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Acute and chronic leptin effect upon in vivo and in vitro rat thyroid iodide uptake

Elaine de Oliveira [a], Aline Teixeira Silva Fagundes [a], Isabela Teixeira Bonomo [a], Flavio Henrique Curty [a], Magna Cottini Fonseca Passos [b], Egberto Gaspar de Moura [a], Patrícia Cristina Lisboa [a,*]

[a] Department of Physiological Sciences, Roberto Alcantara Gomes Biology Institute, State University of Rio de Janeiro, Rio de Janeiro, Brazil
[b] Department of Applied Nutrition, Nutrition Institute, State University of Rio de Janeiro, Rio de Janeiro, Brazil

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Abstract

Leptin has stimulatory effects on the hypothalamic-pituitary-thyroid axis and on deiodinases activities. Here, we evaluated the effect of leptin injection upon in vivo and in vitro thyroid ^{125}I uptake (RAIU). We designed two experiments: acute leptin (LepA) with a single dose of leptin (8 μg/100 g BW/sc), and chronic leptin (LepC), injected with the same dose of LepA, once a day, for 6 days. In parallel, control groups were saline-injected. For in vivo study, part of the animals were injected with ^{125}I (3700 Bq) and killed after 15 or 120 min. In vivo thyroid RAIU was not changed in LepA animals. However, LepC animals showed higher in vivo thyroid RAIU (15 min:+130% and 120 min:+72%; $p<0.05$). For in vitro study, the other animals were killed and their thyroids were incubated with ^{125}I. Thyroids explants from LepA and LepC groups presented lower thyroid ^{125}I content (−32% and −29% $p<0.05$, respectively). The amount of our data suggest that, in vitro, leptin causes a direct inhibition of the rat thyroid RAIU, but in vivo, the effect of leptin was different according to the treatment period, which indicates that other indirect mechanisms are involved in the in vivo leptin chronic stimulation of the thyroid gland.
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Keywords: Leptin; Rats; Iodide uptake; Thyroid function

Introduction

Leptin is a hormone produced mainly by adipose tissue. It acts by maintaining the size of the adipose tissue through hypothalamic action, controlling the appetite and energy balance (Zhang et al., 1994). Thyroid hormones (TH) are important to metabolism regulation and also to body weight control. In animals and humans, some leptin effects on energy balance may be, directly or indirectly, mediated by the hypothalamus–pituitary–thyroid axis (Ahima et al., 1996; Legradi et al., 1997; Flier and Maratos-Flier, 1998; Friedman and Halaas, 1998; Ahima et al., 2000; Seoane et al., 2000; Ortiga-Carvalho et al., 2002; Rosenbaum et al., 2002). The majority of the leptin effects on the thyroid axis indicate that leptin acts mainly by TRH up-regulation (Legradi et al., 1997; Nillni et al., 2000).

The presence of leptin receptor long isoform (Ob–Rb) in the thyroid of adult female rats (Nowak et al., 2002) and in FRTL-5 cells (Isozaki et al., 2004) suggests a direct effect on the thyroid gland.

Iodide is essential for TH biosynthesis. An efficient and specialized system ensures iodide for the thyroid gland. The sodium-iodide symporter (NIS) is a membrane protein which mediates the active transport of iodide into the thyroid and other tissues, but the organification of iodide only occurs in the thyrocyte (Dai et al., 1996). TSH stimulates NIS gene

* Corresponding author. Departamento de Ciências Fisiológicas—5° andar. Instituto de Biologia—Universidade do Estado do Rio de Janeiro. Av. 28 de setembro, 87-Rio de Janeiro, RJ, 20551-030—Brazil. Tel.: +55 21 25876134; fax: +55 21 25876129.
E-mail addresses: pclisboa@uerj.br, patricialisboa@pq.cnpq.br (P.C. Lisboa).

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expression and increases iodide uptake by thyroid cells (Kaminsky et al., 1991; Riedel et al., 2001).

An interesting aspect to consider is the role of some cytokines upon NIS function. TNF-α and IL-1α inhibit both basal and TSH-induced NIS gene expression. TNF-α, IL-1α and IFN-γ also inhibit iodide uptake in FRTL-5 cells (Ajjan et al., 1998). In fact, some cytokines are involved in the suppression of TH production and metabolism in non-thyroid diseases (Bornstein et al., 1998; Torpy et al., 1998). In FRTL-5 cells, Isozaki et al. (2004) observed that leptin suppressed TSH-induced NIS mRNA expression. The main restriction of the FRTL-5 cells studies is that they are unable to organify iodide efficiently and to form colloid.

Considering that some cytokines directly inhibit NIS function and moreover the presence of leptin receptors in rat thyroid cells has already become evident, in our present study, we investigated the role of leptin upon NIS function in the rat thyroid. Hence, this study was designed to evaluate the effect of acute and chronic experimental hyperleptinaemia on the thyroid iodide uptake of fed adults rats, in vivo and in vitro.

Materials and methods

Animals

Three-month-old adult Wistar rats were kept in a room with controlled temperature (25±1 °C) and an artificial dark–light cycle (lights on from 7:00 a.m to 7:00 p.m.). Our protocol was approved by the Animal Care and Use Committee of the Biology Institute of State University of Rio de Janeiro, who based their analysis on the principles described in the Guide for the Care and Use of Laboratory Animals (Bayne, 1996).

In the *acute experiment*, male rats were divided into two groups ($n=8$/group):

LepA received a single subcutaneous injection (200 µl) of 8 µg/100 g body weight (BW) of recombinant mouse leptin (provided by the National Hormone and Pituitary Program, Harbor-UCLA Research and Education Institute, Los Angeles, CA).
C received a single saline injection (200 µl).

In the *chronic experiment*, male rats were divided into two groups ($n=8$/group):

LepC received subcutaneous injection (200 µl) of recombinant mouse leptin (8 µg/100 g BW), daily, in the course of 6 consecutive days.
C received saline for the same period (200 µl).

Both acute and chronic models were used for in vivo and in vitro analysis and rats were sacrificed by decapitation 120 min after leptin or saline injection.

In vivo thyroid ^{125}I uptake (RAIU)

It was demonstrated that thyroid uptake after 15 min of ^{125}I injection reflects iodide uptake (NIS function), and after 2 h, reflects iodide uptake and organification (NIS function+thyroid peroxidase [TPO] activity) (Ferreira et al., 2005).

LepA, LepC and its respective controls were ^{125}I (2.22×10^4 Bq) injected and were killed by decapitation after 15 or 120 min. Thyroid glands were carefully excised, and weighed. The RAIU was individually evaluated in a gamma counter (Cobra Auto-gamma, Packard Instrument Co., IL, USA). Results were expressed as % ^{125}I uptake/mg of thyroid gland.

In vitro thyroid RAIU

Time-course study. In a first step, we performed a time-course study of RAIU from thyroid explants of adult euthyroid rats, in order to establish in vitro NIS function. Glands were sliced and incubated in a minimum essential medium (MEM-SIGMA, MO) enriched with 5 mUI/ml of TSH, 0.1 µM of KI, 2 mM of glutamine and 25 mM of NaHCO3, at 37 °C, 95% O_2/5% CO_2 atmosphere in a Dubnoff metabolic shaker (100 cycles/min), as previous reported (Moura et al., 1990). The concentration of TSH and KI, as well as, the ^{125}I amount were established based on dose–response curve (Okamura et al., 1979, Yoshinari and Taurog, 1986). ^{125}I (3,700 Bq) was added and after 15, 30, 60, 90 and 120 min, thyroid ^{125}I content was counted (Cobra Auto-gamma, Packard Instrument Co., Downers Grove, IL, USA). The time-course experience was performed twice. Data were reported as % ^{125}I uptake/mg of thyroid gland.

Direct leptin effect on rat thyroid NIS function. To investigate leptin effect on in vitro NIS function, we carried out an experiment with thyroid explants from adult male euthyroid rats. Glands were incubated in MEM with 5 mUI/ml of TSH, 0.1 µM of KI, 2 mM of glutamine and 25 mM of NaHCO3, in a shaker at 37 °C with a carbogenic atmosphere, without or with leptin in different concentrations (10^{-9} M, 10^{-7} M and 10^{-5} M) for 30 min. Then, ^{125}I (3,700 Bq) was added for 120 min. The incubation was stopped, and the gland ^{125}I content was counted to estimate in vitro NIS function. Data were expressed as % ^{125}I uptake/mg of thyroid gland.

In vitro NIS function in hyperleptinaemia model. Thyroid explants from LepA, LepC and their respective controls were quickly dissected out. Each gland was sliced and immediately carried to Erlenmeyers containing 2 ml MEM with TSH, KI, glutamine and NaHCO3, at 37 °C in a Dubnoff metabolic shaker and carbogenic atmosphere at the same conditions of the previous experiment. After 30 min of pre-incubation, we added 100 µl of ^{125}I (3,700 Bq) and after 2 h, the ^{125}I tissue content was evaluated in a gamma counter. Medium aliquots were kept to measure TH and estimate its secretion. Data were expressed as % ^{125}I uptake/mg of thyroid gland.

Hormones levels determination (radioimmunoassay)

All measurements were performed in a single assay.

Serum TSH was measured by specific RIA, using a kit for rat TSH supplied by the National Institute of Health (NIH, USA) and expressed in terms of the reference preparation provided (RP-3). The intra-assay variation was 2%.

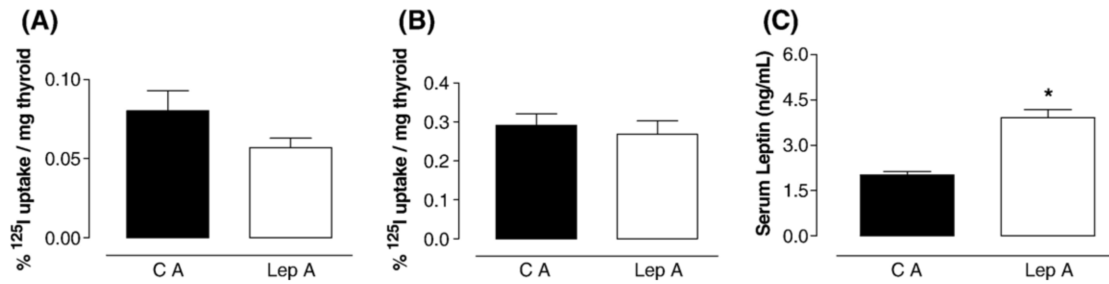

Fig. 1. Fifteen minutes (A) and two-hour (B) thyroid ^{125}I content in vivo in adults rats which received a single saline (black bar) or leptin (8 μg/100 g BW) (white bar) injection. Serum leptin levels of rats (C). Results are expressed as the mean ± SEM of 8 rats in each group.

Serum and medium free (f) T4 and T3 were measured by RIA using commercial kits (ICN Pharmaceuticals, Inc, NY). The intra-assay variations were 4% (T3) and 3% (T4), respectively.

Leptin was measured by RIA kit (Linco Research, Inc., Missouri, USA) which measures both rat and mouse leptin with an assay sensitivity of 0.5 ng/mL and a range of detection from 0.5 to 50 ng/mL. The intra-assay variation was 3.1%.

Statistics

Data were expressed as mean ± SEM, and their statistical comparisons were done using the unpaired test of Mann–Whitney (RAIU and TSH) or Student's *t* test (free thyroid hormones and leptin), with significance level set at $p < 0.05$.

Results

In vivo study

Acute leptin treatment did not affect in vivo thyroid RAIU at 15 or 120 min of ^{125}I administration (Fig. 1a and b). As showed in Table 1, LepA group presented higher serum TSH (+66%, $p < 0.05$), fT3 (+66%, $p < 0.05$) and fT4 (+34%, $p < 0.05$) concentrations. This group also presented hyperleptinaemia (+95%, $p < 0.05$—Fig. 1c).

Chronic leptin treatment increased, significantly, in vivo thyroid RAIU after 15 min (+1.3 fold, Fig. 2a) and 2 h (+33%, Fig. 2b) of ^{125}I injection. LepC did not show changes in serum TSH and TH (Table 1), but showed higher serum leptin concentrations (+75%, $p < 0.05$— Fig. 2c).

Time-course of in vitro NIS function

To evaluate the time-course of ^{125}I uptake from thyroid explants of normal rats (NIS function), we previously carried out incubations in distinct periods (15, 30, 60, 90 and 120 min). We demonstrated that before 120 min of ^{125}I incubation, there is no significant ^{125}I influx to thyroid tissue, suggesting that in vitro NIS function only begins after 2 h (Fig. 3).

Table 1
Serum TSH, T3 and T4 of rats after acute (A) or chronic (C) leptin treatment

Hormones levels	C A	Lep A	C C	Lep C
TSH (ng/ml)	1.2±0.07	2.0±0.21*	1.5±0.21	1.2±0.28
FT3 (pg/ml)	1.5±0.23	2.5±0.35*	2.4±0.24	3.3±0.47
FT4 (ng/dl)	1.7±0.07	2.3±0.18*	2.1±0.15	2.3±0.18

Values represent mean ± SE. *$p < 0.05$ ($n = 8$).

Leptin effect on rat in vitro NIS function

Euthyroid males presented lower thyroid RAIU (120 min of ^{125}I incubation) after leptin incubation at 10^{-9} M (−39%—$p < 0.05$, Fig. 4), showing that leptin inhibits the in vitro NIS function. The other leptin concentrations also caused lower thyroid RAIU (10^{-7} M:−35% and 10^{-5} M:−27%), however, without reaching the statistical significance.

In vitro NIS function in hyperleptinaemia models

Thyroid explants from LepA and LepC showed lower in vitro NIS function (−32% and−29% respectively, $p < 0.05$, Fig. 5a and b). Those glands presented higher T4 in vitro secretion (LepA:+125% and LepC:+200%—$p < 0.05$), however, no change in medium T3 levels (Figs. 6 and 7).

Discussion

Here, we demonstrated that leptin increases NIS in vivo, probably by an indirect mechanism, since the in vitro effect seems to be inhibitory.

Most of the studies about leptin action upon the thyroid function were performed on fasted animals. They concluded that the main leptin action is on the hypothalamus, since the decrease of TRH levels or its mRNA expression is overcome by leptin administration (Legradi et al., 1997; Nillni et al., 2000; Harris et al., 2001).

Data are scarce regarding the direct action of leptin on the thyroid gland. A study in FRTL-5 cells showed that leptin stimulates thyroid cells proliferation, similar to TSH, but inhibits NIS and thyroglobulin mRNA expression stimulated by TSH (Isozaki et al., 2004). These results demonstrated that leptin can present both stimulatory and inhibitory effects on thyroid function, probably by different intracellular mechanisms.

In the present study, there was no change on ^{125}I thyroid uptake in the period of 15 min or 2 h after acute leptin injection. However, ^{125}I uptake of isolated thyroid of LepA rats was lower than controls. Thus, it is possible that, in vivo, the higher serum TSH observed in this group could be up-regulating NIS function, counterbalancing the direct inhibitory effect of leptin.

In the LepC group, in vivo thyroid ^{125}I uptake was higher after 15 min or 2 h from leptin administration. This effect seems

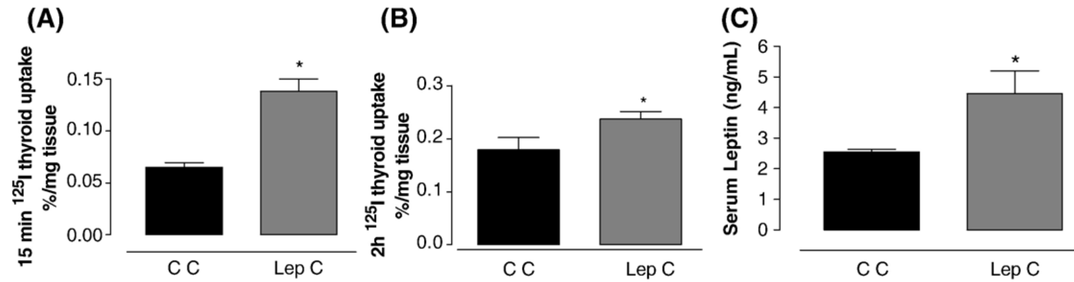

Fig. 2. Fifteen minutes (A) and two-hour (B) thyroid 125I content in vivo in adult rats which received daily saline (black bar) or leptin (8 μg/100 g BW) (gray bar) injection for 6 consecutive days. Serum leptin levels of rats (C). Results are expressed as the mean ± SEM of 8 rats in each group, $*p<0.05$.

to be TSH-independent, since its serum levels were normal in this group. However, given the pulsatility of the TSH release (Brabant et al., 1991), leptin could alter the pulsatile secretion of TRH-TSH (Mantzoros et al., 2001) without significant changes in TSH levels. In addition, an increase of TSH receptor expression or higher TSH bioactivity might be responsible for increasing iodide uptake. In fact, leptin increases TRH secretion that might lead to increased TSH bioactivity. It is possible that, in vivo, LepC animals have developed a partial thyroid resistance to leptin, abolishing its stimulatory effect upon hormonal secretion. The lack of leptin RAIU suppression could explain the finding of an increase of RAIU in vivo; however, we cannot discard the possibility that chronically in vivo leptin administration could increase an unknown NIS stimulatory factor.

When we studied the ^{125}I uptake on isolated thyroid tissue of LepC rats, we detected lower NIS function. In addition, we demonstrated, for the first time, that leptin directly inhibits the in vitro NIS function in rat thyroid explant.

In this study, we detected two distinct situations. First, in vivo leptin treatment reduces (acute) or increases (chronic) ^{125}I uptake at 15 min. Second, leptin addition inhibited NIS function on the thyroid slice system. Therefore, it is possible that in vivo, by stimulation of TRH-TSH secretion, leptin increases thyroid ^{125}I uptake and, this indirect effect surpass its direct inhibitory effect on NIS function. To better understand this process, the evaluation of the ^{125}I uptake from thyroid explants of hyperleptinaemic rats was valuable because it demonstrates that both acute and chronic hyperleptinaemic models presented lower in vitro NIS function, when free of endogenous regulatory substances, supporting the hypothesis of a direct inhibitory effect of leptin upon NIS function.

Despite, leptin could directly down-regulate NIS function, it can increase thyroid follicular proliferation (Isozaki et al., 2004), which could help to explain the observed higher in vivo ^{125}I uptake. Besides, as leptin is a cytokine, it is important to mention that TNF-α, IL-1α and IFN-γ have an inhibitory effect on NIS expression and on iodide uptake in FRTL-5 cells (Ajjan et al., 1998). In transgenic mice whose INF-γ is over-expressed, this cytokine increases TPO and TSH receptor expression, and decreases NIS expression (Carrasco, 1999), showing different effects in the same tissue.

Nowak et al. (2002) reported the presence of the Ob–Rb in the thyroid of normal female rat. In this study, leptin treatment for 6 days increased thyroid volume and serum thyroid hormones, but reduced serum TSH. These authors did not investigate male rats. In our study, the same period of leptin treatment in male rats did not change gland weight, neither serum thyroid hormones nor TSH. Some authors observed the sex steroid effects upon hypothalamic-pituitary-thyroid axis (Mason et al., 1996; Lisboa et al., 1997; Moreira et al., 1997; Borges et al., 1998; Correa da Costa et al., 2001; Lisboa et al., 2001; Moura et al., 2001; Moreira et al., 2005). Then, it is possible that gender influences leptin action upon thyroid function.

The direct in vivo effect of leptin on the thyroid can be more evident in female (Nowak et al., 2002), perhaps for a difference of number in the Ob–Rb. Although there was no report about the detection of Ob–Rb on the thyroid of male rats, in this

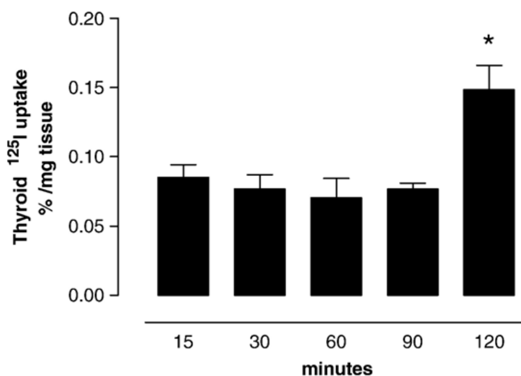

Fig. 3. In vitro thyroid ^{125}I content from adult euthyroid rats after 15, 30, 60, 90 and 120 min of ^{125}I addition in the medium. Results are expressed as the mean ± SEM of 8 rats in each group, $*p<0.05$.

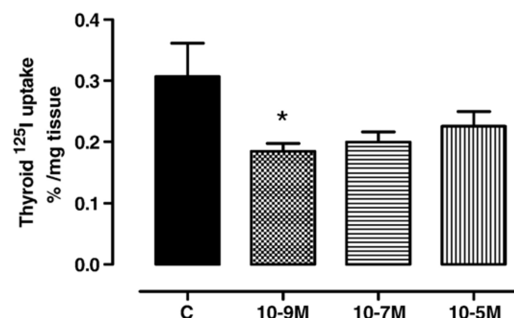

Fig. 4. In vitro thyroid ^{125}I uptake (120 min) from adult euthyroid male rats after incubation with different leptin concentrations. Results are expressed as the mean ± SEM of 8 rats in each group, $*p<0.05$.

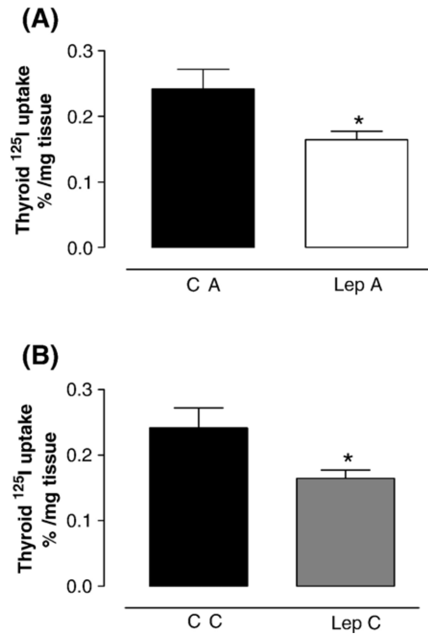

Fig. 5. A—In vitro ^{125}I uptake of thyroids from rats treated with a single saline (black bar) or leptin injection (white bar). B—In vitro iodide uptake of thyroids from rats which received saline (black bar) or leptin injection (gray bar) for 6 consecutive days. Results are expressed as the mean <0.05.

present study, data from in vitro experiments evidenced a direct leptin action on thyroid of adult male rats.

Thyroid explants from LepA animals showed higher in vitro T4 secretion, but no change in medium T3 level. Thus, the increased serum T3 observed in the in vivo experiment can be originated by the conversion of T4 to T3 in peripheral tissues, since some authors demonstrated that leptin increases liver deiodinase activity (Cusin et al., 2000; Lisboa et al., 2003). We also observed that LepC group presented higher in vitro T4

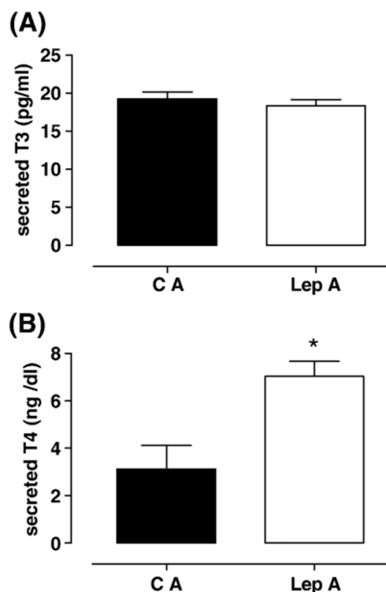

Fig. 6. Medium T3 (A) and T4 (B) levels from thyroid explants of rats acute leptin-treated. Data represent means ± SEM, $*p<0.05$ ($n=8$).

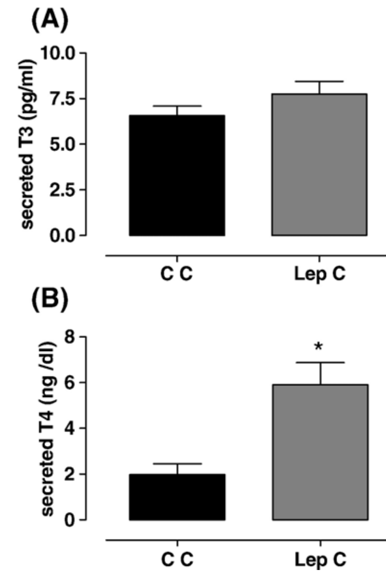

Fig. 7. Medium T3 (A) and T4 (B) levels from thyroid explants of rats chronic leptin-treated. Data represent means ± SEM, $*p<0.05$ ($n=8$).

secretion, and no change in T3, but this effect was not observed in the in vivo experiment. Thus, we demonstrated that leptin seems to exert a stimulatory effect on T4 secretion, even in thyroid explant, that is TSH independent.

In conclusion, in this study we have showed that leptin presents an inhibitory effect on NIS function on the thyroid gland, both acutely and chronically, demonstrated previously only in FRTL-5 cells. However, the chronic leptin exposure causes a RAIU increment in vivo, probably as a consequence of hypothalamic-pituitary stimulation. Our findings show that complex and distinct mechanisms may be involved in the direct and indirect action of leptin on thyroid function.

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