A Study of Age-Dependent Changes in Thyroid Function Tests in Adults

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Total serum thyroxine (T_4), triiodothyronine (T_3), T_3 resin uptake (T_3U), thyrotrophin (TSH), and reverse T_3 (rT_3) were measured in 209 healthy adults 20–89 yr old. Mean T_4 values for men were stable throughout life, but in females under age 60, T_4 values were significantly higher than in older women. Values for T_3U in males were significantly higher than in females throughout all decades, although females had a significant increase in T_3U after age 60. TSH values increased significantly in females over age 60. Throughout all decades, males had stable TSH levels that were slightly higher than the female results before age 60 and lower thereafter. Mean serum T_3 declined similarly for both sexes with increasing age, although not to the extent previously reported. Men had significantly higher mean rT_3 values over all decades than females, although female rT_3 levels decreased after age 50 whereas males maintained stable values. The physiologic reasons for these findings may be due to sex-related changes in binding proteins and alterations in metabolic clearance rates, production, and degradation of these hormones with increasing age.

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Although there have been numerous reports concerning the effects of aging on thyroid function tests, conflicting data have been presented about total thyroxine (T_4), free T_4, and free triiodothyronine (T_3) values in the elderly (*1–5*). Several observers, however, have noted a decline in total T_3 in subjects over 60 (*6–11*). Among the possible explanations for the fall in total T_3 are: (a) a decline in thyroidal T_3 secretion, (b) an increase in T_3 turnover rate, (c) a decline in TBG (*12*), and (d) a decrease in peripheral conversion of T_4 to T_3 (*6*). If the latter explanation were further substantiated, it would appear that serum reverse (3,3′,5′-triiodothyronine, or "rT_3") may also change with aging. There are considerable clinical data (*13,14*) suggesting that levels of rT_3, a metabolically inactive hormone, are increased in some situations where T_3 values are diminished in euthyroidism. It has been suggested that an alteration in the peripheral conversion pathway of T_4 to T_3 exists in some of these circumstances (*14*). The present study, using a sensitive radioimmunoassay, investigates the effects of aging on rT_3 in a large population of healthy individuals. Also, the effects of age on T_4, T_3, free thyroxine index (FT_4 index), T_3 resin uptake (T_3U), and thyrotrophin (TSH) are re-evaluated.

METHODS

Two hundred twenty-five healthy, free-living adults aged 20–80 were studied. None had a history of thyroid disease, goiter, or medications known to alter thyroid function. No subject had undergone recent iodinated contrast studies. None was acutely ill, was suffering from wasting diseases, or had liver disease or renal failure. All were ambulatory; none was hospitalized, and all were well at the time of examination. The majority were white and most were of similar middle-class socioeconomic background.

Subjects over age 60 were carefully screened with a thorough medical questionnaire and a brief physical

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examination to rule out overt thyroid disease or goiter. The majority of these participants were recruited at senior citizen recreation centers where thyroid screening was done.

There were 107 females and 102 males studied. Of the original 225 participants, 16 were eliminated from the study. Two were removed for findings compatible with evidence of elevated thyroxine-binding globulin (TBG), with elevated T_4, and inappropriately decreased T_3U; five for low TBG (low T_4 with inappropriately elevated T_3U); two for incidental findings of hypothyroidism (low T_4 and elevated TSH); six for evidence of limited thyroid reserve of unknown nature (normal T_4 with elevated TSH); and one for hyperthyroidism (elevated T_4, T_3U, and T_3).

Thyroid function was assessed by the methods described below for total T_4, T_3, T_3U, and TSH. Adult normal ranges were previously determined from analysis of samples from 130 healthy, ambulatory, free-living adults, aged 20–40 yr (not subjects used in the present study).

Total T_4 was measured by a modification of the method of Premachandra et al.* (*15*). This radioimmunoassay uses sodium trichloroacetate as the extractant solution and a polyurethane sponge to separate the antibody-T_4 complex from free T_4. Normal range was 5–12 μg/dl (mean ± 2 s.d.), with intra-assay coefficient of variation (CV) of 4.8% and interassay CV 7.0% in the normal range.

T_3 resin uptake was estimated by modified standard techniques† (*16*). The established normal range is 25–35%, with intra-assay CV of 2.0% and interassay CV 3.1% in the normal range.

Free thyroxine index was calculated as the product of the total T_4 concentration and T_3U, with the normal range 1.25 to 4.20.

Radioimmunoassay for TSH was performed by the modified method of Odell et al.† (*17,18*) using polyethylene glycol to separate antibody-bound TSH from unbound TSH. Values between 0 and 7 μU/ml comprised the normal range, with intra-assay CV of 5.2% and interassay CV of 7.4% in this range.

Triiodothyronine (T_3) was measured by the Immo Phase T3RIA kit‡, a specific technique using ANS (8-anilino-1-naphthalene sulfonic acid) to block binding of T_3 to TBG (*19*), and antibody coated to porous glass particles as the separation technique (*20*). Normal range was 90–200 ng/dl (mean ±2 s.d.); intra-assay CV of 7.4%, and interassay CV of 9.6% in this range were found.

The radioimmunoassay for reverse T_3 done in our laboratories was specific for the L-isomer. This assay used bovine gamma globulin as the carrier serum and a double-antibody technique for separation.‖ The estimated normal range (Serono) was 90–350 pg/ml. In our laboratory intra-assay CV was 9.6%, and interassay CV was 10.2%.

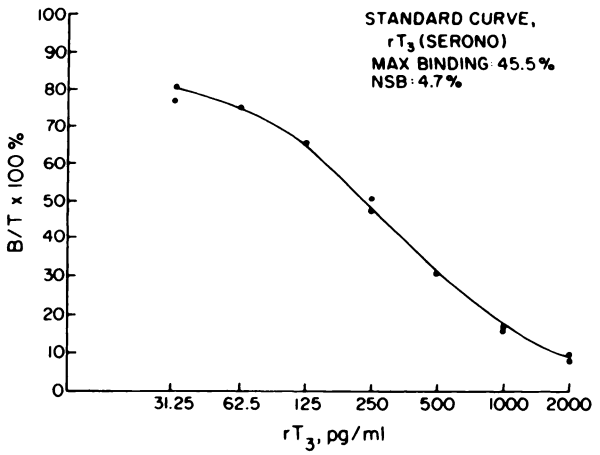

FIG. 1. Standard curve for reverse T_3 (pg/ml) radioimmunoassay (Serono Laboratories).

Figure 1 shows a typical standard curve for measurement of rT_3 used in this study. This curve was derived by reacting Serono antiserum for L-rT_3 with known L-rT_3 standards. Unpublished data by Olivieri for Serono showed insignificant cross reactivity of the rT_3 antiserum with L-T_4 (0.08%), L-T_3 (0.0024%), L-3,5-T_2 (0.0006%), or L-3,3′-DIT (0.032%). Recovery of added rT_3 was between 89 and 100%.

All measurements were run in duplicate and all samples were run in dual assays, with samples equally distributed according to age. Distribution of the levels of thyroid hormones were shown to be Gaussian. The data were analyzed for statistical significance by the appropriate t-test method (*21*). Where significant difference existed, correlations of sex and age were generated using a standard computer program.

RESULTS

Table 1 presents a summary of the means for total serum T_4, T_3U, FT_4 index, TSH, total T_3, and reverse T_3, for males and females separately and as combined groups by decades. Table 2 divides the subjects into groups with members 20–59 and 60–89 yr old, and compares differences in the findings by age and sex (unpaired t-test).

Total T_4. There was a statistically significant ($p < 0.05$) difference in T_4 values between males and females of all ages. This resulted from the significantly higher T_4 levels in women under 60 compared with men in the same age group (7.90 ± 1.34 μg/dl compared with 7.20 ± 1.39 μg/dl, $p < 0.01$, Table 2). Moreover, serum total T_4 concentrations were significantly higher in women under 60 yr old than they were in their older counterparts (7.90 ± 1.34 μg/dl compared with 7.39 ± 1.35 μg/dl, $p < 0.05$, Table 2). These findings for females also are depicted in Fig. 2. Men had relatively stable T_4 levels for

TABLE 1. THYROID FUNCTION BY DECADES. COMPARISONS OF GROUPED DATA, MALES VS. FEMALES*

Sex	Age (yr)	n (subjects)	T_4 (RIA) (μg/dl)	T_3U (%)	FT_4 index	TSH (μU/ml)	T_3 (RIA) (μg/dl)	rT_3 (pg/ml)
Males	20–29	16	7.5	32.2	2.436	2.72	146.0	263.3
	30–39	13	7.3	32.3	2.363	2.93	137.1	265.0
	40–49	9	6.7	30.9	2.067	2.91	135.7	220.8
	50–59	13	7.0	30.9	2.096	3.01	138.8	247.4
	60–69	24	7.5	31.8	2.379	2.05	136.1	228.6
	70–79	22	7.0	30.7	2.128	2.82	120.9	239.4
	80–89	5	7.2	31.4	2.234	2.60	111.4	288.4
Group totals ±s.d.		102	7.2 ±1.2	31.5 ±2.3	2.261 ±0.37	2.65 ±1.42	133.6 ±19.1	245.7 ±46.9
Females	20–29	10	8.4	28.5	2.386	1.94	142.6	227.4
	30–39	12	7.4	30.5	2.254	2.27	131.3	207.0
	40–49	10	8.1	29.1	2.334	2.63	138.8	217.7
	50–59	12	7.7	27.9	2.150	2.53	145.8	216.9
	60–69	30	7.6	29.9	2.300	3.44	125.7	198.4
	70–79	26	7.2	30.0	2.160	3.37	125.0	194.3
	80–89	7	7.4	29.7	2.180	3.14	139.9	181.6
Group totals ±s.d.		107	7.6 ±1.4†	29.4 ±2.4‡	2.235 ±0.37	2.96 ±1.53	132.1 ±23.3	203.9 ±41.3‡
Males and females	20–29	26	7.9	30.8	2.421	2.42	144.7	249.4
	30–39	25	7.4	31.4	2.310	2.61	134.3	237.2
	40–49	19	7.5	29.9	2.212	2.76	137.3	219.2
	50–59	25	7.3	29.5	2.210	2.77	142.2	232.8
	60–69	54	7.5	30.7	2.310	2.82	130.3	211.8
	70–79	48	7.1	30.4	2.151	3.12	123.1	215.2
	80–89	12	7.3	30.4	2.200	2.92	128.0	226.1
Group totals ± s.d.		209	7.4 ±1.3	30.5 ±2.5	2.242 ±0.37	2.81 ±1.48	132.8 ±21.4	224.3 ±48.8

* Males vs. females compared by unpaired t-test; where symbols are not shown, $p > 0.05$.
† $p < 0.025$.
‡ $p < 0.0005$.

all decades without significant changes, and beyond age 60 there were no significant differences between men and women.

T_3U. Serum T_3U values also differed significantly in males and females (Table 2). Males had slightly higher values than those in females throughout all decades [(31.5 ± 2.3)% against (29.4 ± 2.4)%, $p < 0.0005$]. Females, however, showed a small but significant increase in the T_3U after age 60, rising from a mean of (29.01 ± 2.45)% to (29.93 ± 2.31)%, $p < 0.05$, whereas men demonstrated no age-related changes.

FT_4 index. For the 107 males and 102 females studied, the mean values for the T_4 index were 2.235 ± 0.37 and 2.261 ± 0.37, respectively. There were no statistically significant differences by age or sex.

TSH. Males over the entire age range had a mean TSH value of 2.96 ± 1.53 μU/ml, while the mean for females was 2.65 ± 1.42 μU/ml—values that were not significantly different (Table 1). Females over age 60, however, had significantly higher serum TSH levels than younger women (3.379 ± 1.58 μU/ml against 2.347 ± 1.26 μU/ml, $p < 0.05$, Table 2). These data are shown in Fig. 3. Males demonstrated a slight but insignificant fall in TSH after age 60. From Table 2, it can be seen that serum TSH concentrations in males were higher than those of females before age 60, and significantly lower thereafter ($p < 0.005$).

T_3RIA. Values for T_3 showed a gradual decline by decade in both sexes (Fig. 4). There were no appreciable sex differences. Both males and females had a highly

TABLE 2. COMPARISON OF THYROID FUNCTIONS IN NORMAL SUBJECTS, AGES 20–59 VS. AGES 60–89*

Sex	Age (yr)	n (subjects)	T_4 (RIA) (μg/dl)	T_3U (%)	FT_4 index	TSH (μU/ml)	T_3 (RIA) (μg/dl)	rT_3 (pg/ml)
Males	20–59	51	7.20 ±1.39	31.70 ±2.42	2.266 ±0.33	2.878 ±1.27	140.1 ±16.9	252.2 ±49.2
	60–89	51	7.24 ±1.38	31.30 ±2.19	2.257 ±0.37	2.436 ±1.53	127.1 ±19.2‖	239.1 ±43.9
Females	20–59	44	7.90 ±1.34	29.01 ±2.45	2.274 ±0.30	2.347 ±1.26	139.5 ±23.1	216.8 ±39.0
	60–89	63	7.39 ±1.35†	29.93 ±2.31†	2.229 ±0.42	3.379 ±1.58†	127.0 ±22.2‡	194.8 ±40.8‡
Males and females	20–59	95	7.53 ±1.33	30.24 ±2.61	2.270 ±0.32	2.629 ±1.29	139.8 ±19.9	235.8 ±48.0
	60–89	114	7.32 ±1.37	30.50 ±2.34	2.231 ±0.39	2.957 ±1.62	127.0 ±20.8‖	214.7 ±47.5‡

* All values ± s.d. Comparisons by unpaired t-test; where symbols are not shown $p > 0.10$.
† $p < 0.05$.
‡ $p < 0.005$.
‖ $p < 0.0005$.

significant decline in T_3 when groups 20–59 were compared with groups 60–89 yr old. Mean serum T_3 values in both sexes under age 60 were 139.8 ± 19.9 ng/dl in contrast to 127.0 ± 20.8 ng/dl for the older combined group, $p < 0.0005$, Table 2.

Reverse T_3. There were age- and sex-related changes in rT_3. The mean serum rT_3 concentrations in males were significantly higher than those of females in all age groups (see Table 1). Female rT_3 levels significantly decreased after age 60 from a mean of 216.8 ± 39.0 pg/ml (ages 20–59) to 194.8 ± 40.8 pg/ml (ages 60–90), ($p < 0.0005$). Figure 5 demonstrates the age-related changes in rT_3 in females. In contrast, rT_3 values in males do not show a similar decline in later years and there are generally stable values throughout life (Table 2).

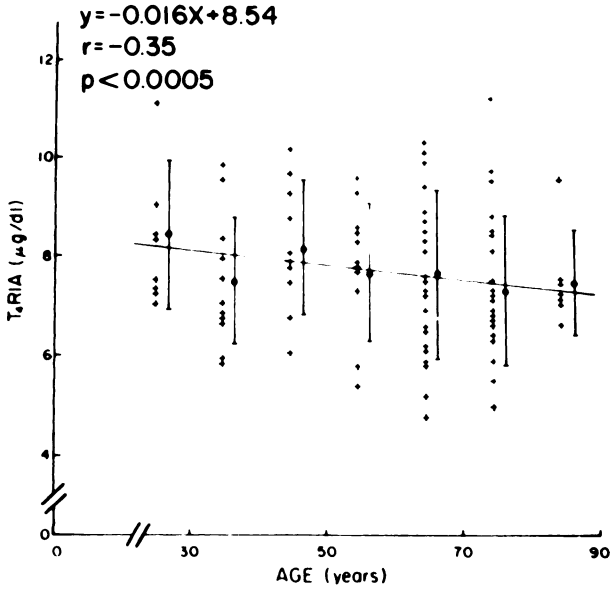

FIG. 2. T_4 values (μg/dl) for females aged 20–89 by decade, show weak negative correlation ($r = -0.35$, $p < 0.0005$). Means (heavy circles) ± standard deviations are shown. A given point may represent more than one observation.

DISCUSSION

This investigation examined the relationship between aging and various parameters of thyroid function in 209 healthy adults, 20 to 89 yr old. Interestingly, an additional 16 subjects were identified with laboratory findings compatible with occult thyroid disease or TBG abnormalities. Most of these subjects were in the older age group, suggesting that thyroid screening may be appropriate in the elderly.

The findings from this study substantiated some earlier observations (*5–11*), but they also added new information concerning age-related changes in thyroid function tests. Moreover, these results describe age- and sex-related changes in serum rT_3 concentrations in adults.

Mean total serum T_4 values in females before age 60 were higher than serum T_4 in males of the same age group, but declined slightly after age 60, becoming comparable with male levels, which were stable

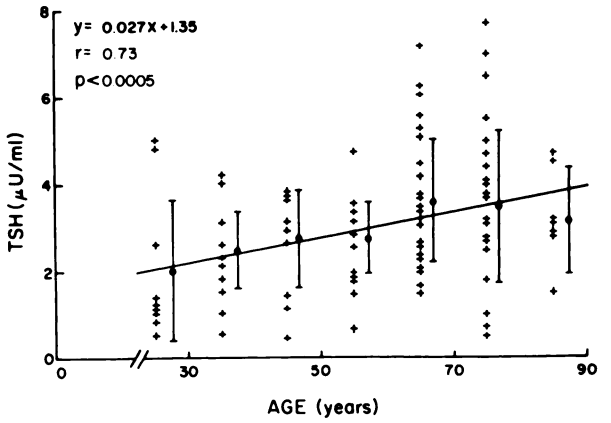

FIG. 3. TSH (μU/ml) for females aged 20–89 by decade, demonstrates significant ($p < 0.0005$) positive correlation. Means (heavy circles) ± standard deviations are shown. A given point may represent more than one observation.

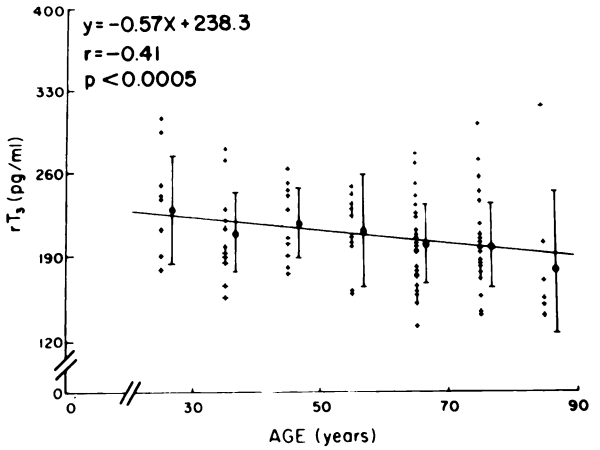

FIG. 5. Reverse T_3 values (pg/ml) for females aged 20–89 by decade, has weak negative correlation ($r = -0.41$, $p < 0.0005$). Means (heavy circles) ± standard deviations are shown. A given point may represent more than one observation.

throughout life. Hesch et al. (*1,3*), Herrmann et al. (*2*), and Bermudez et al. (*13*), however, observed unexplained lower T_4 levels (although within normal limits) in the elderly. Findings similar to our data were noted by Hansen et al. (*9*) in their study of 111 normal subjects. Since free thyroxine index did not change with age in their study, they suggested that the findings in females may have resulted from a decline in estrogen-dependent TBG concentrations after age 60, a theory in agreement with our findings. This explanation is in part supported by the observations of Braverman et al. (*22*) that in 20-to-30-year-old females TBG levels were slightly but significantly higher than in males of the same age. Others have reported that T_4 concentrations do not vary with age (*5,6*), and both Gregerman et al. (*23*) and Braverman et al. (*24*) in earlier studies detected no changes in PBI values in males aged 20–90. Furthermore, Gregerman noted that thyroxine levels remained stable throughout life through a combination of homeostatic adjustments including a decline in T_4 distribution space, a decrease in T_4 turnover rate, and an increase in T_4 degradation rate (*23*). Hesch found that alterations in TBG—which declined in middle age and then increased late in life—did not account for the lower T_4 values observed in his subjects over age 80 (*3*).

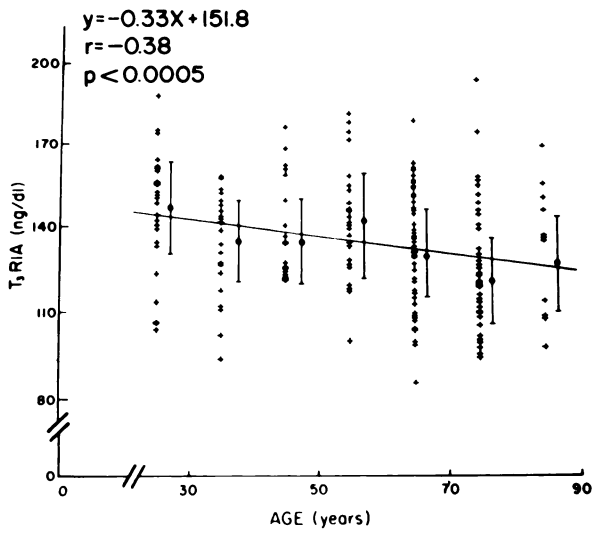

FIG. 4. T_3 values (ng/dl) for males and females aged 20–89 by decade, have weak negative correlation ($r = -0.38$, $p < 0.0005$). Means (heavy circles) ± standard deviations are shown. A given point may represent more than one observation.

The results for T_3 resin uptake values correspond well with the thyroxine findings, with T_3U unchanged for males and slightly but significantly increased for postmenopausal females. These results suggest that slight declines in TBG occur in females after age 50, due most likely to a fall in circulating estrogens. Our results support Braverman's study of sex-related changes in TBG (*22*), which concluded that premenopausal females had slightly higher TBG levels than male counterparts, and that their T_3U values were significantly lower than those of males. In further support of these data, Hansen et al. (*9*) observed an increase in T_3U in women over age 40, from a mean of (27.6 ± 4)% to (30.3 ± 3.3)%.

The free thyroxine index, a parameter well correlated with free thyroxine concentration (*25*), showed no age- or sex-related changes. Hansen et al. (*9*) and Bermudez et al. (*13*), using similar calculations, reached the same conclusion. Other studies using direct measurements have not completely agreed with the calculated findings. Herrmann et al. (*2*) found that free T_4, as well as total T_4, was normal but significantly lower in a group of 77 euthyroid subjects aged 65–92, when compared with adults 20–64 yr old. Lemarchand-Béraud and Vannotti (*26*), using a dialysis method, noted probably significant increases in free T_4 in their small series of nine subjects

over age 70. From previous studies and the data compiled in 209 subjects in this investigation, it can be concluded that changes in free T_4 are small and that values stay within the normal range into old age.

There were significant changes in serum TSH. Although overall male and female serum TSH concentrations were normal and showed no difference in values, older females had significantly higher TSH values than women under age 60. The mean serum TSH in males, although higher than that of females under 60, was lower than values in women over 60. Other investigators have noted similar findings. Jeske et al. (*7*) found that 20% of their euthyroid subjects over age 70 had slight increases in TSH. Lauridsen (*27*) and Lemarchand-Béraud and Vannotti (*26*) reported unexplained age-related TSH increments in both sexes, although values remained within normal limits. Since FT_4 indices were stable and normal in both sexes, regardless of age, it is unlikely that differences in free T_4 account for the change. However, it is possible that free T_3 levels might be mildly lower in older females, resulting in higher serum TSH. Both Hesch et al. (*1*) and Herrmann et al. (*2*) reported decrements, unrelated to sex, in free T_3 in elderly normal subjects, and Bermudez et al. (*13*) noted a decrease in free T_3 index in a small group of older normals. Moreover, Hesch noted that TSH, which was unmeasurable in many younger subjects, could be measured (and was normal) in most of the older group. Another possibility, although not substantiated, is that TSH turnover rates may decrease with age in females. Also, males may produce and release less TSH with advancing years. Snyder and Utiger's findings (*28,29*) of relative hyporesponsiveness of TSH to thyrotropin-releasing hormone (TRH) only in older males supports this explanation. Azizi et al. (*30*) noted that women aged 50–63 were significantly more responsive to i.m. TRH at 4–5 hr than were their male counterparts. Moreover, males aged 50–63 were noted to be less responsive to 2 mg of i.m. TRH than were men aged 20–33, given the same dose.

Triiodothyronine levels were found to decrease with age in both sexes. These findings agreed with most previous studies, but the decline in T_3 was not as profound as previously reported (*1,6–9,11*). Pawlikowski et al. (*4*) noted variable findings and Westgren et al. (*5*) found that T_3 decreased in subjects only after age 80. The reason for the decline in serum T_3 is unknown. It is not likely that lower serum T_3 values in old age are the result of a decrease in TBG. Braverman et al. (*24*) demonstrated that TBG increases in the elderly, a fact that Hesch et al. (*1*) confirmed in their study of older normal subjects with low total and free T_3. A possible explanation may be decreased thyroidal T_3 production and release. It is also possible that the degradation rate of T_3 is increased in old age. Wenzel and Horn (*31*), however, suggested from kinetic data in elderly men that T_4 disposal and T_4-to-T_3 peripheral conversion rates were decreased. Nevertheless, a definitive study of T_4 monodeiodination and T_3 kinetics in aging has not been performed, and it is possible that the lowered T_3 is a result of a combination of these factors. Also, intracellular T_3 concentrations in the aged are unknown.

Reverse T_3 exhibited interesting age-related changes. Males demonstrated stable values, which were normal but significantly higher than those of females of similar age. Women over age 60 had normal but significantly lower rT_3s than younger females. The data from the few studies examining variation of rT_3 with age conflict with our results. Nicod (*32*) found that 20 allegedly healthy geriatric hospital patients had slight increases in rT_3 compared with the relatively stable values observed in a younger population. It is possible that this small number of subjects were suffering from chronic illnesses that alter T_3 and rT_3 dynamics. The wide fluctuations in rT_3 concentration noted by Nicod in patients over age 70 were much greater than the variations observed in this investigation. In another study, in a large normal group Fisher et al. (*33*) found small increases in rT_3 throughout childhood and adolescence.

There are many possible explanations for these observations on rT_3. The decline in female rT_3 with age may be secondary to a decrease in TBG resulting from a fall in estrogen levels postmenopausally. It is widely accepted that rT_3 is bound to TBG, and several studies using different methods for measuring rT_3 (*34–36*) demonstrated that the hormone increased during pregnancy, probably secondary to the effects of high circulating estrogens on TBG. Moreover, Chopra et al. (*36*) noted that rT_3 was elevated in five subjects known to have high TBG. Also, it is possible that differences in rT_3 kinetics exist between males and females. Thyroidal rT_3 secretion in men, although low, may be greater than in women. Alternatively, females may have increased degradation of rT_3 (presumably in the liver and kidney) to account for the declining values that were observed.

The current data do not favor increased peripheral conversion of T_4 to rT_3 as an explanation of these results, since rT_3 did not increase with age whereas T_3 levels fell. In most other situations where altered peripheral conversion has been suspected, T_3 has declined while rT_3 has increased (*13,14*). The current data suggest that the age-related changes in rT_3 probably reflect independent alterations in its production, clearance, and degradation. It is still possible, however, that through an independent process, T_4 peripheral conversion to T_3 decreases without an increase in peripheral conversion to rT_3.

FOOTNOTES

* Abbott Laboratories, Chicago, IL.
† Mallinckrodt Res-O-Mat T_3 Micro Test, St. Louis, MO.
‡ Corning Medical Laboratories, Medfield, MA.
‖ Serono Laboratories, Boston, MA.

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