

Dietary isoflavone supplementation for food allergy: A pilot study



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Clinical Implications

- Isoflavones, genistein, and ipriflavone suppressed *ex vivo* allergen-induced basophil degranulation in food-allergic subjects. Short-term dietary supplementation of over-the-counter genistein and ipriflavone did not suppress effector cell (basophil and mast cell) functions in food-allergic subjects.

TO THE EDITOR:

A variety of soy isoflavone supplements are widely available over-the-counter. Soybean isoflavones (eg, genistein, daidzein) are popular natural products for the treatment of various forms of cancer, cardiovascular, renal and other disorders.¹ Currently genistein is listed as an investigational drug in at least 12 open studies in www.clinicaltrials.gov. There is emerging evidence that isoflavones are beneficial in allergic diseases. Although there are a number of *in vitro* results reported on the potential of isoflavones to regulate cell functions relevant to allergy, very limited/circumstantial evidence exists that directly links the beneficial role of isoflavones and allergy.^{1,2} Soy supplementation reduced eosinophil leukotriene-C4 synthesis, airway inflammation, and improved lung function in asthmatic patients.^{3,4} The high intake of soy-containing foods is associated with reduced prevalence of allergic rhinitis.⁵ However, a recent randomized clinical trial showed no benefit from consumption of isoflavone supplements in subjects with poorly controlled asthma.⁶ In this context, the potential benefits of isoflavones have never been tested in food allergy other than our published reports in mouse models demonstrating a suppression of peanut allergy symptoms and mucosal immune responses.^{2,7,8}

Genistein is available as a nonprescription over-the-counter dietary supplement as soy extracts and in pure forms, marketed for relief from menopause-associated night sweats, mood swings, and bone health, in spite of inconclusive evidence of effectiveness.¹ Ipriflavone, a synthetic derivative of daidzein, has been in clinical use for decades and is available in 22 countries as a prescription medication for the treatment of osteoporosis with an excellent safety record. In addition to inhibiting bone resorption, ipriflavone may have therapeutic effects on several other disorders.⁹ In the United States, ipriflavone is available as a nonprescription or over-the-counter supplement in most drugstores. We tested the efficacy of these over-the-counter genistein (i-Cool, DSM Nutritional Products, Heerlen, Netherlands) and ipriflavone (Ostivone, TSI USA Inc., Missoula, Mont) supplements in suppressing food-allergic symptoms in a human *ex vivo* model system. We also conducted a proof-of-principle study to test the effector cell responsiveness *in vivo* to allergenic foods in food-allergic

subjects. We assessed biomarkers of allergic response including skin prick tests, serum food-specific antibody levels, and basophil degranulation in subjects receiving these supplements.

Basophil degranulation assays were performed with fresh whole blood obtained from healthy volunteers and preincubated with titrated quantities of genistein and ipriflavone for 30 minutes followed by stimulation with anti-IgE (see Materials and Methods in this article's Online Repository at www.jaci-inpractice.org). Genistein and ipriflavone suppressed *ex vivo* basophil degranulation in a dose-dependent fashion (Figure E1, A, available in this article's Online Repository at www.jaci-inpractice.org). To further elucidate the effect of isoflavones on basophils from food-allergic subjects, whole blood from peanut-allergic (n = 6) (Figure E1, B) and milk-allergic (Figure E1, C) subjects were preincubated with 100 μ M genistein, ipriflavone, or genistein+ipriflavone for 30 minutes followed by incubation with peanut or milk extracts, respectively (along with controls, data not shown) and processed as above. Basophil degranulation from both peanut- and milk-allergic subjects was suppressed by *in vitro* allergen+isoflavone treatment when compared with allergen-treatment alone. Whereas genistein and ipriflavone individually have a modest effect on allergen-induced basophil degranulation, the combination genistein+ipriflavone significantly suppressed basophil degranulation (Figure E1, B and C, available in this article's Online Repository at www.jaci-inpractice.org). These data indicate a synergistic effect of genistein and ipriflavone in suppressing basophil degranulation *ex vivo*.

To further investigate whether the basophil-suppressive effect of isoflavones can be translated into potential clinical utilization, we tested the effect of oral supplementation of genistein and ipriflavone in adult food-allergic subjects (allergic to peanuts and tree nuts). In this proof-of-principle study, we recruited a total of 15 food-allergic subjects, randomized (2:1) to receive 30 mg genistein per day plus 200 mg ipriflavone 3 times a day for 4 weeks (n = 8). No supplements were provided to the control group (n = 6). This study was approved by the institutional review board (IRB) and all subjects signed informed consents (IRB approval number: HS#: 13-00258). A total of 15 adult subjects were recruited in this randomized study. The eligibility criteria included physician diagnosed food allergy or convincing clinical history of food allergy and skin prick tests positive to the allergenic food (diameter of wheal > 3 mm) or detectable serum food-specific IgE level (ImmunoCAP > 0.35 kU_A/L) (ImmunoCAP, ThermoFisher, Waltham, Mass). The clinical characteristics of the subjects are provided in Table E1 (available in this article's Online Repository at www.jaci-inpractice.org). One subject withdrew from the study after visit 1. All subjects were asked to avoid a list of soy-containing foods and dietary soy supplements including soy burgers, soy milk, or beverages fortified with soy proteins, soy bars, raw-, roasted-, or boiled soy beans, edamame, miso soup, etc. for the duration of the study. Subjects in the treatment arm were provided with a 4-week supply of dietary supplements by the physician during the initial visit. No supplements were provided to the control group. There were no adverse reactions to test supplements reported during the study.

Effector cell responsiveness (*ex vivo* basophil degranulation, and prick-skin testing to assess mast cell degranulation) and IgE

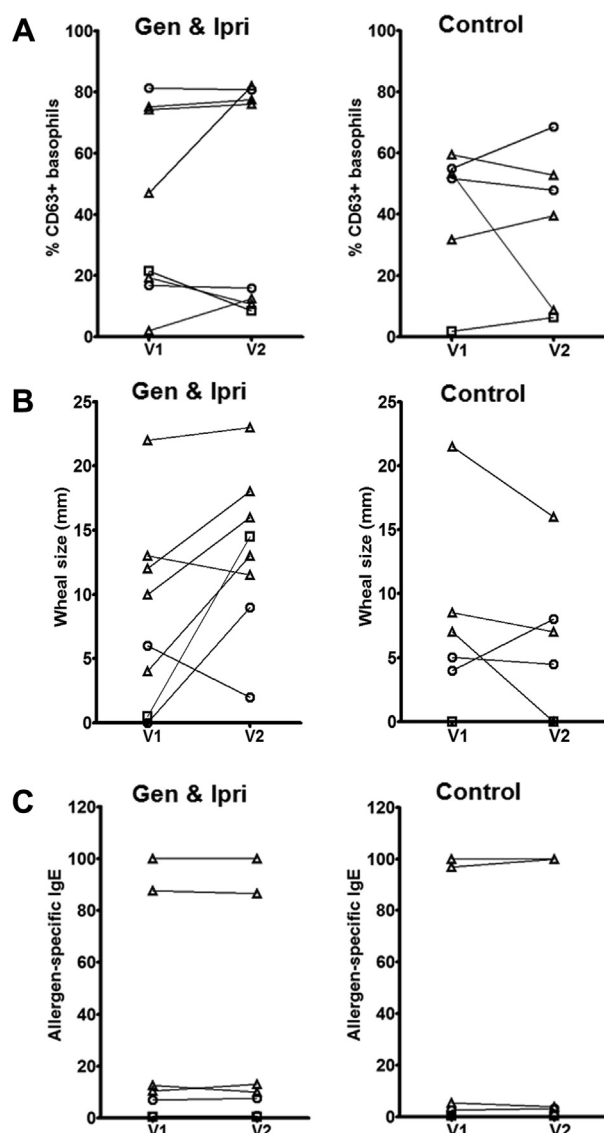


FIGURE 1. (A) Basophil degranulation was performed in food-allergic subjects with respective allergen extracts at visit 1 (v1) and visit 2 (v2). (B) The allergen-induced wheal size measurements (1:20 SPT allergen extract minus saline controls) and (C) allergen-induced IgE were determined at visit 1 and visit 2 (Δ , peanut; $\circ$, walnut; and $\square$, cashew). *Gen*, Genistein; *Ipri*, ipriflavone.

levels (allergen-specific and total) were tested at baseline (week 0) and after 4 weeks of supplementation. When stimulated with appropriate allergen (peanut, cashew, or walnut), no significant changes in basophil degranulation was observed in either the treatment ($P = .54$) or control subjects ($P = .58$) (Figure 1, A). Prick skin test results represented as allergen-induced wheal size measurements (1:20 allergen extract SPT minus saline controls) (Figure 1, B) and allergen-specific IgE levels (Figure 1, C) were inconclusive and did not show any significant differences between visits and between test subjects and controls. All clinical lab results were normal between visits 1 and 2 for both test subjects and control groups. The plasma genistein levels were

detectable and significantly higher at visit 2 in subjects who received supplementation (Figure E2, available in this article's Online Repository at www.jaci-inpractice.org). In conclusion, short-term genistein and ipriflavone supplementation for 4 weeks did not suppress effector cell responses in food-allergic subjects. We intended to recruit a total of 30 peanut-allergic adult subjects for this study. However, the recruitment was stopped after an interim analysis for futility as it was apparent that the isoflavone supplementation did not show any effect on basophil degranulation.

In this study, we followed the duration and the suggested dosages from the manufacturer, which was comparable to prior reports of physiological levels. When compared with animal studies (where the mice are continuously fed with an isoflavone diet⁸), this dose is very small and the duration is short (approximately 1 month). It is possible that at this dose, the systemic isoflavone concentrations were not sufficient or the absorption was not adequate to elicit a suppressive reaction as observed in a recent randomized trial of an isoflavone supplementation study for asthma.⁶ Higher doses are not recommended due to potential side effects/phytoestrogenic properties. These previous studies and our current results clearly demonstrate the limitations of efforts to translate the *in vitro*-cellular and animal studies into clinical applications.

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Conflicts of interest: H. A. Sampson has received research support from David H. and Julia Koch Research Program in Food Allergy Therapeutics; is employed by and has stock options in DBV Technologies. The rest of the authors declare that they have no relevant conflicts of interest.

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ONLINE REPOSITORY MATERIALS AND METHODS

Basophil activation test

Basophil degranulation was performed with different concentrations of allergens. The allergen concentration that induced maximal basophil degranulation at visit 1 was chosen for comparison at visit 2. Whole blood (250 μ L) was cultured with respective food allergenic extracts (100 μ g/mL) for 30 minutes, along with control stimulations such as anti-IgE, N-Formylmethionyl-leucyl-phenylalanine (FMLP), or medium alone. Degranulation reactions were stopped by adding 20 mM EDTA and stained for anti-CD63 and basophil-specific markers followed by flow cytometry. Basophil degranulation is measured as % fold change in CD63^{hi} cells in the basophil gate. Basophils were identified as CD123+ CD203c+ lin- (CD3, CD14, CD19, CD41) events and assessed for activation by expression of CD203c (ENPP3) and CD63 (LAMP-3), which are markers for piecemeal and classical degranulation, respectively. CD63 is a marker of degranulation by virtue of its localization on the basophil granule membrane that is fused with the plasma

membrane during classical degranulation, resulting in the appearance of a population of CD63⁺ basophils. The primary outcome of this assay is the percentage of CD63⁺ basophils after incubation with varying concentrations of allergenic food protein.

End point titration prick skin testing

Prick skin testing was performed with serial 10-fold dilutions of food allergen extract at the baseline visit and after 4 weeks of supplementation with isoflavones. The participants were off antihistamines for an appropriate length of time (5 half-lives of antihistamine) before the test. The starting concentration was the standard food extract (1:20) with serial dilutions of 1:200, 1:2,000, 1:20,000, 1:200,000 wt/vol. The endpoint is the last concentration of food extract to result in a wheal of 3 mm greater than the negative control. We defined the mean wheal diameter as the average of the largest diameter and the corresponding midpoint diameter. All wheals were outlined in ink and then transferred to the permanent record with transparent tape.

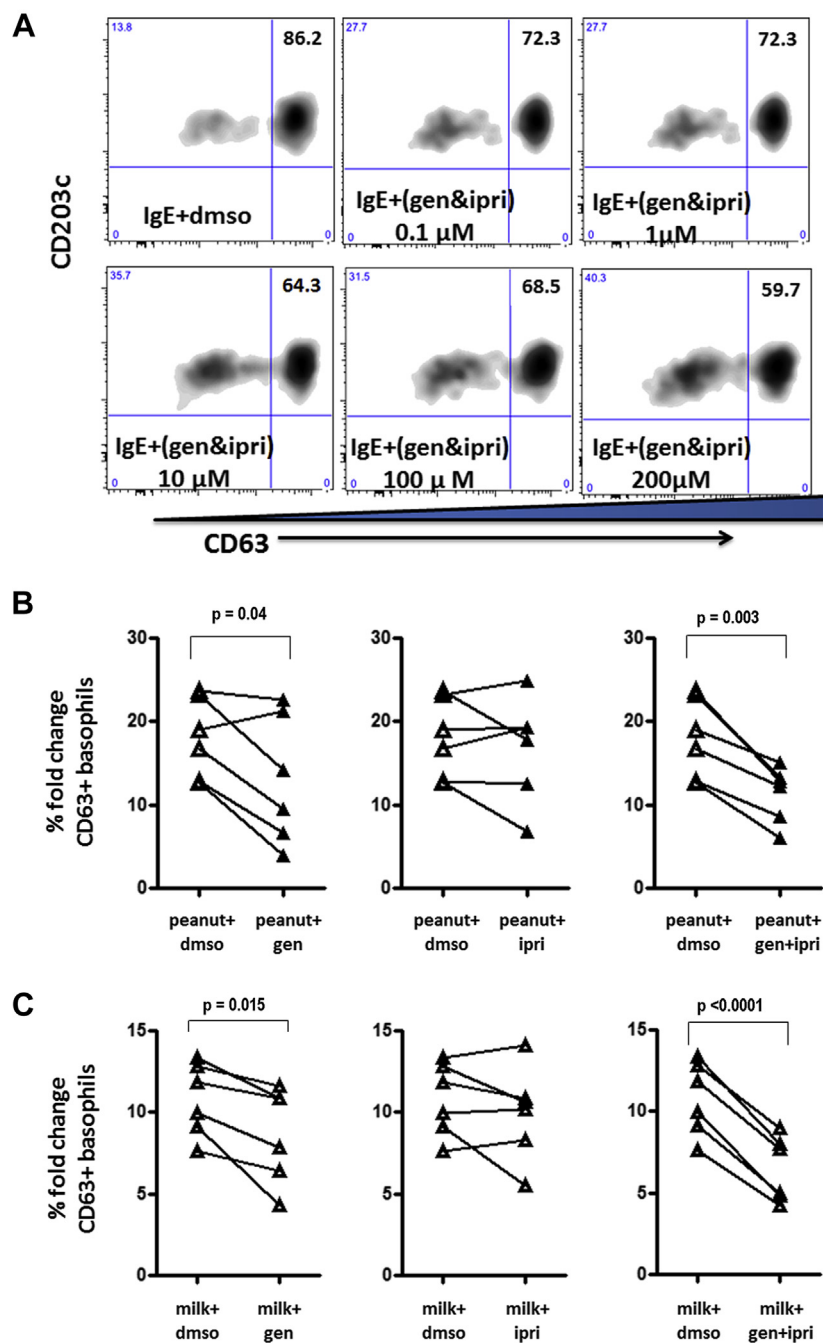


FIGURE E1. **A**, Whole blood from a healthy volunteer was preincubated with indicated concentrations of genistein+ipriflavone for 30 minutes. Basophil degranulation assays were performed by flow cytometry. The axes of the density plot indicate the mean fluorescent intensities of anti-CD63 and anti-CD203c in gated basophils respectively. CD203c is a marker of basophil activation. The expression of CD63 (a marker of intracellular vesicles) on the basophil membrane is a measure of degranulation. The numbers on the upper-right quadrant represent the percentage of degranulated basophils (CD203c⁺CD63⁺). Genistein and ipriflavone suppressed ex vivo basophil degranulation in a dose-dependent fashion. The suppression ranged from 16% for 0.1 μ M to 30% in 200 μ M isoflavone treatment. **B**, Whole blood from 6 peanut-allergic subjects was preincubated with 100 μ M genistein, ipriflavone, or genistein+ipriflavone for 30 minutes. Basophil degranulation assays were performed by flow cytometry in the presence of peanut extract. The mean % fold change of CD63 expression was reduced to 11.3 in peanut+genistein and ipriflavone treated basophils compared with 18 for peanut+dms0 alone ($P = .003$) (P values from the 2-tailed paired t -test). **C**, Basophil degranulation assay was performed in whole blood from 6 milk-allergic subjects as in (**B**), in the presence of milk extracts. The mean % fold change of CD63 expression in milk+genistein and ipriflavone was reduced to 6.43 from 10.77 for milk+dms0 alone ($P < .0001$). Whereas genistein and ipriflavone individually have a modest effect on allergen-induced basophil degranulation, the combination genistein+ipriflavone significantly suppressed basophil degranulation (**B**, **C**). *dms0*, Dimethylsulfoxide (carrier for isoflavones); *gen*, genistein; *ipri*, ipriflavone.

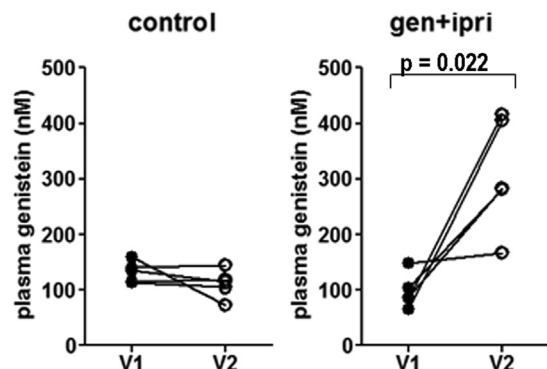


FIGURE E2. Plasma genistein estimation by HPLC. Plasma samples were mixed with equal volume of helix pomatia glucuronidase (10,000 units/mL) and incubated for 18 hours at 37 °C to convert glycosides to aglycones. The proteins were precipitated by mixing with dichloromethane, followed by extraction with acetonitrile at 50 °C. HPLC was performed using a Waters Alliance 2695 HPLC system (Waters Corp). The separation was performed on a ZORBAX SB-C18 column (Agilent Technologies). Genistein peaks are distinguished by its characteristic absorption at 254 nm, by using pure compound as control. Genistein content in plasma was estimated by calculating the area under the curves of chromatogram and extrapolated using the standard curve of pure compound. Shown here are the calculated plasma genistein levels in control and test subjects on visits 1 and 2.

TABLE E1. Clinical characteristics of the subjects

Test	Age (y)	Gender	Allergies	Specific IgE		Total IgE	
				Visit 1	Visit 2	Visit 1	Visit 2
MS02	36	Male	Peanut	17.6	N/A	94	107
MS04	31	Male	Peanut	87.6	86.4	343	432
MS05	23	Female	Cashew	0.47	0.53	116	135
			Walnut	6.86	7.61		
MS09	18	Male	Peanut	>100	>100	1317	1330
MS10	22	Male	Peanut	10.4	12.9	490	487
MS13	19	Male	Peanut	12.5	10	83	51
MS15	35	Male	Walnut	0.7	0.8	388	442
Control							
MS03	19	Male	Cashew	<0.35	0.37	62	84
			Walnut	2.63	3.17		
MS06	22	Male	Peanut	5.36	3.91	18	14
MS08	23	Male	Peanut	96.8	>100	818	1242
MS12	18	Male	Walnut	<0.35	0.39	47	79
MS14	18	Female	Peanut	>100	>100	635	677

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