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Administration of physiologic levels of triiodothyronine increases leptin expression in calorie-restricted obese rats, but does not influence weight loss

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Abstract

Obesity has become a major public health problem, most commonly treated via dietary restriction to promote weight loss. Although leptin and thyroid hormones are involved in the regulation of energy balance, the role of these hormones after the stabilization of weight loss remains unclear. This study was designed to analyze the effect of thyroid hormone on sustained weight loss and leptin gene expression in obese animals after a loss of 5% to 10% of body weight. Thirty-day–old male Wistar rats were separated into 4 groups: control, obese, calorie restriction (CR), and calorie restriction with triiodothyronine administration (CRT). The obese group had increased weight and adiposity, leptin and insulin levels, and leptin gene expression. Dietary restriction in the CR group resulted in decreased body weight and adiposity, diminished leptin, and increased thyroid hormone receptor β expression. The CRT group, submitted to dietary restriction with concomitant administration of a physiologic triiodothyronine dose, had thyroid hormone receptor β expression at levels comparable with those observed in the control group and simultaneously increased leptin expression as compared with that in the CR group, suggesting that thyroid hormone modulates leptin expression under conditions of calorie restriction. Increased leptin expression in the CRT group did not result in increased circulating leptin or a statistically significant reduction in body weight during the treatment period. These data provide impetus for further study, as a longer treatment period may result in increased circulating leptin and, thus, further reduction in body weight during calorie restriction in an obesity model.

1. Introduction

Obesity is a multifactorial chronic disease, which has become a major problem in public health [1-3] because it is associated with other disorders such as high blood pressure, dyslipidemia, type 2 diabetes mellitus, cardiovascular disease, and neuroendocrine alterations [4,5]. Obesity has reached epidemic proportions in the United States. Between 1999 and 2004, the prevalence of obesity continued to increase in adults, as well as children and adolescents [6].

Calorie restriction is the most common strategy used to treat obesity [7,8], and a reduction of 5% to 10% in body weight has been suggested by the National Institutes of Health as an initial goal for weight loss. However, studies have shown that, after this initial loss, further weight loss decelerates and maintaining the initial reduction is a major challenge [9].

Studies have shown that, after the initial loss of 5% to 10% of body weight, leptin concentration is decreased. This reduction may be a result of body fat loss [10] or of interactions with other hormones, such as insulin [11,12], glucocorticoids [13,14], or thyroid hormone [15]. Pinkney et al [16] suggested that, during calorie restriction, leptin and thyroid hormones may decrease in parallel. According to Cao et al [17], long- and short-lasting calorie restriction

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decreases type 1 iodothyronine deiodinase (*DioI*) expression, which may result in the low levels of circulating triiodothyronine (T_3) found in rodents submitted to calorie restriction.

Hence, this study sought to evaluate how the administration of physiologic T_3 levels may influence leptin expression, circulating leptin levels, and continued weight loss in calorie-restricted obese rats.

2. Materials and methods

2.1. Animals and experimental protocol

Thirty-day–old male Wistar rats (weighing approximately 100 g) were housed in individual cages in an animal room under controlled temperature and lighting (12-hour light-dark cycle). The animals were divided into 4 groups: control (C; n = 5)—fed with commercial chow ad libitum; obese(OB; n = 5)—given cycles of hypercaloric diets [18] for 23 weeks; calorie restriction (CR; n = 5)—given a hypercaloric diet for 15 weeks and then submitted to 25% food restriction (commercial chow) for 8 weeks; and calorie restriction with T_3 administration (CRT; n = 5)—given a hypercaloric diet for 15 weeks, submitted to 25% food restriction (commercial chow) for 4 weeks, and then given, in addition to food restriction, a physiologic dose of T_3 (0.5 μg/100 g body weight, intraperitoneally, daily) for 4 weeks (Fig. 1). After 23 weeks, the rats were killed by decapitation between 8:00 and 10:00 AM; and their blood was collected from the trunk. Serum was obtained after centrifugation of blood at 3000 rpm for 10 minutes and stored at −80°C. Retroperitoneal white adipose tissue was collected from each experimental group. Tissue portions of approximately 300 mg were quickly frozen in liquid nitrogen and then stored at −80°C until required for analysis. The experimental protocol was approved by the Committee of Ethics in Animal Experimentation of the Botucatu School of Medicine–UNESP.

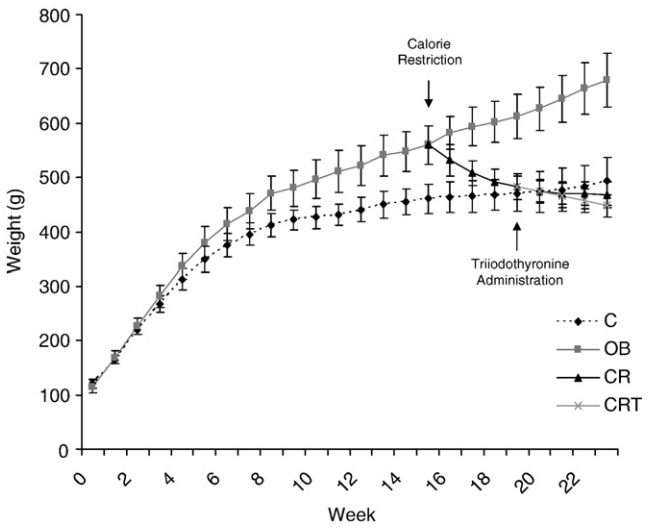

Fig. 1. Body weight development, in grams, in a 23-week experimental period. Five animals were used per group. The CR group received a hypercaloric diet until the 15th week; after that period, it was submitted to caloric restriction. The CRT group also received a hypercaloric diet until the 15th week; it was then submitted to caloric restriction and, from the 19th week, received a T_3 dose of 0.5 μg/100 g of weight. Data are expressed as means and confidence intervals of 95%.

2.2. Body weight and energy intake

The body weight and calorie intake were monitored throughout the entire experiment. Metabolic efficiency (ME) was calculated to analyze an animal's capacity to convert consumed food energy into body weight; this calculation used weight gain by the animals divided by total energy ingested (kilocalories), multiplied by 100 [19].

2.3. Body fat and protein assessment

The animals were anesthetized, decapitated, and thoracotomized; and their viscera were discarded. After carcass drying, fat was extracted by a Soxhlet Extraction Device (Corning Inc, Lowell, MA); and body protein was determined by quantitation of nitrogen [18].

2.4. RNA isolation and reverse transcription

Total RNA was extracted from white adipose tissue by the TRIZOL method (GibcoBRL, Sao Paulo, Brazil). RNA concentration was determined by the absorbance at 260 nm, and all the samples had an absorption ratio of approximately 2.0. One microgram of RNA was used for the synthesis of 20 μL of complementary DNA by SuperScript II First-Strand Synthesis System for RT-PCR (Invitrogen, Sao Paulo, Brazil).

2.5. Measurement of gene expression by quantitative real-time polymerase chain reaction

The real-time reverse transcriptase polymerase chain reaction (PCR) method with an Assay-on-Demand Gene Expression Product (Applied Biosystems, Foster City, CA) used unlabeled PCR primers and a TaqMan MGB probe (FAM dye-labeled) optimized to work with the TaqMan Universal PCR Master Mix (P/N 4304437) in a StepOne Plus PCR system (Applied Biosystems). This method was used to quantitatively measure leptin, thyroid hormone receptor β (TRβ), and cyclophilin messenger RNA (mRNA) expression. All samples were assayed in triplicate. The mRNA contents were normalized to cyclophilin mRNA levels, and differences in expression were determined by the Ct method described in the ABI user's manual (Applied Biosystems).

2.6. Determination of leptin, insulin, and total T_3 levels

Plasma concentrations of leptin and insulin were measured by rat-specific enzyme-linked immunosorbent assay kits (Linco Research Inc, St. Charles, MO). Serum concentration of total T_3 was determined at the Rhesus Laboratory, São Paulo, Brazil, using radioimmunoassay kits.

Table 1
Food, calorie, and water intake, and ME of C, OB, CR, and CRT animals at the last week of treatment

Groups	Variables			
	Intake (g)	Intake (kcal)	Water ingestion (mL)	ME
C	22.62 ± 2.44[b]	83.47 ± 9.02[b]	34.57 ± 3.52[a]	9.79 ± 4.70[b]
OB	18.99 ± 2.53[a]	109.58 ± 14.58[c]	45.71 ± 5.65[b]	16.27 ± 2.43[c]
CR	17.00 ± 0.03[a]	62.73 ± 0.12[a]	33.86 ± 6.79[a]	-7.04 ± 4.55[a]
CRT	17.01 ± 0.01[a]	62.77 ± 0.02[a]	32.00 ± 4.61[a]	-13.44 ± 5.34[a]

Values that do not share a common superscript are significantly different at $P < .05$, determined by ANOVA and complemented by the Tukey test. Data are expressed as means ± standard deviation.

2.7. Statistical analysis

Analysis of variance (ANOVA) was used for data analysis, complemented by Tukey test. Weight development was evaluated according to a 95% confidence interval. To evaluate the association between leptin and TRβ expression, Pearson correlation coefficient was determined. Data are expressed as mean ± standard deviations at a level of significance of 5%.

3. Results

3.1. Body weight, energy intake, and ME

At the end of the experimental period, the OB group (679.50 ± 49.25 g) weighed significantly more than the C, CR, and CRT groups (Fig. 1). A significant weight decrease was observed in the CR (12.57%) and CRT (18.33%) groups. No difference was found between the C, CR, and CRT groups (493.12 ± 44.43, 467.56 ± 23.28, and 448.32 ± 20.41 g, respectively) at the end of the experiment. Food ingestion in the OB, CR, and CRT groups was smaller than that in the C group. However, calorie intake and ME in the OB group were statistically higher when compared with those in the other groups. The CR and CRT groups had a significantly smaller food and calorie intake than did the C group (Table 1).

Table 2
Analysis of carcass of C, OB, CR, and CRT animals at the end of the experimental period

Groups	Carcass composition					
	Water		Fat		Protein	
	(mL)	(%)	(g)	(%)	(g)	(%)
C	221 ± 8.4[a]	57 ± 3.8[ab]	56 ± 31[a]	14 ± 7.2[a]	99 ± 11[ab]	20,3 ± 4[ab]
OB	275 ± 24[b]	52 ± 2.6[a]	132 ± 27[b]	25 ± 4.4[b]	109 ± 11[b]	16,2 ± 2,2[a]
CR	232 ± 8[a]	62 ± 2.7[b]	32 ± 19[a]	8.5 ± 4.5[a]	99 ± 8[ab]	21,6 ± 2,6[b]
CRT	220 ± 11[a]	61 ± 2.7[b]	33 ± 11[a]	9 ± 2.8[a]	96 ± 8[a]	21,4 ± 1,5[b]

Values that do not share a common superscript are significantly different at $P < .05$, determined by ANOVA and complemented by Tukey test. Data are expressed as means ± standard deviation.

Table 3
Plasma levels of leptin and insulin, and serum levels of total T_3 of C, OB, CR, and CRT animals at the end of the experiment

Groups	Concentrations		
	Leptin (ng/mL)	Insulin (ng/mL)	T_3 total (ng/mL)
C	4.82 ± 2.37[a]	1.13 ± 0.35[a]	65.28 ± 7.04[a]
OB	15.73 ± 6.05[b]	3.07 ± 0.55[b]	75.09 ± 15.55[a]
CR	2.81 ± 0.65[a]	1.00 ± 0.45[a]	52.94 ± 16.66[a]
CRT	3.80 ± 0.89[a]	0.83 ± 0.30[a]	64.79 ± 13.92[a]

Values that do not share a common superscript are significantly different at $P < .05$, determined by ANOVA and complemented by Tukey test. Data are expressed as mean ± standard deviation. Reference range for total T_3: 64 to 86 ng/dL. Leptin and insulin were compared with the C group.

3.2. Body fat and protein

The hypercaloric diet significantly increased the fat and decreased the protein percentage in the animals in the OB group when compared with those in the other groups. The decrease in relative protein content in the OB group was a consequence of total body weight. Body fat and protein of the CR and CRT animals were similar to those of the C group (Table 2).

3.3. Leptin, insulin, and total T_3 levels

Plasma leptin levels in the OB group were increased by 327% compared with those in the C group and by 561% compared with those in the CR and CRT groups. No difference in plasma leptin levels was found between the C, CR, and CRT groups. The plasma insulin concentration showed increases similar to those observed with leptin and was significantly higher in the OB group compared with the other groups. There was no statistical difference between the groups in terms of total T_3 serum concentrations. However, the CR group had levels below the reference range (64-86 ng/dL) (Table 3).

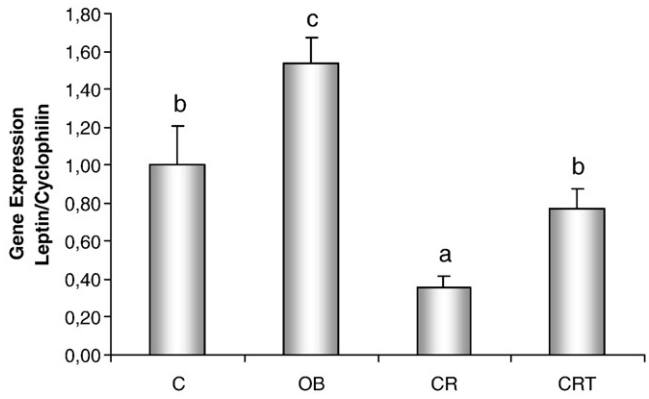

Fig. 2. Relative leptin expression for the different groups. Samples from 5 animals per group were assayed in 3 different PCR assays, and then a mean of the triplicate (for each animal) was used to get the mean of the group (n = 5). Values that do not share a common letter are significantly different at $P < .05$, determined by ANOVA and complemented by the Tukey test.

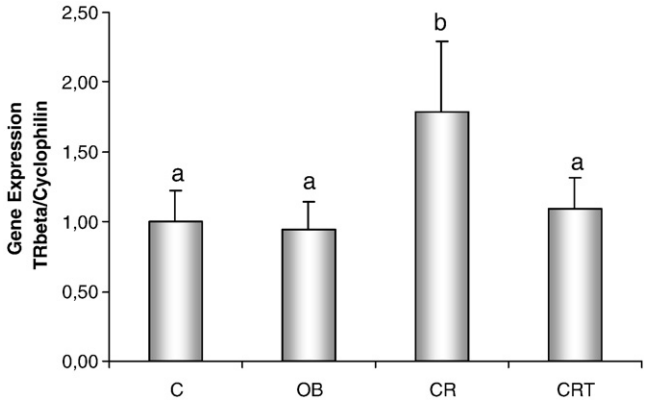

Fig. 3. Relative TRβ expression for the different groups. Samples from 5 animals per group were assayed in 3 different PCR assays, and then a mean of the triplicate (for each animal) was used to get the mean of the group (n = 5). Values that do not share a common letter are significantly different at $P < .05$, determined by ANOVA and complemented by the Tukey test.

3.4. Leptin and TRβ gene expression in adipose tissue

Alterations in leptin and TRβ expression in the adipose tissue of C, OB, CR, and CRT animals are presented in Figs. 2 and 3, respectively. After 23 experimental weeks, leptin expression, as compared with the C group, increased by 54% in the OB group and decreased by 64% in the CR and by 23% in the CRT group. The TRβ expression increased by 79% in the CR group as compared with the C group; and no statistical difference was found between the C, OB, and CRT groups. The animals receiving T_3 had a significant increase in leptin expression and a significant decrease in TRβ expression when compared with the CR group.

In this study, it was not possible to show a significant correlation between leptin and TRβ expression in the adipose tissue. However, there was an inverse relation between leptin and TRβ expression ($r = -0.184$, $P > .05$; as determined by Pearson correlation coefficient).

4. Discussion

Leptin and thyroid hormones are involved in the regulation of energy balance [15]. Ever since it was reported that leptin increases basal metabolic rate [20], various studies have investigated the possible relationship between leptin and thyroid hormone levels; however, the reported results are conflicting. Whereas some studies show no association between leptin serum concentration and thyroid hormone levels [21-23], others report a negative [24,25] or a positive correlation [26,27].

The hypercaloric diet induced weight gain and adiposity, and resulted in a 76.6% increase in carcass fat as compared with the C group (Table 2). Although the OB group ingested a significantly smaller chow amount, its calorie intake was higher than that in the C group. In agreement with published studies, the hypercaloric diet increased the ME in the OB group [1,28,29].

The OB group had high leptin levels (Table 3), which is explained by the increase in body fat [1,30-32]. This group also developed hyperinsulinemia [10,33] associated with high glucose concentrations, showing peripheral resistance to insulin (data not shown). Previous studies have also demonstrated that diet-induced obesity increases leptin expression when compared with other groups [34-36]. This finding suggests a positive correlation between adipose tissue and leptin expression [37].

Our current knowledge of the role of alterations in T_3 serum concentrations associated with weight gain remains unclear. Whereas some studies show an increase [23,38], others found no alterations [31,39]. In our study, we did not find significant differences between the 3 experimental groups and the control group (Table 3).

An animal model that permits the study of metabolic alterations after stabilized weight loss has been proposed based on observations in humans. Stabilization seems to occur after 5% to 10% weight loss in humans [40]. Therefore, the experimental model for this study was defined by weight stabilization after 9% weight loss.

Calorie restriction significantly reduced body weight and adiposity by reducing body fat by 66.3% [8,41-43]. Calorie restriction can be considered a safe clinical method for weight loss because fat mass decreases but protein content remains stable [44], resulting in a decreased risk for other diseases and increased longevity [45]. In this study, calorie restriction increased the relative protein content compared with the OB group, but not when compared with the C group, demonstrating that 25% food restriction for 8 weeks did not affect body protein content.

Low leptin levels increase the activity of the hypothalamic orexigenic signals, which stimulate appetite and suppress energy expenditure [46]. Hence, when weight loss occurs, there is a reduction in leptin levels that is proportional to body fat loss. As expected, there was a significant decrease in leptin expression in animals submitted to a calorie restriction (without T_3 administration) (Fig. 2) [10,37]. In agreement with previous studies [8,30,47-49], during calorie restriction, leptin plasma concentration decreased compared with the OB group.

There were no significant differences for total T_3 levels during calorie restriction [21,23]. However, there was a decrease in T_3 levels in CR group compared with the reference range (Table 3). The reduced levels of circulating T_3 in calorie-restricted animals may be due to the down-regulation of the *DioI* gene [17]. Araujo et al [44] showed a reduction in hepatic deiodinase activity during calorie restriction, which could be normalized in a dose-dependent manner by T_4 replacement, demonstrating that a decreased *DioI* activity is secondary to a decrease in serum thyroid hormone levels during calorie restriction. Normalization of T_4 levels restores serum T_3 and *DioI* activity, and increases body protein loss.

Previously, T_3 administration has been shown to increase weight loss during calorie restriction [38,50,51]; however, supraphysiologic doses were used in these studies. In this study, a physiologic T_3 dose was used in the T_3-treated group to increase the levels to normal values. Weight loss in the CRT group was similar to that in the CR group, indicating that a physiologic T_3 dose does not increase the loss of body weight or body protein during calorie restriction.

Our findings suggest that administration of physiologic dose of T_3 could be used safely in the treatment of obesity because, at this level, T_3 increases the expression of leptin, a hormone with central actions that cause a reduced food intake and an increase in energy expenditure, but does not decrease body protein. However, potential effects of exogenous administration of T_3 on the myocardium and bone need further evaluation.

Normal leptin levels reduce appetite and increase energy consumption by activating catabolism and impeding anabolic signals [46]. Triiodothyronine administration for 4 weeks did not influence leptin plasma levels in animals of the OB group compared with the CR and C groups. Although plasma levels were not altered compared with the CR group, leptin gene expression was increased in the OB group (Fig. 2). One can speculate that the leptin plasma concentration would have increased if the treatment had been maintained for a longer period.

Experiments performed in our laboratory indicate that TRβ is more abundant than TRα in white adipose tissue (data not shown). We observed an up-regulation in TRβ gene expression in CR animals; in contrast, TRβ expression was unchanged in OB and CRT animals (Fig. 3). Therefore, T_3 administration returned TRβ expression to levels observed in the C group and simultaneously increased leptin expression when compared with the CR group. Hence, our results suggest that physiologic levels of thyroid hormone are necessary for appropriate leptin expression. Despite this, it was not possible to show a significant correlation between leptin and TRβ expression.

In summary, this experimental animal model of diet-induced obesity demonstrated increased leptin serum concentration and leptin expression in the adipose tissue. Calorie restriction modified several physiologic markers associated with obesity. Animals subjected to calorie restriction had a decrease in leptin serum concentration and leptin expression, and an increase in TRβ expression. Physiologic T_3 substitution in calorie-restricted animals resulted in increased leptin expression and decreased TRβ expression, thus suggesting that thyroid hormone modulates leptin expression under conditions of calorie restriction.

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