

## Tissue-specific deiodinase regulation during food restriction and low replacement dose of leptin in rats

R. L. Araujo, B. M. Andrade, M. L. da Silva, A. C. F. Ferreira, and D. P. Carvalho

Laboratório de Fisiologia Endócrina Doris Rosenthal, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

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**Araujo RL, Andrade BM, da Silva ML, Ferreira AC, Carvalho DP.** Tissue-specific deiodinase regulation during food restriction and low replacement dose of leptin in rats. *Am J Physiol Endocrinol Metab* 296: E1157–E1163, 2009. First published February 10, 2009; doi:10.1152/ajpendo.90869.2008.—The relationship between thyroid function and leptin has been extensively studied; however, the mechanisms underlying the changes in thyroid hormone economy that occur during caloric deprivation remain elusive. Our goal was to evaluate the thyroid function of rats submitted to 40% food restriction after chronic leptin replacement. Caloric restriction for 25 days led to significantly reduced serum leptin, thyroid-stimulating hormone (TSH), thyroxine ( $T_4$ ), and triiodothyronine ( $T_3$ ) and increased serum corticosterone, while liver, kidney, and thyroid type I deiodinase (D1) and brown adipose tissue (BAT) type II deiodinase (D2) activities were decreased and hypothalamic D2 was significantly increased. Interestingly, thyroid iodide uptake was unchanged by caloric restriction, but thyroperoxidase (TPO) activity was significantly reduced. Leptin replacement for the last 10 days of caloric restriction normalized serum leptin and TSH levels, but serum  $T_4$  and  $T_3$  levels and thyroid D1 and TPO activities were not reestablished. Also, a negative effect of leptin administration on  $Na^+I^-$  symporter function was detected. Liver and kidney D1 and hypothalamic and BAT D2 were normalized by leptin, while pituitary D2 was significantly decreased. In conclusion, a tissue-specific modulation of deiodinases might be implicated in the normalization of thyroid function during leptin replacement in food-restricted rats. Although leptin restores the hypothalamus-pituitary axis during food restriction, it exerts a direct negative effect on the thyroid gland; thus normalization of serum thyroid hormones might depend on changes in deiodinase activities and the long-term thyroid stimulation by TSH to counterbalance the direct negative effects of leptin on the thyroid gland.

hypothalamus; pituitary; thyroid; caloric deprivation; thyrotropin

MODERATE REDUCTION in caloric intake promotes well-known systemic effects such as body and fat mass reduction; however, homeostatic mechanisms impair further weight loss after longer periods of food restriction (40, 41). Studies in rodents suggest that the reduction in serum leptin levels that occurs during weight loss signals to the central nervous system, leading to decreased energy expenditure (1, 2, 3, 5). This rapid fall in serum leptin in response to starvation also suppresses immunity, reproductive, and thyroid functions through central mechanisms (1, 16, 21, 23, 46).

It is well documented that food restriction exerts profound effects on the hypothalamic-pituitary-thyroid axis, resulting in low plasma thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) levels that seem to be secondary to decreased thyrotropin-releasing hor-

mone (TRH) and thyroid-stimulating hormone (TSH) secretion (4, 29, 38). The relationship between thyroid hormones and leptin has been extensively studied over the past few years; however, many results are still conflicting, and some aspects have not been investigated so far. Apart from the central effects of leptin, decreased serum thyroid hormone during caloric deprivation could also be related to reduced liver type I deiodinase (D1) activity, as previously suggested (10, 15). However, we have recently shown (6) that low replacement doses of  $T_4$  during food restriction restore serum  $T_3$  and liver D1 activity, suggesting that decreased liver D1 is a consequence rather than the cause of reduced serum  $T_3$  during food restriction. Apart from the central effects of leptin on the hypothalamic-pituitary axis, a possible direct effect of this hormone on the thyroid gland or tissue-specific deiodinase modulation during food restriction might also occur.

Recently, elegant studies in humans submitted to 8 wk of food restriction showed that low-dose leptin replacement during maintenance of reduced body weight reversed the effects of sustained weight reduction on the circulating concentrations of thyroid hormones, without normalizing TSH (41). These observations indicate a direct stimulatory effect of leptin on the thyroid gland or on the peripheral metabolism of  $T_4$  and  $T_3$  in humans with reduced body weight. On the other hand, recent studies have shown direct inhibitory effects of leptin on the ability of thyrocytes to take up iodide in rat thyroid cells (27, 37).

The transport of iodide is a fundamental step in thyroid hormone biosynthesis and is catalyzed by the  $Na^+I^-$  symporter (NIS), an intrinsic membrane protein localized at the basolateral membrane of thyrocytes (20). Leptin directly inhibits iodide uptake induced by TSH in FRTL-5 cells (27) and in thyroids from fed adult rats (37). Thyroperoxidase (TPO) is a key enzyme in thyroid hormone biosynthesis that catalyzes iodide oxidation, organification, and the coupling of iodothyrosines to produce  $T_3$  and  $T_4$  (39, 44). Thus leptin might also directly modulate both NIS function and TPO activity during caloric restriction. It remains to be determined whether direct effects of leptin on the thyroid gland and on peripheral deiodinase activities might participate in the normalization of serum thyroid hormone levels during caloric restriction.

Therefore, our aim was to evaluate thyroid function and D1 and D2 in food-restricted rats treated with replacement doses of leptin to determine whether leptin normalization could exert direct actions on the thyroid or on the peripheral metabolism of  $T_4$  and  $T_3$ .

### MATERIALS AND METHODS

**Animals.** Adult male Wistar rats were housed at controlled temperature (23°C) with daily exposure to a 12:12-h light-dark cycle and free

Address for reprint requests and other correspondence: D. P. Carvalho, Instituto de Biofísica Carlos Chagas Filho, CCS-Bloco G-Cidade Universitária, Ilha do Fundão, Rio de Janeiro, 21949-900, Brazil (e-mail: dencar@biof.ufrj.br).

access to water and standard rat chow. This investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Pub. No. 85-23, revised 1996) and was approved by the Institutional Committee for Evaluation of Animal Use in Research (CAUAP). All animals were individually housed for a 1-wk acclimation period, and baseline control food intake was assessed.

**Twenty-five days of food restriction.** Food intake was assessed over 7 days for each rat by offering food ad libitum and weighing the quantity of chow consumed. After this period, the ad libitum (control, C) group had free access to food and the food-restricted (R) group received 60% of their individual baseline intake for 25 days, so that food was 40% restricted. During the period of food restriction the rats were weighed every 2 days.

**Leptin treatment.** Fifteen days after the beginning of food restriction, food-restricted (R) rats were randomly assigned to receive chronic leptin treatment (RL group) by two daily subcutaneous injections [recombinant rat leptin, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)] or phosphate-buffered saline (PBS buffer pH 7.4). A leptin dose of 10  $\mu\text{g}/100\text{ g}$  body mass was administered for the last 10 days of food restriction, every 8:00 AM and 5:00 PM. After the experimental period (25 days), the animals were killed by decapitation and blood was collected from the trunk. The animals were killed 3 h after the last leptin administration. Serum was obtained after centrifugation of blood at 1,500  $g$  for 20 min and stored at  $-20^\circ\text{C}$  until specific radioimmunoassay (RIA) to determine serum TSH,  $T_3$ ,  $T_4$ , corticosterone, and leptin. Liver and kidney tissue samples and thyroids were dissected out and stored at  $-70^\circ\text{C}$  until processing for D1 activity. Hypothalamus, pituitary, and brown adipose tissue (BAT) were dissected out and stored at  $-70^\circ\text{C}$  until processing for D2 activity.

**Iodide uptake: NIS activity.** We demonstrated previously (22) that the measurement of radioiodide uptake 15 min after [ $^{125}\text{I}$ ]NaI administration (short-term iodide uptake) reflects iodide transport through the NIS without the influence of *in vivo* thyroid iodine organification activity. Thus, to evaluate *in vivo* NIS function with thyroid radioiodine uptake measurements, the animals received Na- $^{125}\text{I}$  (250,000 cpm ip, Amersham, Little Chalfont, UK) 15 min before decapitation. The thyroids were removed and weighed. The radioactivity of the thyroid glands was measured with a gamma counter (LKB) and expressed as percentage of total  $^{125}\text{I}$  injected per milligram of thyroid.

**Thyroperoxidase preparation and activity.** TPO extraction from rat thyroids was performed as previously described (24, 33). Rat thyroids were minced and homogenized in 0.5 ml of 50 mM Tris-HCl buffer, pH 7.2, containing 1 mM KI with an Ultra-Turrax homogenizer (Staufen, Germany). The homogenate was centrifuged at 100,000  $g$  and  $4^\circ\text{C}$  for 1 h. The pellet was suspended in 0.5 ml of digitonin [1% (wt/vol)] and incubated at  $4^\circ\text{C}$  for 24 h to solubilize TPO. The digitonin-treated suspension was centrifuged at 100,000  $g$  and  $4^\circ\text{C}$  for 1 h, and the supernatant containing solubilized TPO was used for the assays. Protein content was determined by the method of Bradford (13).

The TPO iodide oxidation activity was measured as previously described (14, 33, 34, 35). The assay mixture contained 1.0 ml of freshly prepared 50 mM sodium phosphate buffer, pH 7.4, containing 24 mM KI and 11 mM glucose and increasing amounts of solubilized TPO. The final volume was adjusted to 2.0 ml with 50 mM sodium phosphate buffer, pH 7.4, and the reaction was started by the addition of 10  $\mu\text{l}$  of 0.1% glucose oxidase (Boehringer grade I). The increase in absorbance at 353 nm ( $A_{353\text{nm}}$ ; triiodide production) was registered for 4 min on a Hitachi spectrophotometer (U-3300). The change in  $A_{353\text{nm}}$  per minute ( $\Delta A_{353\text{nm}}/\text{min}$ ) was determined from the linear portion of the reaction curve and related to protein concentration. One unit of activity corresponds to  $\Delta A_{353\text{nm}}/\text{min} = 1.0$ .

**Serum leptin, corticosterone, TSH, and total  $T_3$  and  $T_4$ .** Serum leptin concentrations were measured with a specific RIA for rat leptin obtained from Linco Research (St. Charles, MO). The kit uses

$^{125}\text{I}$ -labeled rat leptin and a rat leptin antiserum to determine the level of serum leptin by the double antibody/polyethylene glycol (PEG) technique. Intra- and interassay coefficients of variation were 3.3–6.8% and 4.1–2.0% respectively, and the sensitivity was 0.5 ng/ml with a 100- $\mu\text{l}$  sample size.

Serum corticosterone concentrations were measured with the ImmuChem  $^{125}\text{I}$  RIA obtained from MP Biomedicals (Orangeburg, NY). The kit is specifically designed for use in laboratory mice and rats and contained  $^{125}\text{I}$ -labeled corticosterone and rabbit corticosterone antiserum to determine the levels of serum corticosterone by the double antibody/PEG technique. Intra- and interassay coefficients of variation were 4.4–10.3% and 6.5–7.1% respectively, and the sensitivity was 7.7 ng/ml with a 100- $\mu\text{l}$  sample size.

Serum TSH levels were evaluated by a specific RIA obtained from NIDDK (Bethesda, MD) and expressed in terms of the reference preparation 2 (RP-2). Intra- and interassay coefficients of variation were 7.7 and 6.5%, respectively, and the sensitivity was 0.63 ng/ml.

Serum total  $T_3$  and  $T_4$  concentrations were measured with commercial radioimmunoassay kits ( $T_3$ : DLS-3100 Active, sensitivity 4.3 ng/dl, inter- and intra-assay coefficients of variation 4.2–6.0% and 5–6.5%, respectively;  $T_4$ : DLS-3200 Active, sensitivity 0.4  $\mu\text{g}/\text{dl}$ , inter- and intra-assay coefficients of variation 7.1–7.4% and 2.9–5.1%, respectively) based on the presence of specific antibodies adhered to the internal surface of propylene tubes.

All procedures were carried out according to the recommendations of the kits.

**Type I iodothyronine deiodinase activity.** Liver, kidney, and thyroid D1s were evaluated as previously described (8, 24). In short, tissue (liver or kidney) samples of 25 mg or one thyroid were homogenized in 1 ml of 0.1 M sodium phosphate buffer containing 1 mM EDTA, 0.25 M sucrose, and 10 mM dithiothreitol, pH 6.9. Homogenates (30  $\mu\text{g}$  of protein from liver or kidney and 15  $\mu\text{g}$  of protein from thyroids) were incubated in duplicate for 1 h at  $37^\circ\text{C}$  (water bath) with 1  $\mu\text{M}$   $rT_3$  (Sigma), equal volumes of  $^{125}\text{I}$ -labeled  $rT_3$  (PerkinElmer Life Sciences, Boston, MA) previously purified with Sephadex LH-20, and 10 mM dithiothreitol (USB) in 100 mM sodium phosphate buffer containing 1 mM EDTA, pH 6.9, in a reaction volume of 300  $\mu\text{l}$ . Blank incubations were carried out in the absence of protein. The reaction was stopped after incubation at  $4^\circ\text{C}$  followed by the addition of 100  $\mu\text{l}$  of fetal bovine serum (Cultilab) and 200  $\mu\text{l}$  of trichloroacetic acid [50% (vol/vol)]. The samples were centrifuged at 8,000  $g$  for 3 min, and the supernatant was collected for measurement of  $^{125}\text{I}$  liberated during the deiodination reaction. Protein concentration in the homogenates was measured by the Bradford method (13) after incubation of homogenates with NaOH (2.5 N). The specific enzyme activity was expressed as picomoles of  $rT_3$  deiodinated per minute per milligram of protein.

**Type II iodothyronine deiodinase activity.** One pituitary gland, hypothalamic tissue surrounding the third ventricle, or 25 mg of BAT was homogenized in 0.1 M sodium phosphate buffer containing 1 mM EDTA, 0.25 M sucrose, and 10 mM dithiothreitol, pH 6.9. Homogenates (50  $\mu\text{g}$  of protein from pituitary or 100  $\mu\text{g}$  of protein from hypothalamus and 150  $\mu\text{g}$  of protein from BAT) were incubated in triplicate for 3 h at  $37^\circ\text{C}$  with 1 nM [ $^{125}\text{I}$ ] $T_4$  (Perkin Elmer Life and Analytical Sciences), 1 mM propylthiouracil (PTU), and 20 mM dithiothreitol (USB) in 100 mM sodium phosphate buffer containing 1 mM EDTA, pH 6.9, in a reaction volume of 300  $\mu\text{l}$ , as previously described (8, 25). Blank incubations were carried out in the presence of  $T_4$  in excess (100 nM). The reaction was stopped at  $4^\circ\text{C}$  in an ice bath with the addition of 100  $\mu\text{l}$  of fetal bovine serum (Cultilab) and 200  $\mu\text{l}$  of trichloroacetic acid [50% (vol/vol)] followed by vigorous agitation. The samples were centrifuged at 8,000  $g$  for 3 min, and the supernatant was collected for measurement of  $^{125}\text{I}$  liberated during the deiodination reaction. Protein concentration was measured by the method of Bradford (13) after incubation of homogenates with NaOH (2.5 N). The specific enzyme activity was expressed as femtomoles of  $T_4$  deiodinated per minute per milligram of protein.

**Statistical analyses.** Results are expressed as means  $\pm$  SE. Body mass, fat content, serum total  $T_3$  and  $T_4$ , corticosterone, and leptin concentrations, and deiodinase activities were analyzed by one-way analysis of variance (ANOVA), followed by Bonferroni multiple comparison tests. The results of serum TSH, NIS function, and TPO activity, which do not follow a normal distribution, were analyzed by Kruskal-Wallis test followed by Dunn's multiple comparison tests. Statistical analyses were performed with GraphPad Prism software (version 4, GraphPad Software, San Diego, CA). Differences were considered significant when  $P < 0.05$ .

## RESULTS

**Body composition: mass and fat contents.** After 25 days of food restriction (R group), final body mass was significantly reduced compared with the C group (Table 1,  $P < 0.01$ ) and the low dose of leptin administered during the last 10 days of food restriction did not produce any further body mass change (Table 1). Retroperitoneal and epididymal fat pads were significantly decreased in R compared with C groups (Table 1,  $P < 0.01$ ), and no further reductions in these fat compartments were observed with leptin treatment (Table 1).

**Serum leptin, corticosterone, TSH, and total  $T_3$  and  $T_4$ .** Serum leptin concentration was significantly reduced (Table 2,  $P < 0.01$ ) after 25 days of food restriction, the dose of leptin administered was able to restore its concentration to the serum levels found in control animals, and serum leptin was significantly higher in the RL group in relation to the R group (Table 2).

Serum corticosterone concentrations were significantly increased (Table 2,  $P < 0.01$ ) after 25 days of food restriction, whether or not the animals received leptin (Table 2).

Serum TSH,  $T_4$ , and  $T_3$  concentrations were significantly reduced after 25 days of food restriction (Table 2,  $P < 0.01$ ). Leptin replacement was able to completely restore serum TSH concentrations in the RL group. However, leptin replacement did not normalize serum  $T_4$  levels, which remained significantly decreased in the RL group (Table 2). On the other hand, the change in serum  $T_3$  induced by prolonged food restriction was partially reversed by leptin. Serum  $T_3$  decreased from  $87.30 \pm 4.04$  to  $40.95 \pm 2.94$  ng/dl in food-restricted rats (Table 2,  $P < 0.01$ ), while leptin treatment for 10 days partially restored serum  $T_3$  to  $61.59 \pm 2.51$  ng/dl (Table 2,  $P < 0.05$ ).

**Type I iodothyronine deiodinase activity.** Thyroid and liver D1 activity were significantly reduced after 25 days of food restriction (Fig. 1A,  $P < 0.01$ ). Leptin replacement normalized liver and kidney D1 activities, although serum  $T_4$  and  $T_3$  remained low (Fig. 1, A and B). Therefore, liver and kidney D1 in the RL group were significantly higher than in

Table 1. *Body mass and fat compartments in control, food-restricted, or food-restricted and leptin-replaced Wistar rats*

	Initial BM	Final BM	Retroperitoneal Fat	Epididymal Fat
C	300 $\pm$ 4.97	339 $\pm$ 5.90	1.67 $\pm$ 0.13	1.71 $\pm$ 0.14
R	299 $\pm$ 4.92	269 $\pm$ 2.92*	0.66 $\pm$ 0.09*	0.23 $\pm$ 0.06*
RL	298 $\pm$ 5.34	265 $\pm$ 3.03*	0.63 $\pm$ 0.12*	0.30 $\pm$ 0.07*

Results are expressed as means  $\pm$  SE of  $>10$  animals/group. Fat compartments are expressed as g/100 g of body mass. BM, body mass (g); C, control; R, food restriction; RL, food restriction + leptin. Leptin was replaced for the last 10 days of food restriction (10  $\mu$ g/100 g body mass twice a day). \* $P < 0.01$  vs. C.

Table 2. *Serum leptin, TSH,  $T_4$ ,  $T_3$ , and corticosterone in control, food-restricted, or food-restricted and leptin-replaced Wistar rats*

	Leptin, ng/ml	TSH, ng/ml	$T_4$ , $\mu$ g/dl	$T_3$ , ng/dl	Corticosterone, ng/ml
C	7.45 $\pm$ 0.34	1.20 $\pm$ 0.12	6.88 $\pm$ 0.31	87.30 $\pm$ 4.04	139.4 $\pm$ 23.15
R	1.85 $\pm$ 0.30*	0.71 $\pm$ 0.02*	4.21 $\pm$ 0.23*	40.95 $\pm$ 2.94*	315.6 $\pm$ 27.6*
RL	6.45 $\pm$ 0.05	1.05 $\pm$ 0.12†	5.06 $\pm$ 0.37*	61.59 $\pm$ 2.51*†	390.1 $\pm$ 33.26*

Results are expressed as means  $\pm$  SE of 7–12 animals per group. Leptin was replaced for the last 10 days of food restriction (10  $\mu$ g/100 g body mass twice a day). TSH, thyroid-stimulating hormone;  $T_4$ , thyroxine;  $T_3$ , triiodothyronine. \* $P < 0.01$  vs. C; † $P < 0.01$  vs. R.

the R group. However, thyroid D1 activity remained reduced in leptin-treated animals, although serum TSH was normalized (Fig. 1C).

**Type II iodothyronine deiodinase activity.** Hypothalamic D2 activity was significantly increased after 25 days of food restriction (Fig. 2A,  $P < 0.05$ ), and leptin treatment normalized D2 activity in food-restricted animals (Fig. 2A). The increase in hypothalamic D2 found during food restriction might be implicated in a higher local generation of  $T_3$  and thus decreased TRH secretion.

Pituitary D2 activity was not changed by food restriction, but leptin administration to food-restricted animals led to a significant reduction in pituitary D2 activity in relation to both C and R groups (Fig. 2B,  $P < 0.05$ ). Pituitary D2 reduction produced by leptin treatment in food-restricted rats might explain, at least in part, the normalization of serum TSH, which might be secondary to decreased local production of  $T_3$  in the thyrotroph.

D2 activity in BAT was significantly reduced after 25 days of food restriction (Fig. 2C,  $P < 0.05$ ), and the replacement dose of leptin restored D2 activity in the RL group to control levels (Fig. 2C).

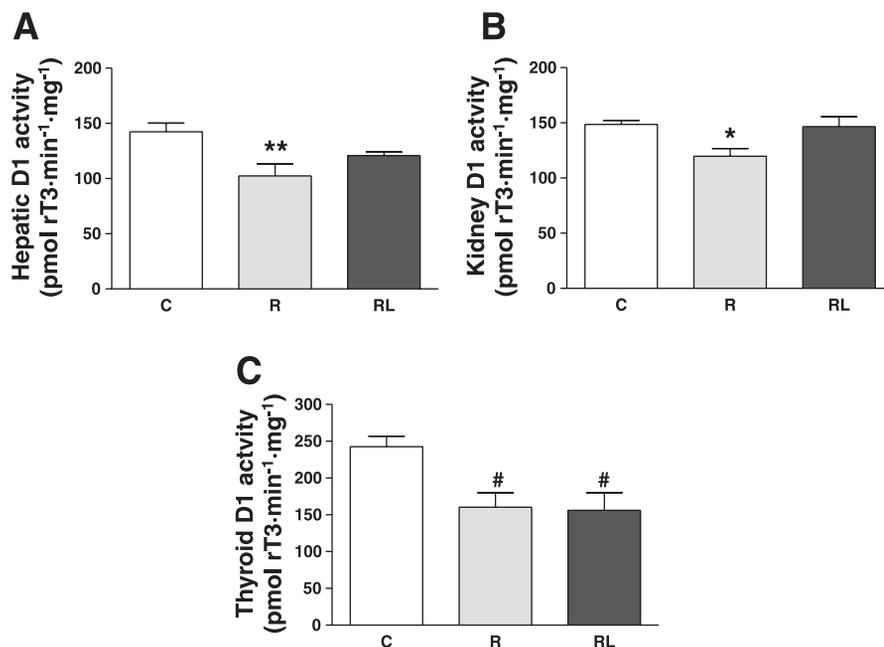
**Iodide uptake: NIS activity.** Food restriction did not alter thyroid iodide uptake (Fig. 3A), even in the presence of reduced serum TSH. A significant decrease in thyroid iodide uptake was detected in leptin-treated rats (Fig. 3A,  $P < 0.05$ ), showing that leptin exerts a direct negative effect on NIS function in vivo, regardless of normal serum TSH.

**Thyroperoxidase activity.** TPO was significantly decreased after 25 days of food restriction (Fig. 3B,  $P < 0.01$ ), and leptin replacement to the RL group did not restore TPO activity (Fig. 3B). These data show that restoration of serum TSH is not sufficient to normalize TPO in food-restricted animals treated with leptin.

## DISCUSSION

We show here that 25 days of 40% food restriction leads to reduction in body mass, retroperitoneal and epididymal fat compartments, serum leptin, TSH,  $T_4$ , and  $T_3$ , with increased corticosterone, just as previously reported (6). Interestingly, the replacement of leptin did not lead to further body mass and fat compartment loss in rats. Other studies that compared ad libitum-fed control animals with ad libitum-fed leptin-treated animals observed that leptin (0.5  $\mu$ g $\cdot$ g body wt<sup>-1</sup> $\cdot$ day<sup>-1</sup> to 10  $\mu$ g/day) significantly reduced food intake (33%–50%) and, as a consequence, was able to determine body and fat mass loss (7, 15, 43). In the present work, we administered a lower dose of

Fig. 1. Type I iodothyronine deiodinase (D1) activity in control (C), food-restricted (R) and food-restricted with leptin replacement (10  $\mu\text{g}/100$  g body mass twice a day for 10 days; RL) rats. *A*: liver D1 (C,  $n = 7$ ; R,  $n = 7$ ; RL,  $n = 7$ ). *B*: kidney D1 (C,  $n = 7$ ; R,  $n = 7$ ; RL,  $n = 7$ ). *C*: thyroid D1 (C,  $n = 7$ ; R,  $n = 7$ ; RL,  $n = 7$ ). Results are means  $\pm$  SE. \* $P < 0.05$ , \*\* $P < 0.01$  vs. C and RL groups; # $P < 0.05$  vs. C group.

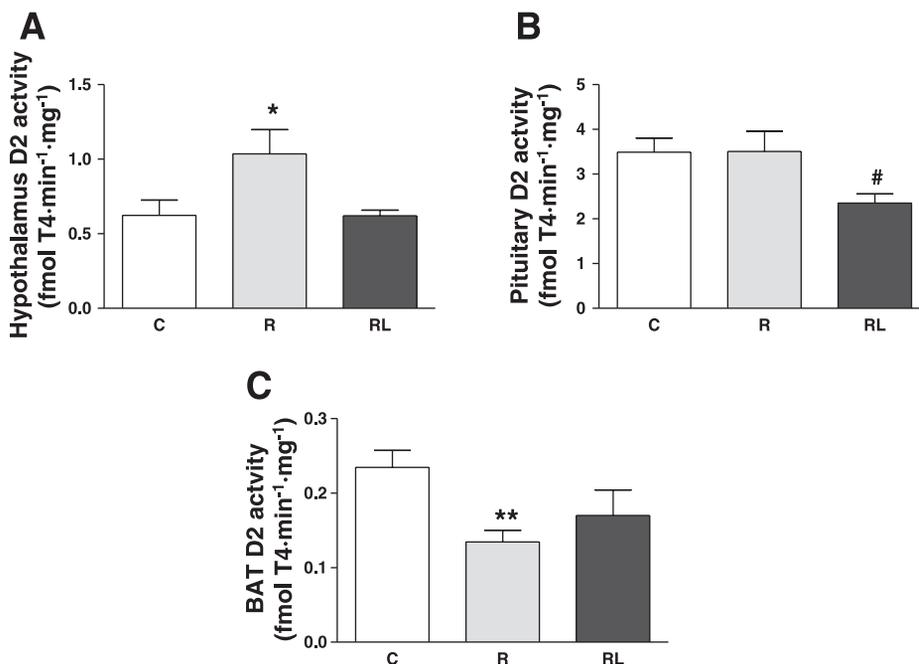


leptin that was just able to normalize serum leptin in food-restricted animals. Boozer et al. (12) evaluated the effect of a low dose of leptin in the treatment of diet-induced obesity and observed that this strategy was not efficient to determine further weight and fat loss. Therefore, it is important to emphasize here that the expected effects of exogenous leptin (decrease in food intake and increase in energy expenditure) largely depend on the nutritional status of the animal. The purpose of the present study was to further characterize the thyroid alterations induced by caloric restriction and to determine the effect of leptin replacement under these circumstances.

Ten days of leptin replacement in food-restricted animals did not normalize serum  $T_4$  concentrations; however, serum  $T_3$

concentrations were significantly increased by exogenous leptin administration. Since serum  $T_3$  concentrations decreased 50% by food restriction and leptin replacement led to an increment of 17%, without changes in serum  $T_4$ , we hypothesize that the mechanism related to normalization of serum  $T_3$  by leptin during food restriction can also be related to changes in the peripheral metabolism of  $T_4$ , apart from the known effects of leptin in the hypothalamic-pituitary axis. These results differ from studies in humans (41), which described significant increments in both serum  $T_3$  and  $T_4$  after leptin administration, without significant changes in circulating TSH; however, there could be differences due to species-specific effects, the dose used and the period of time of leptin treatment, and also the different nutritional status of the individuals.

Fig. 2. Type II iodothyronine deiodinase (D2) activity in C, R, and RL (10  $\mu\text{g}$  leptin/100 g body mass twice a day for 10 days) rats. *A*: hypothalamic D2 (C,  $n = 5$ ; R,  $n = 6$ ; RL,  $n = 7$ ). *B*: pituitary D2 (C,  $n = 6$ ; R,  $n = 6$ ; RL,  $n = 7$ ). *C*: brown adipose tissue D2 (C,  $n = 7$ ; R,  $n = 7$ ; RL,  $n = 7$ ). Results are means  $\pm$  SE. \* $P < 0.05$  vs. C and RL groups; # $P < 0.05$  vs. C and R groups; \*\* $P < 0.05$  vs. C group.



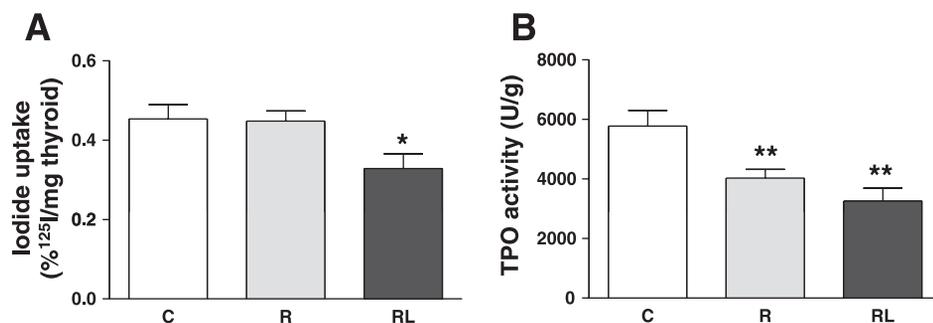


Fig. 3. Thyroid function in C, R, and RL (10  $\mu$ g leptin/100g body mass twice a day for 10 days) rats. A:  $\text{Na}^+/\text{I}^-$  symporter (NIS) function (C;  $n = 5$  R;  $n = 5$  RL;  $n = 5$ ). B: thyroperoxidase (TPO) activity (C;  $n = 18$  R;  $n = 21$  RL;  $n = 10$ ). Results are means  $\pm$  SE. \*  $P < 0.05$  vs. C and R groups; \*\* $P < 0.01$  vs. C group.

Studies evaluating the effects of exogenous leptin on the decreased thyroid economy during short term-fasting in animal models (24 to 72h) are conflicting. In 1996, it was shown (1) that serum thyroxine decreased within 48 h of fasting and a single intraperitoneal dose of leptin could attenuate the fall in thyroxine but did not restore it to control levels. Chronic leptin treatment could also prevent the fall in  $T_4$  determined by 70 h of fasting (3). On the other hand, other studies (11) have shown that leptin (20  $\mu$ g/0.5 ml twice a day) did not restore the decreased serum  $T_3$  and  $T_4$  in 24-h-fasted mice, despite serum leptin normalization. Lujan et al. (31), using rhesus monkeys submitted to a prolonged dietary regimen, also observed that leptin infusion for 16 wk did not restore serum  $T_3$ . These conflicting data show that different approaches lead to controversial results. Therefore, further studies are needed in order to understand the influence of leptin on the thyroid axis during fasting and food restriction, which correspond to quite different physiological conditions. Boelen et al. (11) showed that refeeding after 24 h of fasting in mice resulted in marked increase in serum leptin levels, which were not different compared with control mice; however, it did not result in a complete recovery of serum  $T_4$  and  $T_3$  levels. These results indicate that normalization of leptin levels does not always parallel restoration of serum thyroid hormone levels, suggesting that other peripheral signals may play a role in the regulation of thyroid gland economy during caloric deprivation.

During food restriction, in addition to decreased leptin, serum corticosterone is significantly increased (9, 28). Some authors found that leptin administration is able to reverse the increased serum corticosterone (1, 17); however, in the present study serum corticosterone remained elevated in the leptin-treated rats. We can hypothesize that the ability of leptin to restore serum thyroid hormones during a situation of negative energy balance might depend on the concerted normalization of serum corticosterone levels that did not occur in the present study.

Therefore, considering that in our experimental model the difference between R and RL groups corresponds to serum leptin levels, with sustained decreased serum  $T_4$  and  $T_3$  and increased serum corticosterone, we can infer that the changes in deiodinase activities were mainly secondary to leptin normalization. Here, the fall in serum  $T_3$  concentrations determined by food restriction was diminished in the RL group compared with the R group, and leptin was able to restore D1 activity in liver and kidney as well as D2 activity in BAT, which might contribute to the increase in serum  $T_3$  observed in the RL group. Our data are in accordance with reports that show an important stimulatory effect of leptin on peripheral D1

and D2 activities (15, 30). On the other hand, decreased D1 in the thyroid was not restored by leptin replacement, regardless of normal serum TSH. Since serum corticosterone remained high in the food-restricted animals treated with leptin, we suggest that corticosterone might directly influence thyroid D1 activity. Thus during food restriction the decreased thyroid D1 activity might be related to the diminished serum TSH, such as observed for TPO, or to increased serum corticosterone. Neither TPO nor thyroid D1 was normalized by leptin replacement, probably because of a direct effect of corticosterone that was not counterbalanced by the normal serum TSH. However, another possibility for the lack of normalization of serum  $T_4$  and  $T_3$ , thyroid D1, TPO, and NIS in the presence of normal serum TSH could be related to alterations in the glycosylation of the molecule and thus impaired bioactivity, just as previously demonstrated in sick euthyroid syndrome (32). The sustained decreased serum  $T_4$  and  $T_3$  might be a result of increased peripheral metabolism of  $T_4$  and  $T_3$  during food restriction, just as recently reported in other situations, such as myocardial injury (36, 42).

A previous study (17, 18) showed that the combination of low serum leptin and high serum corticosterone during food deprivation leads to increased hypothalamic D2 activity, and as a consequence decreases TRH expression and TSH secretion during fasting. As shown here, prolonged food restriction also increased hypothalamic D2 activity, which was normalized by leptin administration, notwithstanding the fact that serum corticosterone remained elevated. Thus leptin replacement per se is able to restore serum TSH during food restriction, possibly through the decrease in hypothalamic D2 activity. These results are consistent with the notion that increased hypothalamic D2 activity during food deprivation results in elevated local  $T_3$  production, which in turn decreases TRH expression and as a consequence serum TSH (17, 18). It has recently been demonstrated (19) that D2 is present in glial cells that are in direct contact with neurons coexpressing neuropeptide Y (NPY), agouti-related protein (AgRP), and uncoupling protein 2 (UCP2). The increased D2 during fasting induces  $T_3$ -mediated UCP2 activation, which results in increased excitability of NPY/AgRP neurons. These neurons from the arcuate nucleus might be important for the regulation of TRH secretion by the paraventricular hypothalamus (19).

It is well known that serum TSH is the most important stimulator of all steps of thyroid hormone biosynthesis, including iodide uptake (22, 26, 45). Despite the restored serum TSH in the RL group we observed that iodide uptake was inhibited, while in the R group NIS function was not altered, showing a direct negative effect of leptin on NIS regulation. Interestingly,

we show that during food restriction serum TSH might not be so important for the regulation of thyroid iodide uptake. The inhibitory effect of leptin on TSH-induced iodide uptake has already been shown in FRTL-5 cells and in the thyroids of adult rats (27, 37). However, the inhibitory effect of leptin on NIS function in vivo during food restriction has never been described.

In summary, during negative energy balance, independent of serum TSH the thyroid gland seems to be directly affected. Decreased serum leptin levels contribute to the suppression of the hypothalamic-pituitary axis; however, the direct effect of leptin on the thyroid per se seems to be mainly inhibitory. Despite the fact that a replacement dose of leptin was able to restore serum TSH, leptin normalization in the serum of food-restricted animals caused impaired thyroid iodide uptake and was not effective in restoring thyroid D1 and TPO activities. It remains to be elucidated whether increased serum corticosterone might play a role in thyroid gland physiology under these circumstances. On the other hand, leptin positively regulates liver and kidney D1 and BAT D2, which might be related to the increase in serum T<sub>3</sub> in leptin-treated food-restricted animals.

In conclusion, apart from its central action regulating the hypothalamic-pituitary-thyroid axis, leptin exerts direct peripheral effects on both thyroid gland and deiodinase activities.

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