

Gut Microbial Metabolism of Plant Lignans: Influence on Human Health

Seth C. Yoder*, Samuel M. Lancaster^{†,‡}, Meredith A.J. Hullar*
and Johanna W. Lampe*

*Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA [†]Department of Genome Sciences, University of Washington, Seattle, WA, USA [‡]Molecular and Cellular Biology, University of Washington, Seattle, WA, USA

INTRODUCTION

Lignans are organic compounds synthesized by plants and are characterized by the union of two cinnamic acid residues by β -linkage on the C8 of each propyl side-chain.^{1–3} They are found in over 70 plant families and are found throughout the plant tissue, in roots, rhizomes, stems, leaves, seeds and fruits.^{4,5} Large amounts of lignans and other polyphenols are also found in the knots of soft wood trees, mainly in the form of hydroxymatairesinol.^{6,7} Lignans are structurally similar to lignins, three-dimensional polymers that intercalate with cellulose, hemicellulose, and pectin to form the rigid cell wall of plant cells.^{1,8} Moreover, both lignans and lignins are created by the same initial phenylpropanoid pathway and are synthesized from monolignols (derived from either phenylalanine or tyrosine), but ultimately enter distinct biochemical pathways.^{2,9} Indeed, while lignins are ubiquitous in the plant kingdom, not all plants produce lignans.

Interestingly, despite having been identified over 120 years ago,⁷ the botanical properties of lignans still remain unclear. Lignans are thought to play a role in defending the plant against pathogens and pests because they possess some antifungal, antimicrobial, antiviral, and even insecticidal properties.^{4,10,11} For example, the lignan haedoxan A isolated from *Phryma leptostachya* has insecticidal properties comparable to that of pyrethrins. Additionally, matairesinol and its related metabolites have been shown to reduce fungal growth of *Fomes annosus* in *Picea abies*.^{5,12}

Given the prevalence of lignans in the plant kingdom, they are not surprisingly found in many common foods. The lignans typically present in foods are matairesinol (MAT), pinoresinol (PINO), medioresinol (MED), lariciresinol (LARI), sesamin (SES), syringaresinol (SYR), secoisolariciresinol (SECO), and the glycosylated form of SECO, secoisolariciresinol diglucoside (SDG). Flaxseed is the richest known source of plant lignans with approximately 300,000 $\mu\text{g}/100\text{ g}$ fresh weight, the majority of which is in the form of SDG.¹³ Other seeds with high lignan concentration include sesame seeds (104,446 $\mu\text{g}/100\text{ g}$), cloudberry seeds (43,876 $\mu\text{g}/100\text{ g}$), hemp seeds (32,473 $\mu\text{g}/100\text{ g}$), and blackberry seeds (23,310 $\mu\text{g}/100\text{ g}$).¹⁴ Cereal grains, including rye, wheat, barley and oats also contain moderate to high amounts of lignans.^{13–16} Brassica vegetables, such as kale, cabbage and Brussels sprouts, as well as other vegetables and fruits, such as asparagus, broccoli, garlic, apricots, prunes, and dates, contain moderate amounts of lignans.^{13–17} Lignans are even found in small amounts in common beverages, including black tea, green tea, coffee, soy milk, fruit juices, beer and wine.^{13,18,19}

CONVERSION OF PLANT LIGNANS TO ENTEROLIGNANS BY GUT BACTERIA

Despite their antimicrobial properties, plant lignans can be metabolized and converted to enterolignans (entero- from Greek *enteron* meaning “intestine”) by bacteria residing in the intestine.²⁰ Also referred to as mammalian lignans, enterolignans were discovered independently by two research teams at almost the same time. The two major enterolignans produced by mammalian gut bacteria are enterolactone (ENL) and enterodiol (END). In 1979, Setchell and Adlercreutz first identified what turned out to be ENL, one of the major enterolignans excreted in the urine of both pregnant and non-pregnant women.²¹ A year later, Stich et al. published an article in *Nature* detailing similar findings; they also had isolated what later was identified as ENL from urine in both pregnant and normally ovulating women.²² Axelson et al. went on to demonstrate in humans that plant lignans are dietary precursors of enterolignans.²³

Not long after their discovery, it was confirmed that ENL and END were produced by intestinal bacteria. Axelson and Setchell first conducted a study in 1981 comparing germ-free rats and conventional rats showing that the presence of bacteria was necessary for enterolignan formation.²⁴ Later that same year, Setchell et al. demonstrated in humans that urinary enterolignan excretion falls immediately and significantly following antibiotic treatment.²⁵ Borriello et al. confirmed the metabolism of plant lignans to ENL and END in vitro using human stool samples.²⁰

Following ingestion of SDG or similarly glycosylated lignans, such as PINO diglucoside or sesaminol triglucoside (STG), the sugar moieties are hydrolyzed in the large intestine by *O*-linked deglycosylation, forming SECO and the other aglycones.^{26,27} Studies simulating digestion in the stomach and small intestine show that SDG remains intact in the equivalent of these parts of the gut, suggesting that acid hydrolysis and human intestinal enzymes, such as lactase phlorizin hydrolase (LPH; EC 3.2.1.62), do not play a major role in this initial hydrolysis.^{26,28} It is not until the artificial ascending colon, which is inoculated with gut microbes, that SECO is first detected.²⁸

In converting SDG to ENL four reactions must take place (Figure 7.1). First, SDG must be converted to SECO by *O*-linked deglycosylation, and secondly SECO is converted to the intermediate dihydroxyenterodiol (DHEND) by *O*-linked demethylation. From this point, DHEND may be converted to END by dehydroxylation, and ultimately to ENL by dehydrogenation of END. Alternatively, DHEND may undergo dehydrogenation to construct a lactone ring, thus forming a second intermediate dihydroxyenterolactone (DHENL), which can then be dehydroxylated to form ENL.^{26,27,29–31}

Several investigators have evaluated the capacity of various specific gut bacteria to carry out the reactions necessary for conversion of glycosylated lignans to enterolignans (Table 7.1). Clavel *et al.* determined that *Bacteroides fragilis*, *Bacteroides ovatus*, *Clostridium cocleatum*, *Clostridium saccharogumia*, *Clostridium ramosum*, and *Bacteroides distasonis* are capable of this *O*-deglycosylation, with the first four able to completely deglycosylate SDG within 20 hours of the experiment.^{32–34} Roncaglia *et al.* found ten *Bifidobacterium* strains capable of hydrolyzing SDG.³⁵ Once deglycosylated, SECO can then be demethylated to produce its intermediate DHEND. Clavel *et al.* demonstrated the ability of *Butyribacterium methylotrophicum*, *Eubacterium callanderi*, *Eubacterium limosum*, and *Blautia producta* to catalyze this reaction.^{32,34} However, Clavel also noted that the presence of the SECO demethylating species *E. callanderi* and *B. methylotrophicum* in the human intestinal tract has not been reported. (*Blautia* is a newly classified genus, and the *B. producta* taxon replaces *Peptostreptococcus productus* and *Ruminococcus productus* to which some older articles refer.)⁴⁰ Jin and Hattori also recently identified *Clostridiaceae bacterium* END-2 (originally referred to simply as “strain END-2”) with the ability to demethylate SECO.³⁶ Following demethylation, *Eggerthella lenta* and *Clostridium scindens* can act on the intermediate by removing a hydroxyl group from each aromatic ring to form END.³⁴ *Lactonifactor longoviformis* and the aforementioned strain END-2 perform the final step in ENL production by fashioning the lactone ring.^{33,36} Alternatively, *L. longoviformis* may create the lactone ring subsequent to SECO demethylation, in which case END does not form but rather a second intermediate, DHENL, which may then continue on to form ENL.²⁶

Studies have shown that SES is also capable of being converted to END and ENL in humans, rats, and in vitro incubations, although the SES biotransformation pathway is still underdetermined.^{41–44} Due to SES's methylenedioxy functional groups, it is thought that SES follows a conversion pathway (Figure 7.2) different than that of the other major lignans (Figure 7.1). In addition, the organisms responsible for converting SES are not as extensively researched as those involved in SDG conversion. Zhu *et al.* recently showed that when STG, isolated from sesame cake and the most abundant lignan glycoside in sesame seed, was fermented with human intestinal bacteria, the phylogenetic groups *Bifidobacteria* and *Lactobacillus-Enterococcus* were significantly greater in the STG samples compared to the controls.⁴⁵

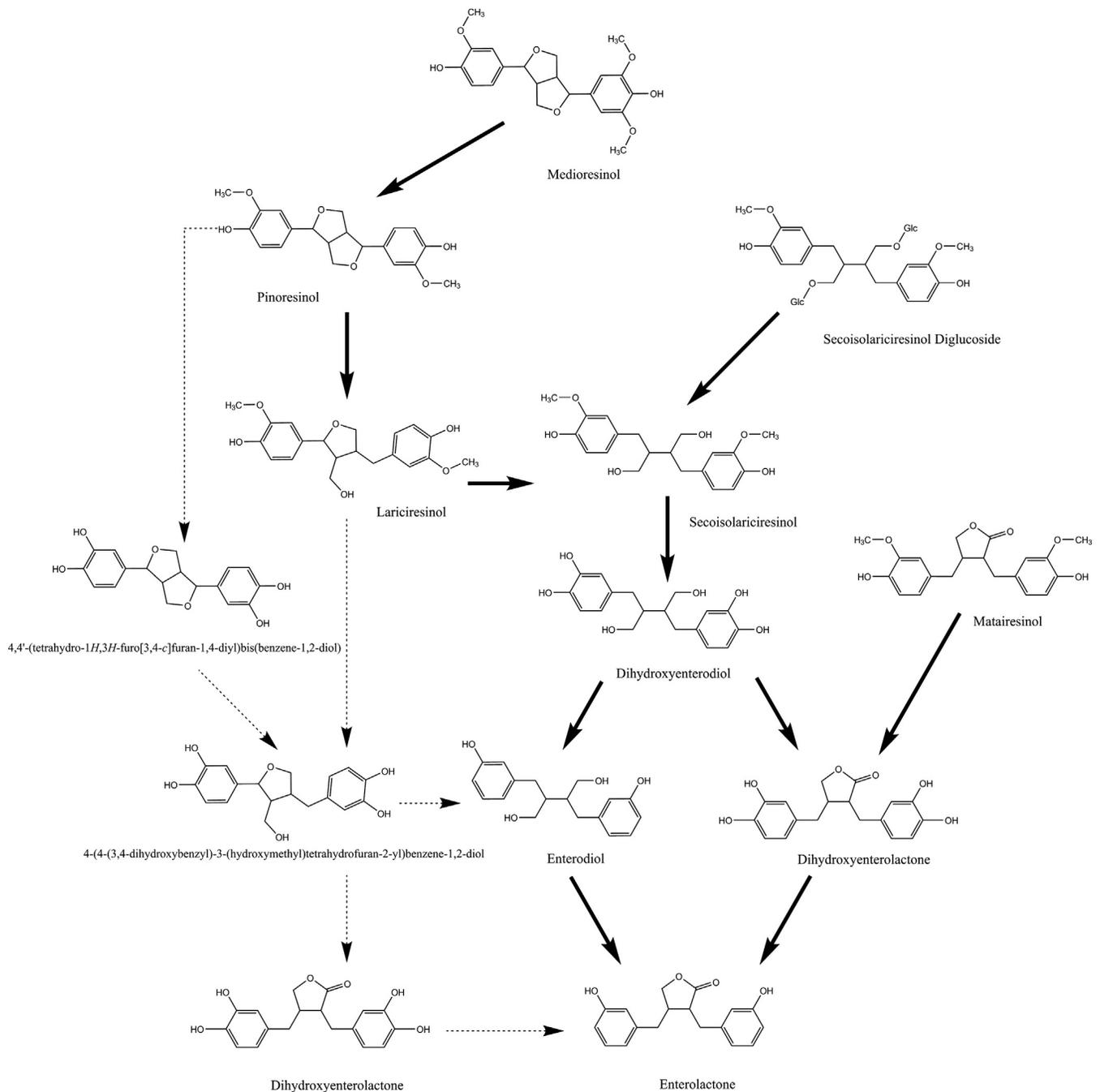


FIGURE 7.1 Conversion pathways of the common plant food lignans to the enterolignans enterolactone and enterodiols. Solid arrows indicate known pathways. Dashed arrows indicate theoretical pathways. Adapted from Clavel *et al.*²⁶

Both END and ENL enantiomers are produced by bacteria, and the human exposure to the enantiomers results from the interaction of the initial type of substrate and the composition of the bacterial consortia. For example, (–) ENL predominates in serum when humans consume their habitual diets, but when supplemented with flaxseed the (+) ENL form increases substantially with comparatively modest increases in (–) ENL.⁴⁶ Additionally, the forms of END and ENL that have been isolated as the result of incubating SDG with intestinal bacteria produce (+) END and (+) ENL. However, if the lignans arctiin or PINO diglucoside are used as a substrate (–) END and (–) ENL are produced.⁴⁷ Subsequently, some bacteria have shown enantiomeric selectivity when converting lignans. *Ruminococcus* sp. END-1 enantioselectively converts (–) END to (–) ENL, while the strain END-2 converts (+) END to (+) ENL.³⁸ *Eggerthella* sp. SDG-2 can convert (+) dihydroxyenterodiols (DHEND)

TABLE 7.1 Known Organisms Involved in Lignan Conversion

Organism(s)	Function	Reference
<i>Bacteroides fragilis</i>	Deglycosylation	Clavel et al. (2006) ^{32, 34} Clavel et al. (2007) ³³
<i>Bacteroides ovatus</i>		
<i>Clostridium cocleatum</i>	Deglycosylation	Roncaglia et al. (2011) ³⁵
<i>Clostridium saccharogumia</i>		
<i>Clostridium ramosum</i>		
<i>Bacteroides distasonis</i>		
<i>Bifidobacterium bifidum</i> WC 418		
<i>Bifidobacterium breve</i> WC 421		
<i>Bifidobacterium catenulatum</i> ATCC 27539		
<i>Bifidobacterium longum</i> subsp. <i>infantis</i> ATCC 15697		
<i>Bifidobacterium longum</i> subsp. <i>longum</i> WC 436		
<i>Bifidobacterium longum</i> subsp. <i>longum</i> WC 439		
<i>Bifidobacterium pseudocatenulatum</i> WC 401		
<i>Bifidobacterium pseudocatenulatum</i> WC 402		
<i>Bifidobacterium pseudocatenulatum</i> WC 403		
<i>Bifidobacterium pseudocatenulatum</i> WC 407		
<i>Butyrivacterium methylotrophicum</i>		
<i>Eubacterium callanderi</i>		
<i>Eubacterium limosum</i>		
<i>Blautia producta</i>	Demethylation	Jin and Hattori (2010) ³⁶
<i>Clostridiaceae bacterium</i> END-2		
<i>Eggerthella lenta</i> <i>Clostridium scindens</i>	Dehydroxylation	Clavel et al. (2006) ³⁴
<i>Lactonifactor longoviformis</i>	Dehydrogenation	Clavel et al. (2007) ³³
<i>Clostridiaceae bacterium</i> END-2	Dehydrogenation	Jin and Hattori (2010) ³⁶
<i>Enterococcus faecalis</i>	Reduction	Xie et al. (2003) ³⁷
<i>Eggerthella lenta</i>	Reduction	Clavel et al. (2006) ³²
<i>Ruminococcus</i> sp. END-1	Dehydrogenation of: (–) END to (–) ENL	Jin et al. (2007) ³⁸
<i>Clostridiaceae bacterium</i> END-2	Dehydrogenation of: (+) END to (+) ENL	Jin et al. (2007) ³⁸
<i>Eggerthella</i> sp. SDG-2	Dehydroxylation of: (+) DHEND to (+) END; (–) DHENL to (–) ENL	Jin et al. (2007) ³⁹
<i>Eubacterium</i> sp. ARC-2	Dehydroxylation of: (–) DHEND to (–) END; (+) DHENL to (+) ENL	Jin et al. (2007) ³⁹

DHEND, dihydroxyenterolactone; DHENL, dihydroxyenterodiol; END, enterodiol; ENL, enterolactone.

to (+) END, but not (–) DHEND to (–) END. Oddly, *Eggerthella* sp. SDG-2 converts (–) DHENL to (–) ENL, but not (+) DHENL to (+) ENL. Evidently, *Eubacterium* sp. ARC-2 can biotransform what *Eggerthella* sp. SDG-2 can not by converting (–) DHEND and (+) DHENL to (–) END and (+) ENL, respectively. However, the bacterium could not transform (+) DHEND and (–) DHENL.³⁹ Jin *et al.* also recently demonstrated the ability of *Eubacterium* sp. ARC-2 and *Eggerthella* sp. SDG-2 to transform the lignan trachelogenin (isolated from safflower seeds) to currently unnamed compounds.⁴⁸

The capacity of specific bacteria to metabolize other plant lignans is less well known. Nonetheless, Xie *et al.* discovered *Enterococcus faecalis* capable of reducing PINO to form LARI.³⁷ Furthermore, *E. lenta* is capable of transforming both PINO and LARI to SECO.³² In addition, Clavel *et al.* reported that *B. producta* is also capable of demethylating other lignans including LARI, PINO and MAT. Considering that the enterolignans, regardless of the plant lignan source, have been associated with some health benefits in human population studies,^{31,49–61} further studies are warranted which elucidate both the microbiome and the microbial metabolic pathways associated with the production of enterolignans. The potential for a gut microbial community to product END and ENL is also affected by the plant lignan substrate. Heinonen *et al.*⁶² showed a range of conversion of plant-derived lignans to END and ENL, ranging from no conversion of isolariciresinol to 100% conversion of LARI. Similarly, production of ENL from arctigenin glucoside and MAT ranged from 5 to 62%, respectively. These results further support the importance of the interaction of the plant lignans and the gut microbial community in dictating enterolignan exposure.

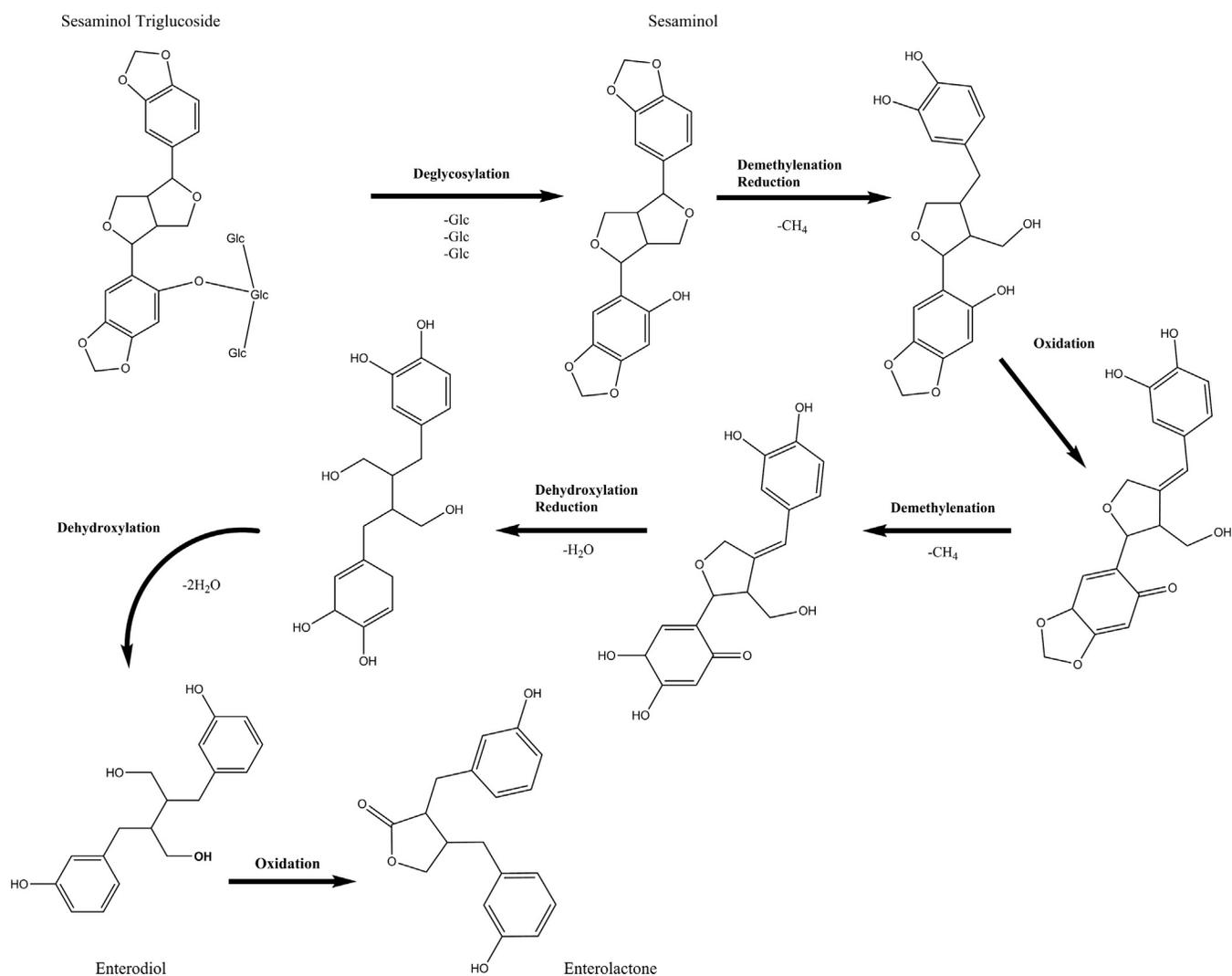


FIGURE 7.2 Overview of potential conversion pathway of the glycosylated sesame seed lignan, sesaminol triglycoside, to the enterolignans. Adapted from Jan et al.⁴³

ASSOCIATIONS BETWEEN LIGNAN EXPOSURE AND HUMAN HEALTH

Plant lignans, and particularly their metabolites, the enterolignans, have been shown to have a range of biological activities.^{27,31,58,63,64} The structural similarity of END and ENL to 17 β -estradiol, a common sex hormone, allows the enterolignans to bind to estrogen receptor alpha (ER α) and exert weak estrogenic or anti-estrogenic effects, which is why they were first classified as “phytoestrogens”^{63,65–67} However, both in vitro and in vivo studies have identified a variety of other mechanisms by which enterolignans may affect the risk of several chronic diseases. These mechanisms include anti-proliferative, anti-inflammatory, and apoptotic effects.^{10,51,58,59,66,68} Several review articles have been published examining the available evidence on lignans and their effects on human health.^{31,49–61} Here we summarize the major conclusions of these reviews and provide further updates on the human studies.

Cancer

The three types of cancer most extensively explored in relation to lignan exposure are breast, colorectal and prostate cancer, to which lignans are linked with cancer prevention. Research concerning other cancers, however, is sparse. The handful of human studies on endometrial cancer and lignans tends toward a null association,^{49,69–71} and a nested case-control study conducted within the European Prospective Investigation

into Cancer and Nutrition–Norfolk (EPIC-Norfolk) study indicated no associations between lignans and gastric cancer.⁷² Gastric and endometrial cancer notwithstanding, the overall evidence tends to support an inverse association between lignans and several common cancers.

In 2005, Webb and McCullough⁵⁹ reviewed the existing literature on dietary lignan exposure and cancer. They concluded that *in vitro* and animal studies supported a role for lignan-rich foods and extracted lignans in modulation of carcinogenesis in colon, breast and prostate; however, they pointed out that the results of the few available epidemiologic studies were inconsistent. Since 2005, additional prospective cohort studies have evaluated the association between dietary lignan intake and cancer risk, relying on self-reported measures of intake such as food frequency questionnaires (FFQ) and food records. With the advent of more sensitive and less expensive assays for END and ENL, investigators have also measured urinary and blood END and ENL, which provide an objective and internal measure of lignan exposure. In 2010, Adolphe et al. reviewed the literature on the health effects of SDG specifically and suggested that the data from animal models support a protective effect of flax lignans against colon, lung and mammary tumors.⁶⁶

Colorectal Cancer

Several epidemiologic studies have examined the association between lignan or enterolignan exposure and colorectal cancer. A case-control study conducted in Canada examined the association between intake of lignans, as measured by FFQ, and risk of colorectal cancer in 1095 cases and 1890 controls.⁷³ The researchers found that the highest dietary lignan intake (> 0.255 mg/day) was associated with a significant reduction in colorectal cancer risk (OR 0.73; 95% CI 0.56–0.94). In 2006, a Dutch retrospective case-control study found that plasma END was associated with a significantly reduced risk of colorectal adenoma (OR 0.53; 95% CI 0.32–0.88) when examining incident cases.⁷⁴ Incident cases were defined as those with a histologically confirmed colorectal adenoma but no history of any type of polyps. ENL was also associated with reduced risk, but to a lesser extent (OR 0.63; 95% CI 0.38–1.06).

A case-cohort study conducted in Denmark similarly examined plasma ENL and colorectal cancer and found significantly lower incidence rate ratios (IRR) for colon cancer among women for each doubling of plasma ENL concentration (IRR 0.76; 95% CI 0.60–0.96).⁷⁵ Interestingly, however, rectal cancer in men was associated with an increased risk for every doubling of plasma ENL concentration (IRR 1.74; 95% CI 1.25–2.44).⁷⁵

Breast Cancer

Conclusions based on human epidemiologic studies are variable for the role of lignans in premenopausal versus postmenopausal breast cancer risk. In their 2005 review, Webb and McCullough⁵⁹ concluded, at that time, that the most support for a role of lignans in cancer prevention was observed for premenopausal breast cancer. Following this, the results of two meta-analyses published within a year of each other provide a more systematic evaluation of available studies. A 2009 meta-analysis of 11 studies (four cohort studies and seven case-control studies) assessing lignan exposure and breast cancer risk determined that, though borderline, there was no association between plant lignan intake and breast cancer risk (combined OR 0.93; 95% CI 0.83–1.03) when the relevant observational studies were pooled.⁵⁷ However, when the studies were divided by menopausal status, they found a significant reduction in breast cancer risk among the postmenopausal women (combined OR 0.85; 95% CI 0.78–0.93; $p < 0.001$). This effect was not observed with pre-menopausal women (combined OR 0.97; 95% CI 0.82–1.15, $p = 0.73$). Where data were available, the investigators also systematically examined ENL concentrations in the blood and estimated enterolignan exposure using values produced from food by *in vitro* fermentation models. Although there was a significant inverse correlation between enterolignan exposure and breast cancer risk (combined OR 0.73; 95% CI 0.57–0.92), the association was no longer significant (combined OR 0.82; 95% CI 0.59–1.14) when blood ENL levels were considered. The authors noted that there was marked heterogeneity between the studies they reviewed making broad conclusions difficult.

Another meta-analysis which included 21 studies (11 prospective cohort studies and 10 case-control studies) concluded that plant lignan intake was not associated with overall breast cancer risk, but if the results are separated by menopausal status, postmenopausal women with a high plant lignan intake have a significantly reduced risk of breast cancer (pooled RE 0.86; 95% CI 0.78–0.94).⁵⁰ A similar association was found when four studies were examined assessing dietary enterolignans (pooled RE 0.84; 95% CI 0.71–0.97). In total, these meta-analyses suggest that menopausal status is an important factor to consider in associations of dietary lignans, enterolignans and risk of breast cancer.

More recent studies continue to support the hypothesis that enterolignans have a protective effect on breast cancer, but that this is dependent on menopausal status. A follow-up study of 2653 postmenopausal breast cancer

patients diagnosed between 2001 and 2005 concluded that both high estimated ENL (HR 0.60; 95% CI 0.40–0.89; p trend = 0.02) and high estimated END were associated with higher overall survival (HR 0.63; 95% CI 0.42–0.95; p trend = 0.02).⁷⁶ Moreover, a retrospective cohort study of 300 breast cancer patients concluded that higher serum ENL levels were associated with both decreased all-cause mortality and decreased breast-cancer mortality.⁷⁷ At 5 years, patients with serum ENL levels ≥ 10 nmol/L demonstrated lower all-cause and breast cancer-specific mortality (HR 0.49; 95% CI 0.27–0.91; $p = 0.025$ and HR 0.42; 95% CI 0.22–0.81; $p = 0.009$, respectively). At 10 years and beyond, however, this association became non-significant (all-cause death: HR 0.65; 95% CI 0.40–1.03; $p = 0.07$ and breast cancer-specific (HR 0.67; 95% CI 0.39–1.14; $p = 0.1$). After stratifying the results by menopausal status, the association between serum ENL and both all-cause mortality and breast-cancer mortality decreased and remained statistically significant at 10 years for postmenopausal women (all-cause death: HR 0.48; 95% CI 0.28–0.82; $p = 0.007$ and breast cancer-specific: HR 0.52; 95% CI 0.29–0.94; $p = 0.03$, respectively).⁷⁷ After adjusting for menopausal status, these studies show a protective effect of ENL suggesting that different mechanisms may be important to consider in pre- and postmenopausal women.

A case-control study conducted in women in Ontario, Canada, examined the relationship between breast cancer and phytoestrogen intake using a FFQ.⁷⁸ The study included 2438 women with breast cancer and 3370 controls and results were stratified by tumor type, menopausal status, and phytoestrogen intake as an adult versus adolescent. Among women with estrogen-receptor-positive and progesterone-receptor-positive (ER+/PR+) tumors, both adult intake of lignans (OR 0.83; 95% CI 0.68–1.00) and adolescent intake of lignans (OR 0.86; 95% CI 0.73–1.00) were associated with reduced risk of breast cancer. After separating the results by menopausal status, the associations in premenopausal women become either null or not significant; however, the association with adolescent lignan intake among postmenopausal women in the ER+/PR+ subgroup remained (OR 0.78; 95% CI 0.64–0.95).⁷⁸

McCann et al. reported on a case-control study analyzing not only lignan intake but also the various types of plant lignans and their relationship to breast tumors. They found that the women in the highest tertile of lignan intake had a 40–50% lower odds of breast cancer compared to those in the lowest tertile of lignan intake.⁷⁹ This result was regardless of menopausal status; however, premenopausal women had the strongest associations with PINO and LARI while postmenopausal women had the strongest associations with MAT.

Animal models have been used to examine the underlying mechanism of action between lignans, enterolignans and breast cancer. In animal models, SDG has been shown to promote cell differentiation in mammary glands, delay the progression of *N*-methyl-*N*-nitrosourea-induced mammary tumorigenesis, and produce beneficial mammary gland structural changes during gestation and lactation.⁶⁶ More recently, SES was shown to decrease cell proliferation and increased apoptosis of MCF-7 tumors in mice.⁸⁰ In addition, a study in germ-free rats showed that, although END and ENL production did not influence the occurrence of breast cancer, the number of tumors, size of the tumors, and tumor cell proliferation decreased, and tumor cell apoptosis increased in rats that were conventionalized with a lignan-converting bacterial consortia. These studies suggest that lignans and bacterially produced enterolignans influence anticancer effects.⁸¹

Prostate Cancer

The evidence regarding the role that lignans may play in prostate cancer is varied. On the one hand, reviews by McCann et al. and Saarinen et al. cite several *in vitro* and animal studies that demonstrate the ability of lignans to act as chemopreventive agents in prostate cells.^{60,61} Indeed, recent evidence confirms that ENL, even at concentrations achievable *in vivo* following the intake of lignan precursors, inhibits the proliferation of early-stage prostate cancer cells.⁸² However, data available from human studies are inconsistent; some evidence suggests a benefit, while other studies found null associations between lignans and prostate cancer. Since those reviews were published, two case-control studies have been published that actually show higher lignan intake is associated with an increased risk of prostate cancer.^{83,84}

A nested case-control study from EPIC-Norfolk examined lignans and prostate cancer.^{83,85} Participants completed 7-day food records, which were used to estimate daily lignan intake. In addition to estimating intake of the plant lignans MAT and SECO, the investigators also estimated intake of preformed enterolignans from dairy and other animal products. This was the first study to consider animal sources of enterolignans in the overall estimate of lignan exposure. Given that mammals, particularly ruminants, are capable of producing large amounts of enterolignans in their rumen, some animal products, particularly dairy foods, contain enterolignans.⁸⁶ The study included 204 prostate cancer cases and 812 controls. Estimated mean (\pm SD) intake of preformed enterolignans were 20 μ g/day (± 9) among cases and 18 μ g/day (± 9) among controls in this EPIC-Norfolk sample.⁸³ In an age-adjusted model, total lignan intake was not associated with prostate cancer (OR 1.05; 95% CI

0.81–1.36; $p = 0.72$). However, total enterolignan intake (which included the non-lignan phytoestrogen equol) was positively associated with prostate cancer (OR 1.41; 95% CI 1.12–1.76; $p = 0.003$) as were equol (OR 1.43; 95% CI 1.14–1.80; $p = 0.002$) and ENL (OR 1.39; 95% CI 1.12–1.71, $p = 0.003$) individually. However, after additional adjustment for covariates, such as age, height, weight, physical activity, social class, family history of prostate cancer, and daily energy intake, the associations became non-significant, suggesting that the lignan effect was confounded with other variables.

Another case-control study, conducted in Jamaica, consisted of 175 newly diagnosed prostate cancer cases and 194 controls.⁸⁴ The researchers evaluated the relationship of urinary phytoestrogens with total cancer and tumor grade. ENL was measured from spot urine samples. Higher concentrations of ENL were positively associated with both total prostate cancer (OR 1.85; 95% CI 1.01–3.44; $p = 0.027$) and high-grade disease (OR 2.46; 95% CI 1.11–5.46; $p = 0.023$).

The small sample size and limitations inherent in a case-control design advocates caution in espousing the results without follow-up studies, but differential findings based on tumor grade, speak to the importance of considering the heterogeneity of cancers and the potential for different effects depending on tumor grade. To date, only a few studies have been statistically powered to be able to consider the association between lignans and cancers, stratified by tumor type or stage.

Cardiovascular Disease

A review of lignans and cardiovascular disease risk published in 2010 by Peterson et al. summarized the findings of both randomized controlled trials (RCTs) and observational studies.⁵⁵ While some of the RCTs showed no effects, many showed favorable effects to blood pressure, C-reactive protein, and lipid profiles. The authors also discussed both observational studies examining lignan intake as well as observational studies examining serum ENL; however, similar to the studies of cancer outcomes, the results of the studies of cardiovascular disease are mixed, and it is difficult to draw definitive conclusions. Five of the 11 observational studies showed decreased cardiovascular risk with either increasing dietary intakes of lignans or increased concentrations of serum ENL, while five studies were described by the authors as having borderline significance, and one was null.⁵⁵ The authors noted that a limitation to the capacity to conduct a systematic review of the interventions was the differences in experimental protocols that were used between the studies.

Since that review was published, a 2012 cross-sectional study found that urinary enterolignans were inversely associated with serum triglyceride levels and positively associated with HDL (“good”) cholesterol.⁸⁷ The authors used three models: an unadjusted model, a model adjusted for age, race/ethnicity, education, income, urinary creatinine (log-transformed), and a third model further adjusted for smoking and alcohol categories, menopause status, use of hormone replacement therapy, BMI, physical activity, and dietary intake of saturated fat, cholesterol and fiber. The results were statistically significant in all three models, with higher urinary excretion of enterolignans (7.84 $\mu\text{mol/L}$) corresponding to lower serum triglycerides (-0.18 mmol/L) compared to the low levels of enterolignan excretion (0.54 $\mu\text{mol/L}$) in the third model ($p = 0.003$). High excretion corresponded to 0.06 mmol/L greater serum HDL in the third model ($p = 0.009$).⁸⁷

Other Health Effects

Given the various actions of enterolignans *in vivo*, there is the potential for these compounds to influence other aspects of health. For example, Pietrofesa et al. tested the effect of isocaloric diets containing three levels of lignans from flaxseed in mice treated with X-rays.⁸⁸ The lignan component was designed to mimic the amount of lignans ingested from a 0%, 10% and 20% whole-grain flaxseed diet. The lignan diets significantly mitigated radiation-related animal death (controls demonstrated 36.7% survival 4 months after the treatment compared to 60–73.3% survival in mice fed 10–20% lignans). Additionally, lung fibrosis, radiation-induced lung injury (measured via bronchoalveolar lavage), and inflammation was decreased in the mice fed flaxseed lignans.⁸⁸ This would suggest that dietary lignans may help mitigate adverse effects in individuals exposed to radiation.

INTERINDIVIDUAL DIFFERENCES IN LIGNAN METABOLISM

Studying the relationship between lignan exposure and disease risk in human populations is challenging and complex. A variety of foods are sources of lignans and these are not eaten in isolation. Traditionally, cuisines that

include dietary sources of lignans also include other foods that may be considered healthy or less healthy. Trying to tease out the association between intake of high-lignan foods in general, which are often also high-fiber foods, or intake of specific lignans, and disease risk is difficult.

In relation to the study of breast cancer, but relevant to other observational studies described in the previous section, Sonestedt and Wirfält described several factors that may contribute to the ambiguous results that have so far been obtained in epidemiologic studies focusing on ENL.⁸⁹ They note that several factors play a role in enterolignan exposure, including the composition of the gut microbial community, dietary intake of lignan-containing foods, antibiotic use, smoking status and constipation. The authors also point out the difficulty in accurately and reliably determining enterolignan exposure and the complicated role that genetic factors play in both cancer development and the actions of ENL.

Even under controlled conditions, substantial interindividual variation has been reported regarding enterolignan production. Kuijsten et al.⁹⁰ showed in a pharmacokinetic study that a variety of plasma profiles arise with a set dose of SDG, with some individuals producing high amounts of both ENL and END, or higher amounts of one enterolignan as compared to the other (e.g., high ENL, low END or vice versa), or very low amounts of both. All 12 participants ingested the same dose of SDG per kg body weight; however, in five subjects the area under the curve (AUC) of ENL was more than twice that of END; in five others, the AUC of ENL was only one- to two-times the AUC of END; in two participants, the AUC of END exceeded that of ENL; and in one participant, ENL concentrations barely increased. Mean (\pm SD) AUC for END and ENL were 966 ± 639 and 1762 ± 1117 nmol/L.h, respectively, the large SD reflecting the wide variation in individual results. Kuijsten et al. noted that the cumulative excretion of END and ENL accounted for about 40% of ingested SDG; however, individual percentages were also variable. Much of this variation is proposed to be due to differences in gut microbial community across individuals.⁹⁰ The different patterns of END and ENL appearance in circulation and the range of cumulative excretion values suggest differential capacity of the gut microbes to manage the various steps in SDG metabolism (Figure 7.3), with some unable to effectively hydrolyze the SDG to SECO and others being ineffective at converting END to ENL.

The variation in enterolignan production by gut microbial communities from different individuals is also clearly demonstrated using *in vitro* incubations. Incubating fresh fecal samples with a flaxseed extract (630 μ mol/L) for 72 h, Possemiers et al.⁹¹ found that the production of END and ENL differed strongly among 100 individuals. END was produced in varying amounts in 63% of the samples, whereas ENL was only produced in 39% of the samples. Further, END and ENL production were positively correlated and were associated with higher β -glucuronidase activity, supporting the importance of the capacity to carry out the initial steps in SDG conversion.

Another potential source of variation in urine or blood measures of ENL and END, may stem from interindividual differences in biotransformation of the enterolignans⁹² although no controlled evaluation of the effects of genetic variants in biotransformation enzymes on lignan availability has been conducted. In humans, plant lignans and their metabolites are efficiently conjugated with glucuronic acid or, to a lesser extent, sulfate. Conjugation takes place in the gut epithelium and liver by UDP-glucuronosyltransferases (UGT) and sulfotransferases, and the conjugates are excreted in urine and bile. Those that are re-excreted in bile undergo enterohepatic recycling.⁹³ In urine, 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%).⁹⁴ Colon cell lines have been shown to rapidly glucuronidate enterolignans, suggesting that the majority of conjugation of lignans likely occurs in the colon.⁹⁵ Further, hepatic microsomal oxidation is also much slower compared to glucuronidation, suggesting that oxidation products of END and ENL are minor metabolites of enterolignans.⁹⁶ Effects of enterolignans on intestinal transporters have not been evaluated, but SES has been shown to increase the mRNA expression of several transporters, thereby possibly affecting absorption of lignans and other phytochemicals.⁹⁷

In addition to the variation in microbial and host metabolism, several physiologic, demographic and lifestyle characteristics of individuals appear to influence the production of enterolignans *in vivo*, including constituents of diet and other non-dietary factors.

Diet

Diet plays a large role in enterolignan production, both due to the differences in food content of the precursors, but also due to effects of food matrices and other dietary factors on availability and conversion of plant lignans to enterolignans. An early study by Adlercreutz et al. evaluated urinary enterolignan excretion in women consuming macrobiotic, omnivorous and lactovegetarian diets and reported that followers of macrobiotic diets, as compared to the lactovegetarians, had substantially greater END and ENL excretion.⁹⁸ The lactovegetarians in turn, as compared to the omnivores, had greater excretion of enterolignans.

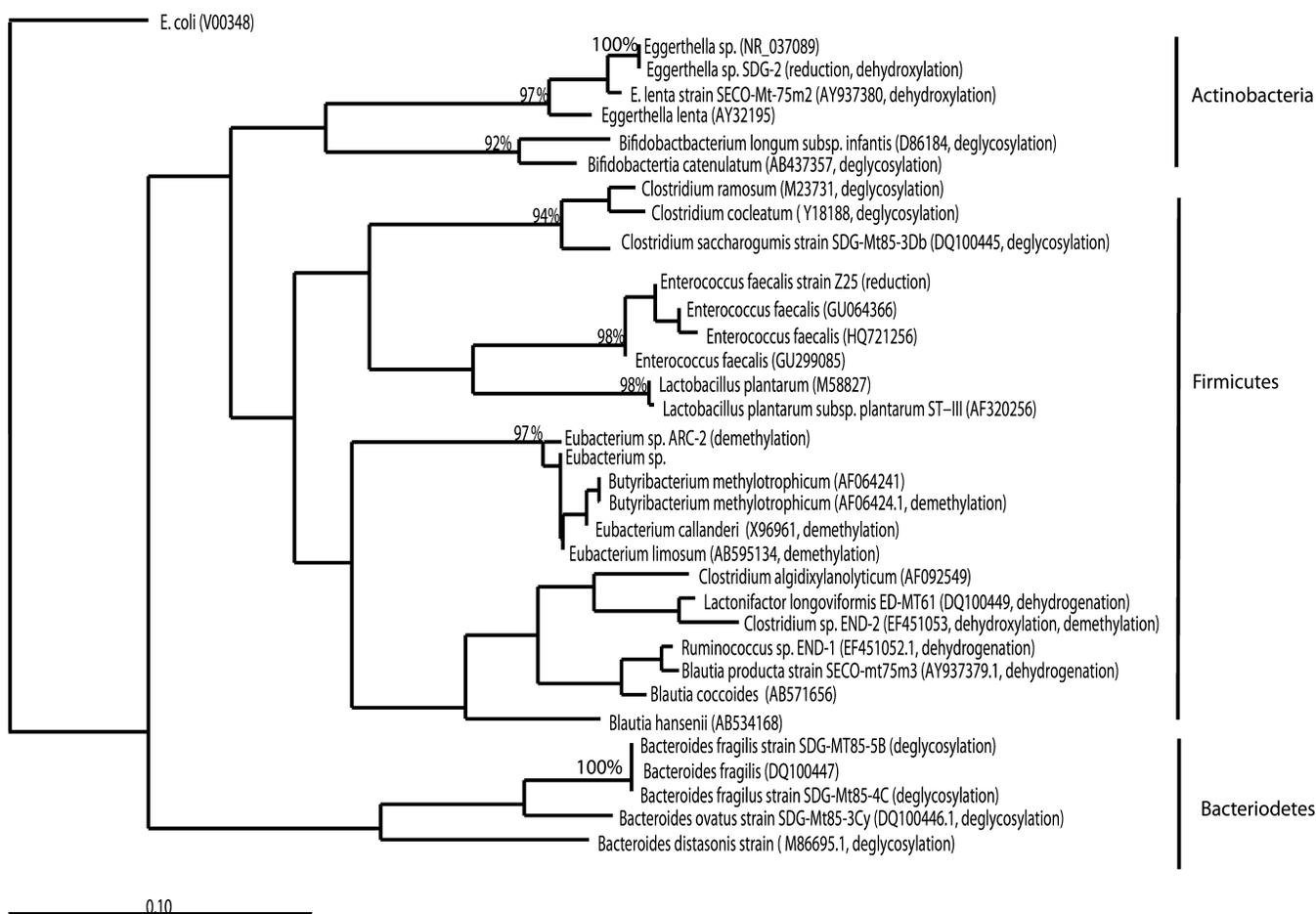


FIGURE 7.3 Phylogenetic tree of lignan-converting bacteria showing that the organisms involved in enterolignan production are distributed across Phyla. Phylogenetic distances were calculated using Neighbor Joining with bootstrap values in percentage (using 1000 replicates) in ARB (www.arb-silve.de) on aligned 16 S rRNA genes from pure cultures of bacteria associated with enterolignan production. The metabolic reactions involved in enterolignan production catalyzed by the bacteria and the accession number for the 16S rRNA gene sequence is in parentheses (see Table 7.1 for more information). Ten percent change in sequence indicated by bar.

Since that early work, many dietary intervention studies have been conducted, further solidifying the link between diet and enterolignan production. Fruits, vegetables, and berries have been demonstrated to increase ENL production.^{99–101} Rye, a rich source of plant lignans, has been shown to increase ENL production in three dietary intervention studies.^{102–104} However, a similar intervention study involving rye products showed no such increase in ENL production.¹⁰⁵ The reason for this discrepancy is unclear; however, the authors note that their participants were generally younger and may have consumed more dietary fiber in their habitual diets than participants in the other studies. This may have contributed to faster lignan transit through the gut, and therefore incomplete biotransformation of the plant lignans.¹⁰⁵ Other dietary trials involving lignan-rich flaxseed and sesame seed revealed significant ENL production.^{41,42,106–109}

Moreover, it appears that the food matrix and food processing may affect the bioavailability of lignans. In a randomized, crossover study, 12 participants were fed whole, crushed and ground flaxseed daily (0.3 g/kg body weight). The results indicated that, compared to ground flaxseed, the mean relative bioavailability of enterolignans from whole flaxseed was 28% and crushed flaxseed was 43%.¹¹⁰ Another dietary study found no effect of baking on the urinary or plasma concentrations of enterolignans when participants were fed ground flaxseed mixed in applesauce or ground flaxseed baked in bread and muffins, suggesting that these compounds are heat stable.¹⁰⁹

Although it has yet to be studied systematically in humans, the presence of dietary fiber may affect the production of enterolignans. In vitro fecal suspensions incubated with 1 g each of extractable and non-extractable fractions of rye produced starkly different results.¹¹¹ The extractable fraction contained mainly soluble fiber and

46 mg/100 g of total plant lignans. The non-extractable fraction contained mainly insoluble fiber and 13 mg/100 g of total plant lignans. Protein and starch amounts were very similar among the fractions. After 48 h of incubation, suspensions containing the non-extractable fraction produced significantly more ENL. The extractable fractions rapidly produced short-chain fatty acids (SCFAs) which quickly decreased the pH of the samples below 5.0. The non-extractable fractions, on the other hand, produced SCFAs at a much slower rate, thus allowing pH to stay in a more neutral range favoring ENL production. The extractable fraction also contained more ferulic giving the samples containing it a lower initial pH.¹¹¹ This suggests that the type of fiber consumed with the lignan may affect its bioavailability.

Sex Differences in Enterolignan Production

The evidence regarding sex differences in lignan metabolism is mixed, but there appears to be a tendency for women to produce more enterolignans than men. Several cross-sectional studies examining the associations between diet and enterolignan production show no sex differences.^{87,112–115} However, a cross-sectional study of 2380 Finnish men and women showed that women had higher concentrations of serum ENL than did men.¹¹⁶ Indeed, plasma ENL and END both appeared earlier and also achieved a higher maximum concentration in women compared to men when following a single SDG dose (1.31 $\mu\text{mol/kg}$ body weight).⁹⁰ Jacobs et al. conducted a feeding study involving a whole-grain diet and found that women had a higher mean baseline serum ENL concentration, although the rise in serum ENL after the whole-grain diet was similar in men and women.¹¹⁷ Another randomized cross-over trial that involved feeding men and women flaxseed and measuring urinary lignan excretion showed no differences between men and women.¹¹⁸ Similar trials involving a lignan dose have showed no difference in enterolignan production between men and women.^{102,110} In contrast, in one controlled feeding study, in which vegetables were fed, men excreted more ENL than women during the experimental diets.¹⁰¹ The tendency here for women to produce more enterolignans than their male counterparts may be explained by Clavel et al. who noted that women tended to harbor more ENL-producing and END-producing bacteria.¹¹⁹ Further, women have been shown to have longer gastrointestinal transit times than men¹²⁰; this longer residence time of fiber-containing foods and plant lignans in the gut may further contribute to greater enterolignan production.

Other Factors Associated with Enterolignan Production

Physiologic and sociodemographic factors have also been shown to be associated with enterolignan levels. Because of the importance of the gut microbial community to enterolignan production, the use of oral antimicrobials is inversely associated with serum ENL concentrations. In a cross-sectional study, Kilkkinen et al. showed, in a sample of 2753 Finnish men and women, that ENL concentrations in antimicrobial users, as compared to non-users of antimicrobials, was significantly lower, even if the time elapsed since antimicrobial treatment was 12–16 months prior to blood sampling.¹²¹ The number of antimicrobial treatments also correlated inversely with ENL concentration.

Gut residence time appears to be another factor that influences ENL production, although most studies rely on non-quantitative measures to assess this factor. For example, Kilkkinen et al., also in a Finnish population, observed positive associations in men with serum ENL concentration and constipation, perhaps demonstrating an association between longer residence time and more ENL production.¹¹⁶ The same study also found that, in women, serum ENL concentration was positively and independently associated with age and constipation, while ENL was inversely associated with smoking. Moreover, female subjects of normal weight had a significantly higher ENL concentration than their underweight or obese counterparts. Other studies in European populations have supported Kilkkinen's findings, reporting inverse associations between body mass index (BMI), smoking, and frequency of bowel movements and plasma ENL concentration.^{115,122}

Recently, the association between sociodemographic and other lifestyle variables and urine ENL levels was evaluated in a large sample of men and women ≥ 20 years in the US. In a subset ($n = 3000$) of the 2003–2006 National Health and Nutrition Examination Survey (NHANES), which collects cross-sectional data on the health and nutritional status of the U.S. population, Rybak et al. found that age, income and physical activity were positively correlated with urinary ENL.¹²³ Further, similar to the studies in European populations, smoking and BMI were inversely correlated with urinary ENL. Despite these associations, the selected sociodemographic and lifestyle variables only explained a limited amount of the total variability ($R^2 \leq 4\%$), suggesting that, compared to other factors, their effects on enterolignan levels are modest.

CONCLUSIONS

Human exposure to END and ENL, bioactive metabolites resulting from the gut microbial metabolism of a variety of plant lignans, is highly variable. From the research available to date, we know that it is dependent on several environmental and physiologic factors including: the types of plant lignans consumed; the foods and food matrices of which the lignans are a part; intake of other foods or medications that may alter gut microbial activity (e.g., dietary fiber, anti-microbial drugs); gut microbial community composition and activity; and gut transit time and factors that affect it. Given this range of factors, characterizing lignan exposure is challenging. Relying on database measures of plant lignan intake does not capture the contribution of the gut microbial community to END and ENL exposure. In contrast, measuring urinary or circulating END and ENL provides an internal measure of exposure to the bioactive compounds, but as Sonestedt and Wirfält point out, also provides a biomarker of dietary lignan intake, a biomarker of a healthy lifestyle and possibly a marker of gut microbial capacity.⁸⁹ It is the complexity of these measures as biomarkers that may contribute to the uncertainties related to the association between lignans and chronic disease risk in observational studies. With the advent of new techniques that allow for rapid and efficient characterization of gut microbial community, adding information on the gut microbial community structure to the statistical models may allow us to better characterize the modifying effect of microbes on lignan–disease risk associations.

Acknowledgements

This work was supported in part by US National Cancer Institute grants U01 CA161809 and U01 CA162077, and Fred Hutchinson Cancer Research Center.

References

1. Ayres D, Loike J. *Lignans: Chemical, Biological and Clinical Properties*. Cambridge: Cambridge University Press; 1990.
2. Umezawa T. Diversity in lignan biosynthesis. *Phytochem Rev*. 2003;2:371–390.
3. Hearon WM, MacGregor WS. The naturally occurring lignans. *Chemical Rev*. 1955;55:957–1068.
4. Pan J-Y, Chen S-L, Yang M-H, Wu J, Sinkkonen J, Zou K. An update on lignans: natural products and synthesis. *Nat Prod Rep*. 2009;26:1251–1292.
5. Gang DR, Dinkova-Kostova AT, Davin LB, Lewis NG. Phylogenetic links in plant defense systems: lignans, isoflavonoids, and their reductases. In: Hedin PA, Hollingworth RM, Masler EP, Miyamoto J, eds. *Phytochemicals for Pest Control*. Washington, DC: American Chemical Society; 1997:59–89.
6. Mäki-Arvela P, Holmbom B, Salmi T, Murzin DY. Recent progress in synthesis of fine and specialty chemicals from wood and other biomass by heterogeneous catalytic processes. *Catal Rev*. 2007;49:197–340.
7. Holmbom B, Eckerman C, Eklund P, et al. Knots in trees – a new rich source of lignans. *Phytochem Rev*. 2003;2:331–340.
8. Cesarino I, Araújo P, Domingues Júnior AP, Mazzafera P. An overview of lignin metabolism and its effect on biomass recalcitrance. *Braz J Bot*. 2012;35:303–311.
9. Davin LB, Jourdes M, Patten AM, Kim K-W, Vassão DG, Lewis NG. Dissection of lignin macromolecular configuration and assembly: comparison to related biochemical processes in allyl/propenyl phenol and lignan biosynthesis. *Nat Prod Rep*. 2008;25:1015–1090.
10. Cunha WR, Luis M, Sola RC, et al. Lignans: chemical and biological properties. In: Rao V, ed. *Phytochemicals – A Global Perspective of Their Role in Nutrition and Health*. Rijeka, Croatia: InTech; 2012:213–234.
11. Harmatha J, Dinan L. Biological activities of lignans and stilbenoids associated with plant–insect chemical interactions. *Phytochem Rev*. 2003;2:321–330.
12. Lewis NG, Kato MJ, Lopes N, Davin LB. Lignans: diversity, biosynthesis, and function. In: Seidl PR, Gottlieb OR, Kaplan MAC, eds. *Chemistry of the Amazon*. Washington, DC: American Chemical Society; 1995:135–167.
13. Milder IEJ, Arts ICW, Van de Putte B, Venema DP, Hollman PCH. Lignan contents of Dutch plant foods: a database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. *Br J Nutr*. 2005;93:393–402.
14. Smeds AI, Eklund PC, Willför SM. Content, composition, and stereochemical characterisation of lignans in berries and seeds. *Food Chem*. 2012;134:1991–1998.
15. Smeds AI, Eklund PC, Sjöholm RE, et al. Quantification of a broad spectrum of lignans in cereals, oilseeds, and nuts. *J Agric Food Chem*. 2007;55:1337–1346.
16. Peñalvo JL, Haajanen KM, Botting N, Adlercreutz H. Quantification of lignans in food using isotope dilution gas chromatography/mass spectrometry. *J Agric Food Chem*. 2005;53:9342–9347.
17. Blitz CL, Murphy SP, Au DLM. Adding lignan values to a food composition database. *J Food Compos Anal*. 2007;20:99–105.
18. Mazur WM, Wähälä K, Rasku S, Salakka A, Hase T, Adlercreutz H. Lignan and isoflavonoid concentrations in tea and coffee. *Br J Nutr*. 1998;79:37–45.
19. Nurmi T, Heinonen S, Mazur W, Deyama T, Nishibe S, Adlercreutz H. Lignans in selected wines. *Food Chem*. 2003;83:303–309.
20. Borriello SP, Setchell KD, Axelson M, Lawson AM. Production and metabolism of lignans by the human faecal flora. *J Appl Bacteriol*. 1985;58:37–43.

21. Setchell KDR, Adlercreutz H. The excretion of two new phenolic compounds (Compound 180/442 and Compound 180/410) during the human menstrual cycle and in pregnancy. *J Steroid Biochem.* 1979;11:xv–xvi.
22. Stitch SR, Toumba JK, Groen MB, et al. Excretion, isolation and structure of a new phenolic constituent of female urine. *Nature.* 1980;287:738–740.
23. Axelson M, Sjövall J, Gustafsson BE, Setchell KDR. Origin of lignans in mammals and identification of a precursor from plants. *Nature.* 1982;298:659–660.
24. Axelson M, Setchell KD. The excretion of lignans in rats – evidence for an intestinal bacterial source for this new group of compounds. *FEBS Lett.* 1981;123:337–342.
25. Setchell KD, Lawson AM, Borriello SP, et al. Lignan formation in man—microbial involvement and possible roles in relation to cancer. *Lancet.* 1981;2:4–7.
26. Clavel T, Doré J, Blaut M. Bioavailability of lignans in human subjects. *Nutr Res Rev.* 2006;19:187–196.
27. Lampe JW, Atkinson C, Hullar MA. Assessing exposure to lignans and their metabolites in humans. *J AOAC Int.* 2006;89:1174–1181.
28. Eeckhaut E, Struijs K, Possemiers S, Vincken J-P, Keukeleire DD, Verstraete W. Metabolism of the lignan macromolecule into enterolignans in the gastrointestinal lumen as determined in the simulator of the human intestinal microbial ecosystem. *J Agric Food Chem.* 2008;56:4806–4812.
29. Wang LQ, Meselhy MR, Li Y, Qin GW, Hattori M. Human intestinal bacteria capable of transforming secoisolariciresinol diglucoside to mammalian lignans, enterodiols and enterolactone. *Chem Pharm Bull.* 2000;48:1606–1610.
30. Wang L. Mammalian phytoestrogens: enterodiols and enterolactone. *J Chromatogr, B: Anal Technol Biomed Life Sci.* 2002;777:289–309.
31. Landete JM. Plant and mammalian lignans: a review of source, intake, metabolism, intestinal bacteria and health. *Food Res Int.* 2012;46:410–424.
32. Clavel T, Borrmann D, Braune A, Doré J, Blaut M. Occurrence and activity of human intestinal bacteria involved in the conversion of dietary lignans. *Anaerobe.* 2006;12:140–147.
33. Clavel T, Lippman R, Gavini F, Doré J, Blaut M. *Clostridium saccharogumia* sp. nov. and *Lactonifactor longoviformis* gen. nov., sp. nov., two novel human faecal bacteria involved in the conversion of the dietary phytoestrogen secoisolariciresinol diglucoside. *Syst Appl Microbiol.* 2007;30:16–26.
34. Clavel T, Henderson G, Engst W, Doré J, Blaut M. Phylogeny of human intestinal bacteria that activate the dietary lignan secoisolariciresinol diglucoside. *FEMS Microbiol Ecol.* 2006;55:471–478.
35. Roncaglia L, Amaretti A, Raimondi S, Leonardi A, Rossi M. Role of bifidobacteria in the activation of the lignan secoisolariciresinol diglucoside. *Appl Microbiol Biotechnol.* 2011;92:159–168.
36. Jin J-S, Hattori M. Human intestinal bacterium, strain END-2 is responsible for demethylation as well as lactonization during plant lignan metabolism. *Biol Pharm Bull.* 2010;33:1443–1447.
37. Xie L-H, Akao T, Hamasaki K, Deyama T, Hattori M. Biotransformation of pinoresinol diglucoside to mammalian lignans by human intestinal microflora, and isolation of *Enterococcus faecalis* strain PDG-1 responsible for the transformation of (+)-pinoresinol to (+)-lariciresinol. *Chem Pharm Bull.* 2003;51:508–515.
38. Jin J-S, Kakiuchi N, Hattori M. Enantioselective oxidation of enterodiols to enterolactone by human intestinal bacteria. *Biol Pharm Bull.* 2007;30:2204–2206.
39. Jin J-S, Zhao Y-F, Nakamura N, et al. Enantioselective dehydroxylation of enterodiols and enterolactone precursors by human intestinal bacteria. *Biol Pharm Bull.* 2007;30:2113–2119.
40. Liu C, Finegold SM, Song Y, Lawson PA. Reclassification of *Clostridium coccoides*, *Ruminococcus hansenii*, *Ruminococcus hydrogenotrophicus*, *Ruminococcus luti*, *Ruminococcus productus* and *Ruminococcus schinkii* as *Blautia coccoides* gen. nov., comb. nov., *Blautia hansenii* comb. nov., *Blautia hydroge*. *Int J Syst Evol Microbiol.* 2008;58:1896–1902.
41. Coulman KD, Liu Z, Hum WQ, Michaelides J, Thompson LU. Whole sesame seed is as rich a source of mammalian lignan precursors as whole flaxseed. *Nutr Cancer.* 2005;52:156–165.
42. Peñalvo JL, Heinonen S-M, Aura A-M, Adlercreutz H. Dietary sesamin is converted to enterolactone in humans. *J Nutr.* 2005;135:1056–1062.
43. Jan K-C, Hwang LS, Ho C-T. Biotransformation of sesaminol triglucoside to mammalian lignans by intestinal microbiota. *J Agric Food Chem.* 2009;57:6101–6106.
44. Liu Z, Saarinen NM, Thompson LU. Sesamin is one of the major precursors of mammalian lignans in sesame seed (*Sesamum indicum*) as observed in vitro and in rats. *J Nutr.* 2006;136:906–912.
45. Zhu X, Zhang X, Sun Y, et al. Purification and fermentation in vitro of sesaminol triglucoside from sesame cake by human intestinal microbiota. *J Agric Food Chem.* 2013;61:1868–1877.
46. Saarinen NM, Smeds AI, Peñalvo JL, Nurmi T, Adlercreutz H, Mäkelä S. Flaxseed ingestion alters ratio of enterolactone enantiomers in human serum. *J Nutr Metab.* 2010. Available from: <http://dx.doi.org/10.1155/2010/403076>.
47. Jin J-S, Hattori M. Further studies on a human intestinal bacterium *Ruminococcus* sp. END-1 for transformation of plant lignans to mammalian lignans. *J Agric Food Chem.* 2009;57:7537–7542.
48. Jin J-S, Tobo T, Chung M-H, Ma C, Hattori M. Transformation of trachelogenin, an aglycone of tracheloside from safflower seeds, to phytoestrogenic (–)-enterolactone by human intestinal bacteria. *Food Chem.* 2012;134:74–80.
49. Adlercreutz H. Lignans and human health. *Crit Rev Clin Lab Sci.* 2007;44:483–525.
50. Buck K, Zaineddin AK, Vrieling A, Linseisen J, Chang-Claude J. Meta-analyses of lignans and enterolignans in relation to breast cancer risk. *Am J Clin Nutr.* 2010;92:141–153.
51. Cardoso Carraro JC, Dantas MI de S, Espescht ACR, Martino HSD, Ribeiro SMR. Flaxseed and human health: reviewing benefits and adverse effects. *Food Rev Int.* 2012;28:203–230.
52. Cassidy A, Hanley B, Lamuela-Raventos RM. Isoflavones, lignans and stilbenes – origins, metabolism and potential importance to human health. *J Sci Food Agric.* 2000;80:1044–1062.
53. Cornwell T, Cohick W, Raskin I. Dietary phytoestrogens and health. *Phytochemistry.* 2004;65:995–1016.
54. Gikas PD, Mokbel K. Phytoestrogens and the risk of breast cancer: a review of the literature. *Int J Fertil Women's Med.* 2005;50:250–258.

55. Peterson J, Dwyer J, Adlercreutz H, Scalbert A, Jacques P, McCullough ML. Dietary lignans: physiology and potential for cardiovascular disease risk reduction. *Nutr Rev*. 2010;68:571–603.
56. Bedell S, Nachtigall M, Naftolin F. The pros and cons of plant estrogens for menopause. *J Steroid Biochem Mol Biol*. 2014;139:225–236.
57. Velentzis LS, Cantwell MM, Cardwell C, Keshtgar MR, Leathem AJ, Woodside JV. Lignans and breast cancer risk in pre- and post-menopausal women: meta-analyses of observational studies. *Br J Cancer*. 2009;100:1492–1498.
58. Touré A, Xueming X. Flaxseed lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components, and health benefits. *Compr Rev Food Sci Food Saf*. 2010;9:261–269.
59. Webb AL, McCullough ML. Dietary lignans: potential role in cancer prevention. *Nutr Cancer*. 2005;51:117–131.
60. McCann MJ, Gill CIR, McGlynn H, Rowland IR. Role of mammalian lignans in the prevention and treatment of prostate cancer. *Nutr Cancer*. 2005;52:1–14.
61. Saarinen NM, Tuominen J, Pylkkänen L, Santti R. Assessment of information to substantiate a health claim on the prevention of prostate cancer by lignans. *Nutrients*. 2010;2:99–115.
62. Heinonen S, Nurmi T, Liukkonen K, et al. In vitro metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *J Agric Food Chem*. 2001;49:3178–3186.
63. Aehle E, Müller U, Eklund PC, Willför SM, Sippl W, Dräger B. Lignans as food constituents with estrogen and antiestrogen activity. *Phytochemistry*. 2011;72:2396–2405.
64. Kurzer MS, Xu X. Dietary phytoestrogens. *Annu Rev Nutr*. 1997;17:353–381.
65. Mueller SO, Simon S, Chae K, Metzler M, Korach KS. Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor alpha (ERalpha) and ERbeta in human cells. *Toxicol Sci*. 2004;80:14–25.
66. Adolphe JL, Whiting SJ, Juurlink BHJ, Thorpe LU, Alcorn J. Health effects with consumption of the flax lignan secoisolariciresinol diglucoside. *Br J Nutr*. 2010;103:929–938.
67. Penttinen P, Jaehrling J, Damdimopoulos AE, et al. Diet-derived polyphenol metabolite enterolactone is a tissue-specific estrogen receptor activator. *Endocrinology*. 2007;148:4875–4886.
68. Saleem M, Kim HJ, Ali MS, Lee YS. An update on bioactive plant lignans. *Nat Prod Rep*. 2005;22:696–716.
69. Lof M, Weiderpass E. Epidemiologic evidence suggests that dietary phytoestrogen intake is associated with reduced risk of breast, endometrial, and prostate cancers. *Nutr Res*. 2006;26:609–619.
70. Zeleniuch-Jacquotte A, Lundin E, Micheli A, et al. Circulating enterolactone and risk of endometrial cancer. *Int J Cancer*. 2006;119:2376–2381.
71. Bandera EV, Williams MG, Sima C, et al. Phytoestrogen consumption and endometrial cancer risk: a population-based case-control study in New Jersey. *Cancer Causes Control*. 2009;20:1117–1127.
72. Zamora-Ros R, Agudo A, Luján-Barroso L, et al. Dietary flavonoid and lignan intake and gastric adenocarcinoma risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Am J Clin Nutr*. 2012;96:1398–1408.
73. Cotterchio M, Boucher BA, Manno M, Gallinger S, Okey A, Harper P. Dietary phytoestrogen intake is associated with reduced colorectal cancer risk. *J Nutr*. 2006;136:3046–3053.
74. Kuijsten A, Arts ICW, Hollman PCH, van't Veer P, Kampman E. Plasma enterolignans are associated with lower colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev*. 2006;15:1132–1136.
75. Johnsen NF, Olsen A, Thomsen BLR, et al. Plasma enterolactone and risk of colon and rectal cancer in a case-cohort study of Danish men and women. *Cancer Causes & Control*. 2010;21:153–162.
76. Buck K, Zaineddin AK, Vrieling A, et al. Estimated enterolignans, lignan-rich foods, and fibre in relation to survival after postmenopausal breast cancer. *Br J Cancer*. 2011;105:1151–1157.
77. Guglielmini P, Rubagotti A, Boccardo F. Serum enterolactone levels and mortality outcome in women with early breast cancer: a retrospective cohort study. *Breast Cancer Res Treat*. 2012;132:661–668.
78. Anderson LN, Cotterchio M, Boucher BA, Kreiger N. Phytoestrogen intake from foods, during adolescence and adulthood, and risk of breast cancer by estrogen and progesterone receptor tumor subgroup among Ontario women. *Int J Cancer*. 2013;132:1683–1692.
79. McCann SE, Hootman KC, Weaver AM, et al. Dietary intakes of total and specific lignans are associated with clinical breast tumor characteristics. *J Nutr*. 2012;142:91–98.
80. Truan JS, Chen J-M, Thompson LU. Comparative effects of sesame seed lignan and flaxseed lignan in reducing the growth of human breast tumors (MCF-7) at high levels of circulating estrogen in athymic mice. *Nutr Cancer*. 2012;64:65–71.
81. Mabrok HB, Klopffleisch R, Ghanem KZ, Clavel T, Blaut M, Loh G. Lignan transformation by gut bacteria lowers tumor burden in a gnotobiotic rat model of breast cancer. *Carcinogenesis*. 2012;33:203–208.
82. McCann MJ, Rowland IR, Roy NC. Anti-proliferative effects of physiological concentrations of enterolactone in models of prostate tumorigenesis. *Mol Nutr Food Res*. 2013;57:212–224.
83. Ward HA, Kuhnle GGC. Phytoestrogen consumption and association with breast, prostate and colorectal cancer in EPIC Norfolk. *Arch Biochem Biophys*. 2010;501:170–175.
84. Jackson MD, McFarlane-Anderson ND, Simon GA, Bennett FI, Walker SP. Urinary phytoestrogens and risk of prostate cancer in Jamaican men. *Cancer Causes Control*. 2010;21:2249–2257.
85. Ward HA, Kuhnle GGC, Mulligan AA, Lentjes MAH, Luben RN, Khaw K. Breast, colorectal, and prostate cancer risk in the European Prospective Investigation into Cancer and Nutrition—Norfolk in relation to phytoestrogen intake derived from an improved database. *Am J Clin Nutr*. 2010;91:440–448.
86. Kuhnle GGC, Dell'Aquila C, Aspinall SM, Runswick SA, Mulligan AA, Bingham SA. Phytoestrogen content of foods of animal origin: dairy products, eggs, meat, fish, and seafood. *J Agric Food Chem*. 2008;56:10099–10104.
87. Peñalvo JL, López-Romero P. Urinary enterolignan concentrations are positively associated with serum HDL cholesterol and negatively associated with serum triglycerides in U.S. adults. *J Nutr*. 2012;142:751–756.
88. Pietrofesa R, Turowski J, Tyagi S, et al. Radiation mitigating properties of the lignan component in flaxseed. *BMC Cancer*. 2013;13:179.
89. Sonestedt E, Wirfält E. Enterolactone and breast cancer: methodological issues may contribute to conflicting results in observational studies. *Nutr Res*. 2010;30:667–677.

90. Kuijsten A, Arts ICW, Vree TB, Hollman PCH. Pharmacokinetics of enterolignans in healthy men and women consuming a single dose of secoisolariciresinol diglucoside. *J Nutr.* 2005;135:795–801.
91. Possemiers S, Bolca S, Eckhaut E, Depypere H, Verstraete W. Metabolism of isoflavones, lignans and prenylflavonoids by intestinal bacteria: producer phenotyping and relation with intestinal community. *FEMS Microbiol Ecol.* 2007;61:372–383.
92. Lampe JW, Chang J-L. Interindividual differences in phytochemical metabolism and disposition. *Semin Cancer Biol.* 2007;17:347–353.
93. Setchell K, Adlercreutz H. Mammalian lignans and phytoestrogens. Recent studies on their formation, metabolism, and biological role in health and disease. In: Rowland IR, ed. *Role of the Gut Flora in Toxicity and Cancer.* London: Academic Press; 1988:316–345.
94. Adlercreutz H, Van der Wildt J, Kinzel J. Lignan and isoflavonoid conjugates in human urine. *J Steroid Biochem Mol Biol.* 1995;52:97–103.
95. Jansen GHE, Arts ICW, Nielen MWF, Müller M, Hollman PCH, Keijer J. Uptake and metabolism of enterolactone and enterodiol by human colon epithelial cells. *Arch Biochem Biophys.* 2005;435:74–82.
96. Jacobs E, Kulling SE, Metzler M. Novel metabolites of the mammalian lignans enterolactone and enterodiol in human urine. *J Steroid Biochem Mol Biol.* 1999;68:211–218.
97. Okura T, Ibe M, Umegaki K, Shinozuka K, Yamada S. Effects of dietary ingredients on function and expression of P-Glycoprotein in human intestinal epithelial cells. *Biol Pharm Bull.* 2010;33:255–259.
98. Adlercreutz H, Fotsis T, Bannwart C, et al. Determination of urinary lignans and phytoestrogen metabolites, potential antiestrogens and anticarcinogens, in urine of women on various habitual diets. *J Steroid Biochem.* 1986;25:791–797.
99. Stumpf K, Pietinen P, Puska P, Adlercreutz H. Changes in serum enterolactone, genistein, and daidzein in a dietary intervention study in Finland. *Cancer Epidemiol Biomarkers Prev.* 2000;9:1369–1372.
100. Mazur WM, Uehara M, Wähälä K, Adlercreutz H. Phyto-estrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry-meal in human subjects. *Br J Nutr.* 2000;83:381–387.
101. Kirkman LM, Lampe JW, Campbell DR, Martini MC, Slavin JL. Urinary lignan and isoflavonoid excretion in men and women consuming vegetable and soy diets. *Nutr Cancer.* 1995;24:1–12.
102. Juntunen KS, Mazur WM, Liukkonen KH, et al. Consumption of wholemeal rye bread increases serum concentrations and urinary excretion of enterolactone compared with consumption of white wheat bread in healthy Finnish men and women. *Br J Nutr.* 2000;84:839–846.
103. Vanharanta M, Mursu J, Nurmi T, et al. Phloem fortification in rye bread elevates serum enterolactone level. *Eur J Clin Nutr.* 2002;56:952–957.
104. McIntosh GH, Noakes M, Royle PJ, Foster PR. Whole-grain rye and wheat foods and markers of bowel health in overweight middle-aged men. *Am J Clin Nutr.* 2003;77:967–974.
105. Kristensen MB, Hels O, Tetens I. No changes in serum enterolactone levels after eight weeks' intake of rye-bran products in healthy young men. *Scandinavian Journal of Food & Nutrition.* 2005;49:62–67.
106. Knust U, Spiegelhalter B, Strowitzki T, Owen RW. Contribution of linseed intake to urine and serum enterolignan levels in German females: a randomised controlled intervention trial. *Food Chem Toxicol.* 2006;44:1057–1064.
107. Lampe JW, Martini MC, Kurzer MS, Adlercreutz H, Slavin JL. Urinary lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. *Am J Clin Nutr.* 1994;60:122–128.
108. Kurzer MS, Lampe JW, Martini MC, Adlercreutz H. Fecal lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. *Cancer Epidemiol Biomarkers Prev.* 1995;4:353–358.
109. Nesbitt PD, Lam Y, Thompson LU. Human metabolism of mammalian lignan precursors in raw and processed flaxseed. *Am J Clin Nutr.* 1999;69:549–555.
110. Kuijsten A, Arts ICW, van't Veer P, Hollman PCH. The relative bioavailability of enterolignans in humans is enhanced by milling and crushing of flaxseed. *J Nutr.* 2005;135:2812–2816.
111. Aura A-M, Myllymäki O, Bailey M, Penalvo JL, Adlercreutz H, Poutanen K. Interrelationships between carbohydrate type, phenolic acids and initial pH on in vitro conversion of enterolactone from rye lignans. In: Salovaara H, Gates F, Tenkanen M, eds. *Dietary Fibre: Components and Functions.* Wageningen, The Netherlands: Wageningen Academic Publishers; 2007:235–245.
112. Adlercreutz H, Honjo H, Higashi A, et al. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am J Clin Nutr.* 1991;54:1093–1100.
113. Horner NK, Kristal AR, Prunty J, Skor HE, Potter JD, Lampe JW. Dietary determinants of plasma enterolactone. *Cancer Epidemiol Biomarkers Prev.* 2002;11:121–126.
114. Lampe JW, Gustafson DR, Hutchins AM, et al. Urinary isoflavonoid and lignan excretion on a Western diet: relation to soy, vegetable, and fruit intake. *Cancer Epidemiol Biomarkers Prev.* 1999;8:699–707.
115. Milder IEJ, Kuijsten A, Arts ICW, et al. Relation between plasma enterodiol and enterolactone and dietary intake of lignans in a Dutch endoscopy-based population. *J Nutr.* 2007;137:1266–1271.
116. Kilkkinen A, Stumpf K, Pietinen P, Valsta LM, Tapanainen H, Adlercreutz H. Determinants of serum enterolactone concentration. *Am J Clin Nutr.* 2001;73:1094–1100.
117. Jacobs DR, Pereira MA, Stumpf K, Pins JJ, Adlercreutz H. Whole grain food intake elevates serum enterolactone. *Br J Nutr.* 2002;88:111–116.
118. Cunnane SC, Hamadeh MJ, Liede AC, Thompson LU, Wolever TM, Jenkins DJ. Nutritional attributes of traditional flaxseed in healthy young adults. *Am J Clin Nutr.* 1995;61:62–68.
119. Clavel T, Henderson G, Alpert C-A, et al. Intestinal bacterial communities that produce active estrogen-like compounds enterodiol and enterolactone in humans. *Appl Environ Microbiol.* 2005;71:6077–6085.
120. Lampe JW, Fredstrom SB, Slavin JL, Potter JD. Sex differences in colonic function: a randomised trial. *Gut.* 1993;34:531–536.
121. Kilkkinen A, Pietinen P, Klaukka T, Virtamo J, Korhonen P, Adlercreutz H. Use of oral antimicrobials decreases serum enterolactone concentration. *Am J Epidemiol.* 2002;155:472–477.
122. Johnsen NF, Hausner H, Olsen A, et al. Intake of whole grains and vegetables determines the plasma enterolactone concentration of Danish women. *J Nutr.* 2004;134:2691–2697.
123. Rybak ME, Sternberg MR, Pfeiffer CM. Sociodemographic and lifestyle variables are compound- and class-specific correlates of urine phytoestrogen concentrations in the U.S. population. *J Nutr.* 2013;1–9.

This page intentionally left blank