SPECIAL GUEST EDITOR SECTION

Assessing Exposure to Lignans and Their Metabolites in Humans

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Phytoestrogens occur naturally in plants and are structurally similar to mammalian estrogens. The lignans are a class of phytoestrogen and can be metabolized to the biologically active enterolignans, enterodiol, and enterolactone by a consortium of intestinal bacteria. Secoisolariciresinol diglucoside (SDG), a plant lignan, is metabolized to enterodiol and, subsequently, enterolactone. Matairesinol, another plant lignan, is metabolized to enterolactone. Other dietary enterolignan precursors include lariciresinol, pinoresinol, syringaresinol, arctigenin, and sesamin. Enterolignan exposure is determined in part by intake of these precursors, gut bacterial activity, and host conjugating enzyme activity. A single SDG dose results in enterolignan appearance in plasma 8–10 h later—a timeframe associated with colonic bacterial metabolism and absorption. Conjugation of enterolignans with sulfate and glucuronic acid occurs in the intestinal wall and liver, with the predominant conjugates being glucuronides. Controlled feeding studies have demonstrated dose-dependent urinary lignan excretion in response to flaxseed consumption (a source of SDG); however, even in the context of controlled studies, there is substantial interindividual variation in plasma concentrations and urinary excretion of enterolignans. The complex interaction between colonic environment and external and internal factors that modulate it likely contribute to this variation. Knowledge of this field, to date, indicates that understanding the sources of variation and measuring the relevant panel of compounds are important in order to use these measures effectively in evaluating the impact of lignans on human health.

Phytoestrogens such as the isoflavones and lignans are naturally occurring compounds found in plants. They are structurally similar to estrogens, can bind to estrogen receptors, and elicit a weak estrogenic response. The relationship between phytoestrogen exposure and disease risk has received substantial attention in the last decade. However, measuring exposure to phytoestrogens in human populations remains a challenging task. Many studies have focused on soy isoflavones, but data from observational and intervention studies also support a potential effect of lignan intake on human health. As such, reliable methods for measuring exposure to lignans are needed if we are to assess their safety and efficacy in humans.

Lignans are biologically active compounds with properties and activities encompassing many areas, including antioxidant, antitumor, weak estrogenic, and antiestrogenic properties, and inhibition of enzymes involved in hormone metabolism (reviewed in ref. 1). Observational and intervention studies in humans have suggested that lignan consumption is associated with favorable effects on hormone levels and metabolism, and reductions in risk of cardiovascular disease, osteoporosis, diabetes, and renal disease (2–9). Inverse associations between urine or serum levels or dietary intakes of lignans and breast cancer risk also have been reported in some (10–14), but not all (15–17), studies. Consumption of plant lignans also has been assessed in relation to other cancers, and inverse associations have been reported between lignan consumption and cancers of the endometrium, ovaries, and thyroid (18–20).

Plant lignans, such as secoisolariciresinol diglucoside (SDG) and matairesinol (MAT), are metabolized by intestinal bacteria to the enterolignans (also known as mammalian lignans) enterodiol (END) and enterolactone (ENL). END can be further metabolized to ENL. Wide interindividual differences in lignan metabolism have been reported (6, 21–25), but the reasons for such variation and the ultimate impact this may have on human health have not been fully evaluated. The complex interaction between the colonic environment and external and internal factors that modulate it likely contribute to interindividual variation in lignan metabolism. Here, we outline the dietary sources of plant lignans, describe the intestinal bacterial and human metabolism of lignans, and discuss factors that influence lignan metabolism and exposure in humans. Current knowledge of this field indicates that understanding the sources of variation and measuring the relevant panel of

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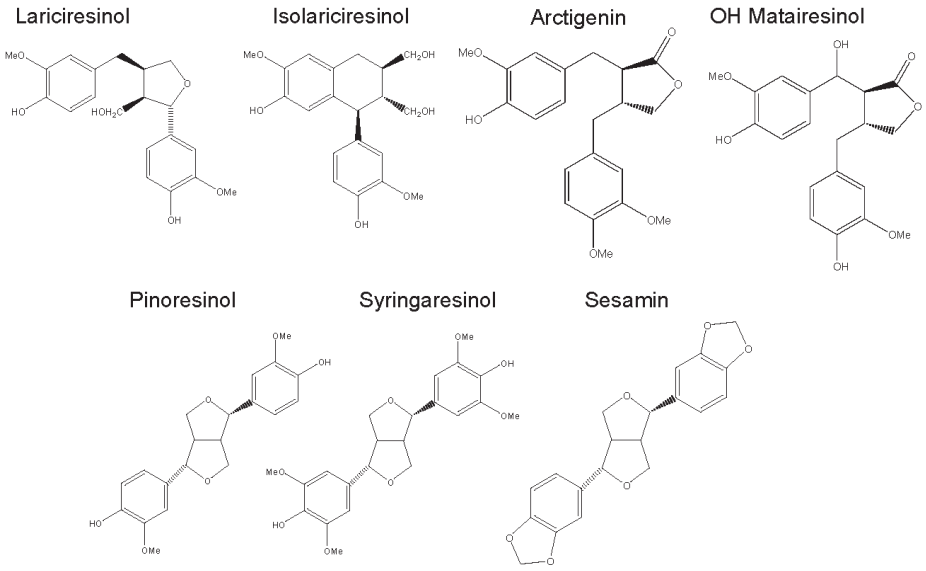

Figure 1. Plant lignan structures.

compounds are important in order to use these measures effectively in evaluating the impact of lignans on human health.

Dietary Sources of Plant Lignans

The lignans are a group of diphenolic compounds produced by plants (Figure 1). Many of these are found in measurable quantities in a wide variety of plant foods. The oilseeds (e.g., flax, soy, rapeseed, and sesame); whole-grain cereals (wheat, oats, and rye); legumes; and various vegetables and fruit (particularly berries) are rich sources of lignans (26–28). The most concentrated sources of ligans known are flaxseed and sesame seed (27–30). To date, much of the focus has been on the secoisolariciresinol (SECO) and MAT content of plant foods. In most plant foods, SDG is present in higher amounts than MAT (28). Several lignan databases have been developed based on the SECO and MAT content of commonly consumed foods and are used to estimate lignan exposure from food frequency questionnaires or food records in population-based studies (31–33).

Recently, Milder and colleagues (34) expanded the lignan database by measuring lariciresinol and pinoresinol in addition to SECO and MAT in a variety of plant foods commonly consumed in The Netherlands. They reported that 105 of 109 foods tested contained measurable amounts of lignans; lignan content ranged from 0–301 000 μg/100 g fresh weight. Importantly, in almost all products, lariciresinol and pinoresinol were in higher concentrations than SECO and MAT. Application of the new database to the estimate of lignan intake in a sample of Dutch men and women showed that lariciresinol and pinoresinol contributed to 75% of lignan intake, whereas SECO and MAT accounted for only 25% (35). These data suggest that studies that have relied solely on the SECO and MAT content of plant foods as an indicator of lignan intake have substantially underestimated the plant lignan content of the human diet.

Intestinal Bacterial Metabolism of Plant Lignans

Lignans are present in plants primarily as glycosides and, upon ingestion, the sugar moieties are hydrolyzed to release the aglycones. Hydrolysis can be carried out by both bacterial β-glucosidases and β-glucosidases in the human gut mucosal brush border (36). The aglycones are absorbed or can be metabolized further by bacteria in the gut to the enterolignans. MAT and SECO can be dehydroxylated and demethylated to form ENL and END, respectively (Figure 2; 37). Bacterial oxidation of END also occurs to yield ENL but, while ENL can be reduced chemically to END, this reverse reaction does not appear to occur in vivo (38). The role of intestinal bacteria in the conversion of plant lignans to the metabolites found in mammals is well illustrated by the fact that germ-free animals do not produce END and ENL (39, 40), and that individuals without an intact colon have low plasma and urinary lignan levels (41). Furthermore, in vitro incubation of purified lignans or lignan-containing foods with human feces results in the production of END and ENL (26, 42, 43).

MAT and SECO were long assumed to be the major plant lignans that could be converted to END and ENL; however, more recently several studies have shown the capacity of gut bacteria to convert other plant lignans to enterolignans (37, 44). Heinonen et al. (37) reported that plant lignans, including pinoresinol diglucoside, 7-hydroxymatairesinol, and lariciresinol, could be converted to END and ENL. The efficiency of conversion of plant lignan precursors to END and ENL in 24 h incubations with human fecal inocula varied greatly depending on the compound and ranged from 0 to 100% (Table 1). These data suggest that several plant lignans are important contributors to enterolignan exposure in humans and may help to explain the

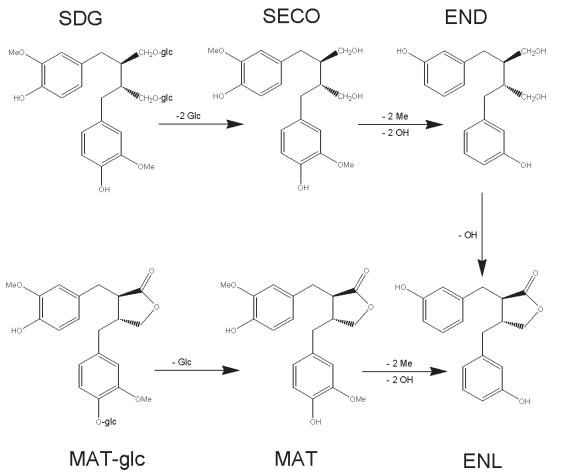

Figure 2. Bacterial conversion of secoisolariciresinol diglycoside (SDG) and matairesinol glycoside (MAT-glc) to enterodiol (END) and enterolactone (ENL).

poor correlations between estimates of lignan intakes as SECO and MAT and amounts of enterolignans excreted in urine.

Bacterial strain-specific metabolism of SDG suggests specialization within the gut microbial communities for different aspects of lignan metabolism. Fecal suspensions used in in vitro incubations are capable of converting SDG to ENL. The reactions involved in SDG metabolism to END include hydrolysis of the glucoside, followed by demethylation of the methoxy groups, and dehydroxylation (44, 45). Wang et al. (44) reported the isolation of *Peptostreptococcus* sp. SDG-1 and *Eubacterium* sp. SDG-2 that were responsible for SECO demethylation and dehydroxylation, respectively. In addition, dehydroxylation by *Eubacterium* sp. SDG-2 was found to be stereospecific for specific intermediates in SECO metabolism (44). Clavel et al. (45) identified *P. productus* SECO-Mt75m3 and *Eggerthella lenta* SECO-Mt75m2 as the demethylating and dehydroxylating strains. Strains of colonic bacteria have been isolated that transform both pinoresinol diglucoside and SDG to END by different pathways, although neither of these organisms metabolize SDG all the way to ENL (44). Furthermore, strains capable of metabolizing SDG do not necessarily perform similar metabolism of other plant lignans. For example, ENL is produced from arctiin by fecal suspensions in in vitro incubations (46). However, the *Peptostreptococcus* sp. SDG-1 and *Eubacterium* sp. SDG-2 that are responsible for SECO metabolism fail to metabolize intermediates in arctiin degradation. The conversion of END to ENL, compared to SECO to END, is associated with a less varied group of bacteria; individuals with higher ENL production had higher proportions of bacteria belonging to the *Atopobium* group and the species *P. productus* and *Clostridium coccoides*, whereas a discrete group of END-producing bacteria could not be identified by correlative analyses (45). Studies have reported that urinary excretion of ENL is very low or nonexistent in some study participants challenged with SDG (23, 24, 47); these results suggest that there may a subpopulation of individuals that lacks the bacteria or appropriate intestinal environment necessary for oxidation of END to ENL.

Sequencing and phylogenetic analysis of the 16S rRNA gene has lead to the discovery of a high diversity of gut bacteria in the absence of culturing (48–50). Complimentary gut community fingerprinting techniques, such as terminal restriction fragment length polymorphism (tRFLP) analysis (51), offer rapid methods to screen for interindividual differences in gut microbial community. We analyzed the fecal microbial community from 2 individuals before and after anaerobic incubation for 5 days with and without SDG in brain-heart infusion media at 37°C (Figure 3). Both individuals produced ENL from SDG. These data show distinct changes in the fecal microbial community composition after in vitro incubation from the initial communities (triangle versus diamonds and squares; Figure 3). The tRFLP peaks negatively correlated ($r > 0.5$) with Axis 1, and the fecal community prior to incubation are 78, 138, 220, and 258 bp (putative *Bacteroides*). In addition, differences developed between the communities from the 2 individuals after in vitro incubation (open and closed diamonds and squares). The tRFLP peaks positively correlated ($r > 0.5$) with Axis 1, and the fecal community after in vitro incubation were peaks 207 bp and 235 bp (putative *B. distasonis*). Peaks positively correlated with Axis 2 include 199, 297, and 305 bp (putative *Peptostreptococcus* sp.), and 376 bp. These findings are in agreement with others (44, 45) that showed a *Peptostreptococcus* sp. was involved in the biotransformation of SECO to intermediates in the metabolic pathway resulting in ENL production. These data also support the view that a microbial consortium, involving several different bacterial species, is required for biotransformation of SDG to ENL. Our preliminary data suggest that community analysis of fecal bacteria in conjunction with in vitro incubations will help identify members of a microbial

Table 1. In vitro conversion of plant lignans to enterodiol (END) and enterolactone (ENL) after 24 h incubation with human fecal microbiota[a]

Compound	Conversion to END, %	Conversion to ENL, %
Matairesinol	0	62
Secoisolariciresinol	50	21
Pinoresinol diglucoside	32	19
Syringaresinol diglucoside	0.4	3
Arctigenin glucoside	0	5.5
7-Hydroxymatairesinol	0.2	15
Isolariciresinol	0	0
Lariciresinol	55	46

[a] Adapted from Heinonen et al. (ref. 37).

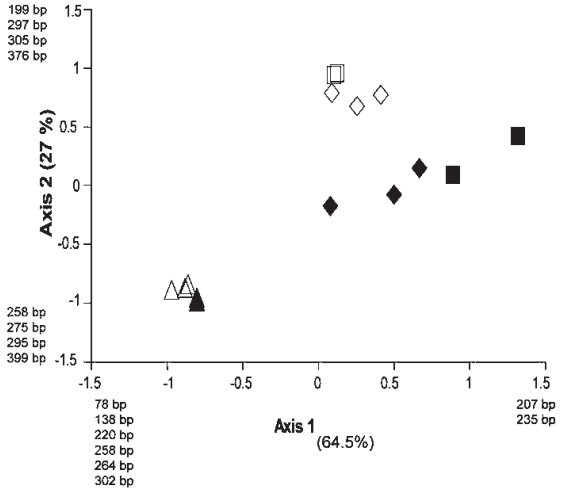

Figure 3. The change in the composition of the fecal microbial community before and after in vitro incubation with and without SDG. Axes 1 and 2 represent the multispecies fecal bacterial communities in multi-dimensional space. Open and closed symbols indicate the samples from 2 different ENL-producing participants. The initial communities are indicated by triangles. Diamonds indicate the microbial community that developed after 5 days of anaerobic incubation with SDG. Squares indicate the microbial community that developed after 5 days of anaerobic incubation without SDG. The tRFLP peaks, indicated in base pairs (bp), were significantly correlated with the axes (r > 0.5).

consortium responsible for the biotransformation of SDG to ENL and can also be used to characterize the interindividual variation in the human gut bacterial response to plant lignan exposure.

Human Biotransformation of Enterolignans

Once absorbed, lignans and their metabolites are efficiently conjugated either with glucuronic acid or, to a lesser extent, sulfate. Conjugation takes place in the intestinal epithelium and liver by UDP-glucuronosyltransferases (UGT) and sulfotransferases, and the conjugates are excreted in urine and bile. Those that are re-excreted through the bile duct are deconjugated by bacterial β-glucuronidase and can undergo enterohepatic recycling (38). The major proportion of lignans excreted in urine are conjugated; ENL and END are excreted primarily as monoglucuronides (95 and 85%, respectively), with small percentages being excreted as monosulfates (2–10%) and free aglycones (0.3–1%; 52). The impact of enterocyte efflux proteins–adenosine triphosphate (ATP)-dependent transporters that export a variety of conjugated and unconjugated compounds out of cells, such as multidrug resistance associated protein 2 (MRP2)–on END and ENL absorption has not been evaluated. Lignans, as well as other phytochemicals, can modulate MRPs (53) such that their own absorption may be affected, particularly at high doses.

Upon incubation with human liver microsomes, ENL and END can both undergo aliphatic and aromatic hydroxylation at various positions, producing an array of novel secondary oxidation products (54). However, only aromatic oxidation products of ENL and END have been identified in urine as conjugates and account for ≤5% of total lignans (55). This apparent minimal handling by Phase I enzymes has been proposed to be due to rapid glucuronidation in the gut epithelium (56) and the liver (57).

Measurement of Enterolignans in Biological Fluids and Tissues

Numerous analytical methods have been developed for the detection and quantification of plant lignans and enterolignans in biological fluids and tissues using a variety of chromatographic and nonchromatographic techniques. These include gas chromatography (GC) with mass spectrometry (MS), liquid chromatography (LC) with MS or tandem MS (MS/MS), LC with electrochemical detection or coularray, and radioimmunoassay or time-resolved fluoroimmunoassay (reviewed in refs. 58–60). As summarized by Kuijsten et al. (61), detection limits in serum or plasma have been reported for time-resolved fluoroimmunoassay of ENL (0.35 nM), for GC/MS of ENL and END (0.2–1.0 nM), for LC/MS for ENL and END (0.15–3.5 nM), and for LC-coularray (1.9–2.1 nM). Circulating concentrations of free, unconjugated enterolignans are low. Therefore, sample preparation methods routinely include enzymatic hydrolysis steps using β-glucuronidases and sulfatases to convert the conjugated enterolignans to the aglycones, such that "total" ENL and END are measured.

Enterolignans appear in circulation approximately 8–10 h after ingestion of plant lignans. With supplementation of pure SDG [1.31 μmol/kg body weight (bw)], END reached its maximum plasma concentration at 14.8 h and ENL reached its maximum at 19.7 h; mean residence time for END was 20.6 h and for ENL was 35.8 h; and maximum plasma concentrations for END and ENL were 73 and 56 nmol/L, respectively (47). Similarly, sesame seed supplementation (50 g) resulted in appearance of the enterolignans after 8 h, and plasma END and ENL concentrations at 24 h were 699 and 567 nmol/L, respectively (30). Despite the relatively long residence times, the high enterolignan concentrations that can be achieved with plant lignan supplementation, and the sensitive methods able to detect low concentrations of END and ENL, circulating concentrations reported in various populations are often quite low and may be lower than the quantification limit of the assay (Table 2). For example, in a sample of 193 men and women in Seattle, WA, Horner et al. (65) reported a mean ± standard deviation (SD) fasting ENL concentration of 24.7 ± 26.1 nmol/L, with 17% of the sample having concentrations below 1.2 nmol/L (the quantification limit of the assay). The biological significance of these relatively low concentrations has not been fully explored in humans. In estrogen-dependent (MCF-7) human breast cancer cells, phytoestrogens,

including ENL, inhibited DNA synthesis at high concentrations (20–90 μM) but induced DNA synthesis at low concentrations (0.1–10 μM), suggesting that, at concentrations generally reported in humans, enterolignans may stimulate, rather than inhibit, cell growth (59, 67). Few studies have assessed binding affinities of enterolignans for estrogen receptors (ERs) α and β but, in one study, the IC_{50} (the ligand concentration yielding 50% inhibition of binding of fluorescein-labeled estradiol to ER) of ENL was 6.7 ± 4.3 μM and 39 ± 22 μM for ERα and ERβ, respectively, and relative binding affinities (relative to diethylstilbestrol, which was set to 100) were 0.07 and 0.01, respectively (68).

In contrast to the enterolignans, plant lignans reach their maximal blood concentrations within 2 h after supplementation and have elimination half-lives ranging from 2 to 6 h (30). Circulating plant lignan concentrations also are relatively low compared to the enterolignans produced from them, and levels are often undetectable in samples collected under habitual dietary conditions (30). Urine collections over 24 h may be more useful; excretion of a panel of 6 plant lignans was in the range of 0.2 to 3 μmol/day (69). Nonetheless, measurement of the plant lignans, without the enterolignans, would not provide a comprehensive measure of overall lignan exposure.

Factors Influencing Availability and Microbial Metabolism of Lignans

Using data from observational studies, investigators have attempted to identify the factors that contribute to circulating or urinary levels of END and ENL. Intake of whole grains, berries, and other plant foods high in dietary fiber or lignan-precursors are often associated with serum ENL concentrations; however, usually only a small amount of the variance can be explained by the dietary, physiologic, and lifestyle factors evaluated. For example, in Finnish men, intake of whole-grain products and fruits and berries and reports of constipation explained 3% of the variation, whereas in Finnish women, body mass index (BMI), smoking, age, constipation, and intake of vegetables explained 14% of the variation (63). Serum ENL was also correlated with dietary fiber intake in women in the United Kingdom, but only accounted for 5% of the variance (66). Among American men and women, age, sex, BMI, and intakes of dietary fiber, alcohol, and caffeine explained 22% of the variation (65).

Several factors can influence gut microbial community composition and enzyme activity which, in turn, could account for some of the observed interindividual differences in lignan metabolism in humans. For example, diet can influence the gut habitat characteristics, such as pH and redox potentials (70), which could influence the location, composition, and activity of the gut microbiota. In a feeding study (71), the bacterial community composition and extracellular enzyme activity were altered by the consumption of different oligosaccharides in the control diet. Thus, intake of various sources of dietary fiber, irrespective of their lignan content, may influence production of enterolignans via effects on the composition and activity of the gut microbiota. Use of oral antimicrobials also alters the gut microbial community. Kilkkinen et al. (72) reported that serum ENL concentrations were significantly lower in individuals who used oral antimicrobials up to 12–16 months before serum sampling than in nonusers. ENL concentrations were associated with the number of treatments and the time from last treatment.

Transit time of material through the large intestine is an important factor affecting the availability of dietary components to the host, primarily because colonic bacterial fermentation can influence circulating concentrations of compounds produced by colonic bacteria (37, 44). Gut transit time influences the amount and composition of the metabolites produced in the lumen by affecting where the fermentation takes place, the availability of substrate, the

Table 2. Plasma or serum concentrations of total (i.e., after enzymatic deconjugation) enterolignans among humans consuming their habitual diet; data are from observational studies or baseline measures from intervention trials

Reference	Population	Sample size	Enterodiol[a], nmol/L, range	Enterolactone[a], nmol/L, range
Stumpf et al. (62)	Finland	85 Men and women	—[b]	12.2
Kilkkinen et al. (63)	Finland	1168 Men and 1212 women	—	Men, 13.8 (0–95.6); women, 16.6 (0–182.6)
Hong et al. (64)	Korea	10 Men	7.5 (Mean)	27.4 (Mean)
Horner et al. (65)	Seattle, WA	193 Men and women	—	14.0 (Geometric mean); ND[c]–155
Grace et al. (66)	Norfolk, UK	284 Women	1.3 (Geometric mean); ND–26.8	12.8 (Geometric mean); ND–1302
Kuijsten et al. (61)	The Netherlands	807 Men and women	1.0	9.2

[a] Median, unless otherwise indicated.

[b] — = Not measured.

[c] ND = Not detected or below the quantification limit of the assay.

composition of the resident microbial community, and the efficiency of microbial metabolism. All of these factors potentially impact the availability of lignans that enter into enterohepatic circulation and undergo microbial metabolism to the enterolignans. Continuous culture systems inoculated with human fecal microbiota have been used to investigate the effects of transit time on biomass, metabolism, enzyme activity, and composition of the microbial communities (73). In a 3-stage reactor, when the retention time was lower (equivalent to faster gut transit), biomass increased and the minimum doubling time of bacterial cells decreased (73) in a manner similar to feeding studies that sped up gut transit time with fiber supplements (74). The observation from a population-based study that constipation is positively associated with serum ENL concentrations suggests that longer residence time in the colon allows for more extensive conversion of the plant lignans and END to ENL (63).

Sex differences in gut transit time influence colonic function and the composition of the microbial community inhabiting the gut ecosystem, which could affect lignan metabolism. In a controlled dietary fiber-feeding study, men, compared to women, had faster intestinal transit times and fermented less fiber (75). Sex steroid hormones alter the susceptibility and resistance of the host to different bacterial species (76, 77), and thereby may affect the composition of the gut microbial community. As such, differences in sex steroid hormones that influence the presence and activity of certain bacteria in the gut may influence lignan metabolism. Clavel et al. (45) reported that women had higher concentrations of both END- and ENL-producing organisms than did men, but this could not be explained by quantitative differences in dominant bacterial groups. In dietary intervention studies, sex differences in serum and urine ENL levels have been reported in some studies (45, 47, 78), but not in others (79).

Summary and Future Considerations

Plant lignans are metabolized by a consortium of intestinal bacteria to the enterolignans END and ENL. Substantial interindividual differences in their metabolism occur, even under controlled dietary conditions, but the reasons for such variation and the ultimate impact this may have on human health have not been determined. Studies available, to date, suggest that ENL is generally more effective than END in terms of, e.g., displacing estradiol and testosterone from sex steroid binding protein and inhibiting human aromatase in vitro (24). Furthermore, the overall level of lignan exposure that is important in relation to health and disease is not known. Improvements in the sensitivity of assays has furthered the use of enterolignans, particularly ENL, as biomarkers of dietary exposure; however, although several factors (e.g., diet, antibiotic use, and constipation) have been identified as contributing to circulating lignan concentrations, very little of the associated variance can be explained. Serum END concentrations are often very low, and in population-based studies are often below assay detection limits, which makes the task of evaluating the importance of one enterolignan over another (i.e., END vs ENL) all the more challenging.

In order to evaluate the safety and efficacy of lignans in humans, we need to better understand the factors that affect overall exposure to them, by: (*1*) establishing more comprehensive dietary databases to estimate plant lignan exposure in population-based studies; (*2*) identifying the variables that influence plant lignan metabolism by intestinal bacteria; (*3*) determining the biologic importance of the Phase I oxidative products relative to the parent enterolignans; (*4*) evaluating the impact of genetic polymorphisms in the Phase II enzymes and efflux proteins on elimination of enterolignans; and (*5*) developing approaches that integrate gut microbial metabolism with microbial fingerprinting techniques to elucidate the role of the gut bacterial community in lignan metabolism. This more comprehensive understanding will facilitate study of the impact of these compounds on human health and guide measurement strategies.

References

(1) Raffaelli, B., Hoikkala, A., Leppala, E., & Wähälä, K. (2002) *J. Chromatogr. B* **777**, 29–43

(2) Hallmans, G., Zhang, J.X., Lundin, E., Stattin, P., Johansson, A., Johansson, I., Hulten, K., Winkvist, A., Aman, P., Lenner, P., & Adlercreutz, H. (2003) *Proc. Nutr. Soc.* **62**, 193–199

(3) Brooks, J.D., Ward, W.E., Lewis, J.E., Hilditch, J., Nickell, L., Wong, E., & Thompson, L.U. (2004) *Am. J. Clin. Nutr.* **79**, 318–325

(4) Kim, M.K., Chung, B.C., Yu, V.Y., Nam, J.H., Lee, H.C., Huh, K.B., & Lim, S.K. (2002) *Clin. Endocrinol. (Oxf.)* **56**, 321–328

(5) de Kleijn, M.J., van der Schouw, Y.T., Wilson, P.W.F., Grobbee, D.E., & Jacques, P.F. (2002) *J. Nutr.* **132**, 276–282

(6) Hutchins, A.M., Martini, M.C., Olson, B.A., Thomas, W., & Slavin, J.L. (2001) *Nutr. Canc.* **39**, 58–65

(7) Haggans, C.J., Hutchins, A.M., Olson, B.A., Thomas, W., Martini, M.C., & Slavin, J.L. (1999) *Nutr. Canc.* **33**, 188–195

(8) Vanharanta, M., Voutilainen, S., Lakka, T.A., van der Lee, M., Adlercreutz, H., & Salonen, J.T. (1999) *Lancet* **354**, 2112–2115

(9) Velasquez, M.T., & Bhathena, S.J. (2001) *Am. J. Kidney Dis.* **37**, 1056–1068

(10) Ingram, D., Sanders, K., Kolybaba, M., & Lopez, D. (1997) *Lancet* **350**, 990–994

(11) McCann, S.E., Muti, P., Vito, D., Edge, S.B., Trevisan, M., & Freudenheim, J.L. (2004) *Int. J. Canc.* **111**, 440–443

(12) Linseisen, J., Piller, R., Hermann, S., & Chang-Claude, J. (2004) *Int. J. Canc.* **110**, 284–290

(13) Pietinen, P., Stumpf, K., Mannisto, S., Kataja, V., Uusitupa, M., & Adlercreutz, H. (2001) *Canc. Epidemiol. Biomarkers Prev.* **10**, 339–344

(14) Dai, Q., Franke, A.A., Jin, F., Shu, X.O., Hebert, J.R., Custer, L.J., Cheng, J., Gao, Y.T., & Zheng, W. (2002) *Canc. Epidemiol. Biomarkers Prev.* **11**, 815–821

(15) Kilkkinen, A., Virtamo, J., Vartiainen, E., Sankila, R., Virtanen, M.J., Adlercreutz, H., & Pietinen, P. (2004) *Int. J. Canc.* **108**, 277–280

(16) den Tonkelaar, I., Keinan-Boker, L., Veer, P.V., Arts, C.J., Adlercreutz, H., Thijssen, J.H., & Peeters, P.H. (2001) *Canc. Epidemiol. Biomarkers Prev.* **10**, 223–228

(17) Zeleniuch-Jacquotte, A., Adlercreutz, H., Akhmedkhanov, A., & Toniolo, P. (1998) *Canc. Epidemiol. Biomarkers Prev.* **7**, 885–889

(18) McCann, S.E., Freudenheim, J.L., Marshall, J.R., & Graham, S. (2003) *J. Nutr.* **133**, 1937–1942

(19) Horn-Ross, P.L., Hoggatt, K.J., & Lee, M.M. (2002) *Canc. Epidemiol. Biomarkers Prev.* **11**, 43–49

(20) Horn-Ross, P.L., John, E.M., Canchola, A.J., Stewart, S.L., & Lee, M.M. (2003) *J. Natl. Canc. Inst.* **95**, 1158–1164

(21) Adlercreutz, H., Fotsis, T., Heikkinen, R., Dwyer, J.T., Goldin, B.R., Gorbach, S.L., Lawson, A.M., & Setchell, K.D.R. (1981) *Med. Biol.* **59**, 259–261

(22) Adlercreutz, H., Fotsis, T., Bannwart, C., Wähälä, K., Mäkelä, T., Brunow, G., & Hase, T. (1986) *J. Steroid Biochem.* **25**, 791–797

(23) Lampe, J.W., Martini, M.C., Kurzer, M.S., Adlercreutz, H., & Slavin, J.L. (1994) *Am. J. Clin. Nutr.* **60**, 122–128

(24) Nesbitt, P.D., Lam, Y., & Thompson, L.U. (1999) *Am. J. Clin. Nutr.* **69**, 549–555

(25) Cunnane, S.C., Hamadeh, M.J., Liede, A.C., Thompson, L.U., Wolever, T.M.S., & Jenkins, D.J.A. (1995) *Am. J. Clin. Nutr.* **61**, 62–68

(26) Thompson, L.U., Robb, P., Serraino, M., & Cheung, F. (1991) *Nutr. Canc*. **16**, 43–52

(27) Mazur, W. (1998) *Baillieres Clin. Endocrinol. Metab.* **12**, 729–742

(28) Adlercreutz, H., & Mazur, W. (1997) *Ann. Med.* **29**, 95–120

(29) Meagher, L.P., Beecher, G.R., Flanagan, V.P., & Li, B.W. (1999) *J. Agric. Food Chem.* **47**, 3173–3180

(30) Peñalvo, J.L., Heinonen, S.M., Aura, A.M., & Adlercreutz, H. (2005) *J. Nutr.* **135**, 1056–1062

(31) Valsta, L.M., Kilkkinen, A., Mazur, W., Nurmi, T., Lampi, A.M., Ovaskainen, M.L., Korhonen, T., Adlercreutz, H., & Pietinen, P. (2003) *Br. J. Nutr.* **89**, S31–S38

(32) Horn-Ross, P.L., Barnes, S., Lee, M., Coward, L., Mandel, J.E., Koo, J., John, E.M., & Smith, M. (2000) *Canc. Causes Contr*. **11**, 289–298

(33) Boker, L.K., Van der Schouw, Y.T., De Kleijn, M.J., Jacques, P.F., Grobbee, D.E., & Peeters, P.H. (2002) *J. Nutr.* **132**, 1319–1328

(34) Milder, I.E., Arts, I.C., van de Putte, B., Venema, D.P., & Hollman, P.C. (2005) *Br. J. Nutr.* **93**, 393–402

(35) Milder, I.E., Feskens, E.J., Arts, I.C., de Mesquita, H.B., Hollman, P.C., & Kromhout, D. (2005) *J. Nutr.* **135**, 1202–1207

(36) Rowland, I., Faughnan, M., Hoey, L., Wähälä, K., Williamson, G., & Cassidy, A. (2003) *Br. J. Nutr.* **89**, S45–S58

(37) Heinonen, S., Nurmi, T., Liukkonen, K., Poutanen, K., Wähälä, K., Deyama, T., Nishibe, S., & Adlercreutz, H. (2001) *J. Agric. Food Chem.* **49**, 3178–3186

(38) Setchell, K.D.R., & Adlercreutz, H. (1988) in *Role of the Gut Flora in Toxicity and Cancer*, I. Rowland (Ed.), Academic Press, London, UK, pp 316–345

(39) Axelson, M., & Setchell, K.D.R. (1981) *FEBS Lett.* **123**, 337–342

(40) Bowey, E., Adlercreutz, H., & Rowland, I. (2003) *Food Chem. Toxicol.* **41**, 631–636

(41) Pettersson, D., Åman, P., Knudsen, E.B., Lundin, E., Zhang, J.-X., Hallmans, G., Härkönen, H., & Adlercreutz, H. (1996) *J. Nutr.* **126**, 1594–1600

(42) Rickard, S.E., Orcheson, L.J., Seidl, M.M., Luyengi, L., Fong, H.H.S., & Thompson, L.U. (1996) *J. Nutr.* **126**, 2012–2019

(43) Borriello, S.P., Setchell, K.D.R., Axelson, M., & Lawson, A.M. (1985) *J. Appl. Bacteriol.* **58**, 37–43

(44) Wang, L., Meselhy, M.R., Li, Y., Qin, G., & Hattori, M. (2000) *Chem. Pharm. Bull.* **48**, 1606–1610

(45) Clavel, T., Henderson, G., Alpert, C.A., Philippe, C., Rigottier-Gois, L., Dore, J., & Blaut, M. (2005) *Appl. Environ. Microbiol.* **71**, 6077–6085

(46) Xie, L.H., Ahn, E.M., Akao, T., Abdel-Hafez, A.A., Nakamura, N., & Hattori, M. (2003) *Chem. Pharm. Bull. (Tokyo)* **51**, 378–384

(47) Kuijsten, A., Arts, I.C., Vree, T.B., & Hollman, P.C. (2005) *J. Nutr.* **135**, 795–801

(48) Woese, C.R. (1987) *Microbiol. Rev.* **51**, 221–271

(49) O'Sullivan, D.J. (2000) *Curr. Issues Intest. Microbiol.* **1**, 39–50

(50) Backhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., & Gordon, J.I. (2005) *Science* **307**, 1915–1919

(51) Liu, W.T., Marsh, T.L., Cheng, H., & Forney, L.J. (1997) *Appl. Environ. Microbiol.* **63**, 4516–4522

(52) Adlercreutz, H., van der Wildt, J., Kinzel, J., Attalla, H., Wähälä, K., Mäkelä, T., Hase, T., & Fotsis, T. (1995) *J. Steroid. Biochem. Mol. Biol.* **52**, 97–103

(53) Lania-Pietrzak, B., Michalak, K., Hendrich, A.B., Mosiadz, D., Grynkiewicz, G., Motohashi, N., & Shirataki, Y. (2005) *Life Sci.* **77**, 1879–1891

(54) Jacobs, E., & Metzler, M. (1999) *J. Agric. Food Chem.* **47**, 1071–1077

(55) Jacobs, E., Kulling, S.E., & Metzler, M. (1999) *J. Steroid. Biochem. Mol. Biol.* **68**, 211–218

(56) Jansen, G.H., Arts, I.C., Nielen, M.W., Muller, M., Hollman, P.C., & Keijer, J. (2005) *Arch. Biochem. Biophys.* **435**, 74–82

(57) Dean, B., Chang, S., Doss, G.A., King, C., & Thomas, P.E. (2004) *Arch. Biochem. Biophys.* **429**, 244–251

(58) Lampe, J.W. (2003) *J. Nutr.* **133**, 956S–964S

(59) Wang, L.Q. (2002) *J. Chromatogr. B* **777**, 289–309

(60) Wilkinson, A.P., Wähälä, K., & Williamson, G. (2002) *J. Chromatogr. B* **777**, 93–109

(61) Kuijsten, A., Buijsman, M.N., Arts, I.C., Mulder, P.P., & Hollman, P.C. (2005) *J. Chromatogr. B* **822**, 178–184

(62) Stumpf, K., Pietinen, P., Puska, P., & Adlercreutz, H. (2000) *Canc. Epidemiol. Biomarkers Prev.* **9**, 1369–1372

(63) Kilkkinen, A., Stumpf, K., Pietinen, P., Valsta, L.M., Tapanainen, H., & Adlercreutz, H. (2001) *Am. J. Clin. Nutr.* **73**, 1094–1100

(64) Hong, S.J., Kim, S.I., Kwon, S.M., Lee, J.R., & Chung, B.C. (2002) *Yonsei Med. J.* **43**, 236–241

(65) Horner, N.K., Kristal, A.R., Prunty, J., Skor, H.E., Potter, J.D., & Lampe, J.W. (2002) *Canc. Epidemiol. Biomarkers Prev.* **11**, 121–126

(66) Grace, P.B., Taylor, J.I., Low, Y.L., Luben, R.N., Mulligan, A.A., Botting, N.P., Dowsett, M., Welch, A.A., Khaw, K.T., Wareham, N.J., Day, N.E., & Bingham, S.A. (2004) *Canc. Epidemiol. Biomarkers Prev.* **13**, 698–708

(67) Wang, C., & Kurzer, M.S. (1997) *Nutr. Canc*. **28**, 236–247
(68) Mueller, S.O., Simon, S., Chae, K., Metzler, M., & Korach, K.S. (2004) *Toxicol. Sci.* **80**, 14–25
(69) Nurmi, T., Voutilainen, S., Nyyssonen, K., Adlercreutz, H., & Salonen, J.T. (2003) *J. Chromatogr. B* **798**, 101–110
(70) Dave, R.I., & Shah, N.P. (1998) *J. Dairy Sci.* **81**, 2804–2816
(71) Tannock, G.W., Munro, K., Bibiloni, R., Simon, M.A., Hargreaves, P., Gopal, P., Harmsen, H., & Welling, G. (2004) *Appl. Environ. Microbiol.* **70**, 2129–2136
(72) Kilkkinen, A., Pietinen, P., Klaukka, T., Virtamo, J., Korhonen, P., & Adlercreutz, H. (2002) *Am. J. Epidemiol.* **155**, 472–477
(73) Macfarlane, G.T., Macfarlane, S., & Gibson, G.R. (1998) *Microb. Ecol.* **35**, 180–187
(74) Stephen, A.M., Wiggins, H.S., & Cummings, J.H. (1987) *Gut* **28**, 601–609
(75) Lampe, J.W., Fredstrom, S.B., Slavin, J.L., & Potter, J.D. (1993) *Gut* **34**, 531–536
(76) Taylor-Robinson, D., & Furr, P.M. (1990) *Epidemiol. Infect.* **105**, 163–168
(77) Tsuyuguchi, K., Suzuki, K., Matsumoto, H., Tanaka, E., Amitani, R., & Kuze, F. (2001) *Clin. Exp. Immunol.* **123**, 428–434
(78) Kirkman, L.M., Lampe, J.W., Campbell, D.R., Martini, M.C., & Slavin, J.L. (1995) *Nutr. Canc*. **24**, 1–12
(79) Juntunen, K.S., Mazur, W.M., Liukkonen, K.H., Uehara, M., Poutanen, K.S., Adlercreutz, H.C., & Mykkanen, H.M. (2000) *Br. J. Nutr.* **84**, 839–846