

Changes in Serum Enterolactone, Genistein, and Daidzein in a Dietary Intervention Study in Finland¹

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Abstract

Phytoestrogens are plant-derived compounds that may have cancer-protective properties. The purpose of the study was to see how enterolactone, daidzein, and genistein serum concentrations reflect major changes in the diet of Finnish subjects. Phytoestrogen concentrations were measured by time-resolved fluoroimmunoassay after hydrolysis and extraction in samples from 85 middle-aged subjects who were part of a 12-week dietary intervention study carried out in North Karelia, Finland. In the baseline and the switchback periods, the subjects consumed their habitual Finnish diet, which is high in saturated fat and low in polyunsaturated fat and vegetables. During the 12-week intervention period, the proportion of dietary energy derived from fat was reduced from ~39% to 23%, and the consumption of vegetables, fruit, and berries was markedly increased. Enterolactone concentrations were measured during the baseline, intervention, and switchback periods. The median concentration of enterolactone rose from 12.2 to 19.5 nmol/l ($P = 0.002$) during the low-fat, high-vegetable diet. Daidzein and genistein concentrations were very low and did not change during the intervention. At baseline, 65% of the population had a low serum enterolactone concentration of <15 nmol/l. During the intervention period, this proportion fell to 34%. These major differences in serum enterolactone concentrations suggest that enterolactone may be used as a biomarker of a healthy diet containing plenty of vegetables, fruit, and berries.

Introduction

Several migrant studies point out the importance of environmental factors in the development of many types of cancer, including breast and prostate cancers (1–3). There is epidemi-

ological evidence that the intake of vegetables, particularly green and yellow ones, fruit and berries, legumes (4–6), and whole-grain cereals (7) is inversely associated with an increased risk of these major hormone-dependent cancers. The mechanisms by which these food items reduce the risk of the diseases are still unclear. Among many other important micronutrients, they contain plenty of hormone-like phytoestrogens. Phytoestrogens comprise several classes of chemical compounds including lignans and isoflavonoids. These compounds have been shown to influence not only estrogen metabolism and activity, but also protein synthesis, growth factor action, angiogenesis, and malignant cell differentiation and proliferation (8). A high intake of phytoestrogens has been postulated to provide protection from many chronic diseases, and a high serum concentration or urinary excretion of phytoestrogens has been associated with a decreased risk of breast cancer (9–11) as well as coronary heart disease (12).

Quite recently, convenient and inexpensive methods based on time-resolved immunofluorescence for the assay of enterolactone, daidzein, and genistein in plasma (13–15) and urine (16) have been developed. To investigate whether the assays could be used in dietary intervention studies, we used material from a study carried out in North Karelia, Finland, in 1983. The present study was done on preserved serum samples from an intervention study aimed at reducing serum cholesterol and blood pressure (17, 18). The purpose of the study was to investigate the effect of major changes in diet on serum phytoestrogens in a free-living population.

Materials and Methods

Participants. The dietary intervention study was carried out in two semi-rural communities (Kitee and Tohmajärvi) in North Karelia, Finland, in the spring of 1983. The aim of the study was to investigate the effects of dietary modification on blood pressure and the serum lipids (17, 18). The participating families had been identified initially through the countrywide hypertension register or through local risk-factor screenings (17). Persons ages 35 to 49 years, with known borderline or mild hypertension, and their spouses were invited to participate. Couples who volunteered and gave their written, informed consent filled in a questionnaire and, if no exclusion criteria emerged, underwent a medical examination. Finally, 43 families were included. One person with major health problems was excluded during the study. Thus, the final study group comprised 85 middle-aged subjects who had no health problems and were not under antihypertensive treatment.

Diets. The subjects underwent a baseline period of 2 weeks, a 12-week intervention period, and a 5-week switchback period (17, 18). During the baseline and switchback periods, all of the families were asked to eat their usual diets. The local North Karelian diet used to be high in saturated fat derived from whole milk products and meat, and low in polyunsaturated fat and vegetables. After the baseline period, the families changed

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their diet for a 12-week intervention period so that the proportion of energy derived from fats was reduced from 39 to 23%. The families were randomly divided into two groups, and the polyunsaturated/saturated fatty acid (P:S) ratio was increased from 0.2 to 0.9 in group I and from 0.2 to 1.2 in group II. The difference in the P:S ratio was achieved by providing the families with different fat spreads. Group I received margarine high in polyunsaturated fats, and group II received a mixture of butter and margarine. Despite the fat reduction, the total calorie intake was kept at almost the same level, the average being 2600, 2300, and 2800 kcal for each period, by increasing carbohydrate and protein intake (18). Strategic food items of the intervention period were skim milk, lean meat, and low-fat sausages and cheese. The subjects were encouraged to have a higher consumption of grain products as a carbohydrate source, as well as vegetables, berries, and fruit. The strategic food items were provided free of charge. After the intervention period, the families changed back to consume their habitual diet, which they followed for 5 weeks.

The intervention diet was accomplished through intensive counseling by nutritionists. The nutritionists met with the subjects at the clinic when the subjects came in for study measurements and made home visits at least twice a week. The subjects kept a careful food consumption record for 7 days during the baseline, for 12 days during the intervention, and for 10 days during the switchback period. Food weights and volumes were measured, and the types of food and drink were described in detail. During the home visits the nutritionists brought the food items of strategic importance, advised the families in the practical management of their diets, and checked the food consumption records. The subjects were encouraged to use vegetables, fruits, and berries as much as possible. They were instructed to favor grain products not only as bread but also as porridge.

The study protocol concerning changes in foods was identical to an earlier study in the same area (19). The consumption of dairy products, cereal products, meat, and eggs did not change significantly, whereas the consumption of vegetables, fruit and berries, fish, and low-fat cheese increased.

Methods. A fasting venous blood sample was taken at the end of each period and in the middle of the intervention period. The lipid analyses were carried out in 1983. Separate serum samples were collected for hormone assays, but only those in one region were originally used for this purpose and the results were published separately (20). The rest of these samples were stored unfrozen at -20°C until their analysis for phytoestrogens. The analytical method used is based on time-resolved immunofluorescence. The methods for enterolactone (13, 14), daidzein and genistein (15) have been published recently.

Enterolactone concentrations were measured in all 85 subjects in all four samples, except for one missing sample in the switchback period. Daidzein and genistein concentrations were measured in the samples collected during the baseline period and after 12 weeks of intervention.

Briefly, the method used is as follows. The serum samples (200 μl) were diluted with 200 μl of hydrolysis reagent containing 0.1 M acetate buffer (pH 5), sulfatase 2 units/ml, and β -glucuronidase 0.2 units/ml. The samples were hydrolyzed overnight. After hydrolysis, free phytoestrogens and the hydrolyzed conjugates were extracted twice with 1.5 ml of diethyl ether. Diethyl ether was evaporated to dryness, and the dry residues were measured by fluoroimmunoassays using the VICTOR 1420 multilabel counter and the DELFIA platewasher and plateshaker (Wallac, Turku, Finland). For the final assay, ex-

tracts corresponding to 20 μl of serum were used. The antisera used were prepared by coupling BSA to 5'-carboxymethyl ether of enterolactone or to 4'-*O*-carboxymethyl daidzein or genistein before immunization in rabbits. The fluorescence label was europium coupled to the same synthesized derivative of the compounds as used for preparation of the antisera. The analyses were performed in antirabbit microtitration strips. All of the samples were analyzed in duplicate, and all of the batches of samples were analyzed with two quality control serum samples going through the whole method and three controlling the immunoassay step only. The interassay CV³ for enterolactone were 9.4 and 11.8% in the concentrations of 16.0 and 32.3 nmol/l, respectively. The mean intra-assay CV and the concentrations were 11.4% (10.9 nmol/l) and 9.3% (78.3 nmol/l). Because the traditional Finnish diet does not contain any good sources of isoflavonoids (any soy products), the concentrations of daidzein and genistein were very low, with most of them being below the lowest point of the standard curve (1.0 nmol/l for daidzein and 1.5 nmol/l for genistein). To be able to measure the concentrations, the volume of the buffer added to the samples after extraction was only one third of the original sample volume. As a result, concentrations of the compounds were measurable from all samples. The interassay CV and control sample concentrations for daidzein were 11.2% (12 nmol/l) and 18.8% (294 nmol/l); for genistein they were 15.2% (20 nmol/l) and 4.5% (680 nmol/l). The mean intra-assay CVs for daidzein and genistein were 8.4% and 6.4%, respectively.

Statistical analyses were accomplished using one-way ANOVA. Logarithms of the values were used. Post Hoc Test LSD (least significant difference) was used to determine which means differed from each other. The differences were considered different at $P < 0.05$. The statistical analyses were performed using SPSS package program version 9.0 (SPSS, Inc., Chicago, IL).

Results

The median concentration of enterolactone rose from 12.2 (95% CI, 10.4–19.3) nmol/l to 19.5 (16.1–31.5) nmol/l ($P = 0.002$), 60%, during the intervention period compared with the baseline, and it was highest at the end of the intervention period. After the 6-week switchback period, the concentrations had decreased only a little, to 17.6 (95% CI, 14.6–26.5) nmol/l ($P = 0.18$). The range of the concentrations was wide, from 0.9 to 85.2 nmol/l. The medians and the CIs of serum enterolactone concentrations at the end of each period and in the middle of the intervention period are presented in Table 1.

The distribution of enterolactone concentrations at the baseline and during and after intervention is seen in Fig. 1. Almost two thirds of the subjects (65%) had concentrations <15 nmol/l, and only 11% had concentrations >30 nmol/l before the intervention. After intervention only one third (34%) of the population had an enterolactone concentration <15 nmol/l and one third (33%) had a concentration of >30 nmol/l.

The median daidzein and genistein concentrations did not change during the intervention. The median daidzein concentrations in the baseline period and after 12 weeks of intervention were 1.21 (95% CI, 1.04–4.14) and 1.41 (1.32–3.03) nmol/l ($P = 0.2$), and the median genistein concentrations were 1.48 (0.93–2.58) and 1.51 (1.16–2.18) nmol/l ($P = 0.3$), respectively. Despite the very low median concentrations, the

³ The abbreviations used are: CV, coefficient(s) of variation; CI, confidence interval.

Table 1 Medians and 95% CIs of serum enterolactone, daidzein, and genistein in the baseline, intervention, and switchback periods

	Baseline period	Intervention		Switchback period
	Median (95% CI)	6 wk	12 wk	
Enterolactone, nmol/l	12.2 (10.4–19.3)	17.2 (13.9–27.2) ^a	19.5 (16.1–31.5) ^b	17.6 (14.2–26.5)
Daidzein, nmol/l	1.5 (1.0–4.1)	ND ^c	1.5 (1.3–3.0)	ND
Genistein, nmol/l	1.2 (0.9–2.6)	ND	1.4 (1.2–2.2)	ND

^a $P = 0.03$, compared with the baseline.

^b $P = 0.002$, compared with the baseline.

^c ND, not determined.

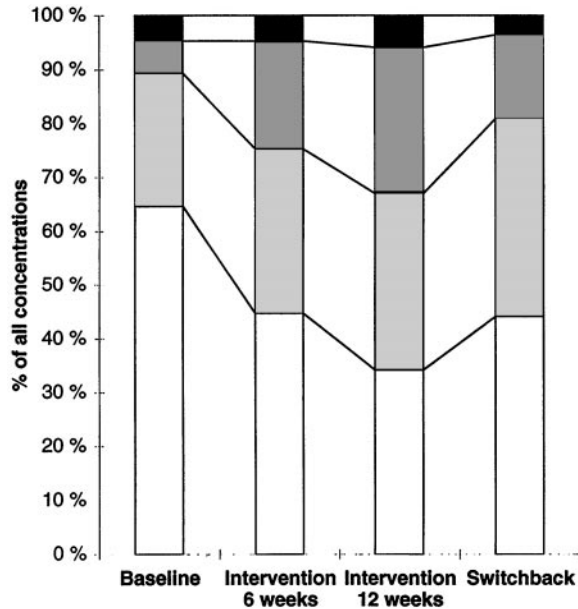


Fig. 1. Distribution of enterolactone concentrations in percentages before, during, and after a dietary intervention in North Karelia, Finland, in 1983 (3). ■, >45 nmol/l; ■, 30–44.9 nmol/l; ■, 15–29.9 nmol/l; □, 15 nmol/l.

variations of daidzein and genistein concentrations were wide, up to almost 60 nmol/l.

Discussion

The subjects' diets during the intervention period contained more vegetables, berries, and fruit than their habitual one, whereas the intake of grain products remained about the same (19). The fat intake was also lower. These dietary changes were clearly reflected in the serum concentration of enterolactone. Good sources of enterolactone precursors are various grains, nuts, seeds, berries, legumes, and some vegetables (*e.g.*, carrots, broccoli; Refs. 8, 21). The rise in the concentrations of enterolactone after a higher consumption of vegetables, fruit, and berries during the intervention period is in agreement with previous studies on the relation between dietary fiber from vegetables, fruit and berries, or legumes and the urinary excretion of enterolactone (22). Recently, Hutchins *et al.* (23) showed that, in experimental conditions, lignan excretion is increased in a dose-dependent manner after consumption of vegetables. In addition, in a study by Lampe *et al.* (24), there was a correlation between total vegetable and fruit intake and urinary excretion of enterolactone.

This is the first population-based intervention study on

lignans, which indicates that such dietary modifications resulting in higher serum enterolactone concentrations are possible to achieve in free-living, ordinary people. The earlier studies were based on 72-h urine samples. A serum sample is much more convenient to collect compared with 24- or 72-h urine samples. It seems, however, that the differences in lignan intake can be seen in serum, as well.

The diet the subjects consumed during the intervention period contained less fat [23% of energy supplied by fat, compared with their habitual diet (39%)]. It has been shown in rats that the absorption of lignans from the intestine is decreased if the intake of fat is increased.⁴ As far as we know there have been no such studies conducted in humans. In the present study, it is impossible to separate the effects of the increased intake of vegetables, fruit, and berries from those of decreased fat intake during the intervention period on serum enterolactone concentrations. Additional studies are needed to determine the role of fat in lignan metabolism.

Daidzein and genistein are found in large amounts especially in soybeans and processed soy products, as well as in smaller amounts in some other legumes (8, 25). The isoflavones are good markers for a soy diet (26–28), but in this population, soy does not belong to the habitual diet. This can be seen in the very low serum concentrations of daidzein and genistein in Finns in both this and previous studies (29, 30).

Despite the increased intake of lignans, almost half of the study population after 6 weeks of intervention and a third of the population after 12 weeks of intervention had a low serum enterolactone concentration of <15 nmol/l. There could be several reasons for this. Enterolactone concentrations in serum reflect the intake of its precursors over the several days before, and it is possible that some subjects did not follow the instructions every day or that they adopted the intervention diet progressively during the study. However, these explanations seem unlikely, because the changes in the average composition of the diets (18), as well as the changes in blood lipids (17) and blood pressure levels (18), had occurred already after 6 weeks of intervention. Another possibility is that their intestinal microflora could not produce enterolactone from its precursors because of a recent intake of antibiotics (31) or because of a different or less abundant microflora. We have found differences of up to about 10-fold between subjects in their capabilities to produce enterolactone from the same amount of rye bread precursors.⁵ It is likely that insufficient microflora is the reason why many subjects have very low serum values of enterolactone. The ability of the microflora to produce en-

⁴ Hallmans, G., Zhang, J.-X., Lundin, E., Bergh, A., Sylvan, A., Bylund, A., Widmark, A., Stattin, P., Damber, J.-E., Åman, P., Johansson, A., and Adlercreutz, H. Results presented at The International Conference on Diet and Prevention of Cancer May 28–June 2, 1999, in Tampere, Finland.

⁵ H. Adlercreutz, personal communication.

terolactone after a standard meal high in lignans has been shown to increase after 1 week of high lignan consumption (32). Occasional intake of whole-grain bread or another good source of enterolactone does not influence the enterolactone level in the body to any great extent because the change in the composition of the intestinal microflora as seen after the regular intake of fiber-rich grain products (33) has not occurred. In the present study, the enterolactone serum concentrations were highest at the end of the intervention period, indicating that the adaptation of microflora takes at least 3 months.

We conclude that enterolactone may be a good biomarker of a healthy diet, reflecting major changes in the diet of Western populations. A high serum enterolactone concentration indicates a regular and plentiful intake of whole-grain products, vegetables, fruit, and berries. The rise seen in serum enterolactone concentrations is associated with beneficial changes in blood lipid profile (17) as well as reduced blood pressure levels (18). The dietary changes resulting in increased enterolactone production are possible to achieve in ordinary, free-living families.

References

- Stanford, J. L., Herrinton, L. J., Schwartz, S. M., and Weiss, N. S. Breast cancer incidence in Asian migrants to the United States and their descendants. *Epidemiology*, 6: 181–183, 1995.
- Ziegler, R. G., Hoover, R. N., Pike, M. C., Hildesheim, A., Nomura, A. M. Y., West, D. W., Wu-Williams, A. H., Kolonel, L. N., Horn-Ross, P. L., Rosenthal, J. F., and Hyer, M. B. Migration patterns and breast cancer risk in Asian-American women. *J. Nat. Cancer Inst.*, 85: 1819–1827, 1993.
- Shimizu, H., Ross, R. K., Bernstein, L., Yatani, R., Henderson, B. E., and Mack, T. M. Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. *Br. J. Cancer*, 63: 963–966, 1991.
- Potter, J. D., and Steinmetz, K. Vegetables, fruit and phytoestrogens as preventive agents. In: B. W. Steward, D. McGregor, and P. Kleihues (eds.), *Principles of Chemoprevention*, pp. 61–90. Lyon, France: IARC, 1996.
- Block, G., Patterson, B., and Subar, A. Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer*, 18: 1–29, 1992.
- Wu, A. H., Ziegler, R. G., Horn-Ross, P. L., Nomura, A. M. Y., West, D. W., Kolonel, L. N., Rosenthal, J. F., Hoover, R. N., and Pike, M. C. Tofu and risk of breast cancer in Asian-Americans. *Cancer Epidemiol. Biomark. Prev.*, 5: 901–906, 1996.
- Jacobs, D. R., Marquart, L., Slavin, J., and Kushi, L. H. Whole-grain intake and cancer: An expanded review and meta-analysis. *Nutr. Cancer (Phila.)*, 30: 85–96, 1998.
- Adlercreutz, H., and Mazur, W. Phyto-oestrogens and Western diseases. *Ann. Med.*, 29: 95–120, 1997.
- Ingram, D., Sanders, K., Kolybaba, M., and Lopez, D. Case-control study of phyto-estrogens and breast cancer. *Lancet*, 350: 990–994, 1997.
- Adlercreutz, H., Heikkinen, R., Woods, M., Fotsis, T., Dwyer, J. T., Goldin, B. R., and Gorbach, S. L. Excretion of the lignans enterolactone and enterodiol and of equol in omnivorous and vegetarian postmenopausal women and in women with breast cancer. *Lancet*, 11: 1295–1299, 1982.
- Zheng, W., Dai, Q., Custer, L. J., Shu, X., Wen, W., Jin, F., and Franke, A. A. Urinary excretion of isoflavonoids and the risk of breast cancer. *Cancer Epidemiol. Biomark. Prev.*, 8: 35–40, 1999.
- Vanharanta, M., Voutilainen, S., Lakka, T., van der Lee, M., Adlercreutz, H., and Salonen, J. Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study. *Lancet*, 354: 2112–2115, 1999.
- Adlercreutz, H., Wang, G., Lapcik, O., Hampl, R., Wähälä, K., Mäkelä, T., Lusa, K., Talme, M., and Mikola, H. Time-resolved fluoroimmunoassay for plasma enterolactone. *Anal. Biochem.*, 265: 208–215, 1998.
- Stumpf, K., Uehara, M., Nurmi, T., and Adlercreutz, H. Changes in the time-resolved fluoroimmunoassay for plasma enterolactone. *Anal. Biochem.*, 278: 1153–1157, 2000.
- Wang, G., Lapcik, O., Hampl, R., Uehara, M., Al-Maharik, N., Stumpf, K., Mikola, H., Wähälä, K., and Adlercreutz, H. Time-resolved fluoroimmunoassay of plasma daidzein and genistein. *Steroids*, 65: 339–378, 2000.
- Uehara, M., Lapcik, O., Hampl, R., Micliola, H., Al-Maharik, N., Mäkelä, T., Wähälä, K., and Adlercreutz, H. Rapid analysis of phytoestrogens in human urine by time-resolved fluoroimmunoassay. *J. Steroid Biochem. Mol. Biol.*, 72: 273–282, 2000.
- Kuusi, T., Ehnholm, C., Huttunen, J. K., Kostiaainen, E., Pietinen, P., Leino, U., Uusitalo, U., Nikkari, T., Iacono, J. M., and Puska, P. Concentration and composition of serum lipoproteins during a low-fat diet at two levels of polyunsaturated fat. *J. Lipid Res.*, 26: 360–367, 1985.
- Puska, P., Iacono, J. M., Nissinen, A., Vartiainen, E., Dougherty, R., Pietinen, P., Leino, U., Uusitalo, U., Kuusi, T., Kostiaainen, E., Nikkari, T., Seppälä, E., Vapaatalo, H., and Huttunen, J. K. Dietary fat and blood pressure: an intervention study on the effects of a low-fat diet with two levels of polyunsaturated fat. *Prev. Med.*, 14: 573–584, 1985.
- Pietinen, P., Dougherty, R., Mutanen, M., Leino, U., Moisio, S., Iacono, J., and Puska, P. Dietary intervention study among 30 free-living families in Finland. *J. Am. Diet. Assoc.*, 84: 313–318, 1984.
- Hämäläinen, E., Adlercreutz, H., Puska, P., and Pietinen, P. Diet and serum sex hormones in healthy men. *J. Steroid Biochem.*, 20: 449–464, 1984.
- Mazur, W. Phytoestrogen content in foods. *Bailliere's Clin. Endocrinol. Metab.* 12: 723–742, 1998.
- Adlercreutz, H., Höckerstedt, K., Bannwart, C., Bloigu, S., Hämäläinen, E., Fotsis, T., and Ollus, A. Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). *J. Steroid Biochem.*, 27: 1135–1144, 1987.
- Hutchins, A. M., Lampe, J. W., Martini, M. C., Campbell, D. R., and Slavin, J. L. Vegetables, fruits, and legumes: effect on urinary isoflavonoid phytoestrogen and lignan excretion. *J. Am. Diet. Assoc.*, 95: 769–774, 1995.
- Lampe, J., Gustafson, D., Hutchins, A., Martini, M., Li, S., Wähälä, K., Grandits, G., Potter, J., and Slavin, J. Urinary isoflavonoid and lignan excretion on a Western diet: relation to soy, vegetable, and fruit intake. *Cancer Epidemiol. Biomark. Prev.*, 8: 699–707, 1999.
- Mazur, W., Duke, J. A., Wähälä, K., Rasku, R., and Adlercreutz, H. Isoflavonoids and lignans in legumes: nutritional and health aspects in humans. *J. Nutr. Biochem.*, 9: 193–200, 1998.
- Adlercreutz, H., Honjo, H., Higashi, A., Fotsis, T., Hämäläinen, E., Hasegawa, T., and Okada, H. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am. J. Clin. Nutr.*, 54: 1093–1100, 1991.
- Karr, S., Lampe, J. W., Hutchins, A. M., and Slavin, J. L. Urinary isoflavonoid excretion in humans is dose-dependent at low to moderate levels of soy-protein consumption. *Am. J. Clin. Nutr.*, 66: 46–51, 1997.
- Seow, A., Shi, C-H., Franke, A. A., Hankin, J. H., Lee, H-P., and Yu, M. C. Isoflavonoid levels in spot urine are associated with frequency of dietary soy intake in a population-based sample of middle-aged and older Chinese in Singapore. *Cancer Epidemiol. Biomark. Prev.*, 7: 135–140, 1998.
- Adlercreutz, H., Markkanen, H., and Watanabe, S. Plasma concentrations of phyto-oestrogens in Japanese men. *Lancet*, 342: 1209–1210, 1993.
- Adlercreutz, H., Fotsis, T., Watanabe, S., Lampe, J., Wähälä, K., Mäkelä, T., and Hase, T. Determination of lignans and isoflavonoids in plasma by isotope dilution gas chromatography-mass spectrometry. *Cancer Detec. Prev.*, 18: 159–171, 1994.
- Adlercreutz, H., Fotsis, T., Bannwart, C., Wähälä, K., Mäkelä, T., Brunow, G., and Hase, T. Determination of phytoestrogen metabolites, potential antiestrogens and anticarcinogens, in urine of women on various habitual diets. *J. Steroid Biochem.*, 25: 791–797, 1986.
- Nesbitt, P., Lam, Y., and Thompson, L. Human metabolism of mammalian lignan precursors in raw and processed flaxseed. *Am. J. Clin. Nutr.* 69: 549–555, 1999.
- Cummings, J., and Englyst, H. Gastrointestinal effects of food carbohydrate. *Am. J. Clin. Nutr.*, 61 (Suppl.): 938S–945S, 1995.