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**Clinical Research Article**



# Thyroid Hormone Homeostasis in Levothyroxine-treated Patients: Findings From ELSA-Brasil

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## Abstract

**Context:** The effectiveness of levothyroxine (LT4) in restoring thyroid hormone (TH) homeostasis, particularly serum thyroxine (T4) and triiodothyronine (T3) levels, remains debatable.

**Objective:** This work aimed to assess TH homeostasis in LT4-treated individuals using data from the Longitudinal Study of Adult Health in Brazil (ELSA-Brasil) study.

**Methods:** The ELSA-Brasil study follows 15 105 adult Brazilians (aged 35-74 years) over 8.2 years (2008-2019) with 3 observation points assessing health parameters including serum thyrotropin (TSH), free T4 (FT4), and free T3 (FT3) levels. We analyzed 186 participants that initiated treatment with LT4 during the study, and 243 individuals continuously treated with LT4 therapy.

**Results:** Initiation of therapy with LT4 resulted in an 11% to 19% decrease in TSH, an approximately 19% increase in FT4, and a 7% reduction in FT3 serum levels (FT3 dropped >10% in ~40% of the LT4-treated patients). This was associated with an increase in triglyceride levels and utilization of hypolipidemic and antidiabetic medications. Participants continuously treated with LT4 exhibited a stable elevation in serum FT4 and a reduction in serum FT3 and TSH levels. While 115 participants (47.3%) had at least 1 serum FT4 levels above the control reference range (>1.52 ng/dL), 38 participants (15.6%) had at least 1 serum FT3 below the reference range (<0.23 ng/dL).

**Conclusion:** The present results challenge the dogma that treatment with LT4 for hypothyroidism restores TH homeostasis in all patients. A substantial number of LT4-treated patients exhibit repeated FT4 and FT3 levels outside the normal reference range, despite normal TSH levels. Further studies are needed to define the clinical implications of these findings.

**Key Words:** hypothyroidism, levothyroxine, TSH, thyroxine, triiodothyronine

**Abbreviations:** BMI, body mass index; BP, blood pressure; D2, type 2 deiodinase; ELSA-Brasil, Longitudinal Study of Adult Health in Brazil; FT3, triiodothyronine; FT4, free thyroxine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LT4, levothyroxine; TH, thyroid hormone; TSH, thyrotropin.

Hypothyroidism is a common condition caused by insufficient secretion of thyroid hormones (THs) that affects approximately 8% of the population according to patient claims and national survey data (1, 2). Treatment for hypothyroidism was developed in the 1890s and consisted of the administration of desiccated animal thyroid extract, which contains thyroxine (T4) and triiodothyronine (T3). The discovery that humans can activate T4 to T3 in peripheral tissues obviated the use of T3. Subsequently, levothyroxine (LT4)—at doses that normalized serum TSH levels—became the preferred treatment for hypothyroidism among clinicians (3).

Today, there is a consensus that LT4 monotherapy is sufficient to restore TH homeostasis. The most recent American Thyroid Association guidelines for the treatment of hypothyroidism did acknowledge that serum T3 levels might not be restored in all LT4-treated patients but did not recommend that serum T3 be monitored in their follow-up (4). However, controversy in this area remains given its potential implication for the use of combination therapy (T4 + T3) (5).

Several studies in animal models have pointed to insufficient T3 levels during LT4 treatment. LT4-treated rats have been found to have relatively lower serum and tissue T3 levels despite normalization of serum TSH levels (6). Subsequent studies revealed that the expression of T3-dependent genes and the activity of key metabolic pathways were not normalized in LT4-treated rats (7), confirming that restoring serum T3 levels is a precondition for achieving tissue euthyroidism. These findings have occurred in the context of evidence suggesting that the hypothalamus-pituitary-thyroid axis is hardwired to preserve normal T3 levels in the circulation (8). Studies in iodine-deficient rats (9, 10), humans (11), and more recently, the utilization of genetically modified mice (7, 12, 13), led to the discovery that serum T3 levels are well preserved in these situations, despite mild/moderate iodine deficiency or inactivation of all 3 deiodinases.

These studies in rodents were not sufficient to challenge the effectiveness of monotherapy with LT4 given its absolute clinical success with millions of patients with hypothyroidism

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worldwide. Nonetheless, evidence that treatment with LT4 in humans fails to restore TH homeostasis has been available since the 1960s and 1970s (14, 15). However, most studies were cross-sectional, included a relatively small number of patients, and were not universally reproduced (16). More recently, 2 studies involving large numbers of LT4-treated patients compared to well-matched controls showed that serum T4 is relatively higher and serum T3 relatively lower at every TSH level quintile distributed within the normal range (17, 18).

In this study, we aimed to examine TH homeostasis over chronic treatment with LT4. We used clinical data available from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). We hypothesized that free T4 (FT4) serum levels would be persistently elevated and free T3 (FT3) levels persistently decreased over the 8.2-year study period. We examined TH homeostasis both as a function of LT4 treatment initiation (ie, pre-LT4 vs post-LT4) and throughout continuous treatment compared to matched euthyroid controls.

## Materials and Methods

### The Brazilian Longitudinal Study of Adult Health Study Population

ELSA-Brasil is a prospective cohort of more than 15 105 Brazilian civil servants aged 35 to 74 years from 6 Brazilian cities. The cohort of participants is formed from individuals working at 5 federal universities and 1 research institute. The sample comprises volunteers (76%) and actively recruited participants (24%), with the latter being recruited from lists of civil servants. Civil servants were chosen as the source of the ELSA-Brasil study population to minimize losses to follow-up related to geographic mobility, and post hoc analyses have shown that the prevalence of behavioral risk factors and chronic conditions are similar to a nationally representative sample of the Brazilian population (19, 20). At the time of this study, participant data were available at 3 individual assessments over the study period: baseline (or wave 1) (2008-2010), wave 2 (2012-2014), and wave 3 (2017-2019). At each wave, participants completed a variety of health questionnaires, reviewed medication lists, underwent standardized clinical measurements, and provided venous blood samples for analysis. Further comprehensive details regarding the cohort can be located elsewhere (20-23). The study was reviewed and approved by the ethics committees of all participating Brazilian institutions, and written informed consent was obtained from all study participants. The study protocol was reviewed by the University of Chicago Institutional Review Board and granted exempt status.

### Study Design

The primary objective of this study was to measure TH levels in patients taking LT4 for the treatment of hypothyroidism to test the hypothesis that treatment with LT4 restores TH levels. All ELSA-Brasil participants who underwent thyroid function testing at all 3 study waves were included in the study. Those who were eligible for selection for the treatment groups had a reported history of hypothyroidism at baseline or during the study period and reported LT4 as an active medication. All other participants were considered for the control groups. To exclude abnormalities in TH homeostasis attributed to overtreatment or undertreatment with LT4, all LT4-treated participants with TSH levels outside the normal reference range (0.4-4.0 mIU/L) were excluded from the study. Also, any participants with a reported history of thyroid cancer were excluded. Any participants eligible for the control population with at least one TSH level outside the normal range were also excluded to avoid the inclusion of untreated thyroid disease in the control (ie, euthyroid) population.

The first approach was to identify participants who started therapy with LT4 for hypothyroidism during the study (not at baseline), that is, for whom data were available before and after LT4 was started (pre-LT4 vs post-LT4 study). This led to the formation of 2 groups (started LT4 between the first and second waves 1 and 2 or between the second and third waves), which were analyzed separately and as a single pooled group. Of note, due to the restriction imposed that TSH levels must be within normal limits, all of these participants had normal TSH levels in the pre-LT4 study wave. As a result, all of the included participants developed hypothyroidism and started treatment during the interim time period between study waves. This allowed for the within-individual comparison of the last known period of euthyroidism vs the first known period under LT4 treatment. Differences in TH levels in euthyroid controls over the corresponding time period were also determined. The second approach was to identify participants who were taking LT4 and reported a history of hypothyroidism at baseline and continued treatment during all 3 waves of the study (continuous LT4 study). These individuals were compared to euthyroid controls not taking LT4 for the duration of the study.

In the pre-LT4 vs post-LT4 study, the control group was generated using a propensity-score-based matching method using a nearest-neighbor approach with a 1:4 ratio, balancing sociodemographic covariates: age, sex, and race. An individual control population was created for each pre-LT4 vs post-LT4 group before pooling. This ensured the appropriate number of control individuals were selected from waves 1 and 2. Covariates were matched at baseline (either wave 1 or wave 2). For the continuous LT4 study, a similar matching approach was used, but balanced covariates included age, sex, race, education, and comorbidities (diabetes, obesity, hyperlipidemia, myocardial infarction, stroke, kidney disease, liver disease, and hypertension).

### Primary and Secondary Outcomes

The primary outcome was TH homeostasis, measured by serum TSH, FT4, FT3 levels, and FT3/FT4 levels. Specifically, we sought to determine the difference in thyroid function levels before and after LT4 initiation, and between those continuously on LT4 throughout the study period and euthyroid controls. From the density plots of the control TH data, we constructed unique ELSA cohort TSH, FT4, and FT3 reference ranges (±2 SDs from the mean) to determine the proportion of the LT4-treated population outside this range. These study-specific ranges may be more appropriate than the standard reference range given that the iodine nutrition status in some ELSA subgroups may be inadequate (24). We also measured available cardiovascular health markers (systolic and diastolic blood pressure, lipid parameters, body mass index [BMI], and use of antihyperlipidemic and oral antidiabetic medications).

### Blood Biochemistry

Venous blood samples were collected from 6:30 to 9 AM after an overnight fast. Participants were instructed to take their

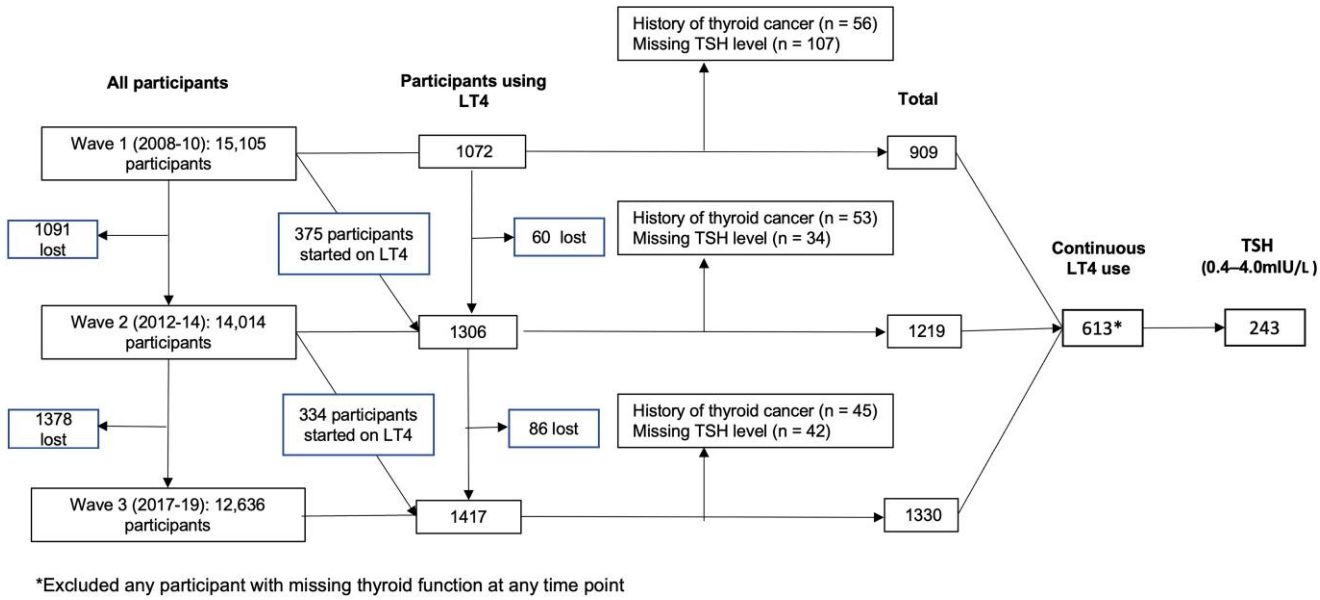


**Figure 1.** Study flowchart. The boxes contain the number of participants. We identified 243 participants continuously using levothyroxine (LT4) for whom all clinical and laboratory data were available.

daily medications (including LT4) after sample collection. TSH (normal range, 0.4-4.0 mIU/L), FT4 (0.93-1.7 ng/dL), and FT3 levels (0.20-0.44 ng/dL) were quantified using a third-generation immune-enzymatic assay (Roche Diagnostic).

### Statistical Analyses

Descriptive analyses were used to characterize the LT4 and control groups. Continuous variables were summarized using the mean and SD, and categorical variables as absolute counts and percentages. Pre-LT4 vs post-LT4 comparisons of thyroid function and cardiovascular health markers were conducted using paired *t* tests and McNemar chi-square tests, as appropriate. For the continuous LT4 study, we stratified by study wave to control for the effect of aging over the study period. Individual and mean thyroid function levels of LT4-treated participants and controls were plotted linearly over the study period (ie, spaghetti plots) to visualize change over time. Density plots were constructed to determine the cumulative proportion of participants with thyroid function tests within the reference range over the study duration. Data collection, manipulation, and statistical testing were conducted using R statistical software (version 4.2.3) with support from the "matchit" package.

## Results

### Thyroid Hormone Levels Before and After Therapy With Levothyroxine

The cohort of participants in the ELSA study varied from 15 105 in the first wave (2008-2010), to 14 014 in the second wave (2012-2014) and 12 636 in the third wave (2017-2019) (Fig. 1). For the pre-LT4 vs post-LT4 analysis, we identified 375 LT4-treated participants with hypothyroidism in the second wave who did not have thyroid disease and were not taking LT4 in the first wave. Of these, 99 had normal serum TSH levels during the first and second waves (Supplementary Table S1) (25). Similarly, we identified 334 LT4-treated participants in the third wave who were not taking LT4 in the second wave, of whom 87 participants had normal serum TSH levels during the second and third wave (see Supplementary Table S1) (25). Altogether, a total of 186 new LT4 users (81% females, 58% White) were identified, with a mean age of 53 (±9.1) years before LT4 use. Of note, the prevalence of liver disease before and after LT4 use was 11.8%, which was slightly higher than the baseline rate in the ELSA population (8.6%) (20). Mean liver function parameters (aspartate transaminase, alanine transaminase, and γ-glutamyl transferase) were within the reference ranges (Supplementary Table S2) (25). The overall rate of initiation of LT4 treatment during the first to second wave interval was 2.67%, and 2.63% during the second and third waves (see Fig. 1), with an average LT4 dose of approximately 65 mcg/day (±40 mcg/day).

The average serum TSH level decreased by 19% after LT4 initiation between the first and second waves and by about 11% between the second and third waves (Fig. 2A). In both groups, FT4 increased about 19% after LT4 initiation and FT3 decreased about 7% (Fig. 2B and 2C). These changes remained when we analyzed the combined group of 186 participants (1st → 2nd, and 2nd → 3rd waves) (Table 1; Fig. 2D-2F). In the combined group, nearly 60% of the participants exhibited a drop in serum FT3 after they initiated therapy with LT4, of which the decrease was greater than 10% in approximately 40% of the participants (see Fig. 2F). In contrast, serum FT3 levels increased by more than 10% in approximately 10% of the participants (see Fig. 2F). There was no correlation between the drop in serum FT3 and serum levels of TSH or FT4 (data not shown). The 2 euthyroid, matched control groups exhibited minor changes in serum TSH, FT3, and FT3/FT4 levels during the same period that, although statistically significant (see Fig. 2, Table 1, and Supplementary Table S3) (25), were relatively small compared to the LT4 group.

Next, we examined cardiovascular health markers to determine if downstream clinical changes manifested in association with changes in TH homeostasis. Triglyceride levels increased

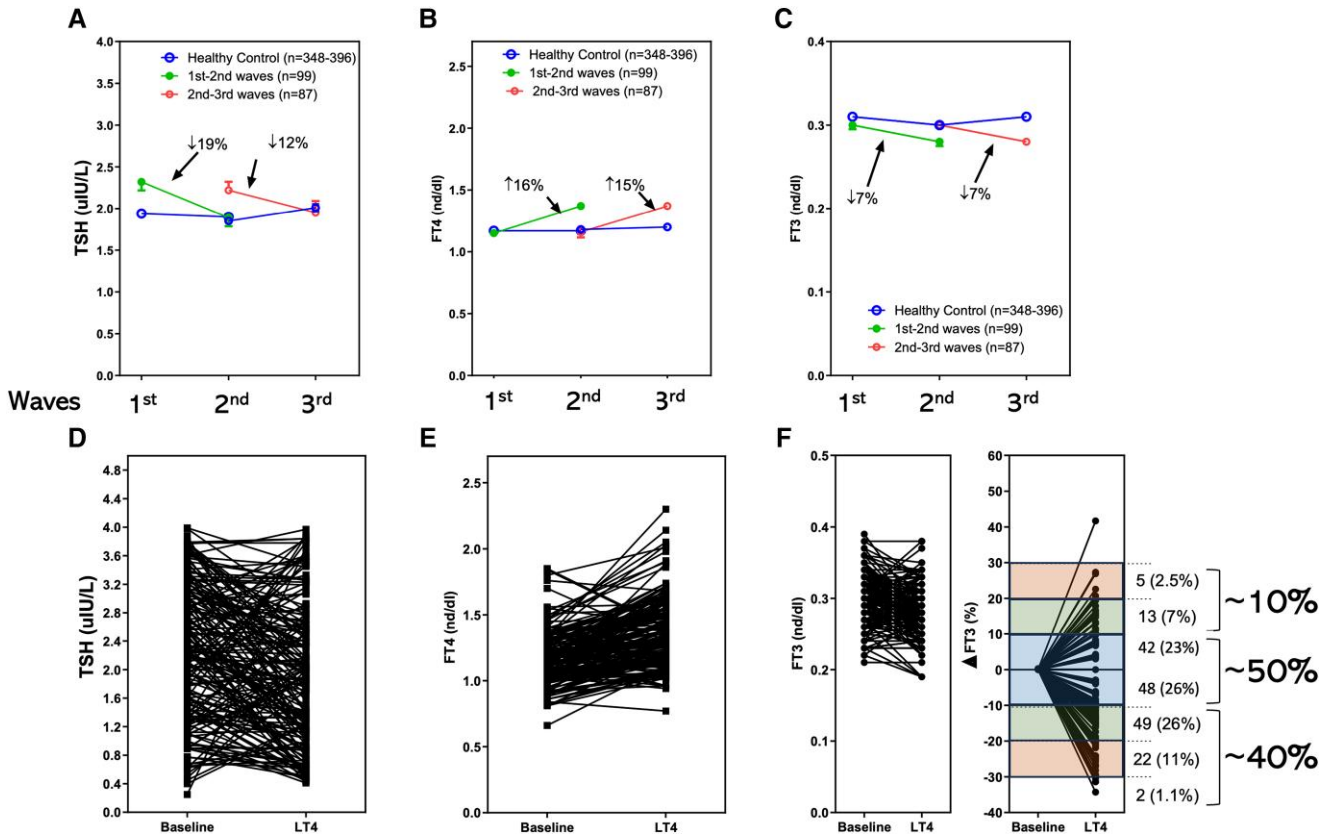


**Figure 2.** Serum thyrotropin (TSH), free thyroxine (FT4), and free triiodothyronine (FT3) in ELSA-Brasil participants who initiated use of levothyroxine (LT4) during the study period. A to C, Data for age-, sex-, and race-matched control populations are also shown in lines with 3 points. The lines with two points indicate participants who started LT4 between the first wave and the second wave or participants who started LT4 between the second to third waves. Plots in D to F show individual participant data after the pooling of both LT4 groups. Plot F shows the percentage change from baseline (before LT4 treatment was started); the rectangles indicate the graded changes in individual serum FT3 levels.

**Table 1. Serum thyrotropin, free thyroxine, and free triiodothyronine in all participants before and after levothyroxine therapy with euthyroid-matched controls**

| Thyroid function | LT4 group (n = 186) | | | Control group (n = 744) | | |
|---|---|---|---|---|---|---|
| | Baseline | After LT4 | *P* | Baseline | Next wave | *P* |
| TSH, mean (SD) | 2.28 (0.95) | 1.91 (0.98) | <.001 | 1.90 (0.73) | 1.95 (0.76) | .025 |
| FT4, mean (SD) | 1.15 (0.20) | 1.37 (0.26) | <.001 | 1.17 (0.16) | 1.18 (0.16) | .064 |
| FT3, mean (SD) | 0.30 (0.03) | 0.28 (0.04) | .001 | 0.30 (0.04) | 0.30 (0.04) | .152 |
| FT3/FT4, mean (SD) | 0.26 (0.05) | 0.21 (0.05) | <.001 | 0.26 (0.04) | 0.26 (0.04) | .002 |

Controls were matched by age, sex, and race using a nearest-neighbor approach with a 4:1 ratio. Normal reference range: TSH: 0.4 to 4.0 mIU/L; FT4: 0.9 to 1.7 ng/dL; FT3: 0.20 to 0.4 ng/dL. Units: age (years), TSH (mIU/), FT4 (ng/dL), FT3 (ng/dL).
Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; LT4, levothyroxine; TSH, thyrotropin.

in the LT4 group (123 vs 130 mg/dL; $P = .033$), but were not different in the control group (Table 2). Conversely, low-density lipoprotein cholesterol decreased in the LT4 group (117 vs 114 mg/dL; $P = .018$). This was accompanied by increased use of both antihyperlipidemic and oral antidiabetic medications (seen in the LT4 and control groups). BMI also increased in both the LT4 and control groups, although the absolute change in both cases was small (BMI 27.3 vs 27.8; $P = .002$ and 26.7 vs 27.1; $P < .001$, respectively). No significant changes in blood pressure were detected in the LT4 group.

## Thyroid Hormone Levels in Participants Continuously Treated With Levothyroxine

Of the 12 636 participants that remained in the study for all 3 waves, we identified 613 individuals who took LT4 continuously and had thyroid function tests available for each study wave, of whom 243 (39.6%) had TSH levels maintained within the normal range (see Fig. 1). This study cohort was 85% female, 66% White, and had a mean age of 55 years (±8.7 years) (Table 3). Of note, when we compared the included and excluded participants, there were no significant baseline differences in age, sex, race, or prevalence of type 2

**Table 2. Cardiovascular parameters in all participants before and after levothyroxine therapy with euthyroid-matched controls**

| Cardiovascular parameters | LT4 group (n = 186) | | | Control group (n = 744) | | |
|---|---|---|---|---|---|---|
| | Baseline | After LT4 start | *P* | Baseline | Next wave | *P* |
| Antihyperlipidemic, n (%) | 40 (21.5) | 69 (37.1) | <.001 | 114 (15.3) | 169 (22.7) | <.001 |
| Oral antidiabetics, n (%) | 19 (10.2) | 38 (20.4) | <.001 | 56 (7.5) | 89 (12.0) | <.001 |
| Total cholesterol, mean (SD) | 198 (37.8) | 196 (37.6) | .386 | 200 (44.5) | 200 (37.1) | .782 |
| HDL cholesterol, mean (SD) | 55.0 (13.1) | 55.9 (13.7) | .187 | 55.9 (13.1) | 57.2 (14.7) | <.001 |
| LDL cholesterol, mean (SD) | 117 (33.6) | 114 (35.2) | .018 | 119 (31.7) | 118 (32.4) | .533 |
| Triglycerides, mean (SD) | 123 (60.0) | 130 (63.8) | .033 | 126 (73.0)[a] | 123 (73.3)[a] | .696 |
| Systolic BP, mean (SD) | 118 (14.8) | 118 (15.6) | .957 | 117 (15.7) | 119 (16.5) | <.001 |
| Diastolic BP, mean (SD) | 74.4 (9.3) | 74.3 (9.2) | .771 | 74.1 (10.1) | 74.7 (10.1) | .070 |
| BMI, mean (SD) | 27.3 (4.6) | 27.8 (4.6) | .002 | 26.7 (4.6) | 27.1 (4.8) | <.001 |

Controls were matched by age, sex, and race using a nearest-neighbor approach with a 4:1 ratio.
Units: total cholesterol (mg/dL), HDL (mg/dL), LDL (mg/dL), triglycerides (mg/dL).
Abbreviations: BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LT4, levothyroxine.
[a]Triglyceride level from one control participant was not available. Reference ranges: total cholesterol less than 200 mg/dL, LDL less than 130 mg/dL, HDL greater than 40 mg/dL, BMI normal: 18.5 to 25, overweight 25 to 30, obese greater than 30.

**Table 3. Sociodemographic characteristics in levothyroxine-treated participants (n = 243)**

| Characteristics | First wave | Second wave | Third wave |
|---|---|---|---|
| Age, mean (SD), y | 55 (8.7) | 58 (8.7) | 63 (8.8) |
| Female, n (%) | 213 (85) | | |
| Race, n (%) | | | |
| White | 160 (66) | — | — |
| Black | 13 (5.3) | — | — |
| Brazilian indigenous | 2 (0.82) | — | — |
| Asian | 8 (3.3) | — | — |
| Multiple races | 61 (25) | — | — |
| Smoker, n (%) | 16 (6.4) | 16 (6.4) | 14 (5.6) |
| Family annual income, mean (SD), $USD | 14 000 (5400) | | |
| Education level, n (%) | | | |
| No formal education | — | — | — |
| Incomplete middle school | 6 (2.4) | — | — |
| Complete middle school | 3 (1.2) | — | — |
| Incomplete high school | 3 (1.2) | — | — |
| Complete high school | 52 (21) | — | — |
| Incomplete college | 16 (6.4) | — | — |
| Complete college | 50 (20) | — | — |
| Graduate education | 120 (48) | — | — |
| Type 2 diabetes mellitus, n (%) | 43 (17) | 57 (23) | 73 (29) |
| Obesity, n (%) | 61 (24) | 74 (30) | 76 (30) |
| Hypertension, n (%) | 81 (32) | 107 (43) | 121 (49) |
| Antihyperlipidemic medication, n (%) | 69 (28) | 90 (36) | 103 (41) |
| Liver disease, n (%) | 24 (9.8) | 24 (9.8) | 24 (9.8) |

diabetes, obesity, or hypertension (data not shown). As expected, the prevalence of most comorbidities increased with age (between first and third waves, respectively), including type 2 diabetes (17% to 29%), obesity (24% to 30%), and hypertension (32% to 49%). However, the prevalence of liver disease did not vary over time (9.8%), and mean liver function parameters remained within normal limits (Supplementary Table S4) (25).

Overall, TH levels remained relatively stable during the 8.2 years of follow-up in both the LT4 and control groups; however, mean serum FT4 levels were consistently higher and FT3 levels were consistently lower in the LT4 group (Table 4). It is striking that FT4 levels remained within the top 3 quartiles or above the normal reference range (Fig. 3A), whereas serum FT3 levels were predominantly found within the bottom 3 quartiles or below the normal reference range (Fig. 3B). At the same time, the serum FT3/FT4 ratio remained well within the bottom 2 quartiles of the normal reference range (Fig. 3C).

The longitudinal analysis of TH levels in the LT4-treated participants revealed that many individuals had serum FT4 and FT3 outside the normal reference range, despite having normal serum TSH levels (see Fig. 3). To study this further, we analyzed the distribution of serum FT4, FT3, FT3/FT4 ratio, and TSH among all LT4-treated and matched control groups (Fig. 4). Whereas the distribution of serum FT4 and FT3 serum values and FT3/FT4 ratio in both groups exhibited the expected Gaussian curves, the FT4 distribution curve in the LT4-treated participants was shifted to the right (Fig. 4A), whereas the FT3 and FT3/FT4 distribution curves were shifted to the left (Fig. 4B and 4C). No shift was identified in the TSH distribution curve (Fig. 4D).

To study whether the shifts in the TH distribution curves caused participants in the LT4 group to fall off the normal reference range, we compared their TH levels with the normal reference ranges in the matched control group for FT4, FT3, and FT3/FT4 in this study, as defined by their average ± 2SD (the reference range in the clinical assays used have not been validated for the Brazilian population): FT4 (0.86-1.52 ng/dL) and FT3 (0.23-0.37 ng/dL). There were 115 (47.3%) LT4-treated participants with at least 1 occurrence of abnormally high serum FT4 (>1.52 ng/dL). Just under 22% had 2 or more occurrences (Table 5). Conversely, in only

**Table 4. Serum thyrotropin, free thyroxine, free triiodothyronine, and FT3/FT4 ratios in levothyroxine-treated participants compared to euthyroid controls**

| Thyroid function | First wave | | | Second wave | | | Third wave | | |
|---|---|---|---|---|---|---|---|---|---|
| | LT4 (n = 243) | Control (n = 972) | *P* | LT4 | Control | *P* | LT4 | Control | *P* |
| TSH, mean (SD) | 2.1 (1.0) | 1.9 (0.76) | .037 | 1.8 (0.91) | 1.9 (0.77) | .153 | 2.0 (0.94) | 2.0 (0.8) | .412 |
| FT4, mean (SD) | 1.4 (0.22) | 1.2 (0.16) | <.001 | 1.4 (0.22) | 1.2 (0.16) | <.001 | 1.4 (0.24) | 1.2 (0.18) | <.001 |
| FT3, mean (SD) | 0.28 (0.03) | 0.31 (0.04) | <.001 | 0.27 (0.03) | 0.30 (0.04) | <.001 | 0.27 (0.04) | 0.30 (0.04) | <.001 |
| FT3/FT4, mean (SD) | 0.21 (0.04) | 0.26 (0.04) | <.001 | 0.20 (0.03) | 0.25 (0.04) | <.001 | 0.19 (0.04) | 0.25 (0.04) | <.001 |
| LT4 dosage, mean (SD) | 84 (33) | — | — | 84 (30) | — | — | — | — | — |

Controls were matched by age, sex, race, education, and comorbidities (diabetes, obesity, hyperlipidemia, myocardial infarction, stroke, kidney disease, liver disease, hypertension) using a nearest-neighbor approach with a 4:1 ratio. Normal reference range: TSH: 0.4 to 4.0 mIU/L; FT4: 0.9 to 1.7 ng/dL; FT3: 0.20 to 0.4 ng/dL. Units: age (years), TSH (mIU/), FT4 (ng/dL), FT3 (ng/dL).
Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; LT4, levothyroxine; TSH, thyrotropin.

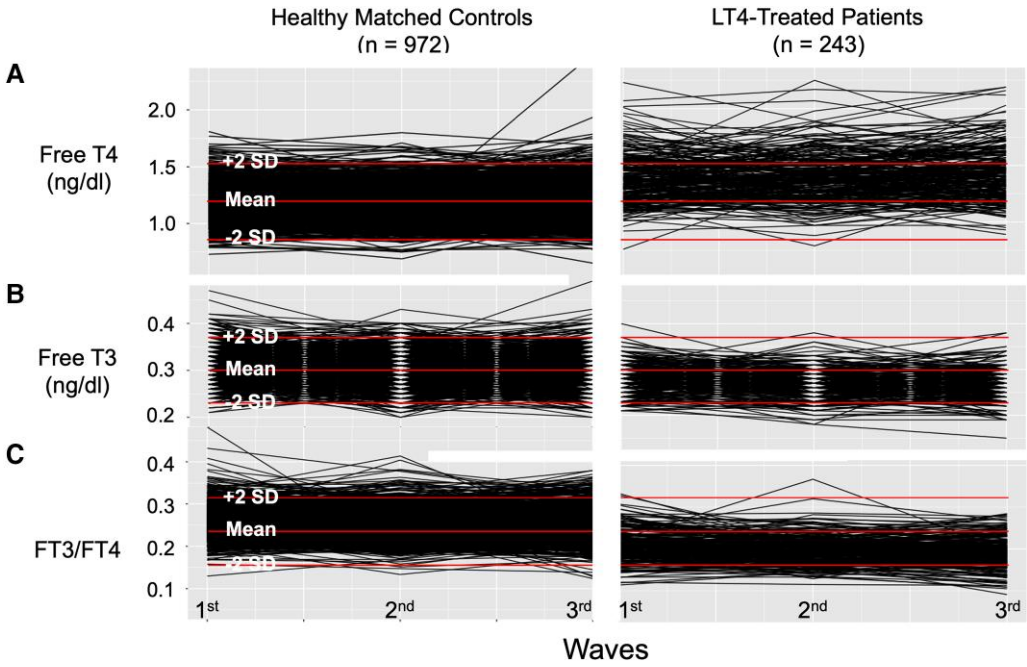


**Figure 3.** Serum free thyroxine (FT4), free triiodothyronine (FT3), and FT3/FT4 ratio in participants continuously treated with levothyroxine (LT4). The lines connect values obtained during 3 waves spanning 8.2 years as indicated. Values for a control population matched for age, sex, race, education status, hypertension, diabetes mellitus, obesity, hyperlipidemia, myocardial infarction, stroke, kidney disease, and liver disease are also shown. The mean ± 2SD are indicated as follows: A, FT4: 1.19 (1.03-1.36); B, FT3: 0.30 (0.27-0.34); C, FT3/FT4: 0.26 (0.22-0.30).

38 (15.6%) of participants, there was at least 1 occurrence of low FT3 (<0.23 ng/dL) observed (see Table 5).

## Discussion

The prospective assessment of TH homeostasis in a large Brazilian cohort followed for approximately 8 years revealed that treatment with LT4 was associated with an approximately 7% reduction in serum FT3 levels, despite an approximately 19% increase in serum FT4 and an 11% to 19% reduction in serum TSH levels. These changes in TH homeostasis remained for years, throughout treatment with LT4. The elevation in serum FT4 was stable, with values near the upper limit of normal throughout the study period. In about 47% of participants, serum FT4 levels were above the normal reference range at least once during the follow-up. An inverse but similar scenario was observed with the serum FT3 levels, which were near or below the lower limit of the reference range for 15.6% of the participants. The initiation of treatment with LT4 was associated with an increase in triglyceride levels despite an increase in the use of cholesterol-lowering medications.

The thyroid gland secretes both T4 and T3 at a ratio of approximately 16:1, which can drop to as low as 8:1 under TSH stimulation (26). The relative increase in T3 secretion, along with the acceleration of T4 to T3 activation (27) via the type 2 deiodinase (D2) pathway, serves to preserve serum T3 levels during conditions of reduced iodine intake as shown in rats (9, 10) and humans (11). Treatment with LT4 in patients with hypothyroidism effectively restores serum TSH levels but slows down the fractional conversion of T4 to T3 (27). Here we show that these changes create conditions for a

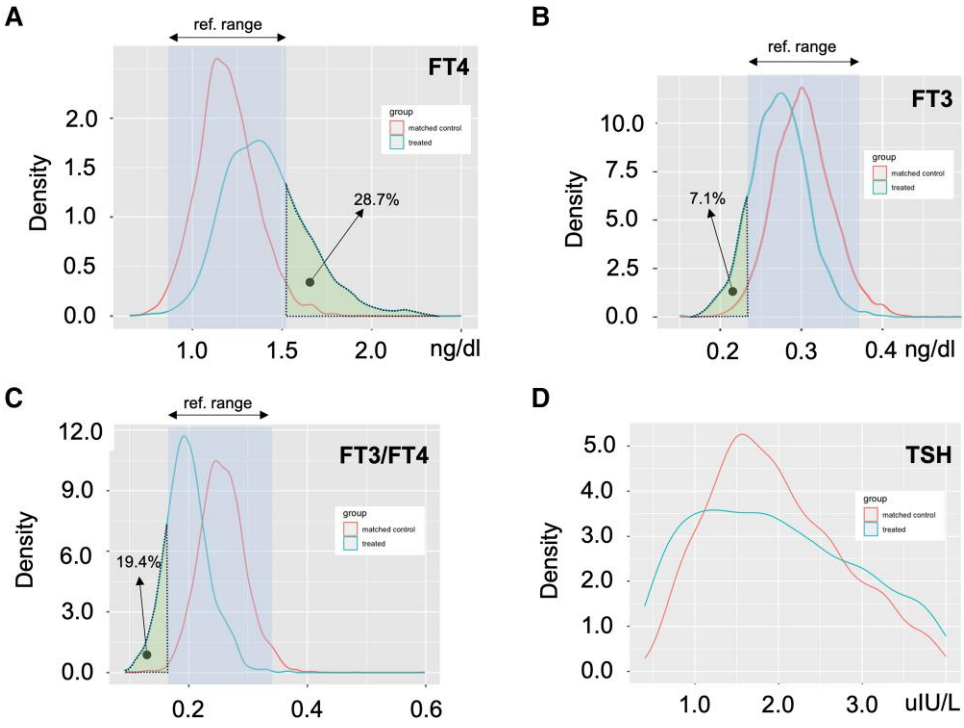


**Figure 4.** Distribution of A, serum free thyroxine (FT4); B, free triiodothyronine (FT3); C, FT3/FT4; and D, thyrotropin (TSH) values among participants continuously treated with levothyroxine (LT4) and matched controls. The blue-shaded areas represent the normal reference ranges. The green shaded areas indicate the participants above (FT4) or below (FT3, FT3/FT4 ratio) the reference range. The reference ranges were defined based on the control population in Fig. 3.

**Table 5. Levothyroxine-treated participants with free thyroxine greater than 1.65 ng/dL or free triiodothyronine less than 0.23 ng/dL**

| Thyroid function threshold | 0 occurrence | 1 wave | 2 waves | 3 waves |
|---|---|---|---|---|
| FT4 > 1.52; n (%) | 128 (52.7) | 52 (21.4) | 41 (16.9) | 22 (9.1) |
| FT3 < 0.23; n (%) | 205 (84.4) | 27 (11.1) | 8 (3.3) | 3 (1.2) |

The reference range in the clinical assays used have not been validated for the Brazilian population, therefore, we estimated reference ranges from the euthyroid control population: FT4 (0.86-1.52 ng/dL) and FT3 (0.23-0.37 ng/dL).
Units: FT4 (ng/dL), FT3 (ng/dL).
Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine.

relative or absolute excess of T4 along with a relative or absolute deficiency in daily T3 production.

Evidence that treatment with LT4 does not restore TH homeostasis first emerged about 50 years ago (15). Nonetheless, LT4 moved rapidly to become the standard of care for the treatment of hypothyroidism, even without randomized clinical trials that assessed its effectiveness and safety (3). In addition to several cross-sectional studies (28), 2 key observational studies examining T3 levels after thyroidectomy are conflicting. The first study prospectively enrolled 50 euthyroid participants who after surgery were prescribed LT4 (29). By the end of the study, serum T3 levels were not different when compared to prethyroidectomy T3 levels, but FT4 levels were higher after therapy with LT4 was initiated (29). The second was a retrospective study of 135 consecutive patients who underwent total thyroidectomy (30). Only in patients with moderately or strongly suppressed TSH levels did serum T3 levels remain within the normal range or above, respectively. Patients with normal TSH levels had lower serum FT3 levels (30). An intriguing recent report supports our conclusions. In the immediate follow-up after total thyroidectomy, patients were randomly assigned to receive therapy with LT4 or LT4 + LT3. After 6 months, the patients receiving LT4 exhibited lower serum T3 levels and higher serum cholesterol levels whereas the ones receiving combination therapy with LT4 + LT3 exhibited normal serum T3 levels, and the cholesterol remained at the presurgical levels (31).

An important remaining point is why treatment with LT4 does not mimic the normal thyroid gland function. The assumption that deiodinase activity alone can supply enough T3 at the tissue level during LT4 treatment may be misguided (16). Here is a potential scenario: Treatment with LT4 increases serum T4 levels, providing substrate for intracellular T3 formation via the deiodinases, mainly D2 (32). In the pituitary gland, this pathway is unimpeded and the higher serum T4 levels result in a relatively greater production of T3 and the suppression of serum TSH (33). However, outside the pituitary gland (eg, skeletal muscle and bone marrow), the D2-generated T3 for the plasma exhibits a self-limiting mechanism (33). This is because T4 induces ubiquitination and degradation of D2, reducing the fractional conversion of T4 to T3 (34). As a result, therapy with LT4 can effectively normalize serum TSH levels while failing to restore serum and tissue T3 levels (16, 35).

Given these findings, the question arises as to the degree to which the persistent modifications in TH homeostasis (lower levels of FT3 and higher levels of FT4) have a long-term effect on patients' clinical status. Animal models have demonstrated an association between LT4 therapy with elevated serum cholesterol levels and a pattern of gene expression compatible with

a reduction in T3 signaling in the liver, brain, and skeletal muscle (7). It is less clear that such a scenario is also present in LT4-treated patients given that T3-responsive biological markers are not readily available for clinical use. In this regard, it is notable that in 2 studies in which surgical thyroidectomy was followed by treatment with LT4 there was an elevation in serum cholesterol levels (36, 37), and a meta-analysis identified elevated serum cholesterol levels in thousands of LT4-treated patients (38). However, the use of serum cholesterol levels alone as a marker of the deleterious effects of LT4 treatment is limited given the observation that statin utilization is increased in the LT4-treated population (18, 39). In the present investigation, we also found a higher utilization of lipid-lowering drugs after patients were started on LT4 (21% → 37%). Most observational studies examining cardiovascular events in LT4 treatment include participants with normal and abnormal TSH levels and many do not report T4 or T3 levels (40). However, a recent study examining a large cohort without thyroidal illness found that both increased FT3 levels and decreased FT4 levels were protective from mortality (41). This would suggest the TH homeostasis changes seen in LT4 treatment may be detrimental to overall health, but data from the LT4-treated population are needed.

The present study is not without limitations. The first is the methodology used to measure serum FT3, which is less reliable than total T3 levels (42). Differences in FT3 levels, even in the aggregate, should be interpreted with caution. Second, there is also an inherent sampling bias when drawing a study population from a single workforce, although this has been done to great effect in other large studies (eg, Nurses' Health Study). The participants in this study have a relatively high household income and access to free health care given their employment status with the federal and state governments in Brazil. Although they have higher education and income compared to the general Brazilian population, they are very similar to people living in metropolitan areas around the state capitals (20). This likely means that the LT4 and control groups had reasonable medical follow-ups outside the study. It is conceivable that the increase in the number of visits associated with the diagnosis and treatment of hypothyroidism might have played a role in the diagnosis of hyperlipidemia and increased prescription of lipid-lowering and antidiabetes drugs. Third, our study included only participants treated with LT4 because the use of TH alternatives (eg, liothyronine and desiccated thyroid extract) is exceedingly rare in Brazil because they are not commercially available. Fourth, because of the requirement of normalized TSH throughout the study period, a significant proportion of continuously treated participants were not eligible for analysis. Because participants were not removed at random, this introduces the potential for sampling bias. However, when we compared the included and excluded participants, there were no significant differences in key sociodemographic or clinical characteristics. Finally, differences in metabolic health markers, including serum cholesterol and blood pressure levels, must be interpreted in context. Despite the study design (pre vs post), the effect of time can introduce key confounders, including those that were observed (weight gain, medication use changes) and unobserved (eg, dietary changes, work stressors, etc).

In conclusion, the present study provides longitudinal evidence that LT4 treatment does not restore TH homeostasis in all patients with hypothyroidism, which could have long-term metabolic consequences. These findings highlight the need for alerting physicians to the shortcomings of therapy with LT4 and for more research focusing on their extended clinical implications.

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## Disclosures

A.C.B. is a consultant for Abbvie, Acella, and Synthonics. G.C.P. is a consultant for Knight, Ipsen, and Bayer. The other authors have no relevant disclosures.

## Data Availability

Some or all data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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