Effect of neonatal hyperthyroidism on GH gene expression reprogramming and physiological repercussions in rat adulthood

Kely de Picoli Souza, Francemilson Goulart da Silva and Maria Tereza Nunes

Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, 05508-900 São Paulo, SP, Brazil

(Requests for offprints should be addressed to M T Nunes; Email: mtnunes@icb.usp.br)

Abstract

The neonatal period (NP) is a critical phase of the development in which the expression pattern of most genes is established. Thyroid hormones (TH) play a key role in this process and, alterations in its availability in the NP may lead to different patterns of gene expression, which might reflect in the permanent expression of several genes in the adulthood. GH gene expression in the pituitary is greatly dependent on TH in the early postnatal life; thus, modifications of thyroid state in NP might lead to alterations in GH gene expression as well as to physiological repercussions in the adult life. This study aimed to investigate this possibility by means of the induction of a neonatal hyperthyroidism in rats (4 µg of 3,5,3'-triiodo-L-thyronine (T3)/100 g body weight, s.c.) for 5, 15 or 30 days, and further evaluation of GH gene expression, as well as its physiological consequences in adult

rats subjected to a transient hyperthyroidism in the first 30 days of life. GH mRNA level was shown to be increased in T3-treated rats for 5 days; when the treatment was extended to 15 or 30 days, the GH mRNA levels were similar to the control group. Moreover, rats treated with T3 for 30 days and killed when 90 days old, i.e., 60 days at the end of the T3 treatment, showed decreased GH mRNA content, body weight, bone mineral density, and lean body mass. In conclusion: (1) T3 effects on GH gene expression depend on the period of life in which the hyperthyroidism is set and on the length of T3 treatment in the NP and (2) transient neonatal hyperthyroidism leads to a lower GH mRNA expression in adult life accompanied by physiological repercussions indicative of GH deficiency.

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Introduction

In mammals, many tissues are not completely developed at birth (Kominami *et al.* 2003, Nakahara *et al.* 2005), which means that the pattern of expression of many genes is not defined entirely. Therefore, the neonatal period (NP) is an important stage of life in which hormonal, neuronal, and metabolic disturbances may influence the program of expression of some genes in the adult life.

Dussault & Labrie (1975) have demonstrated in rats that thyroid hormone serum concentrations are low at birth and increase progressively achieving the adult values approximately at the third week of life, when the maturation of the hypothalamus–pituitary–thyroid (HPT) axis is accomplished. While the set point of the thyroid-releasing hormone (TRH) and thyroid-stimulating hormone (TSH) secretion control is being established (Pracyk *et al.* 1992), the expression of different thyroid hormone receptor (TR) isoforms and the activity of different types of deiodinases in several tissues try to assure a stable supply of thyroid hormones (TH) to tissues, avoiding deleterious effects caused by temporary low or high levels of these hormones (Bates *et al.* 1999, White *et al.* 2001). These adjustments denote the physiological importance of

TH for the body economy at this period, in which the patterns of expression of most genes are being established.

In effect, studies have demonstrated that the induction of a transient hyperthyroidism in rats at NP lead, at the adult life, to a deficit on the myelination of the nerve cells (for review, see Pasquini & Adamo 1994), a decrease in the number of oligodendrocytes by activation of apoptotic mechanisms (Marta *et al.* 1998), alterations in the number and function of the Leydig cells (Teerds *et al.* 1998), a decrease in T4 response to exogenous TSH (Csaba & Nagy 1985), and a decrease in the β -adrenergic receptor's density at myocardium (Novotny *et al.* 1999), among other effects.

Even though these studies show a clear correlation between precocious events induced transiently at the postnatal life and associated repercussions at the adult life, the molecular basis underlying these events is not fully known. In this context, the first month of life is an attractive period to study the transition from the fetal/neonatal to the adult pattern of expression of several genes that are under the control of TH.

Most of the TH effects result from their interaction with TRs (Webb 2004) previously bound to the thyroid hormone response element (Harvey & Williams 2002), located in several target genes. One of them is the growth hormone (GH) gene,

whose expression is induced by 3,5,3'-triiodo-L-thyronine (T3) and reduced in hypothyroid states (Samuels & Shapiro 1976, Volpato & Nunes 1994).

GH is known to exert important physiological actions on the skeletal longitudinal growth as well as on glucose, amino acid, and fatty-acid metabolism (Mauras *et al.* 2000, Robson *et al.* 2002). Impaired GH expression has been associated with disturbed body growth and fuel metabolism (Okada & Kopchick 2001).

The aim of the present study is to investigate the GH gene responsiveness to T3 treatment during the first month of life in rats and whether the transient postnatal hyperthyroidism alters the GH gene expression permanently in the adult life. We further evaluated, in the adult animals, the possible repercussions of a transient postnatal hyperthyroidism in the body weight (BW) and size, body composition, and bone mineral density (BMD), parameters that are under GH control.

Materials and Methods

Animals and treatments

All aspects of animal care and experimentation performed in this study agree with ethical principles in animal research adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Institute of Biomedical Sciences of the University of São Paulo-Ethical Committee for Animal Research (CEEa).

Pregnant Wistar rats were obtained from the breeding colony of the Institute of Biomedical Sciences. They had free access to commercial chow and tap water, in a temperature-controlled room ($23 \pm 1^\circ\text{C}$) with a 12 h light:12 h darkness cycle (lights on at 0700 h). Male Wistar newborn rats, weighing approximately 6 g, were used in this study. Each litter consisted of approximately eight rats per dam and was randomly assigned to one of the two treatment groups: (1) control- or (2) T3-treated group (Hyper).

Treatment of each group was started on the first day (P1) after birth (P0) and was conducted as follows: control rats received s.c. injections of 0.9% NaCl and T3-treated rats received s.c. injections of T3 (Sigma, 4 $\mu\text{g}/100\text{ g BW}$), every day during 5, 15 or 30 days starting one day after birth (P1) and these animals were named as Hyper5, Hyper15, and Hyper30 respectively. After this period, the animals were weighed and killed by decapitation. The pituitary was removed quickly for RNA extraction. To check the efficiency of the T3 treatment, heart ventricle weights and the day pups opened their eyes were determined. Blood samples were collected to evaluate the concentrations of T3 and T4 by RIA (Diagnostic Products Corporation, Llanberis, UK) in sera, using a standard curve prepared with different concentrations of T3 or T4 in iodothyronine-free rat serum.

In order to evaluate the T3 serum clearance rate, an additional study was carried out in rats subjected to T3 or saline for 15 days from birth. It consisted of a time-course

study of T3 serum concentration in which the rats were killed 30 min, 2, 6, and 15 h after the last T3 injection.

For the GH gene-reprogramming studies, the animals were treated with T3 (4 $\mu\text{g}/100\text{ g BW}$, s.c.) for 30 days. The treatment was suspended by 60 days and these animals, named as postnatal transient hyperthyroid rats, had their size (cm), BW (g), body composition (fat and lean mass, g/cm^2), and BMD (g/cm^2) determined. They were killed afterwards and had their GH mRNA expression, T4 and T3 serum levels evaluated. Each experiment included four rats per group and was repeated three times.

The T3 dose used to induce hyperthyroidism (4 $\mu\text{g}/100\text{ g BW}$) was 13 times higher than the physiological replacement dose of T3, which is postulated to be 0.3 $\mu\text{g}/100\text{ g BW}$ (Dillmann *et al.* 1983), and lower than those used in similar studies (Yamazoe *et al.* 1989, Cernohorsky *et al.* 1998).

Total RNA extraction and Northern blot analysis

Total RNA was isolated using the acid guanidinium thiocyanate-phenol-chloroform extraction method (Chomczynski & Sacchi 1987) and quantified by absorbance at 260 nm. Six micrograms of total RNA samples were denatured with formaldehyde-formamide, electrophoresed in 1% agarose gels containing 2.2 M formaldehyde in 1 $\times$ 3-*n*-morpholino-propanesulfonic acid (MOPS) buffer and blotted to a nylon membrane (Nylon-1 membrane; Gibco) by neutral capillary transfer. The membrane was baked at 80 $^\circ\text{C}$ for 2 h in a vacuum oven and pre-hybridized in 50% formamide hybridization solution and 100 $\mu\text{g}/\text{ml}$ denatured salmon sperm DNA at 42 $^\circ\text{C}$ for 4 h (Maniatis *et al.* 1989). Subsequently, the membrane was probed with a ^{32}P -labeled rat GH cDNA by random priming (Random Primers DNA Labeling System kit; Gibco) for 16 h at 42 $^\circ\text{C}$. The membrane was washed under highly stringent conditions and subjected to autoradiography and quantified by phosphor imaging, using the ImageQuant software (Molecular Dynamics, Sunnyvale, CA, USA). All blots were stripped and re-hybridized with a ^{32}P -labeled RNA probe specific for 18S ribosomal subunit (18S rRNA), synthesized by *in vitro* transcription (Maxi Script *in vitro* transcription kit; Ambion, Austin, TX, USA), to correct for the variability in RNA loading. The results were expressed as mean $\pm$ S.E.M. of GH mRNA/18S rRNA ratio.

Body composition and BMD determination

Body composition, lean and fat mass, and BMD were measured by dual energy X-ray absorptiometry using the pDEXA Sabre Bone Densitometer and the pDEXA Sabre Software version 3.9.4 (Norland Medical Systems, Fort Atkinson, WI, USA), both designed especially for small animals (Stikkelbroeck *et al.* 2003). The research mode scan option was used for the measurements. Pixel spacing for the scan was set to 0.5 $\times$ 0.5 mm and the scan speed to 4 mm/s. To limit the scan area, which allows the scans to be performed in a higher resolution mode, the scans were performed from

the first lumbar vertebra to the hind limbs. The analyzed region was the hind body, which includes L2–L6, pelvic bones, hind limbs, and the first four caudal vertebrae. For the scans, the animals were anesthetized with a ketamine–xylazine cocktail and scanned in the prone position. The animals were scanned at 90 days of life (after 60 days, the treatment was completed).

Statistical analysis

The data were subjected to one-way ANOVA, followed by the Student–Newman–Keuls test and Student's *t*-test, when only two groups were compared. They were expressed as means $\pm$ S.E.M. Differences were considered significant at $P < 0.05$.

The data of the time-course study of total T3 serum concentrations of euthyroid and hyperthyroid rats treated with T3 from P1 to P15, after 30 min, 2, 6, and 15 h of a single injection of T3 were entered into two-way ANOVA with time (h) and treatment as the two independent variables, followed by the Student–Newman–Keuls test for multiple comparisons.

Results

The effectiveness of T3 treatment was checked by determining the ratio between heart ventricular weight and body weight (VW/BW), the day of the eye opening (DEO) and T4 serum concentration. Rats treated with T3 (4 μ g/100 g BW, s.c.) for 5, 15, and 30 day-periods from birth presented an increase in the VW/BW ratio, whereas the DEO and T4 levels were decreased, as expected. These data are presented in Table 1 and confirm the induction of hyperthyroidism. In addition, T3 serum concentrations were increased in rats treated with the hormone for 5- and 30-day period being similar to control values after 15 days of the treatment (Table 1).

The following is shown in Figs 1 and 2: (A) the typical Northern blot of one experiment from a total of three

experiments and (B) the quantitative representation of the hybridization of rat GH and 18S rRNA transcripts obtained by densitometric analysis, expressed as mean $\pm$ S.E.M. of GH mRNA/18S rRNA ratios from all the experiments performed. An increase in GH mRNA levels in rats treated with T3 for a 5-day period was observed; however, when T3 treatment was extended to 15 and 30 days, no significant difference in GH mRNA content was detected compared to the control group (Fig. 1). The GH mRNA expression was also estimated in 90-day-old adult rats, which were subjected to a transient hyperthyroidism (4 μ g of T3/100 g BW, s.c.) from P1 to P30 and allowed to grow with no further T3 treatment until P90 (Fig. 2). These rats presented a decrease in GH mRNA content, when compared to the control group. The same occurred with their nose–tail length and BMD, whereas the VW/BW ratio and T4 serum levels remained similar to the control group (Table 2).

Figure 3 illustrates the body composition (lean and fat mass) of rats treated with T3 from P0 to the 30th day of life, when they were killed, as well as at their 90th day of life, following 60 days after the treatment was ended, i.e., after the rats were subjected to transient hyperthyroidism during their first month of life. As observed, 30 days of T3 treatment resulted in a dramatic reduction of fat mass with no corresponding alteration in the lean mass. However, 60 days after the induction of the transient hyperthyroidism by T3 treatment from P1 to P30, the animals presented lower lean mass compared to their respective control, with no significant variation in the fat mass.

Figure 4 shows a time-course study of total T3 serum concentrations (ng/dl) of euthyroid and hyperthyroid animals (treated with 4 μ g of T3/100 g of BW from P1 to P15), after 30 min, 2, 6, and 15 h of a single injection of T3 (4 μ g/100 g of BW). In the hyperthyroid rats, the T3 injection induced an increase of ~ 9 times in T3 serum levels, from 30 min to 2 h, followed by a decrease to half of this value at 6 h of injection and return to the T3 values detected before T3 administration (0 min). The control animals presented a completely different time-course pattern of T3 serum levels, since an increase of

Table 1 Body weight (BW), ventricular weight (VW), ventricular weight to body weight ratio (VW/BW), day of eye opening (DEO) and serum T3 and T4 concentrations of T3-treated rats (4 μ g/100 g, s.c.) for 5, 15, and 30 days. Rats treated with vehicle (NaCl 0.9%) were used as control

Days of treatment after birth	5		15		30	
Groups	Control	Hyper	Control	Hyper	Control	Hyper
BW (g)	14.8 $\pm$ 0.7	13.6 $\pm$ 0.5	24.2 $\pm$ 1.9	22.7 $\pm$ 1.9	87.0 $\pm$ 2.9	71.6 $\pm$ 3.5†
VW (mg)	90.0 $\pm$ 4.0	161.0 $\pm$ 8.0*	107.0 $\pm$ 10.0	152.0 $\pm$ 23.0†	310.0 $\pm$ 20.0	480.0 $\pm$ 20.0*
VW/BW (mg/g)	6.1 $\pm$ 0.3	11.8 $\pm$ 0.3‡	4.4 $\pm$ 0.3	6.8 $\pm$ 0.2‡	3.6 $\pm$ 0.1	6.7 $\pm$ 0.3‡
DEO (postnatal days)	–	–	13	11	13	11
T3 (ng/ml)	0.24 $\pm$ 0.02	0.56 $\pm$ 0.04‡	0.38 $\pm$ 0.05	0.24 $\pm$ 0.05	0.35 $\pm$ 0.03	0.51 $\pm$ 0.04‡
T4 (ng/ml)	28.05 $\pm$ 0.83	13.77 $\pm$ 0.46‡	66.05 $\pm$ 3.27	15.23 $\pm$ 0.91‡	39.52 $\pm$ 0.52	14.20 $\pm$ 1.71‡

Results are expressed by means $\pm$ S.E.M. * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ compared with the control group ($n = 12$ per group).

Figure 1 Northern blot analysis of GH mRNA of rats treated with vehicle (1 and 2) or 4 µg of 3,5,3'-triiodo-L-thyronine (T3)/100 g body weight (BW) (3 and 4), s.c. for 5, 15, or 30 days from birth. (A) Northern blot analysis of GH mRNA levels of one experiment from a total of three experiments of (1 and 2) control and (3 and 4) T3-treated rats. (B) Quantitative representation of hybridization of rGH and 18S rRNA transcripts obtained by densitometric analysis of the exposed films, in arbitrary units (a.u.). Results are expressed as means ± S.E.M. of GH mRNA/18S rRNA ratios. **P* < 0.001 compared with the control group.

~4 times of T3 serum concentration was detected from 30 min throughout 15 h of the T3 administration.

Discussion

GH exerts a significant role on body growth and metabolism in postnatal life. The transcriptional induction of GH gene expression by TH is well documented in this period (Williams *et al.* 1991), but few studies have sought to understand the regulation of GH expression by TH in the NP, as well as the physiological repercussions, in the adult life, of a transient hyperthyroidism induction at early postnatal life.

We reported herein that T3 effects on GH mRNA expression depend on the period of life in which the hyperthyroidism is established and, in the NP, on the length of T3 treatment. We also showed that transient neonatal hyperthyroidism leads to a lower GH mRNA expression in adult life accompanied by physiological repercussions indicative of GH deficiency.

T3 treatment for a 5-day period from birth increased the GH mRNA levels, as occurs in adult rats subjected to the same treatment schedule (Volpato & Nunes 1994). On the other hand, when the T3 treatment was extended to 15 and 30 days, the GH mRNA content did not differ from the values of the control group, a result that diverges from the increased GH mRNA expression observed in adult animals that underwent the same treatment program. T3 serum concentration followed a similar trend of changes observed in GH expression except for rats treated with T3 for a 30-day period in which an increase was observed. The specific responses to T3 treatment for a 15-day period is thought to result from homeostatic adjustments elicited in order to reduce the impact of a high T3 supply to tissues, since the pattern of expression of many genes is not entirely defined yet, and could be influenced by TH, as pointed out earlier.

Therefore, it is not surprising to observe that T3 serum concentrations of T3-treated rats for 15 days were similar to those obtained in the control group, considering that the activity of type-3 deiodinase (D3), an enzyme that converts T3 to T2, is relatively high in neonates (Bates *et al.* 1999) and that D3 is upregulated by T3 (St Germain & Galton 1997, Tu *et al.* 1999). In fact, the time-course profile of the T3 serum

Figure 2 Northern blot analysis of GH mRNA of rats treated with vehicle (1 and 2) or 4 µg of T3/100 g BW (3 and 4), s.c., for 30 days from birth, and allowed to grow with no further T3 treatment until 90 days of life (HyperPNT). (A) Northern blot analysis of GH mRNA levels of one experiment from a total of three experiments, of (1 and 2) control 90 and (3 and 4) HyperPNT. (B) Quantitative representation of hybridization of rGH and 18S rRNA transcripts obtained by densitometric analysis of the exposed films, in arbitrary units (a.u.). Results are expressed as means ± S.E.M. of GH mRNA/18S rRNA ratios. **P* < 0.001 compared with the control group.

Table 2 Nose–anal length (L), BW, VW, VW/BW ratio, serum T4 and T3, and bone mineral density (BMD) of rats treated with vehicle (control) or T3, (4 µg/100 g, s.c.) for the first 30 days of life, and killed at 90th day of life (HyperPNT)

Days of postnatal life	90	
Groups	Control	HyperPNT
L (cm)	25.55 ± 0.34	23.14 ± 0.46
BW (g)	266.93 ± 7.79	211.08 ± 6.18*
VW (mg)	720 ± 20	550 ± 20*
VW/BW (mg/g)	2.70 ± 0.10	2.60 ± 0.09
T4 (ng/ml)	55.02 ± 3.29	67.71 ± 5.80
T3 (ng/ml)	0.31 ± 0.03	0.23 ± 0.05
BMD (g/cm ²)	0.131 ± 0.003	0.113 ± 0.001*

Results are expressed by means ± S.E.M. **P* < 0.001 compared with the control group (*n* = 12 per group).

concentration exhibited by these animals after the administration of a single dose of T3 confirmed the rapid T3 clearance rate. These findings also explain why T3-treated animals presented T3 serum concentration similar to control

Figure 3 Body composition of rats treated with vehicle or 4 µg of T3/100 g BW, s.c., for 30 days (Control 30 and Hyper 30 respectively), and of rats subjected to the same treatment schedule (30 days), but allowed to grow with no further T3 treatment until 90 days of life (control 90 and HyperPNT). The upper and lower panels present the data of lean and fat body mass respectively. Results are expressed as means ± S.E.M. **P* < 0.05, †*P* 0.01 compared with the control group.

Figure 4 Time-course study of total T3 serum concentrations (ng/dl) of euthyroid (squares) and hyperthyroid animals treated with 4 µg of T3/100 g BW from P1 to P15 (circles), after 30 min, 2, 6, and 15 h of a single injection of T3 (4 µg/100 g BW). Results are expressed as means ± S.E.M. **P* < 0.001 compared with the control group.

values at the time of the sacrifice, which occurred 15 h after the administration of the hormone, despite the clear signs of hyperthyroidism (Fig. 4). Indeed, the increased VW/BW ratio, anticipation of the DEO (11th day of life), and decreased T4 serum concentration (Table 1 and Fig. 3) are important physiological indications of the hyperthyroid state, considering the well-known effects of TH inducing cardiac hypertrophy, maturation of the nervous system (Fisher & Lakshmanan 1990, Heron & Rakusan 1996, Yilmazer-Hanke *et al.* 2004), and feedback inhibition of HPT axis (Wolf 2002). In fact, one of the most precocious phenotypic signs of the maturation of nervous system is the time at which the pups' eyes open, which is achieved by the 13th day of life.

The unaltered GH mRNA expression in hyperthyroidism during this specific period of life (15 and 30 days of T3 treatment from P1) was also an intriguing finding. However, it is well documented that, in hyperthyroid states, type-2 deiodinase (D2) activity is decreased in pituitary, central nervous system, and brown adipose tissue (Bates *et al.* 1999, Schneider *et al.* 2001, Volpato & Nunes 2001). Moreover, in the early postnatal life, changes in the expression of TH receptor isoforms are taking place, under TH control (Kaplan & Yaskoski 1981, Silva & Larsen 1982, Bradley *et al.* 1992, Santini *et al.* 1999), which interferes with the pattern of GH gene response to T3 observed in adult rats, in which the adult isoforms predominate. Furthermore, in the NP, the α-2 isoform of TR (TRα 2) is highly expressed, which prevents, in a certain degree, the binding of other TR isoforms (TRα 1, β1, and β2) to the DNA-binding domain (DBD) of the T3 target genes (Bradley *et al.* 1992), and taking into account that T3-binding domain is missing in the TRα 2, this might prevent, in some way, the impact of TH on the expression of many genes in this period of development, in which the patterns of gene expression that will persist in adult life is being settled (Csaba & Nagy 1985).

We assume that the somatotrophs, in particular, could be targeted by this kind of regulation during the period in which the animals were being exposed to high T3 doses, which might explain why the expression of GH gene remained unchanged when the neonatal rats were treated with T3 for longer periods (15 and 30 days). Considering the critical role of GH for growth and development, these adjustments might guarantee an adequate maturation and differentiation of several tissues that occur predominantly during the first month of life (Van der Linden *et al.* 1992, Cernohorsky *et al.* 1998).

The differential response of GH mRNA expression to 5-, 15-, and 30-day period of T3 treatment could be a result of any of the events described above and the causes that elicit these alterations deserve to be further addressed in models, in which somatotrophs could be specifically evaluated. These findings show the complexity of GH gene control by TH and its dependence on the animal age and treatment length. These adjustments might be important later, in adulthood, considering the influence of GH to determine the body lean and fat mass. On the other hand, the induction of hyperthyroidism from P1 to P30 reduced the GH gene expression in adulthood, which was accompanied by physiological repercussions compatible with GH deficiency (Table 2 and Fig. 3).

These findings are consistent with those of Walker & Courtin (1985). These authors demonstrated a decrease of GH content in pituitary of adult rats after induction of HyperPNT. However, they observed a significant decrease in T3, T4, and TSH serum concentrations and suggested that the HyperPNT causes a permanent dysfunction of the HPT axis in adult rats. Bakke *et al.* (1974) also proposed that hyperthyroidism triggered during the NP leads to hypothyroidism in adult rats.

In our study, we cannot ascribe that the decrease of GH mRNA content in adult rats subjected to HyperPNT was due to a permanent dysfunction of the HPT axis, since T3 and T4 levels remained similar to the values of the control group. Furthermore, some genes that are upregulated by TH, like the myoglobin gene (Dos Santos *et al.* 2001, Giannocco *et al.* 2004), actually present an increased expression in this experimental model (data not shown). Thus, it is possible that the discrepancies between the results presented could be explained by differences in the treatment scheme, i.e., length of treatment and T4 administration.

Thereby, lower GH gene expression in the 90-day-old rats could be a consequence of different patterns of expression of TH receptors or of deiodinases in a tissue-specific response that has been established by the exposure of transient hyperthyroidism soon after birth, as already discussed. We assume that the exposure of animals to high doses of T3 in the first weeks of life has triggered adjustments in the set point of several genes, like GH gene, resulting in a long-term variation in its responsiveness to T3. These animals possibly developed a pattern of GH gene expression programmed to respond to high T3 levels, as they were in the physiological range; as a consequence, when the T3 treatment was disrupted and T3

serum concentration returned to its physiological level, somatotrophs responded as if the animals were in a hypothyroid state. It should be stressed that this kind of response seems to be specific for somatotrophs, otherwise we would detect variations in T3 and T4 serum concentrations, which was not the case.

Physiological repercussions indicative of GH deficiency, as diminished BW and length, were also detected in adult rats exposed to hyperthyroidism during the NP, which led us to presume that reduced GH mRNA content effectively led to a lower GH secretion. The decrease in lean body mass (LBM) and BMD is also an indication that GH secretion is impaired, considering the essential role of GH to determine these parameters in vertebrates (Leal Cerro 2004).

The LBM is represented mainly by the skeletal muscles, which are important target of insulin action. In addition, the skeletal muscles are the tissues that continuously exert mechanical forces on bones. Thus, if on one hand, the decreased LBM could influence the glucose homeostasis, then on the other hand, it could attenuate the mechanical forces to the bones, preventing fractures that could take place, since BMD was decreased in animals subjected to transient hyperthyroidism.

Taking together, these results indicate that TH has established a new program for the expression of GH gene and that this molecular modification led to physiological alterations, in the adult life, which could be an adaptation to the new steady-state condition reached.

In conclusion, the present study demonstrates that GH gene expression is regulated by T3 in an age-dependent manner and provides a molecular basis to explain the physiological repercussions observed in growth and metabolism in adult rats that were transiently exposed to hyperthyroidism during precocious postnatal life.

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