Bioavailability of lignans in human subjects

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Dietary lignans are phyto-oestrogens that possibly influence human health. The present review deals with lignan bioavailability, the study of which is crucial to determine to what extent metabolism, absorption and excretion of lignans alter their biological properties. Since intestinal bacteria play a major role in lignan conversion, for instance by producing the enterolignans enterodiol and enterolactone, emphasis is put on data obtained in recent bacteriological studies.

Phyto-oestrogens: Lignans: Enterolignans: Bioavailability: Human intestinal microbiota

Introduction

Phyto-oestrogens are dietary compounds of plant origin that mainly include flavonoids and lignans. Since their chemical structure is similar to those of oestrogens, they have been studied for their involvement in hormone-related disorders, such as reproductive failure and breast cancer (Setchell & Adlercreutz, 1988). Meanwhile, it has become clear that it is crucial to study the bioavailability of phyto-oestrogens to evaluate the relevance of their health effects. The trend to consume increasing amounts of phyto-oestrogen-containing foods in Western countries shows the importance of studying the fate of phyto-oestrogens in the human body. Because abundant data are already available on flavonoids, the present review focuses on lignans.

A prerequisite for investigating lignan bioavailability is to accurately determine their occurrence in foods and to estimate their intake in human populations. Although flaxseed is the main source of lignans (approximately 4 mg/g dried mass), a variety of cereals, fruits, vegetables, legumes and beverages also contain lignans in substantial concentrations (10 ng to 400 µg/g) (Milder et al. 2005a). Thus, lignans are found in a wide range of foods consumed daily in Western countries. Secoisolariciresinol diglucoside (SDG), its aglycone secoisolariciresinol (SECO), and matairesinol (MAT) are the most frequently studied dietary lignans. They have been studied for their possible role in the prevention of breast and prostate cancer (McCann et al. 2005; Thompson et al. 2005) and atherosclerosis (Prasad, 2005). They have also been used as model substrates to assess the bacterial production of the enterolignans enterodiol (ED) and enterolactone (EL) (Borriello et al. 1985), the biological properties of which are proposed to be more potent than those of plant lignans (Brooks & Thompson, 2005; Jacobs et al. 2005). Numerous data presented in the present review deal primarily with SDG. However, it must be emphasised that enterolignans are produced from plant lignans other than SDG and MAT (Axelson et al. 1982), such as arctigenin, arctiin, 7-hydroxymatairesinol, isolariciresinol, lariciresinol (LARI), pinoresinol (PINO), sesamin and syringaresinol (Thompson et al. 1991; Liggins et al. 2000; Heinonen et al. 2001; Xie et al. 2003a,b; Penalvo et al. 2005). Enterolignans are also produced from lignins in rats (Begum et al. 2004). Recent studies agree on the need to expand databases on the lignan content of foods in order to more accurately determine dietary intakes of enterolignan precursors. Milder et al. (2005b) estimated that the total mean intake of LARI, MAT, PINO and SECO is approximately 1 mg/d in Dutch adults. Although the authors did not systematically take into account the influence of food processing on lignan concentrations and that lignan intake may vary between individuals and study populations, an appealing goal remains to know what proportion of this daily ingested mg of plant lignans may be metabolised in the digestive tract, be absorbed and eventually reach target tissues.

The aim of the present article is to provide a comprehensive and critical overview of the current knowledge on lignan bioavailability in human subjects, including data on the production, origin and physiological concentrations of lignan metabolites, with emphasis on the influence of intestinal bacteria on lignan bioavailability.

Abbreviations: ED, enterodiol; EN, enterolactone; LARI, lariciresinol; MAT, matairesinol; PINO, pinoresinol; SDG, secoisolariciresinol diglucoside; SECO, secoisolariciresinol.

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Lignan metabolism in the upper part of the gastrointestinal tract

Host mechanisms, such as saliva action and chewing in the mouth, chemical hydrolysis in the stomach and mucosal enzymic activities, probably influence lignan bioavailability in the upper part of the gastrointestinal tract. However, the relevance of these processes is essentially unknown.

Results obtained in our laboratory showed that SDG was stable after 3 h at 37°C in artificial stomach juice (Clavel et al. 2006a). Similar results were reported for other glycosylated lignans in rat gastric juice (Nose et al. 1992). This agrees with data obtained by Mazur (2000), who found that glycosidic bonds of SDG are difficult to hydrolyse. For lignan extraction from foods, the authors used harsh conditions (2 m-HCl, 100°C, 2.5 h) to obtain SECO. SDG was also stable after 3 h at 37°C in artificial intestinal juice (Clavel et al. 2006a). Thus, it is possible that SDG is not hydrolysed during its passage through the stomach and small intestine.

While deglycosylation of flavonoids by host enzymes occurs in the mouth and small intestine (Walle et al. 2005), studies have not yet been conducted with lignans. Germ-free animal or in situ rat intestinal perfusion (Arts et al. 2004) models could be used to gain basic knowledge on the role of host mechanisms in lignan conversion, such as epithelial deglycosylation and absorption of plant lignans. Penalvo et al. (2004) recovered approximately 2% of the ingested dose of PINO and LARI in the plasma of four individuals 1 h after intake of 50 g sesame seeds. This suggests that a certain amount of ingested plant lignans may be quickly absorbed. This is in agreement with the detection of plant lignans in urine (Bannwart et al. 1989; Lampe et al. 1999; Hutchins et al. 2000; Nurmi et al. 2003). However, mechanisms responsible for uptake of plant lignans in the upper part of the gastrointestinal tract are unknown.

Due to rapid swallowing, it is unlikely that oral bacteria contribute to the conversion of plant lignans. The same applies to the oesophagus and stomach, where physical and chemical conditions do not favour bacterial colonisation. In contrast, at cell densities of approximately 10^7 viable cells/g dried content in the ileum and 10^12 cells/g in the colon, intestinal bacteria are considered to be a major factor that influences lignan bioavailability.

Production of enterolignans by intestinal bacteria

In vitro conversion of plant lignans by faecal slurries (Borriello et al. 1985; Thompson et al. 1991) and studies with germ-free rats (Axelson & Setchell, 1981) have shown that intestinal bacteria are crucial for enterolignan production. To convert SDG to EL, bacteria catalyse four sequential reactions: O-deglycosylation, O-demethylation, dehydrogenation and dehydroxylation (Fig. 1) (Wang et al. 2000). Enterolignan production from LARI and PINO includes one and two additional reduction steps, respectively (Xie et al. 2003b).

Diversity of lignan-converting bacteria and prevalence of enterolignan production

The production of enterolignans from SDG requires the interaction of phylogenetically and functionally distantly related anaerobic bacteria (Fig. 1). The ability to O-deglycosylate SDG has been demonstrated for closely related species of the genera Bacteroides and Clostridium (Clavel et al. 2006b,c). The O-demethylation and dehydroxylation steps underlying SECO conversion are catalysed by several strains of Ruminococcus productus and Eggerthella lenta, respectively (Clavel et al. 2006b). Thus, the presence of different SDG-converting bacteria in the intestine may explain the high prevalence of enterolignan production in human subjects (Clavel et al. 2005). In our laboratory, enterolignans (ED + EL) were produced by all thirty-one human faecal samples tested. One sample from a male adult did not produce EL. Although these data were obtained with SECO only, bacteria involved in enterolignan production from SDG also convert other plant lignans. R. productus not only catalyses the O-demethylation of SECO, but also the O-demethylation of LARI, MAT, PINO and of a variety of other methylated aromatic compounds (Clavel et al. 2006a). E. lenta catalyses both the dehydroxylation of di-demethylated SECO and the reduction of PINO to LARI and of LARI to SECO. These data indicate that enterolignan production from various plant lignans results from a network of reactions (Fig. 1). It should be kept in mind that intestinal bacteria other than the ones identified so far may contribute to the conversion of plant lignans.

Occurrence of lignan-converting bacteria in relation to host factors

Most species mentioned in Fig. 1 are common members of dominant bacterial groups in the human intestine (Finegold et al. 1983; Suau et al. 1999). Culture-based enumerations of SECO-converting bacteria showed that organisms involved in the production of ED occurred at a mean cell density of 6 × 10^5 cells/g faeces (Clavel et al. 2005). In contrast, organisms involved in the production of EL from SECO were detected at a mean cell density of 3 × 10^5 cells/g. Since the occurrence of the latter organisms was related to the amount of EL produced, it is conceivable that variations in the cell density of lignan-converting bacteria explain the marked inter-individual differences observed for enterolignan production (Mazur et al. 2000; Clavel et al. 2005; Kuijsten et al. 2005b). However, this needs to be confirmed by future human intervention studies, in which proportions of lignan-converting bacteria could be compared with concentrations of ED and EL in biological matrices.

Interestingly, women tend to harbour more enterolignan-producing bacteria than men (Clavel et al. 2005). However, this was not associated with variations in proportions of dominant bacterial groups, possibly due to a limited number of samples. Nevertheless, this finding implies that host factors influence the bacterial production of enterolignans. In this context, it is worthwhile to discuss the role of the SECO-dehydroxylation species Clostridium scindens and E. lenta in the dehydroxylation of endogenous compounds that undergo enterohepatic circulation. E. lenta has been studied for its involvement in 21-dehydroxylation of biliary steroids (Feighner & Hylemon, 1980), C. scindens for its ability to 7α-dehydroxylate primary bile acids (Doerner et al. 1997) and to synthesise desmolase and 20α-hydroxysteroid
dehydrogenase (Krafft et al. 1987), both of which are involved in the metabolism of steroid hormones. It is conceivable that sex differences in intestinal levels of steroid hormones, for example, oestrogens, progesterone and testosterone, influence metabolic activities of bacteria capable of converting structurally related dietary compounds. This applies to the ability of \textit{C. scindens} and \textit{E. lenta} to dehydroxylate SECO. The role of progesterone might be of particular interest. Bacterial dehydroxylases are involved in progesterone metabolism (Feighner & Hylemon, 1980). Also, progesterone relaxes smooth muscle tone resulting in a longer intestinal transit time (Bielefeldt et al. 1996). Shoda et al. (1995) and Kilkkinen et al. (2001) reported a positive correlation between intestinal transit and bacterial production of secondary bile acids and between serum EL concentration and constipation, respectively. Some bacteria, including \textit{C. scindens} and strains of \textit{Eubacterium} species, have several bile acid-inducible (bai) genes, which encode enzymes of the bile acid 7α-dehydroxylation pathway (Doerner et al. 1997). Thus, progesterone levels and transit time may influence the conversion of dietary lignans by increasing their availability to bacteria or by inducing bacterial activities, directly or indirectly. However, to draw firm conclusions, it is imperative to characterise enzymes involved in lignan conversion. For example, enzyme expression could be assessed by proteomic analysis in response to the presence or absence of substrate. Another approach would be to screen metagenomic libraries of human intestinal microbiota for bacterial clones catalysing reactions underlying lignan conversion.

\begin{figure}
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\caption{Conversion of the plant lignans pinoresinol (PINO), lariciresinol (LARI), secoisolariciresinol (SECO), secoisolariciresinol diglucoside (SDG) and matairesinol (MAT) by human intestinal bacteria. Bacterial names are identified next to the reactions catalysed by the given organisms. Reactions are: (1) reduction; (2) O-deglycosylation; (3) O-demethylation; (4) dehydrogenation; (5) dehydroxylation. The SECO-demethylating species \textit{Butyrivibrio methanotrophicus} and \textit{Eubacterium callanderi} are not yet known as members of the human intestinal microbiota. (---), Reactions for which no bacteria have been identified so far; ED, enterodiol; EL, enterolactone.}
\end{figure}

Influence of bacterial dehydrogenation on metabolite production

Of the five reactions underlying bacterial production of EL, the dehydrogenation step is of particular interest. First of all, lignan-dehydrogenating bacteria are subdominant members of intestinal microbiota (mean cell density of about $10^5$ cells/g). Only one strain capable of dehydrogenating lignans was identified, namely \textit{Lactonifactor longoviformis} DSM 17459T (Clavel et al. 2006c). More work is needed to quantify this organism in faeces but, considering that EL production is detected in most individuals (Clavel et al. 2005; Kuijsten et al. 2005b), either the prevalence of \textit{L. longoviformis} is high in human subjects or other not yet
identified organisms catalyse the dehydrogenation step underlying EL production.

*L. longoniformis* seems to catalyse only the enantio-specific conversion of (+)-ED to (+)-EL (Clavel et al. 2006c). Xie et al. (2003a) reported the ability of faecal bacteria to produce (+)-EL and (−)-EL from different precursors and proposed that the absolute configuration of lignans is preserved throughout bacterial conversion. This agrees with data obtained in rats by Saarinen et al. (2002). We hypothesise that so-far unidentified bacteria dehydrogenate the (−)-enantiomer of lignans. Since the absolute configuration may influence production rates and biological properties of enterolignans, stereochemistry should be taken into consideration in future studies on bacterial production and biological properties of lignan metabolites. Concerning plant lignans, the (+)-enantiomer of SDG was detected at proportions of more than 90% total SDG in two flaxseed species (Sicilia et al. 2003). However, this ratio may vary in other flaxseed species and in dietary sources other than flaxseed. In a recent study (Knust et al. 2006), (−)-SDG was detected as the major enterolignan precursor in flaxseed, but the authors did not specify the flaxseed species tested. Xia et al. (2001) purified an enantio-specific enzyme from plants of *Forsythia intermedia* and *Podophyllum peltatum* that dehydrogenates (−)-SECO to (−)-MAT. Concerning LARI and MAT, one enantiomer of each was found in large excess in flaxseed (Sicilia et al. 2003), but stereochemistry was not determined. We conclude that the stereochemistry of plant lignans needs to be further investigated in order to accurately estimate physiological levels of active lignan metabolites.

The study of lignan conversion by *L. longoniformis* led to the identification of 2,3-bis(3,4-dihydroxybenzyl)butyrolactone, a new intermediate in the formation of EL from SDG. This shows that enterolignan production from a single precursor may occur through different pathways (Fig. 1). However, it is difficult to make assumptions on the predominance of any of these pathways in vivo, and how this influences conversion rates and biological effects of lignans. Adlercreutz et al. (1993a) found that the ability of 2,3-bis(3,4-dihydroxybenzyl)butyrolactone (referred to as 4,4'-dihydroxy-EL in the study) to inhibit aromatase activity was higher than that of EL. Which lignan metabolites are formed by bacteria in vivo and how they differ between individuals remains a key issue. These metabolites may not necessarily be ED and EL. The presence of certain bacterial groups in faeces might be a good indicator for certain patterns of lignan metabolites and their rate of formation. In the future, in vitro continuous-culture systems could be used to grow mixed cultures of lignan-converting strains under controlled conditions in order to assess how changes in community composition influence the production of lignan metabolites.

**Limitations of bacteriological studies**

Even if bacteriological data are crucial for the study of lignan bioavailability, two major limitations must be pointed out. First, to characterise the mechanisms underlying bacterial conversion of lignans, it is necessary to work not only with pure compounds, but also even with pure enantiomers. However, this makes it difficult to estimate rates of enterolignan production in vivo, since the fate of pure compounds does not necessarily reflect lignan availability from complex food matrices. For example, lignans are primarily found in the outermost layers of seeds and may not be easily accessible to bacteria (Mazur, 2000). Crushing and milling of flaxseed was shown to improve enterolignan bioavailability (Kuijsten et al. 2005a). Furthermore, ester-linked and 3-hydroxy-3-methyl-glutaric-acid-interconnected polymers of SDG have been described in flaxseed (Ford et al. 2001; Kamal-Eldin et al. 2001). Although mucosal and bacterial esterase activities towards phenolic acids have been reported in human subjects (Andreasen et al. 2001) and although enterolignans are produced from lignins in rats (Begum et al. 2004), it is unclear to what extent lignan polymers are hydrolysed in the human intestine. Hence, to assess lignan conversion rates, it would be preferable to use complex food matrices, as done previously by Thompson et al. (1991) and Aura et al. (2006) with faecal slurries. Second, defined mixed cultures catalyse the conversion of SDG to EL in vitro (Clavel et al. 2006b). However, it is difficult to draw any conclusion on the role that each of the identified lignan-converting species plays in vivo. Experiments with gnotobiotic animals could bring to the test the relevance of these in vitro findings. Besides, batch cultures of faecal slurries do not accurately mimic the physico-chemical conditions in the intestine. Depending on the media and incubating conditions used, growth of specific bacterial groups may be favoured. This would lead either to overgrowth of lignan-converting bacteria with ensuing overestimation of corresponding cell densities, or to overgrowth of non-converting bacteria. This may also explain the 60% recovery of enterolignans after incubating SECO with faecal slurries for 48 h (Clavel et al. 2005). Heinonen et al. (2001) reported a comparable recovery (72% of the SECO was converted to enterolignans within 24 h) and noted that the efficacy of conversion varied between plant lignans.

**Enterolignan absorption and blood levels**

**Enterolignan absorption**

Once produced by intestinal bacteria, enterolignans may be efficiently absorbed, conjugated and the resulting metabolites excreted by enterocytes, as proposed by in vitro experiments with human colonic cell cultures (Jansen et al. 2005). EL-sulfate, -glucuronide and ED-glucuronide were detected after exposing cells to enterolignans, but molar proportions were not determined. Conjugation and excretion occurred within 8 h and EL was metabolised or excreted more rapidly than ED. However, these results must be regarded with caution concerning the kinetics of metabolite production, since cancer cell lines might have an increased conjugation and efflux activity. Besides, only two of the three cell lines tested (HT29 and CaCo-2 cells) were responsive to enterolignan exposure.

**Baseline concentrations of lignans in blood**

In most studies on blood levels of lignans, total lignans are measured following hydrolysis of conjugates. However,
conjugation certainly influences the biological properties of lignans, even if conjugation patterns in blood do not necessarily reflect those in target tissues. Based on the analysis of blood samples from twenty-seven women, Adlercreutz et al. (1993b) proposed that the biologically active fraction of enterolignans, including free, mono- and di-sulfated ED and EL, makes up 21–25% of total (conjugated plus unconjugated) enterolignans. The major fraction (approximately 80% total enterolignans) included biologically inactive mono- and di-glucuronides and sulfogluconurides. These ratios and blood concentrations of enterolignans probably depend on study cohorts. Nonetheless, it is proposed that average baseline concentrations of enterolignans (in the blood of subjects on their usual diet) are in the range of 10 to 25 nmol/l (Adlercreutz et al. 1998; Hong et al. 2002; Horner et al. 2002; Grace et al. 2003; Kilkkinen et al. 2003; Valentin-Blasini et al. 2003; Kuijsten et al. 2005a; Low et al. 2005). Recently, the plant lignans LARI and MAT were detected in human serum at concentrations sometimes higher than those of ED and EL (Smeds et al. 2006). For instance, the highest concentration of LARI was 190 nmol/l in the serum of one female subject. On the other hand, SECO was not detected in any of the ten samples tested. Clearly, marked inter-individual differences are observed. In particular, dietary habits influence blood concentrations of lignans. Intake of vegetables, fibres and wholegrain products has been associated with higher EL concentrations (Kilkkinen et al. 2001; Horner et al. 2002). The highest concentration of EL reported in the literature exceeded 1 µmol/l in the blood of one vegan postmenopausal woman (Adlercreutz et al. 1993b). Hence, it has been proposed that blood concentrations of EL are related to the intake of plant lignans (Kilkkinen et al. 2003).

**Blood levels of lignans after dietary intervention**

A growing number of human studies have shown that dietary intervention with lignan-containing foods leads to an increase in blood levels of enterolignans in nearly all individuals (Nesbitt et al. 1999; Jun tunen et al. 2000; Mazur et al. 2000; Stumpf et al. 2000; Jacobs et al. 2002; Tarpila et al. 2002; Kuijsten et al. 2005a). Since most of these studies used amounts of whole grains, oilseeds or fruits that are relevant in terms of daily food consumption, moderate changes in dietary habits may significantly alter blood levels of enterolignans. For instance, Mazur et al. (2000) and Kuijsten et al. (2005a) showed that blood levels of EL significantly increased after a single meal of 500 g strawberries or after daily consumption of approximately 20 g flaxseed for 10 d. Because the type and duration of intervention vary greatly between studies, it is hard to tell whether enterolignan levels in biological matrices reach maximum values beyond a certain ingested dose of dietary precursors or after intake over long periods. During a 12-week intervention favouring intake of lignan-containing foods (Stumpf et al. 2000), the highest concentration of ED occurred during the first 6 weeks (median values were 12-2, 17-2 and 19-5 nmol/l at baseline and after 6 and 12 weeks, respectively; n 85). Conversely, Tarpila et al. (2002) observed a continuous increase during 4 months of intervention with flaxseed (serum EL concentrations were 33, 52 and 70 nmol/l at baseline and after 2 and 4 months, respectively; n 80). However, the participants of the latter study also ingested inulin, which possibly altered microbial activities. Jun tunen et al. (2000) proposed that a daily intake of more than 90 g rye bread (approximately 300 µg lignans) (Milder et al. 2005a) for 4 weeks does not trigger a further increase in blood levels of enterolignans. On the other hand, Nesbitt et al. (1999) observed a dose–response in daily urinary excretion of lignans after intake of 5, 15 or 25 g flaxseed/d for 7 d and did not report a plateau effect at 25 g/d (approximately 75 mg lignans) (Milder et al. 2005a). Thus, we hypothesise that a constant increase in the amount of enterolignans produced in the intestine occurs if dietary intervention is long enough, maybe due to adaptive responses in bacterial activities, which are not necessarily accompanied by changes in bacterial diversity or proportions. However, blood levels of enterolignans may not exceed an individual-specific threshold, due to adaptive responses in host metabolism and excretion mechanisms. The detection of LARI and MAT in blood implies that some plant lignans are rapidly absorbed as such and that the microbial capacities to produce enterolignans can be saturated beyond intake of a certain amount of precursors. However, it is to date not possible to estimate this amount. Future intervention studies in human subjects should assess changes in blood concentration of lignans in relation to changes in intestinal microbiota and should include measurements when dietary treatment is over to determine how durable changes are.

**Blood levels in relation to bacterial conversion of lignans**

Based on results obtained in our laboratory (Clavel et al. 2005) and on a recent pharmacokinetic study by Kuijsten et al. (2005b) who measured enterolignans in blood and urine after a single dose of 500 mg SDG, we can draw a number of conclusions which are relevant to both bacteriological and human studies:

1. Inter-individual differences in cell densities of enterolignan-producing bacteria may explain inter-individual differences in blood concentrations of enterolignans.
2. The detection of enterolignans in the blood of most individuals is most probably linked to the high prevalence of enterolignan-producing bacteria and implies that health effects associated with enterolignans are relevant to all individuals.
3. The predominance of EL ± ED in blood may be partly due to the enterohepatic circulation of ED. Since the ED:EL ratio might be important with regard to health effects, it would be interesting to know whether individuals with low cell densities of lignan-dehydrogenating organisms are those in whom ED is the main metabolite detected after ingestion of SDG.
4. Kuijsten et al. (2005b) found that enterolignans are first detectable in blood 8 to 10 h after dietary intake. Although pharmacokinetic parameters may vary between intake of a single dose of SDG in water and continuous intake of SDG-containing foods, this finding agrees with previous data (Nesbitt et al. 1999;
Mazur et al. (2000) and confirms that enterolignans are primarily produced and absorbed in the colon.

(5) The tendency of women to have higher cell densities of enterolignan-producing bacteria than men (Clavel et al. 2005) agrees with higher blood concentrations of enterolignans in women (Jacobs et al. 2002; Kilkkinen et al. 2003; Kuijsten et al. 2005b). Another sex difference is the earlier appearance of enterolignans in the blood of women (Kuijsten et al. 2005b). However, the cohort size was small in three of the cited studies (n=20) (Jacobs et al. 2002; Clavel et al. 2005; Kuijsten et al. 2005b). Moreover, it is necessary to take into account sex differences in dietary intake and blood volume when comparing blood levels of enterolignans between women and men. In one study involving twenty-one women and eighteen men with slightly elevated serum cholesterol levels, Juntunen et al. (2000) refuted the assumption of significant sex differences in lignan metabolism after rye-bread intake. After a 4-week intervention, women had a non-significant higher mean concentration of EL in serum than men (39.3 v. 28.1 nmol/l) and lignan intake on a per kg body-weight basis did not differ. When assessing sex differences, detailed information on the menstrual cycle should be given too. Early studies showed that enterolignan excretion is associated with pregnancy and the menstrual cycle, although the number of samples analysed was limited (Setchell et al. 1979; Stitch et al. 1980). The possible role of progesterone detailed above would partly explain higher excretion of enterolignans during the mid-luteal phase and early pregnancy. However, a study by Lampe et al. (1994) does not support association between enterolignan excretion and the menstrual cycle. Although more work is required to achieve a consensus in results on this topic, a relationship between lignan and hormone bioavailability is supported by the observed alteration of both the menstrual cycle and levels of sex hormones following flaxseed consumption (Phipps et al. 1993; Brooks et al. 2004).

Beyond the crucial role of bacteria in lignan conversion, the study by Kuijsten et al. (2005b) highlights the importance of host mechanisms in regulating lignan bioavailability. Hepatic metabolism, entero-hepatic circulation and excretion certainly regulate tissue exposure to the lignans produced in the intestinal tract.

**Hepatic metabolism of lignans and tissue concentrations**

Early work in rats suggested that lignans undergo enterohepatic circulation (Axelson & Setchell, 1981). Since then, only two in vitro studies have investigated the production of lignan metabolites by the liver. Jacobs et al. (1999) obtained aliphatic and aromatic hydroxylated metabolites of ED and EL after incubating synthesised enterolignans with human hepatic microsomes. The authors also identified aromatic hydroxylated metabolites in the urine of two women and two men (Jacobs et al. 1999). Using hepatic microsomes too, Niemeyer et al. (2003) detected oxidative metabolites of MAT and SECO. The biological properties of these lignan metabolites remain to be determined and the hepatic conjugation of lignans in human subjects has to be proven.

In a study using a single dose of [3H]SDG in twenty-four female Sprague–Dawley rats (Rickard & Thompson, 1998), the twelve tissues analysed for the presence of radioactivity contained in total about 5% of the recovered dose of radioactivity. Most SDG metabolites were excreted within 48 h after administration. Approximately 30% of the recovered dose was detected in urine and 50% in faeces. The liver was one of the tissues with high concentrations of lignan metabolites. Other tissues with high concentrations were the intestine (mainly the caecum), kidney and uterus. In human subjects, lignan concentrations have been measured in semen, because in the early 1980s entero-

**Lignan excretion**

**Urinary excretion**

Urinary excretion has been the most widely studied aspect of lignan bioavailability. A few key points are summarised here. As in the case of blood levels, urinary excretion of enterolignans is characterised by higher baseline values of EL (100–5000 nmol/d) than of ED (10–1000 nmol/d) (Axelson & Setchell, 1980; Adlercreutz et al. 1995b; Lampe et al. 1999; Hutchins et al. 2000). A dose-dependent increase in ED and EL excretion is observed after dietary supplementation of lignan-containing foods (Lampe et al. 1994; Hutchins et al. 2000) and enterolignan concentrations in urine correlate well with those in blood (Valentin-Blasini...
et al. 2003; Kuijsten et al. 2005b). However, while the mean proportion of free and sulfated enterolignans is approximately 20% in blood (Adlercreutz et al. 1993b), their proportion in urine is below 10% (Axelson & Setchell, 1980; Adlercreutz et al. 1995b). Mono-glucuronidated and sulfogluconuridated enterolignans ranged from 73 to 94% and from 1 to 17% of total urinary enterolignans, respectively. Similar percentages have been observed for the plant lignan MAT and for endogenous oestrogens (Axelson & Setchell, 1980; Adlercreutz et al. 1995b). Interestingly, enterolignans were detected in the urine of a 6-year-old child at approximately 168 nmol/d (Axelson & Setchell, 1980), which shows the potential of a child’s intestinal microbiota to produce enterolignans. In the study by Kuijsten et al. (2005b), the total amount of ED and EL detected in urine was 40% of the ingested dose of 500 mg SDG, the majority of which was excreted within 2 d. The residence time of enterolignans was lower in women than in men (17.3 ± 23.9 h). In earlier studies (Stitch et al. 1980; Setchell et al. 1981), both enantiomers of EL were detected in urine and the authors proposed that urinary EL is racemic.

**Faecal excretion**

Very few data on faecal excretion of lignans are available. In nine omnivorous Finnish women consuming their usual diet, the daily amount of MAT and ED excreted in faeces was approximately 22 and 148 nmol, respectively (Adlercreutz et al. 1995a). The excretion of EL was much higher (1–500 nmol/d). The authors found that faecal excretion of lignans was 2- to 4-fold higher in nine vegetarian women but statistical analyses were not performed. In an intervention study with thirteen women consuming 10 g ground flaxseed/d in addition to their usual diet for a period of three menstrual cycles, Kurzer et al. (1995) observed baseline values of approximately 7, 80 and 640 nmol/d for MAT, ED and EL, respectively. After intervention, the excreted amounts of MAT, ED and EL significantly increased (12, 2-560 and 10 300 nmol/d, respectively). These data, and the fact that urinary excretion of MAT in thirty-one postmenopausal women did not increase after consumption of 10 g flaxseed/d for 7 weeks (Hutchins et al. 2000), suggest that MAT is efficiently converted to enterolignans in the intestine. Alternatively, MAT may be absorbed as such and subsequently metabolised, for example, hydroxylated, by host enzymes.

**Conclusion**

Table 1 summarises important facts on lignan bioavailability in human subjects. The study of lignan bioavailability requires the investigation of lignan absorption, metabolism and excretion at different body sites. The goal is to integrate all data to gain information on physiological concentrations of biologically active metabolites. To reach this goal, more work in needed. For instance, novel host and bacterial metabolites of lignans could be found and their biological properties investigated. Quantitative approaches must be used to determine production rates and ratios of lignan metabolites. Moreover, lignan bioavailability is characterised by marked inter-individuals differences. Future human studies should include intestinal microbiota analysis to help characterising inter-individual differences in the ability to metabolise dietary lignans. Finally, almost all studies on lignans highlight possible beneficial health effects and no adverse effects of lignans have been reported so far (Kulling

### Table 1. Important data on lignan bioavailability in human subjects

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<th>Plant lignans†</th>
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<td>Food content: 0 to 3 g/kg fresh weight</td>
<td>Milder et al. (2005b)</td>
<td></td>
</tr>
<tr>
<td>Mean usual daily intake: about 1 mg</td>
<td>Bannwart et al. (1989); Smeds et al. (2006)</td>
<td></td>
</tr>
<tr>
<td>Maximum usual daily intake: about 78 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detectable in blood and urine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intestinal bacteria are crucial for the conversion of plant lignans in the human intestine</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Detectable in all individuals, EL is not detected in some individuals on their usual diet</td>
<td>Clavel et al. (2005); Clavel et al. (2005b); Adlercreutz et al. (1995a); Lampe et al. (1999); Kuijsten et al. (2005b); Adlercreutz et al. (1993b); Kikkinnen et al. (2001); Lampe et al. (1999); Hutchins et al. (2000); Nurmi et al. (2003); Adlercreutz et al. (1995a); Kurzer et al. (1995); Mazur et al. (2000); Morton et al. (1997)</td>
<td></td>
</tr>
<tr>
<td>EL predominates in blood, urine and faeces of most individuals (about 5- to 10-fold higher amounts than ED)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline concentrations in blood: 10 to 25 nmol/l</td>
<td></td>
<td></td>
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<tr>
<td>Free and sulfate fraction (about 20%)</td>
<td></td>
<td></td>
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<tr>
<td>Baseline urinary excretion: 300 to 3000 nmol/d</td>
<td></td>
<td></td>
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<tr>
<td>Baseline faecal excretion: 100 to 1500 nmol/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary interventions increase blood concentration and excretion</td>
<td></td>
<td></td>
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<tr>
<td>Possible tissue accumulation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EL, enterolactone; ED, enterodiol.

* The Table is meant to give a one-look overview of so-far acknowledged trends. The data list is not exhaustive. Values are given as references and vary between individuals and populations.

† Restricted to lariciresinol, matairesinol, pinioresinol and secoisolariciresinol.

‡ Proportion of food items (n 109) containing detectable amounts of plant lignans.
et al. 1998). However, it must be kept in mind that lignans can interact with highly sensitive hormonal systems. Lignan-containing nutritional supplements should be used with great caution, especially in infants and children or during pregnancy and lactation (Ward et al. 2000). No matter what health effects lignans have, it is essential to study their bioavailability.

References


Grace PB, Taylor JL, Botting NP, Fryatt T, Oldﬁeld MF, Al-Maharkin N & Bingham SA (2003) Quantiﬁcation of


