JCI The Journal of Clinical Investigation

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J Clin Invest. 1966;45(8):1273-1279. https://doi.org/10.1172/JCI105434.

Research Article

Journal of Clinical Investigation
Vol. 45, No. 8, 1966

Observations concerning the Binding of Thyroid Hormones in Sera of Normal Subjects of Varying Ages *

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In man, from childhood through senescence, there occur alterations in several metabolic functions that are regulated in part by the thyroid hormones. Thus, aging is associated with a decrease in basal oxygen consumption per unit surface area and an increase in total serum cholesterol and beta-lipoprotein cholesterol (1–5). Several indexes of thyroid function per se indicate greater thyroid activity in children and adolescents than in young adults and, conversely, less thyroid activity in the elderly. The extensive literature on this subject has been considered in recent publications (6, 7). It has been suggested, therefore, that there exist in childhood and senescence states of physiologic hyper- and hypothyroidism, respectively (4, 6, 8). It is not clear, however, whether this is truly the case. In this regard it may be significant that, in contrast to the situation in clinically recognizable thyroid disease, the serum protein-bound iodine (PBI) varies little, if at all, throughout the life-span of man (6, 9, 10).

Recent studies related to these questions have indicated that the fractional rate of turnover of thyroxine (T_4) in peripheral tissues decreases significantly from childhood through senescence (6, 8, 10–12). Since variations in the binding of thyroid hormones to extracellular proteins greatly influence the kinetics of hormonal metabolism (13–15), the present studies were undertaken to determine whether the changes in the metabolism of T_4 that accompany aging can be ascribed to changes in extracellular binding proteins or to alterations within the tissues themselves.

Methods

Sera were obtained from 99 subjects ranging between 2 and 87 years of age. Except in the prepubertal group, all were males. All donors were in good health. In the older subjects, as would be expected, chronic degenerative disorders were present to a variable degree, but none were ill and all were engaged in their routine daily activities. None were hospital in- or outpatients.[1] No donor of blood was taking any medication known to influence the binding of thyroid hormone in serum. Sera were obtained from freshly drawn blood and were divided into several aliquots, which were quickly frozen and thawed only once before their use in a specific test.

Since it was not possible to carry out any single analysis on all specimens at one time, specimens were divided into decade groups according to the age of their donor. In most instances, an equal number of specimens from each age group was analyzed concurrently. This minimized the possibility that any small differences among measurements in the several age groups could have been due to day-to-day variations in the analytic techniques. Measurements of the per cent of free T_4 in serum, the distribution of "endogenous" T_4 among the binding proteins, and the serum PBI, as well as tests of the *in vitro* resin uptake of ^{131}I-labeled triiodothyronine (T_3) were, with few exceptions, performed in the same sera. For measurement of the T_4-binding capacities of the thyroxine-binding globulin (TBG) and thyroxine-binding prealbumin (TBPA), however, sufficient quantities of these specimens were sometimes not available. Therefore, some additional specimens were obtained from different donors.

Measurements of the per cent of free or unbound T_4 in serum were performed in duplicate by a technique described in detail elsewhere (16). In such analyses, serum is enriched with approximately 0.6 μc ^{131}I-labeled T_4[2] per ml; this results in an increase in stable T_4 concentration of approximately 2 μg per 100 ml. The absolute concentration of free T_4 iodine was calculated as the product

* Submitted for publication November 17, 1965; accepted April 28, 1966.

Supported in part by research grants AM-07917, AM-00267, and AM-09753 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

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[1] The majority of serum samples in the elderly were obtained from personnel of St. Gabriel's Monastery, Brighton, Mass.

[2] Abbott Laboratories, Oak Ridge, Tenn.

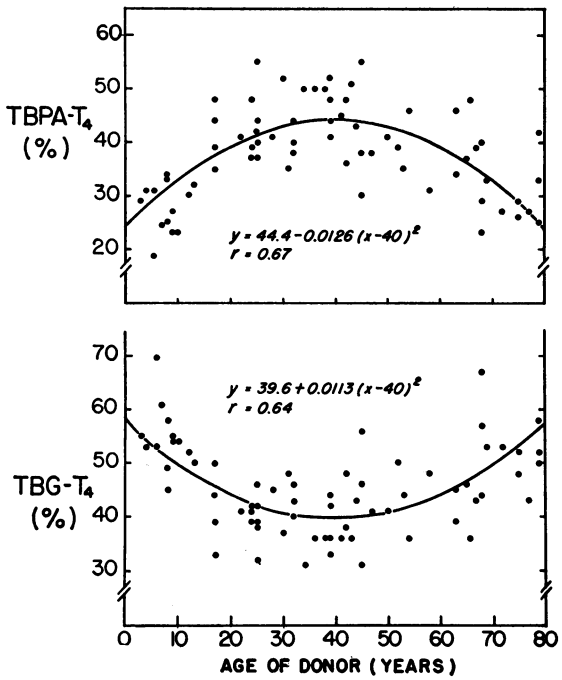

FIG. 1. BINDING OF TRACER CONCENTRATIONS OF THYROXINE (T_4) TO TBPA AND TBG IN SERA OF DONORS OF DIFFERING AGES. r values shown are the coefficients of correlation for the linear relationship between TBPA-T_4 and TBG-T_4, respectively, and values for $(X-40)^2$, where X equals the age of the patient. For both correlations $p < 0.01$. TBPA = thyroxine-binding prealbumin, and TBG = thyroxine-binding globulin.

of the per cent of free T_4 and the PBI. Absolute concentrations of free T_4 were calculated by dividing values for the free T_4 iodine by 0.65. Samples of the sera containing tracer concentrations of ^{131}I-labeled T_4 used in analyses for free T_4 were employed to determine the distribution of endogenous T_4 among the T_4-binding proteins. Paper electrophoretic methods used in the latter analyses and in estimating the T_4-binding capacities of TBG and TBPA have been fully described elsewhere (17). *In vitro* resin uptakes of ^{131}I-labeled T_3 from sera were measured by the technique of Sterling and Tabachnick, and values were compared with those obtained in a pool of normal serum assayed concurrently (18). Serum PBI was measured by a modification of the method of Zak (19). Methods employed in the statistical evaluation of the data are discussed in relation to each group of major findings.

Results

The results obtained are shown in Table I. Although summarized according to decade age groups for purposes of brevity, data were analyzed as a continuum by regression and correlation analysis over the entire range of ages studied (20).

Distribution of tracer quantities of added T_4 in relation to age. When plotted as a scattergram on linear coordinates, data relating age to the proportion of endogenous T_4 bound by TBG and TBPA, respectively, displayed a distinct pattern. The proportion of T_4 associated with TBG was relatively high in the youngest age groups, decreased in the middle age groups, and increased in the older age groups (Figure 1). In general, the data described an approximate parabolic curve, the nadir of which occurred during the fourth or fifth decade. Converse findings were obtained with regard to the proportion of T_4 associated with TBPA.

To test the statistical significance of these apparently complex curvilinear relationships to age, regression and correlation analysis was applied. As would be expected from the biphasic character of the relationships, linear correlations of age vs. the per cent of T_4 bound by TBG or TBPA were very low ($r = 0.05$ and 0.06, respectively). It was therefore necessary to transform the data so that regression analysis could be applied to the curvilinear function. In view of the contour of the curves, conformity to a parabolic relationship was tested. By inspection, the nadir and zenith of the two curves appeared to occur at approximately age 40. Therefore, the curve for the TBG-T_4 proportion could be described by the general formula $Y = a_1 + b_1 (X - 40)^2$, and that for the TBPA-T_4 proportion by the formula $Y = a_2 - b_2 (X - 40)^2$, where X is age, Y is the per cent of T_4 bound by each of the proteins, a_1 and a_2 are constants representing the minimal and maximal values of the two curves, respectively, and b_1 and b_2 are constants. The foregoing equations could then be transformed to the linear form $Y = a_1 + b_1Z$ and $Y = a_2 - b_2Z$, where Z equals $(X - 40)^2$. Individual values for Z were then calculated, and the linear correlation between Y and Z was analyzed; the regression of Y on Z was also calculated by the method of least squares (20). Such analyses revealed highly significant correlations between age and transformed values for the per cent of T_4 bound by TBG and TBPA ($r = 0.64$ and 0.67, respectively; $p \ll 0.01$. Best fit regression equations and the parabolic curves which they generate are shown in Figure 1.[3]

[3] These findings do not necessarily indicate that the true relation between age and the proportion of endoge-

Among individual sera there was observed a highly significant negative correlation between untransformed values for the per cent of tracer T_4 bound by TBG and that bound by TBPA ($r = 0.91$, $p \ll 0.01$). The per cent of added T_4 bound by albumin remained essentially constant in all age groups (linear correlation, $r = -0.03$) and averaged $16.9 \pm 3.7\%$ (mean $\pm$ SD), a value similar to that reported from this laboratory (14).

T_4-binding capacities of TBG and TBPA in relation to age. Measurements of the T_4-binding capacities of TBG and TBPA were performed in sera from 9 donors in the first decade, 11 in the fourth decade, and 15 in the seventh and eighth decades. Many, though not all, of the donors were the same as those whose sera were also analyzed for other aspects of T_4 binding. As compared to the middle age group, children displayed a significantly higher binding capacity of TBG ($p < 0.001$) and a lower binding capacity of TBPA ($p < 0.001$). In general, similar differences from values in the middle age group were noted in sera obtained from the oldest group. TBG was slightly, but not significantly, higher ($0.10 > p > 0.05$) and TBPA significantly lower ($p < 0.01$) in specimens from the elderly subjects. Values for TBG binding capacity in the youngest and oldest age groups did not differ significantly from each other, but values for the T_4-binding capacity of TBPA were significantly lower in the children ($p < 0.01$).

Other studies of hormonal binding in relation to age. No significant linear correlations with age were evident in the values obtained for the per cent of free T_4 ($r = 0.14$), the absolute concentration of free T_4 iodine ($r = 0.05$), the PBI ($r = -0.17$), or resin uptake of T_3 ($r = 0.04$). Similarly, no significant correlations with age were evident when, as had been done with the distribution of endogenous T_4, curvilinear relationships with age were sought.

Discussion

A consideration of data obtained in several laboratories indicates that in man, from childhood through senescence, pronounced changes occur in the peripheral metabolism of the thyroid hormones. In childhood, the fractional rate of peripheral turnover of both T_4 and T_3 is more rapid than in adults (6, 8). Although total daily turnover of T_4 is much less than in adults in absolute terms, it may exceed that of adults when values are expressed in relation to body weight (6, 8). Gregerman, Gaffney, and Shock have demonstrated that during the period from adulthood to senescence, the fractional turnover of T_4 declines still further and total daily turnover of the hormone is decreased, even in relation to body weight (10).[4] These changes in hormonal metabolism are not associated with changes in the concentration of T_4 in the blood, as measured by PBI (6, 9, 10). Gregerman and colleagues suggested that these age-related changes in T_4 metabolism might result from a progressive change in the binding of T_4 in extracellular fluids (10). Increasing T_4 binding, by decreasing the proportion of free hormone, would be expected to decrease the fractional turnover of the hormone (13–15). However, as judged from other states in which hormonal binding is altered, increased binding of T_4 should also lead to an increase in PBI and in the content of the peripheral T_4 pool, with the result that total hormonal turnover remains unchanged (13–15). Therefore, it was clear a priori that increased T_4 binding in advancing age, even if present, could not alone account for age-related changes in hormonal metabolism. Nevertheless, the present studies were undertaken to determine whether changes in hormonal binding contribute to age-related changes in hormonal metabolism. The data seem to clearly indicate that they do not. This conclusion is based on analyses of two measures of over-all hormonal binding, the proportion of free or unbound T_4 in serum and the *in vitro* resin uptake of T_3. Neither function displayed a significant correlation with age, regardless of whether such correlation was sought as a linear function or whether more complex interrelationships were sought. Although some variation in values for the proportion of free T_4 and the resin uptake of T_3 was found in various age groups, such variation was not systematic, and all values

nous T_4 bound by TBG or TBPA is precisely parabolic in nature or that the point of inflection of the curves necessarily occurs at age 40. Rather, the significant correlations merely serve to establish the biphasic nature of the relation between age and T_4 distribution with respect to TBG and TBPA.

[4] When the data of these workers are recalculated in relation to body weight, it does not appear that the volume of distribution of thyroxine, per se, decreases with advancing age.

were essentially within the general normal range for these tests. In primary disorders of T_4 binding, there occur deviations from adult T_4 turnover rates comparable to those seen in childhood and senescence (13–15). However, under these circumstances, values for the per cent of free T_4 and the resin uptake of T_3 are distinctly abnormal (16, 18, 21–23) and clearly different from those obtained in childhood and senescence in the present studies.

It is generally believed that the total concentration of free T_4 in the serum reflects the quantity of hormone available to the tissues (13–16, 24, 25). In most circumstances in which the absolute concentration of free T_4 is altered, other studies have revealed that the absolute rate of turnover of T_4 is similarly abnormal (12, 26, 27). In advancing age, however, T_4 turnover or disposal progressively declines, although as the present studies have demonstrated, the absolute concentration of free T_4 remains unchanged.

The foregoing considerations indicate that the slowing of T_4 turnover that occurs with advancing age cannot be ascribed to changes in the binding interactions of T_4 within the blood. The possibility that, at different ages, such binding interactions might differ significantly *in vivo,* but not *in vitro,* seems quite remote. Current concepts, however, view extracellular binding as a major, but not the sole, determinant of hormonal turnover, since hormonal metabolism could be altered by changes in cellular avidity, penetrability, or degradative and excretory activity with regard to the hormone. It is not clear at present to which of these factors the age-related changes in T_4 metabolism should be ascribed or whether the causative changes occur generally or only within specific organs.

It is possible that the decrease in turnover of T_4 that occurs from childhood through senescence is, in some manner, related to the decrease in basal oxygen consumption that occurs during this period (1–3). It has been proposed that certain metabolic actions of the thyroid hormone may depend upon the metabolism of the hormone (28–30). A progressive decline with advancing age in the activity of mechanisms for hormonal metabolism, although leading to a decrease in hormonal turnover, could result in diminished metabolic effectiveness of the hormone, producing, in a sense, hypothyroidism at the tissue level. Alternatively, it has also been suggested that the turnover of T_4 is somehow linked to and varies with the peripheral metabolic rate (14, 15, 26). Thus, decreas-

TABLE I

*Indexes of thyroid hormone binding in sera from normal subjects of varying age**

Age range	Endogenous T_4 distribution (% added T_4 bound to protein)			T_4-binding capacity		Serum free T_4	PBI	Serum free T_4	Resin T_3 uptake
	TBG	Albumin	TBPA	TBG	TBPA				
				μg T_4/100 ml serum		*% total*	*μg/100 ml*	*mμg/100 ml*	*% normal pool*
2–12	55.0±7.0† (12)	18.2±3.7	26.8±5.5	27.3±3.6 (9)	72±26	0.050±0.007 (7)	6.1±0.7 (7)	4.70±0.74 (7)	101.8± 7.5 (9)
16–20	43.4±7.2 (5)	17.0±3.9	39.6±6.5			0.050±0.015 (7)	5.2±0.7 (6)	3.60±0.77 (6)	100.8± 7.0 (5)
21–30	40.2±4.0 (11)	16.6±3.3	43.1±5.9			0.057±0.015 (10)	5.2±1.1 (10)	4.46±1.41 (10)	104.9± 8.6 (10)
31–40	39.5±5.7 (11)	15.8±1.5	44.6±5.8	21.5±3.6 (11)	183±26	0.055±0.011 (9)	5.2±0.7 (8)	4.27±0.91 (8)	105.8±11.3 (13)
41–50	41.7±7.1 (10)	15.7±2.5	42.7±7.9			0.057±0.008 (9)	5.3±0.9 (8)	4.60±1.14 (8)	104.3±13.3 (12)
51–60	44.6±6.3 (4)	17.7±4.3	37.8±6.4			0.050±0.004 (5)	4.8±0.5 (4)	3.51±0.38 (4)	101.0±11.4 (4)
61–70	47.8±9.7 (9)	15.5±3.1	36.5±8.9	25.3±5.8 (15)	128±60	0.058±0.014 (9)	5.4±0.8 (8)	4.75±1.23 (8)	101.9±15.5 (11)
>70	50.8±4.7 (7)	19.5±7.3	29.7±5.8			0.054±0.008 (7)	5.1±1.2 (5)	4.20±1.34 (5)	107.7± 8.0 (7)

* Abbreviations: T_4 = thyroxine, T_3 = triiodothyronine, PBI = protein-bound iodine, TBG = thyroxine-binding globulin, and TBPA = thyroxine-binding prealbumin.

† Values represent mean ± standard deviation. Numbers in parentheses for each analysis indicate the number of specimens analyzed.

ing energy metabolism with increasing age may produce a secondary decline in thyroid hormone degradation.

In addition, the decreasing basal metabolic rate and slowing of both fractional and total T_4 turnover that accompany advancing age are reminiscent of comparable changes that occur in patients with thyroid insufficiency. In contrast to patients with primary thyroid failure, however, elderly patients display normal values for both the PBI and the absolute concentration of free T_4. Thus, the decreased thyroid function and diminished hormonal secretion that have been demonstrated in elderly individuals probably result, not from failure of the thyroid, but from decreased thyroid stimulation. Whether such decrease is merely the expected response to a decrease in hormonal disposal or whether the hypothalamic-hypophyseal system shares with other peripheral tissues a decreased requirement for thyroid hormone is uncertain. It is apparent, however, both from the complexity and circularity of the foregoing suggested interrelationships and from our present inability to distinguish causes from effects, that many challenging problems remain to be solved in this area of study.

Although the present studies have revealed no change in the over-all intensity of thyroid hormone binding in serum over the range of ages studied, changes in the distribution of tracer quantities of labeled T_4 among the several binding proteins were demonstrated. Thus, the proportion of endogenous T_4 bound by TBG decreased from childhood to adulthood and then increased with increasing age; the proportion bound by TBPA changed reciprocally. At endogenous concentrations of T_4, TBG and TBPA compete for hormone, and shifts of hormone from one protein to the other may reflect a change in the binding activity of only one of the two. On the other hand, it is generally believed that measurements of the binding capacity of one protein are not influenced by changes in the binding capacity of competing proteins. It appears, therefore, that the age-related variation in the distribution of endogenous T_4 observed in the present studies results from reciprocal alterations in the binding activity of both TBG and TBPA, since measured T_4-binding capacities of TBG tended to be higher in childhood and senescence than in young adulthood, whereas binding capacities of TBPA varied inversely.[5] These data in regard to the T_4-binding capacities of TBG and TBPA in the younger age groups are in accord with the findings in most previous studies of this subject. Thus, Meister, as cited by Kunstadter, Buchman, Jacobson, and Oliner (33), found the TBPA-binding capacity in the sera of children to be lower than in adults. In addition, in males, Dreyer and Man found a decline in TBG and increase in TBPA capacities during the transition from adolescence to the adult state (34). In view of the fact that hormones with predominately androgenic or anabolic activity decrease the binding activity of TBG (20, 35) and tend to increase that of TBPA (20, 36), it may be that such age-related changes in the binding proteins are related to changes in gonadal activity. Comparable studies during the prepubertal, child-bearing, and postmenopausal periods in women would serve to clarify this point. It is not clear, however, what physiological function the redistribution of T_4 among the binding proteins may subserve, since significant differences in the function of TBG and TBPA, although postulated to exist (37), have not yet been clearly demonstrated.

Summary

Indexes of thyroid hormone binding have been assessed in sera obtained from 99 normal subjects whose ages ranged between 2 and 87 years. Electrophoretic analyses revealed a highly significant age-related change in the proportion of "endogenous" thyroxine (T_4) bound by thyroxine-binding globulin (TBG). This decreased from childhood to adulthood, reached a nadir in the

[5] In the present studies, a Tris-maleate system, pH 8.6, was the electrophoretic buffer employed. Values for the T_4-binding capacity of TBPA in this buffer are lower than those obtained in other buffer systems used in paper electrophoresis (25, 31). However, comparative studies in this laboratory of a large number of sera in which T_4-binding capacities differed widely revealed that a similar rank order of binding capacities was obtained in Tris-maleate and glycine-acetate buffers, although absolute values in the two buffer systems differed. Furthermore, in disorders in which the T_4-binding capacity of TBPA is abnormal, as in patients with nonspecific illness, the findings in Tris-maleate and glycine-acetate buffers are qualitatively similar (25, 32). It is therefore reasonable to suppose that age-related differences in T_4 binding would also be evident in buffers other than Tris-maleate.

fourth or fifth decade, and then increased with advancing age. The proportion of endogenous T_4 bound by the thyroxine-binding prealbumin (TBPA) displayed a change that was reciprocal to that bound by TBG. These changes were associated with changes in the T_4-binding capacities of TBG and TBPA, as assessed electrophoretically. Thus, specimens obtained from children (first decade) revealed a higher T_4-binding capacity of TBG and a lower T_4-binding capacity for TBPA than did specimens from individuals in the fourth decade. The latter specimens, in turn, revealed a slightly lower T_4-binding capacity of TBG and a higher T_4-binding capacity of TBPA than did specimens from subjects in the seventh and eighth decades.

The age-related change in the distribution of T_4 between TBG and TBPA was not reflected in changes in several indexes of the over-all intensity of thyroid hormone binding. Thus, values for the protein-bound iodine, the proportion and absolute concentration of free T_4, and the *in vitro* resin uptake of triiodothyronine displayed no significant correlation with age. We conclude that the progressive slowing of the peripheral turnover of T_4 that occurs from childhood through senescence does not result from alterations in the binding of thyroid hormone in the plasma, but is probably due to factors associated with the cellular uptake or metabolism of the hormone per se.

Acknowledgments

We are indebted to Mrs. Ann King and Miss Louise Cologiovanni for excellent technical assistance.

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