Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Food Science

Factors influencing interpersonal variability in plasma enterolactone concentration

Faktorer som påverkar interindividuell variation i plasmaenterolakton

Elin Hålldin

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Abstract

Lignans are biologically active diphenolic plant compounds with potential health affecting properties. Some epidemiological studies have demonstrated that a high lignan intake is associated with risk reduction of several chronic diseases such as breast, prostate and colorectal cancer as well as cardiovascular disease. The main sources of lignans are oil seeds (such as flaxseed and sesame seed), whole grains, legumes, vegetables, fruits, berries and some beverages such as coffee, tea and wine. The mammalian lignan enterolactone (ENL) is produced by the intestinal microflora in the upper part of the colon from dietary precursors, and are thereafter absorbed into the circulation. A wide range of plasma ENL concentrations have been observed in human experimental end epidemiological studies, and several factors related to diet and health has been associated with plasma ENL. The aim of this literature review was to identify and evaluate the relative contribution of factors influencing interpersonal variability in plasma enterolactone concentration based on literature extracte according to certain criteria defined by a working group in an ongoing COST-action initiative, the POSITIVe. The total number of human studies fulfilling the search criteria was 96. The main determinants of plasma ENL include lignan intake, intake of lignan-rich foods, composition and activity of intestinal microflora, antimicrobial use, nutrient intake BMI, smoking, sex and age. Composition and activity of the intestinal microbiota seem to be the most critical factor governing interpersonal variability in plasma ENL concentration. Intake of lignan-rich foods, constipation and lifestyle factors such as smoking and BMI explain only a small part of the variation, whereas antimicrobials have a more pronounced effect on plasma ENL. The impact of sociodemographic factors such as age, gender, education level and race/ethnicity may only be confounding factors associated with dietary patterns rather than independent determinants of plasma ENL concentration. The findings of this literature review complement those of earlier studies, and should be taken into account when considering interpersonal variability of lignans in humans.

Keywords: Enterolactone, lignans, interpersonal variability, intestinal microflora, phytoestrogens, plasma.

Sammanfattning

Lignaner är en grupp biologiskt aktiva föreningar i växter med potentiella hälsoeffekter. Vissa epidemiologiska studier har visat att ett högt lignanintag minskar risken att drabbas av hjärt- och kärlsjukdom och vissa cancerformer såsom bröst-, prostata-, tjocktarms- och ändtarmscancer. Lignaner finns främst i oljeväxter (såsom linfrö och sesamfrö), fullkornsprodukter, baljväxter, grönsaker, frukt, bär och vissa drycker såsom kaffe, te och vin. Enterolakton (ENL) är en typ av lignan som bildas av tarmfloran av vissa dietära prekursorer innan de absorberas till blodet. En stor variation av plasmakoncentrationer av ENL har uppmätts i experimentella och epidemiologiska studier och ett flertal faktorer relaterade till kost och hälsa har associerats till denna variation. Syftet med denna litteraturstudie var att analysera faktorer som förklarar interindividuell variation i plasmaenterolakton baserat på vetenskapliga artiklar extraherade inom ett projekt i COST-nätverket POSITIVe. Totalt uppfyllde 96 humanstudier de definierade sökkriterierna. De främsta faktorerna som påverkar plasmakoncentrationen av ENL är lignanintag, intag av lignan-rika livsmedel, aktivitet och sammansättning av tarmfloran, antibiotikaanvändning, näringsintag, *body mass index* (BMI), rökning, kön och ålder. Tarmflorans sammansättning och dess aktivitet har visat sig vara den mest kritiska faktorn som påverkar interindividuell variation i plasmaenterolakton. Intag av lignan-rika livsmedel, förstoppning och livsstilsfaktorer såsom rökning och BMI förklarar endast en liten del av den observerade interindividuella variationen, varav antibiotikaanvändning har en mer betydande effekt. Sociodemografiska faktorer såsom ålder, kön, utbildningsnivå och ras/etnicitet verkar dock endast vara förväxlingsfaktorer (*confounders*) som speglar en individs kostvanor snarare än är oberoende faktorer som påverkar plasmakoncentrationen av ENL. Resultatet av denna litteraturstudie kompletterar tidigare studier och bör beaktas vid undersökning av lignaners variation i människan.

Nyckelord: Enterolakton, lignaner, interindividuell variation, tarmflora, fytoöstrogener, plasma.

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Abbreviations

ARC	Arctigenin
BMI	Body-mass index
ENL	Enterolactone
HMR	7-Hydroxymatairesinol
isoLAR	Isolariciresinol
LAR	Lariciresinol
MAT	Matairesinol
PIN	Pinoresinol
SEC	Secoisolariciresinol
SYR	Syringaresinol
SES	Sesamin

1 Introduction

Lignans are naturally occurring diphenolic plant compounds with potentially favourable effects on human health (Morisset *et al.*, 2009). High plasma concentrations of lignans have been associated with a decreased risk of several chronic diseases such as breast, prostate and colorectal cancer as well as for cardiovascular diseases (Adlercreutz, 2007). It have been suggested that lignans may affect cancer incidence by altering production and metabolism of steroid hormones and through actions at a cellular level (Adlercreutz, 2007). Lignans belong to the phytoestrogens and exhibit both oestrogenic and anti-oestrogenic activities in humans, although their affinities for the oestrogen receptor are approximately 1000-10000 times lower than oestradiol (Rice & Whitehead, 2006). Lignans bind with highest affinity to oestrogen receptor beta (Adlercreutz, 2002).

Lignans are widely distributed in the plant kingdom, and the main food sources are oil seeds (such as flaxseed and sesame seed), whole grains, legumes, vegetables, berries and fruits. Also some beverages are rich in lignans, such as coffee, tea and wine (Milder *et al.*, 2005a). A wide range of lignans exist, although the main plant lignans include secoisolariciresinol (SEC), matairesinol (MAT), pinoresinol (PIN), lariciresinol (LAR), syringaresinol (SYR), sesamin (SES), 7-hydroxymatairesinol (HMR) and isolariciresinol (isoLAR) (Figure 1). The biological effects of lignans are related to their bioactivation to the mammalian lignans enterodiol (END) and enterolactone (ENL) (Figure 1). The production of the mammalian lignans take place in the colon by the action of gut bacteria, and are thereafter absorbed into the circulation (Borriello *et al.*, 1985).

A wide range of plasma ENL concentrations has been found among individuals in epidemiological and experimental studies (Adlercreutz *et al.*, 1993; Kilkkinen *et al.*, 2001; Pietinen *et al.*, 2001), and cross-sectional associations between plasma ENL and factors related to diet and health have been observed (Horner *et al.*, 2002; Kilkkinen *et al.*, 2001). The aim of this study was to identify and evaluate factors influencing interpersonal variability in plasma ENL concentration.

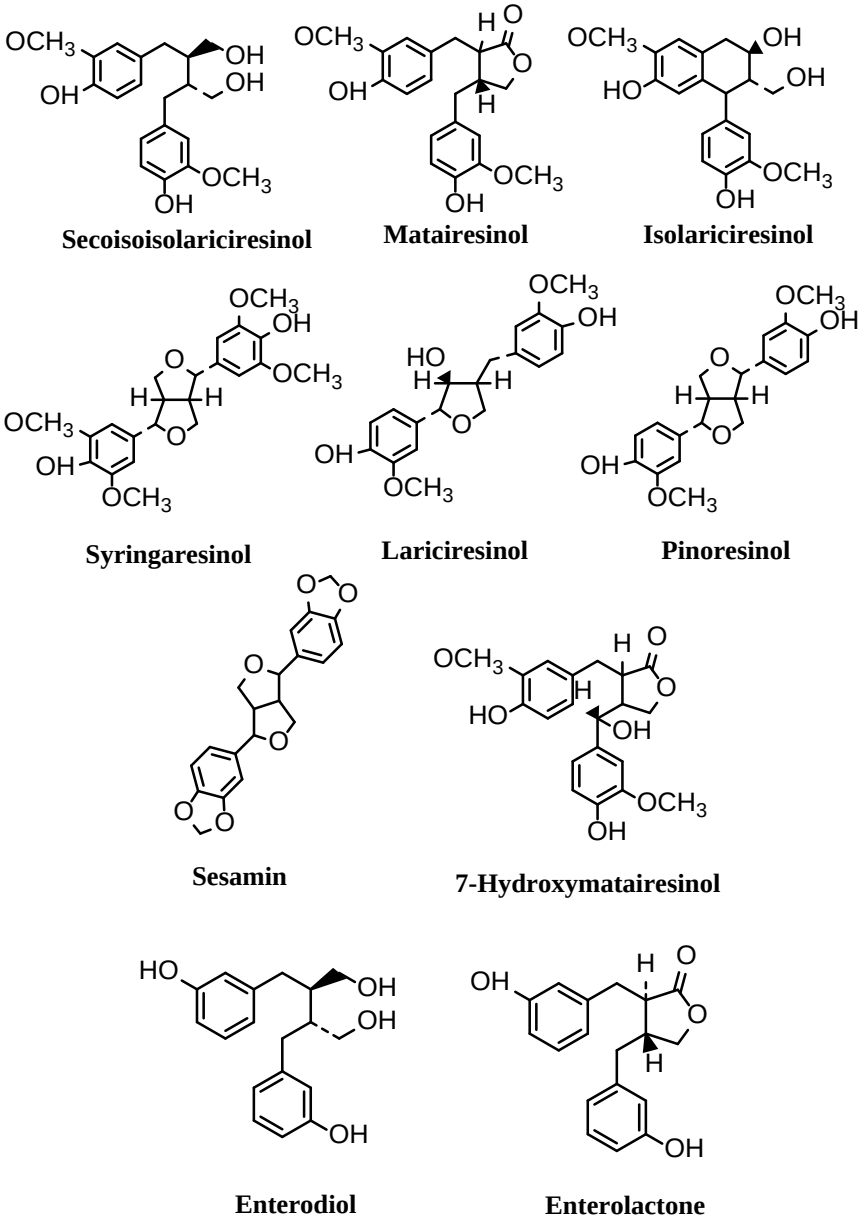

FIGURE 1 Chemical structures of common plant lignans and enterolignans. Modified from Penalvo *et al*. (2005) and Heinonen *et al.* (2001).

2 Materials and Methods

The current study is a literature review, based on scientific literature extracted according to pre-defined search criteria with the aim to identify major determinants of inter-personal variability in the concentrations of lignans in human samples. The literature extraction was conducted by a working group of experts within the COST-network POSITIVe. The literature search was conducted in PUBMED (United States National Library of Medicine) and WEB of SCIENCE.

2.1 Search terms and key words

The search terms and key words used in the literature search included HUMAN* AND (Lignan* OR Secoisolariciresinol* OR Matairesinol* OR Lariciresinol* OR Pinoresinol* OR Syringaresinol* OR Isolariciresinol* OR Arctigenin* OR Trachelogenin* OR Medioresinol* OR 1-Acetoxypinoresinol* OR Secoisolariciresinol di-O-glucoside* OR Sesamin* OR Sesamolin* OR Sesamolinol* OR Sesaminol* OR Sesaminol 2'-O-b-D-glucosyl (1->2)-O-[b-D-glucosyl (1->6)]-b-D-glucoside* OR Sesaminol 2'-O-b-D-glucosyl (1->6)-O-b-D-glucoside* OR Sesaminol 2'-O-b-D-glucoside* OR Sesamol* OR Sesamolinol 4'-O-b-D-glucosyl (1->6)-O-b-D-glucoside* OR 7-Hydroxymatairesinol* OR Isohydroxymatairesinol* OR Secoisolariciresinol-sesquilignan* OR Cyclolariciresinol* OR 7-Oxomatairesinol* OR Todolactol A* OR Conidendrin* OR Hydroxysecoisolariciresinol* OR Nortrachelogenin* OR Lariciresinol-sesquilignan* OR Anhydrosecoisolariciresinol* OR Dimethylmatairesinol* OR Episesamin* OR Episesaminol* OR Sesaminol 2'-O-b-D-glucosyl (1->2)-O-b-D-glucoside* OR Enterodiol* OR Enterolactone* OR Sesaminol 2-O-triglucoside* OR Schisandrin* OR Gomisin D* OR Schisandrol B* OR Tigloylgomicin H* OR Schisanhenol* OR Schisantherin A* OR Gomisin M2* OR Deoxyschisandrin* OR Schisandrin B* OR Schisandrin C* OR 2-Hydroxyenterodiol* OR 4-Hydroxyenterodiol* OR 6-Hydroxyenterodiol* OR 2-Hydroxyenterolactone* OR 4-Hydroxyenterolactone* OR 6-Hydroxyenterolactone* OR 2'-Hydroxyenterolactone* OR 4'-

Hydroxyenterolactone* OR 6'-Hydroxyenterolactone* OR 5-Hydroxyenterolactone* OR 7-Hydroxyenterolactone) AND (Bioavailab* OR pharmacokinetic* OR kinetic* OR ADME OR identif* OR colon microb* OR colon microflora OR gut microb* OR urinary excretion OR biliary excretion OR enterohepatic* OR conjugat* OR Glucuronid* OR sulfat* OR sulphat* OR Mercaptur* OR plasma OR urine OR interindividual varia* OR interpersonal varia* OR intraindividual varia* OR intrapersonal varia*) NOT drug-interactions. Additionally, a document type search was included consisting of the key words (Article OR Book Chapter OR Correction OR Editorial Material OR Letter OR Note OR Proceedings Paper OR Review).

2.2 Literature search and inclusion criteria

The total number of articles fulfilling the search criteria was 443. Of these studies, 96 were human studies that were taken further. The Lignan working group in the network POSITIVe conducted the two first screening processes, where title and abstract were reviewed for relevance, resulting in 69 articles following the criteria, and therefore included in the present analysis. Articles were included based on the following inclusion criteria: 1) Human data relevant to interindividual variation in absorption, distribution, metabolism and excretion (ADME), 2) Include main determinants of interindividual variation in lignan concentrations, 3) Knowledge on proteins/genes involved in ADME of lignans.

The remaining 69 articles were screened in full text and included if relevant according to criteria. A total number of 16 articles were included in the present study. A summary of the inclusion and exclusion of articles from the databases are shown in a flow diagram (Figure 2). Additional articles were retrieved from database links to “similar articles” and from original articles cited in references.

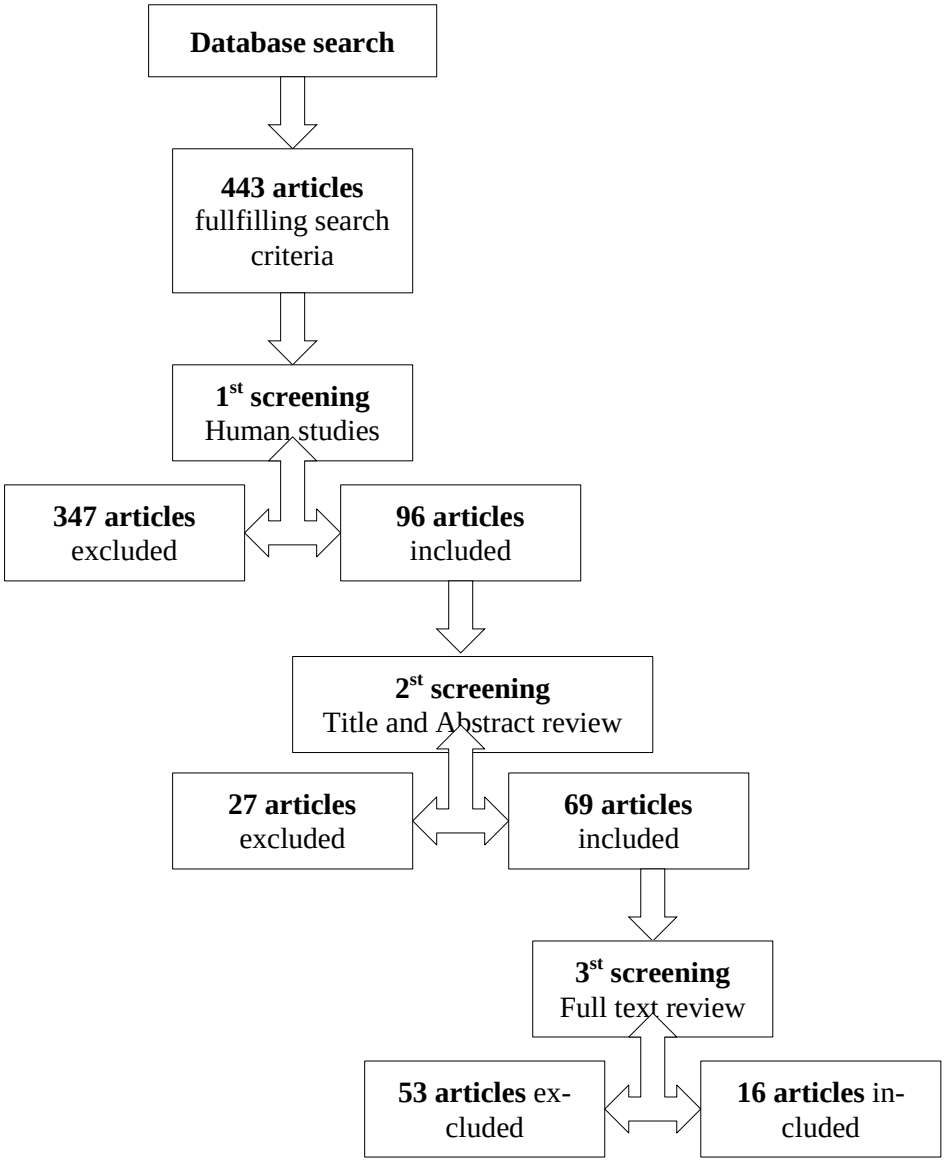

FIGURE 2 A flow diagram illustrating the retrieval process from the electronic databases PUBMED and WEB of SCIENCE.

3 Results and Discussion

3.1 Determinants of plasma ENL concentration

A wide range of factors may influence plasma ENL concentration, as reported by several studies (Table 1). The main determinants reported were lignan intake, intake of lignan-rich food, nutrient intake, composition and activity of intestinal microflora, antimicrobial use, BMI, smoking, sex and age. However, intake of lignan-rich food and the function of intestinal microflora were identified as the most important factors influencing interpersonal variability in plasma ENL concentration.

3.2 Lignan-rich foods

Flaxseed is the richest food source of mammalian lignan precursors, particularly secoisolariciresinol diglycoside (SDG). Intake leads to significant increase in plasma ENL concentration (Atkinson *et al.*, 1993; Lagkouvardos *et al.*, 2015; Morton *et al.*, 1994; Nesbitt *et al.*, 1999).

Sesame seeds have also been found to contain high amounts of lignan precursors, and are the richest source of PIN (Milder *et al.*, 2005a). Peñalvo *et al.* (2005) discovered that sesame seeds contain comparable or even higher concentrations of plant lignans than flaxseeds. The plant lignan sesamin was found in highest concentration in sesame seeds followed by PIN, LAR and HMR, which are all converted to enterolactone (Penalvo *et al.*, 2005). Other important sources of lignans were grain products such as cereals and breads, although many fruits and vegetables contribute with similar concentrations when including LAR and PIN. Alcoholic beverages including wine and beer contain relatively high amounts of lignans, whereas red wine has the highest concentration. Among the non-alcoholic beverages, tea has the highest concentration of lignans followed by coffee and juices (Milder *et al.*, 2005a).

TABLE 1 The main factors influencing interpersonal variability in plasma ENL concentration.

Lignan intake	Intake of lignan-rich food	Intestinal microflora	Nutrient intake	Antimicrobial use	BMI	Smoking	Sex	Age	Reference
			x	x	x		x	x	Adgent *et al.* (2015)
	x								Atkinson *et al.* (1993)
		x							Bolca *et al.* (2009)
			x						Grace *et al.* (2004)
x									Hallund *et al.* (2006)
	x		x		x		x	x	Horner *et al.* (2002)
	x				x	x	x	x	Kilkkinen *et al.* (2001)
				x					Kuijsten *et al.* (2006)
	x	x							Lagkouvardos *et al.* (2015)
	x						x		Linko-Parvinen *et al.* (2007)
	x								MacCann *et al.* (2007)
x	x								Mazur *et al.* (2000)
	x								Morton *et al.* (1994)
x	x		x		x				Morisset *et al.* (2009)
	x								Nesbitt et al. (1999)
x	x								Peñalvo *et al.* (2005)
x									Piller *et al.* (2006)

Several factors have been correlated with plasma ENL. In one study by Kilkkinen *et al.* (2001), determinants of serum enterolactone concentration were examined in a Finnish population of 2380 men and women. Large interpersonal variation in serum ENL was observed (CV of 83 %), where constipation and intake of lignan-rich foods were found to be the main determinants in both men and women (Kilkkinen *et al.*, 2001). Significant positive associations were found between consumption of whole-grain products, fruit and berries and serum ENL in men (Horner *et al.*, 2002; Kilkkinen *et al.*, 2001). In contrast, no significant increase in serum ENL was observed among 16 young healthy men consuming rye-products for 8 weeks, potentially explained by a decrease in transit-time caused by the high fiber intake (Kristensen *et al.*, 2005). Men with constipation have been found to have 23 % higher median serum ENL concentration than men without reported constipation (Kilkkinen *et al.*, 2001). The determinants of serum ENL in men observed by Kilkkinen *et al.* (2001) explained only 2.7 % of the interpersonal variation in serum ENL, suggesting a great impact of unmeasured determinants such as intestinal microflora. In women, the only significant and positively associated dietary determinant of serum ENL concentration was consumption of vegetables (Kilkkinen *et al.*, 2001). Furthermore, age and constipation were also found to be significant predictors positively correlated with serum ENL, whereas BMI and smoking were negative associated with serum ENL among women (Kilkkinen *et al.*, 2001). The determinants of serum ENL in women explained almost 14 % of the interpersonal variation (Kilkkinen *et al.*, 2001).

Plasma ENL have also been found to correlate positively with tea, coffee and alcoholic beverages along with 5 specific plant food groups in a study among 78 men and 115 women, non-smoking Americans. However, no gender-specific associations were found. Two blood samples per subject were obtained with similar results, showing plasma ENL concentrations ranging from 0.6-155.3 nmol/L (Horner *et al.*, 2002). In addition, consumption of a fixed dose of lignans-rich berries have also been observed to cause high interpersonal variations in plasma ENL before and during intervention among 7 subjects consuming a phytoestrogen-free diet (Mazur *et al.*, 2000). The basal plasma ENL concentration ranged from 1.7-22.4 nmol/L (mean= 10.3 nmol/L) before intake of lignan-rich berries. The concentration of serum ENL decreased during the next 4 h (samples taken at 0.5, 1, 2, 4 h after berry meal) ranging from 1.1-21.0 nmol/L (mean=9.9 nmol/L), 1.5-24.1 (mean=10.1 nmol/L), 1.5-19.8 nmol/L (mean=9.2 nmol/L), 0.9-17.4 nmol/L (mean=9.3 nmol/L), respectively. The mean plasma ENL concentration at 24 h was significantly higher than the mean ENL concentrations the first 4 hours, ranging from 2.4-30.2 nmol/L (mean= 20.6 nmol/L) (Mazur *et al.*, 2000).

Moreover, a significant increase and high inter-individual variation in serum ENL was observed among healthy postmenopausal women after a 6-week lignan intervention period (Hallund *et al.*, 2006). After ingestion of 500 mg/d SDG iso-

lated from flaxseed, serum ENL concentration reached 385 ± 67 nmol/L from the basal level of 46 ± 8 nmol/L.

Despite the fact that several dietary factors influence serum ENL, they do not explain much of the variability. The major critical factor governing interpersonal variation is probably the composition and activity of the bacterial flora in the upper part of the colon (Kilkkinen *et al.*, 2001).

3.3 Lignan intake

Plant lignan intake has been positively correlated with serum ENL concentrations (Mazur *et al.*, 2000; Morisset *et al.*, 2009; Piller *et al.*, 2006). Intake and food source of lignans vary by population demographics, depending on habitual dietary patterns (Boker *et al.*, 2002), but also among individuals consuming the same diet over time (Kilkkinen *et al.*, 2001). Other factors causing variability in lignan intake include differences in food composition databases, methodological differences and number of mammalian lignan precursors included in analysis (Peterson *et al.*, 2010). Recent studies tend to have more extensive lignan analysis of foods, resulting in somewhat higher estimations of total lignan intake than earlier studies. The variability of lignan intake estimates between studies may complicate interpretation, especially in meta-analyses (Peterson *et al.*, 2010).

The intake of main dietary lignans in Western populations vary between 0.15-1.1 mg/d, based on food content of SEC and MAT (Boker *et al.*, 2002; Horn-Ross *et al.*, 2001). The main differences in the intake between regions are primarily due to varying lignan content in commonly consumed food (Webb & McCullough, 2005). In Finland, the average intake of lignans was reported to 434 μg/d (Valsta *et al.*, 2003). Another study by Kilkkinen *et al.* (2003) estimated the mean lignan intake of Finnish men and women to 173 μg/d and 151 μg/d, respectively. Lignans were found to be common components of the Finnish diet, but the mean daily intake was comparably low (Kilkkinen *et al.*, 2003). The average lignan intake by women in the United States has been estimated to 578 μg/d, considerably higher than the intakes reported for Finns (de Kleijn *et al.*, 2001). However, food consumption and compiled databases on lignans were not comparable between the studies. Also, the main sources of dietary lignans differed (de Kleijn *et al.*, 2001). In Finland, the main sources of lignans were seeds, cereals, fruit, berries and vegetables (Valsta *et al.*, 2003), while the main source in the USA was fruits (de Kleijn *et al.*, 2001). Furthermore, the mean lignan intake by Dutch women was estimated to 560 μg/d and derived mainly from breads, nuts and seeds primarily constituting of SEC (Linseisen *et al.*, 2004).

Lignan density in the diet has been found to correlate positively with serum ENL concentration (Kilkkinen *et al.*, 2003). However, no association between lignan density and serum ENL existed in subjects who used antimicrobials the

proceeding year (Kilkkinen *et al.*, 2003). LAR and PIN have been found to correlate more strongly to the total lignan intake than SEC and MAT (Milder *et al.*, 2005b). In a representative sample of Dutch women, SEC and MAT only contributed 25 % to the average daily intake of 1241 µg/d, while LAR and PIN contributed with 75 %. Epidemiological studies based on intake of four lignan precursors therefore differ substantially in the classification of subjects, compared to studies only based on SEC and MAT (Milder *et al.*, 2005b).

Several new mammalian lignan precursors were discovered by Heinonen *et al.* (2001), including PIN, SYR, ARC, HMR, isoLAR and LAR. Only LAR and PIN were converted to mammalian lignans, and to some extent, also ARC and isoLAR were metabolized to ENL. Total intake of SEC, MAT, LAR and PIN have been found to correlate more strongly with plasma ENL compared to only SEC and MAT (Milder *et al.*, 2007a). This may imply a more efficient conversion of SEC and MAT into enterolactone.

3.4 Intestinal microflora

Interpersonal variation of plasma ENL have been linked to differences in individual lignan absorption and metabolism by intestinal microflora (Adlercreutz, 1998). Human intestinal bacteria are essential for the conversion of plant lignans to mammalian lignans, which was discovered in the early 1980's (Axelson *et al.*, 1982; Borriello *et al.*, 1985). Lignan-converting bacterial strains were discovered 15 years later (Wang *et al.*, 2000). Intestinal bacteria converting SDG via SEC into mammalian lignans include phylogenetically and metabolically diverse bacteria, primarily members of the dominant human intestinal microbiota (Clavel *et al.*, 2006). The bacterial conversion of dietary SDG involves deglycosylation, demethylation, dehydroxylation and dehydrogenation (Wang *et al.*, 2000). Each biochemical reaction is catalysed by a consortia of bacteria that share metabolic intermediates (Heinonen *et al.*, 2001). However, no single bacteria can completely metabolize SDG to ENL (Clavel *et al.*, 2006).

Several bacteria have been identified to be involved in the deglycosylation step, including strains of *Bacteroides distasonis*, *Bacteroides fragilis*, *Bacteroides ovatus*, *Clostridium cocleatum* and *Clostridium* sp. SDG-Mt85-3Db. The demethylation step has been found to be catalysed by strains of *Butyribacterium methylotrophicum*, *Eubacterium callanderi*, *Eubacterium limosum* and *Peptostreptococcus productus*. The hydroxylation step is catalysed by strains of *Clostridium scindens* and *Eggerthella lenta*, whereas the dehydrogenation step is catalysed by the strain ED-Mt61/PYG-s6 (Clavel et al., 2006).

The metabolism of plant lignans is complex, involving conversion of PIN to LAR which is further metabolized to SEC and MAT, which are directly converted

to END and ENL, respectively. END is thereafter metabolized to yield the oxidised end product ENL (Heinonen *et al.,* 2001). Borriello *et al.* (1985) demonstrated the importance of viable bacteria during conversion of END to ENL by human faecal flora, and that a bacterial concentration of up to 10^3/g faeces were required. Furthermore, depending on the intake of dietary precursor, several metabolic pathways operate to produce END and ENL (Borriello *et al.,* 1985).

The efficiency of different plant lignan precursors conversion into END and ENL varies between 0 and 100 %, based on 24-h incubations with different human faecal inocula (Heinonen *et al.,* 2001). However, some individuals have been reported to produce little or no ENL in response to flaxseed intervention among 9 premenopausal healthy women (Nesbitt *et al.,* 1999). Several studies provide insights of the possibility that a subgroup of individuals may lack the bacteria or appropriate intestinal environment necessary for oxidation of END to ENL (Kuijsten *et al.,* 2005; Lampe *et al.,* 1994; Nesbitt *et al.,* 1999). Wide interpersonal differences in lignan metabolism have also been observed in a number of studies, although the cause of such variation as well as possible health effects have not been fully established (Adlercreutz *et al.,* 1986; Adlercreutz *et al.,* 1981; Cunnane *et al.,* 1995; Hutchins *et al.,* 2001; Lampe *et al.,* 1994; Nesbitt *et al.,* 1999; Rowland *et al.,* 1999).

Axelson *et al*. (1982) offered some important insight into which factors that influence the production rate of END and ENL, including composition of the microflora, the intestinal transit time, and the redox level of the large intestine. The frequency of bowel movement is another factor which has been negatively associated with plasma ENL (Adgent & Rogan, 2015; Johnsen *et al.,* 2004), where a high number of bowel movements decrease the transit time through the colon, resulting in reduced conversion and absorption of lignans (Johnsen *et al.,* 2004).

Clavel *et al.* (2005) discovered that the conversion of dietary lignans derive from catalytic activity of both dominant and subdominant anaerobic bacterial communities in the human intestinal tract. Two-thirds of the individuals in the study had high concentrations of ENL- producing organisms that correlated positively with high concentrations of *Peptostreptococcus productus* and *Clostridium coccoides.* The interpersonal variation in ENL production also depended on high concentration of *Atopobium* group, including *E. lenta,* which correlated positively with high concentrations of ENL-producing organisms (Clavel *et al.,* 2005).

In a recent study by Lagkouvardos *et al.* (2015), 9 healthy male adults were subject to a 1-week flaxseed intervention where gut metabolites and bacterial communities were examined. The results showed that overall diversity and composition of dominant faecal bacteria remained individual specific during the study, and that two abundant species including *Ruminococcus bromii* and *Ruminococcus*

lactaris were positively associated with ENL production (Lagkouvardos *et al.*, 2015).

Lignan metabolism in the human gastrointestinal tract has also been found to be greatly influenced by specific bacteria with enantioselective dehydroxylation and oxidation capabilities (Jin *et al.*, 2007). Clavel *et al.* (2007) observed that the strain ED-Mt61/PYG-s6 exhibit enantiospecific properties, thus only half of the initial concentration of END were converted to ENL in the study. Also, no ENL was detected when the strain was incubated with only (-)-END (Clavel *et al.*, 2007). In addition, Jin et al. (2007) observed enantioselective oxidation of END to ENL by the bacteria, *Ruminococcus* sp. END-1 and END-2. According to newest taxonomic classification, the strain END-2 has been found to be more closely related to *Blautia producta* and *Blautia coccoides* (Liu *et al., 2008).* However, since other strains of *B. producta* are incapable of catalysing dehydrogenation of END it has been suggested that conversion of lignan by bacteria is strain-specific (Clavel *et al.*, 2005).

Interpersonal differences in gut microbial community result in interpersonal variation in plasma ENL (Clavel *et al.*, 2005; Possemiers *et al.*, 2007), where a larger amount of ENL is produced in individuals with higher cell densities of ENL- producing bacteria. Women have been found to have higher abundance of ENL-producing organisms than men (Clavel *et al.*, 2005), but no gender differences in ENL metabolism was observed in a study of ENL concentrations in serum and urine after a rye bread diet (Juntunen *et al.*, 2000). In contrast, Linko-Parvinen et al. (2007) observed a significant increase in serum ENL concentration by whole-grain rye intake only in men. Eeckhaut *et al.* (2008) analysed the metabolism of the plant lignan SDG in human intestinal microbiota from one good and one moderate enterolignan producer. The results demonstrate important differences in enterolignan production, with the conclusion that human intestinal microbiota is subject to large interpersonal variation (Eeckhaut *et al.*, 2008), which is in accordance with other studies (Bolca *et al.*, 2009).

3.4 Use of antimicrobials

The use of oral antimicrobials is associated with decreased serum ENL concentration due to their major impact on the intestinal microflora (Adgent & Rogan, 2015; Kuijsten *et al.*, 2006). In a study by Kuijsten et al. (2006), subjects using antimicrobials had substantially lower median plasma ENL concentration. Among antibiotic users, the mean ENL concentration was 6.9 nmol/L whereas the corresponding concentration among nonusers was 13.6 nmol/L. It has been observed that antimicrobial use before serum sampling result in significant lower serum ENL concentration compared with nonusers and that the effect persist up to 12-16

months (Kilkkinen *et al.*, 2002). Also, number of treatments and the time since the last treatment were found to be associated with serum ENL concentration, whereas the most recent use of antimicrobials were associated with the lowest ENL concentrations. Different antimicrobials may also contribute to variability in serum ENL. The lowering effect of antimicrobials is not influenced by other factors causing variation in plasma ENL, such as consumption of lignan-rich food, smoking, BMI or constipation. Moreover, no effect modification in antimicrobial use by gender has been observed (Kilkkinen *et al.*, 2002). Horn-Ross *et al.* (1997) studied the effect of antimicrobials on serum ENL, but found no significant difference between users and nonusers. However, data on how recently the antimicrobials had been used was not collected.

Previous studies have reported a needed restoration period of 2 weeks for the intestinal microflora to return to normal level after use of antimicrobials (Brismar *et al.*, 1991; Brismar *et al.*, 1993; Eckernas *et al.*, 1991). In contrast, Kilkkinen *et al.* (2002) showed that the ability of intestinal microflora to metabolize lignans normalizes more slowly, suggesting a restoration period of more than one year. In addition, an inverse association between serum ENL and number of antimicrobial prescriptions have been observed, which is in accordance with earlier findings (Edlund *et al.*, 1997). Several possible effects of antimicrobials on intestinal microflora have been suggested, including interference with ENL formation from precursors and interference with enzymatic hydrolysis of ENL conjugates excreted in bile, reducing ENL reabsorption from the gut (Kilkkinen *et al.*, 2002).

Different antimicrobials have various effects on serum ENL concentration, where macrolides have the strongest supressing effect with major impact in both the aerobic and anaerobic microflora. On the other hand, amoxicillin, phenoxymethylpenicillin and cephalosporins have been observed to cause only minor effects on intestinal microflora. Moreover, interpersonal variation may influence the effect of antimicrobials on intestinal microflora (Kilkkinen *et al.*, 2002).

3.5 Nutrient intake

Changes in dietary composition have been found to modulate the gut microbiome composition as demonstrated in several studies (Cotillard *et al.*, 2013; Hullar & Lampe, 2012; Wu *et al.*, 2011). For example, dietary fiber intake has been positively correlated with microbial diversity, and thereby affects the bioavailability of enterolignans (Hullar *et al.*, 2015). Nonetheless, intake of dietary fiber and whole-grain have been significantly associated with higher plasma ENL concentrations in several studies (Adgent & Rogan, 2015; Grace *et al.*, 2004; Horner *et al.*, 2002; Jacobs *et al.*, 2002; Johnsen *et al.*, 2004; Juntunen *et al.*, 2000; Milder *et al.*, 2005b; Milder *et al.*, 2007b; Morisset *et al.*, 2009; Sonestedt *et al.*,

2008). In a study by Horner et al. (2002), dietary fiber accounted for 13 % of the variability in plasma ENL concentration.

Fat intake has been negatively correlated with plasma ENL concentration (Johnsen *et al.*, 2004), although Horner *et al.* (2002) reported no significant association between fat-related variables and plasma ENL. Fat have been suggested to induce an inhibitory effect on the microflora, causing reduced absorption of ENL. Also, higher fat intake has been associated with lower intake of lignan-rich foods (Johnsen *et al.*, 2004).

Energy intake has also been suggested as a determinant of plasma ENL concentration, although two previous studies on determinants for plasma ENL did not adjust for energy intake in multiple analyses (Horner *et al.*, 2002; Kilkkinen *et al.*, 2001).

3.6 Lifestyle factors

Smoking has been negatively correlated with ENL concentration in several studies (Johnsen *et al.*, 2004; Kilkkinen *et al.*, 2001; Kilkkinen *et al.*, 2003; Sonestedt *et al.*, 2008). In addition, smokers have been associated with lower dietary intake of lignans than non-smokers (Milder *et al.*, 2005b). Kilkkinen et al. (2001) found that non- and former smokers had >28 % higher serum ENL concentrations compared to smokers. Similar results were observed by Johnsen et al. (2004) when analysing covariates associated with plasma ENL.

Furthermore, a high BMI has also been inversely associated with plasma ENL (Horner *et al.*, 2002; Johnsen *et al.*, 2004; Kilkkinen *et al.*, 2001; Morisset *et al.*, 2009; Sonestedt *et al.*, 2008). In a similar manner, higher BMI have been associated with lower intake of lignans (Milder *et al.*, 2005b). Kilkkinen et al. (2001) identified BMI as an independent predictor of serum ENL concentration in women but not in men. In the study, normal weight subjects had >23 % higher serum ENL concentrations compared to underweight or obese women. It has been suggested that overweight and obese individuals overestimate their consumption of lignan-rich food, since serum ENL concentrations were significantly lower compared to normal weight subjects even though their reported lignan intake were similar (Kilkkinen *et al.*, 2001).

3.7 Sociodemographic factors

Kilkkinen et al. (2001) observed high interpersonal variation when investigating determinants of serum ENL concentration. In the study, serum ENL showed high variation both between individuals and among gender ranging from 0-95.6 nmol/L (median=13.8 nmol/L) in men and from 0-182.6 nmol/L (median=16.6

nmol/L) in women (Kilkkinen *et al.*, 2001). On the other hand, only a single sample per subject was examined which might be inadequate to attain reliable ENL levels, reflecting the average lignan exposure over time (Hausner *et al.*, 2004). However, due to the large interpersonal variability (CV of 83%), the analytical variation might be of minor importance (Kilkkinen *et al.*, 2001). Lignan density has been observed to be significantly higher in women's diet (Milder *et al.*, 2005b), and might be related to differences in serum ENL among gender. Several studies have included adjustments for sex in statistical analysis (Adgent & Rogan, 2015; Horner *et al.*, 2002; Kilkkinen *et al.*, 2001; Linko-Parvinen *et al.*, 2007).

Age is another significant factor that is positively associated with serum ENL (Adgent & Rogan, 2015; Horner *et al.*, 2002; Kilkkinen *et al.*, 2001). Older individuals as well as women have been observed to have higher ENL levels than younger individuals and men (Kilkkinen *et al.*, 2001). Higher consumption of whole-grain products, fruit and berries by older individuals and women has been suggested to partly explain the differences. In addition have lignan intake been observed to increase with age (Milder *et al.*, 2005b). Moreover have constipation, which is positively related to serum ENL, been observed to be more prevalent among women and older individuals (Harari *et al.*, 1996; Sandler *et al.*, 1990). In contrast, individuals with constipation may have increased absorption of lignans due to slower intestinal motility, also causing increased serum ENL concentration (Kilkkinen *et al.*, 2001).

Education level has been suggested to influence interpersonal variation in plasma ENL (Adgent & Rogan, 2015), although it is likely a result of a healthy lifestyle induced by education level. It have been observed that higher educated individuals choose a healthier lifestyle as a result of increased knowledge of the relation between health behaviours and health outcomes (Kenkel, 1991). Educated individuals have been observed to smoke less, have lower BMI and exercise more compared to non-educated individuals (de Walque, 2007; Kenkel, 1991). A positive association between education and serum ENL concentration have been observed, although the association did not remain significant after adjustment for other factors (Kilkkinen *et al.*, 2001), showing that it is a confounding factor.

Race/ethnicity have also been suggested as a factor influencing interpersonal variation in plasma ENL (Adgent & Rogan, 2015). Hernandez *et al.* (2004) included adjustments for race/ethnicity in the statistical analysis, comparing plasma phytoestrogen levels and dietary phytoestrogen intake. The variability in plasma ENL probably only reflected dietary patterns rather than being a result of race/ethnicity. Furthermore, race/ethnicity was not included as a determinant of plasma ENL in the two previous studies evaluating determinants of plasma ENL concentration (Horner *et al.*, 2002; Kilkkinen *et al.*, 2001).

4 Conclusion

In accordance with earlier findings, composition and activity of the intestinal microbiota seem to be the most critical factor governing interpersonal variability in plasma ENL concentration. Intake of lignan-rich foods, constipation and life-style factors such as smoking and BMI explain only a small part of the variation, whereas antimicrobials have a more pronounced effect on plasma ENL. The impact of sociodemographic factors such as age, gender, education level and race/ethnicity have been observed to influence plasma ENL concentration, although it may only be a reflection of dietary patterns rather than independent determinants of plasma ENL. The findings of this investigation complement those of earlier studies, and should be taken into account when considering interpersonal variability of lignans.

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