
DIETETIC RESEARCH PROJECTS

Effects of Dietary and Supplemental Forms of Isoflavones on Thyroid Function in Healthy Postmenopausal Women

**Tracy Ryan-Borchers, PhD, RD; Boon Chew, PhD;
Jean Soon Park, PhD; Michelle McGuire, PhD;
Lisa Fournier, PhD; Kathy Beerman, PhD**

Because of the health concerns associated with hormone replacement therapy, many women are seeking alternative therapies. Compounds such as soy isoflavones appear to reduce the risk of some age-related diseases and to lessen the severity of menopausal symptoms. However, concerns regarding harmful effects of soy isoflavones on thyroid function have been reported. This 16-week, double-blinded, placebo-controlled trial evaluated the effects of soy isoflavones on thyroid-stimulating hormone in healthy postmenopausal women. The results of this study suggest that isoflavones obtained from either soymilk or supplements have no effect on thyroid-stimulating hormone in well-nourished postmenopausal women. **Key words:** *menopause, soy isoflavones, thyroid function*

MENOPAUSE, defined as the cessation of menstruation resulting from the loss of ovarian follicular activity, is associated with increased risk of several age-related diseases, including coronary heart disease and osteoporosis.

Author Affiliations: *Department of Nutrition, Oregon Health Science University, Portland, Oregon (Dr Ryan-Borchers); and Departments of Food Science and Human Nutrition (Drs Chew, Park, McGuire, and Beerman) and Psychology (Dr Fournier), Washington State University, Pullman.*

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Corresponding author: *Kathy Beerman, PhD, Department of Food Science and Human Nutrition, Washington State University, Pullman, WA 99164 (beerman@wsu.edu).*

Furthermore, symptoms such as night sweats, hot flashes, and mood swings often accompany the early stages of menopause. Many women rely on standard hormone replacement therapy (HRT) to reduce menopausal symptoms and associated health risks. However, there is growing evidence that HRT can increase the risk of developing venous thrombosis, cardiovascular disease, and certain cancers.¹⁻⁴ These studies, which include the Women's Health Initiative, the largest randomized clinical trial conducted to assess long-term use of combined estrogen-progestin HRT, have raised important issues concerning health risks associated with HRT.⁵⁻⁸ Thus, a growing number of women are seeking alternative therapies to alleviate menopausal symptoms most notably soy.^{9,10}

In recent years, health benefits associated with consumption of soybeans have been widely publicized. Some studies have found

that soy-derived biologically active compounds called isoflavones may reduce the risk for certain cancers, cardiovascular disease, and osteoporosis.¹¹⁻¹⁵ In addition to reducing the risk of these diseases, some studies have reported that supplementation with soy products and/or isoflavones lessens the frequency and intensity of menopausal symptoms, particularly hot flashes that often occur at the onset of menopause.¹⁶ Furthermore, improvements in bone mass and reduced bone resorption have also been attributed to the estrogenic properties of isoflavones.¹⁷⁻²⁰ Although studies have failed to demonstrate consistent effects of isoflavones on menopausal health, consumption of soy products and/or isoflavones overall appears to be beneficial.²¹ However, it is important to acknowledge that safety concerns regarding isoflavones have been raised. More specifically, animal models suggest that soy isoflavones may adversely affect human growth, sexual development, reproduction, and thyroid function.²²

Isoflavones are a subclass of flavonoids, a broad group of polyphenolic compounds that have both hormonal and nonhormonal activities.²³ The 3 main isoflavones—daidzin, genistin, and glycitin—are found predominantly in soybeans and other legumes. Isoflavones occur primarily as glycosides, meaning that the bioactive moiety is conjugated almost exclusively to sugars. During digestion, endogenous and bacterial enzymes hydrolyze the glycoside-bound moieties, resulting in the metabolically active aglycone forms—daidzein, genistein, and glycitein. Daidzein can be further converted to equol, which is thought to have even greater biological activity than its parent compound. However, only 30% to 50% of the population appears to be capable of producing equol from daidzein.²⁴ It is possible that the effectiveness of soy and/or isoflavones in treating menopausal symptoms may be somewhat dependent on a person's ability to convert daidzein to equol.²⁵

Isoflavones and their derivatives are structurally similar to human estrogen and have a high-binding affinity to both types of

estrogen receptors ($Er\alpha$ and $Er\beta$), giving them both antiestrogenic and proestrogenic effects.²⁶ Although the proestrogenic actions of isoflavones are believed to contribute to their bone-sparing properties and ability to relieve menopausal symptoms, their antiestrogenic effects are thought to provide protection against hormone-sensitive cancers of the breast.^{27,28} Despite the proposed benefits of isoflavone consumption, health concerns regarding potential harmful effects resulting from long-term soy consumption have been reported.²⁹ One such concern is that consumption of soy products may adversely affect thyroid function.³⁰⁻³²

Hypothyroidism, which results when the thyroid gland releases insufficient amounts of thyroid hormones, has an enormous impact on health. Because thyroid function tends to decline with age and is associated with hormonal transitions, the prevalence of hypothyroidism is greater in women than men, occurring in 6% to 10% of postmenopausal women.^{33,34} For this reason, the American Thyroid Association recommends that adults, particularly women, be screened for thyroid dysfunction by measurement of thyroid-stimulating hormone (TSH) beginning at age 35 years and every 5 years thereafter.³⁵

Much of the evidence for the antithyroid effects of soy and/or isoflavones stems from early animal studies conducted in the 1930s in which rats fed soy-based, iodine-deficient diets developed goiters; enlargements of the thyroid gland resulted from thyroid hormone deficiency.³⁶ However, iodine fortification of the diet was shown to ameliorate this problem. The goitrous effects of soy consumption were also noted during the 1950s and 1960s in infants fed non-iodine-fortified, soy-based formula.^{37,38} This was later found to be essentially prevented by fortification of the formula.

On the basis of the animal and human studies, considerable evidence suggests that soy isoflavones have goitrogenic properties. However, this appears to occur only when iodine intake is inadequate or in those predisposed to thyroid dysfunction. This has been

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demonstrated both in vitro and in vivo studies.³⁹ Studies have shown that genistein inhibits the activity of thyroid peroxidase, an enzyme necessary for the synthesis of thyroxine (T₄) and tri-iodothyronine (T₃).⁴⁰

Given the increased use of soy isoflavones by postmenopausal women today, the potential antithyroid effects of soy and/or isoflavone consumption deserves further investigation. To date, very few controlled intervention trials have been conducted in postmenopausal women, and most of these studies used isolated isoflavones rather than whole soy food products.⁴¹ Therefore, the purpose of the present study was to evaluate the effects of soy isoflavones, obtained from either soymilk or a supplement form, on circulating TSH concentrations in healthy, well-nourished postmenopausal women. We hypothesized that consumption of soy isoflavones, obtained from either soymilk or a tablet form, would not affect TSH production.

PARTICIPANTS AND STUDY DESIGN

This study was conducted as part of a larger research project designed to primarily investigate the effects of soy isoflavones on cognitive function.⁴² Thus, the sample sizes were determined on the basis of expected changes in cognition, rather than thyroid function. Healthy postmenopausal women between 50 and 65 years of age were recruited regionally from the states of Idaho and Washington to participate in this 16-week, double-blinded, placebo-controlled, randomized trial. Potential subjects ($n = 300$) were screened by telephone to determine study eligibility. Women free of major health conditions and who had not menstruated for more than 1 year were deemed eligible for participation. Additional exclusion criteria included the presence of legume allergies, history of smoking, kidney stones, thyroid medication use, and antibiotic therapy within the past 6 months.

Telephone screening resulted in approximately 150 women who were both eligible

and interested in the study. Of the 119 subjects that completed the enrollment process, 5 dropped out for reasons unrelated to the study. After the exclusion of women ($n = 35$) using HRT (an additional control group needed for the larger study), 80 women composed the subset for this secondary investigation. However, adequate amounts of serum were not obtained from 3 subjects, reducing the final sample to 77. The Institutional Review Board of Washington State University approved all study procedures.

Participants provided informed consent prior to beginning a 4-week adjustment diet that minimized the intake of foods containing isoflavones. After this washout period, the 16-week intervention period began. Participants were enrolled in groups, which were then considered as treatment blocks. Within each block (5 in total), they were randomly assigned to receive one of the following treatments: (1) control group ($n = 27$) receiving 706 mL cow milk/d and placebo supplement; (2) soymilk group ($n = 26$) receiving 706 mL soymilk/d and placebo supplement (71.6 ± 3.1 mg isoflavones/d); or (3) supplemental group ($n = 24$) receiving 706 mL cow milk/d and isoflavone tablets (70.0-mg isoflavones/d). Researchers and participants remained blinded to group assignment throughout the study.

The nutrient composition and caloric content of the 2 types of milk were nearly identical (Table 1). The isoflavone content and composition of the supplements (15-mg daidzein, 17-mg genistein, and 3.5-mg glycitein) were formulated and verified by Archer Daniels Midland Co (Novasoy; Decatur, Illinois) to match that of the soymilk. The cow milk was treated with flavoring and coloring agents to resemble the flavor and appearance of the soymilk. The isoflavone content of the soymilk was determined by HPLC (Murphy P, Iowa State University) and is presented in Table 1. Milk products were delivered to the subjects weekly. Placebo tablets were composed of maltodextrin with 10% caramel color, with appearance similar to that of the isoflavone supplements.

[AQ1]

[AQ2]

Table 1. Energy and nutrients supplied by the cow milk and soymilk consumed daily

Component	Cow milk ^a	Soymilk ^b
Carbohydrate, g/d	36	30
Fat, g/d	6	10
Protein, g/d	24	18
Energy, kcal/d	294	282
Calcium, mg/d	900	900
Vitamin D, IU/d ^c	300	360
Isoflavones, mg/d ^d		
Daidzein	...	30.9 ± 1.5 ^e
Genistein	...	37.4 ± 1.3
Glycitein	...	3.6 ± 0.5
Total	...	71.6 ± 3.1

^a1% low-fat cow milk, 706 mL/d; values provided by manufacturer.

^bVanilla Silk soymilk, White Wave, Inc, 706 mL/d.

^cExpressed in international units.

^dCalculated total daily amounts of isoflavone for 706 mL/d soymilk. Data based on analysis of 30 soymilk samples (courtesy of Dr. P Murphy, Iowa State University).

^eMean ± SEM.

Participants were instructed to consume a total of 706 mL/d of "milk" (soymilk or cow milk) for 16 weeks. Milk was consumed in the morning (353 mL) and in the evening (353 mL). The reported iodine content of cow⁴³ and soymilk* is approximately 56 µg/8 oz and 50 µg/8 oz, respectively. Thus, daily consumption (706 mL/d) of cow or soymilk provided subjects with enough dietary iodine (168 and 150 µg, respectively) to meet 100% recommended dietary allowance. Two supplements (isoflavone or placebo tablet) were taken with the milk, twice daily. In addition, all participants were provided daily multivitamin supplements to ensure adequate nutrient intakes. The nutrient content of the multivitamin supplements is presented in Table 2. To maintain proper caloric and nutrient intakes, a dietitian assisted study participants

in making dietary adjustments. Participants met with the dietitian during the baseline period to assist in dietary planning. The dietitian was also available for consultation throughout the entire intervention period. They were instructed to eliminate soy products from their diet throughout the entire study. Compliance was assessed through weekly, 24-hour dietary records and detection of urinary isoflavone metabolites.[†]

BIOLOGICAL SAMPLING AND ANALYTICAL PROCEDURES

Urinary isoflavone concentrations

A 24-hour urine collection was obtained from participants at 0 week (baseline) and week 16. Total urine volume was recorded, and aliquots were frozen at -20°C until analyzed for concentrations of urinary isoflavones and their metabolites. Isoflavone concentrations in urine were measured by HPLC using procedures described by Zhang and Xu.⁴⁴⁻⁴⁶ Briefly, β-glucuronidase-sulfatase H-2 (EC3.2.131, from *Helix pomatia*; Sigma Chemical Co, St. Louis, Missouri) was added to urine (5 mL), and the mixture was incubated at 37°C for 20 hours to release isoflavone aglycones. Samples were then loaded onto Extrelut QE columns (EM Science, Gibbstown, New Hampshire) and extracted with 12 mL ethyl acetate. The eluent was collected and dried under nitrogen gas. Extracted isoflavones were dissolved in 80% methanol in water. An HPLC system (Waters, Milford, Massachusetts) equipped with a photodiode array detector (PDA 996) was used to distinguish the ultraviolet spectra of the specific isoflavone compounds: daidzein (248 nm), equol (280 nm), and genistein (260 nm). Isoflavones were separated in a µ-Bondapak C₁₈ reverse-phase column (Waters; 3.9 mm × 30 cm). The compounds were eluted by using a linear gradient of 40%

[AQ3]

*Iodine content of Vanilla Silk soymilk reported by manufacturer, White Wave, Inc.

[†]To coordinate with the larger study, urinary isoflavones were measured in a subset of subjects (*n* = 41).

Table 2. Nutrient content of multivitamin supplement (per tablet)

Nutrient	Amount	% Recommended dietary allowance
Vitamin A (as acetate)	5000 IU	30
Vitamin C (as ascorbic acid)	60 mg	80
Vitamin D (as cholecalciferol)	400 IU	100 ^a
Vitamin E (as di-alpha tocopheryl acetate)	30 IU	200
Thiamin (as thiamin monoitrate)	1.5 mg	136
Riboflavin	1.7 mg	154
Niacin (as niacinamide)	20 mg	143
Vitamin B ₆ (as pyridoxine hydrochloride)	2 mg	133
Folic acid	400 mcg	100
Vitamin B ₁₂ (as cyanocobalamin)	6 mcg	250
Pantothenic acid (As calcium d-pantothenate)	10 mg	200
Iron (as ferrous fumarate)	18 mg	225

^aAdequate intake.

to 70% methanol in 30 minutes at a flow rate of 1 mL/min. Retention times for the isoflavones were as follows: daidzein 14.9 minutes, equol 16.8 minutes, and genistein 18.5 minutes. The identity of each isoflavone was confirmed by comparing its absorption spectrum with that of a corresponding standard. Isoflavone concentrations were calculated on the basis of standard curves constructed for daidzein (Valeant Pharmaceuticals, Costa Mesa, California), genistein (Calbiochem, San Diego, California), and equol (Sigma-Aldrich).

Serum thyroid stimulating hormone concentration

Fasting blood samples were obtained early morning at baseline (0 week) and following the intervention (week 16). Blood (10 mL) was drawn into evacuated collection tubes, centrifuged (1000×g, 30 minutes, 4°C), and aliquots of the serum were frozen at -80°C until analyzed for TSH. To minimize inter-assay variation, serum samples were batch analyzed for TSH using 2-site sandwich, a direct chemiluminescent technology. Specifically, TSH concentrations were determined by radioimmunoassay using constant amounts of 2 antibodies—a monoclonal mouse anti-

TSH antibody labeled with acridinium ester and, in the second phase, a polyclonal sheep anti-TSH antibody. Samples were analyzed by Pathology Associates Medical Laboratories (Spokane, Washington), a testing facility accredited by the College of American Pathologists and licensed by the State of Washington Department of Health as an approved medical test site.

STATISTICAL ANALYSIS

Data were analyzed by ANOVA using the general linear model procedure of SAS statistical software (SAS Institute, Cary, North Carolina). Protected LSD was used for multiple mean comparisons. The statistical model included block, subject, treatment (control, soymilk, or supplement), period (week 0 and week 16), and the interaction of treatment by period. Treatment block was not found to be significant and, therefore, removed from the model. Statistical significance was set at $P < .05$.

FINDINGS

Participants

Demographic and anthropometric characteristics of participants are presented in

[AQ7]

[AQ4]

Table 3. Characteristics of postmenopausal women by treatment group

Variable	Treatment group			P
	Control (n = 27)	Soymilk (n = 26)	Supplement (n = 24)	
Age, y ^a	55.7 ± 0.8	55.8 ± 0.9	54.8 ± 0.7	.61
Body mass index, kg/m ²	29.4 ± 1.3	27.5 ± 1.2	28.6 ± 1.2	.52
Ethnicity				
Caucasian	25	26	24	
Asian	1	0	0	
Native American	1	0	0	
Number of years since last menstruation ^a	10.1 ± 1.7	7.7 ± 1.3	6.0 ± 1.0	.15

^aMean ± SEM.

Table 3. No significant differences were found by the treatment group with respect to age, body mass index, and number of years since last menstruation. On average, women were 55.5 ± 0.8 years of age, and had not menstruated for 8 ± 1.4 years, and 62% had at least a college degree (data not shown). Average body mass index was 28.5 kg/m² ± 1.2

(range: 20.1–47.6 kg/m²), which did not differ between week 0 and week 16 (*P* = .96).

Effect of treatment on urinary isoflavones

The results of the urinary isoflavone analyses are presented in Table 4. At baseline, total isoflavone concentrations were not

Table 4. Effect of dietary treatment on urinary isoflavones and circulating TSH concentrations at baseline (0 week) and after intervention (16 week)

Variable	Treatment group					
	Control (n = 27) ^a		Soymilk (n = 26) ^a		Supplement (n = 24) ^a	
	0 week	16 week	0 week	16 week	0 week	16 week
Urine						
Total isoflavone, μmol/L ^{a,b}	ND	ND	ND	36.5 ± 7.7 ^d	0.7 ± 0.67 ^c	52.3 ± 7.7 ^d
Daidzein, μmol/L	ND	ND	ND	13.4 ± 15.3 ^d	0.05 ± 0.05 ^c	22.8 ± 12.2 ^d
Gensitein, μmol/L	ND	ND	ND	5.4 ± 0.9 ^d	ND	18.1 ± 3.7 ^d
Equol, μmol/L	ND	ND	ND	17.7 ± 5.7 ^d	ND	11.3 ± 5.6 ^d
Serum						
TSH, mU/L	2.3 ± 0.3	2.3 ± 0.4	2.9 ± 0.3	3.0 ± 0.3	2.5 ± 0.3	2.5 ± 0.3

ND = Nondetectable.

^aIsoflavones measured in a subset sample of 41 subjects (control *n* = 13; soymilk *n* = 15; supplement *n* = 13). No differences were found between baseline TSH values of subjects with and without isoflavone measures (2.5 ± 0.2; *n* = 41 vs 2.6 ± 0.5; *n* = 36, respectively).

^bMean ± SEM.

^{c,d}Means in a row with different superscript letters are significantly different, *P* < .05 (ANOVA followed by a protected LSD for multiple comparisons).

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detectable in any group. Urinary concentrations of daidzein, genistein, and equol were higher ($P < .05$) in the soymilk and supplement groups than in the control group at week 16. Upon completion of the intervention (week 16), there was a significant ($P < .0001$) effect of the treatment group for total isoflavone concentration such that the total isoflavone concentrations were not statistically different between the soymilk and supplement groups (36.5 ± 7.7 and $52.3 \pm 7.7 \mu\text{mol/L}$, respectively; $P = .08$), but were higher than that of the control group ($P < .0001$). There was a significant Treatment \times Period interaction ($P < .001$) for all urinary isoflavones, indicating that the effects of these factors were not independent of each other.

Effect of treatment on serum thyroid stimulating hormone

There was neither an effect of treatment nor period on TSH concentrations among the experimental groups (control, soymilk, and supplement) nor was there an interaction between these variables, indicating that concentrations of serum TSH were within the normal range at baseline (TSH: 0.5–5.0 mU/L)⁴⁷ and remained so following the intervention period in all women. No differences were found in baseline TSH values of participants with and without isoflavone measures (2.5 ± 0.2 ; $n = 41$ vs 2.6 ± 0.5 ; $n = 36$, respectively).

DISCUSSION

Our study is the first to evaluate the effect of both dietary and supplemental forms of isoflavones on thyroid function in postmenopausal women. Data indicate no effect of isoflavones derived from soymilk or supplements on the TSH concentration. Concentrations of all 3 isoflavones—daidzein, genistein, and equol—were elevated in the urine in response to both isoflavone interventions, indicating compliance with the study protocol. These results provide evidence that isoflavone intake at these levels does not

adversely affect thyroid function in healthy, well-nourished postmenopausal women. Although isoflavones derived from soybean products have been found to have antithyroid effects, this does not appear to have clinical importance in well-nourished women.⁴⁸ In this study, both treatments (cow milk and soymilk) provided women with enough iodine to satisfy recommended daily intakes. However, data from the National Health and Nutrition Examination Surveys indicate that in the United States average iodine intake in adult women is 190 to 200 $\mu\text{g/d}$, an amount sufficient to ensure normal thyroid function.⁴⁹ Although iodine tends to be abundant in the food supply, certain subpopulations that exclude iodized salt or fish, and vegan vegetarians may not consume adequate amounts.

Our findings are consistent with Bruce et al, who also investigated the effect of daily isoflavone supplements (approximately 50 mg of soy isoflavones tablets taken 3 times per day) on thyroid function in iodine-repleted postmenopausal women.⁵⁰ In this randomized, double-blinded, placebo-controlled study, thyroid-related hormones (TSH, T_3 , and T_4) were measured at baseline and at 90 and 180 days. These data provided no evidence that consumption of soy isoflavones adversely affected thyroid function. However, study compliance was not verified by measuring plasma or urinary isoflavone concentrations, nor did the study include a dietary intervention.

It is possible that other components of soy foods, rather than isoflavones, may adversely affect thyroid function. For this reason, Duncan et al studied the effects of soy powders with varying amounts of isoflavones on thyroid function.⁵¹ Using a randomized, crossover design, 18 postmenopausal women consumed low- or high-isoflavone content soy powders for 93 days (low isoflavone 1.0 mg/kg/d and high isoflavone 2.0 mg/kg/d). Blood samples were analyzed for a variety of hormones including TSH, T_3 , and T_4 . No effect of low- or high-isoflavone intake was found on thyroid function. However, investigators

in another study fed both men and women 30 g/d of pickled soybeans for 3 months and reported a change in thyroid function.⁵² Although TSH levels increased, little is known about the soy protein and isoflavone content of the treatment, and information pertaining to the iodine intake of participants reported.

It is important to note that laboratory-testing strategies established by the American Thyroid Association for detection of thyroid dysfunction state that the single most reliable test to diagnose all common forms of hypothyroidism is the serum TSH concentration. This is because T₃ measures are poorly correlated with thyroid status, and T₄ metabolism is often reduced in the elderly.^{53,54} This may be why several studies have reported changes in T₃ and/or T₄ concentrations in response to soy intake, but changes were slight and remained within normal ranges.⁵⁵⁻⁵⁷ For these reasons, T₃ and T₄ were not considered in our study and comparisons are, therefore, limited to TSH only.

In conclusion, the results of this study indicate that even in this susceptible population group of postmenopausal women, when iodine intake is likely adequate, isoflavone consumption has no effect on circulating TSH concentration. This finding was similar regardless of whether isoflavones were consumed as part of a food matrix (soymilk) or

in isolation as a supplement. Although the duration of this study was relatively short (16 weeks), the amount of soy isoflavones consumed daily (~70 mg) was considerably more than average intakes in regions of the world where soy products are part of traditional diets.^{58,59} For example, Japanese adults consume on average 7- to 11-g soy protein daily; this is equivalent to 30-50 mg of isoflavones. These amounts are similar to other Asian cultures such as those living in Singapore and Hong Kong.⁵⁹ It is, therefore, reasonable to assume that the amount of isoflavones consumed in this study was sufficient to potentially influence thyroid function. However, this study did not test for the presence of thyroglobulin autoantibodies or antithyroid peroxidase antibodies, both of which are indicators of medical conditions related to thyroid dysfunction.

On the basis of the results from this study, data suggest that increased soy consumption does not increase the risk of hypothyroidism in healthy postmenopausal women. Although iodine status was not evaluated in our subjects, it was assumed that adequate amount of iodine was consumed via milk treatments and as a part of the overall diet. Because soy and/or isoflavones may offer postmenopausal women certain health benefits, more studies are needed to ensure that soy products provide a safe alternative to HRT.

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Authors: Tracy Ryan-Borchers, Boon Chew, Jean Soon Park, Michelle McGuire, Lisa Fournier, and Kathy Beerman

Author Queries

AQ1: Define HPLC.

AQ2: Check whether P. Murphy of Iowa State University provided some personal communication. Also provide the year of communication, if required.

AQ3: Provide location (city, state) of White WaveTM, Inc in footnote 1.

AQ4: Define LSD.

AQ5: Update ref. 42.

AQ6: Provide names of all editors and name of the author of the article from which page 11 is referred in ref. 43.

AQ7: Should "thiamin monoitrate" be "thiamin mononitrate?"