

6th International Symposium on the Role of Soy in Preventing and Treating Chronic Disease

Program Addendum

POSTER CHANGE

The following poster presentation has been moved from Poster Session I, Isoflavone Metabolism to Poster Session II, Other:

Effects of Isoflavone Supplements vs. Soy Foods on Blood Concentrations of Genistein and Daidzein. C.D. Gardner¹, L.M. Chatterjee¹, B.M. Oliveira¹, and A.A. Franke², ¹Stanford Center for Research in Disease Prevention and the Department of Medicine, Stanford University Medical School, USA, ²Cancer Research Center of Hawaii, USA.

POSTER CANCELLATIONS

Poster Session I Cancellations

Cancer

Beneficial Effects of Regular Consumption of Whole Soy Foods on Serum Lipids, Lipid Peroxidation, High Sensitivity CRP and Blood Pressure in Perimenopausal Women: A Randomized, Controlled, Crossover Trial. S. Songchitsomboon, K. Chanda, D. Danboonchant, J. Manonai, J. Hong, and S. Komindr

Isoflavones

Determination of Major Isoflavone Components Based on HPLC Technology among Southern Soybean Varieties in China. J.-M. Sun, F.-X. Han, and A.-L. Ding, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, P.R. China.

Poster Session II Cancellations

Menopausal Symptoms

Soybean Composite Functional Factors Enhanced the Serum Estradiol Concentration and Superoxide Dismutase Activity in Postmenopausal Women. D. Li^{*1,2}, C. Gao², X. Chen², Y. Huang², and X. Li¹, ¹Dept of

Food Sci.& Eng. in Changchun University, Changchun, P.R. China, ²National Research and Popularize Center for Soybean Refined Processing, P.R. China.

Menopausal Symptoms

Impact of Supplementation of Soybean *Chikki* as a Source of Isoflavin on Plasma Calcium Levels of Menopause Women. Ch. Kavitha and K. Krishna Kumari*, ANGR Agricultural University, India.

Other

Soybean Extracts Enhance Elastin in Skin. R. Zhao¹, J.C. Liu², Ch. Bertin³, J.P. Ortonne⁴, M. Seiberg¹, and V. Iotsova-Stone^{*1}, ¹Skin Research Center, Johnson & Johnson Consumer Products Worldwide, USA, ²Global Skin Care Growth Platform, Johnson & Johnson Consumer & Personal Products Worldwide, USA, ³Scientific Affairs, J&J Consumer Europe, France, ⁴CPCAD, Hopital l'Archet II, France.

Other

Studies on Antiradical Action of Herbal Extracts From Seasoning and Their Effect on Foods and Human Health. B. Herskowitz¹, R. Reznik², C. Loudkevitch², R. Segal³, and D. Moraru³, ¹Soglowek Food Industries, Israel, ²Rad Natural Technologies Ltd., Israel, ³University "Dunarea de Jos ", Romania.

NEW POSTER ABSTRACTS

Cognitive Function

Short-Term Soy Consumption Affects Working Memory in Young Females. F. Islam, C. Sparkes, S. Roodenrys, and L. Astheimer*, University of Wollongong, Wollongong, NSW, Australia.

We examined the effect of a short-term high-soy diet on cognitive function in healthy females when their endogenous estradiol levels were low. Thirty healthy young females aged 18-34 years participated in a comparison of cognitive performance at three times: at times of high (mid-luteal) and low estradiol (follicular) levels during their menstrual cycles as well as following 4

days of soy diet supplementation during the early follicular phase. Cognitive testing evaluated working and short-term memory, verbal and spatial memory, spatial manipulation (mental rotation) and verbal fluency. Blood samples were taken on each day of cognitive testing to determine levels of soy isoflavones, sex-hormone binding globulin and endogenous steroid hormones: estradiol, progesterone and testosterone. Hormones and SHBGs were analysed using standard radioimmunoassay (RIA) methods and plasma genistein, daidzein, equol and glycitein were determined by HPLC with electrochemical detection. Results of cognitive tests showed significant effects of soy consumption on measures of both working memory and verbal memory. The relationships between cognitive function and isoflavone and hormone levels will be reported.

ACKNOWLEDGEMENTS

We acknowledge the assistance of Dr. Theresa Larkin for assistance with isoflavone assays.

Other

High Serum S-Equol Content in Red Clover Fed Ewes.

E.A. Mustonen¹, T. Jokela², I. Saastamoinen³, J. Taponen¹, S. Taponen¹, H. Saloniemi³, and K. Wahala^{*2}; ¹Faculty of Veterinary Medicine, Saari Unit, Pohjoinen pikatie 800, FIN-04920, Saarentaus, Finland, ²Faculty of Science, Lab of Organic Chemistry, P.O. Box 55, FIN-00014 University of Helsinki, Finland; ³Faculty of Veterinary Medicine, Section of Animal Hygiene Hämeentie 57 FIN-00014 University of Helsinki, Finland.

One of the most interesting metabolites of red clover or soy isoflavones in mammals is equol. Several in vitro or animal studies have indicated equol to possess more biological activity than the parent compounds daidzein and formononetin present in plants.¹ Most recent research has for instance shown equol to act as an anti-androgen that inhibits prostate growth and hormone feedback in rats.²

In connection with the biological effects of these isoflavones and their metabolites in mammals we have developed a convenient method to analyze isoflavones and their metabolites especially equol in animal serum. We introduce here a simple and efficient quantitative HPLC method to determine isoflavonoid content in the serum of red clover silage fed ewes and for the first time a chiral HPLC analysis of the major serum metabolite (S)-(-)-equol. Equol occurs solely as an (S)-enantiomer in nature as proven in this study. The structure of (S)-equol was verified by the first chiral synthesis of enantiopure (S)-equol.

References

1. C. Atkinson, C.L. Frankenfeld, J.W. Lampe, *Exp. Biol. Med.* 230 (2005) 155-170.
2. T.D. Lund, D. J. Munson, M. E. Haldy, K. D. R.

Setchell, E. D. Lephart, R. J. Handa, *Biology of Reproduction* 70(4) (2004) 1188-1195.

ACKNOWLEDGEMENTS

This work was partially carried out within the EU project PHYTOHEALTH no QLRT-2001-02453. This study does not necessarily reflect the views of the Commission and in no way anticipates the Commission's future policy in this area

Isogenic Soybean with Genetically Altered

Phytoestrogens Content. C. Yesudas, M.J. Iqbal, and D. Lightfoot*, Southern Illinois University, Carbondale, IL, USA.

Soybean seed derived protein products contain compounds that have been shown to have physiological effects on humans and animals that ingest these products. Soy phytoestrogens in combination with soy protein and other matrix components may have beneficial effects in humans. Jointly they reduce the risk of certain cancers and cardiovascular disease, as well as ameliorating symptoms of menopause, including osteoporosis. In contrast, hormone replacement therapy (HRT) with non-plant derived estrogens can have negative effects on cardiovascular health. In non-human animals it is evident that phytoestrogens also affect reproduction, immune function, and growth. Previously we had identified 8 loci (QTL) controlling isoflavone content. Here we report the development of isogenic germplasm with elevated and reduced isoflavones content in an otherwise isogenic matrix. In addition we have sought to isolated genes underlying isoflavones content. The research has lead to the identification of nearly isogenic soybeans, in which phytoestrogen amounts and profiles can be managed for specific needs within the research community, and later the human and animal health industries. This research may expand the usefulness of soy products in the health functional food industries.

Table of some selected soybean genotypes from 100 recombinant inbred lines Near isogenic lines and their phytoestrogen contents showing the QTL associations with beneficial alleles.

Line	QTL Number	Protein%	daidzein	glycitein	genestein	total
52	8	39.2	2181	256	1321	3759
89	0	39.2	1002	125	695	1822

Isoflavone contents for all the aglycones of daidzein (Daid), Genistein (Gen) and Glycitein (Gly) are given in milligrams per kilogram dry weight.

Thanks are due to the OVCR at SIUC for Support to MJI.

Cancer

Randomized, Controlled Intervention Study of Soy Protein Isolate in Men at High Risk for Biochemical Recurrence after Radical Prostatectomy: Design and Accrual

M. Bosland, A. Zeleniuch-Jacquotte, J. Melamed, H. Lepor, S. Taneja, J. Schmoll, H. Watanabe, and P. Walden; NYU School of Medicine, New York, NY, USA.

Various lines of evidence suggest that soy intake may prevent prostate cancer and be beneficial for men with prostate cancer. We designed a double-blind, randomized placebo-controlled clinical trial to determine whether intervention with soy protein reduces biochemical recurrence (PSA failure) in men who are at high risk for PSA failure in the first two years after radical prostatectomy. Eligibility criteria are: High Gleason sum (≥ 8), extra-prostatic extension, seminal vesicle invasion, positive surgical margins, positive lymph nodes, and/or preoperative PSA of ≥ 20 ng/ml. Biochemical recurrence was defined a priori as reaching a PSA of ≥ 0.07 ng/ml (ultra-sensitive assay [Tosoh]) confirmed twice at ≥ 1 month intervals. (Baseline PSA of ≥ 0.07 confers ineligibility). Using literature data and data from NYU, it was estimated that the two-year PSA failure rate in subjects fulfilling these criteria is 30%. With a sample size of 256 evaluable subjects, this study will have 80% power to detect a 50% reduction in PSA failure rate at a 2-sided significance level of 0.05. Given a drop-out of 13%, the accrual goal is 284. Thus far, 153 men have been randomized to soy protein or casein-based placebo (Solae) that are identical in composition, except for protein source, and provide 20 g protein/day with the soy preparation containing ~ 24 -26/day genistein and ~ 40 -43/day total isoflavones. Self-reported compliance is excellent, and serum isoflavones (including equol) are being measured as an independent compliance measure. Thus far, 97 men have completed intervention. All 27 men identified with biochemical failure developed further evidence of recurrence.

ACKNOWLEDGEMENTS

Supported in part by NIH Grant No. CA27790, The Solae Company, and the Fremont Family Foundation. The donation of the intervention materials by The Solae Company is gratefully acknowledged.

Cardiovascular Disease

Soy Protein Consumption and Cardiovascular Disease Risk Factors. N. Tran*, L. Barraj, J. Berman, and T. Alexander, Exponent, Washington, DC, USA.

Experimental and epidemiologic data suggest that soy protein may have cardio-protective effects. In this study we examine the trend of soy protein consumption using food consumption data from the United States Department of Agriculture's (USDA) Continuing Survey

of Food Intake by Individuals (CSFII 96-98) and National Health and Nutrition Examination Survey (NHANES 1999-02). The CSFII is a food consumption survey of private households, designed to provide a multistage stratified area probability sample representative of the 48 contiguous states. The NHANES is an annual, cross-sectional survey, based on a complex multistage cluster survey design to collect information about the health and diet of the civilian, non-institutionalized populations of the United States. The NHANES surveys conduct both in-home interviews and physical examinations focusing on several population groups and health topics, such as reproductive health, cardiovascular disease, mental health, nutrition, oral health, vision, etc. We also investigate the relationship between soy protein and cardiovascular disease (CVD) risks. The NHANES 1999-2002 laboratory component provides data on cardiovascular outcomes including total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, C-reactive protein (CRP), and homocysteine. Using the combined dietary and laboratory data from NHANES, we evaluate the relationship between soy protein intake and cardiovascular health status for population subgroup of males and females >20 years old. Findings for these relationships will be presented.

Phytoestrogens Lower the Blood Serum Atherogenicity in Postmenopausal Women. V. Korennaya¹, V.

Myasoedova¹, N. Nikitina⁴, I. Sobenin³, and A. Orekhov²; ¹Institute for Atherosclerosis Research, Moscow, Russia, ²Institute of General Pathology and Pathophysiology, Moscow, Russia, ³Institute of Experimental Cardiology, Moscow, Russia, ⁴Institute of Physical and Chemical Medicine, Moscow, Russia.

Objective: This investigation was conducted to study the influence of phytoestrogen-rich plant compounds on blood serum atherogenicity in postmenopausal women in ex vivo model.

Methods: Blood serum atherogenicity is defined as the ability of serum to induce cholesterol accumulation in cultured human blood-derived monocytes-macrophages. Apparently healthy postmenopausal women were administered the following plant compounds: extract of grape seeds, grape stems, hop cones, sage and green tea leaves in dosages 50, 100, 250, 500, 1000 mg, and dietary supplement "Phytosoy" (Acropharme, France) in dosage of 35 mg of isoflavones. Blood from women was taken at base line and 2, 4, 6 hours after single oral intake of each of those compounds.

Results: At base line serum taken from women was able to induce 1.3-1.5 fold increase in intracellular cholesterol accumulation in cultured cells. The amount of this cholesterol was taken for 100%. After administration of the extract of grape seeds, grape stems, hop cones, sage and green tea leaves blood serum atherogenicity was lowered by $68 \pm 2\%$, $36 \pm 4\%$, $48 \pm 5\%$, $36 \pm 8\%$, $85 \pm 6\%$ respectively. Soy supplement decreased serum

atherogenicity by $32\pm 3\%$. There was a positive correlation between the antiatherogenic effects of plant compounds and the content of polyphenols ($r=0.975$, $p<0.001$), procyanidins ($r=0.971$, $p<0.001$) and the sum of phytoestrogens (resveratrol, daidzein, genistein, flavone) ($r=0.764$, $p<0.001$). Conclusion: Single-dose intake of botanicals, containing phytoestrogens, suppress serum-induced cholesterol accumulation in cultured cells. We may consider that those plants can be used for the development of natural drug for atherosclerosis prevention in menopausal women.

Effect of Colonic Microflora Enhancement by Fructo-Oligosaccharides on the Hypocholesterolemic Action of Soy.

Julia M.W. Wong, Russell de Souza, Azadeh Emam, Cyril W.C. Kendall, and David J.A. Jenkins. Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, M5S 3E2; The Clinical Nutrition and Risk Factor Modification Center, St. Michael's Hospital, Toronto, Ontario, M5C 2T2, Canada.

Background: Soy isoflavones are postulated as one of the cholesterol lowering components of soy which require hydrolysis by colonic bacteria for absorption and transformation to their physiologically active forms.

Objective: To assess the effect of fructo-oligosaccharides (FOS) as a bifidobacterial prebiotic given with soy protein foods to increase the cholesterol lowering effect of soy protein.

Method: Twenty-three hyperlipidemic subjects completed three 4-week treatments consisting of FOS (10 g/d) and low-fat dairy foods (control), and soy protein (30 g/d) foods with or without FOS (10 g/d) in a randomized controlled crossover study. Fasting blood lipids, blood pressure and body weight were measured at weeks 0, 2 and 4 of each phase, with fecal and urine collections at baseline and at the end of each treatment.

Results: Intake of soy with FOS resulted in a greater reduction in the LDL:HDL ratio compared to their intake alone, in a step wise fashion. LDL:HDL showed a mean (week2&4) increase with intake of FOS alone (0.22 ± 0.10 , $P=0.04$), no change with intake of soy without FOS (-0.05 ± 0.06 , $P=0.46$), and a decrease with intake of soy with FOS (-0.32 ± 0.14 , $P=0.03$), compared to baseline.

Conclusions: Improvements in the lipid profile of individuals consuming soy foods with FOS compared to their intake alone, suggest a possible synergistic effect. Further analysis of urine isoflavones may shed some light on whether soy isoflavones, especially equol production, play a role in the cholesterol lowering of soy.

Isoflavone Metabolism

Biotransformation of Daidzein to S-Equol with Two Intestinal Anaerobic Bacteria. Xiu-Ling Wang^{1,2}, Ho-Jin Kim¹, Ki-Tae Kim³, Hor-Gil Hur⁴ and Su-Il Kim¹, ¹School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea, ²College of Life Science, Agricultural University of Hebei, Baoding 071001, China, ³Takara Korea Biomedical Inc. Research and Development Center, Gyeonggi-Do, 437-020, Korea, ⁴Department of Environmental Science and Engineering, Gwangju Institute of Science and Technology, Gwangju, 500-712, Korea.

We have attempted to produce *S*-equol directly from daidzein by the coculture of two novel anaerobic bacteria, Niu-O16 (J. Biotechnol., 115, p261, 2005) and Julong 732 (Appl. Env. Microbiol., 71, p214, 2005). BHI liquid medium inoculated with both strain Niu-O16 and Julong 732 was incubated for 4 days with 1.2 mM of daidzein in an anaerobic chamber. The bacterial culture broth was sampled every 12 hr and analyzed. Metabolites in the broth were identified on the bases of EI-MS, NMR and UV spectral analysis on HPLC. Enantiomers were separated on chiral stationary-phase HPLC and their $[\alpha]_D$ values were measured to determine their configuration. Almost all of the daidzein in the medium were metabolized within 48 hrs' incubation with 70% conversion rate to equol by the two strains. Dihydrodaidzein (DHD) which is produced only by NiuO16, was observed in the 12hrs culture broth. Also both of the *R*- and *S*-DHD were detected in the broth, only *S*-equol was formed from DHD by the strain Julong 732, indicating that the biotransformation of DHD is highly stereospecific in this strain.

Significantly More Equol in Organic Skimmed Milk.

Eeva Mustonen¹, Saastamoinen Ilkka¹, Antti Hoikkala¹, Tuija Jokela², Juhani Taponen¹, Kristiina Wähälä², and Hannu Saloniemi¹, University of Helsinki, Finland, ¹Faculty of Veterinary Medicine, Department of Clinical Veterinary Sciences, Section of Animal Hygiene and Saari Unit, P.O. Box 57, FIN-00014, Finland, ²Faculty of Science, Department of Chemistry, Laboratory of Organic Chemistry, P.O. Box 55, FIN-00014, Finland.

Legume forages are essential in organic production; red clover and grass-red clover mixtures appear to be most profitable forage legumes for silage production. Red clover is widely cultivated in Finnish organic dairy farms.

The aim of this pilot study was to analyze equol, formononetin and daidzein from commercial cartons of skimmed milk. Twelve cartons of organic milk and four conventionally produced were purchased from ordinary grocery shops between January and March, 2005. We found on average 411 ng/ml of equol, 4 ng/ml of formononetin and 1 ng/ml of daidzein in organic skimmed milk. Conventionally produced milk contained on average

62 ng/ml of equol, and no formononetin or daidzein were found.

Milk samples were analyzed by HPLC. Analysis method was modified from Franke et al (1), Lundh et al (2) and Antignac et al. (3) Isoflavones and metabolites were identified using authentic reference compounds as external standards.

1. Franke, A.A.; Custer, L.J.; Wang, W.; Shi, C.Y. *Proc. Soc. Exp. Biol. Med.* 1998, *217*, 263-273.
2. Lundh, T.J.; Pettersson, H.; Kiessling, K.H. *J. Assoc. Off. Anal. Chem.* 1988, *71*, 938-941.
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