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Decreased Triiodothyronine Binding to Isolated Nuclei from Livers of Preobese and Obese (ob/ob) Mice[1] (42313)

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Abstract. L-Triiodothyronine (T_3) binding to hepatic nuclei from (ob/ob) mice at different ages was examined and compared with that of lean controls. Results showed a significant reduction in T_3 binding in liver nuclei of obese mice at all ages studied. The preobese mice at 2 weeks of age had 27.9% fewer receptor sites/mg DNA compared to lean controls, receptor concentration further decreased to 67.7% at 18 weeks of age. Data presented here demonstrates that the impaired triiodothyronine (T_3) binding to hepatic nuclei present in older (ob/ob) obese mice is an antecedent to the obesity. This report also helps to explain the poor thermoregulation and low oxygen consumption present during the preobese phase of the postnatal development of these animals.

The obese (ob/ob) mouse is an extensively studied animal model of genetic obesity. Numerous metabolic and endocrine disorders have been described in the grossly obese mouse (1). Some of these disorders, such as reduced metabolic rate and hypothermia in response to cold can be detected at a very early age preceding the development of obesity (10–17 days old) (2, 3). Although thyroid hormone is known to regulate metabolic rate (4) and to be essential for survival at lower temperatures (5), these animals are not hypothyroid (6). However, a peripheral impairment of thyroid hormone action, evidenced by a decreased concentration of nuclear triiodothyronine (T_3) receptors in the liver and lung of grossly obese mice, has been demonstrated (7). The purpose of this study was to determine if this decrement in nuclear T_3 receptor concentration in liver precedes the development of obesity and if this decrement changes as the development of obesity progresses. We report a decrease in nuclear T_3 receptor concentration in the liver of preobese mice and that this difference increases as obesity becomes pronounced.

Materials and Methods. All mice used in this study were obtained from Jackson Laboratories, Maine. From 4 weeks of age the visually obese mouse can be separated from lean. Preobese mice at 2 weeks of age were identified on the basis of their thermoregulatory defect in response to cold (2). Two-week-old mouse pups were removed from the nest, housed individually and the initial (at 22.5°C) body temperature was recorded by inserting into the rectum a fine thermocouple (0.6 mm o.d.) attached to a YSI telethermometer. Each pup was then transferred to a 15.5°C cold room and subsequent rectal temperatures were recorded over 15, 30, and 45 min of cold exposure. On the basis of the response some were categorized as "preobese" or lean according to how they tolerated the cold. A few that could not be categorized with certainty were not used in this study. In preliminary studies it was determined that the (ob/ob) genotype could be predicted in 100% of animals. Figure 1 illustrates a typical individual mouse temperature response to cold exposure and the basis of obesity prediction.

The mice were sacrificed under ether anesthesia, the liver excised quickly, blotted, and pooled whenever necessary to get 1.5 g of tissue (as required to provide sufficient nuclei for a six-point saturation isotherm of T_3 binding). Nuclei were prepared as described by Samuels and Tsai (8). DNA content of the isolated nuclei was estimated by Barton's method (9). The

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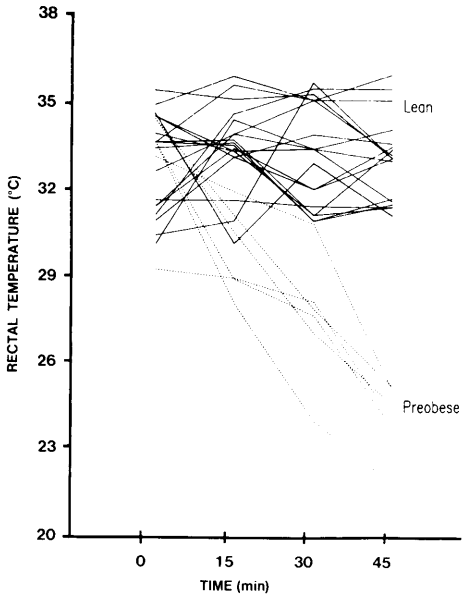

FIG. 1. Individual rectal temperature of 24 two-week-old mice in response to an environmental temperature of 15.5°C. The technique provides differentiation between lean and preobese animals. Borderline animals (two that could not be categorized with certainty) were not used in this study and therefore their genotype was not determined.

method used for tracer T_3 binding to isolated nuclei was first described by Samuels and Tsai (8) and subsequently modified by others (10–12). Isolated liver nuclei containing 50–100 μg DNA in 0.5 ml of STMED buffer (containing 250 mM sucrose, 20 mM Tris–HCl, 1.1 mM $MgCl_2$, 2 mM EDTA, and 5 mM dithiothreitol, pH 7.9 at 22.5°C) were incubated with 0.1 to 5 nM of ^{125}I T_3 (NEN, sp act 750–1200 μCi/μg) at 37°C for 30 min in the presence or absence of 5 μM T_3. After the incubation the reaction was stopped by adding 1.0 ml of washing buffer (10% polyethylene glycol, 0.25% Triton X-100 in STMED) (13). The tubes were thoroughly mixed, then centrifuged at 1500g for 5 min, and the supernatant fraction was discarded. The pellet was washed and its radioactivity (dpm/tube) was measured in a Beckman Gamma 9000. The binding data were analyzed by Reverse Scatchard plot using the program described by Zivin and Waud (14). A typical saturation isotherm and Reverse Scatchard plot is illustrated in Fig. 2.

Results and Discussion. Radiolabeled T_3 binding to liver nuclear receptors of preobese and lean mice at 2 weeks of age were examined. The results indicated a significant reduction in maximum binding (B_{max}) in preobese liver nuclei and not in the affinity of the receptor for the hormone (K_d).

Binding to liver nuclei of obese and lean mice at different ages (4, 6, 8, 12, and 18 weeks old) was also studied. Liver of obese mice had fewer receptors per milligram DNA compared to their lean controls at all ages studied (Table I). The results indicated no change in the affinity of the hormone for the receptors (K_d) (Table I). The preobese mice at 2 weeks of age had 27.9% fewer receptor sites per milligram DNA compared to the lean controls and this receptor concentration further decreased to 67.7% at 18 weeks of age (Fig. 3), a period when weight gain had ceased and obesity was fully developed. Variations due to leakage of nuclear T_3 receptor to the incubation media which might interfere with the results (15) were estimated in preliminary experiments and found to be very low. Thus, the possibility that the observed differences are artifact is highly unlikely.

The findings that the preobese mice at 2 weeks of age had fewer nuclear T_3 receptor sites in liver indicate that this abnormality is

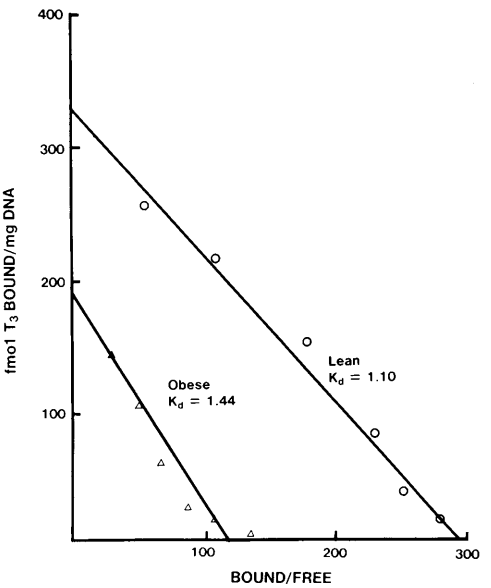

FIG. 2. Representative reverse Scatchard analysis from an experiment of ^{125}I T_3 binding to isolated nuclei from liver of 2-week-old preobese mice and lean controls.

TABLE I. ^{125}I-T_3 BINDING TO HEPATIC NUCLEAR RECEPTOR OF OBESE MICE AND LEAN CONTROLS AT DIFFERENT AGES[a]

Age groups (weeks)	B_{max}[b]		K_d[c]	
	Lean	Obese	Lean	Obese
2 (N = 6)	297.1 ± 28.8	205.3 ± 16.9[d]	1.95 ± 0.32	1.46 ± 0.16
4 (N = 6)	276.4 ± 18.9	204.8 ± 17.1[d]	1.08 ± 0.18	1.17 ± 0.28
6 (N = 6)	299.1 ± 72.2	211.4 ± 53.1[d]	1.79 ± 0.49	1.97 ± 0.65
8 (N = 4)	598.3 ± 65.5	423.5 ± 44.6[d]	2.44 ± 0.53	2.45 ± 0.51
12 (N = 4)	612.1 ± 108.9	396.4 ± 98.2[d]	1.19 ± 0.06	1.16 ± 0.33
18 (N = 5)	675.2 ± 127.9	173.4 ± 9.3[d]	2.15 ± 0.35	1.83 ± 0.26

[a] At 2 weeks the pooled livers were from male and female pups. At all other ages the animals were males.
[b] B_{max} values are means ± SEM expressed as fmole/mg DNA.
[c] K_d values are means ± SEM expressed as nM.
[d] $P < 0.01$ compared to obese animals using t test for paired data between lean and obese.

not a secondary effect of obesity and thus may be a primary cause of the development of obesity. Since thyroid hormone plays a major role in thermogenesis, a decrement in nuclear T_3 receptor concentration could result in a partial refractoriness to actions of T_3 upon thermogenesis in the animal. For example, reduced nuclear T_3 receptor concentration may underlie the decreased response of basal metabolic rate to thyroid hormone and impaired thyroid-induced sodium–potassium-dependent adenosine triphosphatase level observed in these mice (16).

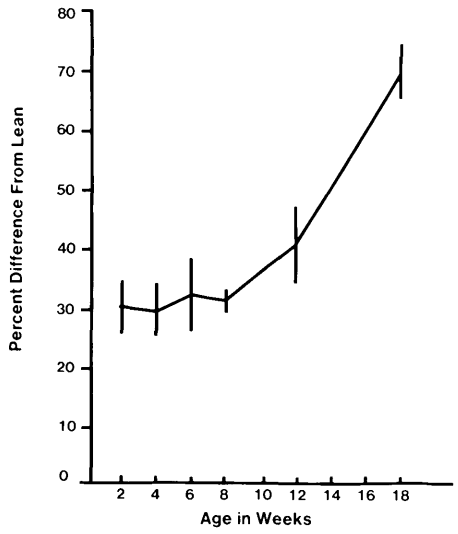

FIG. 3. Percentage difference in hepatic nuclear T_3 receptor concentration between lean and obese mice at several ages. Obesity has not begun to develop at 2 weeks of age; 18-week-old obese animals are grossly obese. (% difference = [B_{max} (lean − obese)/B_{max} (lean)] × 100.

The present data also indicate that the development of obesity may further affect nuclear T_3 receptor concentration since the decrement observed in obese animals increased with age. The substantial decrement in nuclear T_3 receptor concentration in grossly obese adult mice apparently, therefore, results from both congenital and developmental factors. The data presented here helps to explain the poor thermoregulation and low oxygen consumption present in these mice at a very early age preceding the development of obesity. We suggest that the decrement in T_3 receptor present before the development of obesity in the (ob/ob) mouse is a primary determinant of obesity.

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1. Bray DA, York GA. Hypothalamic and genetic obesity in experimental animals: an Autonomic and endocrine hypothesis. Physiol Rev **59**:719, 1979.
2. Trayhurn P, Thurlby P, James WPT. Thermogenic defect in the preobese ob/ob mouse. Nature (London) **166**:60, 1977.
3. Kaplan ML, Leveille GA. Core temperature. O_2 consumption and early detection of ob/ob genotype. Amer J Physiol **117**:912, 1974.
4. Acheson KJ, Burger AG. A study of the relationship between thermogenesis and thyroid hormones. J Clin Endocrinol Metab **51**:84–89, 1980.

5. Gale CC. Neuroendocrine aspects of thermoregulation. Ann Rev Physiol **35**:391–430, 1973.
6. Ohtake M, Bray GA, Azukizawa M. Studies on hypothermia and thyroid function in the obese (ob/ob) mouse. Amer J Physiol **233**:R110, 1977.
7. Guersney DL, Morsihige WK. Na^+ pump activity and nuclear-T_3 receptors in tissues of genetically obese (ob/ob) mice. Metabolism **28**:629–632, 1979.
8. Samuels HH, Tsai JS. Thyroid hormone action. Demonstration of similar receptors in isolated nuclei of rat liver and cultured GH_1 cells. J Clin Invest **54**: 656, 1974.
9. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochemistry **62**: 315–323, 1956.
10. Koerner DM, Surks I, Oppenheimer JH. In vitro demonstration of specific triiodothyronine binding sites in rat liver nuclei. Endocrinol Metab **38**:706, 1974.
11. DeGroot LJ, Torresani J. Triiodothyronine binding to isolated liver cell nuclei. Endocrinology **96**:357, 1975.
12. Surks MK, Koerner D, Oppenheimer JH. In vitro binding of L-triiodothyronine to receptors in rat liver nuclei. J Clin Invest **55**:50–60, 1975.
13. Darling DS, Dickhoff WW, Gorbman A. Comparison of thyroid hormone binding to hepatic nuclei of the rat and a Teleost (Oncorhynchus Kisutch). Endocrinology **111**:1936–1943, 1982.
14. Zivin JA, Waud DR. How to analyze binding, enzyme and uptake data: The simplest case, a single phase. Life Sci **30**:1407–1422, 1980.
15. Lin MH, VanderTuig JG, Romsos DR, Akera T, Leveille GA. Heat production and Na^+/K^+ ATPase enzyme units in lean and obese (ob/ob) mice. Amer J Physiol **238**:E193–E199, 1979.
16. Bernal J, DeGroot LJ. Thyroid hormone receptors: Release of receptors to the medium during in vitro incubation of rat liver nuclei. Endocrinology **100**:648–655, 1977.

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