

Fifth International Symposium on the Role of Soy in Preventing and Treating Chronic Disease

Poster Abstracts¹

Public health and nutritional aspects of soy

Expected Effect on the Overall Diet in the UK from Consumption of 25 g/d of Soy Protein. T. A. Carr and J. I. Harland. Nutrition Consultants, England.

The effect of consuming ~25 g/d of soy protein on the nutrient profile of the diet has been assessed by considering the nutrient profile of soy and soy products, practical examples of substituting soy products for nonsoy products, and soy intake studies. A further consideration is the cost implication of choosing 25g/d of soy protein. Soy is low in fat, in particular saturated fat; is a good source of polyunsaturated fats (linoleic and α -linolenic acid); and does not contain cholesterol. Soy is also an excellent source of protein and contains soluble dietary fiber, micronutrients, and phytochemicals. As such soy fits in well with current dietary guidelines. Practical examples in **Tables 1** and **2** show that substituting soy products for nonsoy products has a favorable effect on nutrient profile. The total amount of energy and fat is higher when semiskimmed milk, low-fat yogurt products, and very lean meat are used and the amount of protein is slightly higher. The main difference is the shift toward the unfavorable saturated fatty acids and low level of PUFA with meat and dairy products. The substitution of up to half the recommended protein intake (1) with 25 g soy protein will tend to reduce the saturated fat content of the diet. This is confirmed in data from the Oxford arm of the European Prospective Investigation into Cancer and Nutrition, where a highly significant trend for reduced saturated fat intake at higher levels of soymilk intake in both pre- and postmenopausal women was reported (2). Protein and carbohydrate intakes are largely unaffected by increased soymilk consumption. Some of soy protein items are cheaper than animal protein alternatives and although soymilk is more expensive, many of the yogurts and dessert items are comparable in cost. Overall there is potential for a healthy eating initiative that need not cost more.

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Soy Consumption of Taiwanese Children in Taipei. K. Hsiao* and P. Lyons-Wall[†]. *National Taiwan Normal University, Chung-Shan Medical University, Taiwan ROC; [†]Queensland University of Technology, Australia.

Soybeans have been a staple food in the traditional Asian cuisine for many centuries. Recent experimental evidence has shown that soy consumption could be a contributing factor to the low rates of heart disease and cancer observed in Asian communities, yet controversy remains as to whether eating soy is beneficial for children—mainly because of the presence in soy of the weakly estrogenic isoflavones. This study examined the range and frequency of intake of soy foods in Taiwanese children in Taipei aged 8–9 y. Total isoflavone intakes were estimated by using a food frequency questionnaire and compared with selected demographic variables. Children consumed a diversity of soy products (20 different items) reflecting the use of soy as a traditional staple food in the Taiwanese cuisine. The most commonly consumed items were soymilk, with a mean intake of 655 g/wk, soft tofu (207 g/wk), and firm tofu (120 g/wk). No associations were found between isoflavone intakes and demographic characteristics such as age, body mass index, and family income. The average daily intake of isoflavones in this study (39 mg/d) is comparable with intakes that have resulted in physiological effects in young women. However, no effects have been reported in children, either anecdotally or in the scientific literature. The potential estrogenic effects of isoflavones may be lowered in children because of the first-pass effect in the liver or the tissues of children may be less sensitive to the effects of isoflavones than tissues of adults.

Using Web-Based Computer Nutrition Education to Effect Attitude Toward Soy Products in an Elderly Population. J. Painter* and J. North[†]. *Eastern Illinois University, IL; [†]University of Illinois Urbana-Champaign, IL.

Soy was introduced into the human diet in China over 4000 y ago. Soy has only recently been introduced into the American diet and many find the beany flavor of soy objectionable. The problem of off-flavors in soy protein products has been a major limitation for its use and was a major topic of discussion at the first World Soy Protein Conference in 1973 and the World Conference on Vegetable Food Proteins in 1978. The perception that soy foods do not taste good is still a problem today. This study

TABLE 1

Composition of various soya products on the UK market (Source: on pack values, June 2002)

	Releat vege mince	Provamel soya fresh	M&S apricot & date cereal bars	Yofu alternative to yogurt peach	Total
Portion size	100 g	250 mL	40 g	125 g	
Energy, kcal	176	115	154	109	557
Protein—soya, g	7	9.3	6.25	4.8	27
Protein—total, g	15.5	9.3	8	4.8	37.6
Fat, g	10	5.3	3.1	2.6	21
Saturated, g	0.7	0.8	1.6	0.4	3.5
PUFA, g	3.1	3.3	0.4	1.6	8.4
Carbohydrates, g	6	7.0	23.1	16.6	53
Sugar, g	1	6.5	13.4	15.5	36
Calcium, mg	?	350	?	125	475
UK cost	22 p	32 p	50 p	32 p	136 p

assesses whether nutrition information delivered via computer can affect how elderly in the United States perceive soy. Elderly subjects (>55 y) were chosen for this study because they are at increased risk for the chronic diseases that soy consumption may ameliorate. All subjects completed a pre- and postintervention survey containing 8 soy preference questions. The survey was validated with a group of elderly people. The traditional treatment group (n = 42) received nutrition information regarding soy in a traditional lecture format. The computer group (n = 32) received the nutrition information via an interactive computer program. The interventions included 3 30-min nutrition education classes conducted over 2 wk. The topics of the classes were what soy is, the health benefits of soy, and cooking with soy. Soy products were tasted at each class. Education delivered via computer significantly ($P < 0.05$) improved the attitudes toward soy foods compared with the traditional teaching method. The negative perceptions of soy appear to be loosely held by this elderly population and are open to change. Computers may provide a cost-effective method of disseminating information regarding the health benefits, acceptability, and use of soy products.

Challenges of Assessing Soy Food and Soy Protein Consumption in European Populations. T. A. Carr* and J. I. Harland†. *T. A. Carr, Nutrition Consultant, London, UK; †J. I. Harland, Nutrition Consultant, London, UK.

Interest in soy protein (SP) intake in Western populations has increased as a result of health claims in the United Kingdom and the United States recommending 25 g/d. Quantifying soy

food and SP intake proves challenging. It is not collected specifically within national food or nutrition surveys. Some published studies have attempted to describe soy product intake; few have quantified SP intake. We assessed SP intake from market research data (MR), isoflavone content of the diet (ISO), and soy food intake from specialist studies (SF). Information relating to SP intake was identified by reviewing the literature using Medline, industry sources, and personal contacts. **Table 3** shows information relating to SP intake from various sources. In the United Kingdom, average SP intake assessed by each of these methods is 0.4, 1–2, and 0.3 (for men) and 0.14 g (for women) for MR, ISO, and SF, respectively. In groups identified as already using soy products, SP intake varies (3–25 g/d). Vegans consume the most. Vegetarians and some meat-eating postmenopausal women achieve half of the 25 g SP/d. These data are limited for the following reasons: 1) Published market research data on soy products are only available for soy dairy alternatives and soy meat replacers. No data are available for other sources of SP (i.e., soy flour, tofu, and isolated soya protein used as ingredients) and the data are only available from UK, German, and French markets. 2) Isoflavone analysis of foods is not uniform and can vary among products depending on growing season and manufacturing. 3) Soy food intake studies have limited assessment of soy food intake to a number of soy food options. 4) Nutrient composition of soy foods in diet analysis databases is limited. 5) The numbers reflect an estimate of SP intake before UK health claim 2002 but also largely reflect intake before U.S. health claim 1999. More recent intakes are likely

TABLE 2

Substituting soya products for nonsoya products results in a favorable nutrient profile

	Very lean minced meat	Semi-skimmed milk	Cereal bar	Low-fat dairy yogurt	Total
Portion size	100 g	250 ml	40 g	125 g	
Energy, kcal	177	115	187	113	592
Protein—total, g	24.7	8.3	4	5.1	42.1
Fat, g	8.7	4.0	8.9	0.9	22.5
Saturated, g	3.8	2.5	1.8	0.5	8.6
PUFA, g	0.3	0.0	2.16	0.0	2.5
Carbohydrates, g	0.0	12.5	24.2	22.4	59
Sugar, g	0.0	12.5	11.2	22.4	46
Calcium, mg	14	300	30.8	188	533
UK cost	60 p	25 p	35 p	32 p	152 p

TABLE 3

Information relating to SP intake from various sources

Study	SP intake/day	Study population
1. UK market research	0.39 g	General population UK
2a. Extrapolation from isoflavone content (European study) (1, 2)	1–2 g	General population
2b. Extrapolation from isoflavone content (European study) (3)	3–11 g	Small groups of soy food users in study
3a. Verkasalo et al. (4) using Food Frequency Questionnaire (FFQ)	25 g (F)	Vegans in study
3b. Verkasalo et al. (5) using FFQ	>12.5 g (F)	Vegetarians in study
	12.5 g (F) from soy milk alone	Vegetarian/vegans in study
	>10 g (F) from soy milk alone	11% postmenopausal non vegetarian women in study
3c. 24 h recall interview of 35,955 participants across Europe (2)	6.8 g (M)	Participants reporting soy food consumption
	2.8 g (F)	
	0.2 g	General population
	8.1 g (M)	Health-conscious participants
	4.6 g (F)	

to be higher from market growth estimates. Recommendations: To better estimate consumption of SP, the national diet and nutrition surveys across Europe should specify and assess all sources of soy food intake; soy intake studies should investigate all sources of soy intake; a validated standard food frequency should be developed (and agreed upon) to estimate soy food intake; and a standardized approach to isoflavone analysis should become uniform. This review should be repeated for the post-July 2002 period to assess SP intake post-health claim in the United Kingdom.

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Framework for the Evaluation of the Effects, Safety, and Claims of Foodstuffs Made from Plants for the Human Diet. I. Vanrullen,* J. L. Berta,* M. H. Loulergue,* R. Anton,† M. Gerber,** J. Guillemain,‡ J. L. Lamaison,†† B. Lesourd,‡‡ I. Margaritis,§ C. Moulis,§§ A. Pichard,## and D. Tomé§§§.

*Agence Française de sécurité sanitaire des aliments (AFSSA), Maisons Alfort, France; †Université Louis Pasteur, France; **Institut national de la santé et de la recherche médicale (INSERM)–CRLC, France; ‡Université François Rabelais, France; ††Faculté de Pharmacie, France; ‡‡Hopital Nord, France; §Faculté des Sciences du sport, France; §§Faculté des Sciences, Sciences Pharmaceutiques, France; §§§Institut National de l'Environnement Industriel et des Risques, France; §§§Institut National Agronomique Paris Grignon, France.

STATE OF THE PROBLEM: For the past few years, the world market for dietary supplements, especially supplements made from plants, has been booming, reaching \$44.8 billion for 2000

in American, European, and Asian markets. However, the scientific evaluation of these products has confronted many difficulties. A new directive (Directive 2002/46/EC) on dietary supplements issued by the European commission in June 2002 includes such substances as plants extracts. Recently, the French Food Safety Agency (Agence française de sécurité sanitaire des aliments) created a team to propose a framework for the evaluation of the safety effects and the claims of foodstuffs made from plants for the human diet. This framework will contribute to the review, at the European level, initiated by the directive. WORKING STEPS AND PROPOSITIONS: The first working step included data collection and analysis for 19 plants of interest to the food industry through detailed monographs compiled in collaboration with industrial partners. The second step is based on the working group's discussion and on an expert review of scientific pharmacology, nutrition, and toxicology data. Using illustrative examples, we highlight the characteristics and the specific difficulties related to this area. Indeed, data are widely based on traditional use for a number of these plants. Traditional use is of specific interest regarding issues about plants, but limitations exist (e.g., lack of history regarding emergent risks). APPLICATION: Data on traditional use data can contribute to the assessment of risk and effect associated with the consumption of these plants in a strictly defined context, which may correspond to a clear standard of claim. However, the more the product use moves away from traditional uses, the more requirements must be increased in terms of demonstrating both safety and physiological effect and, therefore, the claim being made. This evaluation framework is now being applied to plant sources of isoflavones.

Effects of Whole-Milk Intake on Protein Status of Non-Lactose-Intolerant and Nonsymptomatic Lactose-Intolerant Korean Men. S. J. Goh,* S. Kwon,*† B. R. Cha,* Y. J. Hyun,* Y.-B. Lee,† H.-S. Sohn,† C.-W. Chung,† J. H. Lee,* and Y. Jang**.*Department of Food & Nutrition, Yonsei University, Seoul; †Central Research Institute at Dr. Chung's Food Co., Ltd., Chungjoo-Si; **Cardiovascular Genome Center, Yonsei Medical University, Seoul, Korea.

Lactose intolerance (LI) is caused by either congenital or acquired lactase deficiency in the brush border of small intes-

tine. Common clinical symptoms of LI are nausea, abdominal distention, cramping, pain, and watery diarrhea, but there are lactose intolerant individuals without these common symptoms [nonsymptomatic lactose intolerance (NSLI)] who consume dairy products regularly. To investigate metabolic responses of whole-milk consumption, 21 non-LI and 15 NSLI men were recruited and given 3 servings of whole milk per d for 1 mo. Individuals whose secondary postprandial breath hydrogen is >20 ppm after consuming 50 g lactose were defined as LI. Anthropometric (i.e., weight, height), biochemical (i.e., lipid & protein profiles), antioxidative (i.e., catalase, glutathione peroxidase, superoxide dismutase) variables, and oxidative damage to DNA were measured before and after whole-milk intake. Descriptive statistics and Wilcoxon signed ranks test were applied for comparing variables in each group using SPSS for Windows. No significant difference was found in baseline value of total, HDL, and LDL cholesterol; total-to-HDL ratio; albumin; total protein; blood urea nitrogen; and creatinine between the 2 groups (Table 4). After 1 mo of the experiment, LDL cholesterol was significantly increased ($P < 0.05$) in both groups. Additionally, total and HDL cholesterol, albumin, and total protein were increased and creatinine was decreased only in the non-LI group. In conclusion, whole-milk intake may improve the protein status of individuals only if they do not have LI. No benefit to protein levels was found with NSLI individuals. Despite improved protein status for non-LI individuals, increased serum cholesterol levels concern researchers because of possible adverse effect on heart diseases.

Nutritional Changes among Premenopausal Women in a Soy Intervention. S. Acharya, S. Hebshi, A. Williams, C. Oshiro, S. Murphy, and G. Maskarinec. Cancer Research Center of Hawaii, Honolulu, HI.

This study investigated how free-living premenopausal women ($n = 206$) modified food and nutrient intake as a result of consuming soy foods in a 2-y randomized intervention. The intervention group was counseled to consume 2 daily servings of soy and the control group was counseled to maintain its regular diet. Diet was assessed with a validated food frequency questionnaire at randomization and by randomly timed unannounced 24-h recalls every 3 mo. Analyses were based on the 100 intervention and 106 control women who had a minimum of 3 24-h recalls. At randomization, both groups reported similar low mean servings of soy intake (0.15 in the control

and 0.13 in the intervention group). The 2 groups did not differ in body mass index, intake of total energy, or any of the macro- and micronutrients studied. According to the 24-h recalls, the intervention group's mean soy intake increased to 1.87 servings per day and the control group's remained at 0.25 servings per day ($P = 0.0001$). In the intervention group there were significant decreases in intake of dairy products ($P = 0.0002$), percent of energy from saturated fatty acids ($P = 0.002$) and cholesterol ($P = 0.0041$), whereas intake of protein ($P = 0.008$), dietary fiber ($P = 0.0001$), and calcium increased significantly in the control group. These results suggest that consumption of soy foods in the intervention group was associated with improved quality of diet.

Attitudes and Behaviors of Natural Foods Shoppers Versus Mainstream Shoppers Toward Soy Food Consumption. T. Schryver and C. Smith. University of Minnesota, St. Paul, Minnesota, MN.

OBJECTIVE: To examine attitudes and behaviors in soy consumption between soy foods consumers (SC) and nonconsumers (NC). **SUBJECTS:** Fifty-three participants (33 SC, 20 NC), ages 18–91 y, in a mainstream or natural foods grocery store. **VARIABLES:** Focus groups ($n = 7$) included discussions on food purchase factors, lifestyle practices, beliefs about soy foods, conversion to soy consumption, and participant's willingness to change their diets to incorporate soy foods for heart health benefits. **ANALYSIS:** Common themes were identified, coded, and compared using NVivo computer software. **RESULTS:** Barriers to consumption included soy's image problem, a lack of familiarity on how to prepare soy foods, and a perception among NC that soy foods were an inadequate taste substitute for animal-based protein and dairy milk. There was confusion among NC regarding the price, value, availability, and variety of soy foods. SC's conversion to regular consumption was generally initiated by food allergies or intolerances, adoption of a vegetarian lifestyle, or an aversion to dairy and was sustained because they enjoyed the flavor of soy. Although many participants did not know why soy was healthful, others identified it as heart healthy, a good protein, and good for women's health. Some SC had become concerned regarding the controversy surrounding breast cancer and soy consumption. Participants were asked if they would increase their soy consumption if diagnosed with heart disease. Participants agreed that if barriers were removed, they would increase

TABLE 4

Biochemical variables in non-lactose-intolerant and nonsymptomatic lactose-intolerant individuals before and after drinking 3 servings of whole milk daily for 1 mo¹

	Non-lactose intolerant ($n = 21$)			Nonsymptomatic lactose intolerant ($n = 15$)		
	Before	After	P^2	Before	After	P^2
Total cholesterol, mmol/L	5.22 ± 0.22	5.78 ± 0.31	0.018	4.85 ± 0.30	5.13 ± 0.25	0.073
HDL cholesterol, mmol/L	1.30 ± 0.05	1.43 ± 0.07	0.019	1.28 ± 0.06	1.28 ± 0.06	0.778
LDL cholesterol, mmol/L	3.25 ± 0.19	3.66 ± 0.27	0.046	2.94 ± 0.22	3.25 ± 0.15	0.036
Total/HDL ratio	4.01 ± 0.06	4.06 ± 0.10	0.000	3.80 ± 0.07	4.00 ± 0.04	0.001
Albumin, g/L	52.0 ± 0.75	54.3 ± 1.39	0.029	52.9 ± 1.29	52.8 ± 0.57	0.665
Total protein, g/L	79.8 ± 1.54	83.5 ± 2.27	0.026	81.0 ± 2.31	80.7 ± 0.77	0.878
BUN, mg/dL	8.60 ± 0.72	6.70 ± 0.67	0.092	10.2 ± 0.77	9.55 ± 0.74	0.306
Creatinine, μmol/L	50.9 ± 3.85	35.8 ± 3.73	0.012	57.2 ± 5.09	57.2 ± 3.85	1.000

¹ Values are expressed as means ± SE.

² Statistical significance was tested by Wilcoxon signed ranks test for each group. BUN, blood urea nitrogen.

consumption of soy foods. Suggestions were made as to how to increase overall consumption. **CONCLUSIONS:** Improving soy's image and educating consumers on soy foods could increase soy consumption.

Effect of processing on soy and soy food composition

Development of Soy Dishes Culturally Acceptable to Mississippians. J. Huam,* G. T. Bates,* and W. L. Dodson.†
*Alcorn State University, Alcorn State, MS; †Mississippi State University, MS.

The purpose of this research is to develop soy recipes to enhance the use of soy foods that will be culturally acceptable by Mississippians. Soybeans and soybean products are being used extensively by the food industry primarily for their functional characteristics. Although there has been a marked increase in the use of traditional soy food both as a substitute for animal protein and in new food, most Mississippians are still reluctant to incorporate soy foods into their daily diet. Soy foods have gained popularity because of the health benefits associated with their consumption; however, low acceptance of soy foods in the past was partly due to inadequate information on the uses and preparation of soy products and information on its nutritive value. Twenty soy dishes were evaluated by 37–45 sensory panelists from the university who used a 9-point hedonic scale. Average score for each soy dish was tabulated to determine acceptability and ranking. The 3 most favored soy dishes were Peach and Nutty Soy Pudding, Zesty Soy Chili, and Spicy Soybean Soup, which had a mean score of 8.53, 8.32, and 8.20, respectively. Soysational Shake was the least-favored dish with a mean score of 6.70. Compared with the most favored dish, which was rated 9 (like extremely) by 62.2% of the panelists for appearance, 59.5% for flavor, and 56.8% for texture, only 23.8%, 19.0%, and 21.4% of the panelists rated 9 for appearance, flavor, texture, respectively, for the Soysational Shake. This research suggests that the normal resistance to soy foods can be overcome by providing Mississippians with pertinent information and ways to incorporate the maximum nutritional benefits of the product into their daily diet.

Effects of Genotype, Environment and Hydrothermal Treatments on Isoflavone Concentration In Soybean Grains. M. C. Carrão-Panizzi,* K. Kitamura,† A. Kikuchi, A. Simão,‡ S. P. Favoni-Goés,†† and J. M. Mandarino.‡**
*Embrapa Labex-NCAUR, USDA, ARS, Peoria, IL; †Graduate School of Agriculture, Hokkaido University, Japan; **National Agricultural Research Center for Western Region, Japan; ‡Embrapa Soybean, Brazil; ††State University of Campinas, Brazil.

Genetic variability, environmental effects, and processing methodologies affect isoflavone concentrations in soybean grains and food products. Brazilian soybean genotypes were observed to have a significant variability in isoflavone content. Cultivar IAC 100, an insect-resistant cultivar, showed the highest concentration of isoflavones when sown in 2 different locations of Paraná State, Brazil, in 1991, 1992, and 1994. In the same experiments, cultivar BR-36 had the lowest isoflavone content. Growth location and year affect isoflavone concentration. The local average temperature during seed planting is a determinant in isoflavone development. Higher temperatures decrease isoflavone content. Environmental effects are particularly important for the Brazilian soybean pro-

duction region, which includes latitudes ranging from 33°S to the equator. Warm weather in Brazil has decreased the average of isoflavone content of Brazilian soybean cultivars, but specific locations where average local temperatures are lower favor development of isoflavones. In Vacaria (28°30'S latitude), where the average temperature was 19°C, the cultivars FT-Abyara and IAS-5 had 164 and 219 mg isoflavones/100 g, respectively, whereas in Palotina (24°27'S latitude), where the average temperature was 24°C, isoflavone concentrations decreased to 87 and 196 mg/100 g, respectively. To increase the formation of isoflavone aglycones, the most bioavailable compounds, in grain from the soybean cultivars BR 36, FEPAGRO RS-10, and BRS 155, cultivated in 3 locations of Paraná State, Brazil (Londrina, Capanema, and Palmas), in 2000, received hydrothermal treatment at 40, 50, and 60°C for 12 and 18 h. In Palmas (19°C), there was a higher concentration (280 mg/100 g) of total isoflavones than in Londrina (23°C and 140 mg/100 g). The cultivar BRS 155 had the highest content of total isoflavones in Palmas and in Londrina. Nontreated grains of BRS 155 showed an average 4.0 mg aglycones/100 g, which increased to 52 mg/100 g after hydrothermal treatment. Treatment at 50°C for 12 h was the most effective in developing isoflavone aglycones. At 60°C there was a decrease of the aglycones. Malonyl forms, which are thermally unstable, were reduced at higher temperatures.

Effects of Processing on Soy Phytochemicals. C. Wang. Department of NFSH, South Dakota State University, Brookings, SD.

Phytochemicals are defined as nonnutritive biologically active compounds from plants. Soybean has many unique phytochemicals. They include isoflavones, saponins, phytates, phytoosterols, phenolic acids, and trypsin inhibitors. Before the 1990s, some of these phytochemicals were studied as antinutritional factors or even as toxic compounds. However, recent biomedical research has shown that consumption of these phytochemicals might prevent chronic diseases such as cardiovascular disease, cancer, and diabetes. Soybeans are rarely consumed raw and often are processed extensively before consumption. Soybeans are normally processed into soybean oil and protein products. Soy oil is the major vegetable oil consumed in the United States and the world. Soy flour, soy protein concentrate, and soy protein isolate are 3 major soy protein products. Soybeans are also processed into many traditional Asian foods as such soymilk, tofu, miso, and tempeh. Therefore, to harness the benefits of phytochemicals, it is critical to know what happens to phytochemicals during processing. This presentation will summarize the current knowledge on the effects of varied processes on isoflavones, saponins, phytates, and phytoosterols. Some recent findings in the author's laboratory will also be included.

The Effect of Bread Making on Isoflavone Content and Composition in Soy Bread. Y. C. Zhang, S. J. Schwartz, and Y. Vodovotz. The Ohio State University, Columbus, OH.

Isoflavones in soy have been associated with reduced risk of chronic diseases. A highly acceptable soy bread was developed in our laboratory that may serve as a delivery system for isoflavones. Little work has been done on understanding the changes in isoflavones during baking. The objective of this study was to investigate the effect of bread making on the content and composition of isoflavones in soy bread. A soy bread was formulated with soy ingredients (30%). Mixed

dough was proofed at 27°C for 2 h and baked at 165°C for 50 min. The isoflavone content and composition in dough before and after proofing and in the crumb and crust after baking were analyzed using reversed-phase HPLC coupled with a UV spectrophotometer. The total isoflavone content in dough and soy bread was unchanged (3.2 $\mu\text{mol/g}$, dry basis) but their conjugating patterns were altered. Before proofing, dough contained predominantly malonylglucosides (49.1%) and β -glucosides (42.4%) of isoflavones. After proofing, the β -glucosides in fermented dough decreased (\sim -21%) with an increase in aglycones (\sim +20%) and the amount of malonylglucosides remained unchanged. After baking, the malonylglucosides in bread crumb decreased (\sim -15%) with an increase in the β -glucosides (\sim +11%). A slight increase in aglycones of isoflavones (\sim 2%) was also observed. Bread crust contained the lowest amount of malonylglucosides (8.2%) and highest amount of acetylglucosides (20.7%) of isoflavones among all bread samples. The aglycone content in bread crumb and crust were similar (25.6% and 28.0%, respectively). The increase in isoflavone aglycones occurred mainly during proofing (fermentation) as their β -glucosides decreased. Malonyl and acetylglucosides of isoflavones were stable through yeast fermentation. Heat treatment during baking was responsible for the decarboxylation and deesterification of malonylglucosides to form β -glucosides and acetylglucosides. In conclusion, isoflavones are stable through bread making but the distribution of isoflavone derivatives varies.

Effect of Genetics and Environmental Conditions on the Concentration of Soybean Seed Lectins. E. De Mejia,* M. Vasconez,* and R. Nelson[†]. *Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL; [†]USDA-ARS, U.S.

There is an increased interest in the potential health benefits of bioactive proteins from soybeans. Lectins are glycoproteins present in soybeans that selectively bind carbohydrates and are used in medicine in a variety of novel applications. The objective of this research was to determine the lectin concentration and physicochemical characteristics of 144 selected and diverse soybean accessions from the USDA Soybean Germplasm Collection, grown under different experimental conditions. Enzyme-linked immunosorbent assay and gel electrophoresis were used to determine lectin concentration in defatted samples. Lectin concentration ranged from 1.1 to 14.5 mg/g of extracted protein. Because samples were grown at 3 different locations, it was not possible to separate the environmental from the genetic effects. However, >100% differences were observed among accessions of similar maturity grown under the same environment, indicating that major genetic differences do exist. The mean of 23 major ancestral lines of U.S. cultivars was not different from the mean of 16 modern cultivars selected to represent the current diversity of the crop. The highest concentration of lectin was found in the exotic accessions. The lectin concentration was not related to maturity group except that many of the highest concentrations were observed in maturity groups IX and X grown in a subtropical environment. These results show that diversity for lectin concentration does exist within commercial soybean cultivars and that even greater diversity exists within the *Glycine max* species. Thus it is feasible that the levels of this important bioactive protein can be genetically manipulated.

The Effects of Soy-Rich Diet on Lipid Profiles and Lipid Peroxidation in Patients with Systemic Lupus Erythemato-

sus (SLE). D. Warodomwicht,* S. Songchitsomboon,[†] D. Danboonchan,[†] S. Janwityanujit,* J. H. Hong,** and S. Komindr*. *Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [†]Division of Nutritional and Biochemical Medicine, Research Center Mahidol University, Bangkok, Thailand; **School of Food and Life Science, Inje University, Korea.

BACKGROUND. SLE patients have inflammatory events early in the disease, and late in the disease significant morbidity and mortality occur as a result of kidney failure and accelerated vascular disease with heart attack, strokes, and other atherogenic complications. A high prevalence of coronary heart disease occurs in SLE patients, and cohort studies suggested that hyperlipidemia is a risk factor of coronary heart disease in SLE patients. Interest in soy has grown because of its protective effects on cardiovascular disease by lowering blood cholesterol levels. **OBJECTIVE:** To determine whether a soy-rich diet compared with a control diet in female SLE patients is associated with the reduction in both lipid profile and lipid peroxidation. **METHODS:** Eighteen female SLE patients (aged 39.3 ± 11.6 y) in an inactive phase were studied. After a 4-wk run-in period to assess baseline dietary intakes and laboratory values, subjects were assigned to receive a soy-rich ($n = 11$) or control diet ($n = 7$). The subjects were instructed to substitute 25 g soy protein for the same amount of animal protein in their baseline diet for 12 wk under the supervision of a dietitian. Fasting blood samples, blood pressure (3 times by the same person), body weight, height, and body fat were obtained. **RESULTS:** There were no significant differences between groups at baseline. Compared with baseline, no significant reduction in total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, body weight, or blood pressure occurred in either group. Serum plasma glucose concentration was reduced in both groups but the reduction was significant in the control group. **CONCLUSIONS:** This is the first dietary intervention in Thai SLE patients. Because of small sample size, it is not possible to conclude that 25 g soy protein has no benefit on serum lipid reduction in Thai SLE patients. Further study of this effect is needed.

Chemical composition and biological properties of soy constituents

A Novel Extract of Fermented Soy Germ Inhibits Lipopolysaccharide-Induced Tumor Necrosis Factor- α Production In Vitro and In Vivo. W. Pan, G. Blackburn, and J.-R. Zhou. Beth Israel Deaconess Medical Center, Boston, MA.

Tumor necrosis factor (TNF)- α is a potent inflammatory cytokine involved in many pathophysiological conditions including inflammation arthritis. The present study was to determine the effect of a novel extract from soy germs, AglyMax, on inhibiting lipopolysaccharide (LPS)-induced TNF- α production. AglyMax contains 70% soy isoflavone aglycones (70% daidzein, 10% genistein, and 20% glycitein). In the in vitro studies, murine macrophage cells were plated, incubated over night, washed with PBS, and pretreated for 90 min with AglyMax (isoflavones aglycones at 0.1–100 $\mu\text{mol/L}$). Cells were then treated with LPS (40 $\mu\text{g/mL}$) and incubated for 4 h. The supernatants were collected and TNF- α was measured by enzyme-linked immunosorbent assay. AglyMax inhibited LPS-induced TNF- α production by up to 43.7% in a dose-dependent manner. In the animal study, BALB/c mice aged 5–6 wk were randomly divided into 7 groups ($n = 10$); treated for 5 d

with AglyMax, genistein, daidzein, or a genistein-daidzein combination; and challenged with LPS (40 $\mu\text{g}/\text{mouse}$) or vehicle. Blood samples were collected 90 min after LPS challenge, and TNF- α levels were measured. Mice treated with AglyMax at 100–200 mg/kg body weight showed up to a 51.2% reduction of TNF- α production in a dose-dependent manner. AglyMax was more effective than genistein, daidzein, or the genistein-daidzein combination. The results suggest that AglyMax may be used as an anti-inflammation agent.

Enhancing Health-Related Cyclitols in Soy Products. R. L. Obendorf, S. Odorcic, and C. I. Gomes. Cornell University, Seed Biology, Ithaca, NY.

The potential for D-chiro-inositol and D-pinitol to lower blood glucose has stimulated interest in the enhancement of these cyclitols in soy products. *myo*-Inositol is present in all living cells. D-chiro-inositol and D-pinitol are biosynthesized in leaves and transported to seeds where they are stored as their α -galactoside derivatives, fagopyritol B1 and galactopinitols, respectively. This poster reviews recent evidence for the biosynthesis, transport, and storage of health-related cyclitols in soybean and summarizes evidence for enhancing their accumulation in seeds. Pinitol is biosynthesized by conversion of *myo*-inositol to D-ononitol to D-pinitol. Biosynthesis of D-pinitol in leaf tissues is enhanced by water-deficit stress. D-Pinitol accumulates in leaves at all plant growth stages, with highest concentrations in younger leaves near the top of the soybean plant. To date no evidence exists for the biosynthesis of D-pinitol in embryo tissues. D-Pinitol is biosynthesized in maternal (leaf) tissues, transported to seeds, unloaded by seed coats, taken up by embryo tissues, and converted to galactopinitols in the embryo. D-chiro-Inositol follows a similar pattern. *myo*-Inositol is biosynthesized in embryo tissues but is also transported from maternal tissues. In embryo tissues, galactinol synthase converts *myo*-inositol to galactinol that serves as galactosyl donor in the biosynthesis of the raffinose series oligosaccharides. Feeding *myo*-inositol to isolated embryos or leaf-stem-pod explants does not markedly increase accumulation of galactinol, probably because of the transformation of *myo*-inositol to other products, including phytin and cell walls. By contrast, feeding D-pinitol or D-chiro-inositol to isolated embryos or leaf-stem-pod explants markedly increased the accumulation of their α -galactosides in soybean embryo tissues. These observations suggest that upregulating the biosynthesis of these health-related cyclitols may increase their concentrations in soy products.

Chemical Composition and Nutritional Value of Traditional Soy milk. J. L. Peñalvo,*[†] M. C. Matallana,[†] and M. E. Torija.[†] *Institute for Preventive Medicine, Nutrition and Cancer, Folkhälsan Research Center, and Division of Clinical Chemistry, University of Helsinki, Finland; [†]Dpto. Nutrición y Bromatología II: Bromatología. Facultad de Farmacia. Universidad Complutense de Madrid, Spain.

Soybean is a very rich source of essential nutrients and one of the most versatile foodstuffs as shown by the many traditional soy foods that have constituted part of oriental diets for centuries. The most important traditional soy food is soymilk, a water extract of soybeans closely resembling dairy milk in composition and physical appearance. It is important to introduce and to improve the consumption of soy and related products in populations where the supply of nutrients is not sufficient, not just as an alternative to animal protein foods but

as a normal component of the diet. Traditional soymilk is the first option for introducing soy into the diet of new consumers. Because of its mild taste and physical resemblance to animal milk, it can be consumed directly, as intermediate for preparing other soy foods, or as an ingredient in other meals, always maintaining the balanced nutritional pattern of soybean (1,2). The study had 2 goals: to develop a simple method to elaborate soymilk and to characterize its chemical composition and nutritional value. The elaboration process was optimized and a product was obtained that had the highest nutritional and did not include the trypsin inhibitory fraction, the most important antinutritional factor present in nonfermented soy-based foods (3). Standardization of the resulting soymilk was needed in order to have a repeatable production scheme; to that end, different soybean varieties were processed with the mentioned method and the resulting soymilks were analyzed to determine composition, fiber content, and mineral content. The presence of antinutrients such as trypsin inhibitor, phytic acid, and oxalic acid; nonnutritive substances such as oligosaccharides (i.e., raffinose and stachyose); and isoflavones was evaluated. Special attention was paid to the behavior of the mentioned compounds during the elaboration process of soymilk.

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Isoflavone Characterization and Antioxidant Activities of Ohio Soybeans. J. H. Lee,* M. Renita,* S. St. Martin,[†] S. J. Schwartz,* and Y. Vodovotz*. *The Ohio State University/Department of Food Science and Technology; [†]The Ohio State University, Department of Horticulture and Crop Science, Columbus, OH.

Consumption of soybeans and soy products has been associated with reducing the risks of various cancers and inflammatory disease, which may be attributed in part to the presence of isoflavones. Compared with Asian diets, the Western-style diet lacks acceptable products containing large amounts of soy. One strategy to increase the use of soy in this population is to incorporate soy-based ingredients into traditional products such as bread. Systematic characterization of isoflavones and antioxidant activity in soybean will help in selecting varieties with optimal health-promoting effects that can be ultimately used in the manufacturing of bakery products. Isoflavone content in 17 soybean varieties developed and grown in Ohio was determined by a combination of C18 reversed-phased high performance liquid chromatography coupled with a photodiode array detector, and UV-Visible spectrophotometry. Antioxidant activities of soybean extracts were measured using 2,2-diphenyl-1-picryl-hydrazil (DPPH) free radical and a photochemiluminescence (PCL) method. The highest and lowest total isoflavone content in soybean varieties were 304.7 and 111.0 mg/100 g soy, respectively, whereas the average content was 186.3 mg/100 g soy. Antioxidant activities of soybean extracts ranged from 7.51 to 12.18 μmol butylated hydroxytoluene equivalent/g soy using a DPPH method and from 24.00 to 44.40 nmol Trolox equivalent/g soy using a PCL method. No significant correlations between isoflavone content and antioxidant activity were found, suggesting that at least part of the antioxidant activity of soybean extracts may be due to other compounds in the extract.

Isoflavone Concentration in Soy Extracts (Soymilk) Obtained from Soybean Grains, Full Fat Soy Flour, and Isolated Soy Protein. R. da Rodrigues,* M. C. Carrão-Panizzi,[†] and R.H. Moretti**. *Federal University of Pelotas, Food Science Department, Brazil; [†]Embrapa Labex-EUA-USDA, ARS, NCAUR, Peoria, IL; **State University of Campinas, Food Technology Department, Brazil.

Isoflavones have been related to several beneficial effects in humans, such as prevention of cancer, cardiovascular diseases, osteoporosis, and menopause symptoms. These substances occur mostly in soybeans, where their concentrations depend on genetics and on climatic and environmental conditions. In soy products the occurrence and retention of isoflavones vary according to the applied technological processes. Soy extract (soymilk) is the most popular soy-based product for human consumption. It is usually obtained from soybeans by specific technological process or directly by dissolution in water of full-fat soy flour or isolated soy protein. By knowing the isoflavone concentration in the soy extracts, it is possible to estimate the isoflavone intake from these products. Three types of soy extracts with 3% protein were obtained: 1) de-hulled soybean grains were soaked, hot ground, and cooked and the residue separated the residue; 2) full-fat soy flour was dissolved in water; and 3) isolated soy protein was dissolved in water. Isoflavones were determined by high performance liquid chromatography and UV detection, according to Kudou et al. (1). Results for the extracts from soybean grains, full-fat soy flour, and isolated soy protein (HO159/PTI, with 3.4 mg total isoflavones per gram of protein) were, respectively, 225.6, 242.1, and 269.1 mg total isoflavones per 100 g of dried matter and 66:31:3, 62:28:10, and 76:17:7 for the fractions of β -glycosides:malonylglycosides:aglycones. On the basis of these data, it can be concluded that soy extracts are an important source of isoflavones in a diet. Consumption of 250 mL soy extract corresponds to the minimum intake of 23.2 mg isoflavones. (Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo.)

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Lignan Content of Soy-Based Health Supplements. J. L. Peñalvo, S. M. Heinonen, T. Nurmi, and H. Adlercreutz. Institute for Preventive Medicine, Nutrition and Cancer, Folkhälsan Research Center, and Division of Clinical Chemistry, University of Helsinki, Finland.

Lignans, together with isoflavones, are considered to be the representatives of the phytoestrogens. The mammalian lignans enterolactone and enterodiol are metabolically formed by the human intestinal microflora from dietary precursors (1) and constitute an intensively studied group of compounds because of their role in cancer and cardiovascular disease prevention (2,3). The soybean is the richest source of isoflavones, but in addition to these well-known compounds, it also contains plant lignans as a minor fraction (4). Soy-based health supplements appeared in the Western market after the health claims from soy consumption became known, in response to the increase in consumer's health concern. These products were frequently formulated as isoflavone isolates and later as protein isolates after the observation that soy protein was required for the protective effect of isoflavones. The isoflavone content of health supplements has been documented to some extent (5,6) but no study of the plant lignan concentrations in

these products has been presented so far. In this study, levels of plant lignans, secoisolariciresinol, matairesinol, isolariciresinol, pinoresinol, lariciresinol, and syringaresinol were analyzed by gas chromatography–mass spectrometry in 14 commercially available soy-based health supplements. Results show considerable amounts of plant lignans in all the samples, varying from 0.58 to 326 $\mu\text{g/g}$.

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Total Isoflavone Content in Selected European Soy Products. J. L. Peñalvo, T. Nurmi, and H. Adlercreutz. Institute for Preventive Medicine, Nutrition and Cancer, Folkhälsan Research Center, and Division of Clinical Chemistry, University of Helsinki, Finland.

In the past decade many studies have tried to demonstrate the relation between soy consumption and the prevention of various chronic diseases in humans. Soy-rich diets in general or isoflavones in particular have been associated with lower incidence of atherosclerosis, cancer, hypertension, and osteoporosis as well as the alleviation of menopausal symptoms (1–3). As a result of these investigations, the soy-processing industry has grown from the manufacture of alternative dairy products and milk-free infant formulas to a wider range of products embracing all types of tastes and consumer health concerns. The study of the phytoestrogenic action of soy requires a full identification of the total isoflavone content in soy-derived products. Altogether 64 commercially available soy products manufactured in Europe, including health supplements, industrial fortifying agents, dietary supplements, and foods, were analyzed with HPLC using coulometric electrode array detection (4) to characterize their total isoflavone content. Concentrations varied widely among the different groups. Industrial fortifying agents and health supplements contained the highest amounts of total isoflavones (1.20–266 mg/g) and were followed by the dietary supplements used as sports diet enhancers (0.1–0.3 mg/g). The largest variation was found in the soy foods group, with values of 0.003–0.855 mg/g.

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Convenient Synthesis of Isoflavone Calycosin and its Metabolites. Antti Hoikkala and K. Wähälä. Laboratory of Organic Chemistry, University of Helsinki, Finland.

Isoflavonoids are present in various legumes, especially in soy and clover. However, relatively little is known about the metabolism of these compounds in humans, which is of great interest because the activity of the compounds is dramatically altered with alteration of their structure. Verification of previously unknown metabolites requires the synthesis of authentic compounds to be used as standards. Calycosin has been found in different clover varieties and as a metabolic product of daidzein (1,2). We now report the synthesis of the isoflavone calycosin and its possible mammalian metabolites. Analysis of these metabolites by HPLC–time-of-flight mass spectrometry in human samples is underway.

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Cellular and molecular actions of soy—effects on receptors, growth factors, and genes

Nutrigenomic Profiling of Soy Isoflavones: A Comparison of the Effects of Genistein and Daidzein on a Human Gut Epithelial Cell Line Transcriptome. P. Gillies,* H. Smith,[†] B. van Ommen,* and R. Stierum*. *DuPont Nutrition & Health, Newark, DE; [†]Solae, U.S.; **TNO Nutrition and Food Research, The Netherlands.

Gene expression profiling within a bioinformatics platform is a powerful tool with which to explore the molecular effects of nutrients and bioactive compounds in target cells. This nutrigenomic approach offers a comprehensive, albeit temporally defined, survey of genes and biochemical pathways subject to modulation via nutritional bioactive compounds. The information from such studies generates molecular hypotheses to explain recognized or to posit new health benefits. In the studies to be reported, the effects of genistein and daidzein were independently examined in Caco-2 cells, a model of human gut epithelium. Cells were incubated with the test isoflavone at concentrations of 10 and 100 $\mu\text{mol/L}$ for 12 h, RNA was extracted via Trizol/RNeasy procedures, and the transcriptomes were analyzed by using a 16,000-gene oligonucleotide based-array from Operon that included annotated gene sequences. Principal component analysis of mean-centered data provided a global overview of the effects of genistein and daidzein on gene expression. Although the transcriptomes for cells incubated with genistein at 10 and 100 $\mu\text{mol/L}$ were spatially displaced from untreated controls and each other, the transcriptomes for cells incubated with daidzein at 10 and 100 $\mu\text{mol/L}$ overlapped with each other and those of the controls. Univariate analyses of the data identified statistically significant changes in a subset of genes within the isoflavone-modulated transcriptomes. At a concentration of 10 $\mu\text{mol/L}$, genistein and daidzein affected 53 and 27 genes, respectively. Although there was a degree of synchrony in the response of genes to these isoflavones, there were also independent and in some instances oppositional responses. Gene mapping of pathways related to cell cycle and apoptosis suggest the data may be germane to the cancer chemopreventative actions ascribed to isoflavones. The data also underscore the importance of evaluating the collective effects of naturally occurring mixture

isoflavones before ascribing health benefits to specific components therein.

Effects of Soybean Isoflavones on the Expression of Genes Associated with Transforming Growth Factor- β Sensitivity in Cultured Human Breast Cancer Cell Lines. Y.-W. Lee,* K. S. Jin,* Y. H. Kim,* J. S. Kim,** J. I. Kim,[†] and J. C. Hong*. *Department of Biomedical Laboratory Science at Inje University, Gyeongsangnamdo, Korea; [†]Department of Food and Life Science at Inje University, Korea; **Department of Animal Science and Biotechnology at Kyungpook National University, Korea.

It is well known that genistein and daidzein, the 2 major isoflavones in soybean, have a potential anticancer activity through their antiproliferating mechanism. Because transforming growth factor (TGF)- β 1 has been found to have an inhibitory effect on the growth of mammary epithelial cells, the effects of genistein and daidzein on the expressions of TGF- β 1 and its receptor genes, such as TGF- β R1 and TGF- β R2, were investigated by using Northern blot analysis in human breast carcinoma epithelial cell lines—an estrogen receptor (ER)-positive cell line (MCF-7) and an ER-negative cell line (MDA-MB-231)—to see whether the antiproliferating mechanism for their potential anticancer activity is mediated by the changes in the expression of the genes associated with TGF- β sensitivity. In one study, genistein and daidzein were shown to stimulate the expression of TGF- β 1 slightly in both types of cells with different effective doses. Interestingly, daidzein could downregulate the expression of the TGF- β 1 at lower doses when the cells were stimulated to be more proliferative. These results suggested that the extent of TGF- β 1 expression corresponded to their inhibitory effect on cell growth and that the antiproliferative effect of the soy isoflavones could be mediated by the expression of the TGF- β 1 gene. In another study, little or no expression of the TGF- β receptors was found in both cell types, suggesting refractory properties of the cells to the growth inhibitory effect of the TGF- β 1. However, genistein has been shown to stimulate the expression of TGF- β R1 earlier than TGF- β R2 in the MCF-7 (ER+) cells. In addition, the MCF-7 (ER+) cells were more sensitive to genistein than were the MDA-MB-231 (ER-) cells in terms of induction of the receptor genes. Daidzein can also induce the expression of both receptors but less effectively than genistein. These results suggested that the isoflavones may exert antiproliferation activity by inducing the expression of TGF- β receptors in the breast cancer cell lines. Taken together, the soy isoflavones genistein and daidzein may exert their anticancer activity through the reexpression of the TGF- β receptor genes rather than enhancing the TGF- β 1 expression, thereby TGF- β sensitivity could be restored, which in turn inhibits cell proliferation. (Supported by the Korea Science and Engineering Foundation grant R01-2000-000-00187-0.)

Antihormonal Effects of the Soybean Phytoalexin Glyceollin. S. Boue,* M. Burow,[†] B. Shih,* C. Carter-Wientjes,* and T. Cleveland*. *Southern Regional Research Center, USDA, ARS, New Orleans, LA; [†]Tulane University Medical Center, Tulane University School of Medicine, New Orleans, LA.

Soy isoflavones have been well characterized for their estrogenic activities as well as their effects on human health and disease. The types and amounts of these compounds in soy and

other plants are controlled by both constitutive expression and stress-induced biosynthesis. The aim of this study was to identify and characterize unique soy phytochemicals that had not been previously assessed for estrogenic or antiestrogenic activity. Here we describe increased biosynthesis of the isoflavonoid phytoalexin compounds, glyceollins, in soy plants grown under stressed conditions. In contrast to the observed estrogenic effects of coumestrol, daidzein, and genistein, we observed a marked antiestrogenic effect of glyceollins on estrogen receptor (ER) signaling, which correlated with a comparable suppression of 17β -estradiol-induced proliferation in MCF-7 cells. Further evaluation revealed greater antagonism towards ER- α than ER- β in transiently transfected HEK 293 cells. Competition binding assays revealed a greater affinity of glyceollins for ER- α versus ER- β that correlated to greater suppression of ER- α signaling with higher concentrations of glyceollins. Recent *in vitro* data will be presented and the preparation an animal diet enriched with glyceollins for *in vivo* studies will be discussed. In summary, the glyceollins exhibited unique antagonistic effects on ER in both HEK 293 and MCF-7 cells.

Dynamic Profiling of Sex Steroid and Epithelial Growth Factor-Signaling Pathway in Rat Uterus by Genistein. M. S. Cotroneo, W. A. Fritz, and C. A. Lamartiniere. University of Alabama at Birmingham, AL.

The Asian diet, high in soy products, is associated with reduced incidence of hormone-dependent cancers, including endometrial cancer. The primary isoflavone component of soy is genistein, for which we have investigated the time course expression of regulating sex steroid receptor expression and the epidermal growth factor (EGF) signaling pathway in the rat uterus. Female Sprague Dawley rats (aged 21 d) were killed 0, 2, 4, 8, 16, 24, and 48 h after 1 injection of 500 μ g genistein/g body weight (BW); 500 ng estradiol benzoate (EB)/g BW; or the vehicle, dimethylsulfoxide (DMSO). Estrogen receptor (ER)- α decreased after treatment with genistein or EB within 2 h, returning to basal levels within 24 and 48 h, respectively. Subsequently, progesterone receptor was upregulated at 24 and 48 h. This is consistent with the idea of progesterone receptor being a late gene product, indicative of cell differentiation. EGF-receptor (EGFR) expression peaked 16 h after genistein or EB treatment, inversely correlating with extracellular-regulated kinase (ERK) phosphorylation. The upregulation of EGFR in the uterus is contrary to *in vitro* reports of genistein being an inhibitor of protein tyrosine kinases but consistent with our previous demonstration that genistein initially upregulates EGFR expression in the breast. The percentage of phosphorylated EGFR, relative to total EGFR, and phosphorylation of the downstream kinases Raf-1 and MAP kinase/ERK-activating kinases 1 and 2 were reduced, corresponding to decreased phospho-ERK immunolabeling in the stroma, suggesting signal attenuation, and to a signal for cell differentiation. This demonstrates that protein phosphorylation events are more important than receptor protein levels. At 16 h we observed increased uterine weight, epithelial cell height, and cell proliferation in the stroma and epithelium. These effects were inhibited by pretreatment with the antiestrogen, ICI 182,780, suggesting a requirement for ER. Pharmacokinetics analysis showed peak concentrations of genistein and EB at 5.41 and 0.92 h, respectively. These data suggest similar mechanisms of action for genistein and EB in induction of uterine proliferation but with 1/1000 potency. (Supported

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Preparation of Phytoestrogen Disulfates—Potential Sulfatase Inhibitors. K. Wähälä and O. Soidinsalo. Laboratory of Organic Chemistry, University of Helsinki, Finland.

Isoflavonoids are a major group of dietary phytoestrogens possessing a wide variety of biological activities. Recent studies—animal and human—have suggested that a high consumption of phytoestrogens may reduce the risk of breast cancer. The protective effects of phytoestrogens may be due to their role in regulating the synthesis, metabolism, and signal transduction of steroid hormones. The predominant forms of these isoflavonoids and their metabolites in human urine and plasma are the glucuronide, sulfate, and sulfoglucuronide conjugates. Such conjugations make phenolic compounds water soluble and may result in the loss of their biological activity and toxicity. However, it has been shown that daidzein sulfoconjugates are potent inhibitors of the sterol sulfatase enzyme of the peripheral sterol sulfatase pathway, preventing the production of biologically active estrogenic steroids from their inactive sulfoconjugates in mammary tissue (1). We report here an expedient preparation method for disulfates of soy isoflavones and their mammalian metabolites. The new products will be useful as reference compounds in analytical studies of the metabolism, bioavailability, biological activity, and enzyme inhibition of phytoestrogenic isoflavones and their metabolites in humans.

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Soy and prostate cancer

The Specific Role of Isoflavones in Reducing Prostate Cancer Risk. N.B.K. Kumar, K. Allen, D. Riccardi, J. PowSang, J. Seigne, M. Helal, R. Salup, K. Besterman-Dahan & A. Cantor. H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL.

AIM: To evaluate the effectiveness of supplementing a group of early-stage prostate cancer patients with 60 mg soy isoflavones to produce changes in hormonal and proliferative risk variables that are implicated in prostate cancer promotion. **METHODS:** Seventy-six eligible prostate cancer patients with a Gleason score of 6 or below, aged 50 and 80 y, were admitted and supplemented with soy isoflavones (60 mg/d) for 12 wk. Changes in proliferative and hormonal biomarkers were analyzed at baseline and postintervention. **RESULTS:** Of the 76 subjects, 59 completed the 12-wk intervention. Serum free testosterone was reduced or showed no change from baseline to the end of the study in 61% of the subjects in the isoflavone group compared with 33% in the placebo group. Serum total prostate-specific antigen (PSA) decreased or was unchanged in 69% of the subjects in the isoflavone group compared with 55% in the placebo group. However, we did not see an increase in levels of sex hormone binding globulin. Nineteen percent of subjects receiving soy isoflavones reduced total PSA by 2 points or more during the intervention period. **CONCLUSIONS:** Although not statistically significant, these data suggest that surrogate markers of proliferation such as serum total PSA and free testosterone are reduced in a larger number of subjects in the isoflavone-supplemented group compared with the group receiving placebo even in a study of short

duration (12 wk). Thus prolonged consistent isoflavone use may interfere with disease progression or delay the onset of histologic disease.

Clinical Trial of Soy Isoflavone Supplementation Before Radical Prostatectomy in Patients with Localized Prostate Cancer. O. Kucuk,^{*†} F. Sarkar,^{*†} W. Sakr,^{*†} D. Wood,^{**} M. Cher,^{*†} J. Abrams,^{*†} D. D. Doerge,[‡] M. M. Pollak,^{††} Z. Djuric,^{*†} and A. Majumdar^{*†}. *Karmanos Cancer Institute, Detroit, MI; †Wayne State University, Detroit, MI; **University of Michigan, MI; ‡National Toxicological Research Center, FDA, U.S.; ††McGill University, Canada.

Epidemiologic studies have shown an inverse association between dietary intake of soy products and prostate cancer (PCa) risk. We previously observed that soy isoflavone supplements stabilize disease in patients with advanced prostate cancer. To determine the effects of soy isoflavones on newly diagnosed organ-confined prostate cancer, we conducted a small randomized, placebo-controlled clinical trial. Our goal was to use surrogate endpoint biomarkers to investigate the biological effects of soy isoflavone supplementation in patients with localized prostate cancer. Twenty-eight men with newly diagnosed prostate cancer were randomly assigned to receive tablets (Novasoy) containing 200 mg soy isoflavone ($n = 17$) or no supplementation ($n = 11$) for 3 wk before radical prostatectomy. Biomarkers of cell proliferation and apoptosis were assessed by Western blot analysis in benign and cancerous prostate tissues. We assessed oxidative stress markers by measuring levels of peripheral blood lymphocyte DNA oxidation products and plasma 8-isoprostane. Usual dietary intake of nutrients was assessed by a food frequency questionnaire at baseline. Prostatectomy specimens were evaluated for pathologic stage, Gleason score, volume of cancer, and extent of high-grade prostatic intraepithelial neoplasia (HGPIN). Plasma levels of soy isoflavones, insulin-like growth factor-1, insulin-like growth factor binding protein-3, and prostate specific antigen were measured at baseline and after 3 wk of supplementation or observation. There were no significant differences between the intervention and placebo groups with regard to tumor size (82% vs. 70%, <4 cc, $P = 0.64$), involvement of surgical margins, extraprostatic tissues with cancer (50% vs. 33% organ-confined disease, $P = 0.46$), Gleason scores (75% vs 50%, Gleason > 7 , $P = 0.24$), or diffuse involvement of the prostate by HGPIN (94% vs 92%). The data from this small pilot study suggest that soy isoflavones may have different biological effects on different variables. Analysis of clinical and biomarker studies will be presented.

Genistein Differentially Regulates Prostate-Enriched Protein Expression in Prostate Cancer Cells. B. Lazarevic,^{*} ** S. Karlsen,^{*} O. Kucuk,[†] and F. Saatcioglu^{**}. *Aker Hospital, Oslo, Norway; †Wayne State University, Detroit, MI; **University of Oslo, Norway.

Genistein is the most abundant phytoestrogen component of soybeans. It has been implicated in the prevention of hormone-sensitive cancers, including the breast and prostate, because of its various biological activities, such as inhibition of tyrosine kinases and topoisomerases, androgen- and estrogen-receptor binding, and antioxidant properties. Previous studies on the effects of genistein on prostate cancer cells showed that it inhibits the secreted and intracellular levels of the androgen-regulated protein prostate-specific antigen (PSA). Here, we have assessed whether genistein has similar effects on other

prostate-enriched or androgen-regulated genes. Similar to findings with PSA, genistein decreased the levels of kallikrein 4 (KLK4) and NKX3.1 proteins, both of which are androgen-regulated genes highly enriched in prostate expression, in LNCaP prostate cancer cells. In contrast, the expression of the 6 transmembrane proteins of prostate 1 (STAMP1), a highly prostate-enriched protein likely to be involved in endocytic and exocytic pathways, was upregulated in a dose-dependent manner by genistein in LNCaP cells. These data suggest that genistein has variable effects on expression of prostate-enriched proteins. Studies are in progress to dissect the molecular mechanisms that are involved in the effects of genistein on prostate-enriched gene expression and the biological consequences in human prostate cancer.

Soy Isoflavones and Lycopene in the Treatment of Hormone-Sensitive and Hormone-Refractory Prostate Cancer. U. VU Vaishampayan,^{*†} J. J. Forman,^{*†} M. Hussain,^{**} M. Cher,^{*†} E. Pontes,^{*†} J. Fontana,^{*†} K. C. Alluri,^{*} D. Doerge,[‡] F. Sarkar,^{*†} and O. Kucuk^{*†}. *Karmanos Cancer Institute, Detroit, MI; †Wayne State University, Detroit, MI; **University of Michigan, MI; ‡National Toxicological Research Center, FDA, U.S.

Genistein induces apoptosis and inhibits the growth of androgen-sensitive (LNCaP) and androgen-independent (PC3 and VeCaP) prostate cancer cell lines. We conducted a phase II randomized clinical trial to determine the efficacy of lycopene alone or in combination with soy isoflavones in patients with prostate cancer. Eligible patients had to have rising serum prostate-specific antigen (PSA) either after local therapy (group I, hormone sensitive) or while on hormone therapy (group II, hormone resistant). Seventy-one patients had 3 successive rising PSA levels or a PSA value of >10 $\mu\text{g/L}$ at 2 successive evaluations before starting therapy, which consisted of 40 mg soy isoflavone mixture (Solgen) or the same mixture plus 15 mg lycopene (Lyc-O-Mato) twice daily orally for a minimum of 3 mo in the absence of progression. Blood samples were obtained for insulin-like growth factor (IGF)-1, IGF binding protein-3, leukotriene B-4, 8-oxoguanine, 8-isoprostane, nuclear factor- κB , genistein, daidzein, PSA, testosterone levels, and toxicities were assessed monthly. Sixty-one patients were assessable for response, 41 patients in group I and 20 in group II. There were no partial or complete responses. However, most patients achieved disease stabilization similarly in both lycopene-alone (28/34, 82%) and soy-plus-lycopene (20/27, 74%) groups. Hormone-sensitive patients were more responsive to either lycopene (18/21, 86%) or the combination of soy and lycopene (16/20, 80%). In a previous study using soy isoflavones (200 mg/d, Novasoy) we observed 83% and 32% disease stabilization in hormone-sensitive and hormone-refractory groups of patients, respectively. The data suggest that soy isoflavones and lycopene have activity in prostate cancer, particularly in patients with hormone-sensitive disease. Supplementation with lycopene, alone or in combination with soy isoflavones, stabilizes both hormone-refractory and hormone-sensitive prostate cancer. Lycopene (10/13, 76% disease stabilization) appears to be more active than soy isoflavones (4/7, 57% disease stabilization) in hormone-refractory disease.

Potential of Radiation-Induced Cell Killing by Genistein in Prostate Cancer Cells In Vitro. G. G. Hillman, O. Kucuk, M. C. Joiner, B. Marples, Y. Wang, J. L. Wright, K. L. Ellis, M. Yudelev, J. D. Forman, and F. H. Sarkar. Wayne State University, Karmanos Cancer Institute, Detroit, MI.

We showed that genistein, the major isoflavone in soybean, inhibited the growth of human prostate cancer (PCa) cells in vitro by affecting the cell cycle and inducing apoptosis. To augment the effect of radiation for prostate carcinoma, we have investigated the combination of genistein with photon and neutron radiation on PC-3 human PCa cells in vitro. Genistein, at 15 $\mu\text{mol/L}$, caused a significant inhibition in DNA synthesis, cell growth, and colony formation in the range of 40–60%. Pretreatment of cells for 1–2 d with genistein at 15 $\mu\text{mol/L}$ potentiated the effect of low doses of photons (2–3 Gy) or neutrons (1–1.5 Gy) radiation, causing up to 80–95% inhibition in colony formation compared with 50% with radiation alone. This effect was highly reproducible and was more significant if cells were first treated with genistein for a day before irradiation compared with the reverse sequence of cells irradiated first and then treated with genistein. The effectiveness of genistein combined with radiation depended on the continuous exposure of the cells to genistein before and after radiation. The potentiation of radiation cell killing by genistein was also observed with human breast and renal cancer cell lines, suggesting that this effect is not restricted to PC-3 cells and can be applied to other cancer types. Investigating the molecular events by which genistein potentiates radiation-induced cell killing in PC-3 cells, we found that genistein decreases nuclear factor- κB (NF- κB) activity whereas photon radiation induces activation of NF- κB , an early event detectable 30 min postirradiation. Radiation-induced activation of NF- κB activity is completely inhibited by pretreatment of cells with genistein. These data confirm that radiation induces NF- κB activation, and that pretreatment of cells with genistein abrogates this activation. These findings support our hypothesis that treatment of the cells with genistein will block radiation-induced NF- κB activation, leading to a cascade of molecular events driving the cells to an apoptotic pathway and, thus, increasing cell killing.

A Randomized, Controlled 6-Mo Intervention with Soy Protein Isolate in Men with Biochemical Recurrence after Radical Prostatectomy. M. C. Bosland, A. Zeleniuch-Jacquotte, J. Melamed, H. Lepor, S. S. Taneja, J. Schmoll, H. Watanabe, B. Levinson, C. Randolph, and P. D. Walden. NYU School of Medicine, New York, NY.

Several lines of evidence suggest that soy may prevent prostate cancer and may be beneficial for men with prostate cancer. We examined the effect of soy protein on the rise in prostate-specific antigen (PSA) serum levels in men who developed detectable PSA (0.12–1.34 $\mu\text{g/L}$ at baseline) after radical prostatectomy. These men initially had undetectable PSA levels (PSA < 0.1 $\mu\text{g/L}$) and recurred within 3 y after surgery. Sixteen men were randomly assigned to receive soy protein or placebo (Solae-Dupont Protein Technologies) as a daily beverage powder supplement given for 6 mo. One-third of these men were African American. The placebo was a casein-based preparation and both preparations were identical in composition except for the source of protein and soy-specific compounds. They provided 20 g/d of protein and the soy protein supplement contained ~24–26 mg/d of genistein and ~40–43 mg/d of total isoflavones. There was no apparent effect of soy intervention on serum PSA levels, which increased by 28% and 14% in the soy and placebo groups, respectively; this difference was not statistically significant. Although this study is too small to draw definitive conclusions, this finding is in line with other reports of a lack of effects of soy protein on serum PSA in men without detectable prostate

cancer (1,2). However, there was a significant reduction in serum cholesterol over 6 mo in men on soy intervention (–13%) compared with the placebo group (–2%) even though the sample size was small ($P = 0.0482$, 2-sided Mann-Whitney test). This finding is consistent with several reports that soy protein consumption can lower serum cholesterol (2). Self-reported compliance was excellent and there were no adverse events. Serum genistein levels (an independent measure of compliance) increased considerably from baseline ($P = 0.0012$) in the soy group but were not changed in the placebo group. Although these results do not support the notion of that soy consumption is of benefit to men with prostate cancer, they do not exclude the possibility that soy prevents prostate cancer. (Supported by NIH Grants No. CA27790 and M01 RR00096.)

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Soy Consumption, Soy Metabolism, and the Potential Prevention of Prostate Cancer in Caucasian Men. T. Hedlund,* P. Maroni,* P. Ferucci,* R. Dayton,* S. Barnes,† K. Jones,† R. Moore,† Kristiina Wähälä,** L. Ogden,* H. Sackett,* and K. Gray*. *University of Colorado Health Sciences Center, Denver, CO; †University of Alabama at Birmingham, AL; **University of Helsinki, Helsinki, Finland.

The intestinal metabolism of the soy isoflavone daidzein varies among people. Several lines of evidence suggest that the metabolite equol is among the most potent for preventing prostate cancer, although only a minority of Caucasians can produce it. The goals of the current study were 1) to determine whether equol production in Caucasians is linked to the long-term consumption of high amounts of soy and 2) to determine whether prostatic concentrations of 5 isoflavonoids typically exceed serum concentrations in Caucasians consuming soy. Healthy Caucasian men aged 19–65 (some of whom were Seventh-Day Adventists) were recruited from the Denver vicinity. Participants completed a dietary survey estimating their consumption of 34 different soy foods, meat, dairy, and other products. Serum and prostate fluid levels of isoflavonoids were quantitated by HPLC-electrospray ionization-mass spectrometry in 25 high soy consumers and 20 low soy consumers before and after soy consumption. After soy, daidzein was metabolized to *O*-desmethyngolensin in 98% of the men, dihydrodaidzein in 89%, and equol in 20%. Statistical analyses revealed that men who had consumed at least 50 mg/d of isoflavones ≥ 2 y were 4.4-fold more likely to produce equol than men who consumed <50 mg/d ($P = 0.017$). Although vegetarianism was more common among high than low soy consumers, 7 of 9 men who produced equol ate meat a few times per week. Equol production was not correlated with age or the consumption of yogurt, dairy, fruit, or American-style fast food. Daidzein, dihydrodaidzein, *O*-desmethyngolensin, and equol (but not genistein) were present at significantly higher levels in prostate fluid than in serum of most men (median prostate concentrations was 6–13-fold higher). In many cases these concentrations were sufficient to significantly inhibit the growth of prostatic epithelial cells in vitro, suggesting a role not only for equol in preventing prostate cancer but also for several isoflavonoids. In conclusion, these data

indicate that equol production is linked to long-term, high-soy consumption in Caucasians. Although equol may be a potent metabolite of daidzein, it also appears to be a biomarker for people who sustain high total isoflavonoid levels, which may synergistically prevent disease.

Soy and breast cancer

Interactions of Genistein and the Innate Immune System in Breast Cancer. T. D'Alessandro, R. Moore, and S. Barnes. University of Alabama at Birmingham, AL.

During inflammatory responses, activated neutrophils and macrophages are recruited to sites of injury where they produce reactive nitrogen and oxygen species (RNOS). Diets enriched with soy have been associated with a reduced incidence of proinflammatory diseases. Isoflavones (e.g., genistein) found in foods have been implicated in this process because their phenolic group reacts with RNOS; we showed previously that nitration and chlorination of genistein occurs *in vitro*. It has been suggested that some incidences of breast cancer are the result of an inflammatory challenge early in life. This initial event is propagated by later events giving an end result of cancerous lesions. Our aim is to study the interactions of 1) inflammatory cells of the innate immune system and genistein to determine genistein's effect on oxidant production in localized environments and 2) the aforementioned system in the presence of breast epithelial cells to determine whether genistein and its metabolites play a role in modulating breast cancer formation and progression. Studies using human polymorphonucleocytes have provided insight into the formation of chlorinated and nitrated genistein metabolites upon activation with a phorbol ester. The generation of chlorinated products is dependent on phorbol 12-myristate 13-acetate whereas nitrated products need the presence of an NO \cdot generating system such as sodium nitrate (NaNO $_2$). Ongoing studies are investigating the interactions of genistein with macrophages. The focus of these studies is genistein's interaction with the NO \cdot generating system iNOS. Other experiments are being conducted using coculture techniques involving the mouse macrophage cell line RAW 264.7 and either the breast epithelial cell line MCF-7 or MCF-10A. This study focuses on cellular interactions during oxidative stress, and genistein's ability to modulate breast cancer status.

Modulation of Protein Expression Pattern by Isoflavones in Mammary Tumor Cells. J.-S. Kim,* J. S. Lee,* M. K. Sung,[†] Y. K. Kim,** R. N. Yu,[‡] and J.-H. Kim*. *Kyungpook National University, Daegu, Korea; [†]Sookmyung Women's University, Korea; **Kookmin University, Korea; [‡]Ulsan University, Korea.

Soy isoflavones have drawn much attention as potential chemopreventive agents. Although the mode of action of isoflavones has been extensively studied, their precise mechanism for cancer prevention has remained elusive. To address the possible anticarcinogenic mechanism of isoflavones, we examined the protein expression pattern in breast cancer cells incubated with and without isoflavones using 2-dimensional gel electrophoresis, MALDI-TOF, and an NCBI database search. Genistein modulated the expression of several heat shock proteins whereas daidzein did not have a significant effect. Genistein increased the expression of HSP-70, Lasp-1 protein, and triosephosphate isomerase 1 but downregulated uracil DNA glycosylase in MDA-MB-

231 cells. In particular, the posttranslational modification of hsp70/90 appeared to be inhibited by genistein in MCF-7 cells. The hsp70/90 was shifted to higher pI by genistein treatment without a change in the amount of protein. Previous studies suggested that the inhibition of hsp70/90 function led to the degradation of proteins that require this chaperone for conformational maturation, a retinoblastoma-dependent G1 block in cancer cells accompanied by differentiation, and apoptosis. In MCF-7 cells, genistein inhibited the phosphorylation of proteins with a tyrosine residue, such as BiP protein and actin. Meanwhile, the transient knock-out of hsp70 via RNA interference led to the significant decrease in the number of MDA-MB-231 cells, suggesting that isoflavones may exert anticarcinogenic action by regulating the level of intracellular heat shock protein. In conclusion, isoflavones may exert an anticarcinogenic effect by modulating posttranslational modification, expression of heat shock proteins, or both. (Supported by the Korea Science and Engineering Foundation grant R01-2001-00231.)

Phytoestrogens Inhibit Invasiveness of MDA-MB-231 Breast Cancer Cells *In Vitro*. P. J. Magee, H. McGlynn, and I. Rowland. Northern Ireland Centre for Food and Health (NICHE), School of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland.

Metastasis is a major cause of morbidity and mortality in breast cancer. Tumor cell invasion is a crucial step in the metastatic process. It has been proposed that dietary phytoestrogens (including those found in soy) are responsible for the low incidence of breast cancer in Eastern populations such as China and Japan. The aim of this study was to investigate the effects of a panel of phytoestrogens, including isoflavones, lignans, and coumestans, on the invasion of breast cancer cells (MDA-MB-231 and MCF-7) and the noncancerous breast cell line MCF-10A through Matrigel. MCF-7 and MCF-10A cells were not invasive through Matrigel and therefore the effect of phytoestrogens on these cells was not investigated. In comparison MDA-MB-231 cells were highly invasive. The isoflavones genistein, glycitein, daidzein, equol, and O-desmethylandrogensin (O-DMA) and the coumestan coumestrol induced a significant, dose-dependent, inhibition of MDA-MB-231 cell invasion through Matrigel. Most of the isoflavones and coumestrol inhibited invasion by ~30% when present at a concentration of 10 μ mol/L (e.g., O-DMA inhibited invasion by $29.3 \pm 10.3\%$, $P = 0.04$). Inhibition of invasion was apparent with all compounds, even at 2.5 μ mol/L, with O-DMA inhibiting invasion by 31% ($P = 0.032$). At 50 μ mol/L, genistein and coumestrol exerted the most potent inhibitory effects on invasion [decreases of $60.2 \pm 28.5\%$ ($P = 0.00$) and $55.5 \pm 21.1\%$ ($P = 0.001$), respectively]. The effects of the phytoestrogen metabolites equol and O-DMA on invasion were not significantly different from that of daidzein, their parent compound ($P = 0.379$, 0.241 vs. daidzein, respectively). Of the 4 lignans tested only secoisolaricresinol and its metabolite enterodiol induced a significant decrease in cell invasion ($23.7 \pm 12.8\%$, $P = 0.04$; $27.4 \pm 23.5\%$, $P = 0.05$, respectively), but this only occurred at the highest concentration tested (50 μ mol/L). The potent inhibitory effect on MDA-MB-231 cell invasion induced by the isoflavones and coumestrol was not due to cell killing or growth inhibition because cell viability was unaffected (MTT assay). These findings highlight the possible chemopreventive effects of these compounds.

Genistein and Tamoxifen Interaction in MCF10DCIS.com Xenograft Model for Chemoprevention. F. R. Miller,^{*†} L. R. Tait,^{*†} D. Doerge,^{**} F. Sarkar,^{*†} and O. Kucuk^{*†}. *Karmanos Cancer Institute, Detroit, MI; [†]Wayne State University, Detroit, MI; ^{**}National Toxicological Research Center, FDA, U.S.

To validate a preclinical model to assess the activity of chemopreventive agents by using surrogate endpoint biomarkers (SEBs) in ductal carcinoma in situ, we used a xenograft model of human comedo ductal carcinoma in situ recently developed in our laboratory, MCF10DCIS.com. Using our model, we demonstrated a response to tamoxifen, an agent with proven chemopreventive activity in human breast cancer. Growth of MCF10DCIS.com xenografts was inhibited by tamoxifen at 1000 $\mu\text{g/g}$ diet (0.1%). In addition, progression appeared to be inhibited by tamoxifen as evidenced by retention of basement membrane, determined immunohistochemically with collagen type IV and laminin specific antibodies. Genistein (G4660, Solae) at 1000 $\mu\text{g/g}$ in AIN-76A diet achieved serum genistein levels of 1 $\mu\text{mol/L}$ but did not affect growth of MCF10DCIS.com xenografts. The combination of tamoxifen and genistein 4660 (each at 1000 $\mu\text{g/g}$) inhibited growth of the xenografts better than tamoxifen alone but the difference was not significant. This is important in light of a recent report in which genistein abrogated the inhibitory effect of tamoxifen on growth of MCF7 (1). Two SEBs, which were differentially altered by genistein (G4660) and tamoxifen, were matrix metalloproteinase 9 (MMP9) and transforming growth factor (TGF)- β . MMP9 expression by epithelial MCF10DCIS.com cells was decreased by G4660 and increased by tamoxifen. TGF- β was expressed only by stromal cells in xenografts of mice fed control (AIN-76A) diets. G4660 abrogated the expression of TGF- β in the xenograft stroma. In contrast, the epithelial cells in MCF10DCIS.com xenografts from mice fed tamoxifen expressed TGF- β .

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Genistein and Tamoxifen Synergistically Inhibit the Growth of HER2-Overexpressing Human Breast Cancer Cells In Vitro. Z. Mai and J.-R. Zhou. Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.

Although tamoxifen (TAM) is the front-line agent for the treatment and prevention of estrogen receptor-positive (ER+) breast tumors, nearly 40% of estrogen-dependent breast tumors do not respond to TAM treatment. Moreover, the positive response is usually of short duration, and most tumors eventually develop TAM resistance. Overexpression of the *HER2* gene has been associated with advanced and TAM-insensitive or TAM-refractory breast tumor. More effective treatment is needed to enhance the efficacy of TAM treatment. We studied the effects of the soy isoflavone genistein and TAM combination on the growth of the *HER2*-overexpressing BT-474 breast cancer cell line in vitro. Cells were treated with genistein (0–25 $\mu\text{mol/L}$) and TAM (0–5 $\mu\text{mol/L}$), alone and in combination, and the cell growth rate was determined by cell counting. Compared with ER+ MCF-7 cells, BT-474 cells were less sensitive to TAM treatment. On the other hand, BT-474 cells were more sensitive to genistein treatment than were MCF-7 cells. The combinations of genistein and TAM at several concentrations synergistically inhibited the growth of BT-474 cells. Studies using another *HER2*-overexpressing human breast cancer cell line SK-Br-3

showed similar results. Our studies suggest that the soy isoflavone genistein and TAM combination may be used as an effective regimen in the prevention and treatment of *HER2*-overexpressing breast cancer. Further in vivo efficacy evaluation and mechanistic elucidation are warranted. (Supported in part by grant NCCAM/NIH AT00863.)

Paclitaxel-Induced Growth Arrest in Breast Cancer Is Enhanced by Genistein. J. A. Schwartz,^{*†} O. Kucuk,^{*†} and B. S. Gerard[†]. *Karmanos Cancer Institute, Detroit, MI; [†]Wayne State University, Detroit, MI.

To better understand the consequences of long-term soy isoflavone exposure on the molecular signaling events associated with breast cancer, we developed an in vitro cell line model. As part of this model, sublines of the human MCF7 and MCF10AT breast cancer cell lines were generated by chronic exposure to low concentrations of genistein (10 nmol/L). During the characterization of these sublines (designated MCF7G and MCF10ATG), stable changes in cell phenotype conferring altered growth properties and morphologic characteristics were observed. These changes were associated with increased sensitivity to treatment with paclitaxel, a drug commonly used for breast cancer chemotherapy. Specifically, paclitaxel-induced growth inhibition, apoptosis, and G2/M arrest in MCF7G and MCF10ATG cells were twofold greater than those observed in the parental cell lines. Moreover, the changes detected appeared to be stable subline characteristics, as demonstrated by their persistence after removal of genistein from the media. Genistein potentiated the paclitaxel-induced G2/M arrest. In cells that were treated with genistein and paclitaxel, genistein pretreatment potentiated the inhibitory effects on the same parameters in both the subline and parental cells, exceeding the responses to treatment with either agent alone. If the sequence was reversed and genistein treatment followed that of paclitaxel, the G2/M fraction became diminished and very little apoptosis could be detected. One explanation for these findings is that when genistein precedes paclitaxel it leads to S phase entry and paclitaxel-induced arrest. When genistein follows paclitaxel, it promotes G2/M escape and reduces apoptosis. We are also evaluating the effects of genistein on the reduction of paclitaxel resistance. (Supported by NIH Grant DK 54937 to JAS.)

An In Vitro Model of Chronic Soy Isoflavone Exposure. J. A. Schwartz,^{*†} O. Kucuk,^{*†} B. S. Gerard,[†] T. Siramanne,[†] and B. Dryer[†]. *Karmanos Cancer Institute, Detroit, MI; [†]Wayne State University, Detroit, MI.

Tumor cell responses to soy isoflavones have been widely investigated. However, most of the information comes from short-term exposure studies. Little is known about the molecular changes associated with chronic exposure to these agents. Therefore, we have developed an in vitro breast cancer cell line model of long-term genistein exposure. Using the human MCF7 breast cancer cell line, we have established variant sublines by continuous exposure to genistein at 10 nmol/L, 17 β -estradiol at 10 pmol/L, and 4-hydroxytamoxifen at 0.1 $\mu\text{mol/L}$ or charcoal-stripped serum (devoid of hormone) for up to 2 y. The respective sublines, referred to as MCF7G, MCF7+, MCF74OHT, and MCF7HWD cells, have been characterized for a variety of parameters including DNA and ligand binding, transactivation, cell growth, gene and protein expression, and cell cycle distribution. Estrogen receptor- α expression and functional activity have been confirmed for all

of the sublines tested. Differences in estrogen receptor–dependent transactivation, gene expression, cell cycle distribution, morphologic characteristics, and proliferation rates were detected among the sublines. The MCF7G subline had the greatest divergence from the parental cell line. Chronic exposure to genistein caused stable changes in the phenotype of these cells. These changes include enlargement in cell size, the acquisition of serum independence, novel gene expression patterns, karyotype alterations, and increased sensitivity to antitumor agents, which persist even after genistein removal. The results suggest that continuous exposure to low concentrations of genistein may have significant consequences for breast cancer cells in culture. We are investigating the molecular basis for these findings using a series of cell lines and culture conditions.

Breast Enhanced Scintigraphy Test Demonstrates Improvement in Breast Disease After Daily Consumption of Soy Protein. R. M. Fleming, Camelot Foundation, Omaha, NE.

BACKGROUND: Differences in breast tissue can be determined by using breast enhanced scintigraphy test (BEST) imaging. Little work in vivo has been done to determine the effects of soy protein on breast tissue. Our earlier work demonstrated a reduction in inflammatory changes in breast tissue. This work was conducted to examine the effect of daily soy protein consumption on a larger group of women over 1 y. **METHODS:** Sixty-four premenopausal women were studied after initial BEST imaging evaluation revealed fibrocystic changes of the breast. Women were asked to consume a medical-grade soy protein daily, making no other dietary or lifestyle changes during that time. Each woman underwent BEST imaging 1 y later and the results were compared with the initial findings. **RESULTS:** Women and their primary care providers reported a subjective reduction in both breast tenderness and fibrocystic disease. There was a nonstatistical reduction in both the average and maximal breast activity after 1 y of daily soy consumption. There was a statistically significant reduction ($P < 0.01$) in variability of tissue activity after 1 y of soy protein treatment. **CONCLUSIONS:** This is the first in vivo study of effect of soy protein on breast tissue health. The findings are promising and are consistent both objectively and subjectively with a reduction in fibrocystic disease of the breast. Further research is needed to confirm these findings in greater numbers of women and to determine whether soy protein has the same beneficial effect in atypia and breast cancer.

Clinical Trial of Soy Isoflavones Before Breast Cancer Surgery. O. Kucuk,*† F. Sarkar,*† V. Adsay,*† L. Newman,** D. Bouwman,*† Z. Djuric,*† D. Doerge,‡ R. Parchment,*† M. Banerjee,*† and A. Majumdar*†. *Karmanos Cancer Institute, Detroit, MI; †Wayne State University, Detroit, MI; **University of Michigan, MI; ‡National Toxicological Research Center, FDA, U.S.

Dietary intake of soy products has been associated with reduced breast cancer risk. We conducted a randomized placebo-controlled clinical trial of soy isoflavone supplementation to investigate the in vivo effects of soy isoflavones on human breast tissues obtained from lumpectomy and mastectomy specimens. We determined the effects of increased tissue concentration of isoflavones for 3 wk on breast cell proliferation, differentiation, and cell cycle regulatory proteins. Patients with ductal carcinoma in situ or invasive breast cancer were

randomly assigned to take 100 mg soy isoflavone (Novasoy) or placebo daily for 3 wk before surgery. Plasma isoflavone levels were measured in both groups at baseline and after 3 wk. Tissue isoflavone levels were measured on samples from benign breast tissues in both groups. Biomarker studies were performed on surgical specimens by immunohistochemistry and Western blot. There were no differences between the groups with respect to serum levels of 8-isoprostane, a marker of oxidative stress. We also observed no significant differences between the 2 groups in the levels of nuclear factor- κ B activation in peripheral blood lymphocytes. Likewise, immunohistochemical assessment of Akt, epidermal growth factor receptor (EGFR), and HER2-neu expression revealed no significant differences between the groups. Evaluation of the expression of EGFR-related protein, focal adhesion kinase, and angiogenesis markers are being conducted. Ki-67 levels are being measured to evaluate cell proliferation and the TUNEL assay is being used to evaluate apoptosis.

Effect of Soy Isoflavones and Metabolites on Cell Survival in Various Breast Cancer Cell Lines. C. C. Flanagan, P. J. Magee, and I. R. Rowland. Northern Ireland Centre for Diet and Food (NICHE), University of Ulster at Coleraine, Northern Ireland, UK.

Epidemiological studies indicate that the consumption of soy products is associated with a lower risk of breast cancer. Despite the favorable association found between soy and risk of breast cancer, the preventive action of isoflavones in breast cancer needs further investigation. In the present study the effects of the isoflavones genistein and daidzein and the daidzein metabolites equol and O-desmethylangolensin (O-DMA) on cell survival were studied in 2 breast cancer cell lines: MCF-7 [estrogen receptor (ER)- α and ER- β positive] and MDA-MB-231 (ER- β positive). Cells were exposed to the compounds at concentrations of 0, 2.5, 10, and 50 μ mol/L in media containing phenol red (PR) and phenol red–free media (PRF) and the effect of the compounds on cell viability was assessed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. PR appears to stimulate the growth of MCF-7 cells; survival was statistically significant compared with cells tested under PRF conditions ($P < 0.001$). No significant differences were found between PR and PRF growth of MDA-MB-231 cells ($P > 0.05$). Genistein, daidzein, and O-DMA have no statistically significant effect on the growth of either cell line under PR or PRF conditions. Equol had a stimulatory effect on MCF-7 cell growth in PR media at high concentrations ($P < 0.001$) (Fig. 1). Equol also had a stimulatory effect on MDA-MB-231 cell growth in PRF media at high concentrations (50 μ mol/L) but this was not statisti-

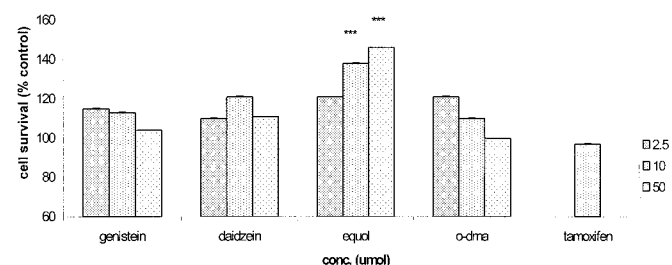


FIGURE 1 Equol significantly increased cell survival in MCF-7 cells under PR conditions. Results expressed as means of independent experiments (4 replicates) \pm SEM.

cally significant ($P > 0.05$). In conclusion, estrogenic compounds in PR medium may stimulate growth of MCF-7 cells. Tamoxifen appears to affect MCF-7 and MDA-MB-231 cells differently. The different effects of daidzein and its metabolite equol in MCF-7 cells suggest that gut microflora metabolism is important in the isoflavones effects and that individual variation in equol production may be biologically significant.

Effects of Soy Protein and Probiotic Consumption on Plasma Isoflavones in Postmenopausal Women with and without a History of Breast Cancer. J. A. Nettleton,* J. A. Greany,* H. Adlercreutz,** K. E. Wangen,* W. Thomas,[†] and M. Kurzer*. *Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN; [†]Department of Biostatistics, University of Minnesota, MN; **Biomedicum Helsinki, Folkhälsan Research Center, Finland.

Soy isoflavones are primarily metabolized by intestinal bacteria. The daidzein metabolite equol has been associated with lower breast cancer risk. Probiotic supplementation has been shown to alter fecal bacteria and enzymes, suggesting that it may be possible to affect isoflavone metabolism and equol production. We hypothesized that consumption of soy with probiotics would increase plasma isoflavone concentrations and equol producer frequency and that effects would differ between breast cancer survivors and women with no cancer history. To test this, 20 postmenopausal breast cancer survivors and 20 control subjects completed 4 6-wk diet periods in a randomized, crossover design: soy protein (S; 26.6 ± 4.5 g/d of protein, 44.4 ± 7.5 mg/d of isoflavones), soy plus probiotics (SP; 10^9 colony-forming units/d of *Lactobacillus acidophilus* DDS + 1 and *Bifidobacterium longum*, 15–30 mg/d of fructooligosaccharide), milk protein (26.6 ± 4.5 g/d of protein), and milk plus probiotics. Plasma genistein, daidzein, equol, and O-desmethylangolensin (O-DMA) were measured by time-resolved fluoroimmunoassay. Soy consumption significantly increased plasma isoflavone concentrations ($P < 0.0001$). Plasma isoflavone concentrations of controls and survivors were not significantly different, although genistein tended to be lower ($P = 0.16$) and O-DMA tended to be greater in survivors ($P = 0.12$). Although plasma genistein, daidzein, and equol concentrations after the S and SP diets were not significantly different ($P > 0.68$), O-DMA concentrations tended to be lower after the SP diet ($P = 0.12$). A clear demarcation between equol producers and nonproducers was not apparent. Plasma concentrations fell into 3 ranges: >10 , 5–10, and <5 nmol/L. Although there were no significant changes in mean equol between the S and SP diets, some subjects' levels were substantially altered. These results show that this strain of probiotic bacteria minimally affects plasma isoflavone concentrations. The observed changes in equol production suggest that equol production within an individual may be more variable than once thought.

Soy and other cancer sites

Inhibitory Effects of Symbiotic *Lactobacterium*- and Yeast-Fermented Soy Extract on Tumor Metastasis and Proliferation. T. Kageura,* M. Suzuki,* T. Moriyama,[†] and T. Ogawa[†]. *Nihon Bio Co., Ltd., Tokyo, Japan; [†]Graduate School of Agriculture Kyoto University, Japan.

People all over the world face serious health problems. The number of patients with neoplasm, allergy, and viral infections involving the immune system is continuously increasing be-

cause our immune systems are getting worse with daily life and exposure to food and endocrine-disrupting chemicals. During our studies for developing immune-response modifiers from functional foods including fermented materials and traditional medicine, we found that symbiotic *Lactobacterium*- and yeast-fermented soy extract (LYS) inhibited tumor metastasis and proliferation by the stimulation of immune systems. METH-ODS: Preparation of LYS: Twelve *Lactobacterium* and 4 yeast strains were added to soy extract, and the fermentation was allowed to proceed statically at 37°C for 72 h. The fermented products were concentrated by freeze drying. Macrophage activation test: Mouse peritoneal macrophages were prepared according to the usual method and treated with the concentrates of LYS to measure nitric oxide production and phagocytic activities. Tumor metastasis test: Female C57/BL/6J mouse were given free access to a normal diet and water containing 1% or 2% LYS for 1 wk before a melanoma injection. After an additional 2 wk, the lungs were removed and total numbers of visible melanoma colonies were counted. RESULTS AND DISCUSSION: By in vitro treatment with LYS, macrophages were significantly activated depending on LYS concentrations. By the addition of 0.3 g/L LYS, production of nitric oxide and phagocytic activities of macrophages were stimulated 15-fold and 3-fold, respectively. Melanoma metastasis in lung was significantly suppressed by LYS in a dose-dependent manner. At 2% LYS, colonies of melanoma were decreased by one-third. These data indicates that LYS inhibits tumor metastasis and proliferation by stimulating the immune systems. LYS is considered to be an immune-response modifier.

Genistein and EGFR Related Protein (ERRP) Potentiate Each Other in Gastric, Colon and Prostate Cancer Cells. U. Kodali,[†] O. Kucuk,*[†] R. Jaszewski,*[†] E. Levi,*[†] D. Dorge,** F. Sarkar,*[†] and A.P.N Majumdar*[†]. *Karmanos Cancer Institute, Detroit, MI; [†]Wayne State University, Detroit, MI; **National Toxicological Research Center, FDA, U.S.

Soy isoflavones may have a role against several malignancies including gastric, prostate, and colorectal cancers. We isolated a negative regulator of epidermal growth factor receptor (EGFR) termed ERRP (EGF receptor related protein), which inhibits proliferation and EGFR activation in cancer cells. ERRP administration inhibits the development of and causes regression of palpable colon cancer tumors in SCID mice. Interestingly, genistein increases ERRP expression in colon cancer cells. To determine whether ERRP and genistein would enhance each other's activity against epithelial cancer cells, gastric (AGS), colon (Caco-2, HCT-116), and prostate (PC-3) cancer cells were exposed to genistein (2–50 μ mol/L), sodium butyrate (2–10 mmol/L), and ERRP (5 μ g/mL) for 48 h. Cells, preincubated with ERRP, were subsequently treated with genistein and butyrate or vice versa. Butyrate was used for comparison as a known differentiation inducer. Proliferation was assessed by MTT assay and alkaline phosphatase was used as marker of differentiation in colon cancer cell lines. Exposure to genistein inhibited proliferation in a dose-dependent manner with 60–70% inhibition with the highest dose. Butyrate induced comparatively greater inhibition. These changes were accompanied by a concomitant reduction in EGFR activation. Preincubation with ERRP caused higher apoptosis than did either genistein or butyrate alone. In colon cancer cell lines, both genistein and butyrate independently induced differentiation. Preincubation with genistein in-

creased the expression of ERRP. Genistein and ERRP potentiated each other in the inhibition of proliferation; promotion of differentiation; and induction of apoptosis in colon, prostate, and gastric cancer cells.

Genistein Does Not Sensitize Human Bone Marrow to XK469 Toxicity at Concentrations that Sensitize BxPC3 Pancreatic Cancer to XK469 Chemotherapy. R. E. Parchment,^{*†} F. Sarkar,^{*†} J. Kassab,^{*†} K. L. Ellis,^{*†} D. Doerge,^{**} and O. Kucuk^{*†}. *Karmanos Cancer Institute, Detroit, MI; [†]Wayne State University, Detroit, MI; **National Toxicological Research Center, FDA, U.S.

Genistein abrogates nuclear factor- κ B (NF- κ B) induction, which occurs in response to XK469, and thereby increases the drug's efficacy against BxPC-3 tumor cells. Therefore, genistein could be a useful adjunct to XK469 chemotherapy. To be clinically useful, however, chemosensitization should be selective for the tumor cells and spare the normal tissues. Genistein sensitization to chemotherapy may occur in tumor cells but not in normal cells, because loss in NF- κ B activity is compensated in normal cells. We evaluated the safety of genistein with and without cytotoxic chemotherapy by exposing human hematopoietic cells to genistein in combination with XK469 at pharmacological concentrations. Drug-induced cytotoxicity was quantified by a clonogenic assay that measures survival of neutrophil-monocyte progenitors named CFU-GM, a target of toxic drug action in vivo. When used with published prediction models (1), this in vitro test correctly identifies the human maximum tolerated dose of 90% of tested drugs within fourfold of the actual maximum tolerated dose. Neither pretreatment nor simultaneous exposure to genistein sensitized human CFU-GM to the in vitro toxicity of XK469. These findings confirm the hypothesis that genistein chemosensitization occurs differentially in malignant cells because they lack compensatory mechanisms for loss of NF- κ B function. The results suggest that genistein may sensitize resistant human tumors to chemotherapy without increasing chemotherapy toxicity to the bone marrow, thereby resulting in a substantial gain in therapeutic index. The CFU-GM test may be useful in selecting chemotherapeutic agents to combine with soy isoflavones in future clinical trials.

1. Parchment, R. E., Gordon, M., Grieshaber, C. K., Sessa, C., Volpe, D. & Ghelmini, M. (1998) Predicting hematological toxicity (myelosuppression) of cytotoxic drug therapy from in vitro tests. *Ann. Oncol.* 9: 357-364.

Genistein Sensitizes Resistant Diffuse Large Cell Lymphoma Cells to Chemotherapy. R. Mohammad,^{*†} A. Al-Katib,^{*†} A. Aboukameel,^{*†} D. Ibrahim,^{*†} D. Doerge,^{**} F. Sarkar,^{*†} and O. Kucuk^{*†}. *Karmanos Cancer Institute, Detroit, MI; [†]Wayne State University, Detroit, MI; **National Toxicological Research Center, FDA, U.S.

Diffuse large cell lymphoma (DLCL) is the most common subtype of non-Hodgkin's lymphoma. The combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) remains the standard therapy for DLCL with a cure rate of ~40%. We developed the cell line WSU-DLCL2 from a patient resistant to CHOP chemotherapy. The antitumor activity of CHOP with or without genistein was evaluated in our WSU-DLCL2 model. In vivo, WSU-DLCL2-bearing SCID mice received genistein alone (800 μ g/(kg \cdot d), orally as gavages for 5 d), CHOP alone ("C," 40 mg/kg, i.v.; "H," 3.3 mg/kg, i.v.; "O," 0.5 mg/kg, i.v.; and "P," 0.2 mg/kg, every day for 5 d, orally), or genistein for 5 d followed by CHOP. Tumor

growth inhibition (T/C), tumor growth delay (T-C), and log10 kill for genistein, CHOP, and genistein followed by CHOP were 33.6%, 19.2%, and 5.2%; 7, 8, and 17 d; and 1.0, 1.2, and 2.6, respectively. To elucidate the mechanism of genistein-induced sensitization of WSU-DLCL2 to CHOP chemotherapy in this xenograft mouse model, we studied the in vitro effect of genistein on growth inhibition, cell cycle, Bax:Bcl-2 ratio, nuclear factor- κ B (NF- κ B) DNA binding, and apoptosis in vitro. At 30 μ mol/L, genistein inhibited the growth significantly, induced G2M arrest, increased Bax:Bcl-2 ratio, decreased NF- κ B DNA binding, and induced apoptosis. Genistein also inhibited NF- κ B DNA binding in vivo whereas CHOP enhanced it. Our results show that genistein has growth modulatory effects on WSU-DLCL2 cells and enhances the antitumor activity of CHOP. We plan to study soy isoflavones in combination with CHOP chemotherapy in a clinical trial in patients with non-Hodgkin's lymphoma.

Protection against Radiation-Induced Mortality in Mice by Soy Isoflavones M. R. Landauer, V. Srinivasan, and T. M. Seed. Armed Forces Radiobiology Research Institute, Uniformed Services University, Bethesda, MD.

Radioprotective agents are compounds that are administered before exposure to ionizing radiation to reduce its damaging effects, including radiation-induced lethality. They have applications in clinical oncology, space travel, radiation site cleanup, radiological terrorism, and military scenarios. The ideal radioprotectant would be nontoxic and would not degrade performance. Genistein and daidzein have been shown to provide protection against nonionizing ultraviolet radiation but not against the more damaging effects of ionizing radiation. We previously reported that multiple oral pharmacological doses of genistein administered before and after gamma irradiation protected mice from radiation-induced lethality. We also reported that a single nontoxic dose of genistein (25-400 mg/kg) injected 1 d before a lethal dose of gamma radiation was radioprotective (1). In nonirradiated mice receiving genistein at 100-400 mg/kg, no adverse effects on locomotor activity, grip strength, motor coordination, body weight, testes weight, or histopathology were observed. When genistein was administered 1 h before irradiation, no radioprotection was found. We now report that in a preliminary study a single subcutaneous injection of a pharmacologic dose (200 mg/kg) of the isoflavone daidzein also resulted in protection from radiation-induced lethality when administered 1 d before gamma radiation but not 1 h before irradiation. There was no significant difference in the radioprotection obtained for genistein and daidzein at the dose tested. These results demonstrate that a single nontoxic subcutaneous administration of genistein or daidzein can protect mice against radiation-induced lethality. (Patent pending.)

1. Landauer, M. R., Srinivasan, V. & Seed, T. M. Genistein treatment protects mice from ionizing radiation injury. *J. Appl. Toxicol.* (in press).

Effects of Soybean Saponin Supplementation on Colon Tumor Development in Carcinogen-Treated F344 Rats. H.-W. Kim,^{*} I.-H. Han,^{*} M.-K. Sung,^{*} R. Yu,[†] J.-S. Kim,^{**} and Y.-K. Kim[§]. *Department of Food and Nutrition, Sookmyung Women's University, Seoul, Korea; [†]Department of Food and Nutrition, Ulsan University, Ulsan, Korea; **Department of Animal Science and Biotechnology, Kyungpook National University, Daegu, Korea; [§]Department of Forest Products, Kookmin University, Seoul, Korea.

Saponins are a group of glycosides composed of a lipid-soluble aglycone moiety attached to water-soluble glycosidic chains. Previous studies showed that soybean saponins suppress the growth of colon tumor cells, and possible explanations for this include inhibition of protein kinase C activation as well as induction of cell differentiation. The objective of this study was to evaluate the efficacy of dietary soybean saponins on the suppression of colon carcinogenesis in rats. Male F344 rats were divided into 3 groups. Animals in group I were fed AIN-76 control diets; animals in groups II and III were fed control diet supplemented with 0.05% soybean saponins. Starting in wk 2, animals in groups I and II were injected once weekly with azoxymethane (AOM) at 15 mg/kg. Half of the animals from each group were killed in wk 11 and aberrant crypt formation was measured; the other half was killed in wk 33 for observation of tumor formation. Results showed that saponin supplementation significantly reduced the number of aberrant crypts, aberrant crypt foci, and aberrant crypt foci with >4 aberrant crypts. The average number of tumors in AOM-treated rats fed control diet was less (2.45 ± 1.36) than that of AOM-treated rats fed saponin-supplemented diet (1.68 ± 1.49) although no statistical significance was found. All animals in the control group had tumors whereas 81.25% of the rats fed a saponin-supplemented diet had tumors. Saponin supplementation itself did not cause any morphological changes in the colon of the rats. These results indicate that the anticancer property of soybeans *in vivo* may partly be attributed to saponins. Mechanisms of the anticancer actions of saponins are currently being investigated. (Supported by the Korea Science and Engineering Foundation grant R01-2001-00231.)

Soy Isoflavone Genistein Inhibits Nuclear Factor- κ B and Sensitizes Human Cancer Cells to Apoptosis Induced by Chemotherapeutic Agents. Y. Li,* K. Ellis,* A. Nedeljkovic-Kurepa,* O. Kucuk,[†] and F. Sarkar*[†]. *Department of Pathology, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI; [†]Department of Internal Medicine, Karmanos Cancer Institute, Detroit, MI.

Carcinogenesis and tumor progression are complex processes involving multiple molecular and cellular pathways. Thus, cancer chemotherapeutic strategies commonly require multiple agents with different modes of action used in combination. However, using multiple agents contributes to added toxicity requiring dose reduction, which may result in reduced tumor response and consequently poor treatment outcome. To overcome such problems, new strategies for combination chemotherapy must be used to increase tumor response to chemotherapy while lowering its toxicity. Genistein, the predominant soy isoflavone, has been shown to inhibit the growth of various cancer cells *in vitro* and *in vivo* without causing toxicity. The antitumor effects of genistein could be in part due to inactivation of nuclear factor- κ B (NF- κ B) pathway. Chemotherapeutic agents are known to induce NF- κ B activity, leading to activation of survival factors and thereby resulting in lower cell killing. We therefore hypothesize that the inactivation of NF- κ B by genistein before the treatment of cells with chemotherapeutic agents will lead to better tumor kill. To test our hypothesis, PC3 (prostate), MDA-MB-231 (breast), and BxPC-3 (pancreas) cancer cells were pretreated with genistein at 30 μ mol/L for 24 h and then exposed to low doses of chemotherapeutic agents for an additional 24–48 h. We found that genistein at 30 μ mol/L combined with

cisplatin at 100 nmol/L, docetaxel at 0.5 nmol/L, or doxorubicin at 50 μ g/L results in significantly greater inhibition of cell growth, as analyzed by MTT assay, compared with any agent alone. Moreover, we found that the combination treatment induced more apoptosis in PC3, MDA-MB-231, and BxPC-3 cells than did a single agent as analyzed by enzyme-linked immunosorbent assay, and these results are consistent with MTT results. We also found that the NF- κ B activity was significantly increased within 2 h of cisplatin, docetaxel, or doxorubicin treatment and that the NF- κ B-inducing activity of these agents was completely abrogated in cells pretreated with genistein. These results are consistent with our hypothesis and clearly suggest that genistein pretreatment inactivates NF- κ B. Together with other cellular effects of genistein, this effect may contribute to increased growth inhibition and apoptosis induced by cisplatin, docetaxel, and doxorubicin in prostate, breast, and pancreatic cancer cells.

Soy and thyroid

Dietary Soy Protein Isolate and Isoflavones Modulate Thyroid Hormone Receptor Expression in Rats. C. W. Xiao, M. R. L'Abbé, S. Gilani, G. Cooke, I. Curran, and S. A. Papademetriou. Nutrition Research Division, Food Directorate, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada.

Soy consumption has been linked to lower incidence of chronic diseases such as cardiovascular diseases, atherosclerosis, and type 2 diabetes. However, the mechanisms involved are poorly understood. The purpose of this study was to examine the effect of soy protein isolate (SPI) and isoflavones on thyroid hormone receptor (TR) expression in rats. In expt. 1, Sprague Dawley rats were fed diets containing casein, SPI, or SPI plus supplemental isoflavones (5–1250 mg/kg diet) for 120, 240, and 360 d. Their offspring (F1) were fed the same diets as their parents for 28, 70, 120, and 240 d. In expt. 2, Sprague Dawley rats were fed diets containing casein or casein plus supplemental isoflavones (50–400 mg/kg diet) for 120 d. The rats were killed at the end of the feeding periods. SPI had no significant effect on either mRNA or protein contents of TR α 1 measured by semiquantitative reverse transcription-polymerase chain reaction and Western blot, respectively. Isoflavones decreased the hepatic TR α 1 level in F1 d 28 male rats. SPI markedly increased hepatic TR β 1 protein but not mRNA steady state levels in both sexes compared with casein. This effect was consistently shown at different stages and generations of the study. Isoflavones had no additional effect on TR β 1 protein content. The tissue distribution analysis revealed the existence of 2 subtypes of TR β 1, 52 kDa and 55 kDa proteins. The 52 kDa TR β 1 was induced by dietary SPI in liver but not in the other tissues measured whereas the 55 kDa receptor was dominantly expressed in thyroid, heart, kidney, testis, and uterus and was unchanged by SPI. The lack of effect of isoflavones on TR β 1 expression was confirmed in expt. 2. Overall, this study demonstrates for the first time that SPI increases the expression of hepatic TR β 1, and exposure to isoflavones inhibits the expression of hepatic TR α 1 in young male rats. The physiological significance of these cellular responses remains to be determined. (Supported by Health Canada.)

Antioxidant properties of soy

Effects of a Soy Protein Diet on Hematology and Oxidant Stress Variables in Rats under Simulated Microgravity. S. Bansal, M. Soulsby, J. Pasley, and P. Chowdhury. University of Arkansas for Medical Sciences, Little Rock, AR.

The effects of a soy protein diet on hematological, oxidant, and antioxidant levels in brain, liver, and pancreatic tissues in an animal model of simulated weightlessness were investigated in the current study. **METHODS:** Rats on a regular laboratory pellet diet or on 9.6% soy protein diet were either not suspended (non-HLS) or hind limb suspended (HLS) by their tails for 14 d. Body weights and food and water intakes were monitored and blood samples were analyzed for hematocrit, red blood cell mass, and hemoglobin before and after suspension. Brain, liver and pancreatic tissues were measured for oxidant and antioxidant levels. **RESULTS:** Body weights of the HLS animals were decreased compared with non-HLS animals ($P < 0.05$). Hematological variables were not different between the non-HLS and HLS groups. Malondialdehyde (MDA) levels in the brain and liver tissues were lower in the non-HLS soy group than the non-HLS group receiving the regular laboratory diet ($P < 0.05$). In HLS groups, however, these levels were higher for both diets, but it was only significant ($P < 0.05$) between the soy non-HLS and the soy HLS group. Basal superoxide dismutase (SOD) levels were not significantly different between the 2 diet groups but were elevated in HLS groups compared with the non-HLS groups ($P < 0.05$). Glutathione levels in the brains of non-HLS soy group were elevated ($P < 0.05$) but were not changed after 14 d of suspension. In contrast, glutathione levels in the livers of soy diet group were generally lower in both HLS and non-HLS animals. Malondialdehyde and SOD levels in the pancreatic tissue were not significantly different between groups. No consistent results were found for pancreatic glutathione levels. **CONCLUSIONS:** Our data suggest that HLS induced a differential tissue-specific oxidative response as determined by MDA, SOD, and glutathione levels. Soy protein in the diet lowered basal peroxide levels but did not affect changes in the peroxide levels induced by HLS. (Supported by a grant from Arkansas Space Grant Consortium.)

Soy Protein with Isoflavones and Isoflavone Extract Decrease Oxidative Stress via Decreased Reactive Nitrogen Species. L. Zhang, M. Anthony, P. Rowe, and J. Wagner. Wake Forest University School of Medicine, Winston-Salem, NC.

OBJECTIVE: To investigate the antioxidant effects of diets containing soy protein isolate with and without isoflavones compared with diets with casein and lactalbumin as the protein source. **METHODS:** 81 cynomolgus monkeys (32 males, 49 females) were randomly assigned to 5 diet groups fed protein from casein and lactalbumin (Cas, $n = 16$), soy containing isoflavones (Soy+, $n = 18$), alcohol-washed soy protein without the isoflavones (Soy-, $n = 15$), semipurified isoflavone extract added to Soy- (IFE, $n = 16$), or the alcohol-extracted fraction of soy protein added to Soy- (AlcExt, $n = 16$). After 24 wk of diet, blood samples were taken to determine plasma cholesterol concentrations, amount of oxidized LDL antibody (OxLDL), and LDL oxidation in response to copper stimulation (males only). Mechanisms for antioxidant activity were assessed by determining myeloperoxidase (MPO), nitrotyrosine, and 8-isoprostane (ISO) con-

centrations. **RESULTS:** All soy groups reduced plasma cholesterol concentrations compared with Cas (all P -values < 0.0001). Soy+ reduced OxLDL by 46% compared with the Cas ($P = 0.007$) and prolonged LDL oxidation lag time by 42% compared with Cas. IFE decreased the total amount of LDL oxidized by copper compared with the other groups (all P -values < 0.005). All soy groups except the AlcExt group had significantly lower concentration of nitrotyrosine compared with Cas (all P -values < 0.01); the Alc Ext group was marginally significant ($P = 0.07$). IFE had a tendency for decreased ISO compared with the other groups (all P -values < 0.06). There was no effect of treatment on MPO. **CONCLUSION:** Soy+ and IFE diets demonstrated the greatest reductions in LDL oxidation. However, all soy groups had lower plasma nitrotyrosine concentrations with no effect on MPO. Thus, reduction in reactive nitrogen species, such as peroxynitrate, is a likely mechanism for reduced oxidative stress with dietary soy consumption.

Antioxidative and Anti-Inflammatory Effect of Genistein through Downregulation of NF κ B Activity in RAW 264.7 Macrophages. C.-Y. Choi, H.-Y. Cho, J.-Y. Park, C.-W. Cho, and Y.-S. Song. Inje University, Kimhae, Korea.

This study was designed to investigate whether genistein may ameliorate oxidative stress and inflammatory status through nuclear factor κ B (NF κ B) activation in the lipopolysaccharide (LPS)-stimulated RAW 264.7 murine macrophage cell line. Treatment of RAW 264.7 cells with genistein significantly reduced LPS-stimulated nitric oxide production in a dose-dependent manner with an IC_{50} of 69.4 μ mol/L. Genistein at concentrations of 50 and 100 μ mol/L reduced prostaglandin E_2 and thiobarbituric acid-reactive substances production, enhancing glutathione level and antioxidant enzyme activities, such as superoxide dismutase and catalase. The specific DNA binding activities of NF κ B on nuclear extracts from treatments with genistein at 50 and 100 μ mol/L were significantly suppressed. These results suggest that genistein has antioxidative and anti-inflammatory activities that suppress intracellular oxidative stress, nitric oxide production, and prostaglandin E_2 production through downregulation of NF κ B activity. [Supported by grant R01-2000-00187 from the Korea Science and Engineering Foundation (KOSEF) and in part by KOSEF through the Biohealth Products Research Center at Inje University.]

Genistein Supplementation Alleviates the Deterioration in Performance and Antioxidant Status of Japanese Quail Associated with Heat Stress. K. Sahin,* M. Onderci,* M. F. Gursu,* N. Sahin,* D. Doerge,† F. Sarkar,† and O. Kucuk†**. *Firat University, Elazig, Turkey; †Karmanos Cancer Institute, Detroit, MI; **Wayne State University, Detroit, MI; ‡National Toxicological Research Center, FDA, U.S.

Genistein, a phytoestrogen found in soybeans, is a powerful antioxidant. We evaluated the effects of genistein supplementation on performance; carcass characteristics; levels of oxidative stress markers (malondialdehyde and homocysteine); and vitamins C, E, and A in Japanese quail (*Coturnix coturnix japonica*) exposed to a high ambient temperature of 34°C. Two hundred and forty Japanese quails (age 10 d) were randomly assigned to 8 treatment groups each consisting of 30 birds. The birds were kept in a temperature-controlled room at 22°C (thermo neutral, TN groups) or 34°C (heat stress, HS groups) for 8 h/d (0900–1700). Birds were fed either a basal

(control) diet or the basal diet supplemented with genistein at 200, 400, or 800 mg/kg diet. Experiments started at age 10 d and continued for 32 d. Measurements were taken on day 42. Heat exposure decreased performance when the basal diet was fed ($P = 0.001$). A quadratic increase in feed intake ($P = 0.01$), body weight ($P = 0.01$), and improvement in feed efficiency ($P = 0.01$) and carcass traits ($P = 0.05$) variables were found in the genistein-supplemented HS groups. Growth rate and feed efficiency also improved in the TN groups. Serum vitamins C, E, and A concentrations increased in the supplemented HS groups ($P = 0.01$) whereas nonsignificant changes occurred in the TN groups. Homocysteine levels in serum and malondialdehyde levels in serum and liver ($P = 0.001$) decreased in all TN and HS groups as dietary genistein supplementation increased. The effects of genistein were greater in HS groups than in TN groups. Results of the present study suggest that supplementation with genistein protects against heat stress by reducing the negative effects of oxidative stress in quail.

Soy and lipid metabolism

Does A Practical Daily Intake of ~25 g Soy Protein Significantly Lower Cholesterol?—A Meta-Analysis of Recent Studies. J. I. Harland* and T.A. Carr[†]. *J I Harland, Nutrition Consultant, Wilts, UK; [†]T.A Carr, Nutrition Consultant, UK.

Many randomized controlled studies evaluated the effect of substantial soy intakes (≥ 40 g) on serum total cholesterol (total-C). In 1995 a meta-analysis (1) was conducted that reviewed published trials and found that at a mean intake of soy protein (SP) of 47 g/d reduced total-C by 0.45 mmol/L. In addition, the amount of SP was a much weaker predictor of the effect on total-C than was initial total-C. Since this time, U.S. and U.K. authorities have reviewed evidence relating to soy and total-C and permitted a health claim indicating that SP may reduce blood cholesterol levels when consumed at a level of 25 g/d. To support the validity of the claim, without projection from higher nonpractical intakes, the effect of lower levels of SP consumption on total-C was evaluated. A search and systematic review of the literature from 1995 to mid-2002 revealed 5 studies including 9 treatments with ~25 g SP or less. These studies represented 340 subjects (men and pre-, peri-, and postmenopausal women) with mean baseline total-C of 4.57–6.26 mmol/L. The studies were all conducted with medium to low fat and saturated fat diets, often NCEP1; in some cases the SP supplemented the diet and in others it was an integral part. A meta-analysis was performed on these treatments, which contained SP in the form of soymilk, tofu, or soy protein isolate (Fig. 2). Average SP intake was 22.5 g (range 14.5–30 g/d). The average reduction in total chole-

sterol was 0.22 mmol/L (3.8%; $P = 0.0003$); LDL-C was 0.17 mmol/L lower ($P = 0.00001$), triacylglycerol was 0.07 mmol/L lower ($P = 0.009$), and HDL was unchanged. Heterogeneity in the data set was tested and found not to be significant. The data from this analysis confirm that in normocholesterolemic or moderately hypocholesterolemic individuals the consumption of a more modest amount of SP (~22.5 g) can lead to a significant reduction in total-C. Healthy individuals consuming ~25 g SP—the level suggested in the various health claims—supports the use of such claims as a public health measure and reinforces the earlier projections of Anderson (1) that at intakes of 25 g SP, total-C would be reduced by 0.23 mmol/L.

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Combining Soy Protein with Phospholipids and Fiber Doubles the Lipid-Lowering Effects Compared with Soy Protein Alone. L. H. Høie,* E.C.A. Morgenstern,[†] J. Gruenwald,[†] H. J. Graubaum,* W. Lüder,[‡] and H.J.F. Zunft^{††}. *Nutri Pharma ASA, Oslo, Norway; [†]Nutri Pharma GmbH, Berlin, Germany; ^{**}Phytopharm Research, Germany; [‡]German Institute of Human Nutrition, Germany; ^{††}Institute for Nutritional Science University of Potsdam, Germany.

Hypercholesterolemia is a major risk factor for cardiovascular disease. Several clinical studies have shown that soy protein, as well as other soy constituents, is effective for lowering plasma lipid concentrations, but direct comparisons of the efficacy of different soy-containing products are rare. This randomized placebo-controlled double-blind study evaluated the lipid-lowering effects of 2 different soy protein supplements, one combining isolated soy protein with soy fiber and phospholipids (Abacor) and one containing isolated soy protein alone (SuproSoy). Over 8 wk, 121 hypercholesterolemic subjects received 25 g soy protein daily from either of the 2 trial substances or 25 g milk protein from the placebo preparation. At the end of the treatment, total cholesterol levels were significantly reduced by 8.0% with Abacor and by 3.4% with SuproSoy and slightly elevated with placebo. LDL cholesterol concentrations were reduced by 9.7% with Abacor and by 5.4% with SuproSoy. Apolipoprotein B was also reduced by both soy products, again with a larger decrease reached with Abacor, whereas the values of HDL cholesterol, triglycerides, homocysteine, folic acid, and vitamin B-12 showed no changes. Study preparations were well tolerated with no adverse events observed. Soy protein supplements proved effective for reducing serum cholesterol levels and are therefore likely to diminish the risk for cardiovascular diseases. The combination of isolated soy protein with soy-derived phospho-

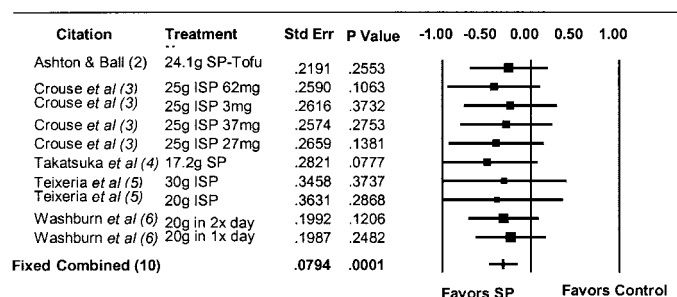


FIGURE 2 Forest plot. Effect of ~25g SP on plasma total cholesterol.

lipids and fibers showed twice the lipid-lowering effect compared with isolated soy protein alone.

Soy Containing Isoflavones and Plasma Lipids: A Randomized Double-Blind Placebo-Controlled Trial. S. Kreijkamp-Kaspers, L. Kok, D. E. Grobbee, and Y. T. van der Schouw. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands.

A meta-analysis comprising 38 intervention studies with soy protein naturally containing isoflavones found a 9.3% decrease in total cholesterol and a 12.9% decrease in LDL cholesterol, which has resulted in the health claim approved by the U.S. Food and Drug Administration that diets low in saturated fatty acids and cholesterol that include 25 g/d of soy protein may reduce the risk of heart disease. Our goal was to investigate whether the improvement in plasma lipids seen with soy protein containing isoflavones can be extrapolated to postmenopausal women. For our double-blind randomized trial we recruited 202 postmenopausal women aged 60 to 75 y. Subjects were randomly assigned to consume either a soy isolate containing 99 mg isoflavones (phytoestrogens) or casein (placebo) for 1 y. At baseline, 3 mo, and the end of the intervention, we measured plasma lipids [total cholesterol, LDL, HDL, triglycerides, and lipoprotein(a)]. Linear regression was used to analyze the differences in change in plasma lipids between the 2 groups during the intervention. The randomization was successful: 153 women (75%) completed the intervention. At baseline total cholesterol was 6.3 mmol/mL (range 3.0–9.8), LDL 4.2 mmol/L (range 1.9–7.0), HDL 1.6 mmol/L (range 1.0–3.0), triglycerides 1.2 mmol/L (range 0.5–2.7), and lipoprotein(a) 0.22 mmol/L (range 0.02–1.58). At 3 mo and 12 mo no significant differences in plasma lipid values were seen between the 2 groups. Our results suggest that the positive effects of soy isoflavones on plasma lipids cannot be extrapolated to postmenopausal women.

The Effect of Soy Germ Isoflavone Extract on Serum Lipids in Postmenopausal Women. Y.-B. Ye,* Y.-X. Su,[†] and M. Verbruggen**. *The First Affiliated Hospital of Sun-Yat-sen University, Guangdong, China; [†]Faculty of Medical Nutrition of Public Health School, Sun Yat-sen University, P.R. China; **Acatis Holding B.V., The Netherlands.

The higher morbidity and mortality of cardiovascular diseases in postmenopausal women are reported to be associated with the deficiency of estrogen. Soy isoflavones have antioxidant activities and are said to be able to decrease LDL cholesterol (LDL-C) and increase HDL-C in some animal and clinical experiments, but this is still controversial and the effective dose is not known. **OBJECTIVE:** To determine the effective dosage and resulting effects of isoflavones extract from soy hypocotyl on serum lipids in postmenopausal women. **DESIGN:** Eighty-seven postmenopausal normocholesterolemic women were randomly assigned, single blind, to 3 treatment groups with daily dosages of placebo or 84 or 126 mg isoflavones of aglycones. Additionally, a positive control group of 10 women was treated daily with estrogen [2.5 mg tibolone (Livial)]. Serum concentration of total cholesterol (TC), total triglyceride (TG), LDL-C, HDL-C, apoprotein AI (ApoAI), and apoprotein B100 (ApoB100) were measured at baseline, midterm (12 wk), and posttreatment (24 wk). The ratios of TC to HDL-C (TC/HDL-C) and LDL-C to HDL-C (LDL-C/HDL-C) were calculated. **RESULTS:** HDL-C at midterm and posttreatment of the 2 isoflavone treatment groups were sig-

nificantly higher than their baseline values ($P < 0.05$), and the mean values at midterm and posttreatment of the 126-mg/d group were significantly higher than those of 84-mg/d group ($P < 0.05$). The percentage changes of HDL-C in the placebo, 84 mg/d, 126 mg/d, and estrogen groups were 1.85%, 6.02%, 11.93%, and 6.38%, respectively, and the mean value of 126 mg/d group was also significantly higher than that of placebo group ($P < 0.05$). TC/HDL-C decreased significantly except for the placebo group. However, there was no significant difference of TC/HDL-C and its percentage change after treatment among the 4 groups. LDL-C/HDL-C of the 2 isoflavones treatment groups after treatment was significantly lower than those of their baseline values, respectively ($P < 0.05$ in 84-mg/d group and $P < 0.01$ in 126-mg/d group). The percentage change of LDL-C/HDL-C in 126-mg/d group (-12.21%) was significantly lower than that of the placebo group (-1.61% ; $P < 0.01$). TC, TG, LDL-C, ApoAI, and ApoB100 in our trial were not changed significantly compared with baseline and were not significantly different among the 4 groups. **CONCLUSION:** The dosage of 84–126 mg/d of isoflavones can increase serum HDL-C and lower LDL-C/HDL-C, which implies the favorable effect of soy isoflavones on lipid metabolism in postmenopausal women. The more effective dosage is 126 mg/d.

Isoflavones in Food with Minimal Soy Protein Reduce Serum Cholesterol and Improve Important Markers of Cardiovascular Risk. C. Clerici,* K. Setchell,[†] M. Pirro,* O. Morelli,* D. Castellani,* V. Giuliano,* G. Sabatino,* S. Orlandi,* S. Asciti,* A. Morelli,* and E. Mannarino*. *Clinica di Gastroenterologia ed Epatologia, Università degli Studi di Perugia, Italy; [†]Clinical Mass Spectrometry, Children's Hospital Medical Center, Cincinnati, OH.

All patients (32 adults) were placed on an Italian equivalent of a Step II diet that typically entails consuming 1 serving (85 g) of pasta per day. After a 2-wk run-in period, each patient was given a pasta fortified with 33 mg total isoflavones daily for 5 wk followed by 5 wk of regular pasta packaged identically. Serum lipids, the urinary isoprostane 8-epi-PGF₂α (a biomarker of in vivo lipid peroxidation), brachial artery flow-mediated vasodilatation by ultrasound, the gold-standard measure of arterial reactivity, blood pressure, serum C-reactive protein (a marker of inflammation often elevated in patients with heart disease), leptin, insulin, and body weight and body mass index were measured at baseline and after 5 wk of consuming the 2 diet regimens. Compliance to the diets was confirmed by measurement of plasma isoflavone concentrations. The diet with isoflavones boosted fasting total isoflavone levels from <1 μg/L at baseline to a mean steady-state concentration of 50 μg/L. Compared with baseline, mean reductions of $5.1 \pm 1.2\%$ ($P < 0.005$) and $6.5 \pm 1.7\%$ ($P < 0.005$) in serum total cholesterol and LDL-cholesterol, respectively, occurred after 5 wk but serum triglycerides and HDL cholesterol did not change. The magnitude of the serum cholesterol reduction was inversely related to the isoflavone concentration. Urinary 8-epi-PGF₂α decreased $37 \pm 10\%$ ($P = 0.008$) from baseline. The lipid changes rebounded to baseline values when patients consumed pasta without isoflavones. Within the limitations of the study design, these data suggest that isoflavones in the presence of only minimal amounts of soy protein are hypocholesterolemic and that the significant reduction in triglycerides typically observed in dietary intervention studies using soy foods, but not observed here, is more likely explained by the action of soy protein. Serum C-reactive protein was signifi-

cantly reduced (-23% , $P < 0.003$) and brachial artery flow-mediated vasodilatation increased by 35% ($P = 0.03$) compared with baseline. No change in serum leptin or insulin was observed, yet small but significant reductions in body weight (mean \pm SEM, -1.9 ± 0.4 kg, $P < 0.004$) and body mass index (-1.64 ± 0.51 , $P < 0.006$) occurred. These studies support a role for isoflavones in reducing serum cholesterol levels in patients with hypercholesterolemia while highlighting the complexity of the mechanism of action of soy food diets on lipid responses. Independent of cholesterol, soy isoflavones have profound effects on reducing inflammation as assessed from the surrogate marker, serum C-reactive protein, reducing lipid peroxidation, and improving brachial artery-mediated vasodilatation, important factors in reducing risk for cardiovascular disease.

Promotion of Soy Foods in a Cardiac Rehabilitation Center. L. Salazar,* J. Huam,[†] E. Varco, and W. L. Dodson*.**

*Mississippi State University, Mississippi Agricultural and Forestry Experiment Station, Mississippi State, MS; [†]Alcorn State University, Alcorn Experiment Station, MS; **Cardiac Rehabilitation Program, Oktibbeha County Hospital, U.S.

The relationship of soy protein to decreased risk of cardiovascular disease is well established; however, the consumption of soy foods in some populations has not kept pace with identified health benefits. The objective of this study was to determine whether an intervention of foods demonstrations with tasting and informal presentations of specific health and nutritional benefits of soy products would increase their consumption by a high-risk population. Forty-one persons (33 males and 8 females) from the Cardiovascular Rehabilitation Program volunteered to participate in the study. The method consisted of 3 phases. The first phase consisted of a survey to determine awareness of key concepts of soy nutrition, current soy usage, and the types of dishes commonly eaten. Salads, cookies, corn bread, soups, and stews were the dishes most frequently consumed and were selected for the inclusion of soy. In the second phase, recipes for tofu salad, cornbread, chocolate chip oatmeal cookie, and tofu burger were developed and evaluated; a commercial soy burger was used as a reference. Nutritional benefits of soy products were presented, and subjects rated the 5 soy foods on a 5-point hedonic scale. The mean ratings were 3.85, 3.70, 4.17, 3.92, and 4.13, respectively. After 2 mo, subjects were surveyed (third phase) to determine changes in awareness and the use of soy products. Approximately 20% of the subjects had tried some of the recipes at home, and 40% had increased their consumption of soy burgers and meat analogues. Consumption of commercial soy burgers was significantly increased. The group showed significant changes in awareness of soy products and health benefits. A 2-mo intervention program with cardiac rehabilitation patients improved the awareness and use of soy products, especially the proprietary items.

Dietary Phytoestrogens and Vascular Function in Dutch Postmenopausal Women: A Cross-Sectional Study. S. Kreijkamp-Kaspers, L. Kok, M. L. Bots, D. E. Grobbee, and Y. T. van der Schouw. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands.

Supplementation with high doses of soy protein has blood lipid lowering effects in animals and in humans. This effect is generally ascribed to the isoflavones in the soy. Phytoestrogens

(estrogen-like compounds in plant foods) have also been shown to improve vascular function when supplemented at a high dose. The effects of low levels of intake that can be achieved through the habitual Western diet remain to be investigated. We conducted a population-based cross-sectional study in 301 postmenopausal women aged 60–75 to investigate the relation between habitual dietary phytoestrogen intake and vascular function. Dietary isoflavone and lignan intake was assessed using a food frequency questionnaire covering the year before enrollment. Both linear and logistic regression models were used. The endpoints were prevalence of hypertension, flow mediated dilatation (FMD), and ankle arm blood pressure index (AAI). In the analyses we adjusted for a wide range of potential confounders. Median isoflavone intake was 0.2 mg in the lowest tertile of intake and 12.9 mg in the highest tertile; median lignan intake was 0.8 mg and 2.1 mg, respectively. High isoflavone intake was associated with a better FMD (difference 1.1% FMD, 95% CI -0.4 , 2.7) and a lower prevalence of hypertension (odds ratio 0.54, 95% CI 0.25, 1.19) although both were not statistically significant. No relation was found with AAI. In conclusion, the results of this study do suggest a protective effect of dietary phytoestrogen intake on vascular function, even at low levels. However, in view of the limited magnitude of the effects, we deem further studies necessary to investigate the exact nature and extent of the cardioprotective effects of phytoestrogens.

Soy Consumption Does Not Alter Homocysteine or Markers of Vascular Inflammation. K. A. Greany,* J. A. Nettleton,* K. E. Wangen,* W. Thomas,[†] and M. S. Kurzer*.

*Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN; [†]Department of Biostatistics, University of Minnesota, MN.

Elevated plasma homocysteine and serum markers of vascular inflammation are associated with increased risk of coronary heart disease. Hormone replacement therapy has been reported to decrease homocysteine and markers of inflammation [E-selectin, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1)] but paradoxically increase C-reactive protein. Treatment with the selective estrogen receptor modulator (SERM) raloxifene has also been shown to decrease homocysteine, sICAM-1, and E-selectin without altering C-reactive protein. We hypothesized that soy would act as a SERM in modulating these risk factors and would decrease homocysteine, E-selectin, and sICAM-1 without an increase in C-reactive protein. Thirty-four postmenopausal women consumed soy protein isolate (25 ± 4 g protein containing 44 ± 7 mg isoflavones per day) and milk protein isolate for 6 wk each in a randomized crossover design. Fasting blood samples were collected at the end of each diet period and endpoints analyzed by enzyme-linked immunosorbent assay. Concentrations of homocysteine, C-reactive protein, E-selectin, sVCAM-1, and sICAM-1 were not different between soy and milk diet treatments. Adjustment for intake of folate, vitamin B-12, and vitamin B-6 did not alter the homocysteine results. These data suggest that decreasing homocysteine concentration and vascular inflammation are not mechanisms by which soy consumption reduces coronary heart disease risk. (Supported by U.S. Army grant no. BC981032, General Clinical Research Center grant no. M01-RR00400 from the National Center for Research Resources, the Minnesota Agricultural Experiment Station, and the Solae Company.)

Phytate-Rich Soy Protein Isolate Reduces Total Homocysteine in Postmenopausal Women. L. N. Clark, H. M. Barwick, D. L. Alekel, K. L. Schalinske, and M. B. Reddy. Iowa State University, Ames, IA.

Cardiovascular disease (CVD) is a major health problem and the risk for this disease increases with age in women after menopause, partially attributable to a rise in circulating total homocysteine (tHcy) and lipids. We aimed to investigate the cardioprotective effect of soy protein isolate (SPI) components phytate and isoflavones. In a double-blind 6-wk intervention study, 55 free-living postmenopausal women (47–72 y) were randomly assigned to 1 of 4 SPI (40 g/d) treatment groups: 1) native phytate, native isoflavone (NP/NI, $n = 14$); 2) native phytate, low isoflavone (NP/LI, $n = 13$); 3) low phytate, native isoflavone (LP/NI, $n = 14$); or 4) low phytate, low isoflavone (LP/LI, $n = 14$). We measured markers of CVD risk: triacylglycerols; total, LDL, and HDL cholesterol; C-reactive protein; and tHcy. Analysis of variance showed that treatment had a significant ($P = 0.045$) effect on reducing only tHcy concentrations but no effect on blood lipids or C-reactive protein. The reduction of tHcy in NP/LI was significantly different from LP/LI treatment (19% vs. 3%) but not from the other 2 treatments (8% for LP/NI, 12% for NP/NI). Although a 10–11% reduction in LDL cholesterol (similar to total cholesterol) was observed with NI compared with a 1–3% reduction with LI treatments, differences among treatments were not significant. Further, contrast coding to test the effect of phytate and isoflavones (NP vs. LP and NI vs. LI) showed that only the native phytate treatments significantly decreased tHcy ($P = 0.015$), but isoflavone treatments did not significantly affect any risk factors assessed. In conclusion, soy protein with native phytate concentrations, regardless of isoflavone content, may protect postmenopausal women from CVD by reducing tHcy. Treatments with native isoflavone content exerted a modest reduction in total and LDL cholesterol; however, future studies with a large number of subjects and longer-term intervention may show a significant effect. (Supported by funding from The Center for Designing Foods to Improve Nutrition, Iowa State University, and SPI from the Solae Company, St. Louis, MO.)

Phytate-Rich Soy Protein Isolate May Protect against Oxidative Stress by Reducing Iron Stores in Postmenopausal Women. H. M. Barwick, L. N. Clark, D. L. Alekel, and M. B. Reddy. Iowa State University, Ames, IA.

Oxidative stress is a major underlying cause of age-related diseases, including cardiovascular, cancer, and neurodegenerative conditions. Oxidative status typically decreases with age whereas body iron stores generally increase with age, especially after menopause. High iron stores may further induce oxidative stress and compromise antioxidant status. The objective of this study was to examine the effect of soy components (phytate and isoflavones) on reducing oxidative stress in postmenopausal women. Free-living subjects ($n = 55$) aged 47–72 y were randomly assigned (double-blind) to 1 of 4 soy protein isolate (SPI; 40 g/d) treatment groups: 1) low phytate, low isoflavone (LP/LI, $n = 14$); 2) normal phytate, low isoflavone (NP/LI, $n = 13$); 3) low phytate, normal isoflavone (LP/NI, $n = 14$); or 4) normal phytate, normal isoflavone (NP/NI, $n = 14$). We measured iron status [serum iron (SI), serum ferritin (SF), transferrin saturation (TS), hemoglobin, and hematocrit] and oxidative stress indexes (protein carbonyls, 8-iso-prostaglandin-f

$< F_2\alpha$, and oxidized low-density lipoprotein) at baseline and 6 wk. Analysis of variance showed that treatment had a modest effect on affecting body iron status ($P = 0.06$) but had no effect on oxidative stress indexes. The NP/LI and NP/NI treatments, respectively, reduced SF by 30% and 13% and TS by 28% and 16%. Furthermore, contrast coding (NP vs. LP and NI vs. LI) to test the effect of phytate and isoflavones revealed that only NP significantly decreased SF ($P = 0.02$), SI ($P = 0.04$), and TS ($P = 0.02$). In conclusion, phytate-rich soy, regardless of isoflavone content, reduced body iron stores and in turn may protect against oxidative stress in aging populations. However, none of the soy treatments had a beneficial effect on oxidative stress. Additional studies with a greater number of subjects or longer duration may be needed to observe a direct effect on oxidative stress. (Supported by funding from the Center for Designing Foods to Improve Nutrition, Iowa State University, and SPI from the Solae Company, St. Louis, MO.)

Effect of Isoflavone Supplementation on Lipid Profiles, Thiobarbituric Acid-Reactive Substances, and Nuclear Factor- κ B Activation in Mildly Hyperlipidemic Women. C.-Y. Choi, S.-H. Ryu, H.-Y. Cho, J.-Y. Park, J.-K. Kim, G.-S. Moon, D.-S. Kim, and Y.-S. Song. Inje University, Kimhae, Korea.

Dietary intake of soy isoflavones has been associated with a decreased risk of cardiovascular disease. One possible mechanism of action is through their antioxidative effect. We found that genistein inhibits the activation of the redox-sensitive transcription factor, nuclear factor- κ B (NF- κ B), in inflammatory macrophages in vitro. The purpose of this study is to examine the effect of isoflavone supplementation for 3 mo on the lipid profile, thiobarbituric acid-reactive substances (TBARS), and NF- κ B activation in mildly hyperlipidemic women. Eight women with triglyceride levels of 1.70 to 2.49 mmol/L participated in this study. After signing an informed consent, subjects completed a food frequency questionnaire and took the study capsules for 3 mo. One capsule contained 100 mg of an isoflavone mixture (Amax NutraSource Inc.; 13.5% daidzin, 26.3% daidzein, 38.6% genistin, 16.1% genistein, 5% glycitin, and trace amount of glycitein). Blood samples were collected at 0, 6, and 12 wk after isoflavone supplementation. Lipid profile, isoflavone, and TBARS levels were determined in plasma. HPLC analysis for isoflavone was conducted using a 0.46 \times 25 cm i.d. C₁₈ reverse-phase column (ZORBAX SB C₁₈, HP) with a mobile phase consisting of 60:40 (v/v) methanol:ammonium acetate (1 mmol/L) at a flow rate of 1 mL/min. NF- κ B activity was determined by an electrophoretic mobility shift assay in peripheral blood mononuclear cells (PBMCs) separated in a Ficoll gradient by centrifugation. Subjects taking isoflavone capsules had lowered triglyceride levels and suppressed NF- κ B activation in PBMCs; their isoflavone levels were 0.28–0.38 μ mol/L during isoflavone supplementation. Furthermore, oxidative stress measured by TBARS was significantly lowered after 6 wk of isoflavone supplementation. None of the study subjects reported any adverse effects or unusual symptoms during or after supplementation. These results support soy isoflavone as a potent hypolipidemic agent and antioxidant that may reduce the risk of cardiovascular disease. (Supported by grant NO. R01-2000-00187 from the Korea Science and Engineering Foundation.)

Dietary Phytoestrogens and Plasma Lipids in Dutch Postmenopausal Women; A Cross-Sectional Study. S. Kreijkamp-Kaspers, L. Kok, M. L. Bots, D. E. Grobbee, and Y. T. van der Schouw. Julius Center *for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands.

After menopause the incidence of cardiovascular disease increases. Phytoestrogens, estrogen-like compounds in plant foods, have been shown to reduce plasma lipids, glucose, and insulin when supplemented at high doses. Little is known about the effects on these endpoints of phytoestrogens in the range of intakes normal for Western diets. We conducted a population-based cross-sectional study in 301 women aged 60–75 y. Dietary isoflavone and lignan intakes were assessed by using a food frequency questionnaire covering the habitual diet the year preceding enrollment. The outcome measures were total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, lipoprotein(a) [LP(a)], fasting glucose, and insulin. We used linear and logistic regression analysis. In the analyses we adjusted for a wide range of potential confounders including lifestyle and dietary factors. Median isoflavone intake was 0.2 mg in the lowest tertile of isoflavone intake and 12.9 mg in the highest tertile of intake. Median lignan intakes were 0.8 and 2.1 mg, respectively. High intake of isoflavones was associated with lower LP(a) levels [odds ratio for high LP(a) = 0.39, 95% CI 0.16; 0.80]. No association with the other plasma lipids, glucose, or insulin was found. In conclusion, the results of this study support the presence of some beneficial effect of dietary phytoestrogens, even at the low levels common in Western populations. However, in view of the limited magnitude of the effect, it is premature to advise postmenopausal women with low phytoestrogen intake to change their diet towards a phytoestrogen-rich diet with the aim of preventing cardiovascular disease.

Soy Isoflavones Supplementation Attenuates Plasma Triglyceride Rise and Nuclear Factor- κ B Activation in Peripheral Blood Mononuclear Cells of Healthy Women during Postprandial Lipemia. C.-Y. Choi, S.-H. Ryu, G.-S. Moon, D.-S. Kim, and Y.-S. Song. Inje University, Kimhae, Korea.

Soy isoflavones have been postulated to protect against cardiovascular disease. One possible mechanism of action is through their antioxidative effect. We found genistein inhibits the activation of the redox-sensitive transcription factor, nuclear factor- κ B (NF κ B) in inflammatory macrophages *in vitro*. The purpose of this study was to examine the effect of isoflavone supplementation on lipid profile, oxidative stress, and NF- κ B activation during postprandial lipemia. Twelve healthy young women, aged 20–25 y, volunteered for this study and were examined via a blood test to exclude any pathological disorder. On 2 different days, all participants received the same fatty breakfast with or without 1 capsule containing 100 mg of a mixture of soy isoflavones (Amax NutraSource Inc.). One capsule of isoflavone mixture contained 13.5% daidzin, 26.3% daidzein, 38.6% genistin, 16.1% genistein, 5% glycitin, and trace amount of glycitein. The fat-enriched breakfast contained 1282 kcal, with 570 kcal (44.4%) as fat. Lipid profile and thiobarbituric acid-reactive substances (TBARS) were determined in blood samples taken at 0, 2, 4, and 6 h after breakfast. NF- κ B activity was determined by electrophoretic mobility shift assay (EMSA) in peripheral blood mononuclear cells (PBMCs) separated in Ficoll gradient by centrifugation; 5 μ g of nuclear proteins extracted from the PBMCs were used

for EMSA. The isoflavone content in the plasma was determined by the methods of King et al. (1) and Record et al. (2). HPLC analysis was conducted a 0.46 \times 25 cm i.d. C₁₈ reverse-phase column (ZORBAX SB C₁₈, HP) with a mobile phase consisting of 60:40 (v/v) methanol:ammonium acetate (1 mmol/L) at a flow rate of 1 mL/min. Subjects receiving the fatty breakfast without isoflavone supplementation had increased NF- κ B activation in PBMCs with the rise of triglyceride level in plasma. However, NF- κ B activation and triglyceride rise after the fatty meal was significantly attenuated in isoflavone-supplemented subjects compared with isoflavone-free subjects. Furthermore, oxidative stress measured by TBARS was significantly lowered at 6 h after breakfast in isoflavone-supplemented subjects. These results provide a new possible mechanism to explain the hypolipidemic and antioxidative effects of isoflavone supplementation in the prevention of cardiovascular disease. (Supported by grant NO. R01–2000-00187 from the Korea Science and Engineering Foundation.)

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Soy Phytoestrogens, Raloxifene, and Endothelial Function in Postmenopausal Women: A Randomized, Placebo-Controlled, Crossover Trial. D. L. Katz,*[†] M. A. Evans,* M. L. Hoxley,* V. Y. Njike,* H. Nawaz,* B. P. Comerford,* and P. M. Sarrel[†]. *Yale Prevention Research Center, CT; [†]Yale University School of Medicine, New Haven, CT.

OBJECTIVE: To compare the effects of soy phytoestrogens and raloxifene on endothelial function in healthy, postmenopausal women. **DESIGN:** Randomized, double-blind, placebo-controlled crossover trial. Subjects ($n = 22$; mean age 58.5 y) underwent endothelial function testing at baseline and after 6 wk of daily soy phytoestrogens (55 mg), raloxifene (60 mg), and placebo in random sequence with intervening 6-wk washout periods. Endothelial function was assessed as flow-mediated vasodilatation (FMD) of the brachial artery using high-resolution ultrasound; digital flux was measured with laser Doppler velocimetry. **RESULTS:** Baseline (pretreatment) FMD was essentially normal at 9.6% (± 6.4). FMD did not change from baseline within any treatment group and no between-group differences were detected. FMD after treatment with soy, raloxifene, and placebo was 8.27% (± 7.7), 10.28% (± 12.3), and 9.5% (± 4.4), respectively. Area-under-the-curve ratios showed no treatment differences for digital velocimetry. **CONCLUSIONS:** In this study of 22 healthy postmenopausal women, neither soy phytoestrogens nor raloxifene enhanced endothelial function. As measured in both the macro- and microcirculations, endothelial function was stable across treatments as were serum lipid values. These findings thus do not reveal a vasoprotective effect of either treatment. However, the cohort had relatively normal endothelial function at baseline. The phytoestrogen treatment in the study was convincingly without benefit. Although cholesterol fell with soy administration, it fell slightly more with placebo. FMD was similarly higher after placebo treatment than after 6 wk of soy. Further study is required to determine if particular subgroups of postmenopausal women derive vascular benefit from the use of selective estrogen receptor modulators or soy phytoestrogens.

Endothelial Function in Response to Sustained Consumption of Soy Protein and Lecithin By Healthy Postmenopausal Women. D. L. Katz,*[†] M. A. Evans,* M. Hoxley,* V. Yanchou Njike,* M. Pearson,[†] and B. P. Comerford*. *Yale Griffin Prevention Research Center, CT; [†]Yale University School of Medicine, Derby, CT.

OBJECTIVE: To assess the effects of soy isoflavone protein concentrate and soy lecithin on endothelial function measured as flow mediated dilation (FMD) of the brachial artery in healthy postmenopausal women. **DESIGN:** Randomized, double-blind, placebo controlled crossover trial. **SUBJECTS/SETTING:** Twenty-five subjects (mean age 61 y; body mass index 25.46) were recruited from the general population of southwestern Connecticut. **INTERVENTION:** Subjects underwent endothelial function testing using high-frequency ultrasound at baseline and after 4 wk of each randomly assigned treatment with intervening 4-wk washout periods. Treatment assignment included soy isoflavone protein (25 g/d) and soy lecithin (20 g/d); 1 active treatment and the alternative placebo; or double placebo. **MAIN OUTCOME MEASURES:** Endothelial function, assessed as FMD of the brachial artery, and serum lipids. **RESULTS:** Twenty-two women completed the trial; baseline FMD (pretreatment FMD) was 8.60 ± 7.20 . No statistically significant ($P > 0.05$) difference was seen in FMD between treatment assignments. A trend was suggested, however, with FMD highest after treatment with both soy protein and lecithin (7.50 ± 9.85) followed by soy protein and placebo lecithin (5.51 ± 10.11), placebo protein and soy lecithin (5.35 ± 6.13), and double placebo (4.53 ± 7.84). Soy isoflavone protein and soy lecithin significantly ($P < 0.05$) increased the HDL-LDL ratio relative to baseline (soy isoflavone protein and soy lecithin, 0.64 ± 0.19 ; soy isoflavone protein and placebo lecithin, 0.58 ± 0.17 ; placebo protein and soy lecithin, 0.65 ± 0.18 ; baseline, 0.49 ± 0.15). **CONCLUSION:** In this sample of healthy postmenopausal women, soy isoflavone protein and soy lecithin significantly improved the lipid profile. A favorable influence on endothelial function by the combination of soy protein and lecithin was suggested but could not be confirmed statistically, possibly because of small sample size, timing of testing, dose, or delivery vehicle (which contained sugar, shown to induce endothelial dysfunction in a dose-

dependent manner). Follow-up study that compensates for these limitations is warranted.

Effect of Soymilk and Whole-Milk Intake on Blood Lipids and Oxidative Damage in Koreans with Hyperlipidemia. H. J. Kim,* J. Y. Kim,* S. Kwon,[†] H.-K. Chung,[†] H.-S. Sohn,[†] C.-W. Chung,[†] J. H. Lee,* and Y. Jang**.*Department of Food & Nutrition, Yonsei University, Seoul; [†]Central Research Institute at Dr. Chung's Food Co., Ltd, Chungjoo-Si; **Cardiovascular Genome Center, Yonsei Medical University, Seoul, Korea.

Soy products offer several cholesterol-lowering components such as soy protein, isoflavones, and dietary fiber. In addition, isoflavones functioning as antioxidants may reduce the oxidative damage that is associated with atherosclerosis. To compare the effects of soymilk and whole milk on serum lipids and oxidative damage to DNA, 50 hyperlipidemic men and menopausal women were recruited and randomly assigned to 3 groups: soymilk (SM), whole milk (WM), and placebo (P). Subjects in each group consumed 3 servings of SM, WM, or P for 12 wk. Anthropometric, biochemical (lipid profiles, proteins, CBC, etc.), and antioxidative variables; oxidative damage to DNA by the Comet assay; and a lipid peroxidation parameter, malondialdehyde were measured before and after the 12-wk trial. Descriptive statistics and Wilcoxon signed ranks test were applied for comparing the variables in each group using SPSS for Windows. No statistical differences in variables were found within the 3 groups between men and women ($\chi^2 = 2.586$, $P = 0.274$). After the trial, subjects in the SM group showed decreases in waist and hip circumferences and malondialdehyde (Table 5). Although there were no differences in LDL cholesterol levels in any group, HDL cholesterol of subjects in the SM group increased significantly. Despite increased apolipoprotein B levels in the other groups, the SM group did not show any increase. Oxidative damage to DNA (i.e., tail moment) showed a downward trend in the SM group but was not statistically significant ($P = 0.053$). In conclusion, long-term soymilk intake may improve the blood lipid profile by increasing HDL cholesterol and by decreasing lipid peroxidation. Additional studies are recommended to

TABLE 5

Lipid profiles, and oxidative damage to DNA and lipid before and after 12 wk intake of soymilk, whole milk, and placebo in hyperlipidemic Korean men and women¹

	Soymilk (n = 20)			Whole milk (n = 10)			Placebo (n = 20)		
	Before	After	P ²	Before	After	P ²	Before	After	P ²
Waist, cm	86.9 ± 6.72	86.0 ± 6.43	0.014	80.1 ± 8.41	80.8 ± 5.83	0.344	86.3 ± 8.01	85.9 ± 7.71	0.147
Hip, cm	95.8 ± 4.96	94.8 ± 5.48	0.022	96.0 ± 2.71	95.3 ± 2.67	0.340	95.0 ± 4.50	95.5 ± 4.79	0.163
HDL, mmol/L	0.93 ± 0.05	0.99 ± 0.05	0.034	1.24 ± 0.02	1.32 ± 0.01	0.109	1.24 ± 0.05	1.28 ± 0.05	0.055
LDL, mmol/L	3.66 ± 0.07	3.66 ± 0.03	0.936	3.79 ± 0.06	3.92 ± 0.08	0.646	3.24 ± 0.03	3.41 ± 0.04	0.156
Apo B, g/L	1.06 ± 0.21	1.09 ± 0.19	0.219	1.07 ± 0.17	1.15 ± 0.17	0.036	1.25 ± 0.20	1.34 ± 0.30	0.014
Tailed DNA ³	9.29 ± 2.88	7.92 ± 2.02	0.147	9.89 ± 2.33	7.92 ± 3.17	0.139	7.65 ± 2.26	6.79 ± 1.80	0.345
Tail moment ⁴	6.78 ± 3.63	5.66 ± 2.56	0.053	8.48 ± 4.71	7.36 ± 5.72	0.767	5.32 ± 2.63	4.27 ± 2.70	0.917
Tail length, μm	54.5 ± 22.6	55.5 ± 15.1	0.398	73.6 ± 33.0	67.6 ± 37.0	0.594	51.2 ± 18.9	50.1 ± 21.6	0.917
MDA, ⁵ $\mu\text{mol/L}$	9.30 ± 4.42	7.98 ± 5.18	0.021	9.21 ± 2.80	9.78 ± 3.28	0.508	5.80 ± 1.58	6.43 ± 3.62	0.911

¹ Values are expressed as mean ± SD.

² Statistical significance was tested by Wilcoxon signed ranks test for each group.

³ % of DNA in the tail.

⁴ % of DNA in the tail × tail length.

⁵ MDA, malondialdehyde.

further investigate the potential benefits of soymilk for preventing DNA damage.

Accumulation of Genistein in Reconstituted Apolipoprotein-Lipid Discoidal Particles. M. H. Kaamanen,* H. Adlercreutz,[†]** M. Jauhiainen,[‡] T. Hakala,* K. Räsänen,* and M. J. Tikkanen*. *University of Helsinki, Department of Medicine, Helsinki, Finland; [†]University of Helsinki, Department of Medicine, Division of Clinical Chemistry, Finland; **Folkhälsan Research Center, Institute for Preventive Medicine, Nutrition and Cancer, Finland; [‡]National Public Health Institute, Department of Molecular Medicine, Finland.

The accumulation of oxidized LDL within the artery wall is one of the primary events in the pathogenesis of atherosclerosis. In our previous studies the dietary intake of soy-derived isoflavones increased LDL's potential to resist oxidation *in vitro* (1) while the underlying mechanism remained unknown. Plasma incubation with [³H]genistein resulted in the formation of lipophilic derivatives, identified as genistein monoesters, which accumulated in LDL and HDL particles (2). In addition, some relatively polar free genistein accumulated in the lipoproteins. In this study, our aim was to explore the structural features, composition, and size of model lipoprotein particles to investigate the potential of genistein to associate with the particles. We incubated different amounts of [³H]genistein (2, 4, 8, and 16 × 10⁵ dpm) for 30 min at +37°C with various reconstituted nascent discoidal particles containing apolipoprotein A-I (apoAI), phospholipids [pyridoxal (PL)] from egg yolk lecithin, and cholesterol (chol). The incubated particles were then purified by Sephadex G-25 gel filtration to remove any unbound genistein and protein content and radioactivity were determined. Our results show that ~0.2% of the added genistein was associated with purified apoAI as well as with the human albumin control. Genistein accumulation was enhanced in apoAI:PL (1:250) particles by >27-fold (from 0.2% to 5.5%) and the degree of accumulation remained the same in the concentration range used. However, the particles containing apoAI, chol, and PL (1 mol:12.5 mol:250 mol) enhanced genistein's accumulation only by eightfold compared with purified apoAI, or ~3.5 times less efficiently than in the absence of cholesterol. Reduced PL component (apoAI:chol:PL were either 1:12.5:125 or 1:12.5:75) enhanced the accumulation less, by 6.5- to 5-fold. The accumulation was enhanced in apoAI and PL particles without chol (1:12.5:75), by 7.5- to 10-fold compared with purified apoAI. In preincubated samples of genistein and apoAI, the association of genistein with apolipoprotein remained after repeated Sephadex G-25 gel filtration. Without incubation, the added genistein was not associated with apoAI in Sephadex G-25. In conclusion, genistein interacts with apoAI and the accumulation is strongly enhanced in reconstituted apolipoprotein-lipid discoidal particles. Genistein accumulated especially in the particles with the largest amount of phospholipids per apoAI in the absence of cholesterol. These results suggest that the lipid components of the particles are significant in the regulation of genistein's accumulation. The accumulation of genistein in lipoproteins may explain the increased oxidation resistance of LDL during intake of soybean isoflavones.

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Inhibitory Effects of Dietary 7S Globulin on Atherosclerosis in Mice Exceed Those of Whole Soy Protein Isolate and Are Independent of LDL Receptors and Plasma Lipoproteins. M. R. Adams,* D. L. Golden,* A. A. Franke,[†] S. M. Potter,** H. S. Smith,** and M. S. Anthony*. *Wake Forest University School of Medicine, Winston-Salem, NC; [†]Cancer Research Center of Hawaii, HI; **Solae Company, U.S.

The hypolipoproteinemic effects of soy can be attributed, at least in part, to its protein component. The 7S globulin, a major soy storage protein, is capable of stimulating the expression of LDL receptors by hepatocytes *in vitro*. Although these effects might be expected to lead to a reduction in cardiovascular risk, the *in vivo* effects of 7S on the cardiovascular system have not been addressed. We determined the effect of 7S globulin and other soy peptide fractions on the development of atherosclerosis in apolipoprotein E null mice and LDL receptor null mice (total n = 416). The comparison groups (differing only by the source of dietary protein) were 1) casein/lactalbumin, 2) isoflavone-replete soy protein isolate, 3) 7S globulin, 4) 11S globulin (the other major soy storage protein), 5) 7S-free soy protein isolate (produced from a cultivar that does not produce 7S globulin), and 6) W008 (a peptide fraction produced by proprietary methods). Aortic atherosclerosis (cholesteryl ester) and plasma lipoprotein cholesterol concentrations were quantified after 4 mo of feeding these diets. Relative to mice fed casein/lactalbumin-based diets, the extent of atherosclerosis was reduced in ovariectomized female mice by all diets containing soy protein. However, relative to mice fed isoflavone-replete soy protein isolate, atherosclerosis was reduced only in mice fed the 7S-containing diet. Reductions averaged 41% and 65% (*P* < 0.05) in male and ovariectomized female apolipoprotein E null mice and 140% (*P* < 0.05) and 39% (*P* < 0.10) in male and female LDL receptor null mice. These effects were not related to variation in isoflavone content of the protein source or plasma lipoprotein cholesterol concentrations. We conclude that a diet rich in 7S globulins has atheroprotective effects that greatly exceed those of isoflavone-replete soy protein isolate. Because these effects did not depend on LDL receptors or influences on plasma lipoproteins, the mechanism of action remains unclear.

Effect of Soybean Isoflavone Aglycone on Serum Cholesterol in Ovariectomized Rats and Healthy Human Volunteers. M. Saito,* T. Izumi,* A. Obata,* M. Yoshimura,* S. Kataoka,* M. Kikuchi,* N. Sato,[†] Y. Fukuwatari,[†] and Y. Ryu[†]. *2nd Department, Research and Development Division, Kikkoman Corp., Noda City, Japan; [†]School of Medicine, Juntendo University, Tokyo, Japan.

PURPOSE: Soybean isoflavone aglycone (IFA) shows many health benefits. This research examined the effect of IFA on serum cholesterol in ovariectomized rats (OVX rats) and healthy human volunteers. We also studied the safety of long-term IFA intake. **METHODS:** In the animal test, Sprague Dawley rats aged 33 wk were divided into 5 groups. A sham operation was performed on rats in group 1 and rats in groups 2-5 received ovariectomies. The rats were fed a calcium-deficient diet from their 34th week. IFAs were orally administered every day by gavage. At the end of test, blood was drawn and serum triglyceride and total cholesterol were measured. The human study was performed in 41 women aged 22-69 y, in accordance with the Helsinki Declaration. The subjects were divided into 2 groups, 18 women (aged 41.5 ± 15.1 y) in the placebo group and 23 women (aged 39.7

± 13.6 y) in the IFA (40 mg/d) treatment group. The subjects took 4 tablets/d for 6 mo. Serum biochemistry measurements were made at the beginning and end of the test. RESULTS: Animals: Compared with the sham operation group, the triglyceride and total cholesterol values of OVX groups increased remarkably. However, in the IFA-treatment groups, triglyceride and total cholesterol concentrations in serum were decreased in a dose-dependent manner. Humans: Total cholesterol did not change after the treatment in both groups. In the placebo group, LDL cholesterol showed a slight change; in the IFA treatment group, LDL cholesterol decreased significantly ($P < 0.05$, day 0: 3.21 ± 0.69 mmol/L) after 6 mo (3.02 ± 0.81 mmol/L). The other biochemical markers in both groups did not change significantly after treatment. CONCLUSION: IFA treatment improved the serum cholesterol level in OVX rats and the healthy humans tested. Moreover, long-term (6 mo) IFA treatment did not show any toxicity.

Hypolipidemic Effect of Dietary Soybean 7S Globulin (β -Conglycinin) Complemented Amino Acids in Obese Mice. M. Maebuchi, M. Machidori, R. Urade, T. Ogawa, N. Maruyama, and T. Moriyama. Graduate School of Agriculture, Kyoto University, Japan.

We investigated whether the hypolipidemic effect of soybean 7S globulin (β -conglycinin), a major component of soybean protein, is caused by the active components derived from 7S globulin or its amino acid composition. 7S globulin supplemented with amino acids to match the composition of casein used as control diet was fed to KK- A^y mice (genetically obese, noninsulin-dependent diabetes mellitus model mouse) for 2 wk, and the clinical variables in serum and the activities of enzymes in liver related to lipid metabolism were measured. Isoflavones and saponins had been removed from the 7S globulin by treatment with 70% ethanol; the amino acid compositions of the washed 7S globulin and the casein were matched to each other by supplementation with amino acids. Triglyceride, cholesterol, insulin, and glucose levels in serum were significantly lower in the 7S globulin diet group (7S-group) and the amino acid-supplemented 7S globulin diet group (7S+AA2-group) than those of amino acid-supplemented casein diet group (casein+AA1-group). Serum leptin concentration tended to be lower in 7S-group and the 7S+AA2-group relative to the casein+AA1-group. In addition, the activity of hepatic fatty acid synthase, a rate-limiting enzyme of fatty acid synthesis, was significantly lower in the 7S-group and the 7S+AA2-group than that of casein+AA1-group. Similar trends were also observed with protein amount and mRNA expression levels. The activities of acyl-CoA oxidase and carnitine-acyl transferase, key enzymes for fatty acid β -oxidation, were significantly higher in the 7S-group than those of the casein+AA1-group and the 7S+AA2-group. These results demonstrated that 7S globulin itself or the active components derived from 7S globulin such as the peptides, but not the amino acid composition of the 7S globulin, was responsible for the hypolipidemic effect. In regard to the regulation of hepatic lipid metabolism by feeding 7S globulin, suppression of fatty acid synthase was caused by the active components, and acceleration of β -oxidation was attributed to the amino acid composition of the 7S globulin. These results suggest that soybean 7S globulin (β -conglycinin) may be an effective food supplement for preventing hyperlipidemia.

Effect of Estrogen Receptor- α on Serum Cholesterol in C57BL6 Mice Fed High-Fat Diets. M. J. Mazur, J. King, M. A. Zakroczymski, P. S. Cooke, and J. W. Erdman, Jr. University of Illinois at Urbana-Champaign, IL.

Consumption of diets rich in soy protein has shown promise as a dietary intervention to reduce blood cholesterol levels. However, the mechanism of soy's cholesterol-lowering effect is not fully understood. This study was designed to determine whether the mechanism requires the presence of estrogen receptor- α (ER- α) for cholesterol reduction. Male (aged ~ 55 d) ER- α knockout and wild type C57BL6 mice were assigned to receive 1 of 3 high-fat, cholesterol-containing diets: casein protein, low-isoflavone soy protein (ISP-), high-isoflavone soy protein (ISP+) ($n = 12$). A control group for each genotype was assigned to a standard laboratory pellet diet to determine usual cholesterol levels in C57BL6 mice ($n = 8$). After 12 wk of feeding, mice were killed and total serum and hepatic cholesterol levels were evaluated. To date, two-thirds of the animals have completed the 3-mo feeding regimen. Although there were some trends, there was no significant difference in either serum and hepatic cholesterol or hepatic lipids due to genotype (ER- α knockout vs. wild type). There was a significant difference in serum cholesterol ($P < 0.0001$), hepatic cholesterol ($P = 0.0016$), and hepatic lipid ($P = 0.0426$) levels of mice due to diet (casein, ISP-, ISP+). Hepatic cholesterol was lower in mice fed ISP+ than either ISP- or casein whereas for serum cholesterol ISP- was higher than both ISP+ and casein. These preliminary results suggest in C57BL6 ER- α knockout and wild type mice that ER- α is not required for dietary modulation of either serum or hepatic cholesterol. These data also suggest that ethanol extraction of soy removes one or more compounds that are hypocholesterolemic to mice. (Supported in part by the Jonathan Baldwin Turner Undergraduate Research Scholarship Program, University of Illinois at Urbana-Champaign.)

Complementary Soy Therapy Prevents Dyslipoproteinemia Associated with Tibolone Treatment of Postmenopausal Cynomolgus Monkeys. S. E. Appt, T. B. Clarkson, M. S. Anthony, and R. W. St. Clair. Wake Forest University School of Medicine, Winston-Salem, NC.

Tibolone is a synthetic steroid used for treating menopausal symptoms and osteoporosis in many countries. A potential disincentive for its use is that it lowers HDL cholesterol (HDL-C) concentrations by $\sim 30\%$ (also reported in monkeys). Our aim was to determine whether coadministration with soy protein isolate containing isoflavones (SPI), which can increase HDL-C, would attenuate tibolone's HDL-C-lowering effects. Plasma LDL plus VLDL cholesterol (LDL+VLDL-C) and HDL function (cholesterol efflux from Fu5AH cells) were also measured. Monkeys ($n = 18$) were randomly assigned to receive 1 of 4 dietary regimens in a crossover design such that all animals receive all diets. Diets contained either casein/lactalbumin (C/L) or SPI as the protein source and then tibolone (Tib, women's equivalent 1.25 mg/d) was or was not added: 1) C/L, 2) C/L+Tib, 3) SPI, and 4) SPI+Tib. The data presented are from the first 3 periods (75%) of the study, but final data will be available for the meeting. As expected, compared with C/L, C/L+tib

TABLE 6

Data from the first 3 periods of the study¹

	C/L	C/L + Tib	SPI	SPI + Tib
HDLC, mmol/L	1.22 ± 0.01 ^a	0.518 ± 0.1 ^b	1.48 ± 0.1 ^a	1.23 ± 0.1 ^a
LDL + VLDLC, mmol/L	7.51 ± 0.52 ^a	8.34 ± 0.51 ^a	4.74 ± 0.54 ^b	4.87 ± 0.51 ^b
Cholesterol efflux, %	28 ± 1.7 ^a	27 ± 1.6 ^a	30 ± 1.7 ^a	29 ± 1.7 ^a

¹ Means with like letters are not significantly different at $\alpha = 0.05$.

treated monkeys had reduced plasma HDLC concentrations ($P < 0.0001$) whereas coadministration with SPI resulted in the conservation of HDLC. Despite the large reductions in HDLC in tibolone-treated monkeys, cholesterol efflux was not reduced. Preventing HDLC reduction with SPI did not increase cholesterol efflux. Plasma LDL+VLDLC was reduced with SPI and SPI+tib ($P < 0.001$) compared with C/L (Table 6). We conclude that coadministration of SPI with tibolone attenuates tibolone's HDLC-lowering effects. Whether this will affect coronary atherosclerosis needs to be determined.

The Isoflavone Glycitein and Group B Soyasaponins Lower Plasma Cholesterol in Female Golden Syrian Hamsters Fed a Diet High in Saturated Fat and Casein. S. Lee, P. Murphy, and S. Hendrich. Iowa State University, Ames, IA.

Soybean isoflavones (daidzein, genistein, or glycitein) and saponins were hypothesized to act as cholesterol-lowering components separate from soy protein. Purified daidzein, genistein, glycitein, (0.9 mmol isoflavone/kg diet), or group B soyasaponins (2.2 mmol/kg diet) were fed to female golden Syrian hamsters (11–12 wk of age, 10 hamsters per group) for 4 wk in a diet containing ~37% of energy as fat (62% from coconut oil), 25% casein, and 0.1% cholesterol. Hamsters fed glycitein or group B saponins had significantly lower plasma total cholesterol (by 16–19%) and LDL cholesterol (by 27–38%) compared with hamsters fed casein ($P < 0.05$). Daidzein and genistein's effects on these lipids did not differ from the effects of either casein or glycitein, but group B saponins lowered LDL cholesterol to a greater extent than did daidzein or genistein. Plasma triglycerides were also significantly less in hamsters fed daidzein and group B saponins compared with those fed casein, with the effects of glycitein and genistein not significantly different from any of the other treatments. There were no differences among treatments in plasma HDL cholesterol level. The percentage of urinary recovery of each isoflavone was glycitein > daidzein > genistein (16:2:1). The excretion of fecal bile acids and neutral steroids were significantly greater in the saponin group compared with the casein group. These data suggest that glycitein's greater cholesterol-lowering effect was due to greater bioavailability as reflected in urinary excretion of glycitein compared with the other isoflavones. Group B soyasaponins seemed to lower plasma total and LDL cholesterol levels by a mechanism involving greater excretion of fecal bile acids and neutral steroids compared with casein. Thus, both soy isoflavones and saponins in nutritionally relevant concentrations contribute to the cholesterol-lowering effects of soy foods and soy protein ingredients and might be useful as purified supplements for cholesterol-lowering as well.

Antihyperlipidemic Effects of Black Bean (*Yak-Kong, Rhynchosia molubilis*) and Soybean in an Ovariectomized Rat Model. S. A. Kang, K. H. Jang, Y. Cho, J. H. Suh, J. A. Han, and R. W. Choue. Kyung Hee University, Seoul, Korea.

This study examined the effects of water extracts of black bean and soybean on lipid metabolism in the osteoporotic model rats created by ovariectomy and a low-calcium diet. The experimental animals were divided into sham operation (SH) or ovariectomy (OVX) groups and then the OVX group was divided into black bean (OB), soybean (OS), and estrogen-treated groups (OE) and treated for 6 and 12 wk. The body weights of OVX group showed a significant increase compared with the SH group at 6 and 12 wk. Body weight gain of the OS and OE groups for 6 wk was decreased compared with the OVX group. The serum leptin levels in the OB, OS, and OE groups were significantly reduced compared with the OVX group. After 6 wk of supplementation, the serum insulin levels in the OVX group were significantly increased compared with the other groups; after 12 wk of supplementation, levels in the OB group were significantly reduced compared with the OVX group. Total cholesterol concentration increased significantly in the OVX group (2.47 ± 0.20 mmol/L) compared with the SH group (1.58 ± 0.47 mmol/L) after 12 wk. The triglyceride levels of the OB (0.78 ± 0.13 mmol/L), OS (1.01 ± 0.06 mmol/L), and OE (1.16 ± 0.19 mmol/L) groups were significantly decreased compared with the OVX (1.63 ± 0.32 mmol/L) group after 12 wk. The HDL cholesterol levels of the OS and OE groups were significantly increased compared with the OVX group after 12 wk. The HDL cholesterol levels of the OB group was increased compared with the OVX group but the difference was not significant. Free fatty acid levels of the OVX group were significantly increased compared with the SH group after 12 wk; the levels of OB and OS groups were increased but not significantly compared with the OVX group. These results demonstrate that black bean and soybean intake have hypolipidemic effects on osteoporosis. Our study suggests that further investigation of *yak-kong* and soybean on preventing weight gain and improving lipid metabolism is warranted.

Dietary Soy Protein Improves Body Composition and Lipids but Not Insulin Sensitivity in Obese, Male Cynomolgus Monkeys. M. Shadoan, J. Collins, and J. Wagner. Wake Forest University School of Medicine, Winston-Salem, NC.

We previously showed that dietary soy protein improves body composition, lipids, and insulin sensitivity in ovariectomized cynomolgus monkeys. Human studies suggest that there may be gender-specific responses to soy protein and soy isoflavones, so we wanted to examine the effect of soy protein on body composition, lipids, and insulin sensitivity in obese, insulin-resistant male monkeys. The study design was a Latin-square, with each monkey ($n = 8$) receiving each of the following

dietary proteins for 10 wk: 1) casein and lactalbumin, 2) soy(+), and 3) alcohol-washed soy(-). The following measures were assessed at the end of each treatment phase: food consumption, body weight, body composition by dual energy X-ray absorptiometry analysis, blood pressure and pulse, lipid and lipoprotein concentrations, and insulin sensitivity by minimal model analysis. Skeletal muscle biopsies taken at the end of each phase were assessed for expression and activity of the insulin receptor by immunoblotting. Soy(-) decreased food consumption, body weight, fat mass, and pulse compared with soy(+). Compared with casein/lactalbumin, soy(-) was also associated with decreased fat mass, total plasma cholesterol, LDL cholesterol, and systolic blood pressure. Fasting leptin concentrations were increased whereas 2-h postfood concentrations were decreased with soy(-) compared with the other 2 dietary proteins. There was no effect of soy protein on insulin sensitivity or skeletal muscle insulin receptor activity despite a reduction in insulin receptor expression with soy protein. The variation in food consumption may have been due to either palatability of the diets or effects on satiety because there were treatment differences in leptin concentrations. Additional studies with greater numbers and of longer duration are needed to further assess these changes.

The Effects of a Soy-Rich Diet on Lipid Profile and Lipid Peroxidation in Perimenopausal Thai Women. S. Songchit-somboon,* K. Chanda,* D. Danboonchan,* C. Manomai,[†] J. H. Hong,[‡] and S. Komindr**.*Division of Nutrition and Biochemical Medicine, Research Center, [†]Department of Obstetric-Gynecology, and **Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand; [‡]School of Food and Life Science, Inje University, Korea.

BACKGROUND: In Thailand, soy is consumed in various forms, including soymilk, tofu, soy sauce, soybean powder, and vegetarian foods. These soy foods have attracted the attention of middle-aged Thai women seeking alternative medicine. This growing interest in the use of soy has been focused on the protective effects of cardiovascular disease and breast, endometrial, and prostate cancers. **OBJECTIVE:** To determine whether incorporating reasonable amounts of soy foods in the habitual diet of perimenopausal women would improve the lipid profile and lipid peroxidation. **METHODS:** Thirty-eight perimenopausal women aged 40–59 y participated in a randomized crossover trial with two 12-wk diets and a 4-wk washout period before and between treatments. The study diets consisted of a control diet (soy-free diet) and an isocaloric soy-rich diet (25 g soy protein in various forms of soy foods containing >50 mg/d of isoflavones substituted for an equivalent amount of animal protein). Subjects were asked to fill in daily food lists of eaten soy. During both study periods, subjects consumed self-selected diets with low-fat and low-cholesterol foods. Fasting blood samples were drawn and blood pressure (3 times by the same person throughout the study) was measured at the start and end of each diet. **RESULTS:** Compared with baseline, a significant reduction in fasting blood sugar, LDL cholesterol (LDL-C), HDL-C, total cholesterol/HDL-C, LDL-C/HDL-C, and diastolic blood pressure was observed after both diets. The soy-rich diet resulted in significantly lower total cholesterol, lipid hydroperoxides, and systolic blood pressure. At the end of the 12 wk, serum total cholesterol was significantly lower in the soy-rich diet than in the control diet ($P = 0.009$). **CONCLUSIONS:** For practical and sustainable outcomes, the substitution of soy-rich diets for

animal proteins was carried out by a partial meal replacement method. Subjects were advised to drink 1 box of soymilk every day and to cook other alternative soy foods according to their own recipes and eat them with other foods for dinner. Although the dietary protein intake of the subjects on soy-rich diet was significantly increased compared with control diet, the cholesterol intake was significantly decreased because of the partial reduction in animal protein and the addition of soy protein. However, the data still showed that blood lipids, lipid hydroperoxides, and blood pressure in these subjects were significantly reduced after 3 mo of the soy-rich diet. Therefore, substitution of soy foods for animal protein may reduce cardiovascular risk in perimenopausal Thai women. (Supported by grants from Thai Health Promotion Foundation.)

Alpha Prime Subunit from 7S Soy Globulin Upregulates Beta-VLDL Liver Receptors in Rats Fed a Cholesterol-Rich Diet. S. Castiglioni,* C. Manzoni,* V. Dani,[†] M. Duranti,[†] P. Morazzoni,** and M. R. Lovati*. *Dept. Pharmacol. Sciences, University of Milano, Italy; [†]Department of AgroFood Molecular Sciences, University of Milano, Italy; **INDENA Scientific Department, Italy.

Recent data concerning the effect of soybean 7S globulin subunits on the upregulation of LDL receptors in HepG2 cells indirectly identify the α' subunit as the candidate responsible for this biological effect. With a recently developed separation technique (patent MI2002 A000147), it became possible to purify the α' subunit from the other 7S globulin components, the α and β subunits, thus allowing us for the first time to evaluate whether this subunit alone is responsible for the upregulation of LDL receptors, as previously suggested. The procedure, based on metal affinity chromatography under dissociating conditions, permits the large-scale isolation of 7S soybean globulin and its α' subunit. The availability of isolated α' subunit from 7S soy globulin prompted us to investigate the influence of this polypeptide on plasma cholesterol and triglyceride levels, as well as on the activity of liver lipoprotein high-affinity receptors in rats fed a cholesterol-rich diet (HC). When administered by gavage to rats fed HC diet, at daily concentrations of 10 and 20 mg/kg body weight, the α' chain significantly reduced plasma cholesterol (-16% and -31%, respectively) and triglyceride (-22% and -33%, respectively) levels as compared with the control group. The activity of liver lipoprotein high-affinity receptors, measured in HC rats treated with the highest dose of α' subunit, showed a remarkable increase of the binding (+96%) as compared with the control group (HC diet alone), thus restoring the receptor activity, normally depressed by HC diet administration. The present results, while confirming our previous in vitro findings on the upregulation of LDL receptors by the α' subunit from 7S globulin, are the first direct in vivo evidence of the effect of soybean 7S globulin α' subunit on lowering plasma cholesterol and triglycerides as well as upregulating β -VLDL receptor in HC rats after oral treatment with this polypeptide.

Soy and obesity, diabetes, and kidney disease

Soy Isoflavones Eliminated Weight Gain Associated with Protein Supplementation in Sedentary Males. A. S. Santo,* R. A. Melton,* H. W. Burton,* J. J. Leddy,[†] R. W. Browne,** S. M. Horvath,* and P. J. Horvath*. *Department of Exercise and Nutrition Sciences, [†]Department of Orthopedics, and **Biotechnical & Clinical Laboratory, University at Buffalo, Buffalo, NY.

Cardiovascular disease (CVD) is the leading cause of death for both men and women in the United States. Increased body mass index (BMI), body weight, percent body fat, and blood pressure are associated with an elevated risk for CVD. Soy protein with and without isoflavones has been associated with a decreased risk for CVD. We hypothesized that ingestion of a protein supplement (soy or casein) would increase BMI, body weight, percent body fat, and blood pressure. Furthermore, we hypothesized the addition of isoflavones to soy would reduce this protein supplement effect. As part of a double-blinded study on postprandial lipemia, 25 male volunteers aged 18–35 y were recruited. BMI (expressed as kg/m²), body weight, percent body fat, and blood pressure averages were 24, 74 kg, 21%, and 113/72 mm Hg, respectively. Subjects were randomly assigned to 1 of 3 protein groups: isoflavone-rich soy, isoflavone-poor soy, or casein. Participants supplemented their normal diet with 25 g/d of protein isolate for 28 d. BMI ($P < 0.02$) increased by 0.27 and 0.30 and body weight ($P < 0.003$) increased by 1.1 and 1.1 kg, respectively, for the casein and isoflavone-poor soy protein groups. No changes in body weight or BMI were seen in subjects consuming the isoflavone-rich soy protein supplement. Percent body fat and blood pressure did not change after any supplementation. Results indicate that isoflavones reduced the amount of weight gain associated with adding a protein supplement to an individual's diet. Blood samples remain to be analyzed for various fasting and postprandial CVD risk factors. (Protein supplements were donated by DuPont Protein Technologies, St. Louis, MO.)

Metabolic and Hormonal Effects of Dietary Soy Phytoestrogens on Young and Mid-Aged Male Rats. L. Bu and E. Lephart. Brigham Young University, Provo, UT.

We previously demonstrated that consumption of dietary phytoestrogens increase brain neuropeptide Y (NPY) levels via diminished serum leptin concentrations from reductions in adipose tissue deposition. In this study, we examined regulatory behaviors in male Long-Evans rats fed 4 different diets containing 0 ppm (AIN), 10 ppm (Phyto-'free'), 200 ppm (Phyto-200), or 600 ppm (Phyto-600) of isoflavones during aging, from age 50 to ~300 d. After 60 d and subsequently 200 d of the diets, food and water intake levels and body weights were recorded. When the rats were ~330 d of age, serum leptin levels and white adipose tissue weights were measured. Body and white adipose tissue weights were highest in the Phyto-'free' group, the AIN and Phyto-200 groups displayed intermediate values, whereas Phyto-600 group showed the lowest body weights. Serum leptin concentrations corresponded to white adipose tissue weights; the highest leptin levels were in the AIN and Phyto-'free' groups whereas the lowest leptin levels were in the Phyto-600 group. Food and water intake was significantly increased in the Phyto-600 group compared with the AIN or Phyto-'free' groups. Because leptin is known to decrease levels of brain NPY, which regulates food intake, it is reasonable to suggest that the significant reduction in WAT deposition in the Phyto-600 group contributed to the significant increases in food and water intake. Conversely, the opposite pattern was observed for these variables in the AIN and Phyto-'free' groups. These data suggest that consumption of dietary isoflavones increases food and water intake while decreasing WAT deposition and thus serum leptin production, implicating alterations in neuroendocrine mechanisms being involved in modifying regulatory behaviors. (Supported by USDA 2002–00798 to EDL.)

Attenuation of Early Renal Injury in Rats with Chronic Renal Disease Is Observed after Only 1 Wk of Soy Protein Feeding. D. E. Fair,* E. Nitschmann,[†] N. Bankovic-Calic,[†] M. R. Ogborn,*[†] H. A. Weiler,*[†] and H. M. Aukema*[†]. *Department of Human Nutritional Sciences and [†]Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada.

Dietary soy protein slows disease progression in animal models of chronic renal injury. To determine how early dietary soy protein feeding alters the progression of chronic renal injury, 58 male and 57 female weanling Han:SPRD-cy rats with genetically determined chronic renal disease were fed diets based on soy protein or casein for 1–3 wk. At the end of these periods, kidneys were taken for analyses. Kidney sections were assessed for fibrosis by staining with aniline blue and for cyst development with hematoxylin and eosin. Fibrous and cyst volumes were quantified by computer image analyses. The rats grew equally well on both diets, but soy protein feeding significantly reduced fibrous volume in male rats by 19% ($P = 0.0455$) after 1 wk and by 40% ($P = 0.0059$) after 3 wk of feeding but did not affect fibrosis development in female rats. Conversely, dietary soy protein reduced renal cyst volumes in female rats by 17% ($P = 0.0352$) after 1 wk and by 28% ($P = 0.0017$) after 3 wk of feeding while reducing cyst volumes in males only after 3 wk (by 34%, $P = 0.0008$). Soy protein feeding also reduced renal enlargement by 27% ($P < 0.0001$) in males after 3 wk of feeding soy protein compared with casein. Hence, the first signs of a beneficial soy protein effect were observed after 1 wk of feeding, with further improvements in histologic changes occurring after 3 wk. In females the early effect was a reduction in cyst expansion whereas in males the early effect was a reduction in the development of fibrosis. This study demonstrates the potential of soy protein to delay renal disease progression in the early stages of renal disease, reinforcing the importance of early detection of renal disease and the utility of early dietary intervention with soy protein in renal disorders. (Supported by the Kidney Foundation of Canada and the Children's Hospital Foundation of Manitoba.)

Preparative-Scale Purification of Soyasaponins for Use in a Murine Model of Polycystic Kidney Disease. F. W. Collins,* D. Philbrick,[†] D. Bureau,** R. Assabgui,* and B. Holub[†]. *Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada; [†]Department of Human Biology & Nutrition Science and **Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada.

In recent times, numerous studies using crude, multicomponent extracts of soy have been used in clinical and animal efficacy trials. Although these results may be indicative of overall effects, in-depth interpretation of the potential mode of action, activity kinetics, dose response, and possible synergism-antagonism interactions becomes more difficult. To help overcome these shortfalls, relatively large amounts of pure substances are needed. In the case of soyasaponins, the lack of pure standards and substantial quantities of individual components has hampered definitive study of their potential therapeutic use in human health. We have developed relatively simple, scaleable purification protocols suitable for both the analytical evaluation and the bulk preparation of gram quantities of soyasaponins from crude extracts and industrial fractionation streams. Novasoy 400 and a prototypical soy molasses fraction (Central Soya) were used for the large-scale

purification of ~5 g Soyasaponin B_b. The process involved first defatting an acidified 80% ethanol solution of the starting material (containing ~5.3% Soyasaponin B_b) using preparative low-pressure column chromatography on Octyl Sepharose in 80% ethanol. The defatted extract was then subjected to ion exchange chromatography on QAE Sephadex A-25 in the formate form and hydrophobic interaction chromatography of the recovered anionic fraction on a hexadecyltrimethylammonium-substituted Sepharose Q Fast Flow column according to U.S. patent application #20020107209. The recovered Soyasaponin B_b was then recrystallized from hot aqueous ethanol at >99.5% purity. Details of the separation and applications of the protocol for process scale production of other soyasaponins will be presented.

Glucose Uptake in Soleus Muscle of Casein and Soy-Fed Female Ovariectomized Sprague-Dawley Rats. C. Stevens, A. Lautenschlaeger, L. McCaskill, and V. Haley-Zitlin. Department of Food Science and Human Nutrition, Clemson University, Clemson, SC.

The effect of soy protein with (soy plus, SP) and without isoflavones (soy minus, SM) versus casein (CS) on soleus muscle glucose transport was compared in 60 female Sprague-Dawley rats (initial age 24 wk) with ovaries intact (Sham) or removed (Ovx). A balanced Teklad diet (isocaloric; isonitrogenous) was pair-fed (20 g/d) for 90 d and free access to water was provided. Animals were housed in wire cages on a 12-h light-dark schedule. After the treatment period, animals were anesthetized with isoflurane (4% mix with O₂ at 1.5–2.0 L/min) and soleus muscles were immediately dissected from both hind limbs with tendons intact. The right soleus was split longitudinally for uptake of 2-deoxy-D-glucose (2DG); the left soleus was frozen in liquid nitrogen and stored at –80°C for analysis of GLUT4/GLUT1 transporter protein and m-RNA. Ovx had significantly greater final body weight than did Sham (349.3 ± 3.37 vs. 289 ± 3.31 g, $P < 0.0001$) and greater average daily feed intake (16.96 ± 0.27 g vs. 15.78 ± 0.27 g, $P < 0.005$) (Table 7). Whether feed intake is responsible for the weights of Ovx or estrogen deficiency contributed to increased lipogenesis is uncertain. Insulin-stimulated glucose uptake was significantly greater in Sham animals ($P < 0.05$). Insulin resistance in Ovx groups is supported by blunted glucose uptake and increased adiposity (data not shown). Diet composition had no significant effect on weight gain (P

= 0.055), feed intake ($P = 0.67$), or glucose uptake ($P = 0.597$). Further analysis will help determine other contributing factors responsible for differences observed. (Supported by DuPont Protein Technologies.)

The Effect of Soy Containing Isoflavones on Body Composition, Handgrip Strength, and Physical Performance in Postmenopausal Women: A Double-Blind Randomized Trial. L. Kok, S. Kreijkamp-Kaspers, D. E. Grobbee, and Y. T. van der Schouw. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands.

The several physiological changes occurring during the process of aging may have serious health consequences, such as increased risk of chronic diseases and disability. It has been suggested that the decline in estrogen level after menopause may be responsible. Estrogen replacement therapy (ERT) may prevent or postpone those changes but is also associated with serious side effects. The goal of this study was to investigate the effects of high-dose isoflavone supplementation on body composition, handgrip strength, and physical performance. We conducted a double-blind randomized placebo-controlled study; 202 postmenopausal women aged 60–75 y were randomly assigned to receive 99 mg isoflavones in 36.5 g soy protein or casein (placebo) per day for 12 mo. Endpoints were body mass index, waist-to-hip ratio, handgrip strength, self-reported functional status, mobility, and physical performance. Handgrip strength was only assessed at the final visit in a subgroup of 88 participants. Linear regression was used to analyze differences in change between the 2 groups during the intervention. The randomization was successful, and 153 (75%) women completed the intervention. In both groups body mass index and waist-to-hip ratio decreased but the change was not statistically significantly different between the intervention groups. Handgrip strength at the final visit was slightly better in the placebo group than in the soy group, but this difference was not statistically significant. Self-reported functional status, mobility, and physical performance all slightly improved during intervention but there were no differences between the groups. The results of the present trial do not support the view that phytoestrogens have favorable effects on body composition, physical performance, and muscle strength in postmenopausal women.

TABLE 7

Results¹

	ShamSM	ShamSP	ShamCS	OvxSM	OvxSP	OvxCS
Initial body weight, g	272.2 ± 3.66 ^a	272.9 ± 5.78 ^a	273.6 ± 3.54 ^a	272.8 ± 5.2 ^a	274.2 ± 5.12 ^a	272.9 ± 4.89 ^a
Final body weight, g	282.3 ± 4.16 ^a	292.63 ± 6.18 ^a	292.18 ± 4.72 ^a	339.69 ± 5.09 ^b	361.5 ± 7.92 ^b	352.4 ± 5.23 ^b
Avg. daily feed intake, g	15.59 ± 0.51 ^a	16.17 ± 0.5 ^a	15.58 ± 0.63 ^a	16.68 ± 0.41 ^b	16.85 ± 0.42 ^b	17.51 ± 0.35 ^b
Basal 2DG uptake, mmol/L intracellular water/20 min	3.53 ± 0.21 ^a	4.43 ± 0.70 ^a	4.25 ± 0.90 ^a	3.97 ± 0.47 ^a	4.37 ± 0.69 ^a	4.46 ± 0.85 ^a
Insulin-stimulated 2DG uptake, mmol/L intracellular water/20 min	4.97 ± 0.59 ^a	5.1 ± 0.92 ^a	5.85 ± 0.56 ^a	4.64 ± 0.39 ^a	4.48 ± 0.56 ^a	4.15 ± 0.32 ^a

¹ Results reported as mean ± SEM. Values with different letters are significantly different, $P < 0.05$.

Effects of A Soy Carbohydrate-Protein Beverage and a Whey Carbohydrate-Protein on Muscle Damage in Collegiate Athletes. P. Bordi, C. Cole, G. Salvaterra, T. Hartman, and J. Paterno. The Pennsylvania State University, University Park, PA.

The objective of this study was to determine the effect of isolated soy protein and isolated whey protein in terms of change of urinary and plasma markers of muscle damage or oxidation, using healthy athletes as subjects. This study was a single-blind randomized crossover trial comparing the effects of soy and whey carbohydrate-protein beverages on exercise-induced muscle damage. The subjects were recruited from a NCAA Division I football team. All subjects participated in total-body weight-training sessions and aerobic-based running sessions. Biological fluids were taken from subjects before and after exercise at baseline, wk 3, wk 4 (after a washout period), and 7 wk. During the treatment periods subjects consumed soy protein or whey protein beverages. Biomarkers were measured before and after exercise. **BIOMARKERS:** Biomarkers included creatine kinase (CK), commonly used for the past 30 y to diagnose muscle damage in various muscle skeletal disorders with no neurological basis, acute myocardial infarction, and cardiac ischemia; lactic dehydrogenase (LDH), a catalytic enzyme used in the reversible conversion of lactic acid to pyruvic acid; and myeloperoxidase (MPO), an iron-containing protein considered a human enzyme, found largely in granules of neutrophils. MPO is the catalyst in the conversion of hydrogen peroxide and chloride ions to hypochlorous acid. **RESULTS:** A test of the fixed effects showed ($P > 0.05$) no significant differences between the 2 groups. Race ($P = 0.0246$), treatment ($P = 0.0003$), start date of intervention ($P = 0.0009$), and pre- or postexercise ($P < 0.0001$) all significantly affected CK. Similar findings are noted for LDH (group effect and race, $P > 0.05$). Treatment ($P = 0.0192$), start date of intervention ($P < 0.0001$), and pre- or postexercise all significantly affected LDH. Baseline measurements for CK and LDH showed no significance ($P > 0.05$) whereas the baseline measurements for MPO showed significance ($P < 0.0001$). **CONCLUSION:** Exercise produced changes in CK, LDH, and MPO in both whey and soy treatment. There was significant difference ($P > 0.0001$), post exercise for CK, LDH, and MPO for soy and whey. When compared by interventions, soy or whey treatment, CK ($P = 0.0003$) and for LDH ($P = 0.0192$) was reported. MPO levels were significantly different at baseline when comparing groups, suggesting a possible advantage in recovery. The group receiving soy as treatment first had statistically significant ($P < 0.0001$) lower MPO values at baseline for the second treatment period when they were crossing over to whey.

Renal Function and Urinary Sodium Excretion Are Not Affected by Dietary Isoflavone Intake in Adult, Normal Male Rats Pair-Fed Diets Based on Casein or Soy Protein. P. Fanti,* J. Stevens,* W. Titlow,† C. Ott,† and B. Jackson.†
*Division of Nephrology, Bone and Mineral Metabolism and †Department of Physiology, University of Kentucky Medical Center, Lexington, KY.

INTRODUCTION: Soy-based vegetarian diets may be superior to animal protein-based diets for preservation of renal function in diabetic patients at risk for nephropathy. Whether this beneficial effect is related to the soy protein itself or to the isoflavones is not known. The recent description of diuretic effects of purified soy isoflavones in rats is consistent with the

TABLE 8

Weight gain and biochemical variables in adult male rats fed semisynthetic diets for 3 wk¹

	Casein group	Soy (-) group	Soy (±) group
Start weight, g	216 ± 3.0	214 ± 2.7	214 ± 2.4
End weight, g	313 ± 4.5	296 ± 6.7	298 ± 3.1
2-wk weight gain, g	97.3 ± 3.1	82.6 ± 5.3	84.1 ± 4.1
s IGF-I, nmol/L	82.6 ± 5.7	79.5 ± 5.4	74.3 ± 5.7
s Urea, mmol/L	5.04 ± 0.3	5.04 ± 0.2	5.18 ± 0.4
s Creatinine, mmol/L	44.2 ± 1.8	53.1 ± 1.8	61.9 ± 1.8
u Nitrogen, mg/d	238 ± 23	262 ± 14	238 ± 22
C _{creat} , mL/min/kg	4.62 ± 0.59	4.21 ± 0.26	4.11 ± 0.39
s Na [±] , mmol	141.2 ± 0.8	142.0 ± 0.8	141.4 ± 0.8
u Na [±] , mmol/d	1.26 ± 0.11	1.32 ± 0.09	1.19 ± 0.11
Fract.Ex. Na [±] , %	0.44 ± 0.03	0.53 ± 0.05	0.49 ± 0.05
s K [±] , mmol	5.7 ± 0.2	6.0 ± 0.1	5.9 ± 0.2
u K [±] , mmol/d	2.25 ± 0.18	2.56 ± 0.14	2.42 ± 0.24
Fract.Ex. K [±] , %	19.6 ± 1.1	24.4 ± 2.1	24.6 ± 2.8

¹ s = serum; u = urine; Fract.Ex. = fractional excretion.

possibility that at least some effects of soy on renal function may be mediated by modulation of renal sodium handling. To start assessing the relative importance of isoflavones vs. soy protein in the prevention of diabetic nephropathy, we conducted preliminary studies in experimental rats. **METHODS:** Twenty-four healthy adult Sprague-Dawley male rats were divided into 3 groups (8/group) and were pair-fed semisynthetic diets that were nutritionally matched except for protein quality and isoflavone content. At the end of 2-wk pair feeding with either isoflavone-free casein diet (Casein group), isoflavone-free soy protein diet (Soy(-) group), or isoflavone-containing soy protein diet (Soy(±) group), metabolic studies were conducted to assess growth rate, serum insulin-like growth factor-I (IGF-I); renal function as expressed by creatinine clearance (C_{creat}); and renal excretion of urea nitrogen, sodium, and potassium. **RESULTS:** As shown in Table 8, the weights were comparable in the groups although a trend was noted toward lower IGF-I in the Soy(±) group. Serum creatinine and urea nitrogen were comparable and no difference was present in urinary excretion or fractional excretion of urea nitrogen, serum creatinine, sodium, and potassium. The C_{creat}, adjusted for body weight, was not different between groups although a trend was noted for lower levels in the Soy(±) group. **SUMMARY AND CONCLUSION:** The isoflavone content of soy protein-based diets did not affect renal function in pair-fed normal rats. In addition, dietary isoflavones did not cause detectable diuretic effects and did not alter renal electrolyte handling. A trend was noted toward lower creatinine clearance and serum IGF levels in the Soy(±) group. These findings do not exclude the possibility that soy isoflavones may cause detectable biological effects after more prolonged administration and/or in the presence of altered renal physiology, such as with impending or established diabetic nephropathy.

Soy Protein Feeding Selectively Reduces Renal Cyclooxygenase-1 (COX-1) Protein Expression in Normal and Diabetic Han:SPRD-cy Rats. H. M. Aukema,*† and M. R. Ogborn.*†
*Department of Human Nutritional Sciences and †Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada.

Dietary soy protein has been shown to retard the progression of injury in several models of chronic renal disease. To investigate potential mechanisms for this beneficial effect on the kidney, we examined the effect of soy protein on the levels of several renal enzymes that regulate eicosanoid and phosphoinositide synthesis and degradation. Our previous studies in the Han:SPRD-cy rat and *pcy* mouse have shown that these signaling pathways are altered in diseased kidneys. In this study, weanling Han:SPRD-cy rats with and without chronic renal disease were fed diets containing either soy protein or casein as the protein source for 6 wk. At the end of the study, renal disease progression was markedly attenuated in the animals fed soy protein, with less histological damage, lower serum urea, and creatinine and higher creatinine clearance than the rats fed casein. Immunoblotting was used to determine the steady state levels of eicosanoid related [cyclooxygenase-1 (COX-1), COX-2, cytosolic phospholipase A₂ (cPLA₂)] and phosphoinositide-related enzymes (cPLA₂, phospholipase C_{γ1}, phosphatidylinositol 4-kinase β) known to be altered in the kidneys of diseased Han:SPRD-cy rats were determined. Consistent with previous reports, the levels of COX-1 and cPLA₂ were higher and the levels of COX-2 were lower in diseased kidneys. Feeding soy protein resulted in a selective reduction in the elevated protein levels of COX-1. Not only did soy protein compared with casein feeding reduce COX-1 protein levels in diseased kidneys, but it also reduced COX-1 in normal kidneys. Neither cPLA₂ nor COX-2 proteins were altered by soy protein feeding. The phosphoinositide-related enzymes were elevated in diseased kidneys but were not influenced by soy protein feeding. The fact that soy protein selectively reduced the level of COX-1 in both normal and diseased kidneys indicates that soy protein mediates its effects at least in part by reducing eicosanoid production by the COX-1 enzyme. (Supported by the Natural Sciences and Engineering Research Council of Canada.)

Comparison of Weight and Lipid Responses to Soy and Milk Meal Replacements. J. Anderson* and L. Hoie[†]. *Metabolic Research Group, University of Kentucky, Lexington, KY; [†]NutriPharma, Oslo, Norway.

Soy protein, as compared with animal protein, has greater hypocholesterolemic effects and may have specific effects to facilitate weight loss and decrease visceral adipose tissue. We compared weight loss and lipid changes with 2 meal replacements (MRs)—a soy-based and a milk-based MR. Ninety-one obese women and men were randomly assigned to receive 1200-kcal diets providing either soy (ScanDiet/Nutriplan) or milk (Slim-Fast) MRs and limited nutrition instruction for 12 wk. Body weight was measured every 2 wk and lipids and waist circumferences were measured at 0, 6, and 12 wk. Subjects averaged 3.8 soy MRs or 1.9 milk MRs daily. With soy MRs subjects lost more weight at each measurement point than with milk MRs. Subjects who completed the study lost more weight at 12 wk with soy (9.1%) than with milk MRs (7.9%) but differences were not significant. Weight responses were similar with last-observation-carried-forward and mixed-model analyses. Reductions in waist circumferences were substantially greater with soy MRs than milk MRs. Serum cholesterol and LDL cholesterol were significantly lower with soy than with milk MR. At 6 wk cholesterol values were decreased 14% for soy vs. 8% for milk MRs ($P = 0.023$) and LDL cholesterol values were decreased 18.4% for soy vs. 8.9% for milk MRs ($P = 0.027$). Significant LDL cholesterol and cholesterol reductions were sustained at 12 wk with soy but not

with milk MRs. MRs use is a potent adjunct to weight loss. At recommended intakes, use of soy MRs promotes more weight loss, greater loss of visceral adipose tissue, and significantly more LDL cholesterol and cholesterol reductions than does use of milk MRs. (Supported by a grant from NutriPharma, Oslo, Norway.)

Evidence That Soyasaponin B₆ Retards Disease Progression in a Murine Model of Polycystic Kidney Disease. D. Philbrick,* D. Bureau,[†] F. W. Collins,** and B. Holub*.
*Department of Human Biology and Nutrition Science and [†]Department of Animal & Poultry Science, University of Guelph, Guelph, Ontario, Canada; **Eastern Cereal & Oilseed Research Centre, Canada.

We reported a lessened cyst growth in the *pcy* mouse model of polycystic kidney disease when mice were fed a soy protein isolate (SPI)-based diet and hypothesized that the soyasaponins may be associated with this therapeutic effect. The effects of feeding a saponin-enriched alcohol extract (SEAE) from soy protein isolate, an isoflavone- and saponin-enriched soy supplement (Novasoy400), or a 99.5% pure soyasaponin B₆ powder on cyst growth are reported here. These studies were carried out in 60-d-old male *pcy* mice in 2 separate 90-d feeding trials. In the first study, mice were fed either a casein-based control diet, a diet in which SPI replaced the casein, or the control diet supplemented with SEAE. In the second study, mice were fed the control diet without soy supplementation or diets supplemented with a soyasaponin plus an isoflavone- and saponin-enriched soy product or a 99.5% pure soyasaponin B₆ powder. Results from study 1 showed that kidney weight, water content, and plasma creatinine and urea levels were markedly reduced in the SEAE-fed animals compared with the control group; likewise, mice fed the SPI-based diet showed a decreased plasma creatinine but only a slightly reduced plasma urea. In study 2, kidney weight, water content, plasma creatinine, and urea levels were significantly reduced in mice fed the soyasaponin B₆ powder and the isoflavone- and saponin-enriched soy supplement compared with controls. We conclude that soyasaponin B₆ can impede kidney enlargement and cyst growth in the *pcy* mouse model of polycystic kidney disease. Studies are underway to determine the most effective therapeutic dose of soyasaponin B₆ for treating this inherited kidney disease.

Effects of soy on reproduction and hormone balance

Effect of Soysselect (a Standardized Soy Extract) on Reproductive and Non Reproductive Tissues in Rats. D. Gallo,* S. Giacomelli,* P. Apollonio,* G.F. Zannoni,[†] C. Ferlini,* A. Riva,** P. Morazzoni,** E. Bombardelli,** S. Mancuso,* and G. Scambia*. *Department of Obstetrics and Gynecology and [†]Department of Histopathology, Catholic University of the Sacred Heart, Rome, Italy; **Indena S.p.A, Milan, Italy.

Hormone replacement therapy (HRT) is currently viewed as a strategy for treating climacteric complaints and preventing osteoporosis; however, because of the augmented risks of breast cancer, stroke, and venous thromboembolism, patients look for alternatives. The efficacy of Soysselect (a standardized soy extract containing 12% w/w isoflavone glucosides) in the treatment of menopausal symptoms has been established in previous clinical studies (1,2). The main objective of the present study was to evaluate the osteoprotective activity of the extract in an experimental model of menopause and to

correlate the effects observed with isoflavone plasma levels; other potential treatment-related effects, such as those on lipid profile and those indicative of toxicity have been also assessed. For the purpose of the study, Sprague Dawley female rats were ovariectomized or sham operated at age 3 mo and subsequently treated for 6 wk. Ovariectomized animals (12/group) received either the soy extract [50 or 100 mg/(kg·d) per os], the vehicle, or β -estradiol [0.5 mg/(kg·d) per os]. At the end of the study rats were killed and blood and organs were collected for subsequent analyses. Bone density analysis showed that soy treatment significantly [$P < 0.01$ at 100 mg/(kg·d)] reduced bone loss in ovarian hormone deficiency. Remarkably, this osteoprotective effect was not accompanied by a stimulatory effect on the uterus, as demonstrated by organ weight data and by histopathological analysis. In immature rats, a lack of uterine stimulatory effect was also observed after administration of the same dosages of the extract. Minor changes were observed in lipid profile of ovariectomized rats after soy administration. Total genistein, daidzein, and equol plasma levels (mean \pm SD) were found to be 0.9 ± 0.5 , 1.5 ± 0.5 , and 2.3 ± 0.8 μ mol/L, respectively, in the low-dose group and 2.6 ± 1.7 , 3.3 ± 1.6 , and 2.3 ± 1.5 μ mol/L, respectively, in the high-dose group. Further studies are now ongoing in our laboratory to thoroughly characterize the pharmacological properties of this soy extract both in terms of therapeutic and safety aspects.

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Effects of Soy Isoflavones on Premenstrual Syndrome. N. Ishiwata,* S. Uesugi,[†] M. Uehara,[†] M. Melby,[†] and S. Watanabe[†]. *Atomi Junior College, Tokyo, Japan; [†]Tokyo University of Agriculture, Japan.

Premenstrual syndrome (PMS) is characterized by psychological and/or physical symptoms during the luteal phase of the menstrual cycle. PMS affects a large segment of the population of reproductive-age women. We studied the effects of 2 isoflavone (IF) supplements on the improvement of PMS symptoms. A total of 242 women, aged 18–21 y, voluntarily participated in a questionnaire study on PMS. Premenstrual symptoms interfering with daily life activity were reported by 86.0% of all subjects. Major symptoms were sleepiness, abdominal pain, backache, headache, breast tenderness, and mental irritability. The severity of PMS was related to menstrual pain and to previous dieting for weight loss. From the questionnaire study, 56 women with PMS were selected to participate in a randomized double-blind crossover intervention trial with IFs and placebo. In the supplement groups, 26 (20 mg/d of IFs) and 28 (40 mg/d of IFs) women completed the study that lasted for 8 menstrual cycles, with 3 menstrual cycles of baseline assessment and 2 menstrual cycles of IF or placebo tablet administration. No significant differences in physical and nutritional characteristics between the groups receiving IFs at 20 and 40 mg/d were observed at entry into the intervention trial. Average IF intake from the diet was 20.7 mg/d. The ratio of estradiol to progesterone at the luteal phase tended to decrease with IF treatment in the subjects with severe PMS. Physical symptoms (particularly headache) were significantly improved in the group receiving 40 mg/d compared with placebo group ($P < 0.05$); in this IF group, increased plasma serotonin concentration (possibly related to decrease in headaches) was

observed. Psychological symptoms did not show significant improvement. Individual personality assessed by the NEO Personality Inventory was related to the severity of PMS. Significant associations between openness and mental irritability, agreeableness and acne and mental symptoms, and conscientiousness and headache were present ($P < 0.05$). We suggest that an IF supplement may be an effective treatment for the improvement of physical PMS symptoms.

Effect of Soy Isoflavone Consumption on Serum Reproductive Hormones in Healthy Young Men. B. L. Dillingham,* B. L. McVeigh,* J. W. Lampe,[†] and A. M. Duncan*. *Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada; [†]Fred Hutchinson Cancer Research Center, Seattle, WA.

BACKGROUND: Inverse associations between soy consumption and prostate cancer risk, along with the contribution of hormones to the etiology of prostate cancer, prompted the current study to determine whether soy isoflavones could alter serum hormones in men. **METHODS:** Thirty-five healthy men (27.9 ± 5.7 y) consumed milk protein isolate (MPI), low-isoflavone soy protein isolate (low-iso SPI; 1.64 ± 0.19 mg isoflavones/d), and high-isoflavone SPI (high-iso SPI; 61.7 ± 7.4 mg isoflavones/d) for 57 d each separated by 4-wk washout periods, in a randomized crossover design. Serum was collected on days 1, 29, and 57 for analysis of reproductive hormones and sex hormone binding globulin (SHBG), and 24-h urine samples were collected at the end of each treatment for analysis of isoflavones. **RESULTS:** Urinary isoflavones were significantly increased by the high-iso SPI relative to the low-iso SPI and the MPI. Serum dihydrotestosterone (DHT) and DHT/testosterone were significantly decreased by the low-iso ($P = 0.036$ and $P = 0.004$, respectively) and high-iso SPIs ($P = 0.047$ and $P = 0.013$, respectively) compared with the MPI at day 57. Other significant effects included a decrease in testosterone by the low-iso SPI relative to the MPI and high-iso SPI at day 29; an increase in dehydroepiandrosterone sulfate by the low-iso SPI relative to the MPI at day 29 and to the MPI and high-iso SPI at day 57; and increases in estradiol and estrone by the low-iso SPI relative to the MPI at day 57. There were no significant effects on free testosterone, androstenedione, dehydroepiandrosterone, androstanediol glucuronide, gonadotropins, or SHBG. **CONCLUSIONS:** Soy protein, regardless of isoflavone content, decreased serum DHT and DHT/testosterone with minimal effects on other androgens, estrogens, gonadotropins, or SHBG in healthy young men. These results provide evidence for some effects of soy on serum hormones that may relate to future prostate cancer risk. (Supported by the American Institute for Cancer Research grant #01B013 and a donation from the Solae Company.)

Estrogen Metabolism in Postmenopausal Women with and without a History of Breast Cancer: Effects of Soy Protein and Probiotic Consumption. J. A. Nettleton,* K. A. Greany,* K. E. Wangen,* W. Thomas,[†] and M. S. Kurzer*. *Department of Food Science and Nutrition and [†]Department of Biostatistics, University of Minnesota, St. Paul, MN.

Soy isoflavones are primarily metabolized by gut microflora. Probiotic supplementation has been shown to alter fecal bacteria and enzymes; therefore, probiotic supplements may enhance isoflavone metabolism and subsequent isoflavone-mediated effects on estrogen metabolism. We hypothesized that soy protein consumption would decrease urinary concentrations of

16 α -hydroxy estrone (16OHE₁), and increase 2-hydroxy estrogens (2OHE) and the ratio of 2-hydroxy to 16-hydroxy (2:16OHE₁) in postmenopausal women and that probiotic supplements would enhance these effects. To test this hypothesis, 20 breast cancer survivors and 20 control subjects were given 4 treatments for 6 wk each in a randomized crossover design: soy protein (0.38 g protein/kg, 0.64 mg isoflavones/kg); soy protein \pm probiotics 10⁹ colony-forming units/d of *Lactobacillus acidophilus* DDS \pm 1 and *Bifidobacterium longum*, 15–30 mg/d of fructooligosaccharide); milk protein (0.38 g protein/kg); or milk protein \pm probiotics. Diets were separated by a 2-wk washout period. Urinary estrogen metabolites were measured by enzyme-linked immunoassay. At baseline, there were no differences in urinary excretion of 2OHE or 16OHE₁ between control subjects and survivors. However, survivors tended to have lower 2:16OHE₁ than did control subjects ($P = 0.10$). Soy consumption tended to increase urinary excretion of 2OHE ($P = 0.07$) and 16OHE₁ ($P = 0.11$) and not affect 2:16OHE₁ ($P = 0.45$). Probiotic supplementation did not independently affect 2OHE, 16OHE₁, or 2:16OHE₁ nor enhance the effects of soy. These results lend support to the hypothesis that a low 2:16OHE₁ increases breast cancer risk. Further, soy consumption in postmenopausal women may increase both 2OHE and 16OHE₁ excretion but does not affect 2:16OHE₁. (Supported by U.S. Army grant no. BC981032, General Clinical Research Center grant no. M01-RR00400 from the National Center for Research Resources, the Minnesota Agricultural Experiment Station, UAS Laboratories, and the Solae Company.)

Neonatal Exposure to the Soy Phytoestrogen Genistein Alters Gene Expression in the Murine Uterus. R. R. Newbold,* E. Padilla-Banks,* and W. N. Jefferson*[†]. *National Institute of Environmental Health Sciences, Research Triangle Park, NC; [†]North Carolina State University, Raleigh, NC.

We have shown that permanent alterations including neoplasia result from developmental exposure to synthetic and naturally occurring estrogens such as diethylstilbestrol or genistein (1). Our findings support the idea that differentiating tissues are uniquely sensitive to perturbation by estrogenic chemicals. The use of soy phytoestrogens has increased dramatically because of the marketing of soy products as nutritional supplements and hormone therapy. Our concern is phytoestrogen exposures that occur during fetal and childhood development such as through soy-based formulas for infants and soy products designed specifically to appeal to children. Thus, we studied the mechanisms involved in the adverse effects of genistein that we previously reported in our animal model. Estrogen-signaling pathways in the uterus were examined in outbred CD-1 mice after subcutaneous injections on days 1–5 with genistein (0.5–50 mg/kg). These dose levels span the range for infants consuming soy-based formula; serum pharmacokinetic analysis documented that the maximum concentrations of total (conjugated plus aglycone) genistein were comparable with those reported from dietary exposures in adult rats or in human infants consuming soy formula. Expression of estrogen receptor (ER)- α , ER- β , and estrogen-responsive genes in the uterus, including lactoferrin (LF), were compared with that of controls at 5, 12, and 19 d. Ribonuclease protection assays and immunohistochemistry showed no expression of ER- β in the uterus of control or genistein-treated mice; however, ER- α was increased after the lowest genistein dose. LF was increased on day 5 after neonatal genistein in a dose-dependent manner and was blocked by the antiestrogen

ICI 182,780, suggesting genistein-induced LF expression was mediated through ER- α because ER- β was not present. Another group of mice was treated with genistein on days 1–5 and then stimulated at 17 d with 3 daily injections of estrogen to mimic puberty. On day 4, ratios of uterine weight to body weight were determined. Mice exposed to genistein at 0.5 mg/kg showed an enhanced response to estrogen at puberty whereas higher neonatal doses dampened effects; altered gene expression paralleled this abnormal uterine wet weight response pattern. Cotreatment with ICI on days 1–5 along with the low dose of genistein blocked the enhanced uterine wet weight response, suggesting this abnormal response was also mediated through ER α . At 2, 4, and 6 mo, fertility was altered in all the neonatal dosed groups. These findings suggest that exposure to genistein during development alters estrogen-responsive genes that may play a role in subfertility and long-term toxicity.

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Role of Phytoestrogens for Menstrual Cycle Symptoms. M. Bryant,* L. Dye,* C. Hill,* J. Powell,[†] D. Talbot,[†] and A. Cassidy**.*University of Leeds, UK; [†]Unilever, UK; **University of Reading, UK.

The endocrine-modulating effects of soy phytoestrogens in healthy premenopausal women are well established. The hormonal effects, which influence menstrual cycle regulation, may be beneficial in cyclical conditions experienced by women, such as premenstrual syndrome (PMS). To date, no controlled intervention trials have examined the use of phytoestrogens for PMS. Given that phytoestrogens exert estrogenic and antiestrogenic effects and have been shown to be anxiolytic, it was proposed that the consumption of phytoestrogen-rich foods may relieve both physical and psychological symptoms of PMS. The effect of phytoestrogen consumption on premenstrual symptoms was examined in a double-blind placebo-controlled crossover study. PMS was confirmed in 23 women after 2 screening cycles. This was followed by the consumption of 100 mg/d of either soy phytoestrogens or placebo in the form of snack bars or powder for 2 complete menstrual cycles. One month of washout was included before the crossover for a further 2 cycles. Symptoms were recorded daily using a 19-item (4-point Likert Scale) questionnaire. Results suggest that consumption of phytoestrogens reduces symptoms of PMS, especially breast tenderness. Corresponding reductions in urinary excretion of estrogen-3-glucuronide were observed in women consuming soy phytoestrogens. This long-term study of soy phytoestrogens suggests potential therapeutic benefits in PMS.

Role of equol

Equol Labeled with Stable Isotopes. T. Jokela and K. Wähälä. University of Helsinki, Finland.

Equol is an inert end metabolite of soy-based daidzein in so-called equol producers (1). It is assumed that only ~30–40% of the adult population excretes equol in urine after consumption of soy foods; however the reasons for this remain to be determined (2,3). Equol possesses estrogenic activity, having an affinity for both estrogen receptors, ER- α and ER- β (4). The antioxidant activity of equol is superior to all other isoflavonoids tested in vitro (5–7). The most sensitive method to quantitate equol in human fluids is the isotope-dilution

mass spectrometry single-ion monitoring technique using a stable isotopically labeled reference compound (1). We present here a new and expedient way to produce an isotopically pure stable d_4 -labeled equol in high yield.

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Equol-Producer Phenotyping in A Free-Living Population in the United States. C. Atkinson,* C. L. Frankenfeld,*[†] W. K. Thomas,* A. Gonzalez,* ** K. Wähälä,[‡] T. Jokela,[‡] S. M. Schwartz,*[†] S. S. Li,* and J. W. Lampe* **. *Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; [†]Department of Epidemiology, University of Washington, Seattle, WA; **Interdisciplinary Program in Nutritional Sciences, University of Washington, Seattle, WA; [‡]Department of Chemistry, University of Helsinki, Finland.

Intestinal bacteria metabolize daidzein to equol and O-desmethylangolensin (O-DMA). Several small studies showed that, when presented with a soy challenge, only ~33–50% of healthy adults produce equol and ~80–90% produce O-DMA. However, determinants of these daidzein-metabolizing phenotypes are unknown. We determined the prevalence of these phenotypes in a large population of men, women, and children ($n = 410$, from within 113 families) and investigated the effect of demographics on manifestation of these phenotypes. All study materials were mailed to participants. To determine daidzein-metabolizing phenotypes, participants consumed a soy bar (~83 mg daidzein) or soy nuts (~10 mg daidzein) on 3 consecutive days and collected a first-void urine sample on d 4. Urine samples were returned by mail, and equol and O-DMA were quantified by gas chromatography–mass spectrometry. In a separate experiment, the stability of equol and O-DMA in urine ($n = 4$) left at room temperature for 14 d was tested; no losses occurred. Demographic data were collected via mailed questionnaire, and general estimating equations were used to determine associations between daidzein-metabolizing phenotypes and demographic variables while controlling for familial correlations. Over 98% of urine samples were received within 14 d of collection (data available for 371; mean = 4 d). Mean age of study participants was 39 y (range 10–95 y), 60% were female, 87% were Caucasian, and 42% and 82% had detectable levels of equol and O-DMA, respectively, in their urine (on-column detection limit = 0.25 ng/ μ L). With detectable levels as the cutoff for each phenotype, a positive association was found between years of education (adults only) and being an equol producer. Age was not associated with the equol-producer phenotype, but increasing age was associated with a decreased likelihood of being an O-DMA producer. Asians were less likely than Caucasians to

be O-DMA producers and, when adjusted for age and sex, being an O-DMA producer was inversely associated with height, weight, and body mass index. Frequency of consumption of fried foods was positively associated with, and caffeinated beverage consumption was inversely associated with, being an O-DMA producer. In conclusion, equol and O-DMA are stable in urine at room temperature for at least 14 d, allowing for the collection of urine samples by mail for phenotype determination. There were no strong predictors of the daidzein-metabolizing phenotypes, but several factors were identified that might contribute. Factors not evaluated here also likely contribute to these phenotypes.

Association with Equol Production and Effect of Isoflavone Treatment on Bone Mineral Density and Climacteric Symptoms in Japanese Women. S. Uesugi,* N. Ishiwata,[†] K. Ouchi,** M. Melby,* and S. Watanabe*. *Tokyo University of Agriculture, Tokyo, Japan; [†]Atomi Junior College, Japan; **MC Medical, Inc., Japan.

The relationship between the effects of isoflavone supplementation on bone mineral density and climacteric symptoms and equol-producing ability was assessed by using a randomized trial of soy isoflavone treatment in Japanese women. A double-blind placebo-controlled crossover trial with an isoflavone supplement (IF) of 40 mg/d was carried out in 42 climacteric Japanese women. A questionnaire and an interview covered climacteric symptoms, health status, dietary and exercise habits, and medical history. The following measurements were made at baseline and after 4 and 8 wk of treatment: anthropometrical measurements, blood pressure, bone mineral density (BMD), blood status, and plasma biochemicals. Urinary isoflavones and metabolites were separately measured from refrigerated samples. The number of equol producers both at baseline and after IF treatment (P-P) was 17 (40.5%), of nonproducers at both periods (N-N) was 12 (28.6%), of producers at baseline who changed to producers after IF (P-N) was 7 (16.7%), and of nonproducers at baseline who became producers after IF (N-P) was 6 (14.3%). Urinary equol concentration was higher after IF treatment than after placebo treatment ($P = 0.06$). There was no significant difference in the average age of each group. The menopausal index score in the P-P group was lower (18 points) than in the other groups (26–31 points). The equol nonproducers at both baseline and after IF treatment had high body fat (30.1%) and high systolic blood pressure (148 mm Hg), and their BMD and climacteric symptoms did not improve with IF treatment. Women who became equol producers after IF treatment (N-P) showed lower plasma LDL at baseline (3.34 vs. 3.39–4.01 mmol/L) and greater improvement in BMD and climacteric symptoms (± 0.14 g/cm³, –5 points) compared with the other groups. Baseline anthropometric and cholesterol levels and effects of IF treatment on cholesterol, BMD, and climacteric symptoms differed according to equol-producing ability.

In Vitro Production of Equol by Human Intestinal Microbiota. K. Decroos, S. Cattoir, N. Boon, and W. Verstraete. Laboratory of Microbial Ecology and Technology, Ghent University, Belgium.

The *in vitro* metabolism of the isoflavone daidzein by human intestinal microbial cultures was investigated, focusing on the formation of the metabolite equol. Four fecal samples from different volunteers consuming a diet containing soy were collected. Microbial cultures were retrieved after removal of

coarse particulate matter. The microorganisms were transferred to brain heart infusion (BHI) growth medium supplemented with daidzein, incubated under anaerobic conditions at 37°C, and sampled every 24 h for 3 d. Samples were analyzed by reverse-phase HPLC with UV detection for daidzein and fluorescence detection for equol. After 72 h the concentration of daidzein had decreased in all the cultures. In one culture no daidzein was found after 72 h and ~50% of the initial added daidzein had been transformed into equol. Plating the latter culture on agar plates and reculturing picked colonies resulted in mixture consisting of a limited number of bacteria producing equol. Denaturing gradient gel electrophoresis analysis revealed that 3 bacterial species were dominant in the inoculum of this bacterial mixture. One extra band appeared after 3 d of incubation, which occurred at the start of equol production, probably representing a strain that plays a major role in equol production. Also shown in this study is that presence of hydrogen gas stimulates the production of equol. The results obtained so far can lead to the isolation and better knowledge of the metabolism of a bacterial strain producing equol and of the environmental conditions in the colon that favor equol production. This in turn can lead to nutritional applications by in vitro production and in vivo stimulation of production through pre- and probiotics of this important metabolite.

Equol and 17 β -Estradiol Enhance Release of MMP-9 from Murine J774 Macrophages and Modulate Its Activity. J. Hwang,*[†] H. H. Hodis,*[†] and A. Sevanian*[†]. *Department of Molecular Pharmacology and Toxicology, School of Pharmacy, and [†]Atherosclerosis Research Unit, School of Medicine, University of Southern California, Los Angeles, CA.

Production of matrix metalloproteinase (MMP) by macrophages causes matrix degradation and atherosclerotic plaque instability. MMPs degrade virtually all components of the extracellular matrix and are essential for vascular remodeling. Although vascular remodeling is beneficial because it allows for adaptation and repair, dysfunctional MMP activity can predispose plaque instability and rupture. J774.1 macrophages were treated with 17 β -estradiol (E₂) at 5 nmol/L or equol at 0.5 μ mol/L for up to 72 h in the presence or absence of ICI 182,780 (100 nmol/L). Cell-conditioned medium was removed after treatments and run on 10% sodium dodecyl sulfate gels containing 1% gelatin. Cell lysates were probed for MMP-9 protein expression by Western blot whereas protease activity in the medium was measured by gelatin zymography. E₂- and equol-treated cells released increased MMP-9 enzyme activity and protein levels; E₂-stimulation was ~3-fold more potent than equol. MMP levels then decreased gradually over 2 d, reaching a plateau over 12 d. MMP stimulation was inhibited by ICI 182,780. However, the specific activity of MMP (units gelatinase activity/g protein) was markedly lower in E₂-treated cells and only marginally lower than controls for equol-treated cells. The reduction in enzyme specific activity by both agents was also prevented in the presence of ICI 182,780, indicating mediation via the estrogen receptor. Increased MMP activity was accompanied by a transient (48 h) increase in gp91phox expression and elevated production of superoxide; however, gp91phox activity decreased to control levels by 72 h. When E₂ or equol was withdrawn from the medium, MMP activity increased over 72 h. These findings show that E₂ and equol induce the expression and activity of MMP-9 via estrogen receptors, but this effect is transient

because a secondary effect of these agents also influences MMP activity, namely that the agents have a suppressive effect on reactive oxygen species (ROS) production, principally mediated by macrophage NADPH oxidase (gp91phox). This suppressive action is not affected by ICI 182,780 and hence classical estrogen receptors. The secondary modulation of ROS also serves to suppress MMP catalytic activity because MMPs are markedly stimulated by ROS.

Metabolism and pharmacokinetics of soy isoflavones

Gender Differences in Isoflavone Metabolism in Nonhuman Primates. M. S. Anthony,* A. A. Franke,[†] M. R. Adams,* J. R. Kaplan,* and T. B. Clarkson*. *Comparative Medicine Clinical Research Center, Wake Forest University School of Medicine, Winston-Salem, NC; [†]Cancer Research Center of Hawaii, Honolulu, HI.

Our previous studies in mice and hamsters revealed that males have higher plasma isoflavone concentrations than do females. Because of the potential importance of isoflavones in the modulation of health effects, we expanded our investigations to include nonhuman primates. The study included male (n = 31), surgically postmenopausal female (Post, n = 20), and premenopausal female (Pre, n = 46) cynomolgus monkeys (*Macaca fascicularis*). All were fed diets with soy protein isolate containing isoflavones at a dose comparable with 155 mg total isoflavones (aglycone units) per 2000 kcal. Diets were fed for 6–12 mo before sampling. Samples were drawn 4 h after feeding, the time of peak blood concentrations for genistein and daidzein. Log-transformed data were analyzed because the distributions were not normal. Isoflavone concentrations, retransformed into original units (nmol/L), are shown (mean \pm SEM) in Table 9. Genistein and daidzein were 5–7 times higher in males and 2–3 times higher in premenopausal females compared with postmenopausal females. Equol in males was ~2 times higher than in premenopausal females. These data suggest that the hormonal milieu may affect isoflavone absorption and metabolism, leading to different end-organ effects in males and females. Notably, data from both humans and nonhuman primates indicate that females may derive more cardiovascular benefits from soy with isoflavones than do males, particularly with respect to vascular function. The current data may suggest a mechanism for those differences and have important implications for studies of optimum dose. Specifically, males may require substantially lower isoflavone doses.

Familial Aggregation of Daidzein-Metabolizing Phenotypes. C. L. Frankenfeld,*[†] C. Atkinson,* W. K. Thomas,* A. Gonzalez,* ** T. Jokela,[‡] S. M. Schwartz,*[†] K. Wähälä,[‡] S. S. Li,* and J. W. Lampe*^{†***}. *Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; [†]Department of Epidemiology and **Interdisciplinary Program in Nutritional Sciences, University of Washington, Seattle, WA; [‡]Department of Chemistry, Laboratory of Organic Chemistry, University of Helsinki, Finland.

Equol and O-desmethylangolensin (O-DMA) are products of intestinal bacterial metabolism of daidzein. In humans, inter-individual variability exists in the capacity to produce these metabolites. The population prevalences of equol producers and O-DMA producers are ~30–50% and 80–90%, respectively, and these phenotypes appear to be stable within individuals over time. Thus, the presence or absence of equol,

TABLE 9

Isoflavone concentrations in male and female nonhuman primates

	Male	Postmenopausal female (Post)	Premenopausal female (Pre)	P-values		
				Male vs. Post	Male vs. Pre	Post vs. Pre
Genistein	301 (-65, +83)	62 (-16, +22)	112 (-20, +25)	<0.0001	0.002	0.10
Daidzein	286 (-69, +90)	41 (-12, +17)	155 (-31, +39)	<0.0001	0.09	0.002
Equol	745 (-166, +213)	678 (-182, +249)	349 (-65, +80)	0.82	0.02	0.08
Total isoflavones	1536 (-283, +347)	911 (-204, +263)	818 (-126, +149)	0.11	0.02	0.72

O-DMA, or both in urine is a marker of a particular intestinal bacteria profile. Little is known about the determinants of these phenotypes but some studies suggest that health effects may be associated with the equol-producer phenotype. Health effects associated with the O-DMA-producer phenotype are unknown. We undertook a population-based study to examine the degree to which daidzein-metabolizing phenotypes aggregate within families. One hundred eight families with at least 3 members from at least 2 generations participated. Participants ($n = 377$) consumed 1 soy protein bar (~ 83 mg daidzein as ~ 78 mg daidzin weight as aglycone units and ~ 5 mg daidzein) daily for 3 consecutive days and collected a first-void urine sample on d 4. Urine samples were analyzed for equol and O-DMA by gas chromatography-mass spectrometry. Intra- and interclass correlations (ICCs) and standard errors (SEs) were calculated. One hundred fifty-five people (41%) were classified as equol producers and 313 people (83%) were classified as O-DMA producers. A small within-individual inverse correlation between equol-producer and O-DMA-producer phenotypes was observed [ICC = -0.08 (SE = 0.11)]. For the equol-producer phenotype, parent-parent correlation [ICC = 0.11 (SE = 0.13), n pairs = 63] was similar to the parent-offspring correlation [ICC = 0.13 (SE = 0.07), n pairs = 288] and to the sibling-sibling correlation [ICC = 0.06 (SE = 0.12), n pairs = 82], suggesting that shared environmental factors may contribute to the phenotype. For the O-DMA-producer phenotype, parent-offspring correlation [ICC = 0.14 (SE = 0.07), n pairs = 286] was positive and less than that of the sibling-sibling correlation [ICC = 0.38 (SE = 0.11), n pairs = 82], and the parent-parent correlation was inverse [ICC = -0.17 (SE = 0.12), n pairs = 63]. For both phenotypes, some differences by relationship subtypes (e.g., brother-brother vs. sister-sister) were suggested. Overall, these results suggest a modest degree of familial aggregation in the O-DMA-producer phenotype and a small degree of familial aggregation in the equol-producer phenotype. Further work is warranted to evaluate the heritable and environmental (shared and unshared) determinants of these daidzein-metabolizing phenotypes.

Isoflavone Excretion Phenotypes Influence Plasma Cholesterol in Golden Syrian Hamsters. M. Renouf, S. Lee, C. Hauck, and S. Hendrich. Iowa State University, Ames, IA.

Previous studies showed a hypocholesterolemic effect of soy isoflavones in Golden Syrian hamsters that may have relevance for humans because of their similarities in cholesterol metabolism. Furthermore, humans exhibit isoflavone absorp-

tion phenotypes apparently influenced by gut microbial degradation of these compounds; when coupled with rapid gut transit time, low degraders excreted more isoflavones in the urine than did high degraders. We hypothesized that hamsters had patterns of isoflavone bioavailability similar to those of humans, which we also proposed to affect plasma cholesterol levels. One-year-old hamsters were divided in 2 groups fed either 320 mg total isoflavones (TI)/kg diet (5 males, 5 females) or 480 mg/kg diet (5 males, 4 females) for 10 d. Urine and feces were collected for 24 h in metabolic cages, and animals were killed under CO₂ after a 16-h fast, when plasma was collected. According to a cluster analysis, hamsters sorted into 2 distinct urinary isoflavone excretion phenotypes: high and low excreters. Mean TI excreted was $44.8\% \pm 5.3\%$ vs. $14.0\% \pm 7.1\%$, mean daidzein (D) was $30.5\% \pm 7.0\%$ vs. $8.6\% \pm 3.8\%$, mean glycitein (G) was $84.3\% \pm 6.8\%$ vs. $28.7\% \pm 13.7\%$, and mean genistein (G) was $35.6\% \pm 7.6\%$ vs. $8.2\% \pm 4.6\%$ of the ingested dose for high vs. low excreters, respectively. Fasting plasma total cholesterol was significantly less in high excreters than in low excreters (e.g., sorted on the basis of TI, 6.12 ± 1.99 mmol/L vs. 8.36 ± 1.56 mmol/L, respectively) regardless of sex. Although females had better bioavailability than males for TI (33.9% vs. 17.6%), D (24.5% vs. 11.8%), and G (27.5% vs. 10%), cholesterol levels compared by sex were not significantly different, seemingly because of great interindividual variability. These data support our hypothesis that Golden Syrian hamsters are good models to study the link between bioavailability and cholesterol-lowering effects of isoflavones. The relevance to humans deserves further exploration.

Highly Sensitive Microscale Mass Spectrometry Methods for Bioavailability Studies of Isoflavones Using Microdialysis. C.-C. Wang,* R. Moore,[†] and S. Barnes*[†]. *Department of Pharmacology and Toxicology and [†]Mass Spectrometry Core Facility, University of Alabama at Birmingham, Birmingham, AL.

There is a great interest in isoflavones and their metabolites because of their potential role in several major chronic diseases. Measurements of isoflavones have been carried out on blood, urine, and tissue extracts by HPLC-mass spectrometry (MS) methods. However, because interstitial fluid (IF) rather than blood surrounds cells, it is more important to analyze IF to understand the physiological role of isoflavones. In vivo sampling techniques, such as microdialysis and ultrafiltration, have been applied to monitoring drug and other exogenous compounds in IF and are suitable for

studying the bioavailability of isoflavones in tissues. However, the limited sample volumes and high salt concentration in samples from these sampling techniques present a challenge for the current HPLC-MS methods for isoflavone analysis. We have described a microscale MS method using a nanoelectrospray interface and membrane-based micro-preconcentrators for the analysis of microliter amounts of isoflavones. Microliters of isoflavones in saline solution can be analyzed directly with this method with detection limits at low femtomole levels. Although normal saline solution is generally used as perfusate for microdialysis sampling, analysis of isoflavone standards spiked in IF sample matrix showed 2–3 times higher signal intensity than did isoflavones in saline solution. Quantitative analysis of isoflavones in IF samples based on a calibration curve from isoflavone standards in saline solution could be questionable. Efforts to generate a reliable standard calibration curve by modifying pH or composition of the matrix for quantitative analysis of isoflavones in interstitial fluids will be presented. Preliminary results from the pharmacokinetic study of isoflavones, such as genistein or daidzein, in breast tissue and aorta in rat models using microdialysis will be discussed.

Determinants of Isoflavone Uptake and Metabolism in Humans. A. A. Franke,* S. A. Hundahl,[†] and L. J. Custer*.
*Cancer Research Center of Hawaii, Honolulu, HI; [†]VA Northern California Health Care System, Mather, CA.

Human dietary isoflavone exposure occurs mainly through soy and 7-O-glucosylconjugates. These glucosides are presumed to be hydrolyzed in the gut to the aglycones before absorption because of the absence of the glycoconjugates in the circulation. We intended to show in vivo evidence that the large interindividual difference in isoflavone bioavailability and recovery is due to the ability of the gut flora rather than the mucosa to metabolize isoflavonoids. Plasma levels and urinary excretion rates of isoflavonoids were measured in subjects with a reduced gut flora as a result of mechanical bowel preparation followed by oral antibiotic treatment. After consumption of a soy drink containing 99 μ mol isoflavone glycosides and 8 μ mol isoflavone aglycones, we found a profound difference in patterns of plasma levels and urinary excretion rates of isoflavonoids after antibiotic treatment vs. control (i.e., before antibiotic treatment): urinary isoflavone recovery was lower by >50% in the first 24-h but higher by 38% in the second 24 h. Urinary excretion rates were highly correlated with plasma levels, confirming our previous findings (1,2). The unknown cause for a biphasic isoflavone appearance pattern in plasma and urine with peak levels at ~1–2 h and again at ~4–6 h is shown to be due to mucosa and gut flora, respectively, by diverse dietary exposure experiments using a variety of isoflavone derivatives. This gives evidence that the gut flora in humans cleaves isoflavone glucosides more efficiently than mucosa glucosidases but also degrades isoflavones in agreement with in vitro studies. It also shows convincingly that isoflavones are taken up in the large intestine. In summary, our results show that the gut flora is the main determinant of isoflavone bioavailability.

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Bioavailability of Dietary Soy Isoflavones in a Rat Model. E. Sepehr,* ** G. Cooke,[†]** P. Robertson,* and G. S. Gilani*.
*Nutrition Research Division and [†]Toxicology Research Division, Health Products and Food Branch, Health Canada, Ottawa, Ontario, Canada; **Departments of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ontario, Canada.

There is considerable interest in consumption of plant-based foods rich in phytoestrogens, more specifically the isoflavones and their derivatives from soy, because of their reported beneficial effects such as their potential to prevent breast and prostate cancer as well as prevent coronary heart disease and osteoporosis. However, information is still insufficient regarding the pharmacokinetics of the absorption and excretion of soy isoflavones. Moreover, data on the effects of sex and age of animals and of the source of isoflavones on their bioavailability are limited. Results from a previous study at Health Canada suggested that the metabolism of dietary isoflavones is significantly affected by the sex of the rats and that the source of dietary isoflavones (such as endogenous or extracted) may have a marked effect on their potency. The present study was conducted to obtain information on the effects of the sex, age, and source of soy isoflavones on their bioavailability in a rat model. Three sources of isoflavones—Novasoy (extracted isoflavones supplement), a mixture of synthetic aglycosides (daidzein, genistein, and glycitein), and a mixture of synthetic glycosides (daidzin, genistin, and glycitin) were administered once only by oral gavage at 20 or 40 mg/kg body weight. The first phase of the animal project has been completed; plasma samples were collected at 0, 10, and 30 min and 1, 2, 8, 24, and 48 h and urine and fecal samples were collected at 0–2, 2–8, 15–24, 24–32, and 32–48 h after dosing. The second phase of the animal project will measure the concentrations of isoflavones and their metabolites in plasma after a single intravenous injection of 2 sources of synthetic aglycosides (daidzein, genistein, and glycitein) and synthetic glycosides (daidzin, genistin, and glycitin). The extent of absorption will be determined for dosage forms by comparing the area under the curve (AUC) of the plasma-concentration time curve after intravenous (i.v.) administration with that after oral administration. The extent of bioavailability will then be calculated as $f = \text{AUC}_{\text{oral}} / \text{AUC}_{\text{iv}} \times 100$. An improved liquid chromatography–mass spectrometry method is being developed to confirm the plasma isoflavone data obtained by the HPLC-UV method used in our previous study. The data will assist in assessing safety, nutritional quality, and health benefits of dietary phytoestrogens.

Metabolism of the Soy Isoflavone Glycitein (7,4'-Dihydroxy-6-Methoxy Isoflavone) by Human Gut Microflora. A. L. Simons, M. Renouf, S. Hendrich, and P. A. Murphy.
Iowa State University, Ames, IA.

Microbial degradation of the soy isoflavone glycitein was investigated in humans by incubating glycitein anaerobically with feces from 13 human subjects (5 men and 8 women, aged 24–54 y) in brain heart infusion medium at 37°C. Samples were taken periodically, extracted with 80% methanol, and fractionated on preconditioned C18 SepPak cartridges using 2,4,4'-trihydroxydeoxybenzoin as an internal standard. Glycitein was analyzed using HPLC at 254 nm. Results showed that at 5 different glycitein concentrations (10, 50, 75, 100,

and 250 $\mu\text{mol/L}$) there was no significant difference between the degradation rates ($k = 0.41 \pm 0.007 \text{ h}^{-1}$, $P = 0.71$). These data suggest that glycitein degradation follows an apparent first-order reaction. When glycitein was incubated in the presence of genistein and daidzein, there was no significant change in the degradation rates ($k = 0.24 \pm 0.04 \text{ h}^{-1}$, $P = 0.37$). Glycitein metabolites were described by liquid chromatography–mass spectrometry (electrospray ionization using negative ionization mode) as dihydro-6,7,4'-trihydroxyisoflavone and 6-hydroxy-*O*-desmethylangolensin. One subject produced daidzein as an additional metabolite of glycitein.

Metabolism of the Soy Isoflavone Glycitein In Vitro and In Vivo. C. E. Ruefer,* E. Donauer,[†] A. Machowetz,* M. Metzler,[†] and S. E. Kulling*. *Federal Research Centre for Nutrition, Institute for Nutritional Physiology, Germany; [†]University of Karlsruhe, Institute for Food Chemistry and Toxicology, Germany.

Phytoestrogens of the isoflavone family are abundant in soybeans, clover, and a variety of beans as secondary plant metabolites. The most common representatives are the isoflavones genistein (GEN), daidzein (DAI), and glycitein (GLY). It is known from various in vitro and in vivo studies that GEN and DAI are extensively transformed by both the gut microflora and the liver (1,2). However, little is known about the metabolism of the isoflavone GLY. We investigated the oxidative metabolism of GLY in vitro using liver microsomes of a male human and of Aroclor-treated male Wistar rats. Furthermore, the reductive metabolism of GLY in vitro under anaerobic conditions was studied using human fecal bacteria as well as bovine rumen fluid. To understand the in vivo metabolism of GLY we analyzed feces and urine of male Sprague Dawley rats after single-dose administration of GLY by stomach intubation. Identification and characterization of the metabolites was carried out by using HPLC with diode-array detection and gas chromatography–mass spectrometry. GLY was converted by human and rat liver microsomes to 8 metabolites, comprising 4 monohydroxylated and 2 monohydroxylated demethylated products, 1 dihydroxylated product, and the demethylation product 6-hydroxy-DAI. Additional hydroxyl groups are almost exclusively introduced into the ortho positions of already existing phenolic hydroxyl groups. The major metabolites are 8-hydroxy-GLY and a monohydroxylated GLY derivative, which we have not yet been able to identify. Using human fecal flora and ruminal microflora to study the reductive metabolism of GLY, we were able to identify 4 metabolites: 6-hydroxy-DAI, which represents the main metabolite in both assays; 6-hydroxy-dihydro-DAI; 6-hydroxy-equol; and 5'-hydroxy-*O*-desmethylangolensin. In rat urine 3 oxidative metabolites (8-hydroxy-GLY, 3'-hydroxy-GLY, and 5,6-dihydroxy-DAI), the demethylated product 6-hydroxy-DAI, and 1 reduced GLY metabolite (6-hydroxy-dihydro-DAI) were identified, whereas in the feces only 2 metabolites were found (6-hydroxy-DAI and 6-hydroxy-dihydro-DAI). There is evidence that further reduced metabolites exist.

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Soy and bone health

A Novel Extract of Fermented Soybean Germs (AglyMax) Promoted Bone Growth in Male Mice Fed a High-Fat Diet. C. Wang,* W. J. Pan,[†] and J. R. Zhou[†]. *Human Nutrition Program, Kentucky State University, Frankfort, KY; [†]Nutrition/Metabolism Laboratory, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.

Some studies have shown that soy isoflavones prevent bone loss in ovariectomized rats and in postmenopausal women. The aglycone forms of isoflavones are more potent biologically because they are more available for absorption than their glucoside forms. Among the soy isoflavones studied, daidzein may be even more effective than genistein in preventing bone loss. However, the effect of soy isoflavones on bone growth is not clear. In this study we used 6-wk-old male mice to determine the skeletal effect of a novel extract of fermented soybean germs (AglyMax) that is rich in daidzein aglycones. Isoflavone aglycones accounted for 30% of the extract by weight with a ratio of daidzein to genistein to glycitein aglycones of 7:1:2. The mice were randomly assigned to 3 groups. Group A was fed a high-fat diet with 60% of its energy derived from fat; group B was fed the high-fat diet supplemented with 6 g AglyMax/kg diet; group C was pair-fed the same amount of the high-fat diet as group B consumed. At the end of the 6-wk experiment, femur bones were analyzed for bone mineral content and density by dual-energy X-ray densitometry (Lunar DPX-IQ). Each bone was also evaluated by a 3-point bending test for its breaking strength. There were no differences between groups A and B in the variables measured, but both groups A and B had higher bone mineral content, bone density, and breaking strength than did group C. Bone calcium and ash content tended to be higher for groups A and B than for group C. These results suggest that the extract of fermented soybean germs promoted bone growth in male mice fed a high-fat diet.

Synergistic Effects of Black Bean (*Yak-Kong*; *Rhynchosia molubilis*) and Levan on the Prevention of Bone Loss in Osteoporotic Model Rats. S. A. Kang,* K. H. Jang,* J. H. Suh,* E. K. Jang,[†] D. K. Ahn,** and S. K. Park*. *Kyung Hee University, Seoul, Korea; [†]RealBioTech Co. Ltd, Korea; **Jaseng Research Institute of Biotechnology & Bioscience, Seoul, Korea.

Phytoestrogens, especially soy-derived isoflavones, are receiving great scrutiny as a food supplement for preventing hormone-dependent diseases such as postmenopausal osteoporosis. In contrast to the common usage of soybean, black bean (*Rhynchosia molubilis*; *yak-kong*) has been used in oriental medicine as an estrogen supplement for preventing postmenopausal osteoporosis. In Korea, several oriental plants including black bean and soybean have been used for bone disease. The purpose of this study was to examine the synergistic effects of the water extracts of black bean and levan (β -2,6 linked fructose polymer, ~6,000,000 Da) on trabecular bone formation and calcium metabolism in the osteoporosis model in rats. At age 12 wk, female Sprague Dawley rats were either sham-operated (SH: $n = 8$) or ovariectomized ($n = 32$). The latter group was subdivided into 4 groups [OVX: $n = 8$; black bean (BB): $n = 8$; Levan: $n = 8$; BB+Levan: $n = 8$]. All 5 groups were fed a low level of calcium (0.08%) and killed after a 6-wk feeding period. After blood samples were collected, the femur and tibia

were immediately excised and muscle was removed, weighed, and stored in fixing solution. Determination of bone density was followed by Gomori staining. The diet of the Levan group contained 10% levan; body weights and tibia weight were not altered by the diet. However, levan intake significantly increased cecum and colonic total weight 2–3-fold ($P < 0.05$). The serum concentrations of calcium and phosphorus were significantly increased by the Levan and BB diets. The serum concentration of alkaline phosphatase and osteocalcin were little changed by the diet. Trabecular bone density was increased by the BB and Levan diets. These findings suggest a curing effect of black bean and levan against bone loss in the OVX rat: intake of black bean and levan may increase bone mineral density of trabecular and serum calcium concentration but not tibia weight. Furthermore, the synergistic effect of black bean and levan may aggravate bone loss, leading to potential health problems in the ovariectomized osteoporotic model rats.

Effects of Dietary Genistein on Nutrient Use and Mineral Status in Heat-Stressed Quail. K. Sahin,* N. Sahin,* M. Onderci,* F. F. Sarkar,[†]** A. Prasad,[†]** D. Doerge,[‡] and O. Kucuk[†]**. *Firat University, Elazig, Turkey; [†]Karmanos Cancer Institute, U.S.; **Wayne State University, Detroit, MI; [‡]National Toxicological Research Center, FDA, U.S.

Genistein inhibits the formation of oxygen free radicals, reduces lipid oxidation, and stimulates antioxidant enzymes and causes alterations in bone metabolism. We evaluated the efficacy of dietary supplementation with genistein on the nutrient use and mineral concentrations in tibia and serum of quail reared under high environmental temperature (34°C). Two hundred forty Japanese quails (aged 10 d) were randomly assigned to 8 treatment groups each consisting of 30 birds. The birds were kept at a temperature-controlled room at 22°C (thermo-neutral, TN groups) or 34°C (for 8 h/d; 0900–1700; heat stress, HS groups). Birds were fed either a basal diet (TN and HS) or the basal diet supplemented with genistein at 200, 400, or 800 mg/kg diet. Experiments started at age 10 d and continued for 32 d. Measurements were taken on d 42. Heat exposure decreased digestibility and bone mineralization when basal diet was fed ($P = 0.001$). Apparent digestibility of dry matter and crude protein was significantly improved by supplementation with low (200 mg/kg diet) and medium (400 mg/kg diet) levels of genistein but was not influenced by supplementation with a high level (800 mg/kg diet) of genistein ($P = 0.05$) in HS birds. The amount of calcium, phosphorus, magnesium, zinc, manganese, and copper in the excreta decreased ($P = 0.01$) whereas calcium, phosphorus, magnesium, manganese, zinc, and copper concentrations in tibia ash increased in HS birds ($P = 0.01$) with genistein supplementation. Calcium and phosphorus concentrations in tibia ash were also increased in TN birds. Genistein supplementation was associated with increased serum calcium, phosphorus, and magnesium ($P = 0.01$) but had no effect on plasma zinc, copper, and manganese ($P > 0.05$) in all groups. Increased serum alkaline phosphatase activity ($P = 0.001$) was associated with increasing dietary genistein in all groups. Effects of genistein were greater in HS birds than in TN birds. In conclusion, genistein supplementation improved the digestibility of crude protein and dry matter, the use of calcium and phosphorus, and bone mineralization.

The Effect of Soy Containing Isoflavones on Bone Mineral Density in Postmenopausal Women: A Double-Blind Randomized Trial. L. Kok, S. Kreijkamp-Kaspers, D. E. Grobbee, and Y. T. van der Schouw. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands.

OBJECTIVE: Osteoporosis is a frequent condition, especially among postmenopausal women because of the decrease in estrogen after menopause. As a result, bone becomes fragile and is more prone to fracture. For a long period of time, estrogen replacement therapy (ERT) was considered a good option for preventing postmenopausal bone loss. However, ERT is associated with serious side effects. Some evidence has shown that phytoestrogens may be able to play an important role in the prevention of bone loss after menopause. The objective of our study was to examine the effect of the intake of high amounts of isoflavones for 1 y on bone mineral density of the left proximal femur and the lumbar spine in postmenopausal women. **METHODS:** For our double-blind randomized trial we recruited 202 postmenopausal women aged 60–75 y. Subjects were randomly assigned to receive either a soy isolate containing 99 mg isoflavones (phytoestrogens) or casein (placebo) for 1 y At baseline and at the end of intervention, bone mineral density of the lumbar spine (L1–L4) and the left hip was assessed by dual-energy X-ray absorptiometry. Linear regression was used to study the difference in change in bone mineral density between both groups. **RESULTS:** For the left hip the placebo group showed a decrease of 0.6% vs. a decrease of 0.05% for the soy group. For the lumbar spine the decreases were 0.27% for the placebo group and 0.39% for the soy group. However, none of the observed changes was statistically significantly different from placebo. **CONCLUSION:** Supplementation of soy protein containing 99 mg isoflavones for 1 y had no effect on bone mineral density of the lumbar spine or the left hip in postmenopausal women aged 60–75.

Effects of Natto (Fermented Soybean; Made from Isoflavone-Rich Soybean) Supplemented with Zinc and Calcium on Bone Density and Bone Metabolic Markers of Postmenopausal Women. I. Koyama,[†] S. Shimanuki,* M. Tezuka,* Y. Kawasaki,* M. Shimizu,** I. Arai,[‡] S. Kakinuma,[‡] and Y. Ozawa*. *Taishi-foods Inc., Miyagi, Japan; [†]Junior College, Saitama Medical School, Japan; **KSO Corporation, Japan; [‡]Aiwa Clinic, Japan.

The effects of the test *natto* made from isoflavone-rich soybeans, supplemented with zinc-fortified baker's yeast and calcium, on bone density and bone metabolic markers were studied in 24 postmenopausal women. The test *natto* was a 40-g pack of *natto* containing 33.5 mg (82.4 μmol) isoflavone, 3.0 mg zinc, and 60.4 mg calcium. The control *natto* was a 40-g pack of *natto* containing intrinsically 22.4 mg (56.0 μmol), 0.8 mg, and 51.6 mg of isoflavone, zinc, and calcium, respectively. A prospective randomized placebo-controlled double-blind study design was used. The subjects were equally divided into 2 groups of 12 subjects each. The subjects in each group ate 1 pack of the test or control *natto* per day for 12 wk. At the end of the test period, the average metacarpal bone densities of the forefingers as well as serum levels of bone-type alkaline phosphatase (BAP) and tartrate-resistant acid phosphatase (TRAP) activities of the subjects in the test group and the control group were measured and statistically compared with each other. No significant changes in the general blood and urine biochemical test values and no changes in calcium,

TABLE 10

Bone densities and BAP and TRAP activities and their variations over the 12-wk test period¹

Group	Before test	After test	Variations
Bone densities, <i>mmAl</i>			
Control	2.298 ± 0.336	2.269 ± 0.334	-0.0292 ± 0.149
Test	2.375 ± 0.265	2.445 ± 0.249*	0.070 ± 0.087**
BAP activities, <i>IU/L</i>			
Control	29.966 ± 14.779	28.203 ± 16.134*	-1.763 ± 2.727
Test	25.583 ± 5.607	26.650 ± 8.417	1.067 ± 4.529**
TRAP activities, <i>IU/L</i>			
Control	7.250 ± 1.193	7.550 ± 1.234†	0.300 ± 0.490
Test	6.917 ± 0.945	6.767 ± 1.032	-0.150 ± 0.737†

¹ Values expressed as mean ± SD, *n* = 12. * *P* < 0.05; significantly different from the test group before the test (Student's *t*-test). ** *P* < 0.10; Different from the control group over the test period (Student's *t*-test). † *P* < 0.10; Different from the control group before the test (Student's *t*-test). ‡ *P* < 0.10; Different from the control group over the test period (Student's *t*-test).

luteinizing hormone in blood, or isoflavone levels in urine were observed over the test period in the test and control groups. Bone density in the test group increased significantly during the test period (*P* = 0.018) whereas no such increase was observed in the control group. The bone density variation in the test group during the test period increased in comparison with that in the control group (*P* = 0.059) (Table 10). No significant increase of BAP activity was observed in the test group over the test period. However, a significant decrease in BAP was observed in the control group (*P* = 0.047). In addition, BAP activity variation increased in the test group over the test period in comparison with the control group (*P* = 0.077). No significant decrease of TRAP activity was observed in the test group, but an increase in its activity was observed in the control group (*P* = 0.057). TRAP activity variation in the test group over the test period was diminished in comparison with the control group (*P* = 0.092). These BAP and TRAP activity results are coincident with the increased bone density in the test group. In conclusion, the test *natto* seemed to be effective in preventing the decrease of bone density in postmenopausal women.

Effects of Soy Protein Supplementation on Spine and Hip Bone Mineral Density in an Older Population in a Randomized Controlled Trial. J. W. Lampe,* ** L. Levy,* S. S. Li,* P. Qu,* K. M. Newton,† and A. Z. LaCroix*†. *Fred Hutchinson Cancer Research Center, Seattle, WA; †Group Health Cooperative, U.S.; **University of Washington, Seattle, WA.

To test the hypothesis that soy protein supplementation preserves bone mineral density (BMD) in men and women, we conducted a controlled parallel-arm double-blinded intervention study with 145 participants aged 50–80 y. Participants were randomly assigned to consume soy beverage powder daily for 12 mo. The active treatment group (+ISO) received soy protein containing 83 mg isoflavones (45.6 mg genistein, 31.7 mg daidzein, and 5.5 mg glycitein), and the comparison group (-ISO) received soy protein containing 3 mg isoflavones. We measured BMD using dual-energy X-ray absorptiometry at the total hip and posterior-anterior spine (L1–L4) at baseline in 22 women (11 +ISO; 11 -ISO) and 123 men (60 +ISO; 63 -ISO); at 6 mo in 16 women (9 +ISO; 7 -ISO) and 99 men (45 +ISO; 54 -ISO); and at 12 mo in 13 women (8 +ISO; 5 -ISO) and 98 men (43 +ISO; 55 -ISO). We used the linear mixed model to test for an isoflavone effect on percentage change in BMD from baseline values in spine and hip separately.

The test was adjusted for sex, age, BMD at baseline, and sex-distribution imbalance (85% men). Among all participants, mean percentage change in BMD (±SE) from baseline in spine was 1.17 ± 0.45 in +ISO and 0.26 ± 0.45 in -ISO (*P* = 0.14) at 6 mo and 1.20 ± 0.46 in +ISO and 0.16 ± 0.44 in -ISO (*P* = 0.10) at 12 mo. Treatment effects on spine BMD were significantly greater in women than men (*P* = 0.01); at 12 mo, in women, mean percentage change from baseline was 0.58 ± 0.70 in +ISO and -1.84 ± 0.86 in -ISO (*P* = 0.05) and, in men, it was 1.32 ± 0.53 in +ISO and 0.31 ± 0.48 in -ISO (*P* = 0.16). By comparison, percentage change in hip BMD was similar in the treatment groups, +ISO and -ISO, and was not different between men and women. Mean percentage change in hip BMD (±SE) from baseline to 12 mo was 0.54 ± 0.38 in +ISO and -0.13 ± 0.36 in -ISO (*P* = 0.20) for all participants. Overall, soy protein with isoflavones tends to preserve spine but not hip BMD in an older population in a randomized controlled trial. (Supported by NIH U01 CA72035 and DuPont Protein Technologies.)

Soy and menopause

The Effect of Soy Containing Isoflavones on Quality of Life in Postmenopausal Women: A Double-Blind Randomized Trial. L. Kok, S. Kreijkamp-Kaspers, D. E. Grobbee, and Y. T. van der Schouw. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands.

Postmenopausal estrogen decline is held responsible for several physical and psychological changes, among which is a decrease in perceived quality of life. A number of trials with estrogen replacement therapy (ERT) showed beneficial effects on quality of life. However, because of known or suspected serious side effects of ERT there is a need for alternatives. The aim of this study was to investigate the effects of a high dose of soy isoflavones on quality of life of postmenopausal women. We conducted a double-blind randomized placebo-controlled trial with soy protein, containing 99 mg naturally occurring isoflavones, or milk protein (placebo) daily for 1 y. At baseline and at final visit, participants filled out the short form of 36 questions (SF-36), the Questionnaire on Life Satisfaction Modules (QLSM), and the Geriatric Depression Scale (GDS). For this trial we recruited 202 postmenopausal women aged 60–75 y. Linear regression was used to analyze the differences in change between groups during the intervention. The ran-

domization was successful and 153 (75%) women completed the intervention. For the placebo group, scores on all dimensions of the SF-36 and the QLSM decreased during the intervention year except for the dimension "role limitations caused by physical problems," which remained stable. The soy group showed increases on 2 dimensions of the SF-36 ("social functioning" and "role limitations caused by physical problems") and on 1 dimension of the QLSM. There were, however, no statistically significant differences in changes of scores between the 2 intervention groups. For the GDS, similarly, no significant differences were found. In conclusion, the findings in this randomized trial do not support the presence of a marked effect of soy isoflavone substitution on quality of life (health status, life satisfaction, and depression) in elderly postmenopausal women.

Daidzein-Rich Isoflavone Aglycones Are Effective in Reducing Hot Flashes in Menopausal Women. L. Khaothiar,* H. Ricciotti,[†] W. Pan,* K. Fadely,[†] J. Prescott,* J. Zhou,* and G. Blackburn*. *Center for the Study of Nutrition Medicine and [†]Department of Obstetrics and Gynecology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.

INTRODUCTION: The undesirable side effects of hormone replacement therapy have led to a search for an alternative therapy for menopausal symptoms. Previous clinical studies using soy or isoflavones produced little to no improvement in hot flashes. The objective of this study is to determine the effect of a novel daidzein-rich isoflavone aglycone extract from soy germ (AglyMax, Nichimo) on hot flash frequency and severity in menopausal women. **METHODS:** A 9-wk open-label feasibility study included 24 menopausal women with 5–14 hot flashes/d. Women discontinued any medication for hot flashes for at least 6 wk. After a 1-wk run-in period all subjects started a daily supplement of 40 mg daidzein-rich isoflavone aglycones for 4 wk. The dose of isoflavone was then titrated to 20, 40, or 60 mg/d for the next 4 wk, depending on response to initial treatment. All subjects recorded their daily hot flash activity. Routine blood count; chemistry; and levels of sex hormones, thyroid hormones, leptin, and insulin were also measured. **RESULTS:** Twenty-one subjects completed the study. Daidzein-rich isoflavone aglycones improved both hot flash frequency and severity. The daily frequency of hot flashes decreased from 8.4 ± 3.8 at baseline to 6.1 ± 3.2 at 4 wk ($P = 0.002$) and 4.7 ± 3.8 at 8 wk ($P < 0.001$). Hot flash composite score (frequency \times average severity) improved from 17.5 ± 11.0 at baseline to 10.8 ± 7.3 at 4 wk (38%) and 8.07 ± 8.9 at 8 wk (54%) ($P = 0.004$ and < 0.001 , respectively). There were no significant changes in blood chemistry or hormone levels. No subject experienced any major side effects. **CONCLUSION:** This study suggests that daidzein-rich isoflavone aglycones may be an effective treatment for menopausal hot flashes.

Soy, brain, and cognition

An Immunohistochemical Study of the Effects of Soy Isoflavones on Aged Rat Brains. Y. B. Lee,* C. W. Chung,* E. Kim,* S. H. Cheon,* H. S. Sohn,* M. H. Won,[†] I. K. Hwang,[†] J. C. Kang,[†] J. Y. Lee,[†] and S. Y. Nam**. *Dr. Chung's Food Co., Ltd., Korea; [†]College of Medicine, Hallim University, Korea; **College of Veterinary Medicine, Chungbuk National University, Korea.

Normal aging is associated with a decline in functions such as sensory and motor response, and sometimes this decline is related to cognitive dysfunction. Cognitive decline appears

with atrophy of cholinergic neurons in specific brain region connected with learning and memory. Recent data suggest that estrogen can protect neurons against various insults or aging process, and soy isoflavones are structurally and functionally similar to estrogen. We investigated the effects of isoflavones on the immunoreactivities of cholinergic enzymes and receptors and delayed matching-to-place (DMP) performance in the normal aging rats. Ten-month-old male Sprague-Dawley rats ($n = 40$) were assigned to 4 groups: CD (control diet), GEN (genistein: 0.1 g/kg diet), ISO-low (isoflavone: 0.3 g/kg diet), and ISO-high (isoflavone: 1.2 g/kg diet). After 16 wk of diet feeding, choline acetyltransferase immunoreactivities of the GEN and ISO-high groups significantly increased in the medial septum area ($P < 0.05$). Choline acetyltransferase immunoreactivities of the GEN, ISO-low, and ISO-high groups significantly increased more than those of the CD group in the hippocampus CA1 area ($P < 0.05$). Acetylcholine receptor immunoreactivities significantly increased in the ISO-low group (156.4%, $P < 0.05$) in the cortex. Muscarinic receptor immunoreactivities significantly increased in the ISO-low (119.5%, $P < 0.05$) and ISO-high (144.8%, $P < 0.05$) groups in the cortex. In contrast, acetylcholine esterase immunoreactivities of the GEN and ISO-low groups significantly decreased in hippocampus ($P < 0.05$). In the DMP water maze task, behavioral performance of the GEN, ISO-low, and ISO-high groups were better across 2, 3, and 4 trials than that of the CD group ($P < 0.05$). Our study showed significant differences among groups not only in the DMP water maze task but also in immunoreactivities of cholinergic enzymes and receptors. We suggest that soy isoflavones can modulate neurotransmission and improve age-related neurodegenerative disease. (Supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea, HMP-00-PT-22000-0036.)

Dietary Phytoestrogens and Cognitive Function in Dutch Postmenopausal Women. S. Kreijkamp-Kaspers,* L. Kok,* D. E. Grobbee,* E.H.F. de Haan,[†] A. Aleman,** and Y. T. van der Schouw*. *Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, [†]Division of Psychonomics, Helmholtz Research Institute, Utrecht University, and **Department of Neuroscience, University Medical Center Utrecht, The Netherlands.

Cognitive function declines after menopause. Estrogen replacement therapy may improve cognitive function but is associated with serious side effects. A possible alternative, phytoestrogens, was effective in improving memory in college students when supplemented for a limited time. The effect of long-term low dose exposure to phytoestrogens as is achieved through the habitual diet is largely unknown. We aimed to study the effect of relatively high dietary intake of isoflavones and lignans within a Western population on cognitive function. We conducted a cross-sectional study in 301 Dutch women aged 60–75 y. Dietary isoflavone and lignan intake was assessed using a food frequency questionnaire covering the habitual diet the year preceding enrollment. Endpoints were memory (Rey Auditory Verbal Learning Test, immediate recall, delayed recall and recognition, Digit Span forward and reversed, and the Doors test), complex attention tasks (Digit Symbol Substitution, and Trailmaking A1, A2 and B), and verbal skills (Verbal Fluency A and N, animals and occupations, and the Boston Naming Task). We used linear regression analysis. In the analyses we adjusted for a wide range of potential confounders including lifestyle and dietary factors.

Median isoflavone intake was 0.2 mg in the lowest quartile of isoflavone intake and 14.6 mg in the highest quartile of intake; median lignan intake was 0.7 and 2.3 mg, respectively. There was no relation with cognitive function and isoflavone intake. High lignan intake was associated with significantly better results on digit symbol substitution (corrected difference between lowest and highest quartile of intake: 17%, P -trend = 0.05) Trailmaking A2 (corrected difference 14%, P -trend = 0.03), verbal fluency a (corrected difference 20%, P -trend = 0.02), verbal fluency occupations (corrected difference 25%, P -trend < 0.01), and Boston Naming Task (corrected difference 19%, P -trend = 0.02). The results suggest that even low-dose phytoestrogens, when incorporated in the daily diet, can improve the cognitive function of postmenopausal women.

Aging and Cognition: Potential Benefits of Soy Isoflavones. C. E. Gleason, C. M. Carlsson, T. Ohrt, A. Slatery, J. Helmers, and S. Asthana. University of Wisconsin, Madison, WI.

Abundant basic science evidence suggests that estrogen has neuroprotective and neuromodulatory effects; however, findings from the Women's Health Initiative (WHI), a large trial using conjugated equine estrogens (CEE), have raised further concerns over the feasibility of traditional CEEs because of the increased risk of serious adverse effects, including cognitive declines. Soy isoflavones may serve as a critically needed alternative therapy to traditional hormone replacement therapy. The few human research projects that have examined the neurocognitive effects of soy isoflavones offer intriguing but as yet preliminary support for isoflavones' beneficial actions on cognition. Careful and systematic study is needed to ascertain the merit of this potential therapeutic. **OBJECTIVES:** This ongoing investigation is evaluating the effects of soy isoflavones on cognition in older men and postmenopausal women. Data from this healthy aging study will be used to design intervention trials in a patient population (older adults with Alzheimer's disease). **HYPOTHESIS:** It is hypothesized that isoflavone supplements will ameliorate age-associated cognitive declines for healthy older adults (i.e., decrease rate of decline, stabilize or enhance cognitive abilities) as compared with subjects on placebo. **SPECIFIC AIMS:** 1) Characterize the cognition-enhancing efficacy of isoflavones. 2) Characterize the dose-response relationship between plasma isoflavones levels and cognition. 3) Identify the adverse effects profile of isoflavone treatment for healthy older men and women. 4) Refine intervention strategies for subsequent trials. **METHODS:** Thirty cognitively healthy older adults will be enrolled

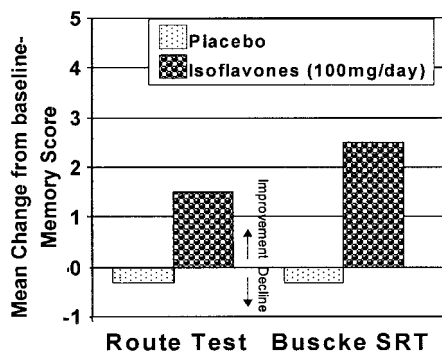


FIGURE 3 Memory measures.

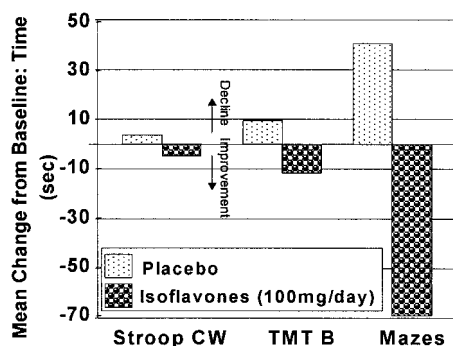


FIGURE 4 Executive function tests.

in this randomized placebo-controlled double-blind parallel-group design clinical pilot study. Subjects will receive either 100 mg/d of soy isoflavones or a placebo for 6 mo. Cognitive evaluations will be conducted at baseline and at 1, 3, and 6 mo after initiation of study medications and 2 mo after termination of treatment. Blood, collected at each treatment visit, will be assayed for plasma isoflavone levels (genistein, daidzein, and equol) and plasma estradiol. **PRELIMINARY RESULTS:** Cognitive data, collected after 1 mo of treatment with isoflavones, are currently available for 8 subjects. These data were used to calculate change from baseline. **Figures 3 and 4** depict these data. Although still preliminary, these data suggest that isoflavone treatments are associated with improvements in memory and executive function when compared with placebo. **SIGNIFICANCE:** Given recent findings from the WHI regarding cognitive effects of CEEs plus progesterone, the need for alternatives to traditional estrogen therapies is all the more pressing. The soy isoflavones may offer men and women a novel and safe alternative therapeutic for cognitive changes associated with normal aging.

A Recipe for Soy Isoflavones Decreasing Anxiety in Mid-Aged Male and Female Rats. E. Lephart,* L. Bu,* K. Setchell,† R. Handa,** T. Lund,** and L. Gonzalez**. *Brigham Young University, Provo, UT; †Children's Medical Hospital Medical Center, U.S.; **Colorado State University, CO.

Dietary phytoestrogens are molecules similar in structure and function to 17 β -estradiol and can bind estrogen receptors. In recent years phytoestrogens have been studied for their health benefit effects on cancers (breast and prostate), cardiovascular disease, osteoporosis, and symptoms associated with menopause (hot flashes). However, little information is available about the influence of dietary phytoestrogens on brain and behavior. In this study, 4 different diets were used: 1) AIN (at <5 ppm), 2) Phyto-'free' (at 10 ppm), 3) Phyto-200 (at 200 ppm), and 4) Phyto-600 (at 600 ppm of isoflavones in the diets). Male and female rats aged 50 d were placed into 1 of 4 diet treatment groups. After ~250 d on the diets, the animals were tested in an elevated-plus maze that was videotaped and later scored (the percentage of entries into and the percentage of total time spent in the open arms). In males the highest levels of anxiety were observed in the AIN and Phyto-'free' groups. Anxiety variables decreased significantly in the Phyto-200 group compared with the AIN and Phyto-'free' groups. The least amount of anxiety was seen in animals fed the Phyto-600 compared with all other groups. In females the pattern of anxiety levels was comparable with that of the male profiles above (by the diet treatments) except that anxiety

levels in the Phyto-200 vs. the Phyto-600 groups were similar (percentage of time in the open arms). However, the number of entries into the open arms in Phyto-200 and Phyto-600 animals were significantly increased compared with the AIN and Phyto-'free' groups. These data suggest that the concentrations of phytoestrogens alone via a dietary source can significantly influence anxiety. Particularly, consumption of diets rich in isoflavones significantly decreased anxiety variables. [Supported by USDA grant USDA 2002-00798 (EDL) and Brigham Young University research office (EDL).]

Phytoestrogens and Cognitive Performance in Premenopausal Women. C. Hill,* L. Dye,* M. Bryant,* J. Powell,† D. Talbot,† and A. Cassidy**. *University of Leeds, UK; †Unilever, UK; **University of Reading, UK.

Evidence to suggest that phytoestrogens may facilitate neurobehavioral actions comes from both animal and human studies. The available preliminary data suggest that the weak estrogenic nature of these compounds plays a role in explaining how sex and menopausal status mediate the effects of soy phytoestrogens on cognitive performance. Certain cognitive domains are sensitive to estrogen, specifically verbal and spatial skills. There is convincing evidence for a cognitive response to estrogen fluctuations across the menstrual cycle. When estrogen levels are high (ovulation and midluteal), performance of tasks that are advantageous to females (verbal ability) is enhanced compared with low-estrogen periods. The reverse pattern is found with tasks advantageous to males (spatial ability). It was therefore hypothesized that cognitive performance will be sensitive to soy-induced hormonal alterations, with sexually dimorphic tasks showing variation according to estrogen levels. A double-blind placebo-controlled trial of 100 mg/d of total soy isoflavones for 2 consecutive menstrual cycles was used in a crossover design. Twenty-three premenopausal women were tested in the follicular and luteal phase of each cycle on various aspects of cognitive performance. Early-morning urine samples were also collected during these phases for hormonal and isoflavone metabolite assessment. The soy intervention resulted in a significant treatment-by-phase interaction for both short-term and long-term verbal memory. During the placebo diet, performance was better in the midluteal phase of the menstrual cycle than in the early follicular phase as expected. The soy diet reversed this trend. Additionally, the soy intervention significantly reduced verbal fluency from baseline compared with placebo. A nonsignificant trend for enhanced spatial performance was also observed during the soy intervention. Hormonal assays suggest that the soy diet affected cognitive performance through central activity, which may not be reflected in peripherally measured estrogen levels.

Increased Aggression and Decreased Affiliation in Adult Male Monkeys after Long-Term Consumption of Diets Rich in Soy Protein and Isoflavones. J. R. Kaplan,* S. Hu,† N. G. Simon,† and M. R. Adams*. *Wake Forest University School of Medicine, Winston-Salem, NC; †Lehigh University, PA.

Estrogen produced by aromatization of gonadal androgen has an important facilitative role in male typical aggressive behavior that is mediated through its interaction with estrogen receptors (ERs) in the brain. Isoflavones found in soybeans and soy-based dietary supplements bind estrogen receptors and have dose- and tissue-dependent effects on estrogen-mediated responses. Although dietary soy isoflavones reportedly reduce

sexual behavior of female rats, it is not known whether aggressive or other social behavior of males is affected or whether any such effects extend to primates. To address these issues, we studied the effects of long-term (15 mo) consumption of diets rich in soy isoflavones on spontaneous social behavior among adult male cynomolgus macaques ($n = 44$) living in 9 stable social groups. There were 3 experimental conditions that differed only by the source of dietary protein: casein and lactalbumin (no isoflavones), soy protein isolate containing isoflavones at 0.94 mg/g protein, and soy protein isolate containing isoflavones at 1.88 mg/g protein. In the monkeys fed the higher amount of isoflavones, frequencies of severe aggressive (67% higher) and submissive behavior (203% higher) and mild submissive behavior (142% higher) were elevated relative to monkeys fed the control diet. In addition, the proportion of time spent by these monkeys in affiliated physical contact with other monkeys was reduced by 68%, time spent in proximity to other monkeys was reduced by 50%, and time spent alone was increased by 30%. These effects were significant whether or not adjusted for baseline (prediet) variations in social behavior. There were no significant differences among conditions in plasma testosterone or estradiol concentrations or in the response of plasma testosterone to exogenous gonadotropin-releasing hormone. The results indicate that long-term consumption of a diet rich in soy isoflavones increases aggressive behavior and reduces indices of social affiliation in male monkeys, effects that are apparently not mediated by alterations in circulating androgens.

A Dietary Soy Supplement Inhibits Proceptive and Receptive Behaviors in Hormone-Primed Female Rats. H. B. Patisaul,* J. Luskin,* and M. Wilson†. *NSF Center for Behavioral Neuroscience and †Yerkes National Primate Research Center, Emory University, Atlanta, GA.

Dietary soy supplements are becoming increasingly popular and are frequently advertised as natural alternatives to estrogen replacement therapy. However, little is known about their effect on the brain or estrogen-dependent behavior. We have examined the effects of a popular soy supplement on sexual proceptivity and receptivity in female rats. HPLC analysis of the supplement identified genistein (3.7 mg/g), daidzein (9.4 mg/g), and glycitein as major phytoestrogen components. Supplement treatment (3.5 g/kg semipurified diet) produced plasma genistein levels in rats similar to those seen in humans consuming a traditional Asian diet. Ovariectomized female rats can be reliably induced into estrus by the sequential administration of estradiol benzoate (10 μ g) followed 48 h later by progesterone (500 μ g). For our study, adult ovariectomized females were treated with either sesame oil, estradiol benzoate, the supplemented diet, or the supplemented diet with estradiol benzoate ($n = 6$ per group). All groups were also given progesterone injections 4 h before testing. Supplement treatment inhibited the lordosis response by 28% in hormone-treated animals compared with hormone-treated controls on a soy-free diet ($P < 0.001$). Similarly, proceptive behaviors were 60% lower in hormone-treated animals on the supplemented diet compared with the hormone-treated controls ($P < 0.001$). Supplement treatment also disrupted pacing behavior, resulting in significantly fewer exits after mounts, intromissions, and ejaculations. The supplemented diet had no effect on any measure of sexual behavior in the absence of estrogen. We previously showed that this soy supplement can disrupt both ER- α - and ER- β -dependent gene expression in the brain and hypothesize that disruption of these estrogen-dependent pro-

cesses in the brain results in the observed changes in female sexual behavior. Collectively, our data suggest that, unlike conventional estrogen replacement therapy, phytoestrogen supplements have antiestrogenic effects in the brain and thus are unlikely an appropriate substitute. (Supported by NSF STC Center for Behavioral Neuroscience IBN-9876754.)

The Relation between Dietary Genistein and Cognitive Function in a Multiethnic Cohort of Midlife Women. M.-H. Huang, G. Buckwalter, T. Seeman, C. Luetters, E. Gold, B. Sternfeld, and G. Greendale. Division of Geriatrics, Geffen School of Medicine at UCLA, Los Angeles, CA.

Isoflavones are naturally occurring selective estrogen receptor modulators and antioxidants, with potential protective effects on cognitive function. However, epidemiologic studies examining the relation between isoflavone intakes and cognitive function are scarce and the results have been mixed. To study this relationship, we analyzed data from the Study of Women's Health Across the Nation (SWAN), a multiethnic, community-based cohort study of women aged 42–52 y at entry. Dietary isoflavone intakes were estimated from an interview-administered food frequency questionnaire at baseline. Intakes of genistein and daidzein were highly correlated ($r = 0.98$); therefore, analyses were done using genistein only. SWAN includes Caucasian, African-American, Hispanic, Chinese, and Japanese women; this analysis was done only in the latter 2 ethnic groups because the others had minimal genistein intake. There were 218 Japanese and women and 211 Chinese women; median intakes of genistein were 6421 and 3538 $\mu\text{g}/\text{d}$, respectively. Cognitive function was measured at 4th follow-up visit and included the East Boston Memory Test, Symbol Digit Modalities Test, and Digits Backward Test. Ethnic-specific general linear models were used to examine relationships between each cognitive test and energy-adjusted genistein intake controlling for age, body mass index, smoking, alcohol, education, language used at interview, and menopause stage. No associations between genistein intake and cognitive function were found in either ethnic group. Our results did not support the protective role of genistein on cognitive function. At present we have outcomes measured at a single time. Isoflavone intake, however, may be associated with cognitive decline over time rather than with these cross-sectionally measured cognitive evaluations. In addition, preventing cognitive decline is more crucial in terms of public health interest. In SWAN, we will collect cognitive function tests at the 7th follow-up visit. This will provide us an opportunity to examine the relation between dietary isoflavone intakes and cognitive decline.

Modulation of Proteins That Associate with $A\beta$ by Dietary Soy Isoflavones. J. Deshane,* X. Sui,* P. Hall,* L. Wilson,*† S. Barnes,*†** and H. Kim*†**. *Department of Pharmacology & Toxicology, †UAB Mass Spectrometry Shared Facility, and **2D Proteomics Laboratory, University of Alabama at Birmingham, AL.

One hallmark of Alzheimer's disease (AD) is the extracellular deposition of amyloid plaques, which are aggregates of fragments of the normal amyloid precursor protein (APP) called $A\beta$. Although increased levels of $A\beta$ are considered pathological, in part because of the pro-oxidant nature of the peptide, little is understood of the events by which this oxidant status is translated into toxic events. Transgenic mice in whose brains the expression of mutated APP results in excessive $A\beta$ levels exhibit memory deficits reminiscent of AD; thus accumulation of $A\beta$ is linked with cognitive impairment. Dietary soy isoflavones previously protected rats against ovariectomy-induced cognitive impairment (1). We hypothesized that soy isoflavones exert their neuroprotective actions in part by attenuating $A\beta$ accumulation. We used a transgenic model of AD, Tg2576 (2), to study the proteins that interact with $A\beta$, hypothesizing that identifying this set of proteins (including those that mediate normal production and clearance of $A\beta$) is crucial to understanding the processes that lead to cognitive impairment. We analyzed brain homogenates of mice (aged 101 wk, $n = 3$ for each group) maintained on either a casein-based (control) diet, soy protein-based diet depleted of the isoflavones (soy-), or intact soy protein-based diet (soy+). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of immunoprecipitates of $A\beta$ revealed several proteins [creatine kinase brain beta chain, β -actin, 14-3-3 family proteins, aldolase-3 (c-isoform), heat shock protein-60 (HSP-60), and a sequence encoded by Riken cDNA2210415M14] that associated with $A\beta$ from mice fed control diet or soy- diet. In the mice fed soy+ diet, however, the amounts of HSP-60, aldolase-3 (c-isoform), and the RIKEN cDNA2210415M14 polypeptide were modulated; moreover, the amount of $A\beta$ in the brains of the tg+ mice on the soy+ diet was attenuated relative to that in the brains from the tg+ mice on the soy- diet. These data indicate that soy isoflavones may have neuroprotective effects via attenuation of $A\beta$ levels as well as by modulation of proteins that interact with $A\beta$.

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