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Review

Inactivation of thyroid peroxidase by soy isoflavones, in vitro and in vivo

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Abstract

Soy-containing foods and dietary supplements are widely consumed for putative health benefits (e.g. cancer chemoprevention, beneficial effects on serum lipids associated with cardiovascular health, reduction of osteoporosis, relief of menopausal symptoms). However, studies of soy isoflavones in experimental animals suggest possible adverse effects as well (e.g. enhancement of reproductive organ cancer, modulation of endocrine function, anti-thyroid effects). This paper reviews the evidence in humans and animals for anti-thyroid effects of soy and its principal isoflavones, genistein and daidzein.
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Contents

1. Introduction

Considerable interest in the putative health benefits from products containing soy isoflavones has recently spawned numerous epidemiological and clinical studies investigating possible cancer

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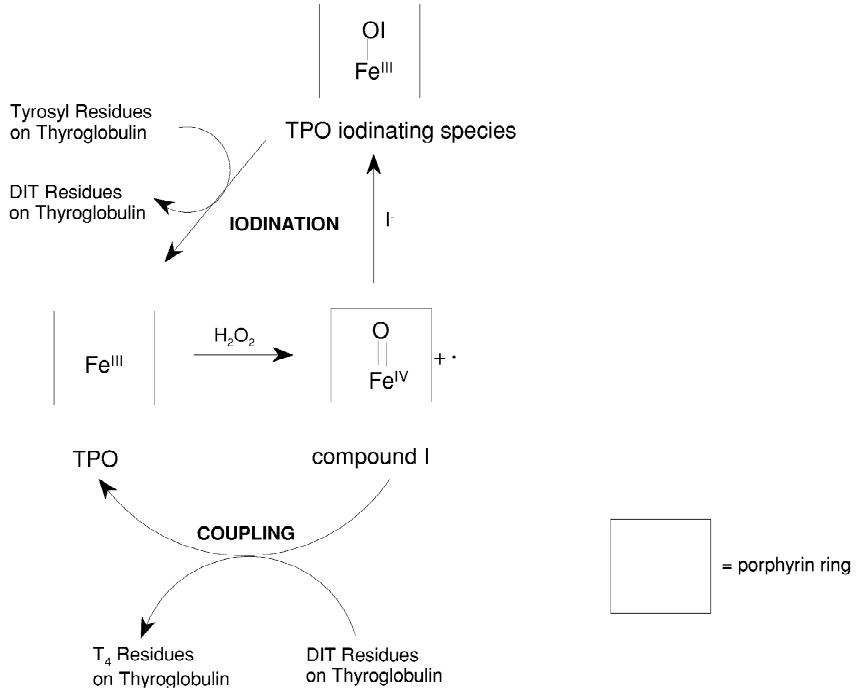
chemoprevention (breast and prostate), relief of postmenopausal symptoms, and osteoporosis amelioration. The preponderance of research investigations on the biological effects of soy isoflavones in humans and experimental animals, both beneficial and adverse, stem from their agonist activity toward estrogen receptors α and β. These activities will not be discussed in this review. However, there may be important interactions between estrogen-signaling pathways and those of the thyroid–pituitary axis, which is the focus of the present review. A significant body of information from in vitro and in vivo investigations indicates that modulation of estrogens or thyroid hormones can have effects on the biological activities of the other hormone. A possible mechanism is the binding of thyroid hormone and estrogen receptors to common response elements of target genes [1]. The result of such binding is either potentiation or inhibition of gene expression and suggests that environmental signals through these two endocrine systems are integrated with important cellular responses [2].

However, the soybean also has a long-standing association with goiter in animals and humans. Many studies have shown the goitrogenic effects of soy in rats and the protective effect of dietary iodide supplementation [3–7]. In a dramatic example of the synergy between dietary iodine and soy, thyroid carcinoma was rapidly induced in rats fed an iodine-deficient diet consisting of 30% defatted soy [8]. Anti-thyroid effects observed in human studies include goiter in infants consuming soy formula and subsequent reversal upon switching to cow milk or iodine-supplemented diets [9–16]. Reports of goiter in infants on soy formulas decreased dramatically after the 1960s, when manufacturers reportedly began iodine supplementation of formulas to mitigate possible anti-thyroid effects. A retrospective epidemiological study by Fort et al. showed that teenaged children with a diagnosis of autoimmune thyroid disease were significantly more likely to have received soy formula as infants (18 out of 59, 31%) when compared to healthy siblings (nine out of 76, 12%) or control group children (seven out of 54, 13%) [17]. However, there were no differences among these three groups with respect to breast-feeding. Ishizuki et al. reported goiter and elevated individual thyroid stimulating hormone (TSH) levels, although still within the normal range, in 37 healthy iodine-sufficient adults without known thyroid disease fed 30 g of pickled soy beans per day for as little as 1 month [18]. Changes in serum thyroid hormone (T3 and T4) levels were not observed. One month after stopping soy consumption, individual TSH values had decreased to the original levels and goiters were reduced in size. Duncan et al. described a statistically significant decrease in T3 levels in 14 premenopausal, but not 18 postmenopausal, women consuming up to 2 mg total of soy isoflavones per kg body weight per day for about 3 months [19,20]. Watanabe et al. recently reported decreased T3/T4 levels during the luteal phase, but increased levels during the follicular phase, of the menstrual cycle in response to soy isoflavone supplementation [21].

This article reviews the literature pertaining to known effects of soy and isoflavones on thyroid function in experimental animals. Rodent studies are recognized as useful risk assessment models for thyroid toxicants, even though significant differences between rodent and human thyroid physiology have been documented [22]. It also attempts to put the body of research on rodent thyroid effects in a context that is useful for predicting potential risks from soy consumption in various human populations.

2. Biosynthesis of thyroid hormones and inhibition by anti-thyroid chemicals

Thyroid peroxidase (TPO) is the heme-containing enzyme found in the apical membrane of thyroid follicular cells that catalyzes the two reactions required for thyroid hormone synthesis (Scheme 1): iodination of tyrosyl residues in thyroglobulin and subsequent oxidative coupling to yield thyroxine (T4) and triiodothyronine (T3). A substantial amount of previous work from this laboratory has demonstrated that inhibition of porcine TPO activity is a common mechanism for many classes of synthetic anti-thyroid compounds [23–27], and naturally occurring flavonoids [28]. Lactoperoxidase (LPO) is a commercially available model enzyme that shares many structural and functional properties with TPO. For this reason, we previously investigated the ability of soy isoflavones to inhibit porcine TPO and LPO activity. Genistein and daidzein were identified



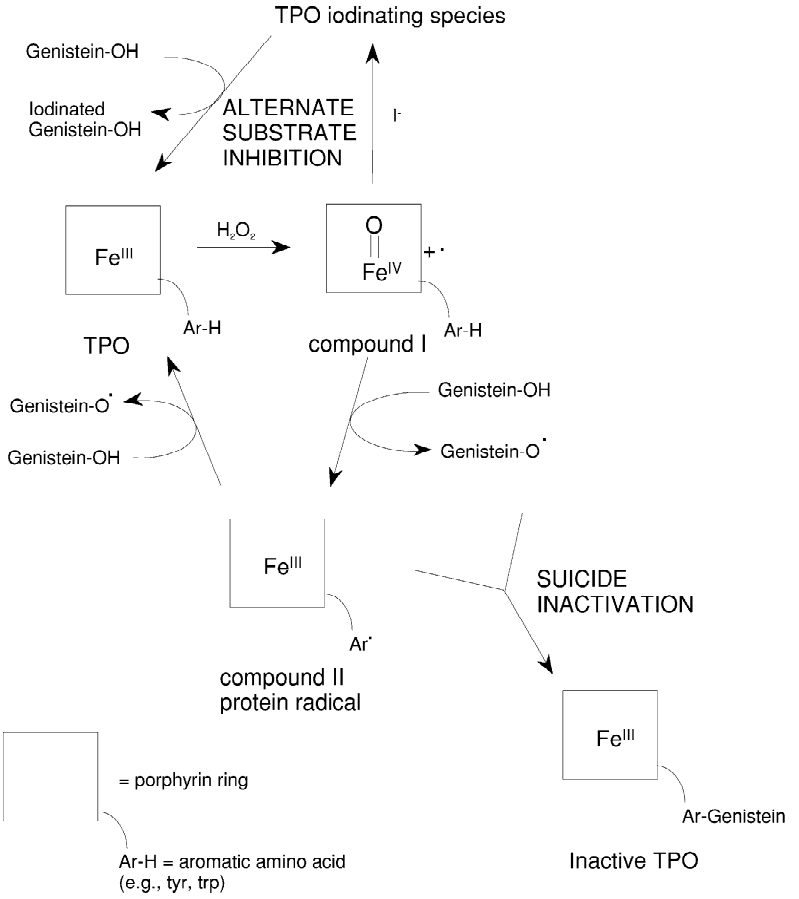
Scheme 1. Proposed mechanism for TPO catalyzed thyroxine synthesis.

as the components in soy that inhibited TPO-catalyzed iodination and coupling [29]. In the absence of iodide, genistein and daidzein are suicide substrates for TPO and LPO as determined by the irreversible loss of both iodinating and coupling activities and concomitant changes in the UV–Vis spectrum of the enzyme. In the presence of iodide, they acted as alternate substrates that blocked tyrosine iodination through preferential formation of iodinated isoflavone derivatives, and thereby dramatically decreased the rate and extent of genistein-mediated loss of peroxidase activity. It was also observed that genistin, the glucoside conjugate, was much less effective in inactivating TPO.

A proposed suicide inactivation mechanism that is consistent with these findings is shown in Scheme 2. The scheme shows the ways in which genistein can intercept reactive enzyme intermediates (i.e. TPO compound I, the protein radical form of TPO compound II and the TPO iodinating species) involved in the iodination and coupling reactions required for thyroxine synthesis [30]. Reaction of compound I with isoflavones would produce a reactive isoflavone radical at the active site along with a radical form of compound II, which could combine to form inactivated enzyme presumably through covalent modification of active site amino acid residues. Consistent with this hypothesis are the covalent binding of approximately 3 mol of radiolabeled genistein per mol of inactivated LPO (unpublished data), the unchanged heme content in inactivated LPO (unpublished data), and the UV–Vis spectral changes observed upon inactivation of LPO and TPO [29].

3. Inactivation of TPO by isoflavones in vitro

Rat microsomal TPO (rTPO), prepared from untreated animals, was used to investigate isoflavone-mediated inactivation in vitro [31]. Fig. 1 shows the time-dependent loss of rTPO activity in the presence of genistein and hydrogen peroxide. The control experiments demonstrate that neither H_2O_2 nor genistein had an effect on enzymatic activity, which is consistent with the suicide inactivation mechanism proposed previously [29]. Kinetic analysis for in-



Scheme 2. Proposed mechanisms for inhibition of TPO by soy isoflavones.

activation of rTPO by genistein showed that the apparent inhibition binding constant, K_i, and the maximal inactivation rate constant, k_{inact}, were 50 n*M* and 0.28 min^{-1}, respectively. Daidzein similarly inactivated rTPO with the corresponding kinetic constants of 143 n*M* and 0.31 min^{-1}. These kinetic parameters, which are consistent with very potent inactivation, were of the same order as those determined for LPO for which it was also determined that the partition ratios for genistein and daidzein were less than 3 (data not shown).

The sensitivity of microsomal rTPO to inactivation by genistein was compared with several related purified solubilized mammalian peroxidases under similar conditions [31]. Incubation of purified bovine LPO, porcine (p)TPO, human (h)TPO, and microsomal rTPO with genistein (10 μ*M*) and H_2O_2 (100 μ*M*) for 5 min produced activity losses of 53±3, 40±6, 62±3, and 66±7%, respectively (mean±SD, *n*=4). These results show that the inactivation of microsomal rTPO was comparable to that produced by genistein with other peroxidases, including hTPO, and suggest that isoflavone-mediated inactivation of TPO is a general phenomenon across mammalian species.

4. Dietary exposure of Sprague–Dawley rats to genistein

The inhibitory effects of genistein and daidzein on purified peroxidases in vitro prompted a further investigation of possible anti-thyroid effects in intact animals. Sprague–Dawley rats were exposed to

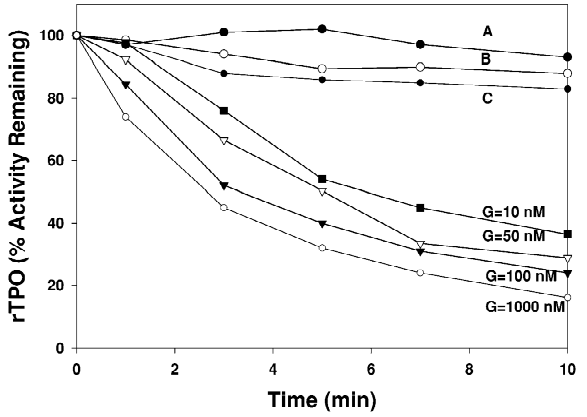


Fig. 1. Inactivation of rat TPO by genistein in vitro. Microsomal rat TPO was incubated with the indicated concentrations of genistein in the presence of H_2O_2 (100 n*M*) at room temperature in 0.1 *M* phosphate buffer, pH 7.0. At various times, aliquots were removed and remaining activity determined using a spectrophotometric guaiacol oxidation assay. (A) Control, rTPO alone; (B) control, rTPO+H_2O_2; (C) control, rTPO+1000 n*M* genistein. Thereafter, G=[genistein] added to TPO and H_2O_2.

genistein in order to investigate the possible disruption of endocrine function in dose range-finding [32] and multiple generation studies (in progress). Genistein was administered at doses of 0, 5, 100, and 500 ppm in soy-free basal diet (total genistein and daidzein ca. 0.5 ppm) to pregnant female rats 4 weeks prior to mating through weaning of pups at postnatal day (PND) 21. The rats were then placed on the same diet consumed by the dam until sacrifice at PND 140. Total blood genistein (i.e. the sum of conjugates and aglycone) concentrations in the PND 140 rats, measured using liquid chromatography electrospray mass spectrometry (LC-ES–MS, see Table 1; [33]), were comparable to those previously reported in several human populations (Table 2; [34–36]. Rats consuming the 500 ppm diet had genistein levels similar to those measured in infants consuming soy formulas [34]. Rats consuming the 100 ppm diet had genistein levels similar to those measured in adults consuming typical Asian diets [35] or soy isoflavone dietary supplements [36]. Rats consuming 5 ppm and control diets had low genistein levels, similar to those in humans consuming a typical Western diet. This information is summarized in Table 1. Significantly higher blood genistein

Table 2
Isoflavone intake and blood concentrations in humans. Estimates of total isoflavone intake[a] and measurements of circulating concentrations were obtained from the cited studies

Population group —Soy form	Total isoflavones[a] (mg/kg per day)[b]	Blood isoflavones (μ*M*)
Adults—Western diet	Very low	Very low
Adults—Asian diet	<1[c]	0.1 –1.2[d]
Adults—soy nutritional supplement	0.7[e]	0.5–0.9[e]
Infants—soy formula	6–9[f]	2–7[f]
Adults—soy cancer supplement	200[g]	?

[a] Total active isoflavones=genistein+daidzein.
[b] Body weight 70 kg.
[c] Soy isoflavone intake from typical Japanese diet estimated at 30 mg/day [21].
[d] From six Japanese men [35].
[e] From three adult volunteers [36].
[f] From seven infants [34].
[g] From label instructions.

Table 1
Genistein consumption and serum levels in Sprague–Dawley rats

Genistein dose (μg/g in diet)[a]	Estimated genistein intake (mg/kg per day)[b]	[Total genistein]	
		Males (μ*M*)[c]	Females (μ*M*)[c]
Basal	0.04	<0.01	<0.01
5	0.4	0.06±0.006	0.10±0.008
100	8	0.59±0.030	0.94±0.21
500	40	6.00±0.65	7.94±2.47

Rats were fed a basal soy-free diet fortified with genistein aglycone at the indicated concentrations and serum levels of total genistein measured using LC-ES–MS as described in Ref. [33].
[a] The basal soy-free diet which contained approximately 0.5 ppm of total genistein and daidzein was fortified genistein aglycone at the indicated level.
[b] Feed consumption and body weights were measured for individual animals and the intake values shown are representative.
[c] Total genistein concentration was measured in serum from male and female rats using LC-ES–MS (limit of quantitation 0.01 μ*M*).

concentrations were observed in female rats (as opposed to the males) in all dose groups, consistent with the significant shorter elimination half-time for genistein measured in males (3.0 vs. 4.3 h, respectively) [33]. Genistein glucuronides were shown to be the predominant circulating metabolites (97–99%) in both rats [37] and humans [36] consistent with extensive first-pass metabolism in the gut [38].

5. Intrathyroidal accumulation of genistein

The concentration of both total and aglycone genistein was measured using LC-ES–MS in thyroids from PND140 rats consuming genistein-fortified diets 12 h after removal from dosed-feed [31]. Fig. 2 shows the results for females and males where the higher average thyroidal levels observed in females reflected the higher average blood concentrations. The fraction of genistein present as aglycone in the thyroid was substantially increased relative to that in blood (18–28 vs. 1–3%). One-way ANOVA analysis showed a significant treatment effect for intrathyroidal total and aglycone genistein over the dose range, and Dunnett's test showed significant differences between the treatment and control groups as indicated in Fig. 2. The doses administered produced serum concentrations of total thyroidal genistein in the range of 0.1–1.2 nmol/g tissue and for the aglycone 0.1–0.3 nmol/g. Using estimates for the aqueous content of thyroid cells of 62%, the concentrations of genistein aglycone were as high as 350 n*M*. Similar results were observed for a number of male and female reproductive organs where total and aglycone genistein levels were higher and the percentage of aglycone was found to be as high as 100% [33]. These data suggest that the lipophilic aglycone form can partition into lipophilic tissues, including the thyroid. No iodinated derivatives of genistein were observed in thyroids or serum using LC-ES–MS (estimated detection limit 0.05 μ*M* in serum, 0.1 nmol/g thyroid). It should be noted that the intrathyroidal genistein aglycone levels shown in Fig. 2 for 100 and 500 ppm dose groups (i.e. 50–300 n*M*) are in excess of those concentrations found to inactivate rTPO in vitro (see Fig. 1 and Ref. [31]).

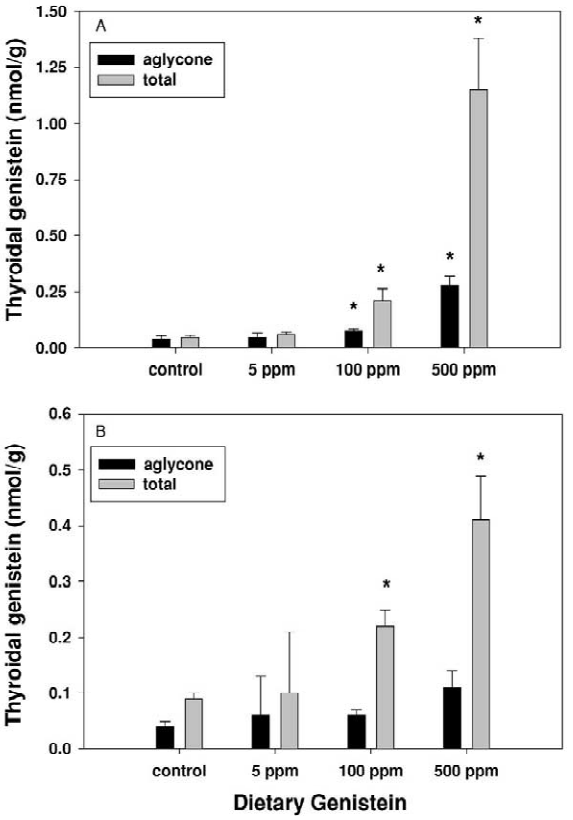


Fig. 2. Dietary consumption of genistein increases intra-thyroidal concentrations of genistein. The concentrations of genistein present in thyroid tissue, both total and aglycone, were determined using LC-ES–MS for female (A) and male (B) rats. Statistical significance relative to the respective control (*) was determined using Dunnett's test ($P<0.05$) as described in Ref. [31].

6. Inactivation of rTPO by dietary genistein in vivo

Fig. 3 shows TPO activity determined in thyroids from the rats fed genistein-fortified diets. In both male and female rats, dose-dependent decreases in rTPO activity were observed. As much as 80% loss of TPO activity was observed in 500 ppm dose females, but of equal importance was the observation that the lowest dose, 5 ppm, produced significant activity losses of 40–55%. Although control rTPO levels were comparable for male and female rats, two-way ANOVA showed a greater loss of activity in females relative to males ($P<0.002$). This finding was consistent with the higher serum and thyroid

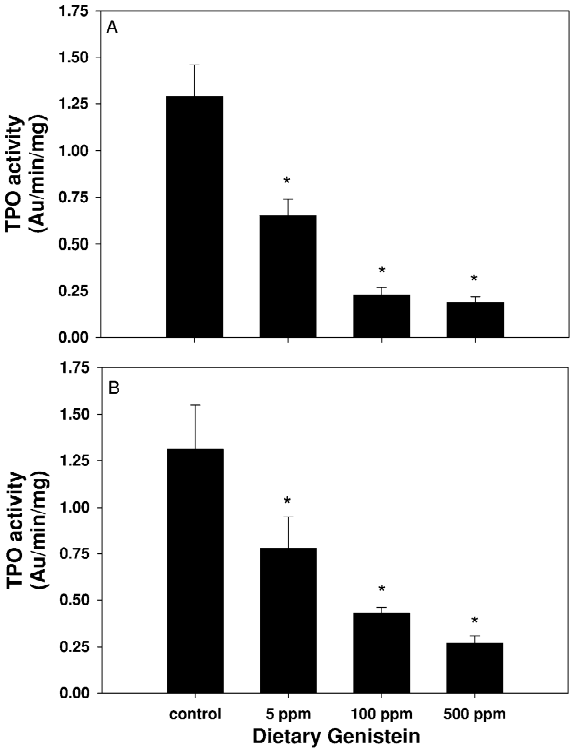


Fig. 3. Dietary consumption of genistein decreases rat TPO activity. Microsomal rTPO activity was measured in thyroids from female (A) and male (B) rats using a spectrophotometric guaiacol oxidation assay. One-way ANOVA demonstrated a significant treatment effect ($P<0.05$) for both males and females and statistical significance relative to the respective control (*) was determined using Dunnett's test ($P<0.05$) as described in Ref. [31].

levels of genistein measured in females. One-way ANOVA showed significant treatment effects for females ($P<0.001$) and males ($P<0.001$) and Dunnett's test indicated that all groups were different from controls ($P<0.03$). Furthermore, because the concentrations of genistein measured in rat thyroids were sufficient for inactivation of rTPO in vitro, it is reasonable to conclude that the reductions observed in TPO activity were due to enzyme inactivation in vivo.

In a previous study, TPO inhibition by isoflavones in vitro was described and conditions were identified under which suicide inactivation or alternate substrate inhibition occurred [29]. The presence of iodide, at concentrations expected in the normal thyroid gland (ca. 28 μM), attenuated the inactivation pathway in vitro. In the present study, no evidence was obtained from LC-ES–MS analysis of serum or thyroids to indicate formation of any of the iodinated genistein species previously characterized in vitro. This finding, coupled with the observation of extensive TPO inactivation in vivo, suggests that the isoflavone-mediated inactivation pathway is favored in the rat thyroid gland over competitive substrate iodination. Therefore, this study with genistein appears to be the first in which chemically-induced loss of TPO activity has been demonstrated both in vitro and in vivo.

7. Inactivation of rTPO by dietary soy in vivo

The previous findings, obtained by fortification of soy-free basal diet with genistein aglycone, were confirmed in a diet comparison study where rats were fed either a standard soy-containing feed (NIH 31 which contains 5% soy meal) or the basal diet (5K96) which contained approximately 60 or 1 ppm total isoflavones, respectively [39]. Soy contains isoflavones as various glucoside conjugates [40] so another study addressed the issue of whether biological effects can be altered by the form of isoflavone consumed in the diet. Reductions in TPO activity of approximately 50% were observed in male and female rats consuming the standard soy diet relative to the control diet (Fig. 4). The magnitude of reduction was commensurate with the measured blood levels of isoflavones. In male rats consuming NIH 31 diet, the average concentrations of total genistein and daidzein were 0.35 ± 0.03 and 0.20 ± 0.02 μM, respectively; for females, the concentrations were 0.62 ± 0.05 and 0.25 ± 0.02 μM, respectively [39]. Serum from rats consuming the basal soy-free diet contained 0.016 and 0.010 genistein and daidzein, respectively [39]. These results show that the form of genistein present in the diet, either aglycone or glucoside conjugates, does not affect the total serum isoflavone concentrations reached at sacrifice or the dose-dependent reduction in TPO content. This finding is consistent with a previous study that concluded administration of genistein, either as the aglycone or the mixture of glucoside conjugates present in soy, had a minimal

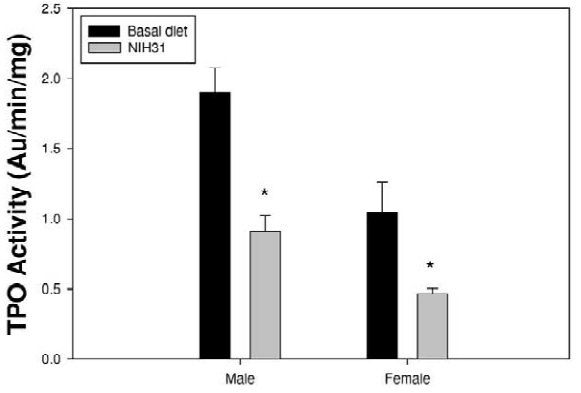


Fig. 4. Measurement of rTPO activity in rats consuming either a standard soy-containing or a soy-free diet. Microsomal TPO was isolated from female and male rats fed NIH 31 diet (ca. 30 ppm each of genistein and daidzein), or the basal diet (ca. 0.5 ppm each of genistein and daidzein) and rTPO activity was measured spectrophotometrically as described in Ref. [31].

effect on total absorption although small differences were observed in the peak concentrations [41]. Thigpen et al. have shown that the isoflavone content of typical rodent diets can vary from less than 5 ppm up to about 500 ppm [42]. These findings suggest the possibility for additive effects on TPO activity between soy in rodent diets and exogenous chemicals being tested for carcinogenicity or other toxicities.

8. Absence of hypothyroid indicators in rats fed genistein

The significant loss of TPO activity in rats consuming genistein-fortified and soy-containing diets made likely a hypothyroid state with concomitant decreased T3/T4 and increased TSH levels. This was predictable given the propensity of rats, particularly males due to their unique thyroid physiology, to anti-thyroid chemicals [22]. However, analysis of sera from all rats in both these studies showed no significant differences in the levels of these hormones relative to untreated controls (data not shown). Furthermore, gland weights and histopathological examination of thyroid sections showed no differences between untreated and 500 ppm genistein dose rats treated identically in a parallel study (not shown). A recent study showed only minor irregularities of the thyroid follicles following administration of a 20% soy diet to rats [43]. These findings were paradoxical in light of the dramatic effects on TPO activity shown in Figs. 3 and 4, although Ishzuki et al. reported goiter and elevated TSH in humans consuming soy in the absence of concomitant changes in T3 or T4 [18].

Thyroid hormone synthesis requires that TPO activity be co-located with its substrates: thyroglobulin, iodide, and H_2O_2, which is produced by the NADPH oxidase [44]. The best evidence suggests that this occurs at the interface between the apical membrane of the follicular cell, which contains TPO and the NADPH oxidase [45], and the lumen in which iodide is accumulated by a sodium iodide symporter present in the basolateral membrane [46] and into which thyroglobulin is secreted [45]. However, histochemical studies have shown that TPO can be more widely distributed in the cell [47]. This leaves open the possibility that an additional pool of TPO could be sequestered such that contact with isoflavones and H_2O_2, but not iodide, is possible. One possible explanation is the vesicles, shown by histochemical analysis to contain TPO [45]; however, it is not clear how isoflavones and H_2O_2, but not iodide, would have access to this pool of TPO. Another possible explanation for the paradoxical findings is that there is a large excess of TPO present in the apical membrane such that even substantial losses have a minimal effect on hormone homeostasis. An observation that is inconsistent with this idea is the coordinated stimulation of TPO gene expression along with that for sodium iodide symporter and thyroglobulin in response to TSH stimulation of thyroid follicular cells [48].

9. Synergism of soy with iodine deficiency in producing hypothyroid effects in rats

Additional insight to the anti-thyroid mechanism of soy and the striking synergism with iodine deficiency has recently come from studies in the rat. Ikeda et al. [43] showed, similarly to Kimura et al. [8], that feeding rats a soy-containing diet (20%

defatted soy bean) produced a severe hypothyroid state characterized by decreased T4, increased TSH and thyroid weight, evidence for increased cell proliferation, and marked histopathological changes from normal gland morphology. This hypothyroid state was observed only when the additional dietary condition of iodine deficiency was included. In addition, marked changes in histopathology of the anterior pituitary from rats fed the combined soy/iodine deficient diet suggested that an unknown component of soy had a direct action on the pituitary gland.

Another report from this group tested the ability of isolated soy isoflavones to act synergistically with iodine deficiency to produce a hypothyroid state [49]. Rats were fed soy-free diets with and without iodine and compared to the 20% defatted soy diet. Further, isolated soy isoflavones (12–18% genistein aglycone, 12–18% daidzein aglycone, 2–4% glycitein aglycone) were added to the respective soy-free diets at levels of 0.2 and 0.04%. It was reported that the 0.04% level of supplementation was equivalent to the amount of isoflavones present in the 20% defatted soy diets. As in the previous study [43], rats receiving the 20% defatted soy diet developed a severe hypothyroid state only in the case of iodine deficiency. However, neither level of isoflavone supplementation, in the presence or absence of iodine deficiency, produced a hypothyroid state. These results suggested that only whole soy, but not soy isoflavones alone, is sufficient to produce a hypothyroid condition in rats under conditions of iodine deficiency. These findings are, however, consistent with isoflavone-mediated TPO inactivation acting synergistically with the unidentified component(s) of soy to produce hypothyroid effects in iodine deficient rats.

10. Possible effects of soy on human thyroid health

The concentrations of total genistein present in rat serum (Table 1) are similar to those in several human populations (Table 2). These similar levels of internal exposure make it reasonable to conclude that human consumption of soy isoflavones could lead to accumulation in the thyroid gland (Fig. 2) and inactivation of hTPO as shown above.

The absence of observed hypothyroid indicators in rats following genistein consumption, despite extensive inactivation of TPO [31], and from mixed isoflavone consumption [49], makes it clear that additional risk factors for thyroid dysfunction, particularly iodine deficiency, are necessary before soy consumption can induce anti-thyroid effects in rats. Although the mechanism is still unclear, a significant amount of previous research has demonstrated the importance of dietary iodine deficiency as a factor in induction by soy of a hypothyroid state in rats [4,8,43,49]. A link between iodine deficiency and soy-induced hypothyroidism in humans comes from the studies of infants on soy formula in whom goiter was reversed upon supplementation with iodine [11]. Additional factors that could influence progression to a hypothyroid state include biochemical impairment of hormone synthesis and metabolism, and exposure to environmental goitrogens (e.g. perchlorate, sulfonamides, glucosinolates, cyanogenic glycosides, flavonoids [50], and persistent halogenated aromatic compounds). However, the results of Ishizuki et al. caution that soy-induced goiter and other hypothyroid indicators can occur in humans in the absence of iodine deficiency [18]. This study reported anti-thyroid effects in 37 healthy, presumably iodine-sufficient adults without known thyroid disease. These effects included goiter and elevated individual TSH levels, although still within the normal range, in 37 healthy adults fed 30 g of pickled soy beans per day for as little as 1 month. Changes in serum thyroid hormone (T3 and T4) levels were not observed. One month after stopping soy consumption, the individual TSH values had decreased to the original levels and goiters were reduced in size.

Soy products are currently heavily marketed to elderly women for relief from the symptoms of menopause, despite the absence of consistent clinical data demonstrating any effects beyond those produced by a placebo [20]. The potential anti-thyroid effects of soy components may be particularly relevant because these women represent the population subgroup in which overt hypothyroidism and a sub-clinical hypothyroid state [51] are most likely to

occur (rates up to 4 and 10%, respectively [52,53]. It is also important to recognize that the incidence of chronic autoimmune thyroiditis increases with age in women and that autoimmune thyroiditis is the predominate risk factor for the development of hypothyroid disease in women [52]. Recent studies showed that dietary genistein caused a potent stimulation of T and B cell-mediated immunity in rats [32,54], a property shared by other estrogenic compounds (e.g. *p*-nonylphenol, ethinylestradiol). In addition, our work suggests that suicide inactivation of TPO by dietary genistein in rats produces a covalently modified form of the enzyme, a potential neoantigen that could trigger immune system recognition [55]. It should be noted that anti-TPO is the major thyroid autoantigen found in human serum [56]. The etiology of thyroid autoimmunity is unknown, but these effects of genistein observed in rats makes it possible that soy consumption could adversely affect the course of autoimmune thyroid disease in women. Furthermore, iodine deficiency is an emerging concern in elderly Americans because consumption of iodized salt, a primary source of dietary iodine, should decrease as increasing information regarding the possible hypertensive effects of high salt intake encourages major reductions. These facts taken together suggest that elderly women should be cognizant of, and monitored for, possible thyroid complications arising from consumption of soy products, particularly when taken in large amounts.

Finally, this information also sheds important new light on the association suggested by the study of Fort et al. between soy formula consumption by infants and development of autoimmune thyroiditis later in life [17]. This study has been criticized in part because of its inability to exclude the possibility that children put on soy formulas were somehow predisposed to autoimmune diseases (e.g. food allergies, presence of viral gastroenteritis). The connection discussed above between stimulation of immune function by genistein, possible neoantigen formation through covalent binding of genistein to TPO, and exacerbation of autoimmune disease provides a plausible explanation for the observations of Fort et al. and suggests that further study of autoimmune thyroiditis in children consuming soy formula is warranted.

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