Vol. 140, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1986 Pages 134-142

TRIIODOTHYRONINE (T3) NEOGENESIS IN LEAN AND OBESE LA/N-cp RATS [1]

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Received August 27, 1986

SUMMARY: Pre-obese LA/N-*cp* rats consumed more food and gained weight more rapidly than their lean littermates, and measures of adipose tissue depots indicated that the excess weight was deposited principally as carcass fat. Serum T_3 concentrations and resting metabolic rates were lower in corpulent than in lean animals, consistent with a greater efficiency of weight gain in those animals. *In vitro* measures of T_3 neogenesis from T_4 were lower in corpulent than in lean animals in liver, kidney, and skeletal muscle and greater in interscapular brown adipose tissue. The intracellular generation of T_3 from T_4 is a fundamental component of the normal adaptive response to alterations in diet and environment, and is an essential prerequisite for the expression of non-shivering thermogenesis. These results are consistent with a functional impairment in the activity of the enzyme T_4-5'-deiodinase in peripheral tissues, and suggest that this impairment is contributory if not causative of obesity in this strain of rat.

INTRODUCTION: The intracellular generation of triiodothyronine (T_3) from thyroxine (T_4) is recognized as an important physiologic function in numerous peripheral tissues (1), where it performs critical roles in genetic expression, the regulation of protein synthesis, and in the regulation of the basal metabolic rate. The role of adaptive changes in T_3 neogenesis in thermogenic tissues is less well defined, but recent studies in the *ob/ob* mouse (2) and in the obese phenotype of the LA/N-*cp* rat (3) indicate that this process may also represent a fundamental component in the

[1] Presented in part at the 70th Annual Meeting, Federation of American Societies for Experimental Biology , St. Louis, MO, April 15, 1986.

0006-291X/86 $1.50

thermogenic response to diet and environment, and which may be defective or improperly regulated in at least some genetic models of obesity(4,5). Several authors have shown that in younger rats, increases in serum T_3 concentration accompany acute cold exposure (2), cafeteria feeding (6), and following the feeding of low protein diets (7), and that these thyroidal responses fail to occur normally in obese Zucker "fatty" rats (8). obese LA/N-*cp* rats, and in the obese phenotype of the *ob/ob* mouse (2). In older lean animals, however, the nutritionally-induced increases in serum T_3 concentration following these stimuli were of lower magnitude (9).

Brown adipose tissue is the only tissue of homeotherms whose primary function is thermogenesis (10). The heat produced by this tissue may be utilized to warm blood in support of homeothermy, or it may be dissapated by the organism when heat production exceeds thermoregulatory needs. This tissue plays a fundamental role in the development and expression of non-shivering thermogenesis, and may increase in quantity and in total thermogenic activity following both environmental and dietary challange (6,11). Permissive quantities of thyroid hormones are essential for the normal adrenergic component of the thermogenic response (12), and in their absence results in an attenuated or impaired thermogenic capacity. Thyroid hormone replacement in thyroidectomized hypothyroid rats results in a return to normal of the adrenergic thermogenic response (13).

The LA/N-*cp* rat is a recently developed congenic strain, where obesity develops as the result of an autosomal recessive trait (14). The obese phenotype demonstrates hyperphagia, hyperinsulinemia and insulin resistance, hypercholesteremia, and hypertriglyceridemia (15,16). Obese animals also tend to be metabolically refractory to the administration of exogenous T_4 but not T_3, and to exhibit impaired thyroidal responses to environmental and dietary manipulation, while their lean littermates respond normally to these stimuli (3). The purpose of the present studies was to

investigate the capacity for T_3 neogenesis from T_4 in several peripheral tissues in the lean and obese phenotypes of the LA/N-*cp* rat, and to determine if the previously observed decreases in serum T_3 concentration in the obese animals of this strain might correspond with the extrathyroidal production of this hormone.

METHODS AND MATERIALS: Groups of lean and corpulent rats aged 4-5 weeks were obtained from our colony, and maintained individually in hanging steel wire-bottomed cages with free access to powdered Purina chow # 5012 and tap water, *ad libitum,* on a reverse light cycle (light 1800-0600 hrs). Animals were weighed weekly with an Ohaus triple beam animal balance. Measures of 6-day food intake, corrected for any spillage, were made on all animals between 5 and 6 weeks of age. At 41 $\pm$ 1 days of age, measures of resting oxygen consumption were made at thermal neutrality (30^{o}C) after an 8 hour fast using the method of Stock (17) as performed in our laboratory (6). At 42 days of age, animals were sacrificed by decapitation, truncal bloods collected for determination of serum triiodothyronine concentration. The liver, left kidney, gastrocnemius muscle, and interscapular brown adipose tissue were rapidly dissected in their entirety, weighed, and an aliquot immediately homogenized in 0.25 M sucrose-0.25mM EDTA buffer. Tissues were homogenized 2 x 10 seconds with a 15 second period in between for recooling with an Astromixer at intermediate speed. Aliquots of the homogenates were incubated for T_3 neogenesis as outlined by Chopra (18) and later modified by Kaplan and Utiger using T_4 as the substrate (2). T_3 content was quantitated with a highly sensitive solid phase radioimmunoassay procedure (19). Tissue protein content was determined by the method of Lowry et al (20) with lysozyme as the standard, and the data were expressed as ug T_3 generated/ug T_4 / gram equivalent of tissue. Data were analyzed with an unpaired T test and other procedures using standard statistical programs (21).

RESULTS: Final body weights and weight gain of the two groups of rats are shown in Table 1, and indicate that corpulent animals were already modestly greater in body weight than were lean rats at the time of sacrifice. Fat pad weights in all white adipose tissue depots and in the interscapular brown adipose tissue (IBAT) depot were modestly heavier in obese than in lean, suggesting that the greater body weights were due to more rapid enlargement of the body fat mass in those animals (Table 2). While the greater mass of the IBAT may be partially due to lipid accretion, the rich brown color of this tissue even in obese animals was suggestive of greater thermogenic activity than is usually observed in older-aged genetically obese animals. IBAT mass was not only greater, but represented a greater proportion of body weight in corpulent than in lean rats (IBAT: BW x 10^{-3} = 3.17 vs 1.96, $p = <0.05$). The overall greater fat mass was due to combined effects of small increases in both adipocyte lipid content and fat cell number per depot, particularly in white adipose depots.

Measures of T_3 neogenesis in homogenates of liver, kidney, gastrocnemius muscle, and in interscapular brown adipose tissue were obtained as described by Kaplan and Utiger(1), and show that the total conversion of T_4 to T_3 was greater in lean than in obese animals in gastrocnemius muscle, and tended to be higher in liver and kidney tissues. In contrast, T_3 formation in brown adipose tissue was greater in obese than in lean animals, consistent with the observation of Kates and Himms-Hagen in normally-housed obese *ob/ob* mice of a similar age (3).

Table 1. Body weights of rats

Group	Initial g	Final g	Gain g/d	Food Intake g/d
Lean	91±5	112±8	5.2±0.21	16.3±1.1
Corpulent	99±21	158±8	8.2±0.3	25.9±1.1
P =	NS	<0.05	<0.05	<0.05

Data are mean ±1 SEM, n = 6 rats/group. Initial and final weights measured at 5 and 6 weeks of age, respectively.

Table 2. Fat pad weights in rats

Group	Epididymal	Retroperitoneal	Dorsal	Sum of 3*	IBAT
Lean	0.40±0.08	0.09±0.04	0.13±0.08	0.60±0.19	0.22±0.04
Corpulent	1.32±0.12	1.18±0.16	3.40±0.36	5.73±0.64	0.50±0.17
P =	<0.01	<0.01	<0.01	<0.01	<0.01

Data are mean ±1 SEM, n = 6 rats/group.
*White Fat depots.

DISCUSSION: The results of this study show that serum T_3 concentrations and rates of T_3 neogenesis from T_4 were significantly lower in skeletal and visceral tissues of obese than of lean rats, but that total T_3 formation in interscapular brown adipose tisssue of obese rats was greater than in their lean littermates. Serum T_3 concentrations and the rates of resting oxygen consumption were also lower in obese than in lean animals, while measures of food intake and of final body weight were greater. These observations are consistent with decreased overall rates of energy expenditure in the obese phenotype, in spite of an apparent attempt by brown adipose tissue to compensate for the generalized decrease in thermogenesis in lean tissues. It is presumed that the excess energy not expended via adaptive thermogenesis in those tissues might then become available for conversion to lipid and stored in adipose tissue. The impairment in thermogenesis reported here is consistent with earlier studies that demonstrated impaired responses to diet and environment (22,23) both in this and in other strains (8,22,23). In those studies, resting oxygen consumption and circulating T_3 concentrations became increased only in lean animals following a regimen of cafeteria overfeeding, and the thermoregulatory responses to a cold challenge were clearly impaired in obese animals (22,23). When Zucker "fatty" rats were fed thermogenic, protein-restricted diets, lean animals exhibited increases in circulating T_3 concentrations and in resting oxygen consumption, while their obese littermates did not

Table 3. Resting oxygen consumption in rats

	Lean	Corpulent	P
ml. O_2/Kg BW $^{0.75}$/min.	16.8±0.4	12.7±0.9	<0.001
n	5	6	

Data are mean ± 1 SEM.

(8). Obese *ob/ob* mice failed to cold adapt and increased their T_3 production in brown adipose tissue only modestly, while their lean littermates were able to increase brown adipose tissue T_3 production from T_4 by over 25-fold and to cold-adapt normally (2). The biochemical role of local T_3 production in response to dietary or environmental manipulation is unknown, but is assumed to be related in some way to thermogenic aspects of energy balance, and probably contribute to the intracellular regulation of protein turnover (24) (TABLES 3 & 4).

The enzyme thyroxine 5'-deiodinase occurs in at least two forms in rats (25), and results in the local, intracellular generation of T_3 in close anatomical proximity to its site of action. This enzyme has been shown to be dependent upon insulin for normal activity in liver (26). In those studies, insulin deficiency markedly reduced circulating T_3 concentrations, which became normalized after restoring insulin treatment. Whether one or both forms of the enzyme are subject to the regulatory effects of insulin are

Table 4. T_3 neogenesis in lean and corpulent rats

Group	Serum T3	Liver	Kidney	Gastrocnemius	IBAT
Lean	0.61±0.08	0.50±0.16	0.84±0.19	1.52±0.03	0.87±0.20
Corpulent	0.38±0.06	0.45±0.17	0.57±0.19	0.99±0.33	1.79±0.46
P =	<0.05	NS	NS	<0.05	<0.05

Data are mean + 1 SEM, n=6 rats/group. Serum T_3 as ng/ml, other data as ug T_3/ugT_4/gram equivalent of tissue/15 min incubation.

unclear. Thus, the hyperinsulinemia and insulin resistance characteristic of generalized obesity and present in this strain of rat (15) may be at least partially responsible for the T_3 on protein degradation and resysthesis (protein turnover) represent an energetically expensive process, and modest decreases in protein turnover activity would be expected to result in a sparing of the lean tissue mass, lower dietary protein requirements, and in energy conservation in peripheral tissues. The heat production inherent in protein synthetic activity represents a significant proportion of the daily energy expenditure, and contributes to obligatory heat production and to maintainence of the body temperature. The greater T_3 neogenesis observed in brown adipose tissue, in concert with the greater mass of this tissue, is presumed to represent a compensatory adaptation of this tissue, which enables the obese animal to maintain normal body temperatures in the presence of a decreased capacity for heat production in other tissues. Because BAT represents only a small proportion of the lean tissue mass in the adult or growing organism, circulating T_3 concentrations remain lower than in lean littermates with normal insulin sensitivity. In other studies, when insulin resistance was decreased via adrenalectomy, T_3 neogenesis in BAT and other peripheral tissues became normalized, and circulating T_3 concentrations returned to normal (27).

The basis for the impaired T_3 neogenesis in peripheral tissues, in concert with greater T_3 production in IBAT in the obese animals of this study could not be determined. While normal insulin action appears to be necessary the regulation of T_4 5'deiodinase activity and of intracellular T_3 production in lean tissues, it seems likely that this process may be under sympathetic control in brown adipose tissue. The sympathetic control of BAT thermogensis is now well established, and Glick et al (28)

have recently shown that individual meals produce an increase in deiodinase activity which temporally corresponds with the sympathetic responses. Longer durations of cafeteria feeding were aparently without effect on deiodinase activity however, for reasons that remain unclear (29). The greater T_3 neogenesis of IBAT is presumed to be a compensatory thermoregulative response to the decreased T_3 production in other tissues, and is quite likely contributory to the maintainence of normal body temperatures in the obese phenotype. In the present study, resting oxygen consumption was greater in lean than in pre-obese animals, consistent with the greater circulating T_3 concentrations. Regardless of the physiologic mechanisms involved in deiodinase regulation in peripheral tissues, the decreased enzyme activity observed is consistent with imparied thermogensis, and in a decreased capacity for energy expenditure in peripheral tissue in the obese phenotype. This decreased capacity for energy expenditure is likely to be contributory if not causative to the development of obesity in this strain of rat.

ACKNOWLEDGEMENTS: The authors wish to thank Joyce Nowacki, Jacqueline McLaughlin, Brenda Jones and Joan Schotte for technical assistance, Dr. Stanley Segall of Drexel University for Institutional Support, and Dr. C.T. Hansen of The Veterinary Resources Branch, Small Animal Section, National Institutes of Health, Bethesda, MD., for breeding stock from which the animals for this study were derived.

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