

Synergistic Effects of High-dose Soybean Intake with Iodine Deficiency, but Not Sulfadimethoxine or Phenobarbital, on Rat Thyroid Proliferation

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The specificity and dose dependence of the synergistic effects of soybean intake with iodine deficiency on the induction of thyroid proliferation were investigated in female F344 rats. In the first experiment, rats were divided into 6 groups, each consisting of 5 animals, and fed a basal diet containing 20% gluten, an iodine-deficient basal diet alone or an iodine-deficient diet containing 0.2%, 1.0%, 5.0% or 25% defatted soybean for 5 weeks. Soybean feeding synergistically induced thyroid hyperplasias with iodine deficiency only at the 25% dose. In the second experiment, rats were also divided into 6 groups, each consisting of 5 animals, and fed a basal diet, a diet containing 20% defatted soybean, 0.025% sulfadimethoxine (SDM), 20% defatted soybean+0.025% SDM, 0.05% phenobarbital (PB) or 20% defatted soybean+0.05% PB for 5 weeks. The SDM treatments significantly ($P<0.05-0.01$) increased the thyroid weights, but this increase rate was less prominent in the SDM+soybean group than in the SDM alone group. The PB treatment was also associated with a tendency for increase in thyroid weight, but again this was smaller in the PB+soybean group than in the PB alone group. Although the SDM or PB treatments reduced the serum triiodothyronine and thyroxine levels and consequently increased the serum thyroid-stimulating hormone (TSH) levels, the soybean feeding did not affect or rather attenuated these changes. Our results clearly indicate that soybean feeding does not synergistically enhance the effects of SDM or PB on the rat thyroid. Thus it can be concluded that soybean intake specifically interacts with iodine deficiency in induction of thyroid proliferative lesions in rats, only at high doses.

Key words: Soybean — Iodine deficiency — Thyroid tumorigenesis — Synergism

Thyroid enlargement due to excessive soybean feeding especially in children and women has been known from epidemiological studies for almost half a century.¹⁻³ It has been also reported that addition of iodine supplements to commercial soy formulas in the 1960s eliminated the prevalence of hypothyroidism in soy-fed infants.⁴ Experimentally, several investigators have also reported the induction of goiters in iodine-deficient rats maintained on a soybean diet.^{5,6} Iodine deficiency is a well-known factor predisposing to thyroid hyperplasias and subsequently thyroid tumors, the mechanism being interpreted as stimulation of follicular cells by increased serum thyroid-stimulating hormone (TSH) following a reduction of thyroid hormone synthesis.⁷ However, the physiological changes underlying the goitrogenic activity of soybean protein in excess and the synergism with iodine deficiency have not been fully elucidated, although we recently found that dietary soybean may primarily stimulate serum TSH release from the pituitary of rats.⁸

It is well known that there are different mechanisms whereby chemicals may promote development of thyroid

tumors in rodents. For example, sulfadimethoxine (SDM) inhibits thyroid hormone synthesis by inhibiting thyroid peroxidase (TPO)⁹ and phenobarbital (PB) induces hepatic microsomal enzymes such as thyroxine (T_4)-uridine diphosphate glucuronosyl transferase (T_4 -UDP-GT) responsible for T_4 clearance in the liver.^{9,10} In both cases, subsequent increase in serum TSH stimulates thyroid follicular cell proliferation. In the present study, the possible synergistic effects of soybean feeding with SDM or PB were investigated in rats. Because it remains unclear whether low doses of soybean can exert any synergistic effects with iodine deficiency, multiple dose levels of soybean were also tested in combination with iodine deficiency with reference to induction of thyroid hyperplasia in rats.

MATERIALS AND METHODS

Animals, chemicals and diet Female 5-week-old specific-pathogen-free F344/DuCrj rats (Charles River Japan Inc., Kanagawa) were maintained in aluminum cages in an air-conditioned room (barrier system) at a temperature of $23\pm 2^\circ\text{C}$ and a relative humidity of $60\pm 5\%$ under a daily cycle of alternating 12 h periods of light and darkness.

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The animals were given ion-exchanged water *ad libitum*. Sodium SDM and sodium PB were respectively purchased from Sigma Chemical Co. (St. Louis, MO) and Iwaki Seiyaku Co., Ltd. (Tokyo). Casein was replaced with gluten or defatted soybean flour as an alternative protein source in the AIN-93G diet (Oriental Yeast Co., Ltd., Tokyo) in order to avoid possible contamination with iodine contained in casein sources.¹¹⁾

Experimental design After a 1-week acclimatization period, in experiment I, rats were divided into 6 groups, each consisting of 5 animals, and fed AIN-93G diet with 20% gluten (group I-1), iodine-deficient diet with 0.2%, 1.0%, 5.0% or 25% defatted soybean (groups I-3, -4, -5 or -6) for 5 weeks (Fig. 1). In experiment II, rats were also divided into 6 groups, each consisting of 5 animals. SDM was administered in their drinking water and PB was supplemented into the diet. They were respectively fed AIN-93G diet of 20% gluten (group II-1), 20% defatted soybean (group II-2), 20% gluten+0.025% SDM (group II-3), 20% defatted soybean+0.025% SDM (group II-4), 20% gluten+0.05% PB (group II-5) or 20% defatted soybean+0.05% PB (group II-6) for 5 weeks (Fig. 1). At week 5, all rats were sacrificed under ether anesthesia. Blood was collected from the aorta. At autopsy, major organs including the thyroid and pituitary were carefully examined macroscopically. They were weighed and fixed in 10% phosphate-buffered formalin, and sections stained with hematoxylin and eosin (H-E) were routinely prepared. For hormone analysis, serum triiodothyronine (T_3)

and T_4 were measured with a radioimmunoassay Riabead kit (Dainabot, Tokyo) and serum TSH with a rat TSH kit (Amersham Life Science, Buckinghamshire, UK).

Statistical analysis Variances of data for body and organ weights, and serum hormones were assessed for homogeneity by Bartlett's procedure. If the variance was homogeneous, the data were examined by one-way analysis of variance (ANOVA) with Student's *t* test multiple comparison procedures. If not homogeneous, they were analyzed by means of the Kruskal-Wallis test followed by the Mann-Whitney *U* test.

RESULTS

No animals died during the experimental period. In experiment I, average body weights were increased in a dose-dependent manner by soybean intake in all the iodine deficiency groups, the difference being significant ($P<0.05$) in those given 1% soybean or more (Table I). Relative thyroid weights (mg/100 g b.w.) were more than twice as high in groups I-2 (16.6 ± 3.6), I-3 (18.2 ± 2.0), I-4 (15.5 ± 1.5), I-5 (16.1 ± 1.2) and I-6 (43.3 ± 8.5) than in group I-1 (7.4 ± 1.3). The increase was 6-fold in group I-6 (Table I). Absolute pituitary weights (mg) were significantly ($P<0.01$) higher in group I-6 (8.40 ± 1.52) than in group I-2 (5.60 ± 0.89).

Table II shows serum hormone data for experiment I. Serum T_3 levels were not affected by the treatments. Serum T_4 levels ($\mu\text{g}/\text{dl}$) were significantly ($P<0.001$) decreased in groups I-2 (2.90 ± 0.34), I-3 (2.76 ± 0.22), I-4

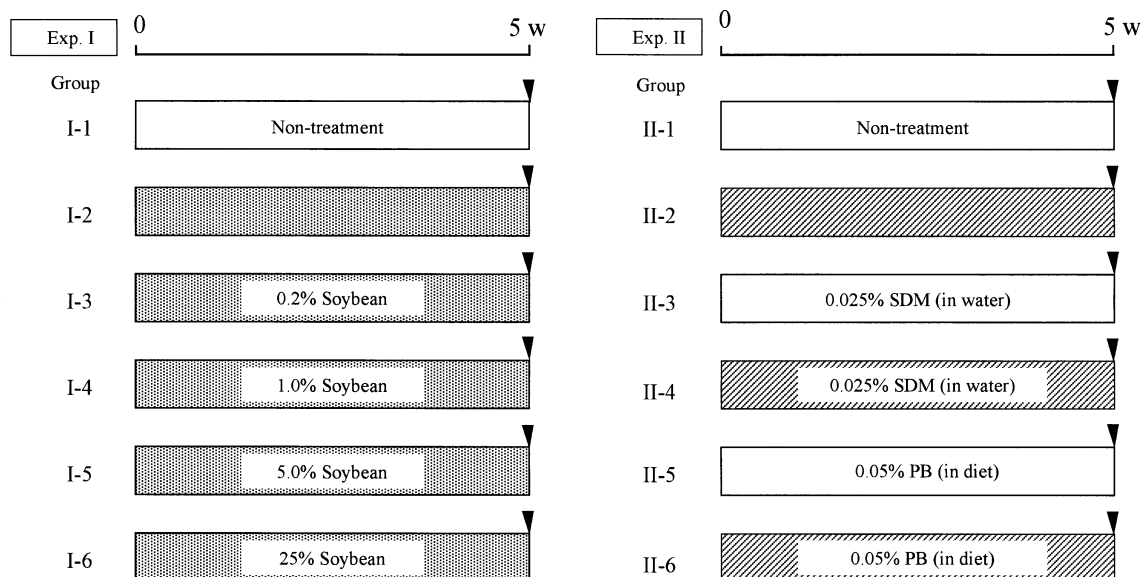


Fig. 1. Experimental design. SDM, sulfadimethoxine; PB, phenobarbital; \blacksquare , iodine-deficient diet; ▨ , soybean diet; \square , basal diet; \blacktriangledown , sacrifice.

Table I. Body and Organ Weights (Experiment I)

	Non-treatment	ID ^{a)}	ID+0.2% Soybean	ID+1.0% Soybean	ID+5.0% Soybean	ID+25% Soybean
Body (g)	116±7	111±5	115±4	120±8 [#]	127±7 ^{###}	129±5 ^{###}
<i>Absolute</i>						
Brain (g)	1.66±0.06	1.64±0.04	1.66±0.06	1.65±0.06	1.69±0.03	1.68±0.02
Pituitary (mg)	7.40±0.55	5.60±0.89 [*]	6.00±1.00	6.80±1.64	7.60±1.14 [#]	8.40±1.52 ^{##}
Thyroid (mg)	8.6±1.8	18.2±4.5 ^{**}	20.8±2.6 ^{**}	18.6±2.3 ^{**}	20.4±1.5 ^{###}	55.8±12.5 ^{###}
Liver (g)	3.70±0.26	3.38±0.21 [*]	3.09±0.04 ^{###}	3.40±0.21 [*]	3.52±0.24	3.72±0.16 [#]
Adrenals (mg)	30.2±5.6	31.0±5.4	30.8±7.8	27.4±5.7	32.8±4.4	35.8±4.0
Kidneys (g)	0.90±0.06	0.86±0.03	0.86±0.07	0.89±0.07	0.91±0.05	0.92±0.04
<i>Relative</i>						
Brain (g/100 g b.w.)	1.50±0.08	1.45±0.07	1.37±0.05	1.34±0.09 ^{**}	1.33±0.09 ^{###}	1.31±0.05 [#]
Pituitary (mg/100 g b.w.)	6.41±0.50	5.13±1.02	5.24±0.84	5.63±1.18	6.00±1.07	6.51±0.97
Thyroid (mg/100 g b.w.)	7.4±1.3	16.6±3.6 ^{**}	18.2±2.0 ^{**}	15.5±1.5 ^{**}	16.1±1.2 ^{**}	43.3±8.5 ^{###}
Liver (g/100 g b.w.)	3.12±0.13	3.08±0.13	2.70±0.06 ^{###}	2.83±0.12 ^{##}	2.73±0.11 ^{###}	2.89±0.05 [#]
Adrenals (mg/100 g b.w.)	26.0±3.4	28.4±5.9	26.8±5.5	22.7±3.7	25.9±1.0	27.8±2.9
Kidneys (g/100 g b.w.)	0.78±0.03	0.79±0.03	0.75±0.03	0.74±0.04	0.72±0.05 [#]	0.71±0.04 ^{##}

Values are mean±SD.

a) Iodine deficiency.

*, **, *** Significantly different from the non-treatment group value at * $P<0.05$, ** $P<0.01$, *** $P<0.001$, respectively.
 #, ##, ### Significantly different from the ID group value at # $P<0.05$, ## $P<0.01$, ### $P<0.001$, respectively.

Table II. Serum T₃, T₄ and TSH Levels (Experiment I)

Group	T ₃ (ng/ml)	T ₄ (μg/dl)	TSH (ng/ml)
I-1. Non-treatment	1.06±0.05	5.74±0.46	5.2±0.6
I-2. Iodine deficiency	1.10±0.00	2.90±0.34 ^{###}	7.3±1.3 [*]
I-3. Iodine deficiency+0.2% Soybean	1.12±0.11	2.76±0.22 ^{###}	6.7±0.6 [*]
I-4. Iodine deficiency+1.0% Soybean	1.00±0.07	2.68±0.31 ^{###}	7.4±0.8 [*]
I-5. Iodine deficiency+5.0% Soybean	1.08±0.08	2.50±0.46 ^{###}	8.2±5.0 ^{**}
I-6. Iodine deficiency+25% Soybean	1.06±0.09	1.81±0.13 ^{###}	45.5±18.6 [#]

Values are mean±SD.

*, **, *** Significantly different from the group I-1 value at * $P<0.05$, ** $P<0.01$, *** $P<0.001$, respectively.

#, ## Significantly different from the group I-2 value at # $P<0.01$, ## $P<0.001$, respectively.

(2.68±0.31), I-5 (2.50±0.46) and I-6 (1.81±0.13) as compared to the group I-1 value (5.74±0.46), and also were significantly ($P<0.001$) lower in group I-6 than in group I-2. In contrast, serum TSH (ng/ml) levels were significantly ($P<0.05$) increased in the iodine-deficient groups I-2, I-3, I-4, I-5 and I-6 as compared to the group I-1 value (5.2±0.6), being significantly ($P<0.01$) higher in group I-6 (45.5±18.6) than in group I-2 (7.3±1.3).

Histopathologically, the most prominent lesion, observed only in the thyroid of group I-6, was marked diffuse follicular hyperplasias almost lacking colloid, indicating synergism of high-dose soybean intake with iodine deficiency. Although mild hypertrophy of follicular cells and slightly decreased colloid due to iodine deficiency were

observed in the thyroids of groups I-2, I-3, I-4 and I-5, there were no significant intergroup differences.

In experiment II, final body weights were significantly ($P<0.001$) increased by the 20% soybean diet, regardless of SDM or PB treatment. In the SDM-treated groups, relative thyroid weights (mg/100 g b.w.) were significantly ($P<0.05$) increased in groups II-3 (29.8±4.2) and II-4 (21.6±4.0) over groups II-1 (12.1±4.2) and II-2 (13.6±3.2), but the increase rate was rather lower in group II-4 (SDM+soybean) than in group II-3 (SDM alone) (Table III). Similarly, the PB-treated groups (groups II-5 and II-6) also showed a tendency for increase in thyroid weight, but again this was smaller in group II-6 (PB+soybean) than in group II-5 (PB alone), the level in the former

Table III. Body and Organ Weights (Experiment II)

	Non-treatment	20% Soybean	SDM ^{a)}	SDM+20% Soybean	PB	PB+20% Soybean
Body (g)	115±6	130±6 ^{***}	117±3	129±5 ^{††}	117±3	134±5 ^{††}
<i>Absolute</i>						
Brain (g)	1.67±0.04	1.68±0.06	1.63±0.05	1.67±0.05	1.59±0.06	1.66±0.04
Pituitary (mg)	10.8±3.6	9.4±4.9	8.4±1.5	10.2±3.0	8.8±1.3	8.2±2.3
Thyroid (mg)	13.8±4.4	7.4±3.5	34.8±4.3 ^{***}	27.8±4.5 [†]	18.8±2.1	17.0±4.0
Liver (g)	3.56±0.31	3.75±0.36	3.28±0.21	3.48±0.11	5.14±0.14 ^{***}	6.01±0.32 ^{##††}
Adrenals (mg)	34.6±6.3	34.4±4.0	32.2±7.3	27.6±3.4	29.0±4.0	33.6±6.0
Kidneys (g)	0.91±0.04	0.92±0.04	0.85±0.03 ^{**}	0.88±0.03	0.90±0.04	0.93±0.02
<i>Relative</i>						
Brain (g/100 g b.w.)	1.45±0.08	1.30±0.05 ^{***}	1.39±0.05	1.30±0.06 [†]	1.36±0.04 [*]	1.24±0.03 ^{††}
Pituitary (mg/100 g b.w.)	9.27±2.67	7.21±3.55	7.18±1.29	7.90±2.23	7.50±0.91	6.07±1.51
Thyroid (mg/100 g b.w.)	12.1±4.2	13.6±3.2	29.8±4.2 ^{***}	21.6±4.0 [†]	16.1±1.6	12.7±3.1
Liver (g/100 g b.w.)	3.07±0.24	2.89±0.22	2.80±0.13 [*]	2.70±0.10	4.39±0.12 ^{***}	4.48±0.32 ^{##}
Adrenals (mg/100 g b.w.)	30.1±5.6	27.4±3.5	27.4±5.6	21.4±3.1	24.8±3.1	25.1±4.6
Kidneys (g/100 g b.w.)	0.79±0.02	0.71±0.02 ^{***}	0.73±0.03 ^{***}	0.68±0.02 [†]	0.77±0.02	0.70±0.02 ^{††}

Values are mean±SD.

a) SDM, sulfadimethoxine; PB, phenobarbital.

*, **, *** Significantly different from the non-treatment value at * $P<0.05$, ** $P<0.01$, *** $P<0.001$, respectively.

#, ## Significantly different from the soybean alone value at # $P<0.01$, ## $P<0.001$, respectively.

†, †† Significantly different from the SDM or PB alone value at † $P<0.01$, †† $P<0.001$, respectively.

Table IV. Serum T₃, T₄ and TSH Levels (Experiment II)

Group	T ₃ (ng/ml)	T ₄ (μg/dl)	TSH (ng/ml)
II-1. Non-treatment	0.98±0.08	4.92±0.42	4.70±0.39
II-2. 20% Soybean	1.06±0.05	5.66±0.55 [*]	5.46±0.51 [*]
II-3. 0.025% Sulfadimethoxine	0.82±0.04 ^{**}	3.82±0.38 ^{***}	8.32±2.10 ^{**}
II-4. 0.025% Sulfadimethoxine+20% Soybean	1.00±0.12 [†]	5.38±0.36 ^{††}	7.52±1.41 ^{##}
II-5. 0.05% Phenobarbital	0.74±0.09 ^{***}	4.36±0.56	9.02±2.90 ^{**}
II-6. 0.05% Phenobarbital+20% Soybean	0.84±0.05 ^{###}	4.42±0.37 ^{###}	7.32±2.13 [#]

Values are mean±SD.

*, **, *** Significantly different from the group II-1 value at * $P<0.05$, ** $P<0.01$, *** $P<0.001$, respectively.

#, ##, ### Significantly different from the group II-2 value at # $P<0.05$, ## $P<0.01$, ### $P<0.001$, respectively.

†, †† Significantly different from the group II-3, -4 or II-5, -6 intergroup value at † $P<0.01$, †† $P<0.001$, respectively.

being comparable to the group II-1 and II-2 values. Relative liver weights were significantly ($P<0.001$) increased in groups II-5 (4.39±0.12) and II-6 (4.48±0.32) as compared to the group II-1 (3.07±0.24) and II-2 (2.89±0.22) values. Relative kidney weights were decreased by 20% soybean feeding, indicating relative change by increased body weight gain.

Table IV shows serum biochemical data for experiment II. Serum T₃ (ng/ml) levels were significantly ($P<0.01$) lower in groups II-3 (0.82±0.04) and II-5 (0.74±0.09) than in group II-1 (0.98±0.08), and also significantly ($P<0.01$) lower in group II-6 (0.84±0.05) than in group II-

2 (1.06±0.05). Serum T₄ (μg/dl) levels were significantly ($P<0.001$) lower in groups II-3 (3.82±0.38) and II-6 (4.42±0.37) than in groups II-1 (4.92±0.42) and II-2 (5.66±0.55), respectively. In contrast, serum T₄ (μg/dl) levels were significantly ($P<0.05$) higher in groups II-2 (5.66±0.55) and II-4 (5.38±0.36) than in groups II-1 (4.92±0.42) and II-3 (3.82±0.38), respectively. Serum TSH levels (ng/ml) were significantly ($P<0.05$) higher in groups II-2 (5.46±0.51), II-3 (8.32±2.10) and II-5 (9.02±2.90) than in group II-1 (4.70±0.39), and also significantly ($P<0.05$) higher in groups II-4 (7.52±1.41) and II-6 (7.32±2.13) than in group II-2.

Histopathologically, in the thyroids of groups II-3 and II-4, moderate hyperplasia with increase in follicular cell height and decrease in colloid accumulation was observed, there being no apparent intergroup differences. In the thyroids of groups II-5 and II-6, mild hyperplasia with decrease in colloid accumulation in small-sized follicles was found, there being again no intergroup differences. In the liver, fatty changes of hepatocytes were seen in groups II-5 and II-6, being especially marked in the latter.

DISCUSSION

Very recently, we have reported that a high intake (20%) of soybean dramatically and synergistically increases the development of thyroid follicular hyperplasias in rats in combination with iodine deficiency.⁸⁾ In the present study, the dose response of the synergistic effects of soybean with iodine deficiency on rat thyroid follicular cell proliferation was examined. As a result, thyroid hyperplasia was only induced at the highest dose (25%) of soybean, suggesting the existence of a threshold. This is extremely important in the risk assessment of soy product consumption, especially in countries or areas of iodine deficiency,¹²⁾ regardless of the species differences between human and rodents, the latter being much more susceptible to thyroid tumorigenesis.¹³⁾

There are two basic mechanisms whereby chemicals produce thyroid gland tumors in rodents. One, classified as genotoxic, involves direct carcinogenic effects at the DNA level in follicular cells. The other, non-genotoxic, involves the so-called negative feedback system of the thyroid-pituitary-hypothalamus in which decreased circulating T_3 or T_4 levels due to disruption of thyroid function or accelerated turnover lead to increased TSH, and prolonged stimulation of follicular epithelium by TSH.^{9, 14, 15)} Many non-genotoxic factors may contribute to decreases in circulating thyroid hormone levels, through inhibition of iodine transport into the thyroid, iodine oxidation or organification defect due to inhibition of TPO, inhibition of thyroid hormone secretion or increased hormone inactivation by hepatic microsomal enzymes such as T_4 -UDP-GT.^{9, 15)} SDM exerts a goitrogenic effect by inhibition of TPO, which catalyzes a reaction essential to thyroid hormone synthesis,⁹⁾ resulting in marked decreases in circulating T_3 and T_4 , increase in TSH¹⁵⁾ and consequent induction of thyroid follicular hyperplasias in rats.^{15, 16)} PB has also been reported to be a thyroid tumor promoter, as shown in a rat initiation-promotion model.¹⁷⁾ PB is a well-known microsomal enzyme inducer that increases UDP-GT, a rate-limiting enzyme in T_4 metabolism¹⁰⁾ as well as a spectrum of cytochrome P-450 isoenzymes.¹⁷⁾ Therefore, it has been concluded that its thyroid tumor promotion is mediated by increase of TSH secretion from the pituitary as a result of the increase of T_4 excretion into the bile by

hepatic microsomal enzymes.¹⁰⁾ In the present study, SDM or PB treatment in fact reduced serum T_3 and T_4 levels, and consequently increased serum TSH levels, although co-administration with soybean did not synergistically enhance but rather attenuated their induction of changes in the thyroid. Therefore, it is likely that excess soybean specifically interacts with iodine deficiency but not with SDM or PB. The different modes of action of those thyroid tumor-promoters must be related to the different interactions with soybean.

Iodine-deficient diets produce enlargement of the thyroid, increase in monoiodotyrosine/diiodotyrosine, and progressive increase in the T_3/T_4 molar ratio¹⁸⁾ (T_3 , containing only 75% as much iodine as T_4 , has 10 times its affinity for thyroid hormone receptors in target cells and correspondingly greater hormonal activity¹⁹⁾). Sustained decrease in intracellular iodine concentration²⁰⁾ due to dietary iodine deficiency inhibits thyroglobulin hydrolysis,²¹⁾ its own organic binding for hydrogen peroxide production,²²⁾ or generation of cyclic AMP.²³⁾ An increase in the uptake of ^{131}I by the thyroid has been demonstrated in an infant on a soy diet.¹⁾ Furthermore, high intake of soy diets in cattle induces goiter via an increased iodine requirement after T_4 depletion through fecal wastage.²⁴⁾ In the present study, although iodine deficiency alone reduced serum T_4 levels and increased TSH levels, the latter was affected by excess soybean, with a tendency for increase in serum T_4 , as demonstrated in our previous study.⁸⁾ These results may suggest primary stimulation of TSH release from the pituitary of rats under certain conditions.⁸⁾ It has been shown that isoflavones, a major active component of soy, inhibit TPO reaction.²⁵⁾ Involvement of phytoestrogenic activity and indirect action through effects on liver function could also play a role in the induction of thyroid proliferative lesions by soybean diet in rats.⁸⁾ Because in the present study the SDM or PB treatment in fact reduced serum T_3 and T_4 and increased TSH levels, but soybean rather attenuated these changes, it is likely that excess soybean intake may induce thyroid proliferation by acting through different pathways, with a possible contribution of the pituitary and/or hypothalamus.

In conclusion, our results clearly indicate that excess soybean intake exerts synergistic effects specifically with iodine deficiency on the development of thyroid hyperplasia in rats, but only at extremely high doses.

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REFERENCES

- 1) Hydovitz, J. D. Occurrence of goiter in an infant on a soy diet. *N. Engl. J. Med.*, **262**, 351–353 (1960).
- 2) Shepard, T. H., Pyne, G. E., Kirschvink, J. F. and McLean, C. M. Soybean goiter. *N. Engl. J. Med.*, **262**, 1099–1103 (1960).
- 3) Pinchera, A., MacGillivray, M. H., Crawford, J. D. and Freeman, A. G. Thyroid refractoriness in an athyreotic cretin fed soybean formula. *N. Engl. J. Med.*, **265**, 83–87 (1965).
- 4) Block, R. J. R., Mandal, H., Howard, H. W., Bauer, C. D. and Anderson, D. The curative action of iodine on soybean goiter and the changes in the distribution of iodo-amino acids in the serum and thyroid gland digests. *Arch. Biochem. Biophys.*, **93**, 15–24 (1961).
- 5) McCarrison, R. The goitrogenic action of soybean and groundnut. *Ind. J. Med. Res.*, **21**, 179–181 (1933).
- 6) Kimura, S., Suwa, J., Ito, B. and Sato, H. Development of malignant goiter by defatted soybean with iodine-free diet in rats. *Gann*, **67**, 763–765 (1976).
- 7) Potter, S. M., Pertile, J. and Berber-Jimenez, M. D. Soy protein concentrate and isolated soy protein similarly lower blood serum cholesterol but differently affect thyroid hormones in hamsters. *J. Nutr.*, **126**, 2007–2011 (1996).
- 8) Ikeda, T., Nishikawa, A., Imazawa, T., Kimura, S. and Hirose, M. Dramatic synergism between excess soybean intake and iodine deficiency on the development of rat thyroid hyperplasia. *Carcinogenesis*, **21**, 707–713 (2000).
- 9) Hill, R. N., Erdreich, L. S., Paynter, O. E., Roberts, P. A., Rosenthal, S. L. and Wilkinson, C. F. Thyroid follicular cell carcinogenesis: a review. *Fundam. Appl. Toxicol.*, **12**, 629–697 (1989).
- 10) McClain, R. M., Levin, A. A., Posch, R. C. and Downing, J. M. The effect of phenobarbital on the metabolism and excretion of thyroxine in rats. *Toxicol. Appl. Pharmacol.*, **99**, 216–228 (1989).
- 11) Reeves, P. G., Nielsen, F. G. and Fahey, G. C. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.*, **123**, 1939–1951 (1993).
- 12) Lind, P., Langsteger, W., Molnar, M., Gallowitsch, H. J., Mikosch, P. and Gomez, I. Epidemiology of thyroid diseases in iodine sufficiency. *Thyroid*, **8**, 1179–1183 (1998).
- 13) McClain, R. M. Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutat. Res.*, **333**, 131–142 (1995).
- 14) McClain, R. M. Thyroid gland neoplasia: genotoxic mechanisms. *Toxicol. Lett.*, **64/65**, 397–408 (1992).
- 15) Capen, C. C. Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. *Toxicol. Pathol.*, **25**, 39–48 (1997).
- 16) Mitsumori, K., Onodera, H., Takahashi, M., Shimo, T., Yasuhara, K., Kitaura, K., Takahashi, M. and Hayashi, Y. Effect of thyroid stimulating hormone on the development and progression of rat thyroid follicular cell tumors. *Cancer Lett.*, **92**, 193–202 (1995).
- 17) McClain, R. M., Posch, R. C., Bosakowski, T. and Armstrong, J. M. Studies on the mode of action for thyroid gland tumor promotion in rats by phenobarbital. *Toxicol. Appl. Pharmacol.*, **94**, 254–265 (1988).
- 18) Fukuda, H., Yasuda, M., Greer, M. A., Kutas, M. and Greer, S. E. Changes in plasma thyroxine, triiodothyronine, and TSH during adaptation to iodine deficiency in the rat. *Endocrinology*, **97**, 307–314 (1975).
- 19) Martin, D. W., Jr., Mayes, P. A., Rodwell, V. W. and Granner, D. K. “20th Harper’s Review of Biochemistry” (1985). Lange Medical Publ., California.
- 20) Wolff, J. Iodide goiter and the pharmacologic effects of excess iodide. *Am. J. Med.*, **47**, 101–124 (1969).
- 21) Bagchi, N., Shivers, B. and Brown, T. R. Studies on the mechanism of acute inhibition of thyroglobulin hydrolysis by iodine. *Acta Endocrinol.*, **108**, 511–517 (1985).
- 22) Nunez, J. and Pommier, J. Formation of thyroid hormones. *Vitam. Horm.*, **39**, 175–229 (1982).
- 23) Van Sande, J., Grenier, G., Willems, C. and Dumont, J. E. Inhibition by iodide of the activation of the thyroid cyclic 3',5'-AMP system. *Endocrinology*, **96**, 781–786 (1975).
- 24) Van Middlesworth, L. Thyroxine excretion, a possible cause of goiter. *Endocrinology*, **61**, 570–573 (1957).
- 25) Divi, R. L., Chang, H. C. and Doerge, D. R. Anti-thyroid isoflavones from soybean: isolation, characterization, and mechanisms of action. *Biochem. Pharmacol.*, **54**, 1087–1096 (1997).