ob	19.4 (18)	9.1 (50)$^{+}$	1.71 (50)	13.4 (50)$^{+}$	2.59 (50)
	ob/ob	19.2 (8)	7.7 (32)	1.36 (32)	10.8 (32)	2.24 (32)
2.5	non-ob/ob	18.8 (8)	8.9 (16)$^{+}$	1.50 (16)*	12.6 (16)	2.35 (16)
	ob/ob	17.8 (7)	7.3 (14)*	2.07 (14)	11.1 (14)*	2.45 (14)
5.0	non-ob/ob	18.5 (9)	8.0 (18)*	1.52 (18)	11.3 (18)*	2.34 (18)
	ob/ob	17.8 (8)*	7.7 (16)	1.51 (15)	10.6 (16)	2.51 (16)
7.5	non-ob/ob	17.9 (9)*	9.0 (18)$^{+}$	2.02 (18)	11.5 (18)*	2.67 (18)
	ob/ob	18.0 (6)	7.0 (14)*	1.50 (14)	10.7 (14)	2.87 (14)
10.0	non-ob/ob	18.3 (9)*	9.3 (18)$^{+}$	2.05 (17)	13.4 (18)$^{+}$	2.74 (18)
	ob/ob	16.9 (8)*	6.7 (16)*	1.42 (16)	11.0 (16)*	2.30 (16)
12.5	non-ob/ob	17.9 (9)*	8.8 (17)	1.70 (17)	13.8 (18)	2.91 (18)
	ob/ob	17.6 (8)*	7.6 (16)	1.29 (16)*	11.1 (16)*	2.80 (16)*
25.0	non-ob/ob	17.6 (8)*	8.9 (18)$^{+}$	0.85 (18)*	11.4 (18)*	1.73 (18)*
	ob/ob	16.2 (6)*	6.6 (12)*	0.82 (12)	9.9 (12)	1.93 (12)
				ANOVA (GLM)		
Phenotype		NS	0.0001	NS	0.0001	NS
T_3		NS	NS	0.0102	0.0235	0.0045
Phenotype x T_3		NS	NS	NS	NS	NS
MSE[2]		3.9706	2.8760	1.1074	6.7850	1.0518

[1]Values are the means of the number of samples in the parentheses. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. [2]MSE = Mean square of the error term from the ANOVA and is used in the protected t-test to determine significant differences between means. *Significantly different from the corresponding 0.0 μg T_3/100 g BW at $P < 0.05$ level, $^{+}$significantly different from non-ob/ob littermates at $P < 0.05$ level.

protein and total diaphragm muscle protein contents were found in saline-treated ob/ob and non-ob/ob mice (Table 1). In general, T_3 treatment did not change total soleus muscle protein in both phenotypes, except in ob/ob mice at 2.5 μg T_3 level that increased protein content. Total diaphragm muscle protein in both phenotypes did not change with T_3 treatment.

Liver weight, total hepatocyte number, and total liver protein. The weight of liver from saline-treated ob/ob mice and their relative liver weight (% liver wt./body wt.) were significantly higher than those of saline-treated non-ob/ob mice (Table 2). All levels of T_3 treatment significantly decreased ob/ob liver weight and relative liver weight, and phenotypic differences were no longer present after the treatment. T_3 treatment did not significantly change liver weight and relative liver weight in non-ob/ob mice. Total number of hepatocytes was not significantly different between saline-treated ob/ob and non-ob/ob mice (Table 2). T_3 treatment significantly decreased the total number of hepatocytes in both phenotypes. Total liver protein was significantly higher in saline-treated ob/ob mice than in saline-treated non-ob/ob mice (Table 2). T_3 treatment significantly decreased total liver protein in both phenotypes, and the differences between ob/ob and non-ob/ob mice were no longer obvious.

TABLE 2

Liver weight, total hepatocytes, and total liver protein in T_3 treated ob/ob and non-ob/ob mice[1]

T_3 (μg/100 g BW)		Body wt. (g)	Liver wt. (g)	Liver wt. (% of BW)	Hepatocytes (x10^6 cells)	Protein (mg/liver)
0.0	non-ob/ob	22.3 (18)	0.97 (18)$^+$	4.36 (18)$^+$	4.808 (18)	206.8 (18)$^+$
	ob/ob	22.6 (18)*	1.15 (18)$^+$	5.10 (18)$^+$	6.203 (18)	239.0 (18)$^+$
2.5	non-ob/ob	20.5 (18)*	0.95 (18)*	4.73 (18)*	5.857 (18)	194.2 (18)*
	ob/ob	20.3 (18)*	0.95 (18)*	4.69 (18)*	4.411 (18)*	206.0 (18)*
5.0	non-ob/ob	20.5 (18)*	0.90 (18)*	4.40 (18)*	2.486 (18)*	195.3 (18)*
	ob/ob	20.2 (18)*	0.97 (18)*	4.85 (18)$^{+*}$	4.433 (18)	210.1 (18)*
12.5	non-ob/ob	20.0 (18)*	0.95 (18)*	4.80 (18)*	2.956 (18)	193.0 (18)*
	ob/ob	19.6 (18)*	0.95 (18)*	4.82 (18)	3.495 (18)*	198.0 (18)*
25.0	non-ob/ob	19.4 (18)*	0.90 (18)*	4.67 (18)	2.012 (18)*	178.8 (18)*
	ob/ob	19.1 (18)*	0.92 (18)*	4.83 (18)	3.635 (18)	190.2 (18)*
			ANOVA (GLM)			
Phenotype		NS	0.0047	0.0015	NS	0.0046
T_3		0.0001	0.0001	NS	0.0147	0.0002
Phenotype x T_3		NS	0.0095	0.0166	NS	NS
MSE[2]		7.04083	0.01517	0.30482	16.5764	1237.89

[1]Values are the means of the number of samples in the parentheses. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. [2]MSE = Mean square of the error term from the ANOVA and is used in the protected t-test to determine significant differences between means. *Significantly different from the corresponding 0.0 μg T_3/100 g BW at $P < 0.05$ level, $^+$significantly different from non-ob/ob littermate at $P < 0.05$ level.

Epididymal and brown adipose tissue weight, cell number, and size. Epididymal fat pad weight in saline-treated ob/ob mice was significantly higher than that of saline-treated non-ob/ob mice (Table 3). T_3 treatment significantly decreased epididymal fat pad weight at all doses of T_3 in ob/ob mice and at higher doses in non-ob/ob mice. However, ob/ob epididymal fat pads always weighed more than non-ob/ob fat pads. The total number of epididymal adipocytes was higher in saline-treated non-ob/ob than in saline-treated ob/ob mice (Table 3). T_3 treatment decreased the number of epididymal adipocytes in both non-ob/ob and ob/ob mice. After T_3 treatment, phenotypic differences in total number of epididymal adipocytes per fat pad pair were no longer apparent.

Dissectable interscapular brown fat pads weights were higher in all groups of ob/ob mice than in corresponding non-ob/ob mice (Table 3). T_3 treatment increased non-ob/ob brown fat pad weight at higher doses but did not affect ob/ob brown fat pad weight. In brown adipose tissues, no differences in total number of brown adipocytes existed between saline-treated ob/ob and non-ob/ob mice (Table 3). T_3 treatment increased only the number of non-ob/ob brown adipocytes. The number of brown adipocytes did not change in ob/ob mice with T_3 treatment.

The size distribution of epididymal adipocytes in saline-treated ob/ob mice was significantly different from that of

TABLE 3

Adipose tissue weight, cell number and average cell size in T_3 treated ob/ob and non-ob/ob mice[1]

T_3 (μg/100 g BW)		Body wt. (g)	Epididymal fat pad wt.(g)	Epididymal fat pad cell no.($x10^6$)	Brown fat pad wt.(g)	Brown fat pad cell no.($x10^6$)
0.0	non-ob/ob	22.1 (20)	0.396 (20)	1.664 (20)	0.122 (20)	3.473 (20)
	ob/ob	23.4 (20)	0.804 (20)+	1.315 (20)+	0.289 (20)+	3.539 (20)
2.5	non-ob/ob	21.2 (20)	0.316 (20)	1.338 (20)*	0.169 (20)	4.535 (20)
	ob/ob	21.1 (20)*	0.631 (20)*+	1.067 (20)	0.303 (20)+	3.331 (20)
5.0	non-ob/ob	21.3 (20)	0.308 (20)	1.405 (20)	0.161 (20)	4.656 (20)
	ob/ob	20.9 (20)*	0.599 (20)*+	1.159 (20)	0.295 (20)+	3.556 (20)*
12.5	non-ob/ob	20.1 (20)*	0.267 (20)*	1.565 (20)	0.188 (20)*	5.649 (20)+
	ob/ob	20.4 (20)*	0.553 (20)*+	1.254 (20)	0.312 (20)+	3.112 (20)
25.0	non-ob/ob	20.5 (20)*	0.254 (20)*	1.009 (20)*	0.200 (20)*	3.715 (20)
	ob/ob	20.1 (20)*	0.521 (20)*+	1.064 (20)	0.332 (20)+	3.796 (20)
			ANOVA (GLM)			
Phenotype		NS	0.0001	0.0015	0.0001	0.0041
T_3		0.0001	0.0001	0.0005	0.0301	NS
Phenotype x T_3		NS	NS	NS	NS	NS
MSE[2]		5.27	0.0201	0.00885	0.249	5.165

[1]Values are the means of the number of samples in the parentheses. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. [2]MSE = Mean square of the error term from the ANOVA and is used in protected t-test to determine significant differences between means. *Significantly different from corresponding 0.0 μg T_3/100 g BW at $P < 0.05$ level, +significantly different from non-ob/ob littermate at $P < 0.05$ level.

non-ob/ob mice (Fig. 1). Saline-treated non-ob/ob mice showed higher proportions of adipocytes in the 40-63 μm diameter range, and saline-treated ob/ob mice had higher proportions in 63-100 μm range. Unlike non-ob/ob adipocytes, ob/ob adipocytes exhibited a bimodal distribution in all treatment groups with high proportions of both small cells and large cells, which is not reflected by average cell size. T_3 treatment resulted in the increase in the proportion of smaller adipocytes and the decrease in the proportion of larger cells in the ob/ob mice. The peak of the distribution in ob/ob mice shifted to the smaller diameter categories but still had higher proportions in the larger cell categories than in non-ob/ob mice.

The brown adipocyte size distribution in ob/ob mice did not exhibit a bimodal distribution (Fig. 2). Saline-treated ob/ob and non-ob/ob mice showed similar distribution patterns, having high proportions of 10-16 μm diameter cells. However, ob/ob mice had more cells in the ranges of 25-40 μm than did non-ob/ob which had higher proportions in the 16-25 μm ranges. T_3 treatment shifted the distribution in non-ob/ob mice towards the ranges of 20-32 μm. In the ob/ob mice, T_3 treatment minimally shift the proportions towards the ranges of 10-25 μm.

The average cell sizes were generated from the frequency distributions for each sample in Fig. 1 and 2. The average

Figure 1 Frequency of adipocyte size distribution in epididymal fat pads from T_3 treated 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 for 20 mice per group. A through J refers to the diameter of cells in ten size categories; A = 25-32 μm, B = 32-40 μm, C = 40-50 μm, D = 50-63 μm, E = 63-80 μm, F = 80-100 μm, G = 100-127 μm, H = 127-159 μm, I = 159-200 μm, J = 200-253 μm. The Wilks' lambda test was used after conducting MANOVA to test significant main effect and interaction. The MANOVA showed significant T_3 effect at $P < 0.0001$, phenotype effect at $P < 0.0001$, and T_3 x phenotype effect at $P < 0.0001$.

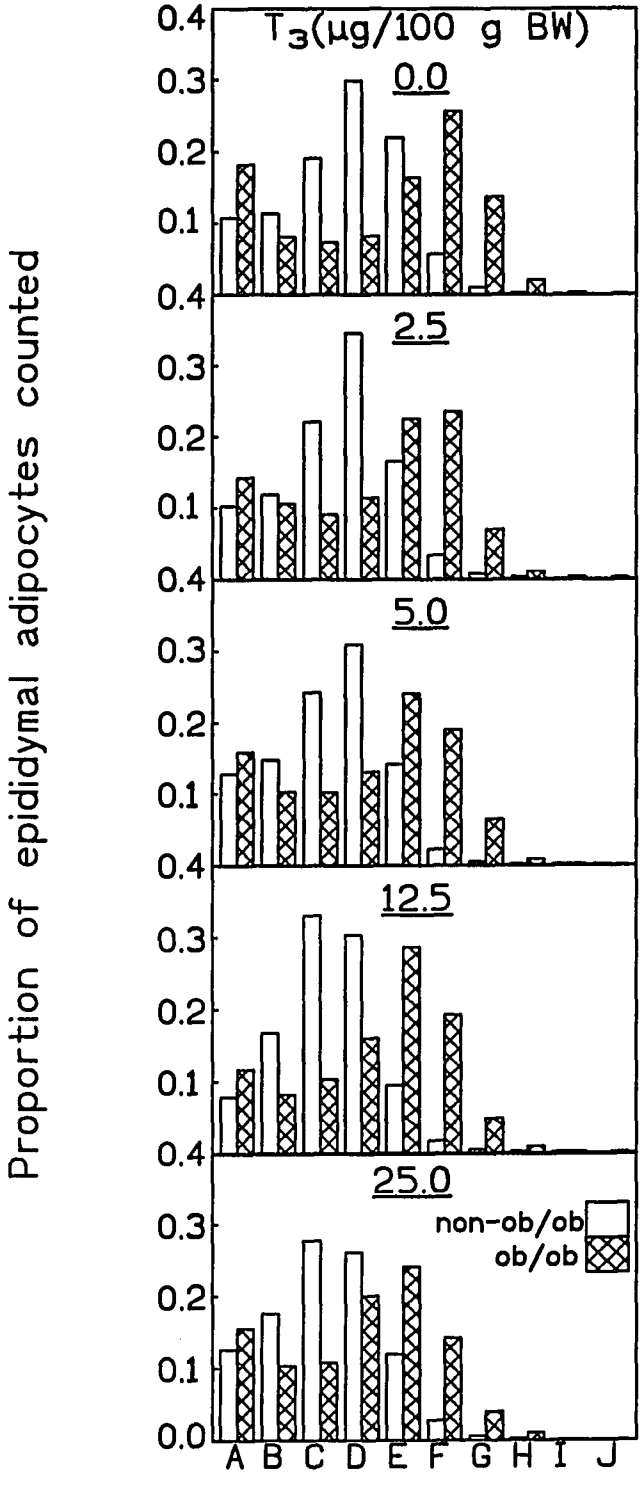
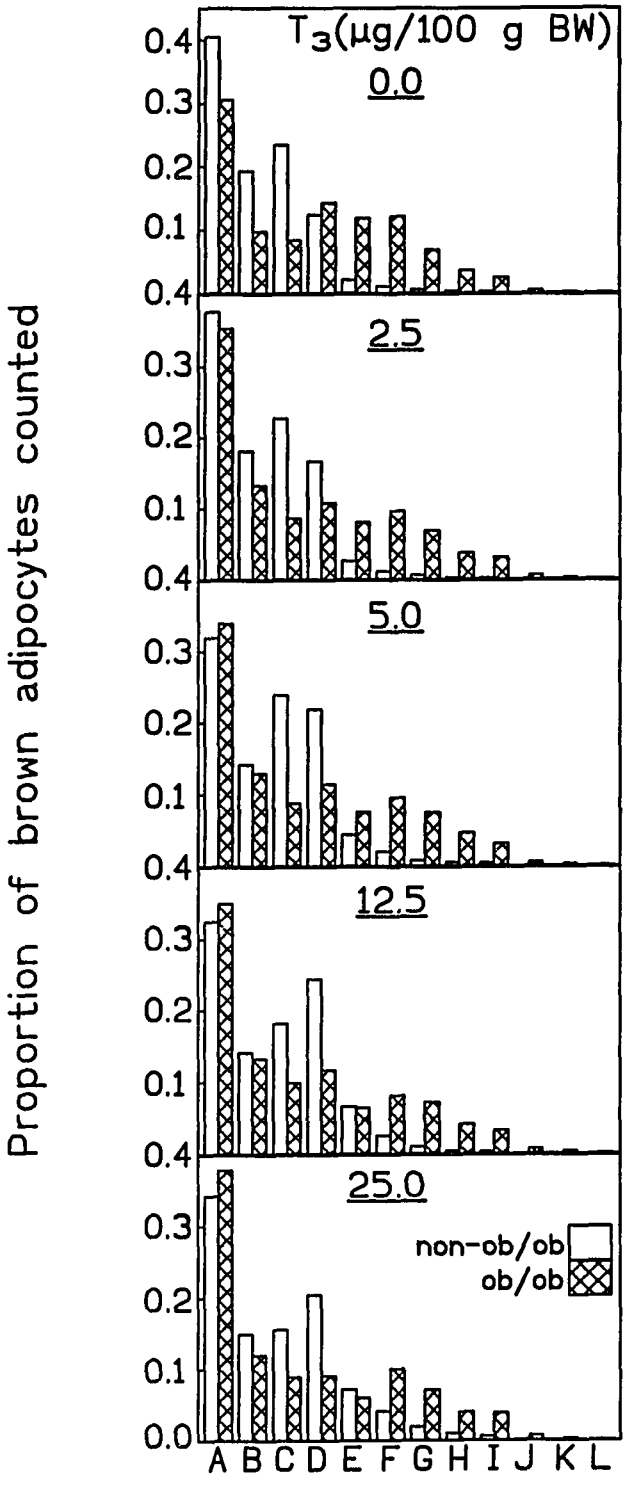

Proportion of epididymal adipocytes counted
T_3(µg/100 g BW)
0.0
2.5
5.0
12.5
25.0
non-ob/ob
ob/ob
0.0
0.1
0.2
0.3
0.4
A B C D E F G H I J

Figure 2 Frequency of adipocyte size distribution in brown fat pads from T_3 treated 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 for 20 mice per group. A through L refers to the diameter of cells in twelve size categories; A = 10-16 μm, B = 16-20 μm, C = 20-25 μm, D = 25-32 μm, E = 32-36 μm, F = 36-40 μm, G = 40-45 μm, H = 45-50 μm, I = 50-63 μm, J = 63-71 μm, K = 71-80 μm, L = 80-100 μm. The Wilks' lambda test was used after conducting MANOVA to test significant main effect and interaction. The MANOVA showed significant T_3 effect at $P < 0.0001$, phenotype effect at $P < 0.0001$, and T_3 x phenotype effect at $P < 0.0001$.


Proportion of brown adipocytes counted
T_3(µg/100 g BW)
0.0
2.5
5.0
12.5
25.0
non-ob/ob
ob/ob
0.0
0.1
0.2
0.3
0.4
A B C D E F G H I J K L

cell size of epididymal adipocytes in saline-treated ob/ob mice was significantly larger than in saline-treated non-ob/ob (Table 4). T_3 treatment decreased the average size of epididymal adipocytes in both phenotypes at all doses of T_3. However, ob/ob mice always had larger cell sizes than did those of non-ob/ob mice. The average size of brown adipocytes in ob/ob mice was larger than non-ob/ob in all groups. T_3 treatment increased the average cell size of brown adipocytes in non-ob/ob mice, and not in ob/ob mice.

Muscle oxygen consumption. In ob/ob soleus muscles, O_2 consumption in saline-treated group was significantly lower than that of non-ob/ob mice. T_3 treatment increased both non-insulin-stimulated and insulin-stimulated O_2 consumption at 7.5 μg/100 g BW and higher levels of T_3 (Fig. 3). Also, T_3 treatment increased insulin sensitivity of soleus muscle O_2 consumption in non-ob/ob mice.

In non-ob/ob diaphragm muscles, T_3 treatment increased non-insulin-stimulated O_2 consumption at 2.5 μg/100 g BW T_3 level, maintained those levels of O_2 consumption, and also increased insulin-stimulated O_2 consumption (Fig. 3). Diaphragm muscles in saline-treated ob/ob mice were not sensitive to exogenous insulin, and insulin-stimulated O_2 consumption was significantly lower than that in muscles in non-ob/ob mice. T_3 treatment increased the sensitivity of ob/ob diaphragm to

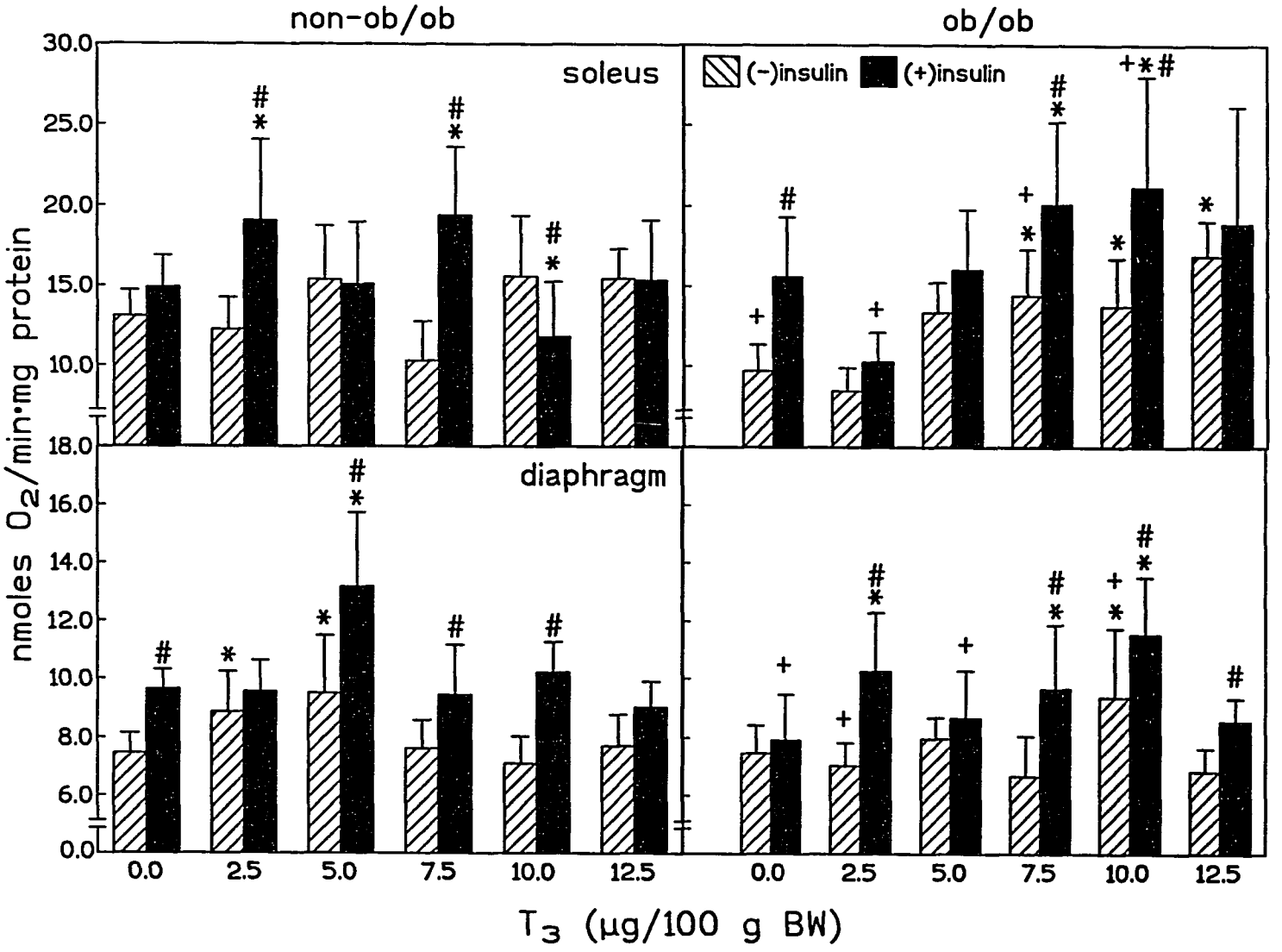
TABLE 4

Average cell size[1]

T_3 (μg/100 g BW)	Epididymal adipocytes (μm)			Brown adipocytes (μm)		
	non-ob/on	ob/ob	*P*	non-ob/ob	ob/ob	*P*
0.0	54.95 (20)	70.03 (20)	< 0.0001	19.28 (20)	26.67 (20)	< 0.0001
2.5	52.94 (20)	66.26 (20)*	< 0.0001	19.87 (20)	25.43 (20)	< 0.0001
5.0	50.74 (20)*	63.44 (20)*	< 0.0001	21.38 (20)*	25.88 (20)	< 0.0001
12.5	49.67 (20)*	64.87 (20)*	< 0.0001	21.83 (20)*	25.37 (20)	< 0.0001
25.0	49.85 (20)*	60.75 (20)*	< 0.0001	22.18 (20)*	25.39 (20)	< 0.0001
		ANOVA (GLM)				
Phenotype		0.0001		0.0001		
T_3		0.0001		NS		
Phenotype x T_3		NS		0.0018		
MSE[2]		34.18		6.621		

[1]Values are the means of the number of samples in the parentheses. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. [2]MSE = Mean square of the error term from the ANOVA and is used in the protected t-test to determine significant differences between means. *Significantly different from the 0.0 μg T_3/100 g BW within phenotypes at $P < 0.05$ level.

Figure 3 Soleus and diaphragm muscles oxygen consumption per unit muscle protein in T_3 treated 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 8-9 mice per group. For soleus muscle, the ANOVA showed a significant insulin effect at $P < 0.05$. Mean square error is 12.180. For diaphragm, the ANOVA showed a significant insulin effect at $P < 0.005$. Mean square error is 1.9845. Significant differences between means were determined by using the protected t-test. *significantly different from the corresponding 0.0 μg T_3/100 g BW at $P < 0.05$, $^{+}$significantly different from non-ob/ob at $P < 0.05$, $^{\#}$significantly different from non-insulin tissue at $P < 0.05$.


non-ob/ob
ob/ob
soleus
diaphragm
(-)insulin
(+)insulin
nmoles O_2/min·mg protein
T_3 (µg/100 g BW)
0.0
2.5
5.0
7.5
10.0
12.5

insulin and also increased both non-insulin-stimulated and insulin-stimulated O_2 consumption.

Hepatocyte oxygen consumption. In saline-treated ob/ob and non-ob/ob mice, no differences in hepatocyte O_2 consumption per 10^6 cells were observed (Fig. 4). T_3 treatment significantly increased hepatocyte O_2 consumption per 10^6 cells in both phenotypes, but to a greater extent in the non-ob/ob mice. Pyruvate showed the greatest substrate-induced respiratory rate, particularly after T_3 treatment. The other substrates did not exhibit substrate-stimulated respiration after T_3 treatment of the mice. When the data were expressed on a whole liver basis, the ob/ob mice had a higher O_2 consumption than did non-ob/ob mice in the saline-treated groups. T_3 treatment significantly decreased whole liver O_2 consumption in both phenotypes (Fig. 4), and differences between phenotypes disappeared. The pyruvate-induced respiration was still higher after T_3 treatment.

Epididymal and brown adipocytes oxygen consumption. Oxygen consumption of saline-treated ob/ob epididymal adipocytes was lower than that of non-ob/ob mice on both a per cell basis and a per whole fat pad basis (Fig. 5). T_3 treatment increased O_2 consumption in both phenotypes, although this difference was not statistically significant ($P > 0.05$). Epididymal adipocyte O_2 consumption was lower in the ob/ob than that in non-ob/ob at all doses of T_3. Pyruvate exhibited the highest

Figure 4 Hepatocytes oxygen consumption per 10^6 cells and per liver in T_3 treated 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 9 mice per group. For O_2 consumption per 10^6 cells, the ANOVA showed significant T_3 effect at $P < 0.0001$, phenotype effect at $P < 0.0001$, T_3 x phenotype effect at $P < 0.0031$ and substrate effect at $P < 0.0001$. Mean square error is 15100.778. For O_2 consumption per liver, the ANOVA showed significant T_3 effect at $P < 0.0001$, phenotype effect at $P < 0.0083$, T_3 x phenotype effect at $P < 0.0001$ and substrate effect at $P < 0.0001$. Mean square error is 27930.16. Significant differences between means were determined by using the protected t-test. *significantly different from the corresponding 0.0 μg T_3/100 g BW at $P < 0.05$, $^+$significantly different from non-ob/ob at $P < 0.05$, $^\#$significantly different from KRB at $P < 0.05$.

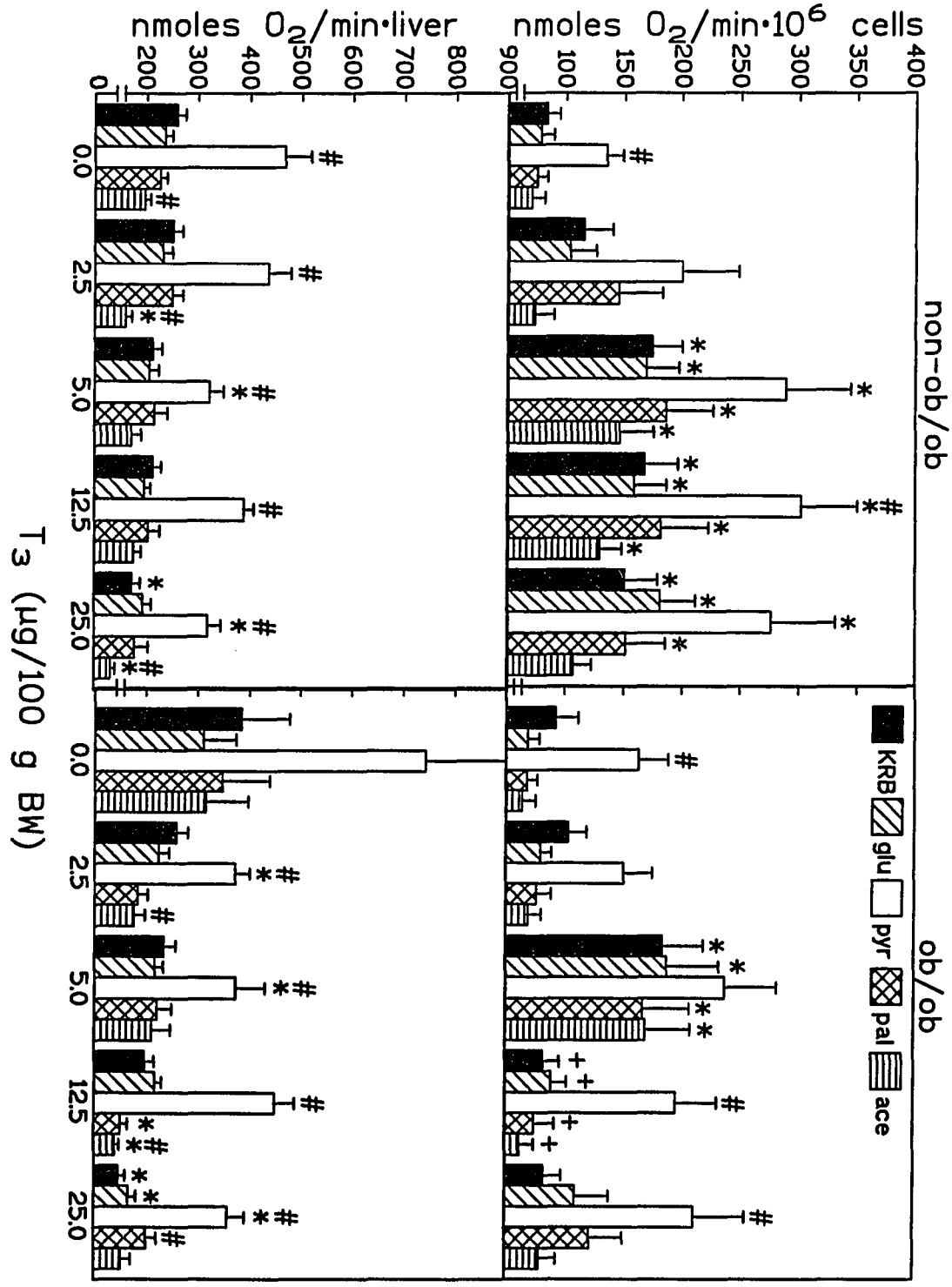
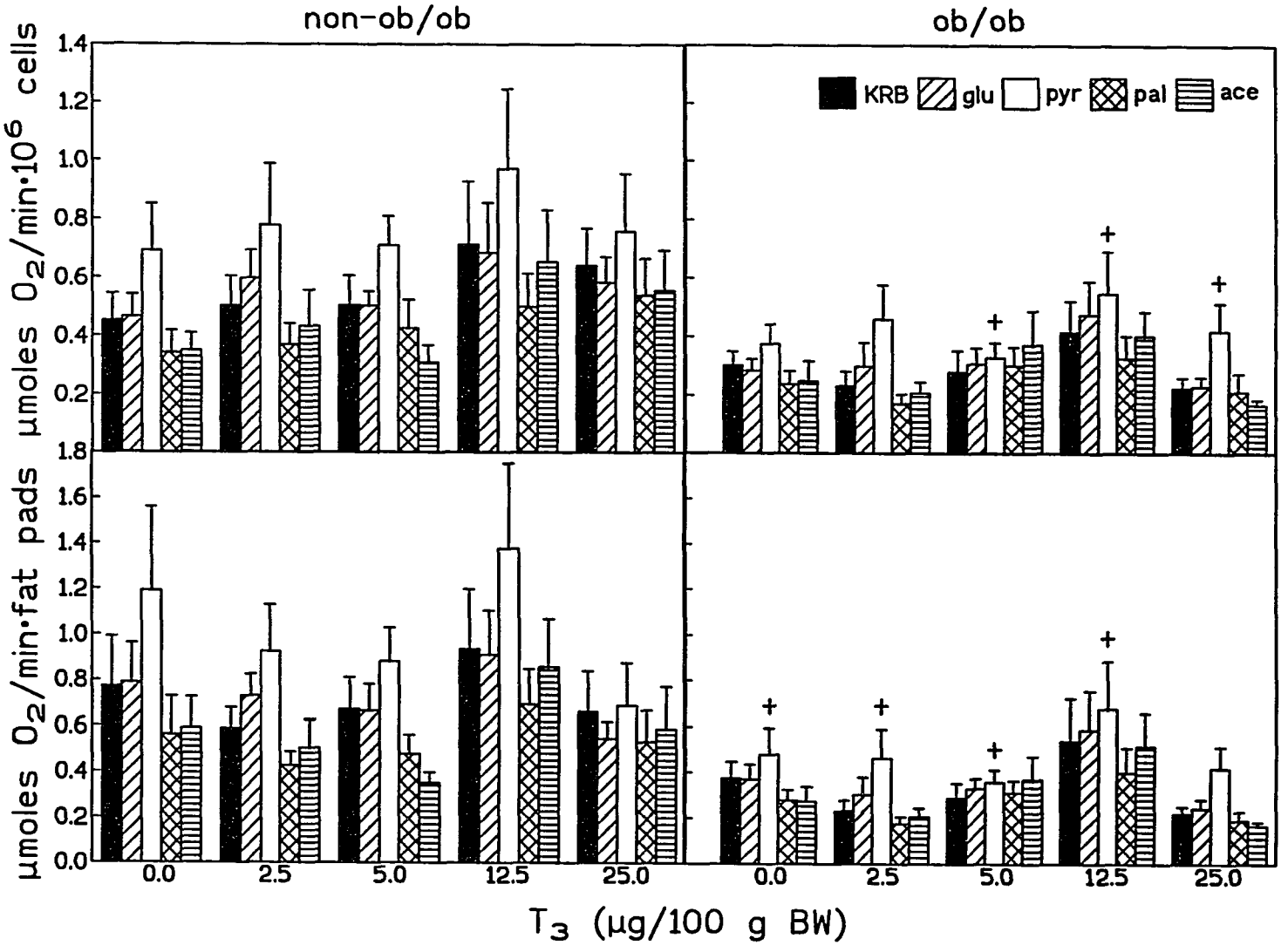

nmoles O_2/min·liver
nmoles O_2/min·10^6 cells
non-ob/ob
ob/ob
KRB
glu
pyr
pal
ace
T_3 (µg/100 g BW)
0.0
2.5
5.0
12.5
25.0

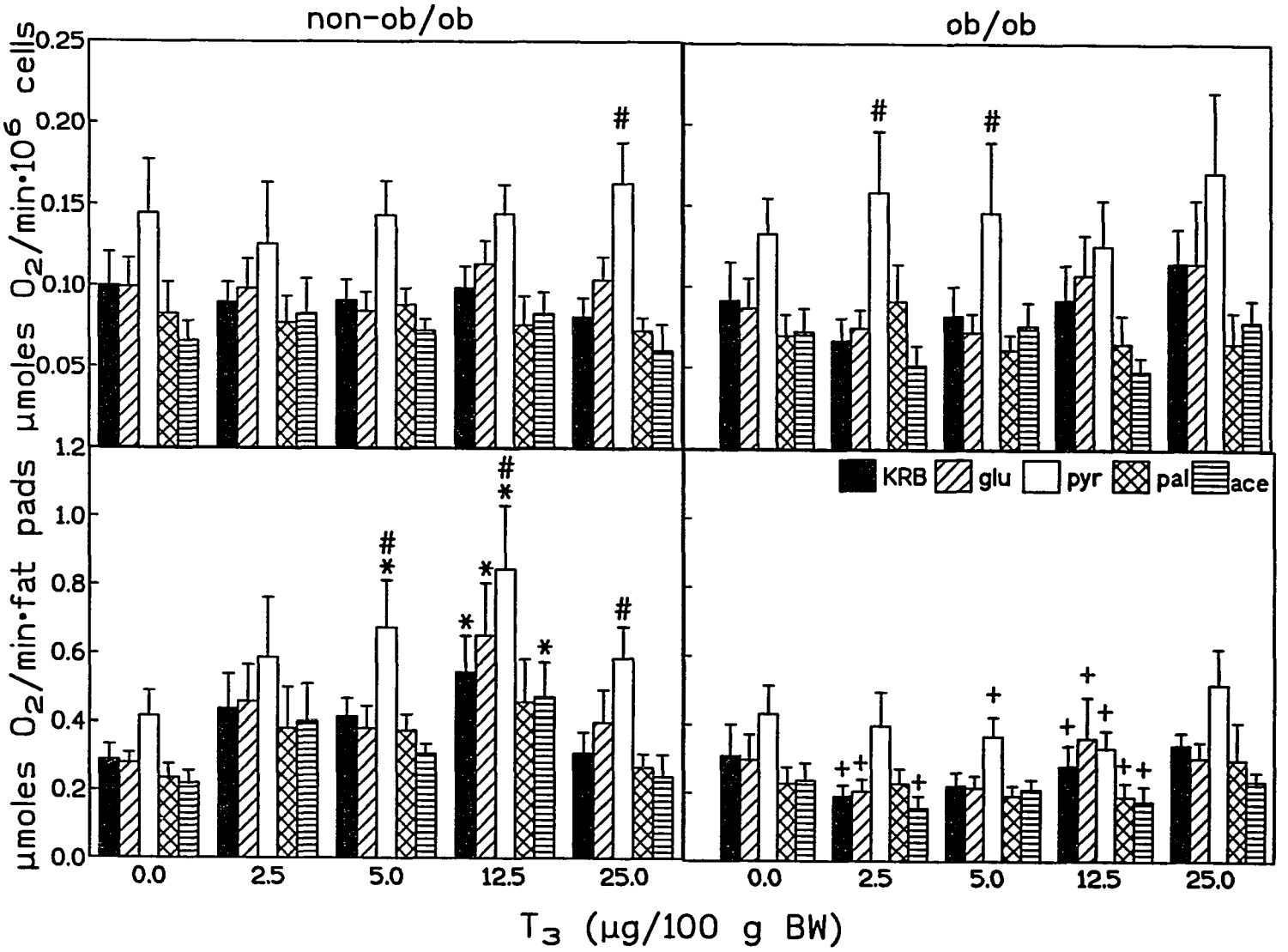
Figure 5 Epididymal adipocytes oxygen consumption per 10^6 cells and per epididymal fat pad pair in T_3 treated ob/ob and non-ob/ob mice at 6 weeks of age. The mice were treated with T_3 from 3 to 6 weeks of age as outlined in Materials and Methods. Values are the means ± SEM for 10 mice per group. For O_2 consumption per 10^6 cells, the ANOVA showed significant T_3 effect at $P < 0.0008$, phenotype effect at $P < 0.0001$, and substrate effect at $P < 0.0001$. Mean square error is 0.1191. For O_2 consumption per fat pad pair, the ANOVA showed significant T_3 effect at $P < 0.0001$, phenotype effect at $P < 0.0001$, and substrate effect at $P < 0.0001$. Mean square error is 0.2243. Significant differences between means were determined by using the protected t-test. $^+$significantly different from non-ob/ob at $P < 0.05$.


non-ob/ob
ob/ob
KRB
glu
pyr
pal
ace
µmoles O2/min·10^6 cells
µmoles O2/min·fat pads
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
0.0
2.5
5.0
12.5
25.0
T3 (µg/100 g BW)

substrate induced O_2 consumption in comparison to other substrates in both phenotypes.

Brown adipocyte oxygen consumption per 10^6 cells was not different in ob/ob and non-ob/ob mice (Fig. 6). However, O_2 consumption per dissectable brown fat pads in ob/ob mice was lower than that in non-ob/ob mice at all doses of T_3. T_3 treatment increased brown adipocyte O_2 consumption dramatically only in the non-ob/ob when expressed on a fat pad basis. The highest substrate induced O_2 consumption was obtained with pyruvate.

Figure 6 Brown adipocytes oxygen consumption per 10^6 cells and per dissectable fat pads in T_3 treated 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 10 mice per group. For O_2 consumption per 10^6 cells, the ANOVA showed a significant substrate effect at $P < 0.0001$. Mean square error is 0.004389. For O_2 consumption per dissectable fat pads, the ANOVA showed significant T_3 effect at $P < 0.0048$, phenotype effect at $P < 0.0001$, T_3 x phenotype effect at $P < 0.0001$, and substrate effect at $P < 0.0001$. Mean square error is 0.06724. Significant differences between means were determined by using the protected t-test. *significantly different from the corresponding 0.0 μg T_3/100 g BW at $P < 0.05$, $^+$significantly different from non-ob/ob at $P < 0.05$, $^\#$significantly different from KRB at $P < 0.05$.


non-ob/ob
ob/ob
µmoles O2/min·10^6 cells
µmoles O2/min·fat pads
KRB
glu
pyr
pal
ace
0.0
2.5
5.0
12.5
25.0
T3 (µg/100 g BW)

DISCUSSION

By 5-6 weeks of age, the obese phenotype is readily apparent by visual inspection, and several metabolic abnormalities are present. The low metabolic rate (Kaplan and Leveille 1974) and abnormal temperature regulation (Joosten and Van der Kroon 1974) evident in preweaning ob/ob mice were likely to be the result of reduced circulating thyroid hormones reported by me (Oh and Kaplan 1993) and by others (Mobley and Dubuc 1979). Also, these mice had fewer liver T_3 receptors per unit DNA than did non-ob/ob at 2 weeks of age, which supports the likelihood that abnormal thyroid hormone action is an early defect (Khan et al. 1986). This early developmental period between 2 and 6 weeks of age might be a critical period in terms of changing metabolic aspects of obesity. In this study, the T_3 treatment had significant effects on oxidative metabolism in skeletal muscles, brown adipose tissue, and liver of young ob/ob mice.

The decrease in body weight in ob/ob mice (Table 1, 2, 3) after T_3 treatment was due mostly to the decrease in body fat. Soleus and diaphragm muscle weights in saline-treated ob/ob mice were lower than those of saline-treated non-ob/ob mice (Table 1). Because skeletal muscle accounts for 40-50 % of body weight in normal weight human, it could be one of the most important metabolic masses (Owen et al. 1978). However,

overall T_3 treatment did not significantly change muscle weight and muscle protein in ob/ob mice.

As expected, epididymal fat pad weight and liver weight in ob/ob mice were greatly decreased after T_3 treatment (Table 2, 3). These changes in above tissue weight after T_3 treatment agree with some of the work of others (Van der Kroon et al. 1981) and with my own past work (unpublished 1993) which reported that total body fat content was decreased but total body protein content did not significantly change. Liver weight and relative liver weight in ob/ob mice were decreased after T_3 treatment possibly by loss of fat content. Total liver protein and total number of hepatocytes were higher in saline-treated ob/ob mice due to the heavier liver (Table 2). T_3 treatment during growth resulted in both lower hepatocyte number and liver protein only in ob/ob mice. This action of T_3 on liver weight and hepatocyte numbers was previously unknown and to my knowledge is first reported here.

The saline-treated ob/ob mice which were not hyperphagic still had larger epididymal fat deposits (Table 3) but had fewer adipocytes and larger cell sizes than did saline-treated non-ob/ob mice (Table 3, 4). This is different from studies in which the ob/ob mice are hyperphagic, which indicates that elevated adipocyte numbers are not necessary for early onset obesity. The earliest indicators of early onset obesity seem to be larger adipocyte size (Kaplan et al. 1980). T_3

treatment resulted in significantly lower epididymal fat pad weight, adipocyte number, and average cell size in ob/ob mice, but these mice still had higher fat pad weights and larger cell sizes than did non-ob/ob mice. Van der Kroon et al. (1981) reported similar results after T_4 treatment in that ob/ob mice receiving the same amount of food as non-ob/ob mice still had higher fat deposition. In the Zucker obese (fa/fa) rat, obesity also develops when hyperphagia is prevented (Berke and Kaplan 1983, Cleary et al. 1980). In adult ob/ob mice, the excessive body fat can be maintained without hyperphagia because of depressed energy expenditure (Himms-Hagen 1983b, Pullar and Webster 1977).

The cell size distribution of epididymal adipocytes was different between ob/ob and non-ob/ob mice. The ob/ob mice showed a bimodal distribution with more cells in the smaller (25-32 μm) and larger (80-100 μm) ranges (Fig. 1). This mode of cell distribution in ob/ob mice suggested that ob/ob mice were making more fat cells (smaller range) while filling fat cells that already exist with fats (larger range). Non-ob/ob mice had a unimodal distribution, having more cells in the 50-63 μm range. T_3 treatment shifted the distribution towards an increased number of smaller cells and a decreased number of larger cells with the peak range of the distribution in the ob/ob mice being in the 63-80 μm range. In non-ob/ob mice, the peak was shifted to the 40-50 μm range. These data are

consistent with the decreased body fat in both phenotypes due to T_3 treatment.

Brown adipose tissue was heavier in saline-treated ob/ob mice than in non-ob/ob mice. T_3 treatment increased brown adipose tissue weight, brown adipocyte number, and average cell size in non-ob/ob mice but not in ob/ob mice (Table 3). Others reported that T_4 treatment increased brown adipose tissue weight in lean but not in ob/ob mice (Knehans and Romsos 1984). Blouquit and Gripois (1990) reported that hypothyroid rats had lower brown adipose tissue weight than did euthyroid rats, and T_3 treatment increased brown adipose tissue weight in hypothyroid rats. These data indicate that the brown adipose tissue in ob/ob mice does not respond to exogenous T_3 as indicated by failure of brown adipocyte number and average cell size to change after the T_3 treatment (Table 3, 4). The cell size distribution of brown adipocytes showed more cells in smaller range in both ob/ob and non-ob/ob mice (Fig. 2). T_3 treatment increased the distribution towards more large cells only in the non-ob/ob mice. These data are consistent with the action of T_3 to increase brown adipose tissue weight, cell size, and number in non-ob/ob mice and support the concept of resistance to the action of T_3 in brown adipose tissue of ob/ob mice.

As previously mentioned, skeletal muscle mass is about 40-50 % of body weight in normal human subjects (Owen et al.

1978) and accounts for at least 50 % of the whole body resting metabolic rate (Tzankoff and Norris 1977). The lower muscle weight in ob/ob mice together with the lower muscle O_2 consumption reported here (Fig. 3) as well as in past reports (Conway and Kaplan 1977, Kaplan and Oh 1991) may account for lower body O_2 consumption (Kaplan and Leveille 1974, Oh and Kaplan 1989). Previously, I reported that T_3 treatment increased both non-insulin-stimulated and insulin-stimulated glucose utilization in muscles from ob/ob and non-ob/ob mice (Oh and Kaplan 1989), but the ob/ob required higher T_3 doses to achieve this stimulation. In the present study, T_3 treatment significantly increased non-insulin-stimulated and insulin-stimulated O_2 consumption in ob/ob soleus and diaphragm muscles, and increased insulin sensitivity in ob/ob diaphragm muscle. Higher doses of T_3 were required in the ob/ob to achieve levels similar with those in the non-ob/ob mice, which is consistent with the concept of T_3 resistance by tissues of ob/ob mice.

DeLuise and Harker (1989) reported that the genetically obese NZO mice had lower soleus muscle O_2 consumption and lower Na^+-K^+-pump activity, which may contribute to the development of obesity. The activation of sympathetic nervous system may provide some link between increased Na^+-K^+-pump activity and energy expenditure by skeletal muscle. In humans, epinephrine-induced thermogenesis is minimal in brown

adipose tissue, which suggests that up to 50 % of the increase in whole body O_2 consumption may take place in other tissues such as skeletal muscle (Astrup et al. 1985). Also, Blaak et al. (1993) reported that both β_1 and β_2 adrenoceptors are involved in the sympathetically mediated thermogenesis whereas α_1, α_2, and β_3 adrenoceptors do not play a role in the healthy human. Because the adrenoceptor population of skeletal muscle mainly consists of β_2 receptors, skeletal muscle is probably an important site of localization of sympathetically mediated thermogenesis (Ligget et al. 1988).

Hepatocyte O_2 consumption per cell was not different between saline-treated ob/ob and non-ob/ob mice (Fig. 4) suggesting that the oxidative capability of ob/ob hepatocytes are the same as non-ob/ob hepatocytes. However, total liver O_2 consumption in saline-treated ob/ob mice was higher than that of non-ob/ob due to the larger liver size and higher number of hepatocytes (Table 2). Higher liver O_2 consumption in ob/ob mice may be the result of increased lipogenic activity. T_3 treatment increased hepatocyte O_2 consumption per cell in both phenotypes. The non-ob/ob mice were more sensitive to exogenous T_3 and achieved higher level of respiration than did ob/ob mice. On a whole liver basis, O_2 consumption was decreased in ob/ob mice after T_3 treatment mainly due to the decrease in liver weight. In the non-ob/ob mice, major changes in whole liver O_2 consumption after T_3

treatment were not evident except at very high doses of T_3. After T_3 treatment, no phenotypic differences between ob/ob and non-ob/ob mice were evident. McBride and Early (1989) reported that total O_2 consumption of isolated hepatocytes was unaffected after T_4 treatment in sheep, but ouabain-sensitive respiration was increased, suggesting that the involvement of Na^+-K^+-ATPase in thyroid hormone-stimulated oxidative metabolism. Except for pyruvate, there was no substrate-induced O_2 consumption in hepatocytes from saline-treated mice. However, T_3 treatment stimulated substrate-induced hepatocyte O_2 consumption. This result agrees with the reports in which T_3 increased substrate-induced (palmitate + lactate) O_2 consumption in isolated rat hepatocytes (Gregory and Berry 1991).

DeGroot et al. (1976) reported no relationship between T_3 receptors and thyroid status in the rat liver. However, it is reported that ob/ob mice had fewer receptors than did non-ob/ob mice at all ages from 2 weeks through 18 weeks of age (Khan et al. 1986). Because the metabolic response to T_3 depends not only on the concentration of hormone but on the number of hormone receptors, ob/ob mice might have both lower thyroid hormone levels and fewer receptors than non-ob/ob mice.

In the present study, T_3 treatment increased hepatocyte O_2 consumption per cell, but total hepatocyte numbers were

decreased, resulting in no change in total liver O_2 consumption. The liver of ob/ob mice was not very responsive to exogenous T_3. Hillgartner and Romsos (1988) reported that decreased hepatic thyroid hormone uptake may contribute to impaired thyroid hormone action in ob/ob mice, because hepatic T_3 production contributes approximately 28 % of the intracellular T_3 in liver.

Epididymal adipocyte O_2 consumptions per cell and per fat pad were lower in ob/ob mice than non-ob/ob mice at all doses of T_3 (Fig. 5). There was no overall effect of T_3 on epididymal adipocyte O_2 consumption. Others report that white adipose tissue is a thyroid hormone-responsive tissue (Cronrath et al. 1988), and T_3 increased malic enzyme activity in explants of human white adipose tissue (Rao et al. 1984). However, oxidative metabolism of white adipose tissue from ob/ob mice in this study was resistant to exogenous T_3, which is consistent with the concept of tissue resistance to thyroid hormone in ob/ob mice.

Brown adipocyte O_2 consumption per cell was not different between ob/ob and non-ob/ob (Fig. 6), suggesting that the oxidative ability of ob/ob adipocytes are the same as non-ob/ob adipocytes. Because ob/ob mice had heavier fat pads than did non-ob/ob but had the same number of cells (Table 3), O_2 consumption per fat pad was lower than that of non-ob/ob mice. T_3 treatment did not change ob/ob brown adipocyte O_2

consumption because it did not change brown fat pad weight, cell number, and cell size. However, in non-ob/ob mice, brown fat pad O_2 consumption increased dramatically after T_3 treatment (Fig. 6) due to the increase in the number of adipocytes (Table 3). Abelenda and Puerta (1990) reported that thyroid hormone did not affect brown adipose tissue significantly when thermogenic requirements are moderate, but participate in the trophic response when thermogenic requirements were intense. This might explain the lack of response of brown adipocyte to T_3 in this study along with tissue resistance of ob/ob mice. The metabolic activity of brown adipose tissue is regulated by norepinephrine via its sympathetic innervation, and thyroid hormone enables the tissue to respond thermogenically to norepinephrine (Himms-Hagen 1984). However, the capacity of these ob/ob mice to respond to norepinephrine by an increase in metabolic rate was reduced (Hogan and Himms-Hagen 1980), and cold-induced increase in brown adipose tissue thermogenesis was very small in ob/ob mice (Kates and Himms-Hagen 1990). Also, ob/ob mice were defective in ß-adrenergic component (Kates et al. 1990). Although the sympathetic innervation of brown adipose tissue seems normal in ob/ob mice (Ashwell and Dunnett 1985), the brown adipose tissue in ob/ob mice may be refractory to norepinephrine or thyroid hormone. These are consistent with

our results that brown adipose tissue of ob/ob mice is resistant to T_3.

From this study, I can conclude that T_3 treatment decreased body weight mostly by a lack of accumulation of body fat, and increased oxidative metabolism in metabolically important tissues studied, which were skeletal muscle, liver, and brown adipose tissue. In ob/ob mice, T_3 treatment significantly decreased body weight along with body fat, but increased oxidative metabolism only in muscles, not in liver or brown adipose tissue. The action of T_3 on ob/ob muscles required higher doses than in non-ob/ob mice. The data in the present report are consistent with the concept of T_3 resistance by muscle and brown adipose tissue in ob/ob mice. The concomitant low circulating levels of thyroid hormones and the tissue resistance to T_3 might be important early defects in this particular obesity syndrome.

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Footnotes

[1]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.

[2]AIN vitamin mix 76, ICN Biomedicals, Costa 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-alpha-tocopherol acetate (250 IU/g), 20 g; cholecalciferol (400,000 IU/g), 250 mg; Menaquinone, 5 mg; sucrose, finely powdered, 972.9 g.

GENERAL DISCUSSION AND SUMMARY

In genetically obese hyperglycemic (ob/ob) mice, the early period of life, prior to gross deposition of fat, is probably critical to the development of the obese syndrome. By 5-6 weeks of age, the obese phenotype is grossly apparent by visual inspection and several metabolic abnormalities are present. Genetic differences in some aspects of metabolism probably are present early and cause abnormal physiological function and subsequent expression of phenotype. The low metabolic rate (Kaplan and Leveille 1974) and abnormal temperature regulation (Joosten and Van der Kroon 1974) evident in preweaning ob/ob mice are likely to be the result of reduced circulating thyroid hormones reported by me in this dissertation and by others (Mobley and Dubuc 1979). Also, these mice had fewer liver T_3 receptors per unit DNA than did non-ob/ob at 2 weeks of age and throughout the adulthood (Khan et al. 1986). This suggests the likelihood that abnormal thyroid hormone action is an early defect. This early developmental period between 2 and 6 weeks of age might be a critical period in terms of changing metabolic aspects of obesity. I proposed that this critical period might be

between 3 weeks of age, immediately after weaning, and 6 weeks of age when the obese phenotype is easily detectable. The possibilities were explored that an early T_3 treatment in this critical period would improve oxidative metabolism and reduce body fat.

The saline-treated ob/ob mice in these experiments clearly showed lower whole body oxygen consumption, lower tissue oxygen consumption in muscle and brown and white adipose tissues but higher oxygen consumption in liver, lower body temperature, lower concentrations of thyroid hormones (T_4 and T_3), and higher body fat at 6 weeks of age as compared with same parameter in saline-treated non-ob/ob mice. T_3 treatment increased circulating T_3 levels to within the normal range, increased animal O_2 consumption, increased body temperature, and decreased body fat of young ob/ob mice without the change in total body protein. The equalized food consumption prevented gross obesity in ob/ob mice during experimental period. T_3 treatment along with restricted food regimen further reduced circulating insulin and glucose level in ob/ob mice.

Contradictory reports exist about concentrations of thyroid hormones in serum 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. Pituitary secretion of TSH is regulated by negative feedback control by T_4 and T_3, and is the principal factor modifying thyroid activity. These experiments showed that ob/ob mice exhibit decreased serum T_4 and T_3 and increased TSH concentrations. Evidently, the pituitary secreted TSH in response to lower circulating levels of thyroid hormones but somehow the thyroid gland could not produce more T_4 or T_3. This lack of thyroid hormone production suggests that there might a defect either in the pituitary-thyroid axis (thyroid gland not responding to the TSH signal from the pituitary) or thyroid gland itself (dysfunctional hormone producing cells).

Individual body composition may influence the whole body metabolism. T_3 treatment decreased body weight mostly by a lack of accumulation of body fat. Because muscle tissues are metabolically more active than fat tissues, the abnormal ratio of protein to fat mass can explain the lower O_2 consumption in the ob/ob mice. The increase in body O_2 consumption after T_3 treatment 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. T_3 treatment increased oxidative metabolism in metabolically important tissues studied, which were skeletal muscle, liver, and brown adipose tissue of non-ob/ob mice. In ob/ob mice, T_3 treatment significantly decreased body weight along with body

fat, but increased oxidative metabolism only in muscle, not in liver or brown adipose tissue. The action of T_3 on ob/ob muscles required higher doses than in non-ob/ob mice. The data in present dissertation are consistent with the concept of T_3 resistance by muscle and brown adipose tissue in ob/ob mice.

The concomitant low circulating levels of thyroid hormones and the tissue resistance to T_3 might be important early defects in this particular obesity syndrome. The importance of these defects to the etiology of obesity is not clearly answered yet. This dissertation has furthered the understanding of the action of thyroid hormone on oxidative metabolism at the early stage of developing obesity in metabolically important tissues especially skeletal muscle and adipose tissues. Thyroid hormone action should be considered in the context of the total physiological setting of the animal because thyroid hormones interact with a large variety of hormonal and metabolic stimuli.

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