# Growth and pituitary content of growth hormone and prolactin in hypo- and hyperthyroid rats

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Se estudiaron el peso y longitud corporal y el contenido hipofisario de hormona del crecimiento (GH) y prolactina (PRL) en *a)* ratas normales (N) alimentadas con purina; *b)* ratas tiroidectomizadas a las que se inyectaron diariamente 0 (T + 0), 0,1 (T + 0,1), 1,8 (T + 1,8) o 25 (T + 25) μg de L-tiroxina/100 g de peso y sometidas a una dieta pobre en yodo (tipo Remington), y *c)* en ratas controles (C) intactas alimentadas con la misma dieta, pero con un suplemento de $KIO_3$ suficiente para proporcionar una concentración plasmática normal de PBI.

En los animales N la longitud corporal y el peso hipofisario correlacionaron con el peso corporal por regresiones de segundo orden, mientras que el contenido hipofisario de GH y PRL mostró un cambio de tipo lineal en relación al peso corporal. La tasa de crecimiento en los animales C fue más reducida que en los animales N, apareciendo más disminuida todavía en las ratas T + 0. Los animales T + 0,1 mostraron una ligera recuperación de la tasa de crecimiento, mientras que en los T + 1,8 fue la misma que en las ratas C. Los animales T + 25 mostraron un peso inferior al de los animales C, a pesar de no haber observado diferencias en el tamaño corporal. En todas las series de animales, con edades superponibles y bajo las diferentes situaciones tiroideas, el contenido hipofisario de GH y PRL no mostró correlación de tipo lineal con la longitud corporal.

Los resultados obtenidos indican que la dieta tipo Remington debe utilizarse en los estudios metabólicos con especial precaución y además ponen de relieve la diferente sensibilidad de las hormonas tiroideas sobre el contenido hipofisario de GH y PRL. Muestran también que para obtener una tasa normal de crecimiento es necesaria la presencia de un balance normal de otras funciones endocrinas.

**Body weight and length and adenohypophysal content of GH and prolactin was studied in normal rats (N) fed purine chow and in thyroidectomized rats daily injected with either 0 (T + 0), 0.1 (T + 0.1), 1.8 (T + 1.8) or 25 (T + 25) μg of L-thyroxine/100 g body weight fed a low iodine content diet of the Remington type and intact controls (C) under the same diet supplement with enough $KIO_3$ to give a normal plasma protein bound iodine concentration. Body length and pituitary weight was correlated in the N animals with body weight by second order regressions while GH and prolactin content in the adenohypophysis of these rats changed lineally with their body length. The growth rate of C was reduced as compared with N and it was further reduced in T + 0. T + 0.1 show a slight recuperation of growth rate while in T + 1.8 the growth rate was the same that in C. T + 25 animals show reduced body weight increase versus C despite of unchanged body length. GH and prolactin pituitary content in all the age matched animals under different thyroidal status were not lineally correlated with their body lengths. The results oblige to call special caution in the used of the Remington type diet for metabolic studies; beside to emphasize the different sensitivities of the thyroid hormones on the GH and prolactin pituitary content they show the need of a normal balance of other endocrine sides to obtain a normal growth rate.**

There exists a direct relationship between age and the functional state of the pituitary, as shown by the changes in the incorporation of labelled amino acids into pituitary proteins of rats[1,2]. These changes are specially marked in the synthesis of both growth hormone (GH) and prolactin (PRL)[3-7]. Variations in thyroid hormone economy of the animals result in intense changes in these parameters. In hypothyroidism there is a reduced pituitary content of both

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GH and PRL[8-15], while in hyperthyroidism there is a normal or reduced GH content[8], whereas PRL content is increased[16,17].

The age of the animals is often quantified as function of the body weight, which does not necessarily follow a parallel change with the body size. This relation is definitely altered in hypo- and hyperthyroidism[18].

In the present study we have tried to establish the relationships between body weight and size in both normal and thyroidectomized rats treated with different doses of exogenous L-thyroxine. As a diet of the Remington type with a low iodine content[19-21] is normally used in studies on thyroid function[22-24], comparisons were carried out between intact animals fed with this diet and others fed regular purina chow. The study was extended to determine the GH and PRL content of the pituitaries from the different groups by using a polyacrilamide gel electrophoresis-densitometric method.

## MATERIALS AND METHODS

Male Wistar rats were surgically thyroidectomized [25](T) at weaning (45-50 g, 30 days of age). The animals were then divided into different groups, being injected daily with either saline (T + 0), 0.1 μg of L-thyroxine/100 g body weight (T + 0,1), 1.8 μg of L-thyroxine/100 g body weight (T + 1.8), or 25 μg of L-thyroxine/100 g body weight (T + 25), and fed a low iodine diet of the Remington type[19,20] for 39-41 days. This diet consisted of corn flour, 6 kg; wheat gluten, 2.5 kg; dried brewer's yeast, 1 kg; NaCl, 0.15 kg and $CaCO_3$, 0,15 kg, having an intrinsic composition of 2.9 % fat, 45.7 % carbohydrates and 30.2 % proteins, corresponding to 3.382 cal./kg. These animals were compared with intact age matched controls (C), injected daily with saline and fed with the same diet, but supplemented with 1.7 μg of $KIO_3$/g. Normal rats (N) of different ages (body weights between 30 to 500 g) did not receive any treatment and were fed with a regular purina chow from Panlab (Spain). The composition of this diet was: 2.5 % fat, 45.1 % carbohydrates and 21.1 % proteins, corresponding to 2.940 cal./kg. The composition of the diets was determined by a specialized laboratory (Laboratorio de Investigación Cerealista, Barcelona, Spain).

All the animals were housed in collective cages in a light cycle and temperature controlled animal room (12 hr on- 12 hr off, and 23 ± 1 °C). They were weighed periodically and at the time of sacrifice their body size was measured from nose to rump. The rats were killed by decapitation and the blood was collected from the neck into heparinized beakers. The protein bound iodine concentration was determined in plasma aliquots[26]. The pituitaries were dissected immediately and weighed. Each adenohypophysis was homogenized in 0.7 ml of 40 % (w/v) sucrose and kept at —27 °C until processing.

Polyacrilamide disc electrophoresis was carried out in 150 μl of anterior pituitary homogenates as described by Davis[27], with minor modifications. Only 7 1/2 % small pore separation gels were used, prepared in tubes of 6 mm i.d. The electrophoresis was run for 45-60 min and later stained with amino black. Fixation of the proteins and destaining of the gels, was carried out with methanol/water/glacial acetic acid (5/5/1, by vol.) for 14 hr and further electrophoresis for 3-4 hr. The protein band associated with GH was identified on the basis of the findings of Lewis et al[15] and Jones et al[6] for the rat. The protein bands associated with albumin and PRL were identified by running parallel tubes with either bovine albumin (fraction V, from Sigma, St. Louis, Mo.) or rat PRL (R.P.1), kindly provided by NIAMDD, as standards. The ratio of the distance of the bands from the «origin» to that of bromophenol blue tracer moving with the front was about 0.26 for GH, 0.44 for albumin and 0.58 for PRL. The intensity of the bands in each gel was determined by densitometry at 600 nm in a Unicam SP 1700 spectrophotometer provided with recorder, according to the area of the peaks. The method was validated by running several albumin (2, 4, 6, 8 and 12 μg/gel) and PRL (15, 30, 40, 50, 70 and 122,5 μg/gel) standards. When the corresponding area values were plotted against the concentrations, linear correlation coefficients were obtained: 0.999 ($p<0.001$) for albumin and 0.992 ($p<0.001$) for PRL.

Statistical comparisons among the groups were carried out by Student's «t» test. Regressions and correlations were determined by standarized methods using a Compucorp 445 computer (Ataio).

## RESULTS

In normal male rats of different ages both body lenghty and pituitary weight were correlated with body weight by highly significant second order regressions (Figs. 1a and 1b): as the animals become older the increase in body weight is followed by a smaller proportional increase in body lenght and pituitary weight. Both the content of GH and of PRL (expressed relative to the albumin concentration of the adenohypophysis), were linearly correlated with body lenght (Figs. 2a and 2b) and with body weight (data not shown).

The rate of the body weight change with increasing age in control rats (C) fed a Remington type diet (low iodine content) supplemented with 1.7 μg of $KIO_3$/g from the end of weaning, was much lower than that of age matched normal rats (N) fed the regular purina chow (Fig. 3). As also shown in Fig. 3, thyroidectomized rats (T + 0) fed the low iodine diet show a slower body weight increase than C rats. This is partially restored by the daily treatment with 0.1 μg of L-thyroxine (T + 0.1), and totally restored to the values of C rats with 1.8 μg of the hormone (T + 1.8).

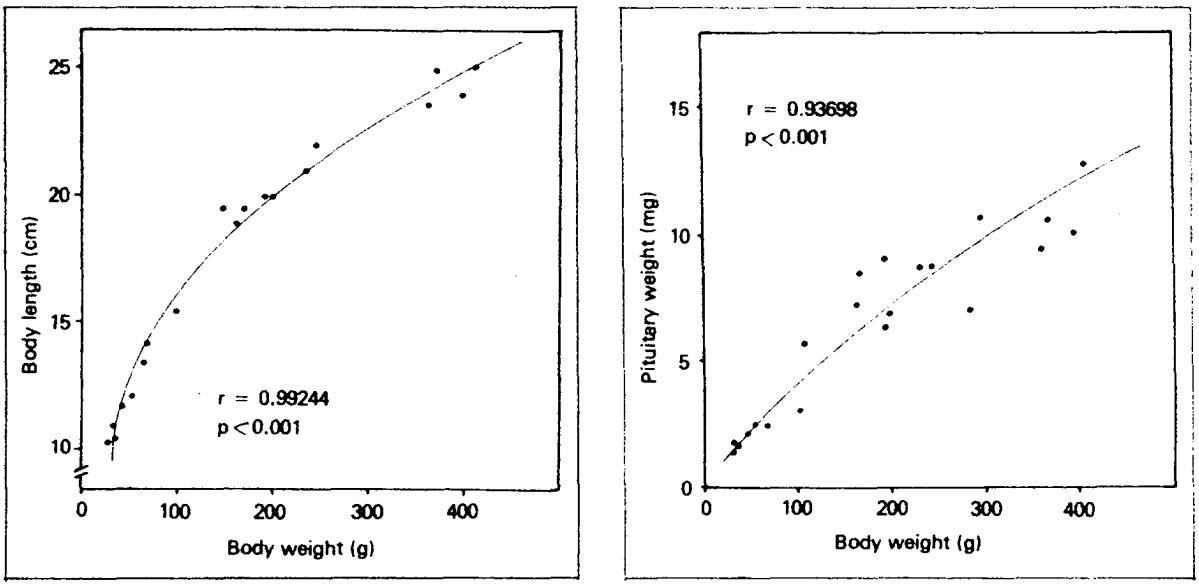


*Fig. 1. Relationships between body weight and body length (Fig. 1a) and body weight and pituitary weight (Fig. 1b) in normal rats of different ages, fed «ad libitum» a laboratory purina chow diet.*

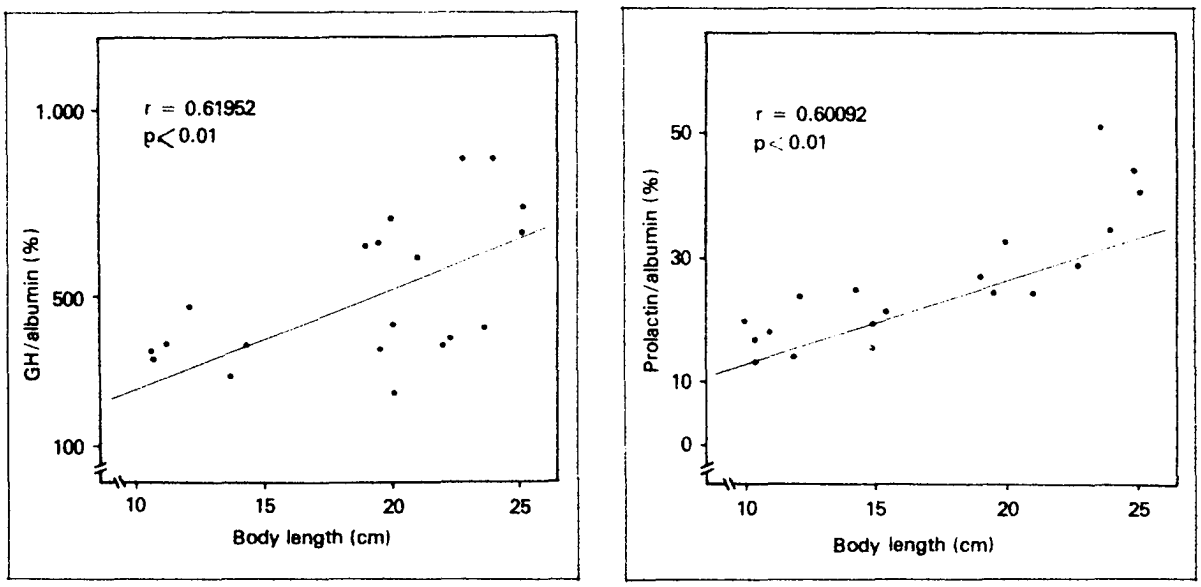


*Fig. 2. Relationships between body length and pituitary GH content (Fig. 2a) and body length and pituitary prolactin content (Fig. 2b) in normal rats of different ages, fed «ad libitum» a laboratory purina chow diet.*

The rate of body weight increase is similar in T + 25 and C rats during the first 30 days of the experiment, but from this time on, the body weight of the T + 25 animals becomes significantly smaller than that of rats from the C group.

At the time of sacrifice (70-75 days of age) the body lenght of the C group was significantly smaller than that of N animals of the same age (Table 1). T + 0 animals show the smallest body lenght. As compared with the C animals, this difference in the size of the thyroidectomized animals decreases in those treated with 0.1 μg $T_4$/100 g and completely disappears in both the T + 1,8 and the T + 25 groups (Table 1). Thus, the differences in body weight were not paralleled completely by those in body lenght, and the body weight to body length ratio was significantly lower in C than in N rats and in T + 0.1 and T + 25 groups than in C animals (Table 1). The body weight-body length ratio was the same for C

**TABLE I. Changes in body weight and size, pituitary GH and prolactin content and plasma protein bound iodine (P.B.I.) concentration in age matched thyroidectomized rats daily injected with different doses of L-thyroxine and intact controls fed under a Remington type diet and normal animals fed a regular laboratory purina chow. The results are given means ± S.E.M. of 6-8 animals group. p. values correspond to the differences of each group vs. the controls (NS = not significant, i.e. p > 0.05).**

| | P.B.I. μg/100 ml | Body wt. g | Body size cm | Ratio w/s g/cm | GH/Alb. % | Prol./Alb. % |
|---|---|---|---|---|---|---|
| Intac controls | 4.93±0.20 | 192.94±4.1 | 19.67±0.27 | 9.81±0.18 | 457.32±40.66 | 17.28±1.73 |
| Normals | 5.39±0.19 | 257.17±4.1 | 20.78±0.33 | 12.40±0.26 | 401.51±59.57 | 17.01±2.42 |
| p | N.S. | <0.001 | <0.05 | <0.001 | N.S. | N.S. |
| Thyroidectomized (0) | 0.34±0.07 | 103.16±6.7 | 14.94±0.40 | 6.81±0.30 | 139.53±13.72 | 4.95±0.49 |
| p | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Thyroidectomized (0.1) | 0.82±0.19 | 163.50±7.8 | 18.11±0.29 | 8.99±0.31 | 109.01±9.34 | 8.53±0.97 |
| p | <0.001 | <0.01 | <0.01 | <0.05 | <0.001 | <0.01 |
| Thyroidectomized (1.8) | 4.95±0.26 | 201.64±7.8 | 19.52±0.34 | 10.29±0.24 | 480.41±41.56 | 17.96±2.19 |
| p | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. |
| Thyroidectomized (25) | 10.15±0.66 | 171.77±4.7 | 19.22±0.17 | 8.94±0.26 | 567.08±57.08 | 27.01±4.7 |
| p | <0.001 | <0.01 | N.S. | <0.01 | N.S. | <0.05 |

( ) = μg of L-thyroxine / 100 g body weight / day

and T + 1.8 groups. The protein bound iodine concentration in plasma does not differ between the N, C and T + 1.8 groups, while, it is significantly reduced in T + 0 and T + 0.1 rats and augmented in T + 25 animals.

The GH content in the pituitary was the same in the N,C,T + 1.8 and T + 25 groups while significantly reduced in T + 0 and in T + 0.1 animals (Table 1). The PRL content in the pituitary dit not differ among N, C and T + 1.8 groups (Table 1 and Fig. 4). It was significantly reduced in the T + 0 and T + 0.1 and augmented in T + 25 versus the C group.

The variation of body size of the animals on the low iodine diet, produced by their different thyroidal status, prompted to study wether the linear correlations found between either pituitary GH or PRL content versus body lenght in normal animals of different ages, was maintained in the age matched thyroidectomized rats and their intact controls. The results are presented in Figs 4a and 4b: as may be seen, neither the GH nor the PRL content shows a linear correlation versus the body lenght of these rats.

The values are actually grouped in two clear populations, one corresponding to the hypothyroid animals (T + 0 and T + 0.1) with the lowest levels of both hormones despite considerable variation in body lenght, and the other to the euthyroid (T + 1.8 and C) and hyperthyroid (T + 25) animals, with a wide variation in hormone concentration and a fairly constant body size.

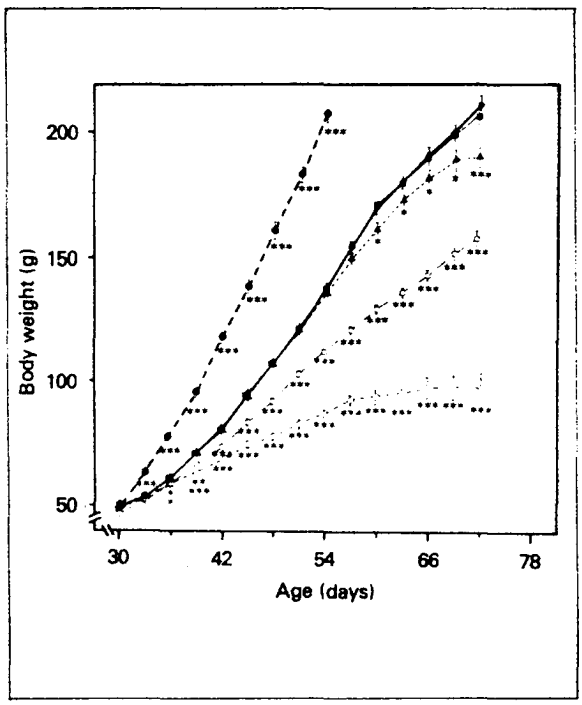


*Fig. 3. Body weight increase with age in normal animals fed a laboratory purina chow diet (•————•) and thyroidectomized rats treated with either 0 (○ — — — ○), 0.1 (□.—.—.—□), 1.8 (■————■) or 25 (▲= = = = = = = ▲) μg of L-thyroxine/100 gm body weight/day, fed a low iodine content diet of the Remington type and their intact controls (●———— ————●) fed under the same diet supplemented with 1.7 μg $KIO_3$/gm. At the 0 day of treatment the animals from all the groups were 30 days old. Asteriks correspond to the statistical differences between each group and the values in the intact controls: * = p<0.05, ** = <0.01, *** = p<0.001. Values are expressed as means ± S.E.M. of 70 - 100 rats/groups.*

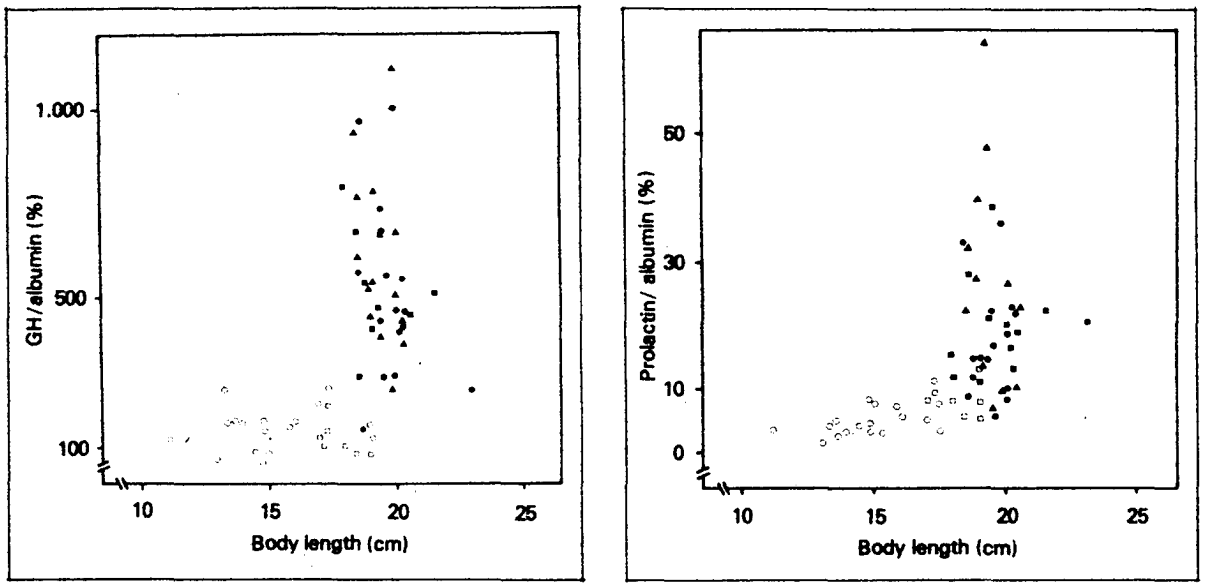


*Fig. 4. Relantionships between pituitary GH content (Fig. 4a) and pituitary prolactin content (Fig. 4b) of age matched thyroidectomized rats treated with either 0 (○), 0.1 (□), 1.8 (■) or 25 (▲) μg of L-thyroxine and their intact controls (•). The values correspond to the same animals of Table I.*

## DISCUSSION

The present study shows that during the prepuberal period the increase in body size of the male rat appears to be proportionally greater than the change in body weight, whereas the opposite occurs in older animals. These relationships are similar to those observed by other authors in other species[28]. There is also a similar relationship between pituitary and body weights. The pituitary content of either GH or PRL is linearly correlated with the body size of the animals, as shown here and observed by other authors[6,4,29,30,31,32]. These relationships are markedly affected by the diet and the thyroidal status of the animals. We have shown here that intact controls on a Remington type diet supplemented with enough $KIO_3$ to obtain a normal plasma protein bound iodine concentration, grow less than on the regular purina chow diet. At each age, the difference between both groups is smaller for the body size than for the body weight, the pituitary content of both GH and PRL being similar. The intrinsic composition of both diets is similar and this suggest that the differences in body weight are not due to changes in the caloric intake of the animals. It is more likely that it is due to a deficiency in some unknown factor in the Remington type diet. These results cautions against the use of this diet for metabolic studies, though it is widely used in experiments on thyroid physiology. We have indeed previously observed that the effects of 48 hr of fasting on various metabolic parameters in euthyroid controls on this diet[23,33], differ from those found in rats on normal purina chow[34,35].

Thyroidectomy produced a decrease in the body weight of the animals which is greater than the decrease in body size, as shown by the fact that the body weight/ lenght ratios are lowest for the T + 0 group. In these animals the metabolic balance is, however, maintained[23,36]. This effect of thyroidectomy might be explained by an increased proportional retention of fat in hypothyroidism[37], which would result in a reduction in the total body density. These alteration are ameliorated by the daily treatment with 0.1 μg of L-thyroxine to the thyroidectomized rats. The pituitary content of GH in the T + 0.1 group is, however, as low as in the T + 0 one; the partial recuperation of body size in these animals would indicate that either the circulating amounts of the hormone has been increased or its effectiveness has been enhanced by the small increase in the amount of thyroid hormones available. The daily injection of 1.8 μg of L-thyroxine normalized all the parameters studied. This shows that the alterations observed in these animals are not produced by the thyroidectomy itself. The daily injection of 25 μg of L-thyroxine to the thyroidectomized

animals increased the plasma protein bound iodine concentration to double than that of the intact controls. These animals mantain a normal GH pituitary content and normal growth. Their body weights are, however, reduced, this is likely to be the result of the net catabolic state of hyperthyroidism[38].

Although pituitary PRL content changes in a very similar way to that of GH, as also shown by other autors[3,12,32,39], their changes are not always parallel, as found here for the T+0.1 and the T+25 groups. In the first group, contrary to the GH, the PRL content of the pituitary was almost double to that found in the T+0 group. This difference must be either the result of a higher sensitivity of the thyroid hormones enhancing the synthesis of PRL than that of GH, or a comparative retention of the new synthetized PRL as compared with the rapid release of GH. The increase of pituitary PRL content found in the hyperthyroid animals (T+25) in the presence of unchanged GH concentration would support the first possibility although greater experimental support is needed to reach a definite conclusion.

The lack of linear relationship between the pituitary content of either GH and PRL versus body size in the age matched animals under different thyroidal status, may be the consecuence of the alterations of other endocrine glands in hypo-and hyperthyroidism besides different levels of thyroid hormones and the pituitary itself, including the pancreas[40,41,42,43], the testicles[44,45], etc. Very probably it is needed the presence of equilibrated amounts and sensitivities of the different hormones that more or less directly affect the growth of the animals, to obtain the direct relationship between the GH and PRL availability and the body lenght observed in the normal animals.

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