# Is a Normal TSH Synonymous with "Euthyroidism" in Levothyroxine Monotherapy?

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**Context:** Levothyroxine ($LT_4$) monotherapy is the standard of care for hypothyroidism.

**Objective:** To determine whether $LT_4$ at doses that normalize the serum TSH is associated with normal markers of thyroid status.

**Design:** Cross-sectional data from the US National Health and Nutrition Examination Survey (2001–2012) was used to evaluate 52 clinical parameters. $LT_4$-users were compared to healthy controls and controls matched for age, sex, race, and serum TSH. Regression was used to evaluate for correlation with serum thyroxine ($T_4$) and triiodothyronine ($T_3$) levels.

**Participants:** 9,981 participants with normal serum TSH were identified; 469 were $LT_4$-treated.

**Results:** Participants using $LT_4$ had higher serum total and free $T_4$ and lower serum total and free $T_3$ than healthy or matched controls. This translated to ~15–20% lower serum $T_3$:$T_4$ ratios in $LT_4$ treatment, as has been shown in other cohorts. In comparison to matched controls, $LT_4$-treated participants: had higher BMI despite report of consuming less calories/day/kg; were more likely to be taking beta-blockers, statins, and anti-depressants; and reported lower total metabolic equivalents. A serum TSH level below the mean in $LT_4$-treated participants was associated with a higher serum free $T_4$ but similar free and total $T_3$; yet those with lower serum TSH levels exhibited higher serum HDL and lower serum LDL, triglycerides, and CRP. Age was associated with serum free $T_3$:free $T_4$ ratio in all participants; caloric intake was associated in $LT_4$-treated individuals.

**Conclusions:** In a large population study, participants using $LT_4$ exhibited lower serum $T_3$:$T_4$ ratios and differed in 12/52 objective and subjective measures.

The ideal therapeutic goal in hypothyroidism would be to restore clinical and biochemical euthyroidism via physiologic thyroid hormone replacement. This concept may seem straightforward, but there are subtleties that have only recently been recognized by the medical community (1, 2). For the last four decades, the standard approach for thyroid hormone replacement in hypothyroidism has been administration of levothyroxine ($LT_4$) at doses that normalize the serum TSH (3). This strategy has been justified with the knowledge that, in humans, the iodothyronine deiodinases in peripheral tissues produce most of the circulating active form of thyroid hormone, triiodothyronine ($T_3$), via conversion from thyroxine ($T_4$) (4). The hypothesis that $LT_4$ 'monotherapy' will maintain an adequate serum pool of $T_4$ and that the iodothyronine deiodinases will then provide physiologic regulation of $T_3$ availability has been held with much conviction (5).

The dogma in clinical thyroidology that $LT_4$ monotherapy at doses that normalize serum TSH is sufficient to restore euthyroidism (1, 2) has come into question as evidence suggests a significant proportion of patients treated with $LT_4$ continue to experience residual symptoms of hypothyroidism, including psychological (6) and metabolic (7) effects. One hypothesis to explain this phenomenon is that serum levels of $T_3$ might not be fully normalized, (8) ie, $T_4$-to-$T_3$ conversion in these patients may be insufficient to restore levels to those achieved when thyroidal secretion of $T_3$ is intact. A second hypothesis is

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based on the fact that in many tissues, intracellular $T_3$ levels cannot be predicted based on circulating thyroid hormone levels due to the actions of the types 2 and 3 deiodinases. Thus, in some tissues a relatively higher serum $T_4$ level could result in enhanced thyroid hormone signaling without affecting circulating $T_3$ levels (9). In contrast, in other tissues the relatively higher serum $T_4$ levels could impair intracellular $T_3$ production via downregulation of a deiodinase pathway (10). In fact, an animal model of primary hypothyroidism supports the hypothesis that $LT_4$ monotherapy does not achieve systemic euthyroidism. Thyroidectomized rodents treated with $LT_4$ at doses that normalize serum TSH exhibit relatively lower serum $T_3$ and higher serum $T_4$ levels as well as markers of hypothyroidism within their brain, skeletal muscle, and liver tissues (10, 11). However studies in humans are necessary given that interspecies differences could limit the translatability of these findings (5). Lastly, symptomatic differences between healthy euthyroid individuals and $LT_4$-treated patients that have normal serum TSH could be independent of serum $T_4$ and/or $T_3$ levels but rather due to multiple other confounders (6, 12).

Hypothyroidism is a prevalent condition (13) and levothyroxine is commonly prescribed; in 2015 levothyroxine was the single most commonly prescribed medication in the US (14). Thus understanding whether all parameters of hypothyroidism are universally restored by $LT_4$ monotherapy has great clinical significance. Here we used publically available data from NHANES, a well-defined, large, cross-sectional population study to evaluate whether individuals on $LT_4$ monotherapy were the same as those not using $LT_4$ in terms of thyroid function tests, thyroid hormone related markers, and to identify clinical factors associated with serum $T_3$:$T_4$ ratios.

## Materials and Methods

### Study Participants

Publically available data was obtained from the US National Health and Nutrition Examination Survey (NHANES), a large, multistage, survey assessing the health and nutritional status of Americans. The eligible population was restricted to individuals ≥ 18 years of age who had serum TSH, free $T_3$, total $T_3$, free $T_4$ and total $T_4$ measured during an NHANES cycle (2001–2002, (15) 2007–2008, (16) 2009–2010, (17) and 2011–2012 (18)). The same assays were utilized to measure serum TSH, free $T_3$, total $T_3$, free $T_4$ and total $T_4$ for all NHANES cycles. Participants were excluded if they had a serum TSH level outside of the reference range (0.24–5.40 mIU/L, n = 450), were pregnant, were taking thyroid-related supplements, methimazole, propylthiouracil, liothyronine, steroids, amiodarone, lithium, desiccated thyroid preparations, antiepileptics or dopaminergic analogues (n = 280; Figure 1). Data were obtained from demographic, questionnaire, and laboratory files; all data were

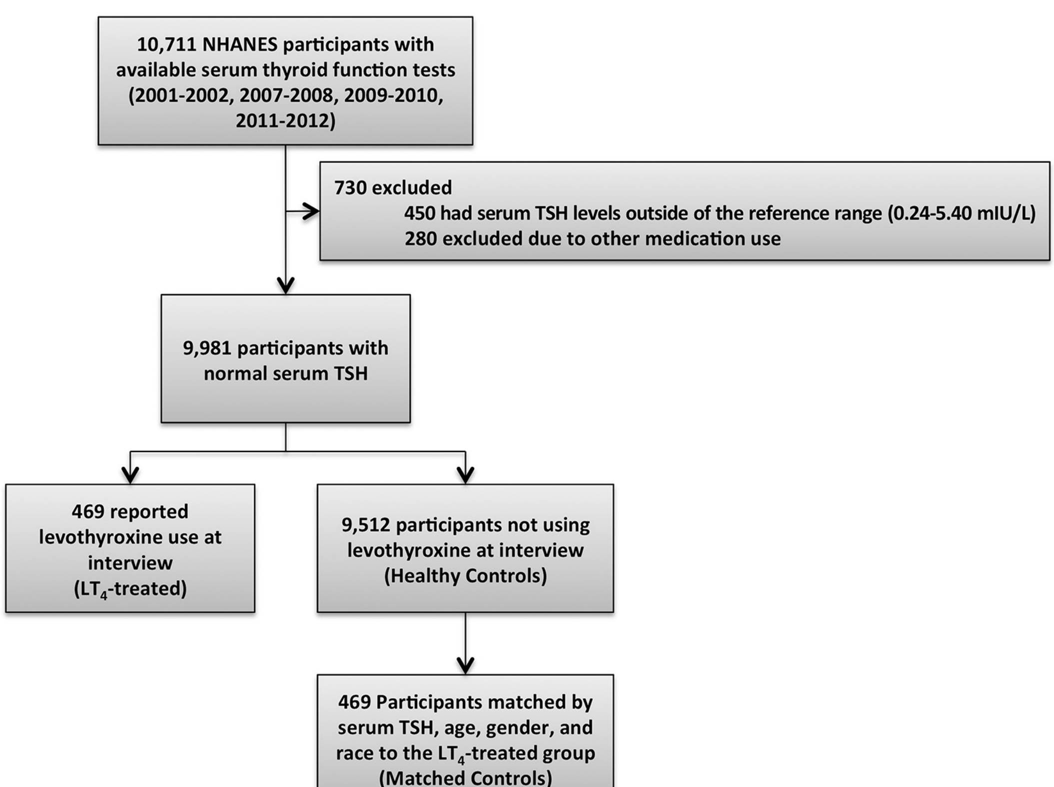


**Figure 1. Study Profile**.

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collected from trained interviewers using validated procedures and questionnaires to minimize bias (19).

### Statistical methods

Analyses were completed with SPSS (version 22.0) (20). Participants were grouped based on report of $LT_4$ use at interview. Two control groups were identified: healthy controls (remaining sample not using $LT_4$) and matched controls, matched to 1:1 for serum TSH, age (both matched within 2 standard deviations of the $LT_4$-treated population's mean), sex, and race/ethnicity to $LT_4$ treated individuals. Differences between groups were compared using $\chi^2$ and Student's $t$ test. Differences in thyroid hormones were compared between individuals below and above the entire population's TSH mean (1.75 mIU/L) within each group.

Pearson's correlation coefficients were calculated to describe the relationship between serum free $T_3$:free $T_4$ ratio and variables. Despite known problems with measurement of serum free $T_3$, (21) this was selected for use in these analyses in an effort to control for estrogen status between diverse study participants. Univariate linear regression was used to determine variables significantly associated with serum free $T_3$:free $T_4$ ratio; unstandardized regression coefficients were reported. Finally, multivariate linear regression was utilized to describe the association of free $T_3$:free $T_4$ ratio while controlling for significant variables; variables identified as significant from the both the $LT_4$ treated and matched controls' univariate analyses were entered into a forward selection model. The current analysis was a cross-sectional examination of serum thyroid levels among $LT_4$-treated vs nontreated individuals and not meant to be representative of the national population. Thus, sample weights were not used to adjust for oversampling of selected groups.

## Results

### $LT_4$-treated participants have a lower serum $T_3$:$T_4$ ratio

From NHANES years 2001–2002, (15) 2007–2008, (16) 2009–2010, (17) and 2011–2012 (18) a total of 9981 participants had a normal serum TSH level and met inclusion criteria for the present studies (Figure 1). Of these, 469 were taking $LT_4$ ($LT_4$-treated) and 9512 were not taking $LT_4$ (healthy controls). In comparison to the healthy controls, $LT_4$-treated participants had ~20% higher serum TSH levels, 10% lower serum free $T_3$ levels, and 15% lower total $T_3$ levels (Supplemental Table 1). In addition, their serum free and total $T_4$ levels were higher than those of healthy controls by about 15%. This resulted in ~25% lower $T_3$:$T_4$ ratios in the $LT_4$-treated participants. The $LT_4$-treated participants also differed significantly in key demographic factors compared to these healthy 'controls': $LT_4$-treated participants were older than those not using $LT_4$, were more likely to be female, and had a different racial-ethnic distribution. This prompted the creation of a group of 'matched controls' – 469 participants who were not using $LT_4$ and were matched by TSH, age, sex, and race were selected from the 9512 healthy controls.

When considering $LT_4$-treated participants vs matched controls, results were consistent; $LT_4$-treated participants exhibited 5%–10% lower free and total $T_3$ serum levels and 10%–15% higher free and total $T_4$ serum levels (Table 1). The serum $T_3$:$T_4$ ratios were approximately 15%–20% less in $LT_4$-treated individuals than in the matched controls.

### Not all clinical parameters are 'normal' in LT4-treated participants

52 parameters possibly associated with thyroid hormone status were assessed in these groups (Table 2). The $LT_4$-treated participants exhibited about 5% higher BMI than healthy and matched controls (Supplemental Table 2 and Table 2). $LT_4$-users had slightly higher systolic and

**Table 1.** Characteristics of adult NHANES participants with normal serum TSH levels, by levothyroxine use.

| | $LT_4$-treated ($n$ = 469) | Matched Controls ($n$ = 469) | p-value† |
|---|---|---|---|
| Age (years) | 64.3 ± 14.1 | 64.1 ± 14.0 | 0.88 |
| Female (%) | 360 (77%) | 360 (77%) | 1.0 |
| Race (%) | | | |
| Non-Hispanic White | 336 (71%) | 336 (71%) | 1.0 |
| Non-Hispanic Black | 36 (8%) | 36 (8%) | |
| Hispanic | 76 (16%) | 76 (16%) | |
| Other | 21 (4%) | 21 (4%) | |
| Serum TSH (mIU/liter) | 2.13 ± 1.32 | 2.15 ± 1.29 | 0.83 |
| Serum Free $T_3$ (pg/mL) | 2.85 ± 0.33 | 3.01 ± 0.39 | <0.0001 |
| Serum Total $T_3$ (ng/mL) | 97.56 ± 20.64 | 108.29 ± 24.89 | <0.0001 |
| Serum Free $T_4$ (ng/mL) | 0.94 ± 0.21 | 0.80 ± 0.14 | <0.0001 |
| Serum Total $T_4$ (ug/dL) | 9.14 ± 1.76 | 8.08 ± 1.56 | <0.0001 |
| Free $T_3$:Free $T_4$ § | 3.18 ± 0.80 | 3.85 ± 0.75 | <0.0001 |
| Total $T_3$: Free $T_4$ § | 109.59 ± 36.30 | 138.46 ± 38.49 | <0.0001 |
| Total $T_3$: Total $T_4$ § | 11.01 ± 2.85 | 13.70 ± 3.37 | <0.0001 |

Data are mean ± SD, n (%). P-value by $\chi$-square (categorical data) or student's $t$ test (continuous data). $LT_4$: levothyroxine; TSH: thyroid stimulating hormone; $T_4$: thyroxine; $T_3$ tri-iodothyronine. *For the comparison of $LT_4$-treated and healthy controls. †For the comparison of $LT_4$-treated and matched controls. §Multiplied × 1000.

**Table 2.** Clinical parameters of adult NHANES participants with normal serum TSH levels.

| | $LT_4$-treated ($n$ = 469) | Matched Controls ($n$ = 469) | p-value† |
|---|---|---|---|
| Objective measures | | | |
| BMI (kg/$m^2$) | 29.8 ± 6.7 | 28.2 ± 6.2 | <0.001 |
| Systolic blood pressure (mm Hg) | 131 ± 22 | 131 ± 22 | 0.80 |
| Diastolic blood pressure (mm Hg) | 68 ± 14 | 68 ± 14 | 0.39 |
| Heart rate (beats per minute) | 72 ± 12 | 72 ± 12 | 0.59 |
| $HbA_{1C}$ (%) | 5.9 ± 0.9 | 5.9 ± 0.9 | 0.54 |
| Fasting glucose (mg/dL) | 106 ± 37 | 104 ± 33 | 0.21 |
| Total cholesterol (mg/dL) | 197 ± 41 | 205 ± 42 | <0.01 |
| HDL (mg/dL) | 54 ± 16 | 57 ± 16 | 0.02 |
| LDL (mg/dL) | 115 ± 35 ($n$ = 175) | 123 ± 37 ($n$ = 183) | 0.03 |
| Triglyceride (mg/dL) | 144 ± 86 ($n$ = 180) | 134 ± 68 ($n$ = 1814) | 0.22 |
| C-reactive protein (mg/dL) | 0.50 ± 0.64 ($n$ = 400) | 0.50 ± 1.00 ($n$ = 402) | 0.94 |
| Ferritin (ng/mL) | 102 ± 106 ($n$ = 103) | 90 ± 102 ($n$ = 103) | 0.35 |
| Creatinine (mg/dL) | 0.93 ± 0.53 | 0.90 ± 0.35 | 0.28 |
| Creatine phosphokinase (IU/liter) | 118 ± 91 ($n$ = 69) | 110 ± 70 ($n$ = 67) | 0.54 |
| Medication use | | | |
| beta-blocker (%) | 175 (37%) | 110 (24%) | <0.0001 |
| Statin (%) | 111 (24%) | 72 (15%) | <0.01 |
| Insulin (%) | 20 (4%) | 14 (3%) | 0.30 |
| Oral hypoglycemic (%) | 59 (45%) | 39 (38%) | 0.25 |
| Anti-depressant (%) | 101 (22%) | 69 (15%) | <0.01 |
| Anti-anxiety (%) | 30 (6%) | 29 (6%) | 0.89 |
| Anti-psychotic (%) | 5 (1%) | 6 (1%) | 0.76 |
| Metabolic equivalents (METs)/Physical parameters | | | |
| Total MET (work and recreational activity) | 2255 ± 3464 | 3167 ± 4803 | 0.01 |
| Work/job requires vigorous activity (%) | 42 (10%) | 27 (6%) | 0.06 |
| Vigorous work MET | 3765 ± 4215 | 6317 ± 7196 | 0.07 |
| Work/job requires moderate activity (%) | 115 (27%) | 128 (31%) | 0.33 |
| Moderate work MET | 2000 ± 2429 | 3046 ± 3368 | <0.01 |
| Walks/bikes for transportation (%) | 75 (18%) | 82 (20%) | 0.55 |
| Transportation MET | 826 ± 952 | 1424 ± 2033 | 0.02 |
| Participates in vigorous recreational activity (%) | 43 (10%) | 38 (9%) | 0.55 |
| Vigorous recreational MET | 658 ± 573 | 688 ± 795 | 0.84 |
| Participates in moderate recreational activity (%) | 173 (41%) | 136 (32%) | <0.01 |
| Moderate recreational MET | 746 ± 595 | 835 ± 841 | 0.28 |
| Cognitive/Well-being parameters | | | |
| Stated health condition (%) | | | |
| Excellent | 26 (6%) | 39 (9%) | 0.38 |
| Very good | 115 (26%) | 111 (26%) | |
| Good | 185 (42%) | 181 (42%) | |
| Fair | 81 (19%) | 80 (19%) | |
| Poor | 31 (7%) | 22 (5%) | |
| Number of days in the past month physical health was not good | 5.6 ± 11.5 | 4.5 ± 8.7 | 0.11 |
| Number of days in the past month mental health was not good | 5.1 ± 11.6 | 4.4 ± 8.8 | 0.32 |
| Number of days in the past month inactive due to physical or mental health | 2.5 ± 7.7 | 1.7 ± 5.7 | 0.12 |
| Physical, mental or emotional limitation kept from working (%) | 82 (18%) | 72 (16%) | 0.38 |
| Experience confusion/memory problem (%) | 62 (13%) | 45 (9%) | 0.08 |
| Limited in activity due to physical, mental or emotional problem (%) | 16 (6%) | 17 (5%) | 0.91 |
| Social factors | | | |
| Smoked at least 100 cigarettes in lifetime (%) | 219 (47%) | 213 (47%) | 0.67 |

(*Continued*)

**Table 2.** Continued

| | $LT_4$-treated ($n$ = 469) | Matched Controls ($n$ = 469) | p-value† |
|---|---|---|---|
| Currently smoking daily (%) | 47 (21%) | 51 (24%) | 0.54 |
| Consumed at least 12 alcoholic drinks per year (%) | 181 (64%) | 178 (62%) | 0.60 |
| Nutrient intake | | | |
| Calories consumed in 24 h recall (kcal/day) | 1761 ± 715 | 1759 ± 927 | 0.98 |
| Calories consumed, adjusted by body weight (kcal/day/kg) | 23 ± 9 | 24 ± 13 | 0.05 |
| % calorie intake compared to DRI for energy | 90 ± 38 | 97 ± 54 | 0.05 |
| Carbohydrate consumed (g) | 217 ± 94 | 219 ± 114 | 0.82 |
| Carbohydrate consumed (%) | 50 ± 11 | 51 ± 11 | 0.39 |
| Protein consumed (g) | 67 ± 31 | 67 ± 35 | 0.87 |
| Protein consumed (%) | 16 ± 4 | 16 ± 5 | 0.95 |
| Fat consumed (g) | 68 ± 36 | 66 ± 41 | 0.59 |
| Fat consumed (%) | 34 ± 9 | 33 ± 9 | 0.33 |
| Selenium intake (mcg) | 52 ± 44 | 52 ± 45 | 0.91 |

Data are mean ± SD, n (%). P-value by $\chi$-square (categorical data) or student's $t$ test (continuous data). $LT_4$: levothyroxine, BMI: body mass index, $HbA_{1C}$: hemoglobin $A_{1C}$, LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol, DRI: dietary reference intake. *For the comparison of $LT_4$-treated and healthy controls. †For the comparison of $LT_4$-treated and matched controls.

lower diastolic blood pressures than healthy controls (Supplemental Table 2), but these differences subsided in the comparison of $LT_4$-treated participants to matched controls (Table 2). Heart rate did not differ between $LT_4$-users and controls, although $LT_4$-users were more likely to be taking beta-blocker medications than controls from either group.

Serum $HbA_{1C}$ levels and fasting glucose values were higher in healthy controls than $LT_4$-treated participants, but these differences were not present in the comparison of $LT_4$-treated participants to the matched controls (Supplemental Table 2 and Table 2). Triglyceride levels did not differ between $LT_4$ users and either control group. Serum LDL, HDL and total cholesterol levels were lower in the $LT_4$-treated group than the matched controls, but more of these participants were taking statin medications (Table 2).

Antidepressant use was more prevalent in $LT_4$-treated participants than healthy or matched controls (Supplemental Table 2 and Table 2). Although more $LT_4$-treated participants were using anxiolytic medications than the healthy controls, there was no difference in distribution of anxiolytic, or antipsychotic use, between $LT_4$-treated participants and matched controls.

Physical activity and metabolic equivalent (MET) assessments were also available in NHANES. In general, $LT_4$-treated participants reported less physical activity via these measures than the healthy controls, but some of these differences were no longer significant in the comparison with matched controls (Supplemental Table 2 and Table 2). $LT_4$-users reported significantly less total, moderate work, and transportation METs than matched controls (Table 2). However, $LT_4$-treated participants reported more participation in moderate recreational activities, 41% vs 32% of matched controls.

Self-report of days in the past month where participants felt that their physical and mental health was 'not good' was more frequent in $LT_4$-users compared to healthy controls, as was report of being inactive due to physical or mental health and frequency of reported problems with confusion/memory (Supplemental Table 2). There was no significant difference in these parameters in the comparison with matched controls, although there was a general trend toward impaired well-being reports in $LT_4$-users (Table 2).

$LT_4$-treated participants consumed less calories per day in a 24-hour dietary recall than healthy controls (Supplemental Table 2). Although $LT_4$-treated participants displayed the same calorie intake compared with matched controls, when adjusted by body weight, $LT_4$-treated participants consumed about 5% less calories per day (Table 2). There were no differences in proportions of carbohydrate, protein, or fat reportedly consumed between the matched controls and $LT_4$-treated participants.

### A lower serum TSH in $LT_4$-treated participants is associated with different metabolic profile but not higher serum $T_3$

The mean serum TSH from the 9981 participants was 1.75 mIU/L. Each participant group ($LT_4$-treated, healthy and matched controls) was further divided into those with serum TSH values above or below this mean (Table 3,

**Table 3.** Thyroid hormone levels of participant groups, by serum TSH.

| | **$LT_4$-treated** (*n* = 469) | | **Matched Controls** (*n* = 469) | |
|---|---|---|---|---|
| | TSH 0.24–1.74 (*n* = 213) | TSH 1.75–5.40 (*n* = 256) | TSH 0.24–1.74 (*n* = 210) | TSH 1.75–5.40 (*n* = 259) |
| Age (years) | 64.1 ± 13.2 | 64.5 ± 14.8 | 64.0 ± 13.4 | 64.3 ± 14.35 |
| Female (%) | 178 (84%) | 182 (71%) † | 181 (83%) | 195 (72%) § |
| Race/Ethnicity (%) | | † | | |
| Non-Hispanic white | 156 (73%) | 180 (70%) | 162 (74%) | 186 (89%) |
| Non-Hispanic black | 19 (9%) | 17 (7%) | 17 (8%) | 21 (8%) |
| Hispanic | 28 (13%) | 48 (19%) | 29 (13%) | 51 (19%) |
| Other | 10 (4%) | 11 (4%) | 9 (4%) | 12 (4%) |
| Serum TSH (mIU/liter) | 0.95 ± 0.43 | 3.11 ± 0.97 † | 0.98 ± 0.41 | 3.09 ± 0.95 § |
| Serum Free $T_3$ (pg/mL) | 2.88 ± 0.34 | 2.83 ± 0.33 | 3.05 ± 0.40 | 2.98 ± 0.38 |
| Serum Total $T_3$ (ng/mL) | 98.04 ± 21.26 | 97.15 ± 20.14 | 111.96 ± 28.38 | 105.32 ± 21.25 § |
| Serum Free $T_4$ (ng/mL) | 0.99 ± 0.21 | 0.90 ± 0.20 † | 0.83 ± 0.14 | 0.78 ± 0.14 § |
| Serum Total $T_4$ (ug/dL) | 9.52 ± 1.80 | 8.84 ± 1.67 † | 8.29 ± 1.48 | 7.92 ± 1.60 § |
| Free $T_3$:Free $T_4$ § | 3.03 ± 0.75 | 3.31 ± 0.81 † | 3.76 ± 0.75 | 3.91 ± 0.75 § |
| Total $T_3$: free $T_4$ § | 104.07 ± 35.09 | 114.19 ± 36.71 † | 138.47 ± 41.09 | 138.45 ± 36.33 |
| Total $T_3$: total $T_4$ § | 10.60 ± 2.63 | 11.34 ± 2.99 † | 13.80 ± 3.85 | 13.61 ± 2.93 |

The mean serum TSH level from the entire population was 1.75 mIU/liter. Participants within each group were then classified as having serum TSH levels above or below this mean, and then thyroid function tests reassessed for each subgroup. Data are mean ± SD, n (%). P-value by $\chi$-square (categorical data) or student's *t* test (continuous data). $LT_4$: levothyroxine; TSH: thyroid stimulating hormone; $T_4$: thyroxine; $T_3$ tri-iodothyronine. *For the comparison of $LT_4$-treated and healthy controls. †For the comparison of $LT_4$-treated and matched controls. §Multiplied × 1000.

Supplemental Tables 3 and 4). Those $LT_4$-users with serum TSH levels below the mean have about 10% higher free and total $T_4$ than those with serum TSH levels above the mean (Table 3). However, serum free and total $T_3$ levels do not differ among $LT_4$-treated participants with serum TSH levels above or below the mean. This resulted in ~10% lower free $T_3$:free $T_4$, total $T_3$:free $T_4$, and total $T_3$:total $T_4$ ratios. In other words, although serum free $T_4$ levels are higher among $LT_4$-users with slightly lower serum TSH levels within the normal range, serum $T_3$ levels (free or total) are unaffected (Table 3).

Despite the lack of difference in serum $T_3$ levels between $LT_4$-users with serum TSH below and above the mean, there were several notable differences between these groups (Supplemental Table 4). $LT_4$-treated participants with lower serum TSH levels had higher serum HDL and lower serum LDL, triglyceride and CRP levels compared to $LT_4$-treated participants with serum TSH levels above the mean; they were also more likely to be using statin medications.

### Factors correlating and associated with the $T_3$:$T_4$ ratio

We next assessed correlation between the 52 clinical parameters possibly associated with thyroid hormone status and the serum free $T_3$:free $T_4$ ratio in participant groups (Supplemental Table 5). No parameter demonstrated a strong correlation with the serum free $T_3$:free $T_4$ ratio. Among the $LT_4$-treated participants, serum triglycerides had the strongest direct correlation with serum free $T_3$:free $T_4$ ratio ($r = 0.30$) and age had the strongest inverse correlation ($r = -0.41$). Assessment for correlation of these 52 parameters with either free $T_3$ (Supplemental Table 6) or free $T_4$ (Supplemental Table 7) was also performed; some factors correlated with free $T_3$ but not free $T_4$, eg, HDL, and some correlated with free $T_4$ but not free $T_3$, eg, triglycerides and antidepressants.

Because these correlations were not strong, we next performed univariate regression analyses to evaluate whether any of the 52 parameters were associated with the serum free $T_3$:free $T_4$ ratio (Table 4 and Supplemental Table 8). In all three groups, age, creatinine, and HDL were negatively associated with the serum free $T_3$:free $T_4$ ratio and BMI, triglycerides, number of calories consumed daily, and grams of fat consumed daily were positively associated. Parameters that were positively associated in both the $LT_4$-treated participants and the healthy controls, but not the matched controls, included smoking history, alcohol consumption and carbohydrate and protein

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**Table 4.** Univariate regression analysis of clinical parameters and the serum free $T_3$:free $T_4$ ratio.

| | $LT_4$-treated ($n$ = 469) | | Matched controls ($n$ = 469) | |
|---|---|---|---|---|
| | Regression Coefficient | p-value | Regression Coefficient | p-value |
| Demographics variables | | | | |
| Age (5 yr increase) | −0.12 | <0.0001 | −0.07 | <0.0001 |
| Female | −0.27 | <0.01 | −0.08 | 0.35 |
| Hispanic | 0.17 | 0.08 | 0.27 | <0.01 |
| Non-Hispanic White | −0.16 | 0.05 | −0.12 | 0.12 |
| Non-Hispanic Black | 0.16 | 0.25 | −0.07 | 0.59 |
| Other race/ethnicity | −0.05 | 0.80 | −0.16 | 0.35 |
| Objective measures | | | | |
| BMI (5 kg/m$^2$ increase) | 0.08 | <0.01 | 0.09 | <0.01 |
| Systolic blood pressure (mm Hg) | −0.01 | 0.02 | −0.01 | 0.41 |
| Diastolic blood pressure (mm Hg) | 0.01 | <0.0001 | 0.01 | <0.0001 |
| Heart rate (beats per minute) | 0.01 | 0.03 | 0.01 | 0.64 |
| HgbA$_{1C}$ (%) | 0.01 | 0.92 | 0.01 | 0.88 |
| Fasting glucose (50 mg/dL increase) | −0.03 | 0.55 | 0.02 | 0.74 |
| Total cholesterol (50 mg/dL increase) | 0.067 | 0.12 | 0.05 | 0.28 |
| LDL (50 mg/dL increase) | 0.04 | 0.64 | 0.05 | 0.52 |
| HDL (50 mg/dL increase) | −0.23 | 0.04 | −0.30 | <0.01 |
| Triglyceride (50 mg/dL increase) | 0.14 | <0.0001 | 0.16 | <0.0001 |
| C-reactive protein (mg/dL) | −0.03 | 0.61 | −0.06 | 0.10 |
| Ferritin (50 ng/dL increase) | 0.01 | 0.85 | 0.07 | 0.12 |
| Creatinine (0.5 mg/dL increase) | −0.11 | <0.01 | −0.25 | <0.0001 |
| Creatinine phosphokinase (50 IU/liter increase) | 0.07 | 0.18 | 0.07 | 0.20 |
| Medication use | | | | |
| beta-blocker | −0.10 | 0.21 | −0.21 | <0.0001 |
| Statin | 0.03 | 0.76 | 0.16 | 0.10 |
| Insulin | 0.17 | 0.35 | −0.06 | 0.78 |
| Oral hypoglycemic | 0.28 | 0.06 | 0.16 | 0.25 |
| Anti-depressant | 0.23 | 0.01 | 0.04 | 0.71 |
| Anti-anxiety | −0.13 | 0.41 | −0.17 | 0.25 |
| Anti-psychotic | −0.23 | 0.52 | −0.24 | 0.45 |
| Metabolic equivalents (METs)/physical parameters | | | | |
| 1000 MET activity (work and rec activity) | 0.01 | 0.46 | 0.01 | 0.31 |
| Work/job requires vigorous activity (%) | −0.01 | 0.96 | 0.06 | 0.71 |
| 1000 Vigorous work MET | −0.03 | 0.39 | 0.03 | 0.21 |
| Work/job requires moderate activity (%) | 0.04 | 0.66 | −0.06 | 0.43 |
| 1000 Moderate work MET | 0.07 | 0.03 | 0.03 | 0.15 |
| Walks/bikes for transportation (%) | −0.10 | 0.34 | −0.04 | 0.67 |
| 1000 Transportation MET | 0.06 | 0.57 | 0.01 | 0.80 |
| Participates in vigorous recreational activity (%) | −0.28 | 0.03 | −0.07 | 0.58 |
| 1000 Vigorous recreational MET | −0.02 | 0.38 | 0.02 | 0.15 |
| Participates in moderate recreational activity (%) | −0.05 | 0.54 | −0.08 | 0.29 |

*(Continued)*

**Table 4.**
Continued

| | | | | | |
|---|---|---|---|---|---|
| 1000 Moderate recreational MET | −0.09 | 0.33 | 0.06 | | 0.40 |
| Cognitive/Well-being parameters | | | | | |
| Excellent/good stated health condition | −0.03 | 0.70 | −0.04 | 0.61 | |
| Poor stated health condition | 0.34 | 0.03 | −0.10 | 0.56 | |
| Number of days in the past month physical health was not good | 0.01 | 0.04 | −0.01 | 0.76 | |
| Number of days in the past month mental health was not good | 0.01 | 0.16 | 0.01 | 0.85 | |
| Number of days in the past month inactive due to physical or mental health | 0.01 | 0.08 | 0.01 | 0.67 | |
| Physical, mental or emotional limitation kept from working | −0.06 | 0.55 | −0.03 | 0.79 | |
| Experience confusion/memory problem | −0.01 | 0.98 | 0.26 | 0.03 | |
| Limited in activity due to physical, mental or emotional problem | 0.36 | 0.08 | −0.04 | 0.85 | |
| Social factors | | | | | |
| Smoked at least 100 cigarettes | −0.02 | 0.82 | 0.02 | 0.79 | |
| Currently smoking daily | 0.27 | 0.04 | 0.09 | 0.50 | |
| Consumed at least 12 drinks per year | −0.20 | 0.04 | 0.06 | 0.51 | |
| Number of alcoholic drinks consumed per day | 0.16 | <0.0001 | 0.11 | 0.01 | |
| Nutrient intake | | | | | |
| 1000 calories consumed in 24 h recall | 0.21 | <0.0001 | 0.09 | | 0.20 |
| Calories consumed, adjusted by body weight (kcal/day/kg) | 0.01 | 0.24 | 0.01 | | 0.68 |
| % calorie intake compared to DRI for energy | 0.01 | 0.24 | 0.01 | | 0.68 |
| 100 grams carbohydrate consumed | 0.11 | <0.01 | 0.05 | | 0.09 |
| Carbohydrate consumed (%) | −0.01 | 0.21 | 0.01 | | 0.35 |
| 50 grams protein consumed | 0.25 | <0.0001 | 0.07 | | 0.15 |
| Protein consumed (%) | 0.01 | 0.87 | −0.02 | | 0.02 |
| 50 grams fat consumed | 0.16 | <0.01 | 0.08 | | 0.06 |
| Fat consumed (%) | 0.01 | 0.68 | −0.01 | | 0.38 |
| Selenium intake (mcg) | 0.01 | 0.98 | −0.01 | | 0.35 |

$LT_4$: levothyroxine, BMI: body mass index, $HbA_{1C}$: hemoglobin $A_{1C}$, LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol, DRI: dietary reference intake.

intake. beta-blocker usage (-) was associated with the serum free $T_3$:free $T_4$ ratio in the healthy and matched controls, but not the $LT_4$-treated participants. Antidepressant usage was positively associated with the serum free $T_3$:free $T_4$ ratio in the $LT_4$-treated participant group alone (Table 4). Many of the 52 clinical parameters were significantly associated with the serum free $T_3$:free $T_4$ ratio in the healthy control group only, including $HbA_{1C}$ (-), fasting glucose (-), LDL (+), and total cholesterol (+) (Supplemental Table 8).

Factors that were identified to be associated with the serum free $T_3$:free $T_4$ ratio in the $LT_4$-treated participants by univariate regression analysis were then assessed in a model of multivariate regression (Table 5 and Supplemental Table 9). In this model, most clinical parameters were no longer significant among the $LT_4$-treated and matched controls. Age was significant in all three groups; for instance in the $LT_4$-treated participants, an age increase of 5 years was associated with a decrease in serum free $T_3$:free $T_4$ ratio of about 0.14 (Table 5). In the $LT_4$-treated participants, calorie consumption was positively associated with the serum free $T_3$:free $T_4$ ratio yet was not significant in either control group. In matched controls, sex was also associated (Table 5). In healthy controls, age, sex, BMI, calorie consumption, creatinine, total cholesterol, and triglycerides were also all associated with the serum free $T_3$:free $T_4$ ratio (Supplemental Table 9).

## Discussion

In comparison to euthyroid individuals not taking $LT_4$, participants taking $LT_4$ with a normal serum TSH exhibited (i) relatively lower serum free and total $T_3$, (ii) relatively higher serum free and total $T_4$, and, consequently, (ii) lower $T_3$:$T_4$ ratios; these relationships were consistent in the comparison with healthy and matched controls. While this phenomenon has been noted in the setting of $LT_4$ monotherapy for the last four decades, (3, 8, 22–29) there are at least two prior, smaller, studies showing that serum $T_3$ levels can be normal in $LT_4$-treated individuals with normal serum TSH (28, 30). While this could be due to the smaller size of the study populations, it is notable that one of these studies is inconsistent with a previous publication from their own group (24).

The major strength of the present studies is the availability of biochemical data as well as markers of quality of life (QOL) in a large population sample to assess for clinical relevance. There were major differences in 7 (out of a total of 21) objective (BMI, total cholesterol, HDL, LDL; beta-blocker, statin and antidepressant use), and 5 (out of a total of 31) subjective (nutrient intake, reported physical activity) clinical parameters between $LT_4$-treated participants and matched controls. While we recognize that these parameters are not specific markers of hypothyroidism and we cannot determine whether they were different between the groups prior to $LT_4$ treatment, this does not mitigate the fact that these data present a strong challenge the dogma that having a normal serum TSH equates with euthyroidism in $LT_4$-treatment.

While it is not clear what underlies the differences in these clinical parameters, the preclinical data indicate an important role played by the suboptimal normalization of serum $T_3$ and/or $T_4$ levels (10). However, the present results revealed that few of the clinical parameters were significantly associated with serum free $T_3$, serum free $T_4$ and/or serum free $T_3$:free $T_4$ ratio by univariate analysis, and the strength of the relationship was not always impressive. Furthermore, statistical significance was lost for most associations in the multivariate analysis. These observations are limited by the cross sectional nature of this

**Table 5.** Multivariate regression analysis identifies clinical parameters associated with the serum free $T_3$:free $T_4$ ratio.

| | **$LT_4$-treated ($n$ = 469)** | | **Matched Controls ($n$ = 469)** | |
|---|---|---|---|---|
| | Regression Coefficient | p-value | Regression Coefficient | p-value |
| Age (5 yr increase) | −0.14 | <0.0001 | −0.10 | <0.0001 |
| Female | – | ns | −0.32 | <0.0001 |
| BMI (5 kg/m$^2$ increase) | – | ns | – | ns |
| Total cholesterol (50 mg/dL increase) | – | ns | – | ns |
| Triglyceride (50 mg/dL increase) | – | ns | – | ns |
| Creatinine (0.5 mg/dL increase) | – | ns | – | ns |
| 1000 MET activity (work and recreation) | – | ns | – | ns |
| 1000 calories consumed | 0.20 | 0.05 | – | ns |
| beta-blocker prescription | – | ns | – | ns |
| Currently smoking daily | – | ns | – | ns |
| Number of alcoholic drinks consumed per day | – | ns | – | ns |

Age was scaled to 5 yr, BMI scaled to 5 kg/m$^2$, total cholesterol scaled to 50 mg/dL, triglyceride scaled to 50 mg/dL, creatinine scaled to 0.5 mg/dL, beta-blocker use, current smoker, number of alcohol drinks consumed per day, total METs scaled to 1000 MET, and calorie intake scaled to 1000 calories. HDL and LDL were omitted due to multicollinearity with total cholesterol. BMI: body mass index, MET: metabolic equivalent.

study but may minimize the potential role for tight maintenance of serum $T_3$ and/or $T_4$ levels in patients with normal serum TSH. As a result, the door is open for other possible explanations, including recognized and unrecognized comorbidities, psychological implications of a chronic illness requiring long-term prescription medication, autoimmune confounders, or increased screenings and treatment in patients who do not feel well (12).

An unrelated byproduct of the present studies was the identification of demographic and biochemical variables that correlate with serum $T_3$:$T_4$ ratios in a large group of normal individuals. Recall that in such group serum $T_3$ and $T_4$ levels as well as the $T_3$:$T_4$ ratio is defined by thyroidal secretion as well as the deiodinase pathways (types 1 and 2). Thus, the multivariate analysis revealed that age, female sex and serum creatinine are negatively associated with serum free $T_3$:free $T_4$ ratio. In contrast, BMI, total serum cholesterol and triglycerides were positively associated with serum free $T_3$:free $T_4$ ratio. At the same time, in $LT_4$-treated individuals, the type 2 deiodinase (D2) is the predominant source of circulating $T_3$, (31) and thus any factor that affects this pathway, and thus the serum $T_3$:$T_4$ ratio, is of great potential clinical relevance. In this regard, in the present investigation we found that in individuals taking $LT_4$, only two variables were significantly associated with serum $T_3$:$T_4$ ratio, namely age and number of calories consumed. The association with age is likely reflecting the fact that skeletal muscle contains D2 and sarcopenia advances with age. At the same time, the association with caloric intake is reminiscent of the fact that insulin stimulates D2-mediated $T_3$ production in the skeletal muscle (32).

There are several limitations to these studies. NHANES is cross-sectional and thus causality cannot be ascertained; it also cannot be determined whether the groups differed prior to treatment with respect to the measured parameters, however, it is reassuring that prevalence of $LT_4$ use in this cohort (about 5%) resembles the prevalence of hypothyroidism that we would expect in an iodine-replete population (13). Participants in these studies were grouped based on their self-reported use of $LT_4$ and there was no availability of records demonstrating previous diagnosis of hypothyroidism. Although hypothyroidism is a very prevalent condition, (13) $LT_4$ is not uncommonly prescribed for euthyroid individuals for other conditions such as fatigue, obesity, and depression, (1) and thus it is possible that the prevalence of these conditions are different between the groups and thus represent a source of confounding. This may not be likely given that euthyroid individuals taking $LT_4$ could exhibit low serum TSH, and thus would have been excluded from these studies. Lastly, there may be additional sources of recall bias or confounding as individuals taking prescription $LT_4$ may report worse QOL because the act of taking/needing a prescription medication may influence their perception of their health; this would more likely influence reporting of subjective variables and would not explain differences in the objective parameters.

In conclusion, NHANES participants with normal serum TSH levels on $LT_4$ montherapy exhibit lower serum $T_3$:$T_4$ ratios than healthy euthyroid controls. $LT_4$-treated individuals have higher BMIs despite reporting lower calorie intake corrected by body weight, report lower physical activity levels, and are more often taking statins, betablockers, and antidepressants. The mechanisms underlying these findings in the $LT_4$-treated individuals remain undefined as we did not observe a significant association in the multivariable analysis with serum free $T_3$, free $T_4$ or free $T_3$:free $T_4$ ratio. Notwithstanding, the concept that establishing a normal serum TSH renders individuals on $LT_4$ monotherapy clinically euthyroid should be revisited and QOL measures should be more highly prioritized in hypothyroidism research and professional guidelines.

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