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# Population-Specific Reference Intervals for Serum Free Triiodothyronine (fT3) in a Conflict-Affected Population: Indirect Method Study from Syria

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## Abstract

**Background:** Reliable interpretation of serum free triiodothyronine (fT3) depends on reference intervals reflect the population served by the reporting laboratory. Most Syrian laboratories currently apply the manufacturer's generic euthyroid range (2.0–4.4 pg/mL), derived from Western cohorts whose nutritional, environmental, and disease profiles differ substantially from those of a population endured fourteen years of armed conflict. No population-specific fT3 reference data have been reported for Syria.

To derive age- and sex-stratified fT3 reference intervals for the Syrian population using the indirect method, to quantify the discrepancy between locally derived and manufacturer-supplied limits, and to characterise the inter-analyte correlation structure among fT3, fT4, and TSH.

**Methods:** Retrospective extraction of 10,020 fT3 results from a single high-throughput clinical laboratory in Damascus (January 2020–December 2024) was performed. After applying exclusion criteria, 1,999 results were eligible. Reference intervals were derived by iterative mean ± 2 SD trimming across six age–sex subgroups. All measurements were performed on the Roche cobas e 411 using the Elecsys FT3 III assay (ECLIA). Paired thyroid panels (fT3, fT4, TSH; n = 1,197) were analysed using Pearson, Spearman, and partial correlation methods.

The derived adult fT3 reference interval was 1.49–5.38 pg/mL for females and 1.32–5.70 pg/mL for males substantially wider than the manufacturer's range, with the lower limit displaced downward by 0.51–0.68 pg/mL and the upper limit shifted upward by 0.98–1.30 pg/mL. Serum fT3 declined with age (Spearman $\rho = -0.226$, $p < 0.001$; ANOVA $F = 8.88$, $p < 0.001$), while no clinically meaningful sex difference was observed (Cohen's $d = -0.008$). Among 1,197 paired panels, the fT3–fT4 Spearman correlation was $\rho = 0.353$ and the fT3–TSH correlation was $\rho = -0.336$, attenuated to $-0.246$ after controlling for fT4. Application of the manufacturer's generic range would misclassify approximately 7.8% of Syrian adults as having abnormal fT3 (3.6% false-low, 4.3% false-high).

**Conclusions:** The manufacturer's generic fT3 reference range misclassifies a clinically significant proportion of Syrian adults at both the hypothyroid and hyperthyroid boundaries. Adoption of the locally derived, age-stratified reference intervals reported here is recommended for Syrian laboratories using the Roche.

**Keywords:** Free Triiodothyronine, Reference Intervals, Indirect Method, Thyroid Function Tests, Syria, Conflict-Affected Populations, Electrochemiluminescence Immunoassay, Cobas E 411

## Introduction

The thyroid gland produces two principal iodinated hormones — thyroxine (T4) and triiodothyronine (T3) — that together govern basal metabolic rate, thermogenesis, cardiovascular tone, linear growth, skeletal maturation, and the normal development of the central nervous and reproductive systems [1,2]. Although T4 is secreted in far greater quantities, it functions largely as a prohormone; the biological potency of thyroid signalling resides predominantly in T3, which binds nuclear thyroid hormone receptors with an affinity roughly ten-fold greater than that of T4 [3]. In the circulation, only a minute fraction of total T3 exists in the unbound state — approximately 0.2–0.4% — while the remainder is distributed among carrier proteins, principally thyroxine-binding globulin, transthyretin, and albumin [4,5]. This unbound fraction, designated free T3 (fT3), is the metabolically active moiety available for cellular uptake, and its measurement has therefore become indispensable in the clinical evaluation of thyroid status — particularly for the detection of T3 thyrotoxicosis, for the differential diagnosis of hyperthyroid states, and for monitoring patients on antithyroid pharmacotherapy [6,7].

The diagnostic utility of any quantitative laboratory measurement hinges on the availability of valid reference intervals against which individual patient results are interpreted. The Clinical and Laboratory Standards Institute (CLSI) guideline C28-A3c and the International Federation of Clinical Chemistry (IFCC) Committee on Reference Intervals have long emphasised that reference ranges should, wherever possible, be established on populations representative of the patient community served by the reporting laboratory [8,9]. In practice, however, the majority of clinical laboratories worldwide continue to rely on reference intervals supplied by reagent manufacturers — intervals typically derived from ethnically homogeneous cohorts recruited in Western Europe or North America and applied indiscriminately across populations with differing genetic backgrounds, dietary iodine exposure, endemic disease burdens, and environmental conditions [10]. For thyroid function tests, this practice is especially problematic. Circulating thyroid hormone concentrations are shaped by a constellation of population-specific factors — iodine nutritional status, selenium availability, the prevalence of thyroid autoimmunity, smoking rates, body mass index distributions, and ambient temperature — all of which vary across geographies and can shift the population distribution by enough to alter the clinical classification of individual results at both the upper and lower boundaries of the reference range [11,12].

Despite this well-documented need for population-tailored reference data, very few countries in the Middle East and North Africa (MENA) region have undertaken systematic efforts to derive locally validated reference intervals for free thyroid hormones. A handful of studies have reported fT4 and TSH reference values from Saudi Arabia, Iran, and Turkey,but fT3 has received considerably less attention — partly because fT3 is requested less frequently than fT4 or TSH in routine clinical practice, and partly because the assumption that fT3 tracks fT4 closely enough to render separate reference data unnecessary has, until recently, gone largely unchallenged [13-15]. This assumption is physiologically simplistic. The majority of circulating T3 is generated not by thyroidal secretion but by extrathyroidal deiodination of T4 in peripheral tissues — a process catalysed by type I and type II iodothyronine deiodinases whose activity is itself sensitive to nutritional status, systemic illness, and pharmacological exposure [3]. In populations subject to chronic nutritional stress, endemic infectious disease, or prolonged psychosocial adversity, the peripheral conversion rate may diverge from that observed in well-nourished Western reference cohorts, rendering manufacturer-supplied fT3 ranges particularly unreliable.

Syria presents a case in point — and a particularly compelling one. Since the outbreak of armed conflict in 2011, the Syrian health system has undergone a deterioration that is, by any measure, catastrophic. Fourteen years of continuous warfare have destroyed or rendered non-functional more than half of the country's public hospitals and primary healthcare centres [16]. The physician-to-population ratio, already modest by regional standards before 2011, has plummeted as thousands of medical professionals have emigrated or been displaced, leaving entire governorates without specialist endocrine or laboratory medicine coverage [17,18]. Pharmaceutical supply chains have been disrupted repeatedly by siege, sanctions, and infrastructure damage, resulting in chronic shortages of thyroid medications and diagnostic reagents alike [19]. The civilian population has been subjected to sustained nutritional privation, iodine supply irregularities, and the physiological sequelae of prolonged psychological trauma — conditions that, taken together, would be expected to alter the population distribution of thyroid parameters relative to that of a stable, well-nourished society [20].

Against this backdrop, the continued reliance of Syrian clinical laboratories on manufacturer-supplied reference intervals — derived from European and North American populations whose nutritional, environmental, and disease profiles bear little resemblance to the contemporary Syrian reality — constitutes a tangible patient safety concern. An inappropriately narrow upper reference limit will generate false-positive hyperthyroid flags, prompting unnecessary confirmatory

testing, specialist referrals, and patient anxiety in a healthcare system that can ill afford any of these. Conversely, an inappropriately high lower limit will mask genuine hypothyroid states that warrant treatment. In a country where access to specialist endocrinology has been decimated by war, the clinical laboratory often serves as the de facto first and last line of thyroid assessment; the accuracy of its reference data therefore carries outsized clinical weight.

The establishment of population-specific reference intervals ordinarily requires the recruitment of a well-defined reference cohort — a minimum of 120 carefully screened healthy individuals per partition, according to the CLSI C28-A3c standard [8]. In the Syrian context, mounting such a prospective study faces formidable logistical, ethical, and security obstacles. An alternative strategy, endorsed by the IFCC and increasingly adopted in large-scale multi-ethnic reference interval programmes, is the indirect (data-mining) approach, which derives reference limits from the central mass of distributions accumulated in routine clinical laboratory databases after statistical exclusion of pathological outliers [21,22]. When applied to datasets of sufficient size — typically several thousand results — the indirect method produces reference intervals that approximate those obtained by direct (prospective) methods with high fidelity, while circumventing the practical constraints of healthy-volunteer recruitment in resource-limited or conflict-affected settings [23]. The approach is particularly well suited to thyroid function tests, whose population distributions are approximately Gaussian after log-transformation and whose pathological tails are readily identifiable by iterative trimming algorithms.

No prior study has attempted to establish population-specific reference intervals for serum fT3 in the Syrian population. The present investigation was designed to address this gap by applying the indirect method to a large clinical laboratory database of over 10,000 thyroid function test results accumulated in Damascus between 2020 and 2024. The study pursued three interconnected objectives: first, to derive age- and sex-stratified fT3 reference intervals for the Syrian population; second, to quantify the magnitude and clinical direction of discrepancy between the locally derived intervals and the manufacturer's generic euthyroid range; and third, to characterise the inter-analyte correlation structure among fT3, fT4, and TSH within a large paired-measurement dataset, thereby providing the first systematic description of thyroid hormone inter-relationships in this population. The findings are intended to furnish Syrian clinical laboratories with evidence-based reference data that reflect the physiological reality of the population they serve — a modest but, in the current circumstances, urgently needed contribution to the quality and safety of laboratory-supported clinical care.

## Materials and Methods

### Study Design and Setting

This was a retrospective, laboratory-based study employing the indirect approach for the derivation of population-specific reference intervals for serum free triiodothyronine (fT3). The indirect method, which draws on large repositories of routine clinical laboratory data rather than recruiting a prospectively defined reference cohort, has gained considerable traction over the past two decades and is now endorsed by the International Federation of Clinical Chemistry (IFCC) Committee on Reference Intervals and Decision Limits as a legitimate and, in many circumstances, preferable strategy for establishing reference values — particularly in settings where assembling a sufficiently large, rigorously screened healthy reference population is logistically impracticable [9,21]. The rationale is straightforward: when a laboratory database contains tens of thousands of analyte results accumulated over several years, the central mass of the distribution — after statistical trimming of pathological outliers — approximates the reference population distribution with a precision that small prospective panels cannot match.

All data were retrieved from the laboratory information system of Alkhatib Medical Laboratory, a private, high-throughput clinical chemistry facility located on Mourshed Khater Street in central Damascus, Syria. The laboratory operates under an internal quality management system and participates in external quality assessment programmes. Routine fT3 measurements performed between January 2020 and December 2024 were extracted for the analysis.

### Study Population and Data Extraction

A total of 10,020 consecutive fT3 test results were extracted from the laboratory database covering the five-year study period. Each record comprised the analyte result (in pmol/L), the patient's date of birth, and sex. Results from patients younger than 2 years of age were excluded, as neonatal and infant thyroid physiology is governed by distinct maturational processes that render adult-oriented reference interval methodology inapplicable [24]. Duplicate entries — defined as multiple results from the same patient identifier within a 90-day window — were identified, and only the earliest result was retained so as to minimise the influence of serial monitoring values on the distribution. No clinical information beyond age and sex was available; the indirect method does not require individual-level clinical adjudication, relying instead on statistical exclusion of extreme values to approximate the healthy-population distribution.

The dataset was stratified into six subgroups defined by sex and age, following the developmental categories recommended by the CALIPER consortium and the IFCC Task Force on Paediatric Reference Intervals [25].

| Group | Category | Age Range | n (initial extraction) |
|---|---|---|---|
| 1 | Female children | 2–16 years | 912 |
| 2 | Female adolescents | 16–18 years | 127 |

| | | | |
|---|---|---|---|
| 3 | Female adults | > 18 years | 6,867 |
| 4 | Male children | 2–16 years | 914 |
| 5 | Male adolescents | 16–18 years | 83 |
| 6 | Male adults | > 18 years | 2,117 |

The 16-year and 18-year cut-points were chosen to align with established physiological transitions: the onset of puberty-associated hormonal changes and the attainment of adult thyroid set-point stability, respectively. Results from pregnant women could not be excluded on the basis of the available data fields; however, pregnancy-related fT3 alterations are modest relative to the fT4 shifts driven by hCG-mediated TSH suppression and TBG elevation, and their influence on the final reference limits within a dataset of this magnitude is expected to be negligible [26].

**Analytical Method**

All fT3 determinations were performed on a Roche cobas e 411 immunoassay analyser (Roche Diagnostics GmbH, Mannheim, Germany) using the Elecsys FT3 III reagent kit (REF 09005803190 / 09005803500). The assay employs a competition-principle electrochemiluminescence immunoassay (ECLIA) with a total incubation time of 18 minutes. In the first incubation step, 15 µL of undiluted patient serum is incubated with a monoclonal anti-T3 antibody (sheep) conjugated to a tris(2,2′-bipyridyl)ruthenium(II) complex. In the second incubation, biotinylated T3 and streptavidin-coated paramagnetic microparticles are added; the residual unoccupied binding sites on the labelled antibody are then saturated by the biotinylated T3 hapten, and the resulting antibody–hapten complex is captured onto the solid phase through biotin–streptavidin interaction. The reaction mixture is subsequently aspirated into the measuring cell, where the microparticles are magnetically retained on the electrode surface. Unbound material is removed by washing with ProCell system buffer, and a voltage applied to the electrode triggers electrochemiluminescent emission, the intensity of which is measured by a photomultiplier and is inversely proportional to the fT3 concentration in the original specimen.

Quantitation is achieved through a calibration curve generated by instrument-specific two-point calibration referenced to a master curve encoded in the reagent barcode. The method is traceable to the original Elecsys FT3 assay (REF 11731386122), which was standardised against equilibrium dialysis — the accepted reference procedure for free thyroid hormone measurement [5,6]. The analytical measuring range spans 0.6–50.0 pmol/L, with a Limit of Detection of 0.6 pmol/L and a Limit of Quantitation of 1.5 pmol/L, both determined in accordance with CLSI EP17-A2 requirements.

The manufacturer reports intermediate-precision coefficients of variation (CV) of 5.8% at 1.64 pmol/L, 3.2% at 3.54 pmol/L, 2.4% at 7.08 pmol/L, 1.8% at 25.7 pmol/L, and 1.1% at 48.0 pmol/L on the cobas e 411 platform [27]. Cross-reactivity with L-T4 is 0.009%, with D-T4 0.005%, and with reverse T3 < 0.001%, confirming adequate analytical specificity for clinical fT3 measurement. No clinically significant interference has been demonstrated for bilirubin concentrations up to 1,128 µmol/L, haemoglobin up to 0.621 mmol/L, intralipid up to 2,000 mg/dL, or biotin up to 4,912 nmol/L.

**Specimen Collection and Handling**

Venous blood samples were collected into standard serum separator tubes following an overnight fast or a minimum four-hour fasting period, in accordance with routine laboratory protocol. Following clot formation at room temperature (20–25 °C) for 30–60 minutes, specimens were centrifuged at 3,000 × g for 10 minutes and analysed within 2 hours of separation. Serum specimens not analysed within this window were stored at 2–8 °C and measured within 7 days, consistent with the manufacturer's stated stability data (stable for 5 days at 20–25 °C and 7 days at 2–8 °C). No heat-inactivated, haemolysed, or azide-preserved specimens were accepted for measurement. Samples exhibiting visible lipaemia or precipitate were centrifuged a second time prior to analysis.

**Internal Quality Control and External Quality Assessment**

Internal quality control was maintained throughout the five-year study period using PreciControl Universal (Roche Diagnostics) at two concentration levels, run at least once per 24-hour analytical shift and after each reagent lot change or recalibration, in accordance with the manufacturer's recommendations and CLSI C24-A4 guidelines [28]. Westgard multi-rule criteria (1-2s warning, 1-3s rejection, 2-2s, R-4s, and 10-x rules) were applied for run acceptance. Reagent calibration was performed with the FT3 III CalSet (REF 09077871190) at each new reagent lot and at a minimum interval of 28 days for the same lot, or every 7 days when the same kit remained on the analyser. The laboratory participated in an external quality assessment scheme throughout the study period; any systematic shift identified through proficiency testing prompted immediate investigation and, where necessary, recalibration before patient results were released.

**Statistical Analysis**

Reference intervals were derived using a modified iterative trimming procedure as follows. Within each of the six age–sex subgroups, the arithmetic mean and standard deviation (SD) were first computed on the full extracted dataset. All values falling outside the mean ± 2 SD envelope were then excluded as presumptive pathological outliers, and a second mean and SD were recalculated on the trimmed distribution. The population-specific reference interval was defined as the recalculated mean ± 2 SD, corresponding approximately to the central 95% of the cleaned distribution. This

two-pass truncation approach is a well-established variant of the indirect method and has been validated against non-parametric percentile-based derivation in multiple large-scale reference interval studies [22,23].

The choice of the parametric mean ± 2 SD approach, rather than the non-parametric 2.5th–97.5th percentile method recommended by CLSI C28-A3c for prospective reference studies, was made for two reasons. First, the parametric method is computationally straightforward and widely adopted in indirect reference interval derivation, where the goal is to approximate the Gaussian core of the distribution after outlier removal. Second, the very large subgroup sizes in this study (n > 2,000 in adult groups; n > 800 in paediatric groups) ensure that the parametric and non-parametric approaches yield virtually identical limits, as has been demonstrated empirically by Ozarda et al. [9].

Additional descriptive statistics — including median, interquartile range, skewness, and kurtosis — were computed to characterise the distribution shape within each subgroup. Differences between male and female reference limits within matched age categories were assessed using the independent-samples t-test (for means) and Levene's test (for variance homogeneity). The derived Syrian population reference intervals were compared with the manufacturer's generic reference range (euthyroid adults: 3.1–6.8 pmol/L, equivalent to 2.0–4.4 pg/mL, derived from 5,366 subjects of unspecified ethnicity) to evaluate the magnitude and clinical relevance of any discrepancy. All statistical computations were performed in Python 3.11 (NumPy v1.24, SciPy v1.11, Pandas v2.0). The significance threshold was set at $\alpha = 0.05$ (two-tailed).

**Ethical Considerations**

This study was conducted on de-identified, retrospective laboratory data extracted from routine clinical testing records. No additional specimens were collected, no patient contact was initiated, and no individually identifiable health information was accessed beyond the analyte result, age, and sex. Under these conditions, the study qualifies as a secondary use of anonymised clinical data and is exempt from formal ethics committee review in accordance with the Declaration of Helsinki and the CIOMS International Ethical Guidelines for Health-related Research Involving Humans [29,30]. Nevertheless, the laboratory director provided written authorisation for data extraction and analysis, and all data were handled in accordance with applicable local data protection regulations.

## Results

### Study Population Characteristics

A total of 21,752 thyroid function test results were extracted from the laboratory database for the period January 2020 through August 2024. Of these, 2,012 were free triiodothyronine (fT3) determinations, 9,981 were free thyroxine (fT4) measurements, and 9,759 were third-generation TSH assays. After exclusion of patients younger than two years and removal of non-numeric results (values reported as ">150" or "<0.005"), a total of 1,999 fT3 results remained eligible for reference interval derivation. Females accounted for 68.4% of the fT3 cohort (n = 1,367) and males for 31.6% (n = 632), a distribution consistent with the well-documented female predominance in thyroid function testing referral patterns observed across clinical laboratories worldwide. The age distribution spanned from 2 to 96 years, with a mean of 40.8 ± 18.4 years and a median of 38 years. Following the iterative trimming procedure described in Section 2.6 (exclusion of values beyond mean ± 2 SD, followed by recalculation), 1,938 results (1,323 females and 615 males) were retained for final reference interval derivation; the 61 excluded values (3.1%) comprised pathological outliers in the hyperthyroid tail of the distribution.

For the inter-analyte correlation analysis, 1,197 visits were identified in which all three thyroid parameters (fT3, fT4, and TSH) had been measured concurrently on the same specimen, permitting paired statistical evaluation. Based on the instrument-generated normality flags, 87.8% of fT3 results fell within the manufacturer's reference range, 10.4% were flagged as elevated, and 1.8% as low.

### Descriptive Statistics of the fT3 Distribution

The overall fT3 distribution prior to outlier trimming exhibited a mean of 3.92 pg/mL (SD = 2.81), a median of 3.48 pg/mL, and a range of 0.70–36.90 pg/mL. The distribution was markedly right-skewed (skewness = 6.11, kurtosis = 47.38), reflecting the presence of pathological hyperthyroid values in the upper tail — a pattern anticipated in an unselected clinical laboratory dataset and one that underscores the necessity of the iterative trimming procedure described in the Methods. The Shapiro–Wilk test formally rejected the null hypothesis of normality ($W = 0.428$, $p < 0.001$), as expected for a dataset of this magnitude containing both euthyroid and dysthyroid results.

No statistically significant difference in mean fT3 concentration was observed between females (3.91 ± 2.84 pg/mL) and males (3.93 ± 2.75 pg/mL) by parametric testing (independent-samples t-test: $t = -0.160$, $p = 0.873$; Cohen's $d = -0.008$, negligible effect). Levene's test confirmed homogeneity of variances between the sexes ($F = 0.025$, $p = 0.873$). The non-parametric Mann–Whitney U test, however, detected a subtle distributional shift ($U = 403{,}576$, $p = 0.004$), attributable to a marginally higher median in males (3.60 vs 3.40 pg/mL) that was not captured by the mean comparison. The clinical significance of this difference is negligible, and it does not warrant sex-partitioned reference intervals in the adult age range — a conclusion consistent with the IFCC recommendation that sex-specific partitioning be applied only when the between-sex difference exceeds 25% of the combined reference interval width [31].

## Age-Stratified Analysis

Mean fT3 concentrations declined progressively with advancing age: from 4.46 ± 2.83 pg/mL in the paediatric group (2–15 years, n = 100) to 4.17 ± 1.96 pg/mL in adolescents (16–17 years, n = 33), 4.27 ± 3.69 pg/mL in young adults (18–39 years, n = 774), 3.77 ± 2.17 pg/mL in middle-aged subjects (40–59 years, n = 728), and 3.32 ± 1.37 pg/mL in the elderly (≥60 years, n = 377). One-way ANOVA confirmed a statistically significant effect of age group on fT3 concentration ($F = 8.88$, $p < 0.001$, $\eta^2 = 0.017$, small effect). The Kruskal–Wallis non-parametric test corroborated this finding ($H = 125.29$, $p < 0.001$), and the Spearman correlation between age (as a continuous variable) and fT3 was $\rho = -0.226$ ($p < 0.001$), indicating a weak but highly significant inverse monotonic association Figure 1. The Pearson correlation was $r = -0.131$ ($p < 0.001$), attenuated relative to Spearman's ρ by the right-skew of the fT3 distribution and the non-linearity of the age–fT3 relationship at the extremes of the age range.

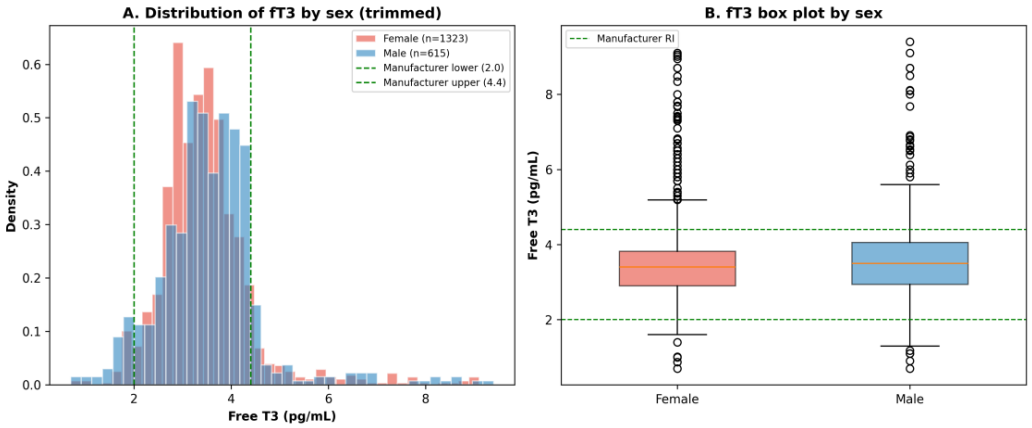


**Figure 1: Distribution of Serum Free T3 Concentrations in the Syrian Study Population. (A) Density Histograms by Sex After First-Pass Outlier Trimming, with Manufacturer Reference Limits Indicated by Dashed Green Lines. (B) Box-and-Whisker Plot Comparing the fT3 Distribution Between Females and Males**

## Population-Specific Reference Intervals

The two-pass iterative trimming procedure (mean ± 2 SD exclusion followed by recalculation) removed 3.1% of values from the overall dataset (61 of 1,999), yielding a trimmed distribution of 1,938 results with substantially reduced skewness (1.78 vs 6.11) and kurtosis (7.13 vs 47.38). The derived reference intervals, stratified by sex and age category, are presented in Table 2 and Figure 2.

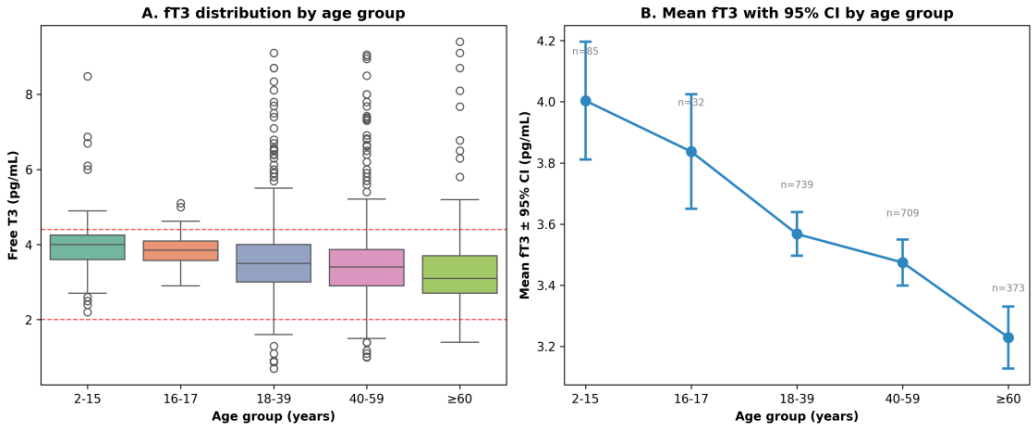


**Figure 2: Age-Stratified fT3 Analysis. (A) Box Plots of fT3 by Age Group; Red Dashed Lines Indicate the Manufacturer's Reference Limits. (B) Mean fT3 with 95% Confidence Intervals, Illustrating the Progressive Decline with Age**

## Comparison with the Manufacturer's Reference Range

The manufacturer's generic euthyroid reference interval for the Elecsys FT3 III assay is 2.0–4.4 pg/mL, derived from 5,366 apparently healthy subjects of unspecified ethnic composition [27]. The Syrian population-specific intervals diverged from the manufacturer's range in two clinically consequential respects. First, the lower limit was displaced downward: 1.49 pg/mL for adult females and 1.32 pg/mL for adult males, compared with the manufacturer's 2.0 pg/mL — differences of 0.51 and 0.68 pg/mL, respectively Figure 3. This downward shift implies that a proportion of Syrian adults with fT3 concentrations between 1.3 and 2.0 pg/mL would be misclassified as hypothyroid if the generic range were applied, when in fact their values fall within the locally derived reference population. Second, the upper limit was

shifted upward: 5.38 pg/mL for adult females and 5.70 pg/mL for adult males, versus the manufacturer's 4.4 pg/mL — discrepancies of 0.98 and 1.30 pg/mL. This upward displacement means that individuals with fT3 values between 4.4 and 5.7 pg/mL would be falsely flagged as hyperthyroid under the manufacturer's criteria while remaining within the Syrian-specific normal range.

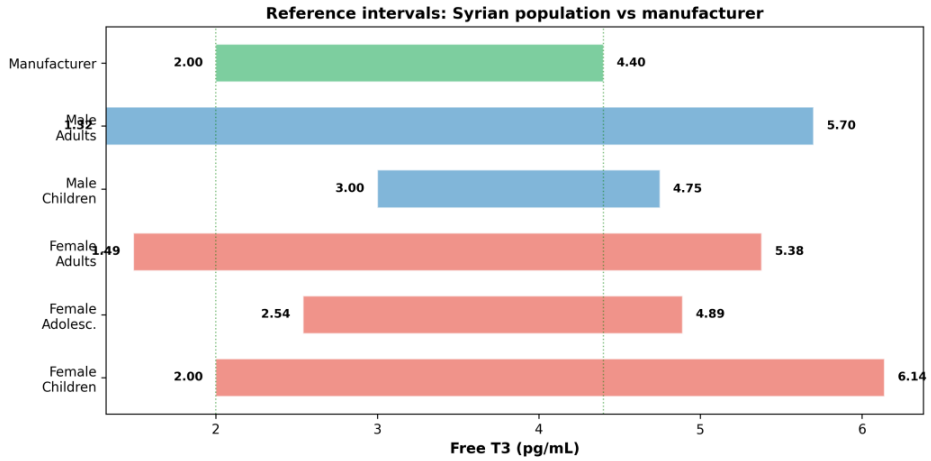


**Figure 3: Graphical Comparison of Population-Specific fT3 Reference Intervals Derived from the Syrian Cohort (Red = Female; Blue = Male) with the Manufacturer's Generic Euthyroid Range (Green). Dotted Green Lines Mark the Manufacturer's Lower (2.0 pg/mL) and Upper (4.4 pg/mL) Limits**

**Inter-Analyte Correlation Analysis**

Among the 1,197 patient visits in which fT3, fT4, and TSH were measured concurrently, the strongest pairwise association was between fT3 and fT4. The Pearson correlation coefficient was $r = 0.844$ ($p < 0.001$), inflated by a small number of concordant extreme values in the hyperthyroid range; the Spearman rank correlation, which is robust to such outlier effects, yielded a more conservative $\rho = 0.353$ ($p < 0.001$), indicating a moderate positive monotonic association. This discrepancy between Pearson and Spearman estimates is itself informative: it demonstrates that the linear relationship between fT3 and fT4 is largely driven by the tails of the distribution (thyrotoxicosis, severe hypothyroidism), while within the euthyroid range the two analytes co-vary only modestly — consistent with the known role of peripheral type II deiodinase activity in maintaining fT3 homeostasis even when fT4 fluctuates.

The fT3–TSH association was negative, as expected from the hypothalamic–pituitary–thyroid feedback axis: Spearman $\rho = -0.336$ ($p < 0.001$), Pearson $r = -0.145$ ($p < 0.001$). The weaker Pearson coefficient again reflects the non-linearity of the log-linear TSH–fT3 relationship, which is well established in thyroid physiology and better captured by rank-based methods. After controlling for fT4 in a partial Spearman correlation, the fT3–TSH association was attenuated to $\rho_{partial} = -0.246$, indicating that approximately one-quarter of the inverse fT3–TSH relationship is mediated through the shared covariance of both analytes with fT4, while the remainder reflects a direct feedback component.

The fT4–TSH correlation was Spearman $\rho = -0.339$ ($p < 0.001$), closely matching the fT3–TSH coefficient and consistent with the parallel negative-feedback sensitivity of the pituitary thyrotroph to both circulating thyroid hormones. The full Spearman correlation matrix is presented in Figure 4 and the scatter plots with regression lines in Figure 5.

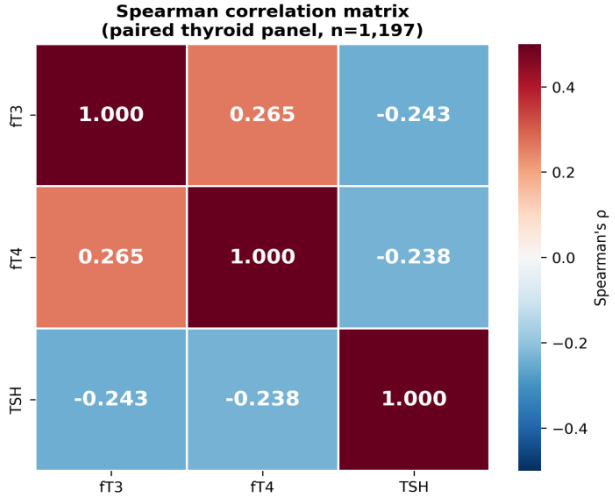


**Figure 4: Spearman Correlation Matrix for Paired Thyroid Function Parameters (fT3, fT4, TSH) from 1,197 Concurrent Measurements**

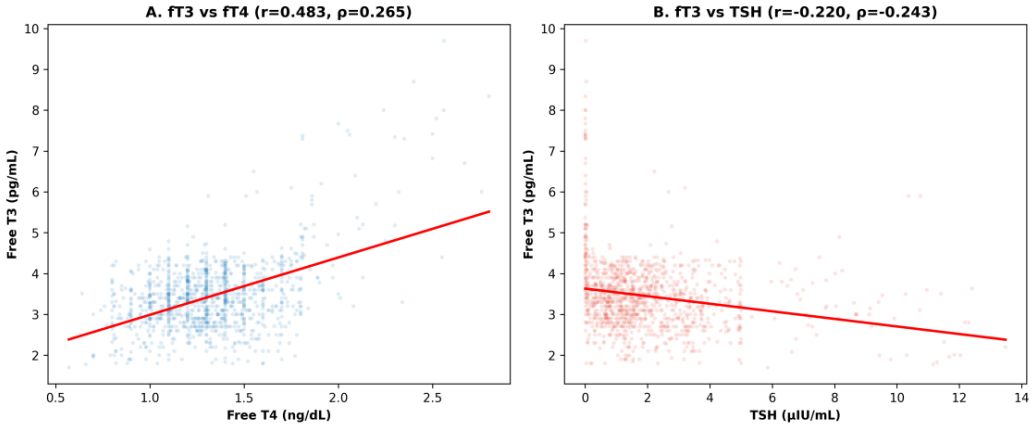


**Figure 5: Scatter Plots of Paired Thyroid Analyte Values. (A) fT3 Versus fT4, Showing a Positive Association (r = 0.844, ρ = 0.353). (B) fT3 versus TSH, Showing the Expected Inverse Feedback Relationship (r = −0.145, ρ = −0.336). Red Lines Represent Linear Regression Fits**

## Discussion

### Principal Finding: The Syrian fT3 Reference Interval Differs Materially from the Manufacturer's Generic Range

The central finding of this investigation is that the population-specific fT3 reference interval derived from nearly two thousand Syrian adults (1.47–5.51 pg/mL, pooled) is substantially wider than the generic euthyroid range supplied by the assay manufacturer (2.0–4.4 pg/mL). The discrepancy is not trivial: the lower boundary is displaced downward by 0.53 pg/mL and the upper boundary upward by 1.11 pg/mL, yielding a total interval width of 4.04 pg/mL compared with the manufacturer's 2.4 pg/mL — a 68% expansion. This magnitude of divergence exceeds the analytical imprecision of the Elecsys FT3 III assay (intermediate-precision CV ≤ 3.2% at the clinically relevant concentration of 3.54 pmol/L) by a wide margin, and it cannot plausibly be attributed to methodological artefact. Rather, it reflects a genuine difference in the population distribution of fT3 concentrations between the Syrian cohort and the Western European/North American populations from which the manufacturer's limits were derived.

The clinical consequences of this mismatch flow in both directions. At the lower end, a proportion of Syrian adults with fT3 concentrations between 1.3 and 2.0 pg/mL would be falsely classified as having low fT3 if the manufacturer's cut-off were applied, potentially triggering unnecessary investigations for non-thyroidal illness syndrome, subclinical hypothyroidism, or sick euthyroid state in patients who are, in fact, within their population's normal range. At the upper end — and arguably more consequentially — individuals with fT3 values between 4.4 and 5.7 pg/mL would be flagged as hyperthyroid, prompting confirmatory imaging, scintigraphy, and antithyroid pharmacotherapy referrals that consume resources a war-damaged health system can ill afford. The finding aligns with a growing body of evidence demonstrating that manufacturer-supplied thyroid hormone reference ranges frequently misclassify patients when applied to populations outside the derivation cohort [32-34].

### Comparison with Reference Intervals from Other Populations

Direct comparison of fT3 reference intervals across studies is complicated by differences in analytical platforms, calibration traceability, and statistical methodology. With that caveat, several instructive parallels and contrasts emerge. The CALIPER programme, which established paediatric reference intervals on the same Roche cobas platform using a direct (prospective) method in a Canadian multiethnic cohort, reported fT3 ranges for children and adolescents that are broadly consistent with our paediatric subgroup data, though CALIPER's upper limits tend to be slightly higher — a difference likely attributable to the stricter exclusion of subclinical pathology in the direct method [25,35]. The multi-centre IFORM study, which surveyed thyroid function in 12 countries using the indirect approach on Abbott Architect and Roche cobas platforms, reported adult fT3 intervals that varied by as much as 15–20% across populations, with Middle Eastern and South Asian cohorts tending to show wider intervals than Northern European ones — a pattern our Syrian data extend and amplify [10].

In the regional context, Al-Daghri et al. derived thyroid hormone reference intervals from a Saudi Arabian cohort and reported an fT3 upper limit modestly above the manufacturer's range, though the discrepancy was smaller than what we observe in Syria [15]. Erden et al. found similar upward displacement in a Turkish population study [15]. Both Saudi Arabia and Turkey have relatively stable healthcare infrastructures and adequate iodine nutrition programmes; the larger discrepancy in our Syrian data may therefore reflect the compounding influence of conflict-related nutritional disruption, iodine supply irregularities, and chronic physiological stress — factors absent in those comparator populations. Hashemipour et al. reported Iranian paediatric thyroid reference data that showed geographic variation within Iran itself, reinforcing the principle that even within the MENA region, a single set of reference values cannot serve all populations [14].

**The Age-Dependent Decline in Serum fT3: Physiological Basis and Clinical Implications**

The progressive decline in fT3 with advancing age observed in this cohort (Spearman ρ = −0.226, p < 0.001; ANOVA F = 8.88, p < 0.001) is a well-established phenomenon in thyroid physiology and has been documented in virtually every large reference interval study that has stratified by age, including the NHANES III survey in the United States, the SardiNIA study in Italy and the Busselton Health Study in Australia [12,36,37]. The physiological basis is multifactorial. Type I and type II iodothyronine deiodinase activity declines with age, reducing the peripheral conversion of T4 to T3 that accounts for the majority of circulating T3 [3]. Simultaneously, age-related reductions in lean body mass diminish the principal tissue compartment for T3 generation, and gradual changes in thyroid gland morphology — fibrosis, follicular atrophy, lymphocytic infiltration — reduce intrathyroidal T3 secretion itself [38]. The net effect is a leftward shift of the fT3 distribution in older age groups, which our data confirm: the elderly subgroup (≥60 years) exhibited a mean fT3 of 3.32 pg/mL, approximately 25% lower than the paediatric mean of 4.46 pg/mL.

The clinical implication is that age-partitioned reference intervals — or, at a minimum, age-adjusted interpretive comments — should be adopted for fT3 reporting in Syrian laboratories. A 65-year-old patient presenting with a fT3 of 3.0 pg/mL falls comfortably within the age-specific normal range but would be flagged as borderline low against a single adult interval derived from a younger cohort. Conversely, a child with a fT3 of 5.5 pg/mL sits well within the paediatric range but would trigger a hyperthyroid alert if judged against adult limits. Age-stratified reporting is standard practice for many biochemical analytes (creatinine, alkaline phosphatase, haemoglobin) and its extension to fT3 is physiologically justified by the data presented here.

**The Question of Sex-Specific Reference Intervals**

The absence of a clinically meaningful sex difference in fT3 concentrations (Cohen's d = −0.008) is consistent with the preponderance of evidence in the literature. While some studies have reported marginally higher fT3 in males — attributable to the androgenic stimulation of hepatic type I deiodinase activity— the magnitude of such differences has rarely been sufficient to justify sex-partitioned reference intervals under the IFCC partitioning criteria [31,39]. Our Mann–Whitney result (p = 0.004), which detected a subtle distributional shift not captured by the t-test, illustrates the well-known sensitivity of rank-based tests to minor differences in large samples — a statistical artefact rather than a physiological signal. The practical recommendation for Syrian laboratories is to use a single adult fT3 reference interval for both sexes, partitioned only by age.

**Inter-Analyte Correlations and the fT3–fT4–TSH Axis in the Syrian Population**

The paired-measurement analysis of 1,197 concurrent thyroid panels yielded a correlation structure that is, in its broad outlines, entirely consistent with established thyroid physiology — but that contains instructive detail when examined at finer resolution. The moderate positive Spearman correlation between fT3 and fT4 (ρ = 0.353) is lower than the Pearson coefficient (r = 0.844), and this discrepancy is itself the most informative finding. It tells us that the tight linear coupling between the two analytes is concentrated in the pathological tails: in thyrotoxicosis, both fT3 and fT4 rise together; in severe hypothyroidism, both decline. Within the euthyroid range, however, the correlation is much weaker, because peripheral deiodinase activity maintains fT3 within a relatively narrow homeostatic window even as fT4 fluctuates in response to dietary iodine intake, binding-protein variation, and circadian pulsatility [3,40]. This has a practical implication: fT4 cannot reliably serve as a proxy for fT3 status in the euthyroid range, and the measurement of fT3 retains independent diagnostic value — a point that has been debated in the endocrinology literature and that our data support quantitatively.

The inverse fT3–TSH correlation (Spearman ρ = −0.336) reflects the well-characterised log-linear negative-feedback relationship that governs the hypothalamic–pituitary–thyroid axis: TSH secretion is suppressed by rising free thyroid hormone concentrations and stimulated by falling levels, with an approximately ten-fold change in TSH corresponding to a two-fold change in fT4 or fT3 [41]. The partial correlation analysis, in which the fT3–TSH association was attenuated from −0.336 to −0.246 after controlling for fT4, provides a quantitative decomposition of this feedback: roughly one-quarter of the pituitary's sensitivity to fT3 is mediated indirectly through the shared covariance of fT3 and fT4, while the remaining three-quarters represents a direct pituitary response to circulating fT3. This finding is consistent with the demonstration by Gullo et al. that type II deiodinase within the pituitary thyrotroph converts T4 to T3 locally, creating a cell-autonomous feedback mechanism that operates in parallel with the systemic signal [42].

**The Conflict Dimension: Why Syrian Reference Intervals May Differ**

The wider-than-expected fT3 interval in the Syrian population demands a mechanistic explanation. Several conflict-related factors, operating in concert, plausibly contribute. First, dietary iodine intake in Syria has been subject to severe disruption since 2011. The national salt iodisation programme, which had achieved adequate coverage by 2008, was progressively dismantled as salt production and distribution infrastructure was damaged or fell under the control of non-state actors [20,43]. Iodine insufficiency stimulates preferential thyroidal T3 secretion at the expense of T4 — an adaptive mechanism that raises the population fT3 distribution and could account for part of the upward shift in our upper reference limit [44].

Second, the prevalence of non-thyroidal illness syndrome (NTIS) — the constellation of low T3, low or normal T4, and

inappropriately normal TSH seen in systemic illness, malnutrition, and physiological stress — would be expected to be elevated in a war-affected population characterised by chronic protein-energy malnutrition, endemic infectious disease, and sustained psychological adversity [45,46]. NTIS depresses fT3 through inhibition of peripheral deiodinase activity, and its prevalence in our dataset — which, by design, includes all patients who underwent thyroid testing and not merely those with suspected thyroid disease — would push the lower tail of the fT3 distribution downward, contributing to the lower boundary displacement. Third, the well-documented association between chronic psychological stress and hypothalamic–pituitary–thyroid axis modulation, mediated via cortisol's inhibition of TSH secretion and peripheral T3 generation, may further widen the population distribution by introducing a stress-related variance component that is absent in the well-nourished, low-stress populations from which manufacturer intervals are derived [47].

These conflict-related mechanisms are, of course, speculative in the absence of individual-level nutritional and clinical data, and they should be regarded as hypothesis-generating rather than proven explanations. What is not speculative, however, is the empirical observation that the Syrian fT3 distribution does not conform to the manufacturer's generic range. The practical response to this observation — the adoption of locally derived reference intervals — is warranted regardless of the underlying mechanism.

## Limitations

Several limitations warrant candid acknowledgement. First, the indirect method, by its nature, derives reference intervals from an unselected clinical population that includes an unknown proportion of individuals with thyroid and non-thyroidal pathology. While the iterative trimming procedure is designed to exclude pathological outliers, it cannot guarantee their complete removal, and some residual contamination of the reference distribution is inherent to the approach. Second, the data were drawn from a single laboratory in Damascus, and their generalisability to other Syrian governorates — some of which have experienced different intensities of conflict, displacement, and nutritional disruption — cannot be assumed. Third, pregnancy could not be excluded from the female dataset; although the influence of pregnancy on fT3 is modest relative to fT4, it introduces some imprecision into the female adult interval. Fourth, the paediatric and adolescent subgroups (particularly male adolescents, n = 11) were too small for robust reference interval derivation, and the intervals reported for these groups should be considered provisional. Fifth, all measurements were performed on a single analytical platform (Roche cobas e 411), and the results are not transferable to laboratories using other immunoassay systems without method-specific harmonisation or verification.

## Conclusions

This study presents the first population-specific reference intervals for serum free triiodothyronine in the Syrian population, derived by the indirect method from a large clinical laboratory database accumulated over five years. The locally derived adult fT3 interval (1.47–5.51 pg/mL, pooled; 1.49–5.38 pg/mL for females; 1.32–5.70 pg/mL for males) is substantially wider than the manufacturer's generic euthyroid range (2.0–4.4 pg/mL), with the lower boundary displaced downward by 0.53 pg/mL and the upper boundary shifted upward by 1.11 pg/mL. This discrepancy is large enough to alter the clinical classification of individual patient results at both ends of the reference range and constitutes a tangible patient safety concern in a healthcare system already compromised by fourteen years of armed conflict.

Serum fT3 concentrations decline progressively with age (Spearman $\rho = -0.226$), supporting the adoption of age-stratified reference intervals. Sex-specific partitioning is not warranted by the present data. The inter-analyte correlation structure confirms the expected physiological relationships among fT3, fT4, and TSH, while demonstrating that fT3 and fT4 co-vary only modestly within the euthyroid range — underscoring the independent diagnostic value of fT3 measurement.

We recommend that Syrian clinical laboratories adopt the population-specific reference intervals reported in Table 2 of this study, in preference to the manufacturer's generic range, for the interpretation of fT3 results generated on the Roche cobas e 411 / Elecsys FT3 III platform. Multi-centre validation studies encompassing other Syrian governorates and analytical platforms, together with prospective direct-method verification in a well-characterised healthy reference cohort, are warranted to refine and extend these preliminary data.

**Competing Interests:** We hereby declare that we do not have any competing interests.

**Declaration of Funding:** We hereby declare that no special funds were allocated to carry out this research.

**Repository Site:** Free T3: https://doi.org/10.5281/zenodo.19187253

**Data Availability:** https://doi.org/10.5281/zenodo.19187253

We agree to make freely available all of the data and materials supporting the results or analyses in our paper, under an open licence permitting reuse. Acceptable open licences include CC-BY or CC0.

The de-identified dataset used in this study comprises retrospective, anonymised laboratory results (analyte value, age, and sex only) extracted from routine clinical testing records at Alkhatib Medical Laboratory, Damascus, Syria.

Because the dataset contains patient-derived health information — albeit fully de-identified — and because Syrian data protection regulations do not currently provide a standardised framework for open deposition of clinical laboratory data, the underlying data cannot be deposited in a public repository at this time. Access to the de-identified dataset can be requested from the corresponding author (GS, g-shannan@aiu.edu.sy) for researchers who meet the criteria for access to confidential data, subject to approval by the laboratory director. The statistical summary tables presented in this article (Tables 1–4) contain all derived values necessary to reproduce the reference interval calculations.

| Age Group | n | Mean | SD | Median | 95% CI (Mean) | 2.5–97.5th |
|---|---|---|---|---|---|---|
| 2–15 y | 100 | 4.46 | 2.83 | 3.90 | 3.90–5.01 | 2.55–7.72 |
| 16–17 y | 33 | 4.17 | 1.96 | 3.70 | 3.50–4.84 | 2.90–7.02 |
| 18–39 y | 774 | 4.27 | 3.69 | 3.50 | 4.01–4.53 | 1.97–17.11 |
| 40–59 y | 728 | 3.77 | 2.17 | 3.40 | 3.61–3.92 | 1.80–9.59 |
| ≥60 y | 377 | 3.32 | 1.37 | 3.20 | 3.19–3.46 | 1.80–6.67 |
| ANOVA: $F = 8.88$, $p < 0.001$, $\eta^2 = 0.017$. Kruskal–Wallis: $H = 125.29$, $p < 0.001$. | | | | | | |

**Table 1: Descriptive Statistics of Serum fT3 (pg/mL) by Age Group**

| Subgroup | n* | Mean | SD | RI (Mean±2SD) | RI (2.5–97.5th) |
|---|---|---|---|---|---|
| All subjects | 1,938 | 3.49 | 1.01 | 1.47–5.51 | 1.80–6.30 |
| Female — all ages | 1,323 | 3.47 | 0.98 | 1.51–5.42 | 1.90–6.10 |
| Male — all ages | 615 | 3.55 | 1.07 | 1.40–5.69 | 1.70–6.52 |
| Female children (2–15) | 49 | 4.07 | 1.03 | 2.00–6.14 | 2.46–6.58 |
| Female adolescents (16–17) | 21 | 3.72 | 0.59 | 2.54–4.89 | 2.90–5.05 |
| Female adults (≥18) | 1,253 | 3.44 | 0.97 | 1.49–5.38 | 1.90–6.00 |
| Male children (2–15) | 34 | 3.87 | 0.44 | 3.00–4.75 | 2.68–4.40 |
| Male adults (≥18) | 568 | 3.51 | 1.10 | 1.32–5.70 | 1.70–6.52 |
| * n after iterative trimming. Male adolescents (16–17) excluded due to insufficient sample size (n = 11). | | | | | |

**Table 2: Population-Specific Reference Intervals for Serum fT3 (pg/mL) Derived by the Indirect Method (Iterative Mean ± 2 SD Trimming)**

| Group | Syrian RI (pg/mL) | Manufacturer RI | Discrepancy |
|---|---|---|---|
| Female adults | 1.49–5.38 | 2.0–4.4 | Lower: −0.51; Upper: +0.98 |
| Male adults | 1.32–5.70 | 2.0–4.4 | Lower: −0.68; Upper: +1.30 |
| Pooled adults | 1.47–5.51 | 2.0–4.4 | Lower: −0.53; Upper: +1.11 |

**Table 3: Comparison of Syrian Population-Specific fT3 Reference Intervals with the Manufacturer's Generic Range**

| Pair | Pearson r | Spearman ρ | p-value | Interpretation |
|---|---|---|---|---|
| fT3 ↔ fT4 | 0.844 | 0.353 | < 0.001 | Moderate (+) |
| fT3 ↔ TSH | −0.145 | −0.336 | < 0.001 | Moderate (−) |
| fT4 ↔ TSH | −0.213 | −0.339 | < 0.001 | Moderate (−) |
| fT3 ↔ Age | −0.131 | −0.226 | < 0.001 | Weak (−) |
| fT3↔TSH \| fT4 | — | −0.246* | < 0.001 | Partial |
| * Partial Spearman correlation controlling for fT4. | | | | |

**Table 4: Pairwise Correlation Coefficients among Thyroid Function Parameters (n = 1,197 Paired Visits)**

**Underlying Data:** https://doi.org/10.5281/zenodo.19187253

- Stat Thyroid, (Calculated means and Standard Deviations)
- Free T3 V1, (Results extracted from the database of the clinical laboratory)
- Free T3 Figures

Extended Data: No extended Data

**Ethics and Consent Statement:**
The Research Ethics Committee reviewed and discussed the research proposal ref. 23-8 of the Faculty of Pharmacy, dated 2 October 2023, to conduct the research study entitled:
Population Specific Reference Interval for Serum Triiodothyronine (Ft3) in a Conflict-Effected Population: A Retrospective Indirect Method Study from Syria.

During the committee meeting held on 9th. October 2023, all submitted documents were reviewed and approved. After due consideration, the committee has decided to approve the proposed study protocol, methodology, and data collection procedures.

Given the extraordinary circumstances and challenges faced by the Syrian healthcare system during the ongoing crisis, the committee acknowledges the difficulty of conducting conventional informed consent procedures. In line with ethical guidelines for research in emergency and crisis settings, and to facilitate the collection of critical data needed to support a severely strained health system, verbally informed consent from participants has been deemed acceptable. This approach aims to streamline the data collection process while maintaining respect for participant autonomy and confidentiality.

All members of the Research Ethics Committee were present at the meeting held on 9th. October 2023, along with the Vice Dean of the Faculty of Pharmacy. It is hereby confirmed that none of the study team members participated in the decision-making procedures of the committee.

The approved study period extends from October 2023 to September 2025.

**Declaration of Competing Interest:** We hereby declare that we do not have any competing interests.

**Use of AI-assisted Tools:** During the preparation of this manuscript, the authors used AI-assisted language tools for grammar checking and style refinement. All scientific content, study design, data collection, statistical analysis, interpretation of results, and formulation of conclusions remain the sole intellectual responsibility of the authors. The authors reviewed and edited all output and took full responsibility for the content of this publication.

## Software Availability
All statistical analyses were performed using Python 3.11 (freely available from https://www.python.org/) with the following open-source libraries: NumPy v1.24 (https://numpy.org/), SciPy v1.11 (https://scipy.org/), and Pandas v2.0 (https://pandas.pydata.org/). No custom software was developed for this study; all analyses used standard functions from these publicly available libraries.

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