TSH enhancement of FT4 to FT3 conversion is age dependent

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Abstract

Objective: We previously reported increasing free T3 (FT3) to free T4 (FT4) ratios as thyroid-stimulating hormone (TSH) increases within the normal range in children. It is not known if this phenomenon is age-related among humans, as previously reported in rats. This study examines the relationships between TSH and FT3/FT4 ratios in different ages.
Design: Retrospective examination of thyroid tests from patients without thyroid disease from community clinics.
Methods: Free T3, free T4, and TSH levels from 527 564 sera collected from patients aged 1 year or greater were studied. Exclusion criteria were the following: missing data, TSH greater than 7.5 mIU/L, and medications that may interfere with thyroid hormone activity. A total of 27 940 samples remaining after exclusion were stratified by age. Samples with available anthropometric data were additionally stratified for body mass index (BMI). Correlations of TSH to FT4, FT3, and FT3/FT4 ratios by age group were examined.
Results: Up to age 40, for each increasing TSH quartile, FT3 and the FT3/FT4 ratio increased and FT4 decreased significantly (for both FT3, FT4 and FT3/FT4 ratio, $P < 0.05$ for every TSH quartile when compared with the 1st quartile, except FT3 in the 30–40 age group). In older age groups, increasing TSH was not associated with increased FT3/FT4 ratio.
Conclusion: As TSH levels increase, FT3/FT4 ratios increase until age 40, but this differential increase does not occur in older age groups. This may reflect a decrease in thyroxine (T4) to triiodothyronine (T3) conversion with age, which may be part of the aging process.

European Journal of Endocrinology (2016) **175**, 49–54

European Journal of Endocrinology

Introduction

In children, we recently reported that within the normal range, the free triiodothyronine (FT3)/free thyroxine (FT4) ratio increases as thyroid-stimulating hormone (TSH) levels increase (1). This effect may be caused by increased deiodination of thyroxine (T4) to triiodothyronine (T3) that has been shown to occur in the cells of the thyroid gland *in vitro* in response to TSH (2, 3). Other explanations of this effect have also been suggested (4). We have recently been able to analyze data from a different dataset of over half a million blood samples from all age groups, which enabled testing whether this finding, i.e. increased FT3/FT4 ratio associated with increasing TSH levels follows true in all ages. Since this dataset belongs to a health maintenance organization, it was possible to cross the results with patient files in order to test for other confounding factors.

Subjects and methods

We studied a database of 527 564 blood samples that had been collected in the Jerusalem area between January 2011 and September 2013 and, as requested by the physicians in community clinics, tested for TSH, FT4, and FT3. We examined only samples taken with the same commercial

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DOI: 10.1530/EJE-16-0007

Published by Bioscientifica Ltd.

kit (Cobas kits used on modular analytics E-170 analyzer, Roche Diagnostics) since we found significant variance between different kits.

Only samples for which all three parameters (TSH, FT4, and FT3) were available were included in the analysis (422 012 samples were excluded due to lack of one parameter, mostly FT3). Exclusion criteria, applied by anonymous data retrieval from Clalit Health Services' electronic charts (using patient identification numbers), were the following: history of any of the following: positive titers of antithyroid peroxidase or antithyroglobulin, past or current treatment with levothyroxine, methimazole, propylthiouracil, recombinant thyrotropin, all antiepileptic drugs, lithium, or glucocorticoids. Also, samples in which TSH levels were above 7.5 mIU/L or below 0.2 mIU/L were excluded since such patients would have been under medical follow-up or evaluation and were more likely to be ill. These exclusion criteria allowed us to minimize the influence of samples withdrawn from patients who may have had altered thyroid physiology due to intrinsic or extrinsic causes. In order to allow for a sufficient increment in TSH levels through the cohort, we used the 7.5 mIU/L cut-off. Moreover, virtually all patients between the upper normal limits and this level have been shown to revert back to 'normal,' so the bias of abnormal thyroid function was thought to be minimal with these samples (5, 6). We also excluded 4569 samples from patients aged 1–20 years, collected between January 2011 and November 2011 in order to avoid overlap with our previous study of these samples. After application of all exclusion criteria, TSH, FT4, and FT3 results for 27 940 samples remained, including 10 227 males and 17 713 females. The final cohort included samples withdrawn from patients between the age group of 1 year and 110 years (mean age 24.07 ± 16.25 years), with a relatively large portion of samples from young subjects. A total of 10 848 were below age 20 (mean age 11.69 ± 5.60 years) and 17 092 were older than 20 years (mean age 35.13 ± 14.54 years).

Additionally, we stratified results by BMI in the different age groups because of reports that these parameters can affect the thyrotropin–thyroid axis (7, 8, 9). A total of 18 664 samples contained anthropometric data sufficient for calculating BMI registered from community clinics in electronic files within 6 months of blood sampling. Subjects with BMI data were divided into four groups according to the World Health Organization (WHO) definitions: (i) underweight (less than the 5th percentile), (ii) normal weight (from 5th to less than the 85th percentile), (iii) overweight (85th to less than the 95th percentile), and (iv) obese (equal to or greater than the 95th percentile).

Each age group (1–10, 10–20, 20–30, 30–40, 40–60, 60–80 years, over 80 years) was divided into four subgroups according to TSH levels – from the lowest through the highest levels of TSH.

Statistical methods

Data are presented as mean ± S.D. unless otherwise reported. Outcomes (FT3, FT4, FT3/FT4 ratio, and TSH) were compared between the different age and weight groups using the *t*-test for independent samples. The *post hoc* Scheffe test for multiple comparisons was

Table 1 Comparison of mean hormone levels and relationship between different age groups upon segregation to TSH quartiles.

	Hormone levels and ratio		
Age/TSH quartile	FT3	FT4	FT3/FT4 ratio
1–10 years ($n=3969$)			
Q1	6.28[a]	17.09	0.37[a]
Q2	6.50[c]	16.87	0.39[a]
Q3	6.56[a]	16.86	0.39[a]
Q4	6.70[c]	16.63[a]	0.41[c]
10–20 years ($n=6879$)			
Q1	5.65[a]	15.66	0.37[a]
Q2	5.73[a]	15.54	0.38[a]
Q3	5.79[a]	15.39[a]	0.38[a]
Q4	5.82[b]	15.30[b]	0.39[b]
20–30 years ($n=8301$)			
Q1	5.06	15.52	0.34
Q2	5.06	15.33[a]	0.35
Q3	5.04	15.28[a]	0.34
Q4	5.14[c]	15.25[a]	0.36[a]
30–40 years ($n=4451$)			
Q1	4.91	15.13	0.34
Q2	4.91	15.08	0.34
Q3	4.89	14.90[a]	0.35
Q4	4.90	14.56[c]	0.37[a]
40–60 years ($n=3284$)			
Q1	4.84	15.18	0.33
Q2	4.80	15.06	0.33
Q3	4.73[c]	14.56[c]	0.34
Q4	4.66[c]	14.24[c]	0.34
60–80 years ($n=693$)			
Q1	4.45	15.26	0.41
Q2	4.49	14.95	0.31
Q3	4.40	14.45[a]	0.34
Q4	4.43	14.36[a]	0.32
>80 years ($n=363$)			
Q1	4.11	15.43	0.28
Q2	3.88	14.91	0.27
Q3	3.75	14.90	0.26
Q4	3.81	14.87	0.27

[a]$P<0.05$ when compared with the 1st TSH quartiles; [b]$P<0.05$ when compared with the 1st and 2nd TSH quartiles; [c]$P<0.05$ for all comparisons between the different quartiles.

Table 2 Comparison of thyroid hormones ratio and relationships between different age groups.

	Age group						
	1–10 years	10–20 years	20–30 years	30–40 years	40–60 years	60–80 years	>80 years
n	3969	6879	8301	4451	3284	693	363
FT3/FT4 ratio[b]	0.39±0.13	0.38±0.22	0.35±0.24	0.35±0.23	0.34±0.15	0.35±0.72	0.27±0.13
Correlation (*r*)							
TSH-FT3/FT4	0.16[a]	0.06[a]	0.04[a]	0.05[a]	0.02	−0.05	−0.08
TSH-FT3	0.16[a]	0.07[a]	0.03[a]	0.00	−0.11[a]	−0.01	−0.12[a]
TSH-FT4	−0.05[a]	−0.07[a]	−0.04[a]	−0.11[a]	−0.18[a]	−0.13[a]	−0.05

r, Pearson's correlation coefficient. [a]$P<0.05$ for significance of correlation; [b]Presented as mean±S.D.

used when comparing different age and BMI groups. Cross-sectional association of serum TSH, FT4, and FT3 concentrations and body mass index was tested using multivariate linear regression models and presented as the Pearson correlation coefficient. For comparison of linear coefficients in the different groups, we used the Fisher r-to-z transformation. All tests were two-tailed and a *P*-value of 0.05 or less was considered to be statistically significant.

Ethics

All data retrieval and analyses were computerized and anonymous. The study was approved by the Clalit Health Services' institutional review board.

Results

The FT3/FT4 ratio increased significantly in the pediatric group with increasing TSH quartile. This trend was present until age 40 and was completely absent in the 40–80 years groups. There was a stepwise significant increase in FT3 with increasing TSH quartile in the pediatric group and young adult until 30 years of age, while no such change in the older groups was found. There was a parallel stepwise decrease in FT4 levels until age 40 (Table 1).

In the pediatric and young adults, until age 40, there was a positive and significant correlation between TSH and FT3/FT4 ratio ($r=0.08$; $P<0.001$), but in the older groups, this correlation decreased to nil as age increased (from 0.04 to −0.08) (Table 2). This trend, i.e. the decreasing correlation with age was linear and significant ($r=-0.94$, $P=0.02$) (Fig. 1). Until 30 years of age, there was a significant positive linear correlation of TSH with FT3 ($r=0.14$; $P<0.001$), while in the above 30 groups, no positive correlation was noted. There was a negative correlation between FT4 and TSH ($r=-0.02$, $P=0.01$) up to age 80 (Table 2).

Separate analysis for the normal-weight group showed similar results to those of the entire study population in the pediatric group and among adults younger than age 30, i.e. FT3 and FT3/FT4 ratio increased with increasing TSH. Differently than for the entire group, above age 30, FT3 and FT3/FT4 ratio did not change with increasing TSH, and FT4 was significantly lower in the higher TSH quartiles (Table 3).

Similar trends were noted when comparing the two genders. In general, both FT4 and FT3 are slightly lower among females for each TSH quartile (data available upon request). Correlations of TSH, thyroid hormone, and their ratios by gender are presented in Table 4. The correlations for the over 80 age group were not significant due to the small numbers of samples available from elderly patients after application of our stringent exclusion criteria. Therefore, this age group is not presented.

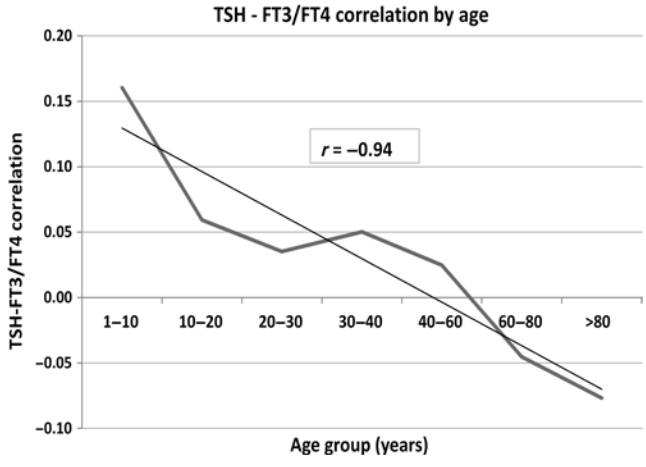

Figure 1
TSH correlation with FT3/FT4 ratio across decades of life. *r*, Pearson's correlation coefficient. *P* value <0.01 for significance of correlation.

Table 3 Comparison of mean hormone levels and relationships between normal weight patients in the different age groups upon segregation to TSH quartiles.

Age/TSH quartile	Hormone levels and ratio		
	FT3	FT4	FT3/FT4 ratio
1–10 years ($n=304$)			
Q1	6.40	16.73	0.39
Q2	6.58	16.88	0.39
Q3	6.64	16.63	0.41
Q4	6.85[b]	15.97[b]	0.44[b]
10–20 years ($n=2598$)			
Q1	5.54	15.71	0.36
Q2	5.67[a]	15.65	0.37[a]
Q3	5.72[a]	15.48	0.38[a]
Q4	5.68[a]	15.45[a]	0.37[a]
20–30 years ($n=3640$)			
Q1	5.05	15.74	0.33
Q2	5.08	15.48[a]	0.33[a]
Q3	5.11	15.49[a]	0.34[a]
Q4	5.18[a]	15.45[a]	0.34[a]
30–40 years ($n=1414$)			
Q1	4.94	15.41	0.33
Q2	4.87	15.05[a]	0.33
Q3	4.90	15.33	0.33
Q4	4.97	14.80[c]	0.35
40–60 years ($n=774$)			
Q1	4.95	15.52	0.33
Q2	4.76	15.37	0.31
Q3	4.68	14.78[c]	0.32
Q4	4.63[a]	14.10[a]	0.33[a]
60–80 years ($n=122$)			
Q1	4.64	14.81	0.32
Q2	4.59	15.14	0.32
Q3	4.66	14.53	0.33
Q4	4.51	13.81	0.33

[a]$P<0.05$ when compared with the 1st TSH quartiles; [b]$P<0.05$ when compared with the 1st and 2nd TSH quartiles; [c]$P<0.05$ for all comparisons between the different quartiles.

Discussion

After our earlier study that looked at the effect of increasing TSH on FT3/FT4 ratio in the pediatric age group, we did the same analysis on an expanded database including a much larger number of children (almost five-fold) and included adult patients (1). The results until age 40 showed that as TSH quartiles increased, the FT3/FT4 ratio increased, as we showed previously for children and in this study, the decrease in FT4 with increasing TSH quartiles was significant (Table 1). We have previously suggested that this phenomenon could be an *in vivo* reflection of the previously reported increase in the *in vitro* activity of deiodinases in response to increasing TSH concentrations (3). Another side-finding was the difference between the genders in thyroid hormone levels that is in agreement with one previous study and differs somewhat from the two others (10, 11, 12).

From our results (Tables 1 and 2), it appears that beyond age 40, the effect of TSH to increase T3 production from T4 becomes less pronounced and may be completely abolished in the elderly. This could reflect either reduction in deiodinase activity with age, development of TSH resistance with increasing age, or a combination of these. In aging rats, there are data showing a decline in the activity of type 1 deiodinase but no similar decline in thyroid hormone receptor expression or activity. Thus, in rats, the prominent age-related change is in the deiodinase and not in the receptor (13). It is unlikely that any alterations in FT3/FT4 ratios are secondary to insufficient iodine since it appears that by and large Israel is iodine sufficient (14).

The findings in this study are particularly intriguing in view of recent research in gerontology. Individuals from families with longevity have lower FT3 and FT4 despite

Table 4 Comparison of TSH and thyroid hormone levels and relationships by gender.

	Age group					
	1–10 years	10–20 years	20–30 years	30–40 years	40–60 years	60–80 years
n						
Males	1876	2343	2539	1763	1280	328
Females	2093	4536	5762	2688	2004	365
Correlation coefficient (*r*)						
TSH–FT3						
Males	0.19[a]	0.02	0.06[a]	−0.06[a]	−0.10[a]	−0.07
Females	0.16[a]	0.08[a]	0.06[a]	0.02	−0.08[a]	0.05
TSH–FT4						
Males	−0.04	−0.08[a]	0.03	−0.14[a]	−0.12[a]	0.00
Females	−0.10[a]	−0.07[a]	−0.01	−0.09[a]	−0.19[a]	−0.13[a]
TSH–FT3/FT4 ratio						
Males	0.15[a]	0.05[a]	0.00	0.12[a]	0.04	−0.03
Females	0.21[a]	0.06[a]	0.05[a]	0.03	0.02	−0.07

[a]$P<0.05$ for significance of correlation.

higher TSH (15, 16). Fabbri and coworkers have recently reported that aging people with a lower metabolic rate have a significantly lower level of multiple morbidities associated with age, i.e. they are healthier than those with a higher metabolic rate (17, 18). If reduced metabolism is in fact a protective mechanism, it may explain why people who have lower FT4 and FT3 and also have a lower FT3/FT4 ratio are still alive at an older age. Interestingly, type 3 deiodinase that protects fetal tissues from excess thyroid hormone action has recently been shown to become activated in damaged tissues such as cancer cells, and is induced by factors associated with stress such as hypoxia-inducible factor-1-alpha (HIF1α), tumor growth factor-β (TGFβ), and others (19, 20). This deiodinase degrades T4 intracellularly to reverse T3, and one could speculate that increasing degradation of this type with age would dampen the effect of TSH on the FT3/TF4 ratio because more T4 is degraded to reverse T3 instead of to T3.

Clearly, much remains to be learned about the significance of the changes we have described before it will be possible to make practical conclusions such as whether thyroid hormone treatment would be beneficial or detrimental to the aging individual. However, in our opinion, clarification of the normal changes associated with aging of the pituitary–thyroid axis is an important step toward such future conclusions.

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

Funding
This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Author contribution statement
All authors fulfill the following four criteria: (i) substantial contributions to conception or design of the work, or the acquisition, analysis, or interpretation of data for the work; (ii) drafting of the work or revising it critically for important intellectual content; (iii) final approval of the version to be published; and (iv) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. In addition, the following were specific contributions of each author: D Strich, G Karavani, and D Gillis: conception and design of the study; S Edri was responsible for acquisition of the data. Each of the coauthors is confident of the integrity of the contributions of all other coauthors.

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Received 4 January 2016
Revised version received 24 March 2016
Accepted 4 May 2016