Dietary Soy Isoflavone Intake, Postmenopausal Estrogen Use and Self-Reported Arthritis in Older Japanese American Women. Madeline Murguia Rice,* Andrea Z. LaCroix,† Johanna W. Lampe,†** Amy Borenstein Graves,‡ Mark Kestin,†**†† Gerald Van Belle,† Barbara L. Drinkwater‡‡ and Eric B. Larson.†** *George Washington University, Department of Epidemiology and Biostatistics, Washington, DC; †University, of Washington, Seattle, WA; **Fred Hutchinson Cancer, Research Center, Seattle, WA; ‡University of South Florida, Tampa, FL; ††Bastyr University, Kenmore, WA; and ‡‡Pacific Medical Center, Seattle, WA.

Hormonal status may influence the onset and progression of several common joint diseases. The objective of this study was to examine the cross-sectional association among dietary soy isoflavone intake, postmenopausal estrogen use and self-reported arthritis in 225 Japanese American women 65 years and older living in King County, Washington. Soy consumption was measured with a 14-item soy food frequency questionnaire. Soy isoflavone intake was estimated by using published isoflavone contents of soyfoods and grouped according to tertiles. Postmenopausal estrogen use and the prevalence of arthritis were ascertained by self-report. In an attempt to consider the timing of events in a cross-sectional analysis, only women who were arthritis-free at menopause onset were included. Ninety-five women reported no arthritis and 130 women reported arthritis onset during their postmenopausal years. In a model including age, weight, soy consumption and estrogen use, dietary soy isoflavone intake was significantly and negatively associated with self-reported arthritis (odds ratio = 0.44 for the highest tertile of isoflavone intake compared with the lowest tertile; $P < 0.05$) and postmenopausal estrogen use was significantly and positively associated with self-reported arthritis (odds ratio = 2.15 for current estrogen users compared with women who had never used estrogen; $P < 0.05$). Further adjustment for bone mineral density and lifestyle characteristics did not change the results. These data suggest that regular soy consumption may be associated with a lower prevalence of arthritis, whereas postmenopausal estrogen use may be associated with a higher prevalence of arthritis. These data were cross-sectional and limited by a subjective self-reported outcome. Longitudinal studies and randomized trials are needed to confirm these results. (Supported by Grant AG09769 and a graduate research supplement from the National Institute on Aging.)

Applications of Soy in Skin Care. Jue-Chen Liu, Miri Seiberg, Jonathan Miller, Juff Wu, Stanley Shapiro and Rachael Grossman. Johnson & Johnson Consumer Products Worldwide Skin Research Center, Skillman, NJ.

It is known from traditional Chinese medicine that topical soy provides skin care benefits. We recently discovered that small soy proteins (soybean trypsin inhibitor and Bowman-Birk inhibitor) partially inhibit skin pigment formation via regulation of a novel protease-activated receptor 2 pathway. On the basis of these findings, stabilized topical soy preparations containing soybean trypsin inhibitor, Bowman-Birk inhibitor and other soy components were developed. Formulations were screened through both biological and physical tests to ensure that biological activities and stability were retained in the formulation. Chemical analysis indicated that these soy preparations contained a complete spectrum of the soy active components needed for skin care. These formulations retained biological activity throughout a shelf life of 2 y and did not irritate the skin. Further pharmacological and clinical studies demonstrated that these soy preparations provided a wide range of skin care benefits. Applications for these preparations include reducing appearance of pigmentation (age spots, melasma and mottled pigmentation), reducing ultraviolet-induced erythema and peeling, reducing appearance of acne-related blotches and erythema, delaying appearance of hair regrowth and smoothing and moisturizing the skin. In summary, research into the fundamental properties of soy has led to naturally safe and effective soy preparations that deliver a wide range of valuable skin care benefits.

Phytoestrogens and Thyroid Cancer Risk in Women. Pamela L. Horn-Ross, K. J. Hoggatt and Marion Lee. Northern California Cancer Center, Union City, CA.

BACKGROUND: Thyroid cancer incidence is three times higher in women than in men. It is one of the five most common types of cancer occurring in young women and in recent Asian immigrants. Female hormones and changeable lifestyle factors (e.g., dietary intake) may be important in thyroid carcinogenesis. METHODS: A population-based case-control study of thyroid cancer in women, 20–74 years old, was conducted in the San Francisco Bay area. Of 817 cases, diagnosed in 1995–1998 (1992–1998 for Asian women), and identified through the cancer registry, 608 (74%) were interviewed. Controls were identified through random-digit dialing and frequency-matched to cases on age and ethnicity. Of 793 eligible controls, 558 (70%) were interviewed. Interviews were conducted in six languages. Phytoestrogen consumption was assessed by a food frequency questionnaire and quantified from a recently developed database. RESULTS: All three classes of phytoestrogens examined (isoflavones, coumestans and lignans) and four of the seven specific compounds were associated with a reduced risk of thyroid cancer. The odds ratio for the highest quintile of total phytoestrogen consumption compared with the lowest was 0.64 (95% confidence interval: 0.41–0.98). Risk reduction was apparent for both white and Asian women. CONCLUSIONS: Despite the association between soybean consumption and goiter, a major risk factor for thyroid cancer, soy-based foods and phytoestrogens in particular seem to be associated with a reduced risk of thyroid cancer in women. Possible mechanisms include antioxidant effects, direct effects on endogenous estrogen and thyroid hormone

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levels, or antiestrogenic effects resulting from competitive binding to the estrogen receptor.

Breast Enhanced Scintigraphy Test Demonstrates Improvement in Breast Inflammation in Women Consuming Soy Protein. Richard Fleming. The Camelot Foundation, Omaha, NE.

BACKGROUND: Breast enhanced scintigraphy testing (BEST) combines myocardial and breast-imaging technologies into a single test that yields diagnostic information about myocardial function and distinguishes between normal breast tissue, inflammatory breast tissue and carcinoma of the breast. Debate exists over the possible effects of soy protein on breast tissue. To determine whether soy protein has any effect on inflammatory changes of the breast, women with inflammatory breast changes as defined by BEST imaging were asked to consume soy protein daily and were restudied 6 mo later. METHODS: BEST imaging was performed on 197 women and 3 men over 2.5 y. There were 104 cases of women with inflammatory changes of the breast with a maximal count activity of 229 ± 50. Of these 87 women, 40 were started on a 24-g/d regimen of soy protein containing 160 mg isoflavones and 1000 mg saponins. Of the 40 women, 30 have been studied both before and after 6 mo of soy protein consumption. RESULTS: Of the 30 women studied to date, the maximal count activity was 245 ± 40 when initially evaluated by BEST imaging. After 6 mo of dietary supplementation with soy protein, the maximal count activity was 202 ± 32. Using a two-tailed *t* test for comparison, we found a statistically significant ($P < 0.05$) difference between the inflammatory changes noted before and after soy protein consumption. No other dietary or lifestyle changes were reported by the women during follow-up evaluation. CONCLUSIONS: BEST demonstrated a reduction in breast inflammation in 30 women consuming daily soy protein during a 6-mo follow-up evaluation. An additional 10 women are currently being studied to determine whether the effect is persistent. Further research is indicated to determine the long-term ramifications of soy protein consumption on breast tissue.

Keynote—Genistein Chemoprevention: Timing and Mechanisms of Action in Murine Mammary and Prostate. Coral A. Lamartiniere,*† Michelle S. Cotroneo,* Wayne A. Fritz,* Jun Wang,* Roycelynn Mentor-Marcel*† and Ada Elgavish.†** *Department of Pharmacology and Toxicology, †UAB Comprehensive Cancer Center, and **Department of Genomics and Pathology, University of Alabama at Birmingham, Birmingham, AL.

Epidemiological reports suggest that Asians eating a traditional diet high in soy have reduced incidence of breast and prostate cancers. We investigated the potential of genistein, the primary isoflavone of soy, to protect against these cancers in animal models. For mammary cancer studies, Sprague-Dawley rats were fed AIN-76A diet ± 250 mg genistein/kg diet. Dimethylbenz[a]anthracene was administered by gavage at d 50 postpartum to induce mammary tumors. Mammary cancer chemoprevention was demonstrated after prepubertal and combined prepubertal and adult genistein treatments. Prenatal- or adult-only genistein exposures did not protect. Dietary genistein did not result in increased incidence of mammary tumors. These experiments demonstrate that timing of exposure to genistein is important for mammary cancer chemoprevention. The cellular mechanism of action was found to be mammary gland differentiation, as shown by whole-mount analysis and β-casein expression. An imprinting effect was shown for epidermal growth factor receptor expression in mammary terminal end buds. For prostate cancer studies, we used two models. The first was a chemically induced prostate cancer rat model. Lobund-Wistar rats were exposed to 0, 25 and 250 mg genistein/kg AIN-76A diet, starting at conception and continuing until necropsy at age 11 mo. Male offspring were injected subcutaneously with flutamide on d 50–66 and with testosterone on d 67–69 and injected in the dorsal prostate with *N*-nitrosomethylurea on d 70. The rats were given testosterone implants starting at d 77. Genistein in the diet inhibited the development of *N*-nitrosomethylurea-induced prostate invasive adenocarcinomas in a dose-dependent manner. For the second prostate cancer model, we used a transgenic mouse model that results in spontaneously developing adenocarcinoma tumor of the prostate (TRAMP). Mice fed phytoestrogen-free diet had well-differentiated (52%), moderately differentiated (18%), and poorly differentiated (31%) prostatic adenocarcinoma tumors by age 28–30 wk. Genistein in the diet (0, 100, 250 and 500 mg/kg AIN-76A) reduced the incidence of poorly differentiated prostatic adenocarcinomas in a dose-dependent manner. mRNA transcripts of androgen receptor, estrogen receptors α and β, progesterone receptor, epidermal growth factor receptor, transforming growth factor-α, insulin-like growth factor-I, and extracellular signal-regulated kinase-1 were elevated in prostates of 12-wk-old transgenic mice compared with nontransgenic mice. Genistein fed to transgenic mice reversed the effect on androgen receptor, estrogen receptor-α, progesterone receptor, epidermal growth factor receptor, insulin-like growth factor-I, and extracellular signal-regulated kinase-1 but not on estrogen receptor-β and transforming growth factor-α mRNA expression. We conclude that dietary genistein protects against mammary and prostate cancers by regulating specific sex steroid receptors and growth factor signaling pathways.

Soy Isoflavones in the Treatment of Prostate Cancer. Maha Hussain,* Fazlul H. Sarkar,† Zora Djuric,* Michael N. Pollak, Mousumi Banerjee,‡ Daniel Doerge,†† Joseph Fontana,*‡‡ Sreenivasa Chinni,† Joanne Davis,† Jeffrey Forman,# David P. Wood§ and Omer Kucuk.*** *Division of Hematology and Oncology, †Department of Pathology, #Department of Radiation Oncology, §Department of Urology and ‡Center for Healthcare Effectiveness Research, Wayne State University and the Barbara Ann Karmanos Cancer Institute, Detroit, MI; ‡‡VA Medical Center, Detroit, MI; **Department of Medicine, McGill University and Jewish General Hospital, Montreal, Quebec, Canada; and the ††Division of Biochemical Toxicology, National Center for Toxicological Research, U. S. Food and Drug Administration, Jefferson, AR.

We previously observed that genistein induces apoptosis and inhibits growth and prostate-specific antigen (PSA) production and secretion in androgen-dependent (LNCaP) and androgen-independent (PC3 and VeCaP) prostate cancer cell lines. To determine the effect of soy isoflavones on serum PSA levels, we conducted a phase II clinical trial in patients with prostate cancer. Eligible patients had prostate cancer and were previously untreated (group I), treated with local therapy (group II), or treated with hormone therapy (group III) and had to have either three successive measurements of rising PSA levels or a PSA of > 10 μg/L (294 pmol/L) at two successive evaluations. No other therapy or micronutrient supplements were allowed. Patients received 100 mg soy isofla-

vones orally twice daily for a minimum of 3 mo in the absence of progression or toxicity. Serum levels of total genistein and daidzein, PSA, testosterone, insulin-like growth factor-1 and insulin-like growth factor binding protein-3 levels were measured and toxicity was assessed monthly. Forty-one patients were enrolled; 4 patients in group I, 18 in group II, and 19 in group III, with a median PSA level of 13.3 μg/L (391 pmol/L). Forty patients were evaluated for PSA response. A total of 226 mo of supplementation was given with a median of 6 mo per patient (range: 1–10 mo). Three of 4 patients in group I, 15 of 18 in group II, and 6 of 19 in group III achieved stable disease. The rate of linear rise in serum PSA levels decreased by 71% (from 14% to 4%, $P = 0.03$) in group II and by 56% (from 27% to 16%, $P = 0.07$) in group III patients. Although significant increases in serum isoflavones were observed after supplementation, no significant changes were observed in serum levels of testosterone, insulin-like growth factor-1, or 5-hydroxy-methyl-deoxyuridine. Interestingly, insulin-like growth factor binding protein-3 level decreased significantly after soy isoflavone supplementation ($P = 0.0007$). Although the mechanism is unclear, soy isoflavones seem to decrease the rate of rise in serum PSA and stabilize the disease in patients with hormone-sensitive as well as hormone-refractory prostate cancer.

Soy Phytoestrogens and Estrogen-Dependent Breast Cancer Growth. Clinton D. Allred, Yound H. Ju, Kimberly F. Allred, Jongsoo Chang and William G. Helferich. Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL.

The estrogenic isoflavones found in soy products have several biological activities. Our research has focused on the effects dietary genistein from various sources on growth of both estrogen-dependent and estrogen-independent human breast cancer cells both in vitro and in vivo. The focus of this presentation will be on the estrogenic effects of the soy isoflavones in vivo. Dietary genistein enhances tumor growth, cell proliferation and expression of the estrogen-responsive gene pS2. We have demonstrated that genistein stimulates growth of estrogen-dependent tumors in a dose-dependent manner. Additionally, we have conducted studies in which various soy component fractions (normalized to genistein content) were fed to mice implanted with estrogen-dependent breast tumors. We have observed that although these diets contain similar amounts of genistein, the form in which genistein is administered can significantly affect tumor growth rate. In summary, genistein can act as an estrogen agonist resulting in proliferation of estrogen-dependent human breast cancer cells in vitro and enhances growth of MCF-7 cell tumors (in vivo) implanted into ovariectomized athymic mice.

Soy Phytoestrogens as an Adjunct to Hormone Replacement Therapy: Implications for Breast Cancer Prevention from Rat Studies. Yasmina A. Paramastri and J. Mark Cline. Wake Forest University School of Medicine, Winston-Salem, NC.

The weak estrogen-agonist activity of soy phytoestrogens (SPE) is unlikely to provide a complete alternative to hormone replacement therapy for postmenopausal women. However, our work has shown dose-dependent interactions of mammalian estrogens and dietary soy phytoestrogens, indicating that maximal benefit may be derived from combining hormone replacement therapy and SPE. This study assessed the effect of this combined strategy on breast cancer prevention in rats. Seventy-two female Sprague-Dawley rats were raised on a phytoestrogen-free diet and at age 50 d were given 80 mg/kg of the carcinogen dimethylbenz[a]anthracene by gavage to induce mammary carcinomas. The rats were then randomly distributed into a 2×3 factorial experiment including two casein-lactalbumin–based diets (either SPE-free or providing 13 mg $\cdot$ kg^{-1} $\cdot$ d^{-1} of SPE) and three hormonal conditions (intact, ovariectomized or ovariectomized plus conjugated equine estrogens [CEE] at 0.033 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}). Doses were scaled from doses for women—0.625 mg CEE/d and 120 mg SPE/d—on a caloric basis. Phytoestrogens were added as a semipurified extract containing genistein (63%), daidzein (33%), and glycitein (4%). Diets were isocaloric and matched for fiber, protein and tocopherols. The rats were examined for tumor onset twice weekly for 200 d. Tumor incidence was greatest in intact rats (100% by 150 d after dimethylbenz[a]anthracene); dietary SPE did not reduce tumorigenesis in these rats. Fewer tumors (50% at the study's end) were found in CEE-treated rats not given SPE; this incidence was reduced to 8% (one tumor in one rat) by adding SPE to CEE. Tumor incidence in ovariectomized rats not given CEE was 25%, and this was not lowered by SPE. In conclusion, mammary tumor promotion by cyclic endogenous ovarian estrogens was not inhibited by SPE in a casein-lactalbumin–based diet. However, tumor promotion by continuous exogenous estrogens was inhibited, suggesting that SPE may have benefits for the breast as an adjunct to hormone replacement therapy.

Soy Enhances Tamoxifen's Cancer Chemopreventive Effects in Female Rats. Andreas I. Constantinou, Haiyan Xu, LynAriane Morgan Lucas and Daniel Lantvit. University of Illinois at Chicago, Department of Surgical Oncology, Chicago, IL.

Tamoxifen is a known effective chemopreventive agent against breast cancer in high-risk women. Studies suggest that soy-containing diets may protect against mammary tumors in female rats. Whether the consumption of soy enhances or negates tamoxifen's protective effects against breast cancer is unknown. The objective of this study was to evaluate the effect of a soy product in combination with tamoxifen against mammary tumorigenesis in female rats. Four groups of 20 female Sprague-Dawley rats were treated with dimethylbenz-[a]anthracene (DMBA) plus the following test agents, each mixed in AIN76A diets: no treatment (DMBA control), tamoxifen (0.125 mg/kg diet), soy protein isolate (SPI, 160 g/kg diet), and tamoxifen plus SPI. A fifth group of 10 female rats was fed the basal diet without being exposed to DMBA. After a 4-mo follow-up, during which the appearance, number, and location of mammary tumors were recorded, we found the following results. Tamoxifen was effective in reducing mammary tumor multiplicity (tumors per rat) from 7.9 in the DMBA control group to 5.6, representing a 29% reduction. A 37% reduction in tumor multiplicity was evident in the group that was fed SPI, and a 62% reduction in the group that was fed tamoxifen plus SPI. Tumor latency was significantly increased only in the tamoxifen plus SPI group. None of these treatments showed any effect in the rate of animal growth or caused any toxicity. SPI, tamoxifen and their combinations altered the relative expression of estrogen receptor-α and -β in the mammary glands and tumors. Purified genistein, alone and in combination with 4-OH tamoxifen, increased the ratio of estrogen receptor-α to estrogen receptor-β mRNA levels in

cultured human breast cancer T47D-A18 cells. These studies show that in rats the chemopreventive efficacy of tamoxifen can be improved by soy and that the mechanism of action involves the relative expression of estrogen receptors.

Quantitation of Soy Phytoestrogens in Human Breast Tissue and Biological Fluids. Julie Maubach,* Herman Depypere,† Rudolphe Serreyn,† Denis De Keukeleire,* Marc Mareel and Marc Bracke.**** *Laboratory of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium; †Department of Gynaecological Oncology, Ghent University Hospital, Ghent, Belgium; and the **Department of Radiotherapy, Nuclear Medicine, and Experimental Cancerology, Ghent University Hospital, Ghent, Belgium.

Substantial public and scientific interest has developed in the possible health benefits of phytoestrogens. The need to further explore the fate of phytoestrogens inside the body, concentrations achieved in different body compartments, and safety of various intakes has, therefore, arisen. Although determination of genistein, daidzein and equol in biological fluids has been extensively documented, little is known about the tissue distribution of these compounds after ingestion (1). Our objective was to quantitate genistein, daidzein and equol in breast tissue—compared with urine and serum levels—in women undergoing breast reductions, after the women had taken an isoflavone tablet containing 100 mg genistein, 37 mg daidzein, and 15 mg glycitein or a placebo tablet for 5 consecutive days before surgery. After sample treatment, including hydrolysis with β-glucuronidase and arylsulfatase and exhaustive removal of fat via solid-phase extraction, separation of the analytes was achieved by isocratic high performance liquid chromatography with UV diode array detection. The method has been fully validated. Soy phytoestrogens were detected in human breast tissue, with equol concentrations being invariably higher than those of genistein and daidzein in the same tissue. To the best of our knowledge, this is the first method described for measuring the concentration of phytoestrogens in human breast tissue. We believe that our data can give valuable insight into the concentrations achieved in mammary tissue after phytoestrogen ingestion and so could increase our knowledge as to which intake levels are necessary for obtaining the beneficial health effects associated with these compounds and their metabolites.

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Effects of an Isoflavone Intervention on Hormones and Mammographic Densities in Premenopausal Women. Gertraud Maskarinec,* Andrew E. Williams,* Adrian A. Franke* and Frank Stanczyk.† *Cancer Research Center of Hawaii, Honolulu, HI; and †University of, Southern California, Los Angeles, CA.

Isoflavones, which are phytoestrogens contained in soyfoods, may affect breast cancer risk. This randomized double-blinded trial with 34 premenopausal women investigated the effect of tablets with 100 mg isoflavones or placebo, given daily, on the ovulatory cycle and on mammographic densities for 1 y. At baseline and at 1, 3, 6 and 12 mo, the subjects donated blood and urine samples ~5 d after ovulation as determined with an ovulation kit. Compliance with the study regimen was confirmed by urinary isoflavone excretion and tablet counts (> 85%). We performed serum assays for estrone, estradiol, estrone sulfate, progesterone, sex hormone-binding globulin and the gonadotropins follicle-stimulating hormone and luteinizing hormone and measured the metabolites estrone glucuronide, 2-hydroxyestrone and 16α-hydroxyestrone in urine. Mammographic densities were assessed at baseline and after 1 y by a computer-assisted method. After log transformation, we applied the method of least squares to fit general linear models to test for an intervention effect. Before randomization, both groups consumed fewer than two servings of soy per week and had similar dietary patterns. Baseline serum and urinary variables did not differ by group. Over the 1-y period, we found no significant differences between treatment groups for any of the measured hormones, measured metabolites or estimated free estradiol. Menstrual cycle length was not affected by the intervention. Exclusion of the 22 nonovulatory cycles did not alter the results. We detected small nonsignificant changes in mammographic characteristics. The findings do not support the hypothesis that isoflavones modify the reproductive cycle of premenopausal women in 1 y. Although the results contradict earlier research, the randomized design and the long treatment period support the validity of this study. However, these findings do not exclude the possibility that other substances in soy or alternative cancer protective mechanisms of isoflavones may lower breast cancer risk in women who consume soy foods regularly.

Effects of Isoflavones on Breast Density, Estradiol and Gonadotrophins: A Double-Blind, Randomized, Placebo-Controlled Trial. Charlotte Atkinson,* Ruth M. L. Warren,† Mitch Dowsett, Nick E. Day* and Sheila A. Bingham.‡** *Medical Research Council Biostatistics Unit, Institute of Public Health, Cambridge, UK; †Department of Radiology, Addenbrooke's Hospital, Cambridge, UK; **Department of Biochemistry, Royal Marsden Hospital, London, UK; and ‡Medical Research Council Dunn Human Nutrition Unit, Cambridge, UK.

One hundred seventy-five women 49–65 y old with Wolfe's P2 or DY mammographic patterns were randomly assigned to receive an isoflavone tablet (40 mg isoflavones; Promensil; Novogen Ltd., Stamford, CT) or placebo daily for 1 y. The primary outcome was mammographic breast density; secondary outcomes were circulating levels of estradiol, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Women taking hormone replacement therapy or with previous breast cancer or breast surgery were ineligible. The percentage densities on mammograms at recruitment and after 1 y of intervention were visually estimated. Estradiol, FSH and LH were measured in fasting blood samples taken at baseline and 1 y; hormonal changes were assessed only in postmenopausal women. There were no baseline differences between treatment groups. At 1 y, breast density in postmenopausal women and in pre- and perimenopausal women (combined into one group) had decreased in both treatment groups but differences between treatments were not significant ($P > 0.01$). When divided into tertiles of age, there was a significantly greater decrease in breast density in the isoflavone group than in the placebo group among women in the highest tertile of age (56–65 y; $P < 0.05$). Among postmenopausal women, estradiol increased nonsignificantly in both treatment groups but the difference between treatments was not significant ($P > 0.05$). FSH and LH decreased significantly in both treatment groups but changes did not differ significantly between treatments ($P > 0.05$). In conclusion, and in contrast with

hormone replacement therapy, clover-derived isoflavones did not increase breast density; a significant reduction in density was seen in women aged 56–65 y. However, sample sizes within tertiles of age were small and a larger study is needed to confirm these findings. There were no treatment effects for estradiol, FSH or LH in postmenopausal women.

Adolescent and Adult Soy Intake and Risk of Breast Cancer in Asian-Americans. A. H. Wu, M. C. Pike and P. Wan. University of Southern California, Los Angeles, CA.

In a study of breast cancer in Asian-Americans residing in California and Hawaii, we (1) reported that risk of beast cancer was reduced with increasing frequency of intake of tofu. However, this study was limited in that our assessment of soy was crude. We have since conducted a population-based case-control study of breast cancer in Asian-Americans in Los Angeles County that was specifically designed to investigate further the association between soy and breast cancer. We report results on 628 Asian-American women, aged 25–74 y, diagnosed with histologically confirmed primary breast cancer. A comparable number of Asian-American women without breast cancer, matched to cases on age, ethnicity and neighborhood of residence, were the comparison group. Intake of soyfoods was influenced by birthplace and Asian ethnicity. For control subjects, soy (isoflavones) intake was highest for Chinese (27 mg/d), intermediate for Japanese (19 mg/d), and lowest for Filipino women (9 mg/d). Intake of soy was more than double for Asian women born in Asia compared with those born in the United States. The risk of breast cancer was significantly influenced by soy intake during adolescence and adult life. After adjusting for age, Asian ethnicity, education and migration history, women who reported soy intake four or more times per week during adolescence showed a 40% reduced risk of breast cancer compared with those who did not consume soy ($P < 0.05$). There was also a significant trend of decreasing risk with increasing quartile level of soy intake during adult life ($P < 0.05$). The reduction in breast cancer risk associated with soy intake was observed in Asian migrants as well as women born in the United States. Soy intake during adolescence and adult life conferred independent protective effects against breast cancer risk in this population.

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Keynote—Hormonal Effects of Soy in Premenopausal Women and Men. Mindy Kurzer. Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN.

Over the past few years, there has been increasing interest in the possible hormonal effects of soy and soy isoflavone consumption in both women and men. Soy consumption has been suggested to exert potentially cancer-preventive effects in premenopausal women, such as increased menstrual cycle length and sex hormone-binding globulin levels and decreased estrogen levels. There has been some concern that consumption of phytoestrogens might exert adverse effects on men's fertility, such as lowered testosterone levels and semen quality. The studies in women have provided modest support for beneficial effects. One cross-sectional study showed serum estrogens to be inversely associated with soy intake. Seven soy intervention studies controlled for phase of menstrual cycle. These studies provided 32–200 mg/d of isoflavones and generally showed increases in menstrual cycle length and decreases in blood concentrations of estradiol, progesterone, midcycle gonadotropins and sex hormone-binding globulin. A few studies also showed increased ratios of urinary 2-(OH) to 16α-(OH) and 2-(OH) to 4-(OH) estrogens. Soy and isoflavone consumption does not seem to affect the endometrium in premenopausal women, although there have been weak estrogenic effects reported in the breast. Thus, studies in women have mostly been consistent with beneficial effects, although the magnitude of the effects is quite small and of uncertain significance. Only two intervention studies reported hormonal effects of soy isoflavones in men. These recent studies in men consuming soyfoods or supplements containing 40–70 mg/d of soy isoflavones showed no effects on either plasma hormones or semen quality. These data do not support concerns about effects on reproductive hormones and semen quality.

The Specific Role of Isoflavones in Estrogen Metabolism in Premenopausal Women. N. B. Kumar, K. A. Allen, D. Riccardi, A. Cantor and C. E. Cox. H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

We demonstrated that supplementing with 40 mg/d of soy isoflavones in a premenopausal group of Western women could increase length of menstrual and follicular cycles and moderately alter steroid hormone levels. Because levels of these steroid hormones and menstrual cycle length are recognized risk factors for breast cancer, this is an important finding. Increase in menstrual cycle length would reduce the number of menstrual cycles in a lifetime, thereby reducing the total number of times the breast is exposed to estrogen. In addition, women will spend more days in the increased follicular cycle lengths when proliferation is at its lowest. If this occurs over a prolonged period with consistent soy consumption, reduction in breast cancer risk potentially can be affected. BACKGROUND: Evidence is increasing that soy isoflavones may play a role in the production, metabolism and bioavailability of sex hormones and their effect on target tissues. The aim of this study was to evaluate the effectiveness of supplementing a group of premenopausal, breast cancer-free women with a dietary supplement of isoflavone (40 mg/d) to produce a change in steroid hormones and menstrual cycle length. METHODS: Sixty-eight consecutively recruited premenopausal, omnivorous women of all races and ethnic groups between 25 and 55 y old were admitted to the study and randomly assigned to an experimental group supplemented with soy (40 mg/d of genistein) or to a control group consuming a placebo for 12 wk. Changes in their anthropometric, nutritional and hormonal biomarkers from early follicular phase were analyzed at baseline and postintervention. RESULTS: As hypothesized, serum-free estradiol and estrone levels decreased moderately in the soy group. Sex hormone-binding globulin increased in 41.4% of subjects in the soy group and 37.5% in the placebo group. Free estradiol decreased in 53.85% of subjects in the soy group and 37.5% in the placebo group. Estrone decreased in 55.56% of subjects in the soy group and 42.86% in the placebo group. Mean menstrual cycle length increased by 3.52 d in the soy group and decreased by 0.06 d in the placebo group ($P = 0.04$) from baseline to the third menstrual cycle. Mean follicular phase increased by 1.46 d in the soy group and 0.14 d in the placebo group ($P = 0.08$). CONCLUSIONS: These data suggest that increased, consistent isoflavone intake affects estrogen metabolism by altering the steroid hormone concentrations and menstrual

cycle length, thereby demonstrating a potential for reducing the risk for breast cancer.

Intestinal Bacterial Conversion of the Soy Isoflavone Daidzein to Equol: Implications for Human Health. Johanna W. Lampe, Heather E. Skor, Charlotte Atkinson and Cara L. Frankenfeld, Fred Hutchinson Cancer Research Center, Seattle, WA.

The isoflavone equol is produced by intestinal bacterial metabolism of the soy isoflavone daidzein. Approximately one-third of people harbor the microbes capable of producing equol from daidzein and studies suggest that having the equol-producer phenotype may impart health benefits. For example, equol-producing women have steroid hormone profiles associated with lower risk of breast cancer and have the greatest lengthening of the menstrual cycle follicular phase with soy supplementation. In a case-control study, higher urinary equol excretion was associated with a lower risk of breast cancer. We are using several approaches to study equol production and to examine the effect of equol-producer phenotype on health. Using a simple in vitro system, we have started to identify factors that affect conversion of daidzein to equol. Fecal inoculate from equol-producing individuals results in conversion of daidzein to equol under anaerobic and aerobic conditions and incubation with certain antibiotics can inhibit equol and dihydrodaidzein (an intermediate metabolite) production. For example, significant amounts of dihydrodaidzein, but no equol, were produced when ampicillin was used, supporting the hypothesis that the conversion of daidzein to equol involves more than one type of bacterium. To test whether genetic factors influence an individual's capacity to produce equol, we are conducting a family-based association study of equol-producer phenotype. We are recruiting 100+ multigeneration families and testing members for equol-producer status; we will use the estimating equation approach to determine whether there is familial aggregation of equol production. Our other studies are designed to determine, within the context of observational studies, the effect of equol-producer phenotype on markers of estrogen exposure; we are using markers of long-term estrogen exposure (e.g., bone mineral density) and estrogen metabolism. We hope that this multipronged approach will help to elucidate the effect of the equol-producer phenotype on human health.

Identification of Isoflavone Metabolites by Gas Chromatography-Mass Spectrometry. Satu-Maarit Heinonen,*† Antti Hoikkala,† Kristiina Wähälä† and Herman Adlercreutz.* *Folkhälsan Research Center and Department of Clinical Chemistry, Biomedicum, University of Helsinki, Finland; and †Laboratory of Organic Chemistry, Department of Chemistry, University of Helsinki, Helsinki, Finland.

Numerous different types of foods as well as tablets contain isoflavones from soy or red clover. The metabolism of daidzein and genistein (the main isoflavones of soy and major metabolites formed from red clover isoflavones formononetin and biochanin A) has been studied to some extent, but detailed studies of the metabolism of other isoflavones present in soy and red clover are lacking. The metabolism of isoflavones was studied in two feeding studies. In the first study six participants included two soybars per day in their normal diet for 2 wk. The main isoflavones of the soybar were characterized by gas chromatography-mass spectometry (GC-MS) as daidzein, genistein and glycitein. In the second study the metabolism of red clover isoflavones was investigated. Seven female participants ingested a single dose of four red clover-based dietary supplements. The tablet was found to contain mainly formononetin and biochanin A; other abundant isoflavones were daidzein, genistein, prunetin, calycosin and pseudobaptigenin. Urine samples collected before and after the isoflavone supplementation were treated and analyzed by GC-MS as described in our previous study. The structures of the isoflavone metabolites identified in urine samples were characterized with authentic synthetic reference compounds. Some of the metabolites for which reference compounds were not available were identified with metabolites formed with 24-h in vitro incubations of pure isoflavone standards with human fecal inoculum. The main oxidative metabolites of daidzein and genistein were 7,3′,4′-; 6,7,4′-; and 7,8,4′-trihydroxyisoflavones and 5,7,3′,4′-tetrahydroxyisoflavone, respectively. The corresponding reductive metabolites (isoflavanones, isoflavans and α-methyldeoxybenzoins) of oxidative metabolites were also isolated and identified in urine samples. The identification of reductive metabolites of glycitein, formononetin and biochanin A is presented for the first time. It is suggested that the biological effects of isoflavones may be mediated by some of the identified metabolites.

Five-Generation Study with Isoflavones of Sexual Dimorphic Behavior and Maturation in Sprague-Dawley Rats. Chai-Won Chung, Sockju Kwon, Yoon-Bok Lee and Heon-Soo Sohn. Dr. Chung's Food, Heungduk-Gu, Chungjoo-Si, Choongchungbuk-Do, Korea.

Isoflavones are similar to estrogen in structural and functional properties. However, there are still arguments that the similarity might cause sexual dimorphic characteristics in males. To investigate the five-generation effects of isoflavones consumption on sexual dimorphic behavior and maturation in Sprague-Dawley rats, 24 males and 24 females [first generation (G1)] were randomly divided into four groups: control (CON, no isoflavones) and three commercially available regimens; cow's milk (CM, no isoflavones), nutritional-modified soymilk and cow's milk mixture (MSC, 15 mg/d isoflavones), and soymilk (SM, 24 mg/d isoflavones). The first offspring of each generation were fed each regimen after weaning, and this was continued through the fifth generation (G2–G5). Spatial memory and open-field activity was measured by the Morris water-maze test and by the open-field activity test using video-tracking system, respectively; body weight increase of dams and pups and reproductive outcome were measured; and the histomorphology of sex organ-related tissues was examined. SPSS for Windows was used for data description and Kruskal-Wallis tests. No offspring were in the CM group because of low hematocrit levels. The exploratory action (rearing and learning) of CON males was less frequent than that of females. The learning activity (G2, 18.5, $P = 0.04$; G3, 11.2, $P = 0.03$; G4, 10.7, $P = 0.02$) of SM males was more frequent than CON and MSC. The higher maternal body weight increase during pregnancy ($P = 0.055$) and longer first parturition day ($P = 0.086$) were shown in MSC over generations. Litter size was significantly higher in G2 MSC (12.9, $P = 0.02$) than in CON (8.42) and SM (8.57). The body weight increase of pups seemed to be low in SM and MSC, but no differences were noted in the survival rate and sex of pups. Other variables will be analyzed and discussed. The data assessed so far revealed no significant sexual dimorphic behavior and maturation differences over generations except the learning frequency of SM males.

Multigenerational Animal Studies of Soy Protein Isolate: Is There Evidence for Adverse Developmental Effects? Thomas M. Badger, Martin Ronis and Reza Hakkak. Arkansas Children's Nutrition Center and Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR.

Three primary proteins are used in infant formulas sold in the United States: soy protein isolate (SPI), bovine casein (CAS) protein and bovine whey (WPH) protein. Formula made with SPI contains high concentrations of many substances not normally found in human breast milk, which has led to concerns over possible adverse health effects in infants consuming formula compared with human milk. Phytochemicals similar to those found in soy infant formula have been reported to cause deleterious effects, such as infertility, thyroid disorders, central nervous system disorders and even death. There is a paucity of data on developmental, physiological, neurophysiological, behavioral, metabolic and molecular effects of soy diets during pregnancy and infancy. We have studied the effects of SPI in short-term, long-term and multigenerational studies in rats because of obvious ethical and safety concerns about studies in infants. Aside from minor differences in body weight gain profiles, SPI-fed rats did not differ from CAS-fed rats in development, organ weights, in vitro hepatic metabolism or reproductive performance. However, puberty in females rats fed SPI diets was accelerated ($P < 0.05$) compared with those fed CAS ($P < 0.05$). Although male rats fed SPI diets had normal serum testosterone levels, female rats fed SPI had altered serum estrogen profiles compared with rats fed CAS ($P < 0.05$). Female rats fed SPI or treated with genistein had reduced incidence of chemically induced mammary and colon cancers ($P < 0.05$) compared with CAS controls. Results from our animal studies and from clinical studies of infants and adults consuming SPI suggest that subtle effects can occur. Determining the long-term health consequence implications of early diet exposure to SPI still awaits further research, but epidemiological and laboratory data suggest potential benefits to soy infant formula rather than adverse effects. (Supported by Arkansas Children's Nutrition Center, a U. S. Department of Agriculture, Agricultural Research Service Program.)

Effects of Developmental Exposure to Genistein, a Soy Phytoestrogen, in an Experimental Animal Model. Retha Newbold. Developmental Endocrinology Section, Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Genistein, a naturally occurring isoflavone, interacts with estrogen receptors and multiple molecular targets. Human exposure to genistein is predominantly through dietary consumption of soy products, but its use as a nutritional supplement and pharmaceutical has dramatically increased in the past few years. This increase is primarily a result of reports suggesting that phytoestrogens, particularly genistein, may lower the risk of severe chronic diseases and protect against various cancers. Although much attention has been given to the beneficial effects of dietary estrogens, it is important to balance these against the potential for deleterious effects, especially if exposures occur during critical early stages of human development. It is well known that the developing fetus is uniquely sensitive to perturbation with estrogenic chemicals; the carcinogenic effect of prenatal exposure to diethylstilbestrol (DES) is a classic example. Because infants and children are exposed to genistein in soy-based infant formulas and soy products marketed especially to children, we investigated the potential of genistein to cause adverse effects using an experimental animal model. This model previously showed an increased incidence of uterine adenocarcinoma in aged mice developmentally treated with DES. Outbred female CD-1 mice were treated on d 1–5 with equivalent estrogenic doses of genistein (50 mg $\cdot$ $kg^{-1} \cdot d^{-1}$) or DES (0.001 mg $\cdot$ $kg^{-1} \cdot d^{-1}$). At 18 mo, the incidence of uterine adenocarcinoma was 35% for genistein and 31% for DES. These data show that genistein can have adverse consequences if exposure occurs during critical periods of differentiation. Thus, the use of soy-based infant formulas in the absence of medical necessity and the marketing of soy products designed to appeal to children with the assumption that there will be no adverse effects deserve further close examination.

Soy and the Thyroid: Can the Effects of Isoflavones Observed in Rodent Studies Guide Human Investigations? Daniel R. Doerge. National Center for Toxicological Research, Jefferson, AR.

In addition to well-described estrogenic effects, considerable scientific evidence from animal and human studies suggests a link between soy consumption and goiter. These studies underscore the critical relationship between iodine status and the thyroid function because iodine deficiency greatly exacerbates antithyroid effects from soy, whereas iodine supplementation is protective. Our earlier published studies in vitro focused on thyroid peroxidase (TPO), the enzyme that catalyzes thyroid hormone synthesis. Genistein and daidzein, the principal soy isoflavones, are suicide substrates for TPO. When Sprague-Dawley rats were fed iodine-sufficient soy-free diets fortified with genistein (0.5–500 ppm) over 5 mo of life, intrathyroidal genistein concentrations were in the range that produces significant inactivation of rat and human TPO in vitro. Furthermore, TPO activity in these rats was reduced in a dose-dependent manner by as much as 80%. These observations showed that genistein inactivates TPO in vivo. However, a hypothyroid effect was not observed: that is, triiodothyronine, thyroxine and thyroid-stimulating hormone serum levels; thyroid weights; and thyroid histopathology were all normal. These results suggest that additional factors may be required for soy to induce overt thyroid toxicity in animals and humans. These factors could include iodine deficiency, additional soy components that act synergistically, other biochemical impairments of hormone synthesis, and additional goitrogenic dietary components. Related studies with Sprague-Dawley rats showed that dietary genistein produced potent dose-dependent stimulation of T and B cell-mediated immunity from either developmental or adult exposures, properties common to several estrogenic compounds. These findings seem relevant to epidemiological associations between soy infant formula consumption and development of autoimmune thyroid disease later in life. The formation of a neoantigen through covalent binding of isoflavones to TPO, combined with stimulated immune function, is a plausible explanation for the increased incidence of thyroid autoimmunity. The notion that exposure to soy can cause immune stimulation is also supported by the recent observations of significantly increased incidences of allergies and asthma in adults who had consumed soy formula as children (1). These results may also be relevant to women consuming soy and isoflavone supplements for putative amelioration of menopausal symptoms. Elderly women have the highest incidences of overt and subclinical hypothyroidism, conditions that are closely associated with thyroid autoimmu-

nity. Furthermore, iodine deficiency is an emerging concern in the elderly because the intake of iodized salt, a principal dietary source, may be reduced for treating hypertension. It will be important to consider these laboratory findings in the design of prospective human clinical studies to confirm or refute antithyroid properties of soy.

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Isoflavone-Rich Soymilk Prevents Bone Loss in the Lumbar Spine of Postmenopausal Women: A Two-Year Study. Eva Lydeking-Olsen,* Jens-Erik Beck Jensen,† Kenneth D. R. Setchell, Mia Damhus* and Trine Holm Jensen.*** *Institute for Optimum Nutrition, Copenhagen, Denmark; †Copenhagen University Hospital, Hvidovre, Denmark; and **Childrens Hospital and Medical Center, Cincinnati, OH.

BACKGROUND: Previous short-term human studies showed a bone-sparing effect of soy protein with isoflavones at 80–90 mg/d. No studies on the long-term effect of soy intake on bone mass have been published. AIM: The aim of the study was to evaluate the effect of 2 y of consumption of isoflavone-rich soymilk (100 mg/d), natural transdermal progesterone (25 mg/d), or both on bone mineral density in the lumbar spine and hip. DESIGN: Postmenopausal white women, mean age of 58 y (range: 41–75 y), were randomly assigned, double-blind, to one of four treatment-groups: isoflavone-rich soymilk (soy^+; $n = 23$), transdermal progesterone (TPD^+; $n = 22$), both (soy^+, TDP^+; $n = 22$), or placebo (isoflavone-poor soymilk, soy^-) and progesterone-free cream (TDP^-; $n = 22$). At baseline and 1 and 2 y, bone mineral content (BMC) and density (BMD) were measured in lumbar spine and hip by using dual-energy X-ray absorptiometry. All participants received a broad-spectrum food supplement (Osforte) containing 680 mg calcium (citrate and carbonate), 300 mg magnesium (aminochelate), 20 mg silicium (sodium metasilicate), 15 mg zinc (aminochelate), 6 mg manganese (aminochelate), 3 mg boron (proteinate), 2 mg copper (aminochelate), 200 mg vitamin C, 40 mg pyridoxine, 200 IU vitamin D-3 and 1 mg vitamin K-1. The soymilk was enriched with calcium to 120 mg/100 mL; with a daily intake of 400–500 mL, total calcium intake from diet, soymilk and food supplement was 1200–1500 mg/d. RESULTS: Of 107 women entering the study, 7 withdrew early, 6 developed intolerance to soy, and 2 developed intolerance to the skin cream. Three were excluded from analysis because of self-reported poor compliance, leaving 89 for analysis, 22–23 per group. The percentage change in lumbar spine BMD and BMC, respectively, did not differ from zero in the soy^+ group (+1.1%, +2.0%) and TDP^+ group (−1.1%, +0.4%) but loss occurred in the soy^- group (+4. 2%, $P = <0$ 01; +4.3%, $P = <0.01$) and the soy^+, TDP^+ group (+2.8%, $P = 0.01$; +2.4%, $P = 0.05$). No significant changes occurred in any group for femoral neck BMD or BMC, indicating that the food supplement, soy protein, or both had a stabilizing effect across the groups. CONCLUSION: Isoflavone-rich soymilk prevented bone loss in the lumbar spine and transdermal progesterone had a bone-sparing effect in postmenopausal women. The combined treatment, however, showed a negative interaction, resulting in bone loss to a greater extent than with either treatment alone but less pronounced than placebo. (Acknowledgments: Soy milk and placebo were provided by ALPRO, Belgium; Proderma and placebo were provided by Phillips Nutritionals, Laguna Hills, CA; Osforte was provided by Genese A/S, Denmark. The study was supported by grants from the Danish Ministry of Health, Fondation Idella, Glunz and Jensen Foundation, Civilingenieur Frode V. Nyegaard and Wife's Foundation, Director E. Danielsen and Wife Foundation, and IMK Foundation.)

Effects of Soy Isoflavones on Calcium Metabolism in Postmenopausal Women. L. A. Spence,* E. R. Lipscomb,* J. Cadogan,* B. R. Martin,* M. Peacock† and C. M. Weaver.* *Purdue University, West Lafayette, IN; and †Indiana University School of Medicine, Foods and Nutrition Department, Indianapolis, IN.

Several lines of evidence suggest that soy isoflavones and related compounds act as estrogen agonists and have beneficial skeletal effects. However, little is known about the metabolic effects of isoflavones and, specifically, about their effects on calcium handling by the body. The aim of this study was to test the hypothesis that soybean isoflavones increase calcium absorption and enhance calcium retention in postmenopausal women. Fifteen postmenopausal women were studied under three different 1-mo controlled dietary interventions in a randomized, crossover design: soy protein enriched with isoflavones (SP), soy protein void of isoflavones (SM), and a casein-whey (CW) control. Dietary calcium was 1106 mg/d and dietary isoflavones were ± 65 mg/d. Serum isoflavones, determined by liquid chromatography-mass spectrometry, were significantly greater ($P < 0.01$) at the end of the SP phase at 879 ± 388 nmol/L compared with 107 ± 37 nmol/L and 21 ± 10 nmol/L at the end of the SM and CW phases, respectively. A combination of metabolic balance methodology and radioisotopic tracer techniques were used to measure calcium absorption, calcium excretion and net calcium retention. Fractional calcium absorption of 0.20 ± 0.05 (SP), 0.20 ± 0.04 (SM), and 0.19 ± 0.06 (CW) was not affected by dietary treatment. Urinary calcium excretion was significantly reduced ($P < 0.05$) with the consumption of soy compared with casein whey: SP, 81 ± 32 mg (2.0 ± 0.8 mmol); SM, 81 ± 42 mg (2.0 ± 1.1 mmol); and CW, 121 ± 69 mg (3.0 ± 1.7 mmol). Net calcium retention was significantly increased ($P < 0.01$) with the consumption of soy compared with casein whey: SP, 3 ± 50 mg (0.08 ± 1.3 mmol); SM, 16 ± 61 mg (0.4 ± 1.5 mmol); and CW, –50 ± 89 mg (−1.3 ± 2.2 mmol). Thus, soy protein, but not isoflavones, reduced urinary calcium excretion and enhanced net calcium retention compared with milk protein. However, neither soy protein nor isoflavones influenced fractional calcium absorption.

Comparative Effects of Soy Isoflavones, Soy Protein and 17β-Estradiol on Calcium and Bone Metabolism in Adult Ovariectomized Rats. D. J. Cai,* D. M. Cullen,† C. H. Turner and C. M. Weaver.*** *Department of Foods and Nutrition, Purdue University, West Lafayette, IN; †Osteoporosis Research Center, Creighton University, Omaha, NE; and **Indiana University, Indianapolis, IN.

Osteoporosis is a disease with fragile fractures in skeletal sites resulting from bone loss. Several animal and short-term human studies indicate that isoflavones may be as effective as estrogen in preventing bone loss. This study compared the effectiveness of soy isoflavones to estrogen replacement therapy in an adult animal model for postmenopausal osteoporosis. Unmated 6-mo-old ovariectomized and sham-operated Sprague-Dawley rats were randomly assigned to nine groups (16 rats per group) and pair-fed for 8 wk. 17β-Estradiol was administered via subcutaneous implants in estrogen replacement therapy groups. Diets consisted of soy protein, casein, or

both, with or without isoflavones. Measurements, including calcium balance, bone densitometry, biomechanics and histomorphometry, were used to investigate calcium and bone metabolism. Total calcium balance and ^{45}Ca retention were measured for the last 2 d after an oral gavage of ^{45}Ca. Serum ^{45}Ca profiles after oral and intravenous doses of ^{45}Ca along with the calcium balance data were used for kinetic modeling analysis. Different regions of the left femur were analyzed for bone mineral density. The proximal and midshaft tibias were processed for bone histomorphometry. Mechanical strength was determined at midshaft and femoral neck of the left femur. After ovariectomy, estrogen prevented bone loss in trabecular bone and suppressed formation on both trabecular and cortical bone surfaces as indicated by bone mineral density and histomorphometry. Isoflavones given alone or as soy protein did not prevent trabecular bone loss. Combining isoflavones with estrogen had no additional benefits. None of the treatments affected significantly either total calcium balance or ^{45}Ca absorption. However, soy protein showed significant effects on reducing urinary loss of calcium regardless of isoflavone levels. We conclude that estrogen, but not isoflavones at the levels tested, prevented bone loss after hormone deficiency.

Does Soy Protein and Its Isoflavones Prevent Bone Loss in Peri- and Postmenopausal Women? Results of a Two-Year Randomized Clinical Trial. Mara Z. Vitolins, Mary S. Anthony, Leon Lenchik, Deirdre R. Bland and Gregory L. Burke. Wake Forest University School of Medicine, Department of Public Health Sciences, Winston-Salem, NC.

Human studies and animal models of experimental osteoporosis have reported that soy protein or its isoflavones may be effective in preserving bone mass. Soy protein with isoflavones has been shown to have modestly beneficial effects on bone mineral density (BMD) in estrogen-deficient conditions. However, long-term studies of soy effects on BMD in humans are lacking. The purpose of this report is to evaluate the effect of dietary soy proteins containing differing amounts of isoflavones on BMD in 241 peri- and postmenopausal women who participated in a double-blind randomized controlled clinical trial. Study participants consumed a dietary soy supplement daily that contained 25 g soy protein with differing doses of isoflavones: $<$ 5 mg/d isoflavones [soy$^-$], 42 mg/d (medium-dose isoflavones), or 58 mg/d (high-dose isoflavones). One hundred seventy-two participants had both baseline and 2-y BMD measurements of the total body and were included in this analysis. All BMD measurements were obtained on a QDR-2000 scanner. In a preliminary unadjusted evaluation, there was an estimated loss in total body BMD of 0.5% in the soy$^-$ group, 0.3% in the medium-dose group, and 0.9% in the high-dose group from baseline to the 2-y measurement (ANOVA, $P = 0.28$). In this study using modest levels of isoflavones, the 42- and 58-mg isoflavone doses were not better than the $<$ 5-mg isoflavone dose in preserving bone. However, given the data on calcium excretion-sparing effects of soy protein, the $<$ 1% changes seen in this study over 2 y may suggest the potential for the preservation of bone by soy protein.

Dietary Proteins: A Pharmacological Tool? Cesare R. Sirtori. Center E. Grossi Paoletti, University of Milano, Italy.

Dietary proteins, according to common knowledge, should be fully destroyed to single amino acids in the gut. A variety of enzyme systems can hydrolyze proteins: trypsin, chymotrypsin, pepsin and others. Hydrolyzed single amino acids, thus, would become building material for tissue proteins as well as a source of energy. However, the picture is not at all clear. Clinical allergy to proteins has shown that small and even large peptides can be well-absorbed in some individuals. Furthermore, cases of selective breakdown of proteins to active peptides have been repeatedly described. Such is the case for casomorphins from milk, a well-recognized breakdown product with potential high-grade analgesic activity and, more recently recognized, an insulin sensitizing effect. Exorphins, breakdown products from wheat, can instead stimulate insulin release. Soy proteins provide a clear example of how dietary proteins may affect one major parameter, that is, cholesterolemia. Fractions of soy protein have been clearly shown to affect LDL receptor regulation in human liver cells, thus providing a unique mechanism of cholesterol reduction. The evaluation of soy globulin mutants not endowed with this property suggests that the 7S soy globulin, or segments thereof, may be responsible for the hypocholesterolemia. A recent stimulating example is provided by blood pressure-regulating peptides. Peptides of 3–6 amino acids are powerful regulators of blood pressure in selected animal models (such as the spontaneously hypertensive rat). Recently, the incorporation of active peptides into dietary proteins, in particular into the α1 subunit of 7S globulins from soy proteins, has been achieved with the hypotensive hexapeptide ovokinin. Such engineered dietary proteins display a powerful blood pressure-lowering activity in the spontaneously hypertensive rat, suggesting that peptides are absorbed and recovered intact in the circulation after ingestion. Dietary proteins, selectively modified by genetic engineering, may offer an exciting tool for human disease treatment.

Effect of Fermented Soybean Extraction on the Production of Nitric Oxide and on Blood Pressure in SHR-SP Rats. X. A. Song,*† K. Ikeda,* M. Takebe,† T. Noguchi,* W. Pan† and Y. Yamori.* *World Health Organization Collaborating Center for Research on Primary Prevention of Cardiovascular Diseases, Kyoto, Japan; and the †Biotics Department, Nichimo, Japan.

The main purpose of our study was to assess the effects of fermented soybean extraction (FSE) containing 30% isoflavone aglycones on the production of nitric oxide and on systolic blood pressure in SHR-SP rats. After the administration of FSE, significant decreases in systolic blood pressure were confirmed in the FSE group compared with the control group. Nitric oxide levels in urine and serum were also significantly higher in the FSE group than in the control group. Brain and ventricular weights were significantly lower in the FSE group than in the control group. Significantly higher levels of eNOS mRNA were also confirmed in the FSE group than in the control group. In conclusion, isoflavone aglycones could decrease systolic blood pressure significantly through an increase in the formation of nitric oxide, and the promotion effects of FSE on the formation of eNOS mRNA were confirmed in SHR-SP rats. If clinically applied without any detrimental effect, isoflavone aglycones are expected to contribute to preventive cardiology.

Soy Supplements with Phytoestrogens Reduce Blood Pressure at Rest and during Stress in Middle-Aged Men. Sheila G. West,* Catherine M. Stoney,† Diane L. Habash, Karen M. Cook† and Julie A. Nelligan.†** *Department of Biobehavioral Health, Pennsylvania State University, University Park,

PA; and the †Department of Psychology and **General Clinical Research Center, Ohio State University, Columbus, OH.

In a recent randomized trial, we showed that pharmaceutical estrogens reduce blood pressure and vascular resistance at rest and during standardized stressors in the laboratory (1). Here, we examined the effects of soy vs. casein protein (30 g/d) on heart rate and blood pressure responses in 26 healthy, nonsmoking men (40–55 y). Soy supplements contained 60 mg isoflavones. Blood pressure, heart rate and vascular resistance were measured at rest and during a 5-min speech stressor and a 2-min cold pressor task. The same measurements were made again 5 wk posttreatment. Analyses were done by subtracting the pretreatment level from the posttreatment level for each variable; pretreatment level was used as a covariate. The effects of soy on diastolic blood pressure and heart rate were larger during stress than at rest, resulting in significant task-protein interactions ($P < 0.02$). For example, soy-related reductions in diastolic blood pressure were most evident during the cold pressor task (−4 vs. +3 mm Hg for soy vs. casein, $P = 0.02$), suggesting a reduction in vascular resistance as a potential mechanism for this effect. Heart rate responses to the speech task (a performance-based stressor) were significantly reduced with soy but not with casein ($P = 0.03$). In contrast, resting SBP was significantly reduced by soy at rest and during both stressors (average change in SBP = −9 vs. −3 mm Hg, for soy vs. casein), with a main effect of protein type for systolic blood pressure ($P = 0.056$). These results suggest that the beneficial vascular effects of soy persist during sympathetic nervous system stimulation. In addition, the blood pressure-lowering effects of soy were also maintained in response to a vasoconstrictive challenge (the cold pressor task). Subsequent analyses of data from this study will examine whether reductions in total peripheral resistance and plasma catecholamines are plausible mechanisms for these effects.

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Aging Inhibits the Beneficial Effects of Estradiol but Not Genistein on Vascular Reactivity: Are Estrogen Receptors Involved? Irma Suparto,* Jamie Fox† and J. Koudy Williams.† *The Institute Pertanian Bogor, Bogor, Indonesia; and †Wake Forest University School of Medicine, Winston-Salem, NC.

Aging inhibits dilation and promotes constriction of arteries. Mammalian estrogens, such as 17β-estradiol (E2), promote vasodilation and inhibit vasoconstriction in younger individuals. The objectives of this study were to determine whether aging inhibits the beneficial effects of E2 and the phytoestrogen genistein on phenylephrine (PE)-mediated constriction and to investigate the potential role of estrogen receptors (ER) in mediating these effects. Thoracic aortic rings of old (1–2 y old) and young (1–2 mo old) wild-type, ER-α knockout (ERKO), and ER-β knockout (BERKO) mice were studied in vessel baths after the rings had been incubated for 24 h in a nutrient solution (control) or a nutrient solution containing E2 (10 nmol/L) or genistein (100 nmol/L). Rings were tested for development of tension to increasing doses of PE. In young mice, E2 reduced constriction to PE by 32% in wild-type ($P < 0.05$ vs. control), 23% in ERKO ($P < 0.05$), and 5% in BERKO mice ($P > 0.05$); genistein reduced constriction to PE by 27% ($P < 0.05$), 19% ($P > 0.1$), and 15% ($P > 0.1$), in wild-type, ERKO, and BERKO mice, respectively. In old mice, E2 did not affect vascular responses to PE ($P > 0.5$). Genistein reduced constrictor responses in old wild-type mice by 38% ($P = 0.12$) any by similar amounts in ERKO and BERKO mice. It is concluded that aging inhibits the effects of E2 more than genistein on vascular response to PE and that although both ER-β and ER-α play a role in modulating E2 responses, ER-β seems to play a greater role.

Neither Isoflavones nor the Alcohol-Extracted Fraction Added to Alcohol-Washed Soy Protein Isolate Restores the Lipoprotein Effects of Soy Protein Isolate. Mary S. Anthony, Robert M. Blair and Thomas B. Clarkson. Wake Forest University School of Medicine, Comparative Medicine Clinical Research Center, Winston-Salem, NC.

Whether the isoflavones are the active component in soy protein for modulation of plasma lipoproteins and atherosclerosis prevention has been debated. Alcohol-washed soy protein isolate (SPI), devoid of isoflavones, has reduced efficacy for lowering LDL cholesterol and increasing HDL cholesterol. Increasing amounts of isoflavones in the same quantity of SPI result in a dose-response lowering of LDL cholesterol. However, purified isoflavones do not affect LDL or HDL cholesterol concentrations. The current study was conducted to determine whether adding an isoflavone extract or the whole alcohol-extracted fraction to alcohol-washed SPI would restore effects on LDL and HDL cholesterol to those of intact SPI. Male and female cynomolgus monkeys ($n = 81$) were randomly assigned to one of five groups: casein + lactalbumin (C/L); alcohol-washed SPI (soy^-); intact SPI (soy^+); soy^- + alcohol-extracted fraction (AlcExt); and soy^- + isoflavone extract (IsoExt). The macronutrient composition of the diets was identical. The isoflavone concentrations (as aglycones) were matched in the soy^+, AlcExt, and IsoExt diets. Diets were fed for 6 mo. LDL and HDL cholesterol were measured monthly. All soy groups had lower LDL cholesterol ($P < 0.0001$) and higher HDL cholesterol concentrations ($P < 0.05$) than did the C/L group. The soy^+ group had a 24% lower LDL cholesterol ($P = 0.002$) and a 22% higher HDL cholesterol concentration ($P = 0.002$) than did the soy^- group. The AlcExt group had a marginally lower LDL cholesterol concentration (13%, $P = 0.11$) and an HDL cholesterol concentration that was not different ($P = 0.3$) from the soy^- group. The IsoExt group had LDL and HDL cholesterol concentrations that were not different from those of the soy^- group ($P > 0.3$). These data confirm that the soy^- is not as effective as soy^+ for lipoprotein improvements and suggest that neither purified isoflavones nor the whole alcohol-extracted fraction added to alcohol-washed SPI can restore all the lipoprotein-modulating properties of intact SPI. Thus, alcohol washing SPI may result in disruption of the protein, protein-isoflavone complex, or some other component of SPI that is important for effects on plasma lipid concentrations.

A Paradoxical Association between Plasma Isoflavone Concentrations on a Soy-Containing Diet and Both Plasma Lipoproteins and Atherosclerosis. Thomas B. Clarkson,* Mary S. Anthony,* Michelle Smith,† Landon Wilson† and Stephen Barnes.† *Comparative Medicine Clinical Research Center, Wake Forest University School of Medicine, Winston-Salem, NC; and the †University of Alabama at Birmingham, Birmingham, AL.

To evaluate the association between plasma isoflavone concentrations and postmenopausal atherosclerosis progression,

surgically postmenopausal cynomolgus monkeys were fed diets for 36 mo containing untreated soy protein isolate with isoflavones (soy^+; $n = 59$) or soy protein isolate that had been alcohol-washed to remove isoflavones (soy^-; $n = 56$). Plasma lipoprotein and isoflavone concentrations were measured (isoflavones by liquid chromatography-mass spectrometry) and atherosclerosis progression was evaluated morphometrically. The soy^+ group compared with the soy^- group had 16% lower LDL cholesterol ($P = 0.003$), 13% lower HDL cholesterol ($P = 0.01$), and marginally inhibited atherosclerosis progression in the iliac (21% lower, $P = 0.10$) and coronary (25% lower, $P = 0.12$) arteries. Further analysis of the association between plasma isoflavones and plasma lipoproteins and atherosclerosis in the soy^+ group used between-animal differences in isoflavone concentrations, which was largely equol. Plasma concentrations of genistein, daidzein and equol were correlated, so total isoflavone concentrations were used. The soy^+ group was divided into tertiles of total plasma isoflavone concentration: < 410, 410–760, and > 760 nmol/L. There were unexpected associations across tertiles of isoflavone concentrations in the soy^+ group; an inverse association with HDL cholesterol (trend, $P = 0.04$); and positive associations with LDL cholesterol (trend, $P = 0.02$), iliac artery atherosclerosis progression (trend, $P = 0.03$), and coronary artery atherosclerosis (trend, $P = 0.01$). We conclude from these data that the effects of dietary isoflavones on plasma lipoproteins and atherosclerosis relate in part to individual differences in metabolism as represented by plasma isoflavone responses. Within the soy^+ group, the directions of associations were opposite to those predicted, whereas for the combined soy^- and soy^+ groups there seemed to be a biphasic response. It remains uncertain whether the level of isoflavone response is causally related to the observed variability in lipids and atherosclerosis or whether such variability reflected the actions of other related but unidentified factors.

Dietary Intake of a Soy Protein Isolate Supplement Improves Nutritional Status in Malnourished Hemodialysis Patients. T. J. Stephenson, B. P. Sawaya and P. Fanti. University of Kentucky Medical Center, Department of Nutrition and Food Science, Graduate Center for Nutritional Sciences and Division of Nephrology, Bone, and Mineral Metabolism, Lexington, KY.

Protein-energy malnutrition is a common complication in maintenance hemodialysis and is a powerful predictor of morbidity and mortality in this population. Inadequate dietary intake is a key contributor to protein-energy malnutrition. Soyfoods, as a rich source of protein, may be a beneficial oral food supplement in preventing malnutrition in end-stage renal disease patients. METHODS: In a recent pilot study 20 chronic hemodialysis patients (age 66 ± 3.1 y; 12 men, 8 women) with a low protein catabolic rate, a marker of malnutrition, were studied. Subjects were provided with a soy protein isolate drink (20 g soy protein) with or without isoflavones at each dialysis treatment for 1 mo. RESULTS: As shown in the **Table 1**, protein catabolic rate and serum albumin were significantly improved after 4 wk of treatment regardless of isoflavone composition. No changes in serum prealbumin were detected. The soy supplement was well-tolerated by the hemodialysis patients and had no adverse effects on phosphorus, calcium, sodium or potassium balance. CONCLUSIONS: This pilot study suggests that a soy protein isolate supplement may be beneficial in reversing malnutrition in hemodialysis patients. This study sets the stage for future broader-scope studies in predialysis, peritoneal dialysis and hemodialysis patients aimed at investigating the potential effect of soy supplementation on nutrition, morbidity and mortality in renal failure.

TABLE 1

Results of a pilot study of 20 chronic hemodialysis patients

	Baseline	Treatment	Posttreatment
Protein catabolic rate, $g \cdot kg^{-1} \cdot d^{-1}$	0.95 ± 0.15[a]	1.14 ± 0.28[b]	1.00 ± 0.23[ab]
Serum albumin, *g/L*	35.3 ± 0.8[a]	36.3 ± 0.7[b]	35.9 ± 0.7[ab]
Serum prealbumin, *mg/L*	291.5 ± 84.6[a]	291.3 ± 88.9[a]	Not determined

Means with any like superscripts are not significantly different at $\alpha = 0.05$.

Effects of Soy Protein on Diabetic Nephropathy and Blood Lipids in Type 2 Diabetes Mellitus. Sandra R. Teixeira,* Kelly A. Tappenden,* William Marshall,† LeaAnn Carson,* Michael Ringenberg and John W. Erdman, Jr.*** *Division of Nutritional Sciences, University of Illinois at Urbana-Champaign, Urbana, IL; †Danville Veterans Administration Medical Center, Danville, IL; and **Veterinary Diagnostic Laboratory, School of Veterinary, Medicine, UIUC, Urbana, IL.

Two studies, one animal and one human, were conducted to evaluate the role of soy protein on diabetic nephropathy and blood lipids in type 2 diabetes. The objective of the animal study was to determine the qualitative and quantitative effects of dietary protein on the progression of diabetic nephropathy in a type 2 diabetes animal model (BKS.cg-m +/+ $Lepr^{db}$). Twenty-four diabetic (db/db) and 24 control (db/m^+) mice were given free access to one of four diets (12% or 20% soy protein or 12% or 20% casein) from 35 d old until they were killed (age 184–217 d). Blood and urine were collected throughout the study to measure biomarkers of diabetes and diabetic nephropathy. The results show that diets rich in soy protein prevent an increase in urinary albumin excretion, which is typically seen in male BKS.cg-m +/+ $Lepr^{db}$ diabetic mice. This suggests an improvement in glomerular macromolecular permeability and indicates slower development of diabetic nephropathy. The objective of the human study was to determine whether incorporating soy protein in the diet of type 2 diabetics with diabetic nephropathy would decrease urinary albumin excretion and improve lipid profile. Fourteen patients were studied in a crossover design. The study consisted of a 4-wk lead-in, two 8-wk interventions separated by a 4-wk washout and a final 4-wk washout. Diets were similar to a step I diet but with 1 g protein·kg body $weight^{-1} \cdot d^{-1}$ during the lead-in and washout periods and with 1.4 g protein · kg body $weight^{-1} \cdot d^{-1}$ during the two interventions. During the 8-wk interventions, 0.5 g protein·kg body $weight^{-1} \cdot d^{-1}$ was either soy protein or casein. Blood and urine were collected at the beginning of each period to measure biomarkers of diabetes, kidney function and blood lipids. The results indicate that the consumption of soy protein instead of casein leads to a statistically significant reduction in urinary albumin excretion

and total-to-HDL cholesterol ratio, whereas HDL cholesterol, plasma arginine and the arginine-to-lysine ratio are significantly increased. (Supported in part by the Illinois Council for Food and Agricultural Research and Protein Technologies International.)

Beneficial Effects of Soy Protein Use on Renal Function in Young Type 1 Diabetic Subjects with Early Diabetic Nephropathy. Tammy J. Stephenson,* James W. Anderson,* David Jenkins,† Cyril Kendall† and Paolo Fanti.* *University of Kentucky Medical Center, Department of Nutrition and Food Science, Graduate Center for Nutritional Sciences and Division of Nephrology, Bone, and Mineral Metabolism, Lexington, KY; and the †University of Toronto, Department of Nutritional Sciences, Faculty of Medicine, Toronto, Canada.

Diabetic nephropathy is the most frequent cause of end-stage renal disease in the industrialized world, accounting for 40% of all end-stage renal disease cases. Dietary intake, including protein amount and quality, seems to affect the progression of renal disease. The specific aims of this research were to determine whether soy protein intake, compared with animal protein intake, in young persons with type 1 diabetes is associated with amelioration of glomerular hyperfiltration, reduction in both total and oxidized LDL cholesterol, and reduction in microalbuminuria. METHODS: Fourteen subjects with type 1 diabetes (age 29.4 ± 2.4 y, duration of diabetes 15.1 ± 2.3 y) under fairly good glycemic control (HbA_{1c} 0.0721 ± 0.0048) were studied. Twelve of the subjects were hyperfiltering (glomerular filtration rate > 120 $mL \cdot min^{-1} \cdot 1.73m^{-2}$) and three had microalbuminuria [urine: 20–200 mg albumin/g creatinine (0.036–0.36 μmol albumin/mmol creatinine)]. After a 4-wk run-in period to assess baseline dietary habits and laboratory values, subjects were instructed to substitute 45–55 g soy protein for the same amount of animal protein in their diet for 8 wk (soy diet) followed by an additional 8 wk of their habitual diet. RESULTS: As shown in **Table 1**, glomerular filtration rate was significantly reduced during the soy diet. In addition, there was a 7% and 9% reduction in total and LDL cholesterol, respectively, after 8 wk of the soy diet. LDL oxidation was measured in a subpopulation of seven participants and was numerically lower during the soy diet than during the baseline and control diets. Dietary protein intake was equivalent for the baseline and soy diets but was significantly reduced during the control diet. Urine sodium excretion was unchanged throughout the study. CONCLUSIONS: Incorporation of soyfoods into the diet of type I diabetes patients with early diabetic nephropathy is well-tolerated and seems to have beneficial effects on glomerular filtration rate and lipid profile.

Effects of Dietary Soy Phytoestrogens on Brain Aromatase, Anxiety Behavior, Neural Structure and Memory. E. D. Lephart,* R. W. Rhees,* T. W. West,* L. Y. Tian,* L. H. Bu,* D. L. Simmons,*† K.D.R. Setchell, H. Adlercreutz‡ and T. D. Lund.*** *Neuroscience Center, Brigham Young University, Provo, UT; †Department of Chemistry and Biochemistry, Brigham Young University; Provo, UT; **Clinical Mass Spectrometry Center, Children's Hospital Center, Cincinnati, OH; and the ‡Institute for Preventive Medicine, Nutrition, and Cancer, Folkhälsan Research Center, University of Helsinki, Helsinki, Finland.

This study examined the influence of phytoestrogens (estrogen-like plant compounds) present in rodent diets (via soy) on brain aromatase, anxiety behavior (in the elevated plus maze), sexually dimorphic hypothalamic nuclei [sexually dimorphic nucleus of the preoptic area (SDN-POA) and anteroventral periventricular nucleus (AVPV)], visual spatial memory (VSM) and determined phytoestrogen, calbindin, and cyclooxygenase-2 levels in the rat brain. In male and female Long-Evans rats fed either lifelong exposure to a high-phytoestrogen diet (Phyto-600) or a phytoestrogen-free diet (Phyto-free), there were no significant alterations in brain aromatase. In the elevated plus maze, phytoestrogens produced marked anxiolytic effects in both males and females. In a subset of rats, at age 80 d one-half were either kept on their original diet (Phyto-600 or Phyto-free) or changed to the opposite diet (Phyto-free or Phyto-600). Males fed (lifelong) or changed to a Phyto-free diet had significantly decreased SDN-POA and increased AVPV volumes compared with males fed (lifelong) or changed to the Phyto-600 diet. The opposite was found in females. Within the radial arm maze, VSM was enhanced in females fed the Phyto-600 diet, whereas in males VSM was inhibited by the same diet. Male rats fed the Phyto-600 diet had significantly higher phytoestrogen concentrations in the frontal cortex, hypothalamus and cerebellum but not in the hippocampus than did males fed the Phyto-free diet. Also, males fed the Phyto-600 diet had decreased calbindin (a neuroprotective agent) and increased cyclooxygenase-2 (an inflammatory factor prevalent in Alzheimer's disease) levels in the frontal cortex compared with males fed the Phyto-free diet. These data suggest that phytoestrogens via a soy diet do not alter brain aromatase, produce anxiolytic effects in the elevated plus-maze, significantly alter volumes of the SDN-POA and AVPV, and significantly alter VSM during adulthood.

Phytoestrogens Enhance Working Memory in a 90-Minute Delayed Matching-to-Place Water Maze Task. Melvenia M.

TABLE 1

Results for the three diets

	Control diet	Baseline diet	Soy diet
Glomerular filtration rate, $mL \cdot min^{-1} \cdot 1.73\ m^{-2}$	159 ± 7.7[a]	143 ± 7.4[b]	161 ± 10.0[a]
Total cholesterol, *mg/dL* [*mmol/L*]	181 ± 10.1[a]	168 ± 8.0[b]	187 ± 9.4[a]
	[4.69 ± 0.26]	[4.35 ± 0.21]	[4.84 ± 0.24]
LDL cholesterol, *mg/dL* [*mmol/L*]	115 ± 9.3[a]	105 ± 7.7[b]	117 ± 8.6[a]
	[2.98 ± 0.24]	[2.72 ± 0.20]	[3.03 ± 0.22]
LDL oxidation, conjugated dienes, *μmol/L*	45.1 ± 3.0	41.0 ± 4.7	43.7 ± 3.9
Urine sodium, *mEq/h*	9.20 ± 1.46	8.00 ± 1.61	7.63 ± 1.59
Dietary protein intake, $g \cdot kg\ body\ weight^{-1} \cdot d^{-1}$	1.30 ± 0.12[a]	1.34 ± 0.11[a]	1.08 ± 0.10[b]

Means with any like superscripts are not significantly different at $\alpha = 0.05$.

Martin,* Gary Dohanich, John A. McLachlan*‡ and Cynthia C. Gulledge.*†** *Environmental Endocrinology Laboratory, Center for Bioenvironmental Research of Tulane and Xavier Universities, Tulane University, New Orleans, LA; and the Departments of †Ecology and Evolutionary Biology, **Psychology, and ‡Deptartment of Pharmacology, Tulane University, New Orleans, LA.

Aging is associated with physical and mental decline. Many menopausal women are encouraged to take hormone replacement therapy but would like an alternative. Studies showing beneficial effects of phytoestrogens have prompted increased public interest and marketing in the United States, but little is known about effects of phytoestrogens on the brain. On the basis of previous studies on estrogens, we hypothesized that phytoestrogens would enhance working memory in aging rats. Ovariectomized retired breeder Sprague-Dawley rats were housed singly and randomly assigned either a phytoestrogen-enriched (Phyto) or -reduced (Free) diet and given 23–25 g of food daily. The Phyto diet had and isoflavone content of 55 μg/g (65% genistein, 33% daidzein, and 2% glycitein) to achieve the isoflavone range of 2.7–3.3 mg $\cdot$ kg body weight^{-1} $\cdot$ d^{-1}. We used a reference-memory water maze task (stationary hidden platform) to measure reference memory and a delayed matching-to-place (DMP) water maze task (hidden platform moved daily) to measure working memory. For the DMP water maze task, rats had either a 2-min delay (easy) or a 90-min delay (difficult) between trial 1 and trial 2 on any given day for 8 d. No significant difference was noted in reference memory performance in the two diet groups. With a 2-min delay, there was also no overall difference between groups in the working memory task. However, with a 90-min delay, we found a statistically significant overall effect of treatment, with the Phyto group performing better across all trials than the Free group, suggesting that phytoestrogens in the diet improved working memory performance in aging female rats in a task with increased memory load.

Proteomics Analysis of Brain Protein Changes Induced by Soy Isoflavones. H. Kim,*† L. Chaves,* P. Hall,* T. DeSilva,*† L. Coward*† and M. Kirk.*† *Department of Pharmacology and Toxicology and †Proteomics Laboratory, Comprehensive Cancer Center Mass Spectrometry Shared Facility, University of Alabama at Birmingham, Birmingham, AL.

Soy isoflavones added to a non-soy protein-based diet protect against ovariectomy-induced cognitive decline in rats as well as against reductions in mRNA of proteins critical for neuronal viability. We showed previously that dietary soy containing normal levels of isoflavones significantly suppressed selected neurodegeneration-relevant brain protein modifications in a primate model of menopause. In this study, we used proteomics technology to study protein changes correlated with the health benefits of soy isoflavones in mammalian brain. The hypothesis was that dietary soy isoflavone intake would either induce changes in proteins that enhance brain function or viability, or attenuate protein changes associated with cognitive decline or neurodegeneration. C57B6 mice were maintained in dietary groups from weaning. One group was given intact soy protein (SOY$^+$) and the other group was given soy protein that had been alcohol extracted, resulting in the depletion of 90% of the isoflavones (SOY$^-$). Aged mice were killed and the proteins in total brain homogenates were resolved by two-dimensional electrophoresis (isoelectric focusing in the first dimension, sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the second dimension). Proteins were detected by staining the gels with colloidal Coomassie blue. Image analysis of the stained gels with PDQuest software (BioRad) indicated that several brain polypeptides were altered in response to soy isoflavones. After in-gel digestion with trypsin, matrix-assisted laser desorption/ionization–time of flight mass spectrometry of the tryptic peptides identified several polypeptides that were altered in amounts or modified in the brains of mice that ingested the diet containing the isoflavones relative to mice that ingested isoflavone-depleted soy protein. These proteins included enolase and hippocampal cholinergic neurostimulatory peptide precursor protein. Thus, ingestion of soy protein affects multiple proteins in mammalian brain, and this is probably due to the isoflavones. This is the first demonstration of proteomics analysis of protein changes induced by dietary soy in a target mammalian tissue. Understanding the roles of these protein changes awaits further experimentation.

The Soy and Postmenopausal Health in Aging (SOPHIA) Study: Overview and Baseline Cognitive Function. Donna Kritz-Silverstein, Denise Von Muhlen and Elizabeth Barrett-Connor. University of California-San Diego, La Jolla, CA.

The Soy and Postmenopausal Health in Aging (SOPHIA) Study is a double-blind, randomized, controlled clinical trial designed to determine the effects of 110 mg isoflavones vs. placebo on cognitive function, hip bone density and menopausal symptoms in postmenopausal women 55–74 y old (mean: 61 y; SD: 5). Of 56 women randomly assigned to treatment, 53 were followed for 6 mo. Women assigned to the treatment group ($n = 27$) took two pills per day containing a total of 110 mg soy-extracted isoflavones. Women assigned to the placebo group ($n = 26$) took two pills per day containing inactive ingredients. The majority were white (83%); 11% were Hispanic. Educational level ranged from 12 to 20 y (mean: 15; SD: 2). There were no significant differences ($P > 0.10$) on baseline characteristics. Several cognitive function tests were administered at baseline and repeated at follow-up, including trails A and B; category fluency; the mini-mental status examination; and logical memory and recall, which is a paragraph recall test assessing immediate and delayed verbal memory. There were no significant differences on any of the cognitive function tests at baseline ($P > 0.10$). Age- and education-adjusted comparisons of the percentage change in cognitive function between baseline and follow-up showed a significantly greater increase (indicating improvement) in category fluency for women in the treatment group compared with women in the placebo group ($P = 0.02$). Although not statistically significant, women in the treatment group showed greater improvement than did women in the placebo group on the other tests of verbal memory (logical memory 1 and 2). In trails B, women in the treatment group showed more improvement than did those in the placebo group ($P = 0.08$, borderline significance). Age-stratified analyses indicated that among older women, those in the placebo group exhibited a decrement in performance on verbal memory tests compared with those in the treatment group, whose performance improved over time. The results suggest that isoflavone supplementation does not have a negative effect on cognitive function. Women using isoflavones showed more improvement in verbal memory, and isoflavone use may help prevent decline in verbal memory, especially for older women. The effects of isoflavone use on cognitive function may be similar to those of estrogen.

Dietary Soy Improves Memory in Humans. Rosanna Duffy,* Nicholas Jarrett,† Emma Fluck,† Karen Casey,* Sandra File† and Helen Wiseman.* *Department of Nutrition and Dietetics, Nutrition, Food and Health Research Center, King's College London, London, UK; and the †Psychopharmacology Research Unit, Center for Neuroscience, King's College London, London, UK.

It is widely theorized that estrogen-related factors may play an important role in cognitive function. Because soy contains high levels of phytoestrogens, these compounds may prove to be important for beneficial effects on cognition. OBJECTIVE: The main objective of this study was to investigate the effects of a diet rich in soy isoflavones on memory and frontal lobe function in healthy young male and female subjects. METHODS: Twenty-seven subjects (15 men and 12 women; mean age 25.5 y) were randomly assigned to the high-soy diet (100 mg total isoflavones/d; $n = 15$) or a low-soy diet (0.5 mg total isoflavones/d; $n = 12$) for 10 wk. Memory and frontal lobe functions were tested using the Cambridge Automated Neuropsychological Test Battery and a series of other cognitive tests. RESULTS: Subjects receiving the high-soy diet improved in tests of episodic memory, showing a significantly greater improvement than the group consuming the low-soy diet, in the numbers of prose items recalled immediately after a short story ($F_{(1,25)} = 5.0$, $P < 0.05$). Subjects consuming the high-soy diet improved significantly compared with those consuming the low-soy diet in terms of the number of pictures recalled after 20 min ($F_{(1,23)} = 4.3$, $P < 0.05$). In the delayed matching to sample task, a task of nonverbal memory, when the 4-s delay was imposed, the high-soy group performed significantly better than the low-soy group ($F_{(1,21)} = 4.4$, $P < 0.05$). These subjects had faster response times after consumption of the high-soy diet after 10 wk, whereas the response times of those subjects consuming the low-soy diet became slower. In tests of semantic memory, there was no effect of diet on category generation, but in the letter fluency task there was a significant sex × diet × day interaction ($F_{(1,23)} = 7.0$, $P < 0.01$). In the tasks assessing frontal lobe function, the group consuming the high-soy diet improved in the number of trials required to complete the task of mental flexibility, involving rule learning, shifting, and reversal, whereas the performance of the low-soy group showed a slight deterioration ($F_{(1,23)} = 4.8$, $P < 0.05$). In the Stockings of Cambridge test there was a significant sex × day × diet interaction ($F_{(1,17)} = 6.0$, $P < 0.05$), because in the five-move test only females consuming the high-soy diet showed an improvement, whereas those on the low-soy diet deteriorated. Males improved their performance regardless of diet. CONCLUSION: A 10-wk period of soy isoflavone consumption resulted in significant improvements in verbal and nonverbal episodic memory and frontal lobe function (1). We are currently investigating the effects of soy isoflavones on cognitive function in postmenopausal women.

1. File, S. E., Jarrett, N., Fluck, E., Duffy, R., Casey, K. & Wiseman, H. (2001) Eating soya improves human memory. Psychopharmacology 157: 430–436.