The long-term regulation of food intake and body weight depends on the availability of thyroid hormones in the brain

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Abstract

OBJECTIVES: We evaluated the contribution of the thyroid hormones to the long-term maintenance of feeding behavior and body weight, while distiguishing their direct central effects from those resulting from the metabolic rate in the peripheral tissues.

METHODS: We assessed the effect of hypothyroidism on the long-term (6 months) regulation of food intake, body weight, and energy expenditure in rats. We then generated the recovery of a euthyroid condition in the brain while maintaining a low T_3 availability for the peripheral organs, i.e. a combined condition of central euthyroidism with peripheral hypothyroidism, with the aid of a pharmacological combination.

RESULTS: Hypothyroidism caused a decrease in the daily food intake, body weight, and body temperature. The food intake and body temperature stabilized at a lower value, whereas body weight kept decreasing at a constant rate. The administration of exogenous T_4 increased food intake and body-weight gain, but had no effect on body temperature.

CONCLUSIONS: The thyroid hormones are necessary for the long-term regulation of energy intake, storage, and expenditure by different mechanisms. The feeding behavior seems to be partially dependent on a direct action of the thyroid hormones on the brain and this effect is independent of the energy expenditure in the peripheral organs. The body weight is closely dependent on the thyroid status and its maintenance seems to involve thyroid action on mechanisms other than feeding and metabolic rate.

ORIGINAL ARTICLE

INTRODUCTION

Long-term body weight results mainly from the balance between energy intake and energy expenditure. Both factors can be regulated independently according to the requirements of the maintenance of some critical variables, such as the energy availability and the size of the energy stores. Both the energy intake and expenditure are controlled by the medial region of the hypothalamus, which plays a key role in the regulation of many variables related to energy and metabolism. Among the effector mechanisms of the hypothalamus, the thyroid axis and its hormones (T_3 and T_4) are traditionally viewed as a mechanism that maintains the general metabolic activity and the body temperature.

One of the major effects of the thyroid hormones (THs) is to stimulate the metabolic rate both in peripheral tissues and in the brain (López *et al.* 2010; Matsumura *et al.* 1992), and thus they have the general effect of promoting energy expenditure and thermogenesis. By increasing the expenditure, the THs create a negative energy balance that reduces the size of the energy stores and the body weight and thus increases the hypothalamic drive to feed. This has been traditionally viewed as the main mechanism by which the THs influence feeding behavior. For instance, hyperthyroidism causes a decrease in body weight along with an increase in food intake (Luo & MacLean 2003). The situations that imply an energy deficit, such as fasting or diabetes mellitus, decrease both the circulating levels of T_3 (Matsumura *et al.* 1992) and the conversion rate of T_4 to T_3 (O'Mara *et al.* 1993).

The causal relationship between thyroid hormone levels, basal metabolism, and feeding behavior is not always evident. Some hyperthyroid subjects show increased feeding that leads to body-weight gain in spite of having a mainly catabolic state (Gurney *et al.* 1970), which suggests that feeding does not compensate solely for the energy deficit caused by the elevated metabolism. The body weight of hypothyroid subjects correlates weakly with the degree of thyroid dysfunction and is not consistent among studies (Hsieh *et al.* 2002; Oge *et al.* 2005; Pinkney *et al.* 1998). Obese subjects show positive correlations between thyroid activity and the Body Mass Index (De Pergola *et al.* 2007; Reinehr & Andler 2002), which indicates that most forms of obesity do not imply a decrease but instead an increase in the activity of the thyroid axis. Whether this effect is a compensatory response to increased body weight or it is itself causative of obesity is not clear. For instance, administration of the active thyroid hormone T_3 in doses too low to cause a metabolic effect have been shown to moderately increase the amount of food taken by euthyroid rats (Kong *et al.* 2004). Taken together, these have been interpreted as indicative that thyroid hormones promote feeding behavior through a direct effect at the central level besides the well-known elevation of the metabolic rate in the peripheral tissues. Although both effects would cause an increase in food intake, they may have opposite consequences on body weight over the long term.

Three intracellular deiodination mechanisms have been described that can modify the availability of T_3 in the target cells with no concomitant change in the circulating levels of the hormone (Gereben *et al.* 2008). Particularly, the selenodeiodinases DI and DII are able to increase the concentration of the active hormone T_3 by deiodination of its relatively inactive precursor T_4. DI is present in most peripheral tissues including liver and kidney (Bates *et al.* 1999) and DII is particularly important in the nerve tissue of the brain (Crantz *et al.* 1982; Sharlin *et al.* 2010). These mechanisms show clear-cut differences in their response to substrate availability and in their susceptibility to blockade by drugs like propyl-thiouracil (PTU) (Gereben *et al.* 2008; Roy & Mugesh 2006; Silva *et al.* 1982).

Our study was aimed at evaluating the role of the thyroid hormones as a mechanism of regulation of the energy balance. We analyzed the effects of long-term thyroid hormone deficiency on food intake and body weight in rats, taking advantage of the local deiodination mechanisms to distinguish the relative contribution of the central and the peripheral actions of these hormones to the alimentary response.

MATERIALS AND METHODS

Male Wistar rats, weighing 300 to 350 g at the beginning of the experiment, were kept individually in wire mesh cages in a temperature (24 ± 1 °C) and light (12-h:12-h, lights on at 0800) controlled environment. Food (powdered laboratory rodent chow 5001, PMI, Minnetonka, MN, USA) and tap water were freely available during the entire experiment.

After a 3-week habituation period, animals were divided in three groups of similar average body weight (4 rats per group). A first group received no treatment and was used as a control group. A second group was treated for the generation of hypothyroidism by administering methimazole (M, 60 mg/kg·day) and PTU (15 mg/kg·day), both dissolved in the drinking water. The drug concentrations were adjusted weekly according to the average daily water consumption. This treatment was maintained for the following 17 weeks. The third group received the same treatment but after 9 weeks a dose of 20 μg/kg·day of T_4, also dissolved in the drinking water, was administered in addition to the antithyroid drugs. The T_4 supplementation was maintained for the following 8 weeks. This treatment was aimed at creating a situation of near-euthyroidism at the central level while keeping a hypothyroid condition at the peripheral tissues. The rationale is, that being blocked both thryroid-hormone synthesis (by methimazole) and DI activity (by PTU), the exogenous T_4 becomes a potential source of T_3 for tissues in which DII activity (spared by PTU) is quantitatively impor-

tant. Because the DII is abundant in the nervous tissue and the T_4 crosses the blood-brain barrier more readily than T_3 (Bernal 2005; Bianco *et al.* 2002), this treatment is expected to increase the availability of the T_3 in the brain while maintaining a T_3 deficiency in most of the peripheral organs.

Daily food intake, colonic temperature, and body weight were measured regularly during the treatment. At the end, samples of trunk blood were taken to determine the total plasma levels of T_3 and T_4 using commercial ELISA kits (Diagnostic System Laboratories, Webster, TX, USA) and blood glucose using Medisense Optium strips (Abbott Laboratories, CA, USA). All the experimental procedures are in accordance with The Guide for the Care and Use of Laboratory Animals of the Mexican Council for Animal Care (NOM-062-ZOO-1999). Every effort was made to minimize the number and potential suffering of the experimental subjects.

Data on food intake, colonic temperature, and body weight were analyzed by means of two-way ANOVA tests followed by Newman-Keuls post-hoc tests. The data on plasma T_3, T_4, and glucose were compared by a Student's *t*-test or one-way ANOVA tests. The significance level was set at 0.05.

RESULTS

Rats treated for 17 weeks with a mixture of methimazole and PTU showed a significant reduction in the circulating levels of both T_3 and T_4. The T_4 concentration decreased to about one half of the control values (153 ± 15 vs. 274 ± 9 ng/mL; $p<0.05$) from the fourth week of treatment and remained at similar values until the end of the experiment. As expected, the restitution of T_4 from the ninth week raised the levels of circulating T_4 to normal values, but because peripheral deiodination was blocked, the circulating T_3 remained at low levels (Figure 1). The glucose levels in blood showed no difference among the three groups (data not shown).

Hypothyroid rats showed a consistent decrease in their daily food intake from the beginning of the treatment. This decrease was more pronounced during the first 3 weeks of the treatment, with the average intake remaining nearly constant from this time on, at about one half of the values of the control group (Figure 2). Interestingly, the restitution of T_4 caused a slight increase in food intake that progressively elevated the values of daily intake to make them different fom those of the rats that remained hypothyroid (from week 10) and similar to those of the control group (from week 16).

Similar to the daily food intake, the body weight decreased because of the treatment with antithyroid drugs. However, contrary to intake, the body weight continued to decrease from the fifth week to the end of the experiment with a nearly constant slope (–7.12; $R = 0.97$; Figure 3). Before the administration of T_4, the third group showed a similar trend in the loss of body weight (slope = –9.2; $R = 0.94$) that turned into an increase immediately after the beginning of the supplementation of T_4. This rate of weight gain was larger than control (slope = 5.19; $R = 0.98$ for the M+P+T_4 group vs. slope = 3.27; $R = 0.86$ for control group) and was maintained to the end of the experiment. As a result of this trend, the average values of the T_4-supplemented group approached those of the control group and became different from those of the hypothyroid group from week 12 on.

The colonic temperature was measured under the same circumstances. Again, antithyroid treatment

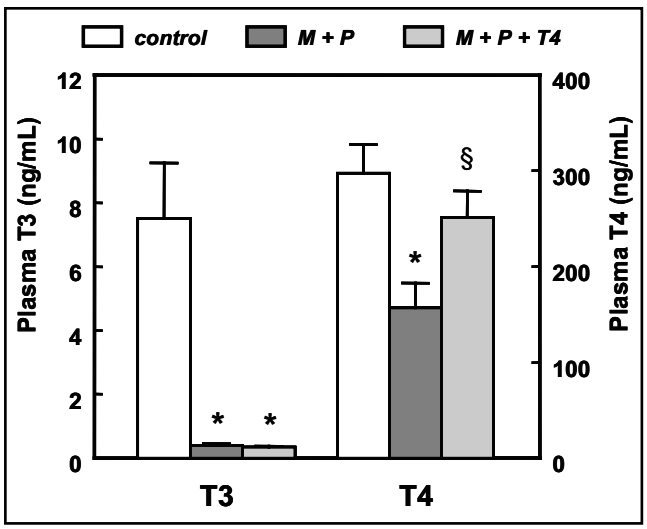

Fig. 1. Total concentrations of thyroid hormones in the trunk blood of the rat measured after 17 weeks of treatment with antithyroid drugs (M+P), antithyroid drugs plus T_4 from the ninth week (M+P+ T_4), or no treatment (control). Data are the mean ± SE ($n = 4$). *$p<0.05$ vs. control; §$p<0.05$ vs. hypothyroid (M+P) group.

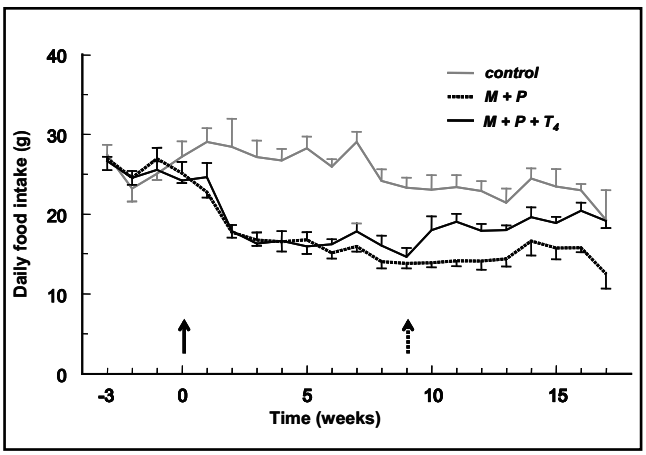

Fig. 2. Daily food intake of rats treated with methimazole and PTU for 17 weeks (M+P), with methimazole and PTU plus T_4 from the ninth week (M+P+T_4), or with no treatment (control). Daily intake values were averaged weekly for each animal, and the data are the mean ± SE of $n = 4$. The solid arrow shows the beginning of the antithyroid treatment for both M+P and M+P+T_4 groups. The dashed arrow shows the beginning of the T_4 supplementation for the M+P+T_4 group.

caused a decrease in the average values that was evident from the third week after the beginning of the treatment ($p<0.05$ vs. control group). Contrary to the other two variables, the body temperature was not stimulated by the supplementation with T_4, so that the temperature values of the supplemented group were not different from those of the hypothyroid group at any time, and these two groups had significantly lower values than the control animals until the end of the experiment (Figure 4).

DISCUSSION

The thyroid hormones (THs) have a general effect of increasing metabolic rate and energy expenditure that cause a negative energy balance and a compensatory increase in feeding. This is traditionally viewed as the main mechanism by which THs affect body weight and food intake. However, not all situations that imply alterations of the thyroid status also cause the corresponding changes in body weight and food intake. Some of the effects of the THs on body weight (López *et al.* 2010) and food intake (Kong *et al.* 2004) seem to be exerted by a direct action at the hypothalamic mechanism that regulates the energy balance.

In our work we evaluated the contribution of the THs to the long-term maintenance of feeding behavior and body weight, while distiguishing their direct central effects from those resulting from the metabolic rate in the peripheral tissues.

The thyroid status depends on both the secretory activity of the thyroid gland and the deiodination activity that takes place in extrathyroid tissues. In our study we caused the decrease of the TH supply by the thyroid gland along with the inhibition of the DI deiodinase, which is the main source of T_3 for most peripheral tissues. This manipulation dramatically decreased the concentration of circulating T_3 and caused a decrease in the metabolic rate of the rats, as evidenced by the drop in colonic temperature. Under these circumstances, we administered exogenous T_4 that is expected to cross the blood-brain barrier (Hagen & Solberg 1974) and to be deiodinated by the DII deiodinase, thus increasing the T_3 supply to the brain tissue. The supplementation with T_4 at a moderate dose (20 μg/kg) elevated the circulating levels of this hormone to a value close to the control, whereas the circulating T_3 remained at low levels. This indicates that the DI blockade was effective in inhibiting the peripheral production of T_3 from the exogenous T_4. A previous report demonstrated that the administration of exogenous T_4 to thyroidectomized rats in a dose that recovers normal plasma T_4-concentration also recovers normal T_3 levels in the brain and brown adipose tissue but not in the plasma or other peripheral organs (Escobar-Morreale *et al.* 1996), even if peripheral deiodination is not inhibited. From this, it can be expected that the combined treatment with a DI inhibitor plus T_4 restitution in this study would cause the T_3 availability in the brain to be significantly higher than in the peripheral circulation.

Under the antithyroid treatment, the rats had a decrease in the daily food intake and body weight gain along with the fall in colonic temperature. These effects are in agreement with the known consequences of thyroid-function impairment. The subsequent supplementation with exogenous T_4 affected these variables differently, causing a clear-cut change in the temporal profile of food intake and body weight and having no effect on the body temperature.

These observations differ from the traditional view, in which body weight and food intake are modified in accordance with the energy requirements that result from the overall metabolic activity, which in turn is directly stimulated by the thyroid hormones. Our

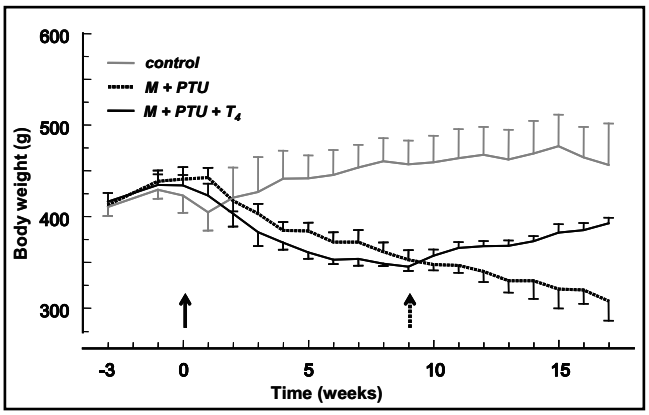

Fig. 3. Body weight of rats treated with methimazole and PTU for 17 weeks (M+P), with methimazole and PTU plus T_4 from the ninth week (M+P+T_4), or with no treatment (control). Data are the mean ± SE of $n = 4$. The solid arrow shows the beginning of the antithyroid treatment for both M+P and M+P+T_4 groups. The dashed arrow shows the beginning of the T_4 supplementation for the M+P+T_4 group.

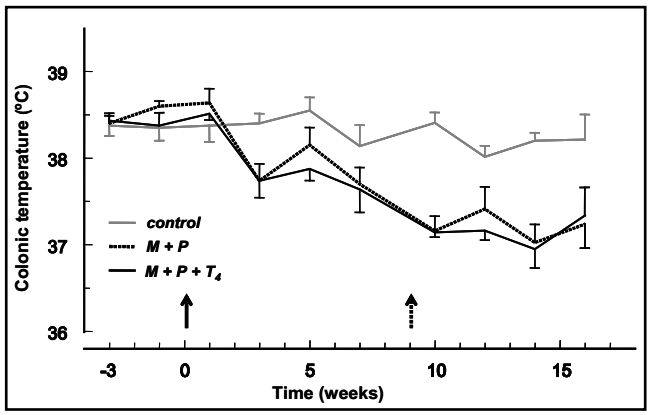

Fig. 4. The colonic temperature of rats treated with methimazole and PTU for 17 weeks (M+P), with methimazole and PTU plus T_4 from the ninth week (M+P+T_4), or with no treatment (control). The data are the mean ± SE of $n = 4$. The solid arrow shows the beginning of the antithyroid treatment for both M+P and M+P+T_4 groups. The dashed arrow shows the beginning of the T_4 supplementation for the M+P+T_4 group.

results suggest that the long-term feeding behavior and body weight are stimulated independently from the overall heat production and not as a consequence of it. This could be interpreted as the effects of thyroid hormones on these two kinds of variables (food intake and body weight on one side and body temperature on the other) being dependent on different physiological processes.

The pharmacological manipulation we used was aimed at impairing the physiological processes dependent on the DI activity, while maintaining those in which DII is involved. Although we did not measure deiodinase activity, from the reported occurrence of DII in brain tissue as compared to the peripheral organs we can hypothesize that this deiodinase would produce different local availability of T_3 in brain and in the periphery. This possibility is in line with the fact that two kinds of TH-mediated responses were observed, then reinforcing the idea that they depend on the thyroid action at different sites. The main contribution to the overall heat production is made by the peripheral organs (Aschoff *et al.* 1971), in which T_3 is derived mainly from DI activity. From this it results that the long-term maintenance of food intake and body weight would presumably be dependent on the production of T_3 in the brain by the DII.

Our data also suggest that the THs participate in the maintenance of body weigh in different ways. Food intake and body weight are causally related, and feeding is known to make the largest contribution to the changes in body weight, when compared to energy expenditure (Westerterp & Speakman 2008). In our study, both variables showed a similar profile, because the antithyroid treatment caused a fall in daily food intake that was followed by a parallel decrease in body weight. Though the food intake reached a minimum after the third week of antithyroid treatment and decreased no further, the body weight decreased at a constant rate until the end of the experiment, with no indication that this trend would be modified afterwards. This suggests that the THs participate in the maintenance of body weight by affecting some other processes besides food intake, quite probably the internal handling of energetic fuels. The known proliferative effects of THs, i.e. adipogenesis (Ying *et al.* 2007), do not seem to mediate this effect on body weight, because in our experiment the weight loss caused by hypothyroidism was reversed by the administration of T_4. Assuming that in our model T_4 is deiodinated mainly in brain, this would indicate a central site of action of TH to revert body weight loss.

Restricted feeding, i.e. access to only a fraction of daily food requirements, also causes a fall in body weight. The weight loss in this case is rapid at the beginning and slows as the condition persists, so that body weight stabilizes at a lower level (Levin & Keesey 1998). For hypothyroidism, a stable level in body weight is not reached in spite of stable values of daily food intake. As long as the two situations can be compared, it seems that the body weight loss caused by undernutrition is subjected to some form of regulatory compensation, whereas that caused by chronic hypothyroidism is not. The T_4 restitution to hypothyroid rats caused an accelerated weight gain as an attempt to reach the ideal body weight according to the age of the rat. These observations fit into a model of regulation of the body weight as proposed previously (Cabanac 2001; Levin & Keesey 1998), and suggest that the THs are an essential part of the mechanism of body weight maintenance acting on the central integration that determines both energy intake and expenditure.

CONCLUSIONS

Our results demonstrate that the THs are necessary for the long-term maintenance of body weight and food intake. This effect is independent of the thermogenic action of the THs and would presumably be mediated by direct action on the brain, probably at the centers of regulation of the energy balance in the hypothalamus.

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