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Lack of Effect of Soy Isoflavone on Thyroid Hyperplasia in Rats Receiving an Iodine-deficient Diet

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We have reported a dramatic synergism between soy intake and iodine deficiency regarding induction of thyroid hyperplasia in rats. Because isoflavones are active constituents of soybeans, in the present study, their possible contribution was examined. Female F344 rats were divided into 8 groups, exposed to diet containing a 0.2% soy isoflavone mixture (SI), 0.2% SI+iodine deficiency (ID), 0.04% SI, 0.04% SI+ID, 20% defatted soybean (DS) alone, 20% DS+ID, ID alone or basal diet alone for 5 weeks. Thyroid weight was not influenced by SI, but was increased by the ID and DS diets with a further significant increment in the DS+ID group ($P<0.01$). Compared to the control value, serum T_4 was significantly ($P<0.01$) increased by 20% DS alone and decreased in all groups given the ID treatment ($P<0.001$). Serum thyroid stimulating hormone (TSH) level was increased by ID, and further enhanced by DS ($P<0.01$) but not SI. Histopathologically, diffuse hypertrophy and/or hyperplasia of thyroid follicles were observed in the ID-treated groups, the severity being enhanced by DS but not SI. Proliferating cell nuclear antigen labeling indices (%) were elevated in the ID diet groups and again enhanced by DS, but not SI. These results thus suggest that isoflavones may not be involved in the mechanisms underlying the synergistic goitrogenic effect of soybean with iodine deficiency.

Key words: Thyroid — Isoflavone — Iodine-deficient — Soybean

Soyfoods have received considerable attention from the viewpoint of their role in disease prevention, especially in relation to heart disease,[1)] osteoporosis[2)] and cancer.[3)] However, soybeans have long been implicated in diet-induced goiter.[4,5)] With regard to soy protein-induced goiter, little is known regarding the mechanisms or the responsible components. Recently, it was reported that isoflavones (genistein, daidzein) inhibit thyroid peroxidase (TPO)-catalyzed reactions essential to thyroid hormone synthesis[6)] and increase phase II enzymes[7,8)] such as glutathione *S*-transferase (GST), quinone reductase (QR) and uridine diphosphate glucuronyltransferase (UDP-GT), which inactivate thyroid hormones.[9)]

Iodine plays a central role in thyroid physiology, being both a major constituent of thyroid hormones and a regulator of thyroid gland function. Iodine-deficient diets are known to produce goiter and promote thyroid carcinogenesis.[10,11)] Recently, we reported dramatic synergism between defatted soybean (DS) intake and iodine deficiency (ID) regarding induction of thyroid hyperplasias in rats,[12)] in which DS substantially influenced thyroid hormone levels with ID within 5 weeks. Because soy isoflavones (SI) are active constituents of soybeans, in the present study their possible influence on the thyroid, alone and in combination with ID, was investigated in rats.

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MATERIALS AND METHODS

Specific-pathogen-free female F344 rats, 4 weeks old, were obtained from Charles River Japan Inc. (Kanagawa) and housed five to a polycarbonate cage with stainless steel wire-mesh as bedding in an air-conditioned animal room (room temperature; 23±2°C, relative humidity; 60±5%, a 12 h light/dark cycle). The animals were given ion-exchanged water and AIN-93G diet (Oriental Yeast Co., Ltd., Tokyo)[13)] *ad libitum*. Casein was replaced with gluten or DS flour as an alternative protein source in order to avoid possible contamination with iodine contained in casein sources. Animals without any abnormal findings after a 2-week acclimation period were selected for the present study. The rats were divided into 8 groups, each consisting of 5 animals with similar initial mean body weights. They received AIN-93G diet, 20% DS, 0.2% SI, 0.04% SI, ID, 20% DS+ID, 0.2% SI+ID or 0.04% SI+ID for 5 weeks. The SI contained more than 30% of aglycones (genistein 12–18%, daidzein 12–18% and glycitein 2–4%) and was obtained from Kikkoman Co., Ltd. (Tokyo). The dose of 0.04% SI used in this study is as high as that contained in 20% DS.

At autopsy, major organs including the thyroid, pituitary, kidney, brain, adrenal and liver were carefully examined macroscopically. After weighing, they were fixed in 10% phosphate-buffered formalin, routinely processed and sections stained with hematoxylin and eosin (H-E) were examined under a microscope. Thyroid proliferative lesions were classified as described in our previous report.[12] For the analysis of thyroid follicular cell proliferation, sections were immunohistochemically stained with anti-proliferating cell nuclear antigen (PCNA) antibody PC-10,[14] obtained from Dakopatts (Glostrup, Denmark). The numbers of PCNA-positive nuclei (PCNA-labeling indices) in 1000 cells in follicular epithelium were counted and expressed as percentage values.

Blood was collected from the abdominal aorta under ether anesthesia for hormone assays. Triiodothyronine (T_3), and thyroxine (T_4) were measured with a RIABEAD radio-immunoassay kit (Dainabott, Tokyo), and thyroid stimulating hormone (TSH) with a rat TSH kit (Amersham Life Science Inc., Arlington Heights, IL).

Variance in data for lesion multiplicities, body weights and organ weights were estimated for homogeneity by means of Bartlett's procedure. If the variance was homogeneous, the data were assessed by one-way analysis of variance (ANOVA) techniques with Student's *t* test for multiple comparison. If not homogeneous, they were analyzed by using the Kruskal-Wallis test followed by the Mann-Whitney *U* test.

RESULTS

Body weights were increased in the 20% DS and ID+20% DS groups ($P<0.01$) as compared to the control and ID alone group values (Table I). Relative organ weights are also shown in Table I. The weight of thyroid glands was increased by the ID diet ($P<0.05$ or 0.01) plus 20% DS ($P<0.01$), but not SI. There were no significant changes in the weights of brain, liver, pituitary, adrenals or kidneys, except for decrease of brain weight in the 20% DS+ID group as compared to the ID alone group value.

Table II summarizes data for thyroid hormone levels. Serum T_3 was significantly ($P<0.05$) elevated by the ID and ID+20% DS treatments as compared to the control value. Serum T_4 was significantly ($P<0.001$) decreased by

Table II. Serum T_3, T_4, TSH Levels in Rats Treated with Soybean or Soy Isoflavone with or without Iodine Deficiency for 5 Weeks

Groups	Hormones		
	T_3 (ng/ml)	T_4 (μg/dl)	TSH (ng/ml)
Control	0.95±0.06	4.52±0.21	5.70±1.00
20% DS	1.00±0.07	5.44±0.48**	6.30±1.46
0.2% SI	0.88±0.04	4.22±0.15	4.94±0.28
0.04% SI	0.86±0.05	4.12±0.29*	5.24±0.39
ID	1.16±0.18*	2.52±0.35***	8.16±2.64$^{\#\#}$
20% DS+ID	1.10±0.12*	2.04±0.17$^{\#***}$	14.20±2.92***
0.2% SI+ID	0.94±0.05$^{\#}$	2.16±0.18***	7.32±0.86
0.04% SI+ID	1.00±0.12	2.76±0.24***	8.72±4.21

Values are means±SD.
*, **, ***: Significant difference from the control value at * $P<0.05$, ** $P<0.01$, *** $P<0.001$, respectively.
#, ##: Significant difference from the ID group value at # $P<0.05$, ## $P<0.01$, respectively.
DS, defatted soybean; SI, soy isoflavone; ID, iodine deficiency.

Table I. Summary of Data for Final Body and Relative Organ Weights of Rats Treated with Soybean or Soy Isoflavone with or without Iodine Deficiency for 5 Weeks

Groups	Organs						
	Body weight (g)	Thyroid[a)]	Pituitary[a)]	Brain[b)]	Liver[b)]	Adrenals[a)]	Kidneys[b)]
Control	122.7±7.5	12.8±2.8	4.9±1.9	1.4±0.1	2.9±0.1	28.7±2.8	0.8±0.0
20% DS	130.8±4.6	14.4±1.7	4.9±1.6	1.3±0.0	2.8±0.2	23.3±3.1	0.7±0.0
0.2% SI	115.8±1.6	11.6±1.3	4.5±1.4	1.4±0.1	3.0±0.0	29.6±3.2	0.7±0.1
0.04% SI	123.0±7.9	10.0±3.0	3.4±1.5	1.4±0.1	3.0±0.2	28.0±1.6	0.8±0.0
ID	115.6±3.5	19.9±3.9*	5.2±1.5	1.4±0.1	3.0±0.1	26.4±3.5	0.8±0.0
20% DS+ID	142.6±5.5$^{\#\#}_{**}$	34.8±10.6$^{\#\#}_{**}$	4.9±1.0	1.2±0.1$^{\#}$	3.0±0.5	26.0±1.8	0.7±0.0
0.2% SI+ID	116.2±4.9	18.8±2.3*	4.3±1.5	1.4±0.0	2.9±0.1	29.7±3.8	0.7±0.0
0.04% SI+ID	119.8±5.6	22.1±5.0**	6.4±0.6	1.4±0.1	2.9±0.1	30.3±3.2	0.8±0.1

Values are means±SD.
*, **: Significant difference from the control value at * $P<0.05$, ** $P<0.01$, respectively.
#, ##: Significant difference from the ID group value at # $P<0.05$, ## $P<0.01$, respectively.
DS, defatted soybean; SI, soy isoflavone; ID, iodine deficiency.
a) mg/100 g body weight, *b*) g/100 g body weight.

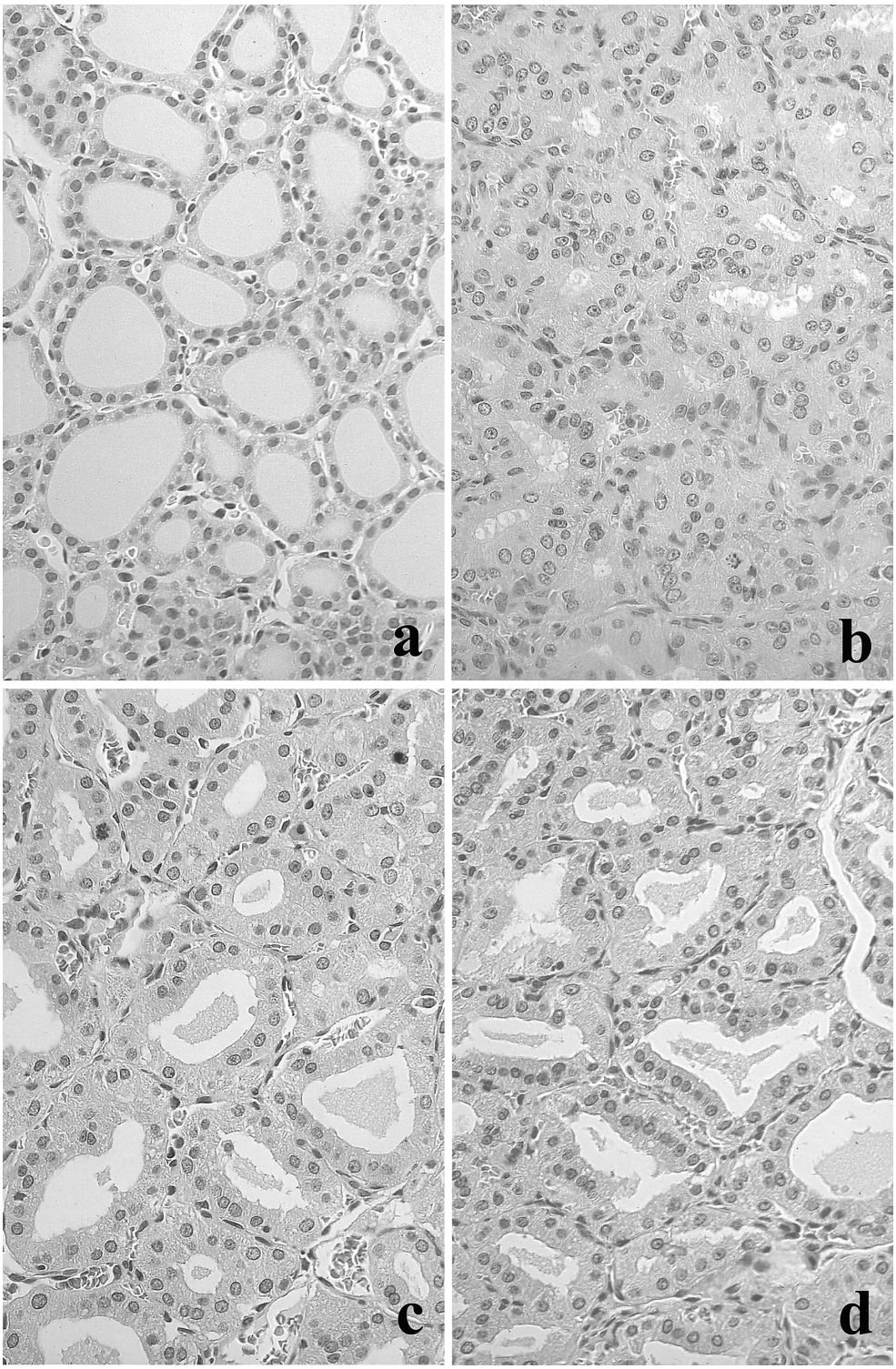

Fig. 1. Thyroid lesions of female rats receiving soybean or soy isoflavone with or without iodine deficiency for 5 weeks. a: Intact thyroid in a control rat. b: Showing severe diffuse hyperplasia in a rat receiving deffated soybean with iodine deficiency. c: Moderate hyperplasia in a rat receiving iodine deficiency alone. d: Moderate hyperplasia in a rat receiving 0.2% soy isoflavone with iodine deficiency. (Original magnification ×100).

Table III. PCNA Labeling Indices in Thyroid

Group	PCNA labeling index (%)
Control	0.09±0.13
20% DS	0.08±0.15
0.2% SI	0.08±0.05
0.04% SI	0.09±0.07
ID	8.44±2.91*
20% DS+ID	12.09±2.41*
0.2% SI+ID	9.40±1.48*
0.04% SI+ID	8.91±2.27*

Values are means±SD.
*: Significantly difference from the control value at * $P<0.001$.
DS, defatted soybean; SI, soy isoflavone; ID, iodine deficiency.

ID as compared to the control value, and enhanced by additional 20% DS but not SI, which in fact caused a decrease at 0.04% SI alone ($P<0.05$). Serum TSH was increased by ID, and further enhanced by 20% DS but not SI ($P<0.01$).

Histopathologically, diffuse irregularity of hypertropic follicular cells and decreased colloid were observed in all of the ID-treated rats (Fig. 1, b, c and d). The most prominent lesion was noted in the thyroid of 20% DS+ID group, in which diffuse follicular hypertrophy and/or hyperplasia and lack of colloid were evident (Fig. 1b). However, in the thyroid of the SI+ID group (Fig. 1d), changes were limited to mild to moderate irregularity of thyroid follicles. The thyroids without the ID treatment did not demonstrate apparent histological changes (Fig. 1a). Table III summarizes data for cell proliferation. The PCNA-labeling indices (%) were elevated by ID but not DS or SI alone. The value for the DS+ID group, but not either of the SI+ID groups, was higher than that for the ID alone group, albeit without statistical significance.

DISCUSSION

The fact of thyroid enlargement due to excessive soybean intake, especially in women and children, has been known for half a century. Soy protein diet has been shown to increase T_4, free T_4 and TSH levels in animals, whereas T_3 is generally not affected.[15–17] Experimentally, several investigators have also reported the induction of goiter in iodine-deficient rats maintained on a soybean diet.[12, 18] In the present study, 20% DS increased the serum T_4 ($P<0.01$) and TSH levels and synergistically stimulated thyroid growth in rats exposed to the ID diet. The mechanisms underlying the co-goitrogenic effect of excess DS and ID remain to be elucidated.

SI, such as genistein and daidzein, which are active constituents of soybeans, exhibit various biologic characteristics possibly associated with anticancer influence, including estrogenic/antiestrogenic[19] and antioxidant effects,[20] inhibition of TPO-catalyzed reactions[6] and elevation of UDP-GT.[7, 8] In addition, a synthetic plant flavonoid (EMD 21388) is known to displace T_4 from its binding protein and thus increase serum free T_4 in rats.[21] The structure (3-methyl-4′,6-dihydroxy-3′,5′-dibromoflavone) of this compound is somewhat similar to that of soybean genistein (4′,5,7-trihydroxyisoflavone). We therefore thought that feeding the isoflavone might have affected the thyroid hormone level and exerted synergistic goitrogenic effects with ID. However, SI did not affect histopathology or thyroid hormone levels except for the slight T_4 decrease in the 0.04% SI alone group with or without ID. These results are in line with a previous study,[22] in which isolated soy protein increased T_4 concentrations, but protein from soy protein concentrate, which was water-extracted and thus contained much higher levels of SI, did not. In addition, in our earlier study, isoflavone (400 ppm) and genistein (250 ppm) did not promote thyroid carcinogenesis due to *N*-bis(2-hydroxypropyl)nitrosamine in male[23] and ovariectomized rats.[24] Thus, these results indicate that inhibition of TPO and metabolic excretion by UDP-GT associated with SI may not be the main mechanisms underlying the goitrogenic effects of soybeans, and suggest that isoflavone alone may not be involved in the mechanism underlying the goitrogenic effects of soybean and the synergism with ID. In addition, SI has an estrogenic effect[19] and estradiol is known to influence thyroid hormone levels.[25, 26] Estrogen acts directly on thyroid tissue via estrogen receptors[27] and is known to enhance extra-thyroidal conversion of T_4 to T_3,[25, 26] resulting in increased secretion of TSH and T_3, but decreased T_4.[25] In addition, 17β-estadiol promotes *N*-methyl-*N*-nitrosourea-induced thyroid carcinogenesis under the influence of ID.[28] However, the estrogenic effect of SI is only weak[19] and SI did not change T_3, T_4 and TSH levels. Thus, it is possible that the estrogenic effect of SI is also not involved in the thyroid effects of soybean.

Although iodine plays a central role in thyroid physiology, the findings of iodine deficiency studies as a whole remain inconclusive.[29] The process of goitrogenesis is likely to be the consequence of an increased TSH stimulation linked to an initial reduction of circulating thyroid hormone caused by iodine deficiency.[11, 12, 18] In rats maintained on a low-iodine diet, serum T_4 levels decrease to very low values, whereas the T_3 level is relatively well maintained.[10, 30] The maintenance of serum T_3 levels in ID rats involves an increase in thyroidal biosynthesis of T_3 relative to T_4.[30] In the present study, iodine deficiency by itself significantly reduced serum T_4 and increased TSH levels, although it rather showed a tendency to increase serum T_3 as compared to the control value. However, this might be a spurious finding, since the control value was below the historical control range in our laboratory

(1.07±0.12 ng/ml). Furthermore, detection of small changes in thyroid hormone concentrations can be confounded by normal variability between animals.[31)]

In conclusion, the present results suggest that isoflavones may not be involved in the mechanism underlying the goitrogenic effects of soybeans and the synergism with iodine deficiency. These effects may result from unknown factors or a combination of constituents acting together.

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