The different requirement of \( L-T_4 \) therapy in congenital athyreosis compared with adult-acquired hypothyroidism suggests a persisting thyroid hormone resistance at the hypothalamic–pituitary level

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Abstract

Background: Levothyroxine (\( L-T_4 \)) is commonly employed to correct hormone deficiency in children with congenital hypothyroidism (CH) and in adult patients with iatrogenic hypothyroidism.

Objective: To compare the daily weight-based dosage of the replacement therapy with \( L-T_4 \) in athyreotic adult patients affected by CH and adult patients with thyroid nodular or cancer diseases treated by total thyroidectomy.

Design and methods: A total of 36 adult patients (27 females and nine males) aged 18–29 years were studied; 13 patients (age: 21.5 ± 2.1, group CH) had athyreotic CH treated with \( L-T_4 \) since the first days of life. The remaining 23 patients (age: 24 ± 2.7, group AH) had hypothyroidism after total thyroidectomy (14 patients previously affected by nodular disease and nine by thyroid carcinoma with clinical and biochemical remission). Patient weight, serum free thyroid hormones, TSH, thyroglobulin (Tg), anti-Tg, and anti-thyroperoxidase antibodies were measured. Required \( L-T_4 \) dosage was evaluated. At the time of the observations, all patients presented free thyroid hormones within the normal range and TSH between 0.8 and 2 μIU/ml.

Results: Patients had undetectable Tg and anti-thyroid antibodies. The daily weight-based dosage of the replacement therapy with \( L-T_4 \) to reach euthyroidism in patients of group CH was significantly higher than that in those of group AH (2.16 ± 0.36 vs 1.73 ± 0.24 μg/kg, \( P < 0.005 \)). Patients of group CH treated with \( L-T_4 \) had significantly higher serum TSH levels than patients of group AH (\( P = 0.05 \)) as well as higher \( FT_4 \) concentrations.

Conclusions: To correct hypothyroidism, patients of group CH required a daily \( L-T_4 \) dose/kg higher than group AH patients, despite higher levels of TSH. The different requirement of replacement therapy between adult patients with congenital and those with surgical athyroidism could be explained by a lack of thyroid hormones since fetal life in CH, which could determine a different set point of the hypothalamus–pituitary–thyroid axis.

Introduction

Thyroid hormone replacement therapy with levothyroxine (\( L-T_4 \)) is the treatment of choice in primary hypothyroidism (1). The goal of this therapy is to achieve patient’s well-being and restore serum TSH to levels within the reference range (1, 2). As \( L-T_4 \) has a narrow therapeutic index, the margin between overdosing and underdosing...
can be small (3). The most common causes of hypothyroidism in the adult age are thyroid autoimmunity, total thyroidectomy for nodular disease or for thyroid cancer, and radioiodine treatment for the cure of hyperthyroidism (4). Primary hypothyroidism present at birth (congenital hypothyroidism (CH)) can be due to a defect in the development of thyroid gland during fetal life. In some of these subjects, thyroid gland is completely absent (athyreosis), whereas in others can exist an ectopic gland or a properly sited but non-functioning gland (5).

The dosage of the replacement therapy with L-T4 reflects the requirement of thyroid hormones in hypothyroid patients, in particular in those with athyreosis that requires a complete substitution of the thyroid function (3, 6, 7, 8). However, in all cases, individual adjustments are required to achieve the appropriate daily dose (1, 2, 3).

When starting L-T4 therapy, one approach is to recommend a dosage based on the patient’s pretreatment serum TSH concentration (9). A second approach, usually used in athyreotic subjects, is to calculate the patient’s starting dosage based on the body weight (3, 10, 11).

Dosage requirement is conditioned by the quantity of residual thyroid function, as is the case for hypothyroid patients affected by Hashimoto’s thyroiditis or by Graves’ disease treated with 131-I, who require a smaller L-T4 dosage to normalize TSH compared with patients who are totally athyreotic (3, 9, 10, 11). Other factors are represented by patient age, body weight, lean body mass (12, 13, 14, 15), patient adherence to L-T4 therapy, and timing of L-T4 administration (16), conditions that affect L-T4 absorption and metabolism (lactose intolerance, celiac sprue, autoimmune gastritis, impaired gastric acid secretion, some medications, food, and beverages) (17, 18).

Several reports documented elevated serum TSH in many patients with CH treated with L-T4, despite clinical euthyroidism and normal serum concentrations of FT4 and FT3 (19, 20, 21). The inappropriately elevated serum TSH levels are more evident during the early months of treatment and, in some instances, the serum TSH levels have remained relatively elevated in treated CH infants, 5 years of age and older, and the elevated levels do not seem accountable to serum FT4 (22). This relative hyperthyrotropinemia in many treated CH children has been attributed to suboptimal therapy or abnormal setting of the T4 negative feedback control of pituitary TSH secretion (22).

The aim of this study was to analyze L-T4 requirement in athyreotic adult patients identified at the neonatal screening for CH compared with adult patients treated by total thyroidectomy and starting L-T4 therapy later in life, after adolescence.

**Subjects and methods**

**Subjects**

A total of 36 patients aged 18–29 years (mean ± s.d. age: 23.0 ± 2.9 years, 27 females and nine males) were recruited retrospectively from patients referred to our Division of Endocrinology of Pisa for athyreotic CH, total thyroidectomy for nodular thyroid disease, and thyroid cancer. For both groups, only patients with undetectable serum thyroglobulin (Tg), anti-Tg and anti-thyroperoxidase antibodies, were selected. The BMI of all patients ranged from 17.8 to 29.2 kg/m² (23.5 ± 3.1 kg/m²). Neck ultrasound and thyroid scintigraphy were performed in all patients showing no thyroid tissue in the anterior cervical area. Patients had a medical history and physical examination before and during the study. Each patient’s medication was documented and patients taking medications known to interfere with L-T4 absorption or alter L-T4-binding proteins were excluded from this study. No atrophic gastritis was clinically evidenced. All of them were treated with the same commercial preparation of L-T4.

Of the total number of patients, 13 were affected by CH due to thyroid agenesis (group CH, age: 21.5 ± 2.1 years, range: 18.3–24.6; nine females and four males, BMI between 17.8 and 29.2 kg/m²). They were detected at neonatal screening for CH in the first days of life and treated with appropriate L-T4 replacement therapy. Afterwards, they began the programmed follow-up therapy in order to keep serum TSH within the normal range and to maintain satisfactory clinical conditions. The agenesis was confirmed by neck scintiscan and ultrasound in infant age. They had physical examination and serum FT4, FT3, and TSH concentrations measured in the first 6 months of life on a monthly basis, then every 3 months until the first year of life and every 6 months until 17–18 years of life. In the adult age, the examination was performed once or twice a year. Growth and development were normal in all cases.

Serum FT4, FT3, and TSH concentrations were measured in 23 adult patients (group AH, age: 24 ± 2.7 years, 16 females and seven males, BMI between 19.4 and 28.7 kg/m²) treated with total thyroidectomy. Of these patients, 14 (subgroup NG, age: 23.6 ± 2.4 years, nine females and five males) were affected by thyroid nodular goiter while nine (subgroup TC age: 24.5 ± 3.0 years, seven females and two males) were affected by thyroid carcinoma. According to the current criteria for the management of thyroid cancer, they were apparently free of disease (23). In particular, basal and recombinant human TSH-stimulated serum Tg and anti-Tg antibodies were
undetectable, indicating no functioning thyroid tissue at the time of the study. Adult patients had a minimum follow-up of 3 years.

**Laboratory evaluation of thyroid function**

Data used for analyses were obtained from all the patients after achievement of a stable biochemical condition of euthyroidism; even if the normal range of serum TSH is between 0.4 and 4 μIU/ml, L-T4 dosage was increased or decreased in order to have serum TSH of 0.8–2 μIU/ml. For all considered parameters, results represent the mean of the values obtained from at least two consecutive annual measurements, once a biochemical euthyroid state was reached with a serum TSH of 0.8–2 μIU/ml.

Morning blood samples were obtained at 24 h after the last ingestion of L-T4 between 0800 and 1000 h in all cases. Serum TSH level was measured by a chemiluminescent method (Immulite 2000, DPC, Los Angeles, CA, USA). Serum FT4 and FT3 levels were measured by an immunometric method (Vitro System, Ortho-Clinical Diagnostic, Rochester, NY, USA). Normal values in our laboratory for adult population are as follows: TSH, 0.4–3.4 mIU/ml; FT4, 7–17 pg/ml (9.0–21.9 pmol/l); and FT3, 2.7–5.7 pg/ml (4.15–8.75 pmol/l). Anti-Tg and anti-thyroperoxidase antibodies were measured using a two-step immunoenzymatic assay (AIA-Pack TgAb and TPOAb; Tosoh, Tokyo, Japan). Serum Tg level was measured using an enzymatic assay (AIA-Pack TgAb and TPOAb; Tosoh, Tokyo, Japan). Serum FT4 and FT3 levels were measured by an immunometric method (Vitro System, Ortho-Clinical Diagnostic, Rochester, NY, USA). Normal values in our laboratory for adult population are as follows: TSH, 0.4–3.4 mIU/ml; FT4, 7–17 pg/ml (9.0–21.9 pmol/l); and FT3, 2.7–5.7 pg/ml (4.15–8.75 pmol/l). Anti-Tg and anti-thyroperoxidase antibodies were measured using a two-step immunoenzymatic assay (AIA-Pack TgAb and TPOAb; Tosoh, Tokyo, Japan). Serum Tg level was measured using an enzymatic assay (AIA-Pack TgAb and TPOAb; Tosoh, Tokyo, Japan).

**Statistical analysis**

The Kolmogorov–Smirnov test was used to assess the normality of data. Statistical tests used to compare groups of subjects included Student’s t-test and ANOVA for difference in mean values of Gaussian distributed variables, while the Mann–Whitney U and Friedman tests were employed for skewed variables (i.e. TSH). Post-hoc analyses were conducted using the Bonferroni correction of significance. A P value > 0.05 was considered statistically significant. Data are expressed as mean ± S.D. or median with interquartile range (IQR).

**Results**

Patient weight, serum FT4, FT3, Tg, anti-Tg and anti-thyroperoxidase antibodies, TSH concentrations, and L-T4 dosage required were evaluated. At the time of the observations, all patients presented free thyroid hormones within the normal range and values of TSH between 0.8 and 2.0 μIU/ml.

The characteristics of the study groups are given in Table 1.

On average, the daily weight-based dosage of the replacement therapy with L-T4 in patients of group CH was significantly higher compared with patients of group AH (2.16 ± 0.36 vs 1.73 ± 0.24 μg/kg per day, P < 0.005, Table 1, Fig. 1A). Although CH patients required a higher amount of L-T4, the serum TSH level was significantly higher in these patients when compared with those of group AH (median 1.80 ± 0.8 vs 1.03 ± 0.67 μIU/ml, P = 0.05, Table 1, Fig. 1B).

Serum FT4 levels were higher in group CH patients when compared with group AH patients (13.2 ± 1.8 vs 11.8 ± 1.9 pg/ml, P = 0.04) (Fig. 1C), whereas there were no differences in the mean levels of serum FT3 between group CH and AH patients (3.7 ± 0.4 vs 3.7 ± 0.5 pg/ml, P = 0.84) (Table 1). When calculating the FT3/FT4 ratio, group CH patients showed lower values than group AH patients (0.285 ± 0.034 vs 0.319 ± 0.049 pg/ml, P = 0.03) (Table 1).

There was no statistical difference between the doses needed by the patients of subgroup NG (1.74 ± 0.2 μg/kg per day) and the patients of subgroup TC (1.70 ± 0.1 μg/kg per day) (Table 1).

When serum FT4 values were plotted against serum TSH, a clear shift on the right and upward direction was observed in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2). The slope of the regression lines of both groups was less steep in CH patients (Fig. 2).

**Discussion**

The aim of L-T4 supplementation in hypothyroidism is to establish a clinical condition without restraints (1, 2, 3). Especially, in young children, L-T4 supplementation should be performed with more accuracy (24). Monitoring of treatment solely based on a patient’s signs and symptoms is not feasible, and supplementation is monitored by measuring thyroid function determinants and comparing these values with those of the reference ranges (24).

The daily replacement dose of L-T4 to cure adult hypothyroidism is, on average, 1.6 μg/kg per day (1, 2, 3, 6, 7, 8). L-T4 is recognized as a drug with a narrow therapeutic index and its dose can be finely adjusted to keep the serum TSH within the specific ranges appropriate for a particular patient’s diagnosis, age, and coexisting medical conditions (3).
In children with CH, the timing of therapy is crucial to neurological outcomes (24). The overall goal of L-T4 replacement therapy in children with CH is to ensure that these patients are able to have growth and mental development that is as close as possible to their genetic potential (24).

Although the total daily requirements of L-T4 are related to body mass (13, 14), unexplained differences can occur among individuals for the same age and body size, even in the absence of functioning thyroid tissue (12, 15). It has been hypothesized that factors such as food, drugs, enteral absorption, sex, time of blood sampling, age, and cigarette smoking influence the serum TSH, FT4, and FT3 concentrations (16, 17, 18). The impact of these factors balanced with genetic factors is unknown (25).

In previous studies, it has been described that, in CH newborns treated with L-T4 substitution therapy during the first weeks, the plasma FT4 can be normalized within the age-specific reference range in 3–4 days, whereas for plasma TSH a period of 3–4 weeks is required (19, 20, 21). Besides, during the first months and years in CH newborns and children treated with L-T4, TSH plasma levels above the reference range are frequently encountered despite serum FT4 within the reference range (19, 20, 21). Kempers et al. (26) described that, in children with CH, establishing FT4 well within the reference range will result in elevated TSH concentrations, whereas TSH concentrations within the reference range can be accomplished by elevated FT4 concentrations. However, these observations were obtained in children and up to adolescents and no clear information in the adult life is available (22–26).

In this study, we demonstrate that patients with CH due to aneuploidy (group CH patients) use a daily L-T4 dose/kg in adult life higher than the amount used by adult patients with post-surgical hypothyroidism (1, 11). In addition, CH patients have higher levels of serum FT4 in spite of levels of TSH higher than that in patients who became hypothyroid in adulthood. These data suggest that, in CH patients, a higher amount of FT4 is required to obtain a normal level of TSH and direct toward a central defect such as a sort of weak central resistance to thyroid hormone in CH patients. Intriguingly, despite the higher serum FT4 levels, the FT4 vs. TSH regression line was less steep in CH patients than in AH patients, confirming partial resistance to thyroid hormone in CH patients, and providing a better explanation for the effect of serum thyroid hormones. The FT4 vs. TSH regression line was less steep in CH patients than in AH patients, indicating partial resistance to thyroid hormone in CH patients.

### Table 1

<table>
<thead>
<tr>
<th>Age, weight, height, BMI, serum FT4, FT3, TSH, and dose of L-T4 (expressed as mean, s.d., and median) in all groups of patients (CH, TC, NG, and AH patients).</th>
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<tr>
<td><strong>CH</strong> (n = 13)</td>
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<td><strong>Weight (kg)</strong></td>
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<tr>
<td><strong>l-T4 (µg/kg per day)</strong></td>
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<td>2.16</td>
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CH, congenital hypothyroidism; TC, patients affected by thyroid cancer and treated by total thyroidectomy; NG, patients affected by nodular goiter and treated by total thyroidectomy; AH, patients with adult hypothyroidism after total thyroidectomy.
levels of CH patients, we found no differences in the mean levels of serum FT3 between group CH and AH patients. As a consequence, CH patients have a lower FT3/FT4 ratio. A possible explanation for this finding may be a defective thyroid hormone metabolism in CH patients. However, the mechanism involved requires further investigation.

Apparently, in L-T4-supplemented patients with CH, the set point of the thyroid’s regulatory system, defined as the individual’s specific combination of mutually dependent FT4 and TSH, differs from adults who became hypothyroid later in life. We decided to select patients with thyroid agenesis or subjects who underwent total thyroidectomy to ensure that no functioning tissue was present in both groups as demonstrated by the absence of serum Tg. In this case, the secretion of both T4 and T3 is negligible. As this was similar in congenital and acquired primary hypothyroidism, it does not explain the difference in the dose of L-T4 to maintain euthyroidism. A fundamental difference between patients with CH and those with acquired hypothyroidism is the timing of onset of thyroid hormone deficiency. Patients with CH have decreased thyroid hormone concentrations during the period when the hypothalamic–pituitary–thyroid system matures toward an integrated system for control of the thyroid hormone state. Therefore, we can hypothesize that the different requirement of L-T4 replacement therapy in patients with congenital thyroid agenesis and surgical hypothyroidism could be explained by a lack of thyroid hormones since fetal life in CH, which could determine a different set point of the hypothalamus–pituitary–thyroid axis.

Imprinting of the feedback control axis has been shown in animal models (27, 28) and in humans (29). Adult rats transiently exposed to high doses of L-T4 during the neonatal period (27) manifest hypothyroidism with decreased TSH concentrations. Similarly, adult rats transiently exposed to L-T4 in the neonatal period (28) show low serum T4 levels, an increased serum TSH level, and impaired response to TRH.

Cavaliere et al. (29) studied 12 patients with congenital goitrous hypothyroidism, ten patients with an ectopic thyroid and onset of hypothyroidism at 3–8 years of age, and six patients with adult-onset hypothyroidism. Their data showed that adult patients with treated CH require larger doses of exogenous L-T4 to block the TSH response to TRH than do treated adult-onset hypothyroid patients. However, in this human model, a residual thyroid activity in goitrous and ectopic CH and also in patients with adult-onset hypothyroidism was probably present, while in our experimental design, CH patients with agenesis or adult hypothyroid patients with undetectable serum Tg were selected. The maternal thyroid status during pregnancy is also relevant in determining impairment in the hypothalamus–pituitary–thyroid axis (30, 31, 32). In fact, the exposure of this axis to a high thyroid hormone concentration, together with thyroid-stimulating antibodies transferred from mothers with Graves’ disease, might alter the axis feedback (31, 32). The occurrence of central hypothyroidism in infants of

**Figure 1**

(A) Daily requirement of L-T4 (µg/kg) in CH and AH patients. The circle indicates a moderate outlier (<3 IQR), while the asterisk indicates an extreme outlier (>3 IQR). (B) Serum TSH concentrations in CH and AH patients. The circle indicates a moderate outlier (<3 IQR). (C) Serum FT4 concentrations in CH and AH patients. CH, congenital hypothyroidism; AH, patients with adult hypothyroidism after total thyroidectomy.

**Figure 2**

Distribution of TSH and FT4 concentrations (logarithmic scale) and regression lines in CH and AH patients.
mothers with Graves’ disease was first described by Matsuura et al. (30). It has been proposed that a prenatal phase of hyperthyroidism that affects the thyroid’s regulatory system can be responsible for the transient central hypothyroidism in the neonates. Fisher et al. (22), in a study carried out to assess feedback control of TSH in treated CH infants, demonstrated that abnormal maturation of the feedback control of TSH secretion is present in 43% of CH young infants and persists in 10% of older children, indicating that, in most children, the resistance improves with age. Our data obtained from agenetic CH patients show that a partial resistance of the feedback mechanism of TSH regulation persists even in adulthood. It was reported by other authors that the presence of relatively higher levels of serum FT4 in our adult CH patients and in the children with CH in the absence of clinical features of thyrotoxicosis could suggest a generalized rather than an isolated central thyroid hormone resistance. However, no studies proving this hypothesis are currently available and the few papers addressing the topic seem rather in favor of a selective central thyroid hormone resistance. In conclusion, adult subjects with CH due to thyroid agenesis need higher doses of L-T4 to maintain adequate levels of thyroid hormones. We believe that this different requirement is due to a different set point of the hypothalamic–pituitary–thyroid axis.

Conflict of Interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Clinical Study

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