THE EFFECTS OF SOYBEAN DIET ON THYROID HORMONE AND THYROTROPIN LEVELS IN AGING RATS

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Objective. To estimate the effect of soybean diet on serum level of thyroid hormone, its metabolites and thyrotropin (TSH) during aging in rats.

Methods. Male Donryu rats were fed laboratory chow containing 40 (Group A) or 10 volume percent (Group B) soybean protein, while controls (Group C) received regular laboratory chow. Groups of 10 animals of each groups were sacrificed by decapitation at the age of 12, 18, 24 and 30 months. Serum total thyroxine (T_4), free thyroxine (FT_4), 3,5,3'-triiodothyronine (T_3), 3,3',5'-triiodothyronine (rT_3) and 3,3'-diiodothyronine (3,3'-T_2) and TSH concentrations were measured by specific radioimmunoassays.

Results. In Group A the level of T_3 decreased significantly at from the age of 18 months, while in other groups such decrease was found only from the age of 24 months. Such changes were closely resembled by these in the level of 3,3'-T_2, while inverse changes were observed in the level of rT_3 which was increased in Group A from the age of 18 months and in the other groups from the age of 24 months. Serum T_4 and FT_4 level was decreased in all groups at the age of 30 months and no changes were observed in the level of TSH.

Conclusions. The findings suggest that the level of T_4, FT_4 and T_3 with its metabolite 3,3'-T_2 stepwise decreased with aging, while that of rT_3 showed inversely and increase. These changes were influenced by the content of soybean protein in the diet, the most rapid changes being found in the group with the high content of such protein.

Key words: Thyroxine – Triiodothyronine – Reverse triiodothyronine – Thyrotropin – Aged rats – Soybean diet

There are several reports on the changes in serum thyroid hormone levels during aging in rats (Sartin et al. 1977; Frolkis et al 1978; Klug and Adelman 1979; Chen and Walfish 1979; Forciea et al. 1981; Jang et al. 1985). However, some data appear to be still contradictory. For instance, Klug and Adelman (1979) reported a decrease of thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3) with aging in rats, while Jang et al. (1985) did not find any changes in T_3 concentrations. From these and other data follows that the possible changes in thyroid hormone concentrations in aged rats still remain to be elucidated.

On the other hand, it is known that even the diet composition may influence the concentration of thyroid hormone in serum (Wartofsky and Burman 1982; Engler and Burger 1984) and, in addition, the diet composition also affects the life span and incidence of diseases with aging (Yu et al. 1982, 1985; Maeda et al. 1989; Iwasaki et al 1988 a,b; Bertani et al. 1989; Jeckel-Neto 1994). From these reports it appears that, in aging rats, the diet composition could affect either the thyroid hormone secretion or metabolism, or both.

The aim of this study was to evaluate the thyroid function in aging rats fed the diets with varying concentration of soy bean protein.

Table 1
Composition of the diets

	Group A	Group B	Group C
Calories/100 g	335	338	326
Crude protein (%)	39.9	17.7	10.2
Crude fat (%)	3.81	3.70	3.85
Crude ash (%)	5.2	6.5	4.9
N free extract (%)	35.3	58.5	62.6
Minerals (%)	3.46	3.42	3.41

Materials and Methods

Animals: Male Donryu rats were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and individually housed in specific-pathogen free (SPF) facility under the temperature (23±1 °C) and illumination control (12:12 h light:dark cycle, lights on at 6.00 h).

The animals were divided into three groups, each of them being fed the diet with various content of soy bean protein (for details see Tab. 1), e.g. Group A 40 %, Group B 10 % and Group C 18 % of total volume as protein. The diets were formulated to provide equal caloric intake for all three groups which has been reported not to influence significantly the nutritional conditions (Yu et al. 1985). The diets for Groups A and B were kindly prepared by Clea Japan, Inc. (Tokyo, Japan) and the diet for the Group C was commercially available rodent chow from the same company. The rats were given tap water *ad libitum*.

The body weight of each animal was recorded weekly during the whole observation period and the animals were examined for external symptoms of diseases. The animals were decapitated under slight ether anesthesia at 12, 18, 24 and 30 months after birth and serum was obtained by centrifugation of collected blood. At autopsy, all organs were examined macroscopically and weighed, the major organs were examined for pathological findings and the animals with tumors were excluded from the final evaluation.

Radioimmunoassay of hormones: Thyroxine (T_4), 3,3',5-triiodothyronine (T_3), 3,3',5'-triiodothyronine (rT_3) and 3,3'-diiodothyronine (T_2) were measured by specific in house radioimmunoassays (Mitsuma 1978; Mitsuma et al. 1976, 1979). TSH was determined by NIAMDD rat TSH kit and free T_4 (FT_4) was measured by commercially available kit.

Table 2
Serum concentrations of T_3, rT_3 and 3,3'-T_2 (means ± S.E.)

	Group A	Group B	Group C
T_3 (ng/dl)			
12 months	66 ± 4	70 ± 4	65 ± 3
18 months	38 ± 2a,b	68 ± 4	64 ± 3
24 months	30 ± 1a	38 ± 3a	40 ± 2a
30 months	31 ± 2a	34 ± 2a	36 ± 2a
rT_3 (ng/ml)			
12 months	12.0 ± 0.6	13.0 ± 0.7	12.9 ± 0.7
18 months	22.0 ± 1.0a,b	13.2 ± 0.6	13.5 ± 0.8
24 months	21.0 ± 0.9a	20.7 ± 0.9a	19.8 ± 0.8a
36 months	19.8 ± 0.9a	19.7 ± 0.8a	19.7 ± 0.7a
3,3'-T^2 (ng/dl)			
12 months	7.3 ± 0.3	7.7 ± 0.3	7.6 ± 0.4
18 months	4.2 ± 0.2a,b	6.8 ± 0.2	7.2 ± 0.3
24 months	2.6 ± 0.1a	3.6 ± 0.1a	3.8 ± 0.2a
36 months	2.4 ± 0.07a	3.0 ± 0.1a	3.4 ± 0.1a

The values are expressed as means ± S.E., the number of animals being 10 for each group and time interval.
a - significant difference between ages ($P<0.001$)
b - significant difference between groups ($P<0.001$)

Statistical evaluation: Mean and standard error of the mean were calculated for each group. The differences between groups were evaluated by analysis of variance.

Results

Serum T_3 and 3,3'-T_2 concentrations showed a decrease with aging during the whole observation period. Their significant decrease in the Group A appeared as early as at 18 months, while that in Groups B and C was found at 24 months (Tab. 2). Serum T_4 and FT_4 concentrations decreased at 30 months in all groups (Tab. 3). In contrast, serum rT_3 concentrations increased significantly at 18, 24 and 30 months in Group A, while such increase in Groups B and C was found after 24 and 30 months (Tab. 2). Mainly due to the decrease of T_4 level the T_3/T_4 ratio decreased at 18 and 24 months in Group A and at 24 months only in Groups B and C (Tab. 4). The rT_3/T_4 ratio increased at 18, 24 and 30 months in the Group A and only at 24 and 30 months in the Groups B and C which was mainly due to the considerable decrease of T_4 level (Tab. 4). However, serum TSH level did not change in any group or time interval (Fig. 3).

Table 3
Serum concentrations of T_4, FT_4 and TSH (means and S.E.)

	Group A	Group B	Group C
T_4 (mg/dl)			
12 months	8.6 ± 0.3	8.4 ± 0.4	8.3 ± 0.4
18 months	8.4 ± 0.4	8.1 ± 0.3	8.0 ± 0.3
24 months	8.0 ± 0.4	8.2 ± 0.3	8.5 ± 0.4
30 months	1.8 ± 0.1a	2.5 ± 0.1a	2.3 ± 0.1a
FT_4 (ng/ml)			
12 months	3.8 ± 0.2	3.8 ± 0.2	3.6 ± 0.3
18 months	3.9 ± 0.3	3.3 ± 0.2	3.0 ± 0.2
24 months	3.5 ± 0.2	3.2 ± 0.2	3.2 ± 0.3
30 months	1.7 ± 0.1a	1.7 ± 0.1a	2.0 ± 0.1a
TSH (ng/ml)			
12 months	210 ± 19	215 ± 21	212 ± 20
18 months	225 ± 21	224 ± 22	226 ± 23
24 months	230 ± 24	227 ± 24	215 ± 21
30 months	220 ± 22	220 ± 21	226 ± 23

The values are expressed as means ± S.E., the number of animals being 10 for each group and time interval.
a - significant difference between ages ($P<0.001$)

Table 4
The T_3/T_4 ration and rT_3/T_4 ratio in serum

	Group A	Group B	Group C
T_3/T_4 x 1000			
12 months	7.8 ± 0.4	8.3 ± 0.4	7.8 ± 0.3
18 months	4.6 ± 0.2a,b	8.4 ± 0.3	7.8 ± 0.4
24 months	3.2 ± 0.1a	4.6 ± 0.15a	4.7 ± 0.2a
30 months	15.6 ± 0.8a	13.6 ± 0.7a	15.6 ± 0.8a
rT_3/T_4 x 1000			
12 months	1.4 ± 0.08	1.5 ± 0.09	1.6 ± 0.1
18 months	2.7 ± 0.15a,b	1.7 ± 0.09	1.8 ± 0.1
24 months	2.6 ± 0.1a	2.5 ± 0.11a	2.3 ± 0.1a
30 months	11.0 ± 0.9a	7.9 ± 0.4	8.5 ± 0.5a

The values are expressed as means ± S.E., the number of animals being 10 for each group and time interval.
a - significant difference between ages ($P<0.001$)
b - significant difference between groups ($P<0.001$)

It may be summarized that most of the changes in Group A which was fed by high protein diet occurred earlier than these in Groups B and C.

Discussion

As far as we are aware, the influence of diet composition on serum thyroid hormone concentrations during aging have not yet been thoroughly evaluated. The present study attempted to evaluate this question in rats fed three different diets with various content of protein. In addition to different diets, the effect of aging was simultaneously evaluated by studying the hormone levels up to the age of 30 months.

It was demonstrated that the level of T_4 and T_3 in serum as well and the T_3/T_4 ratio decreased at the age of 30 months in all groups, while at the same time the level of rT_3 and the rT_3/T_4 ratio increased. These data partly confirmed the previous report in which T_3 and T_4 decreased in aged rats (Klug and Adelman 1979). We also found that the decrease in T_3 concentration occurred earlier in the Group A in which the protein consumption was the highest. This might result either from decreased T_3 secretion from the thyroid or decreased peripheral T_4 to T_3 conversion under simultaneously decreased T_4 level in serum or both. Since in this study the decrease of FT_4 paralleled that of T_4, it appears to be unlikely that even the decrease of thyroxine binding proteins may participate in the decrease of T_4 and T_3 levels at the age of 30 months.

The stepwise deiodination of T_4 to T_3 and rT_3 followed by that of T_3 to 3,3'-T_2 and 3,5-T_2 is due to the effect of deiodinases which are influenced by several external and internal factors including nonthyroidal diseases, diets and starvation (Wartofsky and Burman 1982; Engler and Burger 1984). The decrease of T_3 level in blood associated with elevated rT_3 is called "low T_3 syndrome" which is due to the decreased T_4 to T_3 conversion under simultaneously increased rT_3 production. The present data indicate that low T_3 syndrome is present in aged rats which is further confirmed by simultaneously decreased 3,3'-T_2.

Finally, several authors found nephropathy, tumors and degeneration of muscles in aged rats and reported that high protein diet induced nephropathy which resulted in shorter life span (Yu et al. 1985; Maeda et al. 1985; Iwasaki et al. 1988a; Bertani et al. 1989). Moreover, nephropathy is also known to induce the low T_3 syndrome (Wartofsky et al. 1982; Engler and Burger 1984). Thus, the present data and previous reports taken together suggest that a decrease in serum T_3 concentration may be due to the decrease inm T_4 to T_3 conversion induced by nephropathy in a high protein diet group during aging. Any considerable participation of pituitary thyrotropic function

in this process seems to be unlikely, since no changes in TSH levels were found which is also in agreement with the data by KLUG and ADELMAN (1979). In general, the findings suggest that protein content in the diet influences thyroid hormone metabolism in aged rats.

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Accepted: June 15, 1998