Normal Reference Interval for Thyrotropin Response to Thyroliberin: Dependence on Age, Sex, Free Thyroxin Index, and Basal Concentrations of Thyrotropin

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We measured the thyrotropin response (ΔTSH) to 200 µg of thyroliberin in 131 subjects without thyroid dysfunction or other disease and with basal values for thyroid function that were within the normal reference intervals for our laboratory. By univariate and multivariate statistical methods we found ΔTSH to be significantly influenced by the basal concentration of thyrotropin (TSH0) and the free thyroxin index (FT4I). When the effects of variations in TSH0 and FT4I were eliminated, ΔTSH in men under 40 years of age did not differ from that in women. A decrease in ΔTSH with increasing age was found in men but not in women. Thus a reference interval for ΔTSH should consider TSH0, FT4I, and, in men, age. On the basis of multiple linear regression analysis, we constructed a formula for ΔTSH reference intervals that takes into account individual values for TSH0 and FT4I. The formula should be applicable for women, regardless of age, up to 77 years and for men under 40 years. For older men a correction for the age-related decrease in ΔTSH must be applied.

The thyrotropin (TSH) response to thyroliberin in healthy subjects seems to be related to several different factors. Most investigators agree that it is positively correlated to the dose of thyroliberin (1, 2) and to the basal concentration of TSH (3–5), and women seem to respond better than men (6, 7). Results regarding the influence of age (1, 7, 8) and the concentration of thyroid hormones in serum (5, 7, 9) have been contradictory.

Factors influencing the TSH response to thyroliberin often have been investigated, but in comparatively small groups of subjects. Whether these factors influence the TSH response independently or in combination is not known. Because the quantitative significance of the established relations has not been elucidated in detail, it is not known to what extent they should be considered in the construction of a reference interval for the TSH response to thyroliberin.

We have measured the TSH response to thyroliberin in 131 subjects without thyroid disease and without medication, and tried to evaluate the relative significance of the factors mentioned above. On the basis of this information we suggest some principles for the construction of a reference interval for the TSH response to thyroliberin.

Materials and Methods

Subjects

From subjects examined with the thyroliberin test to exclude possible thyroid disease, we selected 131 in whom no thyroid disorder or other disease was found, either at the time of the thyroliberin test or on follow-up six to 36 months later. Their values for triiodothyronine (T3), thyroxin (T4), and TSH in serum were within the normal reference intervals used in our laboratory. Subjects receiving any medication, including oral contraceptives, were excluded.

The group consisted of 94 women (mean age 45 years) and 37 men (mean age 41 years); the age range was 18 to 77 years.

The thyroliberin test was performed by sampling blood for TSH determination before and 20 and 30 min after intravenous injection of 200 µg of thyroliberin ("Thyrefact"; Hoechst, F.R.G.). The TSH response to thyroliberin (ΔTSH) was calculated as the difference between the basal TSH (TSH0) and the highest TSH value registered after thyroliberin.

Analytical Methods

In the radioimmunassay for TSH we used a rabbit antiserum (Milab, Malmö, Sweden) that had been absorbed with human chorionadotropin–Sepharose 4B to eliminate cross reaction with gonadotropins. Normal rabbit serum was added to the reaction mixture containing antiserum and serum samples or standards, to eliminate the possibility of TSH values being falsely increased owing to antirabbit serum antibodies. After 4–5 h of incubation at 4°C, we added 125I-labeled TSH and incubated again, overnight. Goat antirabbit-IgG in polyethylene glycol 6000/water (5/95 by vol) was added; after this had stood for 30 min at room temperature, the mixture was centrifuged, the supernate decanted, and the radioactivity in the precipitate measured. The reference interval was 0 to 7 milli-int. units/L.

In the T3 assay the binding between T3 and thyroid-hormone-binding globulin was inhibited with 8-anoilino-1-naphthalene sulfonic acid, and a rabbit antiserum (Milab) directed against a conjugate between T3 and human albumin was used. The binding constant was $0.8 \times 10^{16}$ mol and the sensitivity $0.2$ nmol/L. Anti-T3 with 8-anilino-1-naphthalene sulfonic acid and $[125I]T_3$ were added to the samples. After 2–4 h of incubation we added the second antibody, suspended in polyethylene glycol/water as in the TSH method, and centrifuged the samples after 20–40 min at room temperature. The radioactivity in the precipitate was counted after the supernatant fluid was decanted. The reference interval was 1.0–3.0 nmol/L.

T4 was assayed with a rabbit antisera (Milab) against a T4-bovine serum albumin conjugate. In this assay serum samples (or standards), antiserum, 8-anilino-1-naphthalene sulfonic acid, and $[125I]T_4$ were mixed and incubated for 2–4 h at room temperature. Bound and free radioactivity was separated in the same way as in the TSH and T3 methods. After centrifugation the radioactivity was measured in a gamma counter, with automatic calculation of results by use of a spline-function method. The reference interval was 57–154 nmol/L.
Saturation of thyroid-hormone-binding proteins was determined by use of a T3 resin uptake test (10). The free thyroxin index (FTI) and the free triiodothyronine index (FTI) were calculated as the products of the value for the T3 resin uptake test and the concentration in serum of T4 and T3, respectively.

Statistical Methods

Statistical computations were performed with use of a statistical program package.

The reference intervals were determined as mean ± 1.96 standard deviations (SD). Skewness and kurtosis were calculated both with and without different transformations such as log x and xα (α = 2, 3, . . . , 9). If the transformed values had a distribution close to normal, we used the mean and SD of the transformed values in determining the reference interval.

We used two-sided t-tests for independent samples to test whether observed mean values were equal. When there was a statistically significant difference (p < 0.05) in variance between two samples, we used the separate variance estimate of the two-tail probability. Otherwise, the pooled variance estimate was used.

We used the Pearson product–moment formula to determine the coefficient of correlation. Polynomial regression was performed by using multiple regression analysis.

Results

Basal Variables of Thyroid Function

In both sexes there was a skewed distribution of TSH values, lower values being more frequent than the higher ones (Table 1). After logarithmic transformation the distribution was more nearly Gaussian. In women older than 60 years, but not in men, there was a clustering of higher TSH values; values for younger women were similar to those in men (Table 2). The concentrations of T3 and T4 in serum and the FTI and FTI were not significantly correlated with either TSH or age, and did not change consistently with increasing TSH up to 7.5 milli-int. units/L (Table 1) or age (Table 2). The values for T3, T4, FTI, and FTI did not differ significantly between men and women (Table 2). The distribution of serum T3, T4, FTI, and FTI values were nearly gaussian, and the mean values fell near the centers of normal reference intervals of our laboratory.

TSH Response to Thyrokinin

Time–response relations. With increasing ΔTSH the 30-min value for TSH tended to be higher than the 20-min value (Figure 1). Subjects with ΔTSH < 15 milli-int. units/L had a 30-min reading that was significantly higher (p < 0.05) than the 20-min reading, but no such difference was found in subjects with ΔTSH < 15 milli-int. units/L.

Relations to TSH0, thyroid hormone concentrations, age, and sex. ΔTSH correlated significantly with TSH0 (r = 0.66, p < 0.01), and with serum FTI (r = −0.30, p < 0.01), T4 (r = 0.20, p < 0.05), and FTI (r = −0.21, p < 0.05), but not with T3. Student's t-test showed that ΔTSH was significantly higher for women than for men (p < 0.01). There was no significant correlation between ΔTSH and age in women, but in men ΔTSH decreased significantly with age (r = −0.37, p < 0.05).

A more detailed statistical analysis of the dependence of ΔTSH on TSH0 was performed separately for women and men. The resulting regression curves were: (women) ΔTSH = 0.293 TSH0² + 1.79 TSH0 + 6.08; (men) ΔTSH = 0.001 TSH0² + 2.34 TSH0 + 4.01. To quantify the impact of age and sex on ΔTSH, we divided the subjects into three age groups for each sex. We found no significant differences in T4, FTI, T3, or FTI among any of these groups (Table 2). However, TSH0 was significantly greater in older women (Table 2). Because ΔTSH varied with TSH0, the ΔTSH values in different age and sex groups could not be compared directly. To correct for the effect of TSH0 on ΔTSH, we proceeded as follows: By the regression analyses we calculated a predicted mean ΔTSH for any given TSH0, then we could express the observed individual ΔTSH values for women as percentages of the predicted ΔTSH value, which permitted the influence of age in women to be quantitatively assessed independently of variations in TSH0. Expressing the observed ΔTSH values for men as percentages of the predicted ΔTSH calculated from the regression function for women allowed a comparison between the sexes as well (Figure 2). The responses obtained in this way showed only an insignificant variation of ΔTSH with age in women (Figure 2). Not even when we compared the values for women < 55 years with values for women > 55 years did we find any significant difference. The response in men < 40 years did not differ significantly from that in women but was significantly (p < 0.05) less than that in men > 40 years: 73% in the range 40–59 years, and 67% in the range 60–79 years (Figure 2).

Because the distribution of ΔTSH was highly skewed, we performed various data transformations, as described in Statistical Methods, and found the transformation xα the most suitable for further statistical analysis. We therefore used ΔTSH⁻⁰·⁻³ in the remaining statistical analysis.

The significantly lower ΔTSH in older men was compensated for in the following statistical calculations by application of the following factors for age-correction of the male ΔTSH values:

for men 40–59 years, ΔTSH = 1.37 × observed ΔTSH
for men 60–79 years, ΔTSH = 1.49 × observed ΔTSH

We then performed stepwise multiple-linear regression analysis with all 131 cases, using log TSH0, T3, T4, FTI, FTI, and age as independent variables and ΔTSH⁻⁰·⁻³ as dependent variable. Only log TSH0 and FTI contributed significantly to the observed variation in ΔTSH⁻⁰·⁻³. The final regression equation was used to construct a reference region for ΔTSH with upper and lower limits given as follows:

limits = (0.85 log10 TSH0 - 0.003 FTI + 2.163 ± 1.96 · 0.283)⁻⁰·⁻³

Thus the upper and lower limits vary with TSH0 and FTI. The formula is directly applicable to women and to men < 40 years. For men > 40 years the above-mentioned correction should be applied to the observed ΔTSH value before using the formula for limits. Figure 3 illustrates the limits for some different FTI values.

Discussion

The individuals investigated in the present study were selected among subjects who were given the thyrokinin test because of symptoms that might suggest thyroid dysfunction. The selection criteria included values for TSH0, FTI, and FTI within normal intervals of our laboratory and a clinical examination that did not confirm the initial suspicion of thyroid dysfunction, even at a later follow-up. Even if the subjects selected this way cannot be regarded as normals, the results show that they were quite close to "normal." For example, the mean values for serum T3 and T4 in the group were close to the centers of the reference ranges of the laboratory for these parameters, and their TSH0 values were log normally distributed, which corresponds well to the pattern found by Kågedal et al. (11) in a health survey involving 4087 women. In the women, but not in the men, we found a significantly higher TSH0 in subjects with...
over 60 years old, which is in accord with the increasing prevalence of thyroid autoantibodies with age in women but not in men (12, 13). These findings, together with the selection criteria, suggest that the present subjects come close to normality, and the pattern of their TSH response to thyroliberin may be expected to apply to any group of healthy subjects.

We used a thyroliberin dose of 200 μg because a linear log dose–response relation has been found for the TSH response to thyroliberin in the range 6.25–400 μg of thyroliberin (1).
FT₄I, and, in men, age to be related to ΔTSH. The stepwise linear multiple regression analysis showed that only TSH₀ and FT₄I contribute significantly to the observed variation in ΔTSH. By that analysis there was no effect of age, probably owing to the fact that variations with age applied to only a minor subsample of the material, nor of FT₄I, probably owing to its strong correlation with FT₄I. Thus, TSH₀, FT₄I, and, in men, age should be considered in the construction of a reference interval for ΔTSH. As is evident from Tables 1 and 2, TSH₀, FT₄I, and age are not intercorrelated. The well-known correlation between T₄ and TSH₀ in individuals, including hypothyroid subjects (16), thus does not seem to apply in the normal TSH₀ interval.

To quantify the effects of age and sex on ΔTSH, minimizing bias from TSH₀ and FT₄I variations, we expressed the observed ΔTSH values as the percentage of a mean response predicted from the regression function for women with regard to TSH₀. This largely eliminated the effect of variations in TSH₀ and FT₄I did not change significantly with age or sex. These calculations showed a considerable decrease in ΔTSH with age in men but not in women, which should explain the sex-related difference, because men < 40 years old had practically the same ΔTSH as women. This agrees well with earlier studies (1, 7). Thus, when small groups of younger subjects are investigated, a sex-related difference is not detected (e.g., 3) but will appear in larger populations that cover a wider age range (e.g., 7).

The correlation between TSH₀ and ΔTSH in the present group of subjects is similar to that found by others (3, 5). An influence of FT₄I independent of TSH₀ is in agreement with the findings of Sawin and Hershman (5) and of Bastenie et al. (9) and conforms well with the concept that serum T₄ is a more important determinant of TSH release than is T₃ (16). Multiple-regression analysis of the effects of TSH₀ and FT₄I on ΔTSH resulted in a reference interval for ΔTSH with respect to TSH₀ and FT₄I as well. We used data transformation to compensate for the fact that the ΔTSH values were not symmetrically distributed around the regression line between TSH₀ and ΔTSH. The formula can be programmed into a programmable pocket calculator, and the individual reference interval for ΔTSH corresponding to given values for TSH₀ and FT₄I can easily be obtained. This reference interval should apply to women regardless of age up to 77 years, and also to men < 40 years. For older men a correction factor regarding age must be introduced to take into account the decreasing ΔTSH with age in men.

References