Effects of High- and Low-Isoflavone (Phytoestrogen) Soy Foods on Inflammatory Biomarkers and Proinflammatory Cytokines in Middle-Aged Men and Women

David J.A. Jenkins, Cyril W.C. Kendall, Philip W. Connelly, Chung-Ja C. Jackson, Tina Parker, Dorothea Faulkner, and Edward Vidgen

This study sought to determine effects of high- and low-isoflavone soy protein foods on acute-phase proteins and proinflammatory cytokines and whether isoflavone phytoestrogens might act as estrogens, which enhance the immune response. Forty-one hypercholesterolemic men and postmenopausal women underwent three 1-month diets consisting of a low-fat dairy food control phase and high- and low-isoflavone soy food test phases (50 g/d and 52g/d soy protein, respectively, and 73 mg/d and 10 mg/d isoflavone, respectively). Diets were low in saturated fat (<5% of energy) and cholesterol (<50 mg/d). Fasting blood analytes and blood pressure were measured at the start and end of each phase. For the entire group of subjects, no treatment differences were observed for acute-phase proteins or proinflammatory cytokines. However, a significant interaction was noted between diet and sex. Assessing the results of men and women separately, women showed significantly higher interleukin-6 (IL-6) values after the high-isoflavone soy diet ($P = .013$) compared to control values. For women, the difference between the high- and low-isoflavone IL-6 values was significant using the unadjusted data ($P = .048$) but not after adjustment. No significant effects were seen for men or women in C-reactive protein (CRP), serum amyloid A (SAA), or tumor necrosis factor-α (TNF-α). Thus, high levels of isoflavone intake appear to increase serum concentrations of IL-6 in women. This finding may indicate an estrogenic effect of soy isoflavones in enhancing the immune response and provide a possible explanation through enhanced immune surveillance for lower incidence of certain cancers in soy-eating parts of the world.

THE ABILITY TO mount an inflammatory response has both advantages and disadvantages in terms of chronic disease. Classically, advantages of the inflammatory response are seen in terms of combating infection, whereas in autoimmune diseases the inflammation is unwanted. Originally, advantages were also seen in terms of tumor control and "immune surveillance"[1] and more recently in the function of the inflammatory process to reduce growth factor production, including insulin-like growth factors,[2] which have been implicated in tumor promotion.[3] On the other hand, inflammation appears to be increasingly important in cardiovascular disease (CHD) with the demonstration that C-reactive protein (CRP) is a strong independent predictor of CHD in men and women.[4-6]

Estrogens have been shown to stimulate the immune system[7] and raise CRP levels[8,9] and attention has been drawn to the greater propensity of women to suffer autoimmune diseases.[10,11] Soy proteins are associated with phytoestrogens or plant-derived estrogenic substances (isoflavones in the case of soy). It was therefore of interest to determine the effect of soy protein with phytoestrogens present at high or very low levels on inflammatory biomarkers and whether these plant estrogen-like substances produced responses similar to mammalian estrogens.

We therefore assessed the levels of CRP, serum amyloid A (SAA), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in hyperlipidemic men and women who had taken part in a study of high- and low-isoflavone soy foods on blood lipids. The lipid results of this study have been reported elsewhere.[12]

MATERIALS AND METHODS

Study Protocol

The study followed a randomized crossover design in which all 41 subjects participated in three 1-month phases with each phase separated by a minimum 2-week washout period. The 3 phases consisted of a dairy and egg protein phase (control) and 2 soy protein phases, one high and the other low in isoflavones. During all study phases subjects followed their own self-selected National Cholesterol Education Program (NCEP) step 2 diets (<7% energy from saturated fat and <200 mg/d dietary cholesterol),[13] but substituted very low-fat dairy or soy foods, with which they were provided, for the major sources of protein in their customary diets. Subjects were blinded as to the level of isoflavone in the soy foods. Nine men and 6 women started with the control phase first. The respective figures for low- and high-isoflavone soy phases were 8 and 6, and 6 and 5, respectively.

The study was approved by the Ethics Committee of the University of Toronto and St Michael's Hospital and informed consent was obtained from the subjects.

Subjects

We enrolled 73 subjects; 18 withdrew either before or after randomization but before starting the study. Fourteen withdrew during the first and second phases. Forty-one subjects completed all 3 phases (23 men

From the Clinical Nutrition and Risk Factor Modification Center, and the Department of Medicine, Division of Endocrinology and Metabolism, St Michael's Hospital, Toronto, Ontario; Departments of Nutritional Sciences, Biochemistry, and Laboratory Medicine and Pathobiology, Faculty of Medicine, University of Toronto, Toronto, Ontario; and the Laboratory Services Division, University of Guelph, Guelph, Ontario, Canada.

Submitted November 6, 2001; accepted January 3, 2002.

Supported by the Natural Sciences and Engineering Research Council of Canada (Ottawa, Ontario) and Loblaw Brands Ltd (Toronto, Ontario). D.J.A.J. is a Canada Research Chair in Nutrition and Metabolism funded by the Federal Government of Canada.

Address reprint requests to David J.A. Jenkins, MD, Clinical Nutrition and Risk Factor Modification Center, St Michael's Hospital, 61 Queen St E, Toronto, Ontario, Canada M5C 2T2.

0026-0495/02/5107-0016$35.00/0

doi:10.1053/meta.2002.33352

Table 1. Calculated Dietary Intakes (mean ± SE) for Week 4 of the Control and Soy Diet Phases

	Control (n = 40)	Low-Isoflavone Soy (n = 39)	High-Isoflavone Soy (n = 40)
Energy (MJ/d)	7.29 ± 0.31	7.31 ± 0.31	7.97 ± 0.36†
(kcal/d)	1,743 ± 73	1748 ± 74	1905 ± 85
Total Protein (g/d)	88 ± 4	92 ± 3	94 ± 3
(%)	20.7 ± 0.8	21.9 ± 0.9	20.4 ± 0.6
Vegetable protein (g/d)	28 ± 2	88 ± 3‡	90 ± 3‡
(%)	32.8 ± 1.8	96.0 ± 0.5‡	95.9 ± 0.4‡
Soy protein (g/d)	0 ± 0	52 ± 2‡	50 ± 2‡
(%)	0.0 ± 0.0	12.6 ± 1.0‡	11.0 ± 0.5‡
Available carbohydrate (g/d)	263 ± 12	258 ± 13	278 ± 13
(%)	60.4 ± 1.1	58.5 ± 1.1	58.5 ± 0.8
Total dietary fiber (g/d)	27 ± 2	25 ± 2	28 ± 2
(g/MJ)	3.8 ± 0.3	3.4 ± 0.2	3.5 ± 0.2
Total fat (g/d)	32 ± 2	35 ± 2	39 ± 3‡
(%)	16.3 ± 0.6	17.7 ± 0.6	18.2 ± 0.7*
SFA (g/d)	7 ± 1	8 ± 1	9 ± 1‡
(%)	3.5 ± 0.2	4.1 ± 0.2*	4.2 ± 0.2†
MUFA (g/d)	12 ± 1	13 ± 1	15 ± 1‡
(%)	5.9 ± 0.3	6.4 ± 0.3	6.9 ± 0.4†
PUFA (g/d)	10 ± 1	12 ± 1	12 ± 1
(%)	5.4 ± 0.2	6.0 ± 0.3	5.6 ± 0.3
PUFA:SFA (P/S ratio)	1.6 ± 0.1	1.6 ± 0.1	1.4 ± 0.1*
Dietary cholesterol (mg/d)	59 ± 5	68 ± 6	71 ± 6
(mg/MJ)	8.3 ± 0.7	9.4 ± 0.6	9.1 ± 0.6
Alcohol (g/d)	7 ± 2	5 ± 2	9 ± 3
(%)	2.5 ± 0.7	1.9 ± 0.5	2.8 ± 0.7

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

*$P < .050$, †$P \leq .01$, and ‡$P \leq .001$, significance of the difference of either low- or high-isoflavone soy *v* control using the Tukey adjustment in SAS.

and 18 postmenopausal women; age, 62 ± 2 years; body mass index [BMI], 25.3 ± 0.5 kg/m^2). All subjects had elevated low-density lipoprotein (LDL) cholesterol levels on initial assessment (>4.1 mmol/L). Prior to the start of the study, 8 subjects had elevated triglyceride levels (>2.3 mmol/L; range, 2.76 to 4.77 mmol/L). None had clinical or biochemical evidence of diabetes or liver or renal disease, and none were taking hypolipidemic agents. Five women were taking hormone-replacement therapy and 3 were taking levothyroxine. Five men were taking 1 or 2 of the following: β-blocking agents (n = 2), angiotensin-converting enzyme (ACE) inhibitors (n = 1), angiotensin II subtype 1 (ATI) receptor blocker with a calcium channel blocker (n = 1), and calcium channel blocker alone (n = 1). Four women were taking the following drugs: angiotensin II ATI receptor blocker (n = 1), calcium channel blocker (n = 2), and calcium channel blocker with an ACE inhibitor (n = 1). Dosages for all medications for all subjects were held constant throughout the study. Subjects were also asked to maintain their habitual level of physical activity throughout all 3 study phases.

Diets

During study phases the diets were the subjects' self-selected NCEP step 2 diets in which the main protein-containing foods—meats, fish, dairy, eggs, nuts (eg, peanut butter), and legumes—were replaced on the control phase by low-fat dairy products including skim milk, yogurt, cottage cheese, very low-fat Hoop cheese (Western Creamery, Toronto, Canada), processed fat-free cheese slices (Kraft Canada, Don Mills, Canada), and egg substitute (Egg Beaters, Lipton's, Toronto, Canada). On the high- and low-isoflavone soy phases the main protein-containing foods were replaced by low-fat soy milk (0.1% fat) (Sanitarium, Pt Sidney, Australia), soy "hot dogs," "breakfast links," soy "burgers," and "cold cuts" (Yves Veggie Cuisine, Vancouver, Canada), as well as tofu nuggets (Soy City Foods, Toronto, Canada) and tofu burgers (La Soyarie, Hull, Canada). These products were made either from alcohol-washed or non–alcohol-washed soy protein isolate (Protein Technologies International, St Louis, MO), or from tofu, which was made from soy beans selected for their very high or very low isoflavone content (First Line Seeds, Guelph, Canada; Advantage Seed Growers and Processors, Lucknow, Canada). The nutrient profiles of the dairy and soy food substitutions were balanced for fatty acid composition and dietary cholesterol intake by the addition of butter (group mean, 1.1 g/d) and 1 egg (53 g/wk) for an 8.4-MJ (2,000-kcal) soy diet. The corresponding addition on the control diet was an oil mixture providing 0.27 g/d soy oil, 3.2 g/d safflower oil, 1.5 g/d corn oil, and 4.3 g/d canola oil. Foods provided were designed to represent 20% of the subjects' estimated daily total energy intake[14] and the foods provided contributed approximately 13.5% energy as protein expressed as a percentage of daily recorded energy intake (Table 1). Analyses of isoflavones as aglycones in the soy foods indicated that the mean daily intake of isoflavones for the 41 subjects was 10 ±0 mg/d on the low-isoflavone soy food phase and 73 ± 3 mg/d on the high-isoflavone soy food phase.

Soy milks for the study were delivered by courier to the subjects' homes at the beginning of the phase. All other foods were provided on a biweekly basis to be kept refrigerated or frozen by the subjects until consumed. Subjects were provided with self-tarring digital electronic scales on which to weigh all soy and dairy items prior to consumption throughout the study. They also checked off these items as eaten on the weekly lists provided. Scales were used to weigh all food items during the weeks when diet histories were recorded. The subjects' week four 7-day diet histories recorded during the first phase were photocopied and given to that subject at the beginning of all subsequent phases as

the template on which to model their dietary intake. Subjects were also instructed that during the study they should eat no additional soy or dairy foods, legumes, nuts, or viscous fiber sources such as psyllium.

Compliance was assessed by 7-day diet histories, completed checklists of the foods provided on a weekly basis, and the return of any uneaten food items at clinic visits, which were then weighed and recorded.

Every effort was made to ensure that subjects maintained the same body weight throughout the study with appropriate advice given where necessary at each clinic visit.

Measurements

Fasting body weight, blood samples, and blood pressure were obtained at the start and end of each 4-week diet phase. Seven-day weighed diet histories were recorded prior to each phase and on week 4 of each phase and checked with the dietitian to assure accuracy.

Analyses

Serum samples , stored at -70°C were analyzed for CRP by end-point nephelometry (Behring BN100, N high-sensitivity C-reactive protein reagent, Dade-Behring, Mississauga, Canada), SAA (Human SAA Cytoscreen enzyme-linked immunosorbent assay [ELISA] Kit, Catalog #KHA0012, BioSource International, Camarillo, CA), IL-6 (Human IL-6 ,US UltraSensitive Cytoscreen ELISA Kit, Catalog #KHC0063, BioSource International), and TNF-α (Human TNF-α Ultrasensitive ELISA kit, Catalog #KHC3013, BioSource International). For CRP, SAA, IL-6, and TNF-α, the intra- and interassay coefficients of variation were, respectively, 2.4%, 7.7%, 4.3%, and 5.2%; and 3.7%, 10.7%, 6.8%, and 9.7%. Serum lipids were analyzed according to the Lipid Research Clinics protocol[15] for total cholesterol (TC), triglyceride, and high-density lipoprotein cholesterol (HDL-C), after dextran sulfate-magnesium chloride precipitation.[16] All samples from a given individual were analyzed in the same batch. LDL-C was calculated.[17] Full lipid, lipoprotein, and apolipoprotein data are reported elsewhere.[12]

Dietary isoflavone concentrations were measured as 3 aglycones (genistein, daidzein, and glycitein) in foods, which had been freeze-dried. After acid hydrolysis of endogenous isoflavones, aglycones in alcoholic extracts were identified and quantitated by high-pressure liquid chromatography (HPLC)[18,19] using a 600E multisolvent delivery system with a photodiode array detector monitoring at 200 to 350 nm (Waters, Marlborough, MA) and a Nova Pak C18 column (5 μm, 150 mm $\times$ 3.9 mm internal diameter) (Waters) equipped with a C18 guard column. Appropriate isoflavone standards were analyzed. Biochanin A was used as an internal standard, with recovery values ranging from 80% to 100%. Food isoflavone data have been reported elsewhere.[12]

Freeze-dried soy and dairy foods were analyzed in the laboratory using Association of Official Analytical Chemists methods for fat, protein,[20] and fiber[21] with available carbohydrate calculated by difference. The fatty acid composition was determined by gas chromatography.[22] Diet histories were assessed using a computer program based on US Department of Agriculture data[23] supplemented with data from food labels and from results of foods analyzed in the laboratory. The percentage figures for soluble and insoluble fiber were derived from published data.[24]

Statistical Analysis

The results are expressed as means $\pm$ SE. The isoflavone effect was assessed by comparing the 3 treatments using least-squares means test with a Tukey adjustment to determine the significance of differences between treatments.[25] The statistical model included week 4 values as the response variable, treatment and sequence as main effects, treatment by sex as the interaction term, a random subject effect nested within sex by sequence, and baseline as a covariate. SAS version 8 software was used throughout.[25]

RESULTS

Compliance was good. Consumption of the prescribed foods expressed as a percentage of energy for the 3 phases was as follows: control, 97.2% $\pm$ 0.7%; low-isoflavone soy, 96.9% $\pm$ 0.8%; and high-isoflavone soy, 97.8% $\pm$ 0.5%. There were no significant differences between treatments. There were no differences between the control and the soy foods in body weight change (control, -0.3 $\pm$ 0.1 kg; low-isoflavone soy, -0.3 $\pm$ 0.1 kg; high-isoflavone soy, -0.4 $\pm$ 0.1 kg) (Table 2).

In general, blood lipid CHD risk factors were reduced equally on the high- and low-isoflavone soy diets.[12] Expressed as change from baseline, on the control phase, LDL-C was reduced by 2.3% $\pm$ 2.4% (P = .337), on the low-isoflavone phase by 6.4% $\pm$ 2.3% (P = .008), and on the high-isoflavone phase by 7.6% $\pm$ 2.7% (P = .008).[12]

Table 2. Mean $\pm$ SE Week 0 and 4 Values of Acute-Phase Proteins and Proinflammatory Cytokines on the Control, Low-, and High-Isoflavone Diet Periods (N = 41)

		Control		Low-Isoflavone Soy		High-Isoflavone Soy	
	Sex	Week 0	Week 4	Week 0	Week 4	Week 0	Week 4
Body weight (kg)	Men	76.6 $\pm$ 1.9	76.3 $\pm$ 1.8	76.5 $\pm$ 1.9	76.1 $\pm$ 1.8	76.7 $\pm$ 2.0	76.3 $\pm$ 1.9
	Women	64.7 $\pm$ 2.7	64.3 $\pm$ 2.7	64.1 $\pm$ 2.8	64.0 $\pm$ 2.7	64.7 $\pm$ 2.7	64.4 $\pm$ 2.7
Acute-phase proteins							
CRP (mg/L)	Men	1.6 $\pm$ 0.2	2.0 $\pm$ 0.4	3.5 $\pm$ 1.2	2.5 $\pm$ 0.4	2.5 $\pm$ 0.9	2.0 $\pm$ 0.3
	Women	3.4 $\pm$ 1.1	2.7 $\pm$ 0.7	2.4 $\pm$ 0.5	3.1 $\pm$ 0.6	2.8 $\pm$ 0.7	4.5 $\pm$ 1.6
SAA (μg/L)	Men	11.3 $\pm$ 1.5	10.7 $\pm$ 1.2	13.9 $\pm$ 2.6	14.0 $\pm$ 1.6	12.1 $\pm$ 1.9	11.8 $\pm$ 1.4
	Women	17.8 $\pm$ 3.1	21.3 $\pm$ 8.5	14.8 $\pm$ 1.7	35.0 $\pm$ 18.4	14.5 $\pm$ 1.7	31.7 $\pm$ 12.2
HDL-C (mmol/L)	Men	1.20 $\pm$ 0.05	1.11 $\pm$ 0.04	1.19 $\pm$ 0.05	1.13 $\pm$ 0.04	1.18 $\pm$ 0.07	1.13 $\pm$ 0.05
	Women	1.49 $\pm$ 0.09	1.39 $\pm$ 0.09	1.49 $\pm$ 0.09	1.46 $\pm$ 0.09	1.50 $\pm$ 0.09	1.38 $\pm$ 0.08
Proinflammatory cytokines							
IL-6 (pg/mL)	Men	0.89 $\pm$ 0.43	0.93 $\pm$ 0.39	1.14 $\pm$ 0.42	0.99 $\pm$ 0.48	0.88 $\pm$ 0.32	0.88 $\pm$ 0.47
	Women	0.57 $\pm$ 0.16	0.34 $\pm$ 0.06	0.35 $\pm$ 0.05	0.38 $\pm$ 0.07	0.48 $\pm$ 0.09	0.72 $\pm$ 0.16*
TNF-α (pg/mL)	Men	2.05 $\pm$ 0.55	2.08 $\pm$ 0.54	1.80 $\pm$ 0.28	1.73 $\pm$ 0.23	2.01 $\pm$ 0.38	2.16 $\pm$ 0.63
	Women	1.49 $\pm$ 0.11	1.47 $\pm$ 0.11	1.42 $\pm$ 0.11	1.44 $\pm$ 0.08	1.40 $\pm$ 0.09	1.47 $\pm$ 0.13

*P = .013, significance of the difference between control and high-isoflavone soy diets assessed by the general linear model in SAS.

Cytokines and Acute-Phase Proteins

For the entire group of subjects, no treatment differences were observed for acute-phase proteins or proinflammatory cytokines. However, a significant interaction was noted between diet and sex and the data are therefore presented broken down by sex for comparisons between the 3 phases (control, low-, and high-isoflavone) (Tables 2). In women, IL-6 on the high-isoflavones soy diet at week 4 was significantly different from the control week 4 value ($P = .013$). Although the women's week 4 IL-6 value on high-isoflavone soy was not significantly different from the low-isoflavone value after adjustment ($P = .115$), the difference was significant before adjustment ($P = .048$). No other comparison in the data set achieved significance, although there was a tendency for CRP and SAA to be higher after the high-isoflavone soy diet.

DISCUSSION

This study indicates that consumption of high-isoflavone soy foods may increase IL-6 concentrations in serum. Soy isoflavones have been considered to have estrogenic properties and have been classed as phyoestrogens or plant-derived compounds with estrogenic activity. Estrogens have been reported to enhance the immune response,[8] including IL-6 production.[26] We believe this is the first study to suggest a potential nonspecific immune-stimulatory effect of soy phytoestrogens. The implications are broad. On the negative side, soy isoflavones may increase the risk of autoimmune disorders and possibly CHD, where raised CRP levels have been found to be highly predictive of future disease.[3] On the positive side, high-isoflavone soy may prime the immune system to control infections and possibly enhance natural defenses against tumor development.

In terms of overstimulation of the immune response, the effects of soy consumption were numerically very modest when compared to control values. CRP has been strongly associated with increased CHD risk in cohort studies.[6] However, the rise in CRP levels after high-isoflavone soy consumption was not seen in men and was not significant in women. In the absence of a significant increase in CRP, an association between soy isoflavones and increased CHD risk related to an enhanced immune stimulation seems very unlikely. The more so because soy proteins have been shown to reduce a wide range of CHD risk factors, including the LDL-C:HDL-C ratio,[27,28] together with blood pressure,[29] oxidized LDL-C,[30-32] and homocysteine[33] concentrations. Vegetable proteins have been a component of successful CHD regression studies[34,35] and are a notable dietary feature of traditional diets in the Orient, where CHD is uncommon.[36] In terms of increased autoimmune disease, soy and other high-isoflavone–containing foods have not been implicated. Follow-up of children fed soy infant formula revealed no ill effects in adult life,[37] and other reports of soy consumption and disease that have focused on cancer incidence[38] or mental function[39] have not reported excess morbidity or mortality from autoimmune diseases.

In terms of reduction in cancer risk, the main interest in soy has related to hormone antagonistic activity and reduction in risk of hormone-dependent cancers of breast and prostate.[40] We suggest that enhanced immune surveillance may be a further feature of regular soy food consumption. The lower mortality from hormone-dependent cancers in Japan and China is notable.[41] Soy milk consumption has been associated with reduced prostate cancer incidence and mortality.[42] Despite concerns from animal models that phytoestrogens may enhance breast cancer risk,[43] no increase in breast cancer has emerged related to soy consumption.[44] Rather soy-eating parts of the world are notable for low rates of breast cancer.[41]

Both estrogens and phytoestrogens (isoflavone) are antioxidants, and isoflavones have been shown both to reduce circulating levels of oxidized LDL-C[12,30-32] and to reduce in vitro oxidizability of LDL-C.[45,46] This antioxidant activity of soy isoflavones has also been suggested to relate to anticancer properties of soy through reduction in DNA damage.[47] However, if soy isoflavones are immune stimulants as has been determined for mammalian estrogens, then this function together with their antioxidant activity may be another factor tending to reduce cancer risk.

Other effects of soy isoflavones may involve a range of related mechanisms with antitumor implications. In the present study, an increase in IL-6 concentrations was seen after feeding high-isoflavone soy. IL-6 has been shown to stimulate production of metal binding metallothionin, which binds zinc and reduces blood levels.[48] IL-6 also appears to reduce plasma concentrations of insulin-like growth factor-1.[49] These actions would tend to reduce protein synthesis, and cell growth and multiplication. In the longer term, these effects may also contribute to reduced tumorigenesis.[3]

We conclude that soy isoflavones may have a modest action in increasing the cytokine IL-6 even at intake levels where no other endocrine-related effects occur. This effect may be part of a general estrogenic stimulation of the immune response or may be more specific for IL-6. On balance, this action would appear to be a beneficial effect with possible implications in cancer risk reduction, a function that has been ascribed to soy. Further studies are warranted to assess the effect of soy phytoestrogens on IL-6 and in turn its relation to suppression of growth factors such as insulin-like growth factor-1, raised concentrations of which have been associated with a range of cancers including colon, breast, and ovary.[3]

ACKNOWLEDGMENT

The authors wish to thank Loblaw Brands Ltd, Toronto, Ontario; Yves Veggie Cuisine Inc, Vancouver, British Columbia; Protein Technologies International, St. Louis, Missouri; Sanitarium, Pt. Sidney, New South Wales, Australia; Soy City Foods, Toronto, Ontario; La Soyarie, Hull, Quebec; First Line Seeds, Guelph, Ontario; Advantage Seed Growers and Processors, Lucknow, Ontario, for the development and generous donation of soy foods, soy protein isolates, and high- and low-isoflavone soy beans. We sincerely thank Robert Chenaux, Larry Griffin, Sherry Casey, and Judy Coveney of Loblaw Brands Ltd; Yves Potvin and Gerry Amantea of Yves Veggie Cuisine Inc; Belinda Jenks of Protein Technologies International; Greg Gambrill of the Sanitarium; John Esqueval of Soy City Food; Koichi Watanabe of La Soyarie; Jonathon Jenkinson of First Line Seeds; and Roy van Wyk of Advantage Seed Growers and Processors for their very valuable help and advice on this project. We also thank Yu-Min Li, George Koumbridis, Jean-Paul Dini, and Clara Lavandier who provided excellent technical assistance.

REFERENCES

1. Doll R, Kinlen L: Immunosurveillance and cancer: Epidemiological evidence. Br Med J 4:420-422, 1970
2. Wolf M, Bohm S, Brand M, et al: Proinflammatory cytokines interleukin 1 beta and tumor necrosis factor alpha inhibit growth hormone stimulation of insulin-like growth factor I synthesis and growth hormone receptor mRNA levels in cultured rat liver cells. Eur J Endocrinol 135:729-737, 1996
3. Smith GD, Gunnell D, Holly J: Cancer and insulin-like growth factor-I. A potential mechanism linking the environment with cancer risk. BMJ 321:847-848, 2000
4. Rifai N, Ridker PM: High-sensitivity C-reactive protein: A novel and promising marker of coronary heart disease. Clin Chem 47:403-411, 2001
5. Roivainen M, Viik-Kajander M, Palosuo T, et al: Infections, inflammation, and the risk of coronary heart disease. Circulation 101: 252-257, 2000
6. Ridker PM, Hennekens CH, Buring JE, et al: C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 342:836-843, 2000
7. Knoferl MW, Jarrar D, Angele MK, et al: 17β-Estradiol normalizes immune responses in ovariectomized females after trauma-hemorrhage. Am J Physiol Cell Physiol 281:C1131-1138, 2001
8. Ridker PM, Hennekens CH, Rifai N, et al: Hormone replacement therapy and increased plasma concentration of C-reactive protein. Circulation 100:713-716, 1999
9. Cushman M, Legault C, Barrett-Connor E, et al: Effect of postmenopausal hormones on inflammation-sensitive proteins: The Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. Circulation 100:717-722, 1999
10. Beeson PB: Age and sex associations of 40 autoimmune diseases. Am J Med 96:457-462, 1994
11. Gale EA, Gillespie KM: Diabetes and gender. Diabetologia 44:3-15, 2001
12. Jenkins DJ, Kendall CW, Jackson C-J, et al: Effects of high and low isoflavone soy foods on blood lipids, oxidized LDL, homocysteine and blood pressure in hyperlipidemic men and women. Am J Clin Nutr (in press)
13. The Expert Panel: Summary of the Second Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). JAMA 269:3015-3023, 1993
14. The Lipid Research Clinics Population Studies Data Book. Vol II. The Prevalence Study-Nutrient Intake. US Department of Health and Human Services. NIH Publication No. 82-2014, September 1982
15. Lipid Research Clinics: Manual of Laboratory Operations. Lipid and Lipoprotein Analysis (revised 1982). Washington, DC, US Government Printing Office, US Department of Health and Human Services Publication no. (NIH) 75-678, 1982
16. Warnick GR, Benderson J, Albers JJ: Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 28:1379-1388, 1982
17. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499-502, 1972
18. Wang H-J, Murphy P: Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, crop year, and location. J Agric Food Chem 42:1674-7167, 1994
19. Franke AA, Custer LJ, Cerna CM, et al: Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. Proc Soc Exp Biol Med 208:18-26, 1995
20. Association of the Official Analytical Chemists: AOAC Official Methods of Analysis. Washington, DC, Association of the Official Analytical Chemists, 1980
21. Prosky L, Asp NG, Furda I, et al: Determination of total dietary fiber in foods and food products: Collaborative study. J Assoc Off Anal Chem 68:677-679, 1985
22. Cunnane SC, Hamadeh MJ, Liede AC, et al: Nutritional attributes of traditional flaxseed in healthy young adults. Am J Clin Nutr 61:62-68, 1995
23. The Agricultural Research Service: Composition of Foods, Agriculture Handbook No 8. Washington, DC, US Department of Agriculture, 1992
24. Anderson JW, Bridges SR: Dietary fiber content of selected foods. Am J Clin Nutr 47:440-447, 1988
25. SAS Institute: SAS/STAT User's Guide (ed 6.12). Cary, NC, SAS Institute, 1997
26. Herrington DM, Brosnihan KB, Pusser BE, et al: Differential effects of E and droloxifene on C-reactive protein and other markers of inflammation in healthy postmenopausal women. J Clin Endocrinol Metab 86:4216-4222, 2001
27. Anderson JW, Johnstone BM, Cook-Newell ME: Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 333:276-282, 1995
28. Jenkins DJ, Kendall CW, Mehling CC, et al: Combined effect of vegetable protein (soy) and soluble fiber added to a standard cholesterol-lowering diet. Metabolism 48:809-816, 1999
29. Washburn S, Burke GL, Morgan T, et al: Effect of soy protein supplementation on serum lipoproteins, blood pressure, and menopausal symptoms in perimenopausal women. Menopause 6:7-13, 1999
30. Jenkins DJ, Kendall CW, Vidgen E, et al: Effect of soy-based breakfast cereal on blood lipids and oxidized low-density lipoprotein. Metabolism 49:1496-1500, 2000
31. Jenkins DJ, Kendall CW, Garsetti M, et al: Effect of soy protein foods on low-density lipoprotein oxidation and ex vivo sex hormone receptor activity—A controlled crossover trial. Metabolism 49:537-543, 2000
32. Jenkins DJ, Kendall CW, Vidgen E, et al: The effect on serum lipids and oxidized low-density lipoprotein of supplementing self-selected low-fat diets with soluble-fiber, soy, and vegetable protein foods. Metabolism 49:67-72, 2000
33. Hermansen K, Sondergaard M, Hoie L, et al: Beneficial effects of a soy-based dietary supplement on lipid levels and cardiovascular risk markers in type 2 diabetic subjects. Diabetes Care 24:228-233, 2001
34. Ornish D, Scherwitz LW, Billings JH, et al: Intensive lifestyle changes for reversal of coronary heart disease. JAMA 280:2001-2007, 1998
35. Ornish D, Brown SE, Scherwitz LW, et al: Can lifestyle changes reverse coronary heart disease? The Lifestyle Heart Trial. Lancet 336:129-133, 1990
36. Menotti A, Keys A, Kromhout D, et al: Inter-cohort differences in coronary heart disease mortality in the 25-year follow-up of the Seven Countries Study. Eur J Epidemiol 9:527-536, 1993
37. Strom BL, Schinnar R, Ziegler EE, et al: Exposure to soy-based formula in infancy and endocrinological and reproductive outcomes in young adulthood. JAMA 286:807-814, 2001
38. Fraser GE: Associations between diet and cancer, ischemic heart disease, and all-cause mortality in non-Hispanic white California Seventh-day Adventists. Am J Clin Nutr 70:532s-538s, 1999 (suppl 3)
39. White LR, Petrovitch H, Ross GW, et al: Brain aging and midlife tofu consumption. J Am Coll Nutr 19:242-255, 2000
40. Stephens FO: The rising incidence of breast cancer in women and prostate cancer in men. Dietary influences: A possible preventive role for nature's sex hormone modifiers—The phytoestrogens. Oncol Rep 6:865-870, 1999

41. Adlercreutz H, Markkanen H, Watanabe S: Plasma concentrations of phyto-oestrogens in Japanese men. Lancet 342:1209-1210, 1993

42. Jacobsen BK, Knutsen SF, Fraser GE: Does high soy milk intake reduce prostate cancer incidence? The Adventist Health Study (United States). Cancer Causes Control 9:553-557, 1998

43. Hsieh CY, Santell RC, Haslam SZ, et al: Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. Cancer Res 58:3833-3838, 1998

44. Key TJ, Sharp GB, Appleby PN, et al: Soya foods and breast cancer risk: A prospective study in Hiroshima and Nagasaki, Japan. Br J Cancer 81:1248-1256, 1999

45. Kuohung W, Shwaery GT, Keaney JF Jr: Tamoxifen, esterified estradiol, and physiologic concentrations of estradiol inhibit oxidation of low-density lipoprotein by endothelial cells. Am J Obstet Gynecol 184:1060-1063, 2001

46. Wiseman H, O'Reilly JD, Adlercreutz H, et al: Isoflavone phytoestrogens consumed in soy decrease F(2)-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. Am J Clin Nutr 72:395-400, 20000

47. Mitchell JH, Collins AR: Effects of a soy milk supplement on plasma cholesterol levels and oxidative DNA damage in men—A pilot study. Eur J Nutr 38:143-148, 1999

48. Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 340:448-454, 1999

49. De Benedetti F, Alonzi T, Moretta A, et al: Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. J Clin Invest 99:643-650, 1997