ABSTRACT High concentrations of enterolignans in plasma are associated with a lower risk of acute coronary events. However, little is known about the absorption and excretion of enterolignans. The pharmacokinetic parameters and urinary excretion of enterodiol and entero lactone were evaluated after consumption of their purified plant precursor, secoisolariciresinol diglucoside (SDG). Twelve healthy volunteers ingested a single dose of purified SDG (1.31 μmol/kg body weight). Enterolignans appeared in plasma 8–10 h after ingestion of the purified SDG. Entero diol reached its maximum plasma concentration 14.8 ± 5.1 h (mean ± SD) after ingestion of SDG, whereas entero lactone reached its maximum 19.7 ± 6.2 h after ingestion. The mean elimination half-life of enterodiol (4.4 ± 1.3 h) was shorter than that of enterolactone (12.6 ± 5.6 h). The mean area under the curve of enterolactone (1762 ± 1117 nmol/L·h) was twice as large as that of enterodiol (966 ± 639 nmol/L·h). The mean residence time for enterodiol was 20.6 ± 5.9 h and that for enterolactone was 35.8 ± 10.6 h. Within 3 d, up to 40% of the ingested SDG was excreted as enterolignans via urine, with the majority (68%) as entero lactone. In conclusion, a substantial part of enterolignans becomes available in the blood circulation and is subsequently excreted. The measured mean residence times and elimination half-lives indicate that entero lignans accumulate in plasma when consumed 2–3 times a day and reach steady state. Therefore, plasma enterolignan concentrations are expected to be good biomarkers of dietary lignan exposure and can be used to evaluate the effects of lignans.

KEY WORDS: enterodiol • enterolactone • lignans • secoisolariciresinol diglucoside • bioavailability
25 g ground flax. Plasma concentrations of enterolignans started to increase 9 h after intake and were still higher than baseline values after 12 and 24 h. In a study carried out by Mazur et al. (24), enterolactone plasma concentrations started to increase 8 h after consumption of 500 g of strawberries. Furthermore, they limited their consumption of black tea and coffee to a maximum of 2 cups (500 mL) a day. Consumption of selected wheat products (white bread, pasta), white rice, milk products (milk, yogurt, cheese), meat and fish, several fruits (e.g., apple, pear), and vegetables (e.g., cucumber, tomato, paprika, cabbage) was allowed so that, in principle, the intake of micro- and macronutrients was adequate. To ensure an adequate fiber intake, wheat bread with a low lignan content (370 nmol lignans/100 g bread), was supplied daily. Bread is an important source of fiber in the Netherlands. Every day a standard breakfast (low lignans) was provided at the Division of Human Nutrition. Lunch and dinner were also provided on the first 2 d of the study.

**Diet.** To avoid interference from other dietary sources of lignans, the participants started a diet poor in lignans 7 d prior to the study and followed it throughout the experiment. The participants were given a list of lignan-containing foods and beverages and were asked to avoid them. They avoided dried fruits, berries, several vegetables (e.g., asparagus, broccoli, and zucchini), legumes, seeds and nuts (e.g., flax, sesame, and peanut), breakfast cereals, cereal and muesli bars, whole-grain products (e.g., rye bread, whole grain bread, and brown rice), olives, virgin olive oil, herbal tea, grape juice, and orange juice.

**Analytical methods.** Total enterodiol and enterolactone concentrations were measured in plasma and urine after hydrolysis of conjugates using a freshly prepared enzyme mixture of β-glucuronidase; AUC, area under the curve; SDG, secoisolariciresinol diglucoside; f_onset, onset of the plasma curve.

**Materials and Methods**

**Subjects.** The Medical Ethical Committee of the Department of Human Nutrition at Wageningen University approved the study, and all subjects gave their informed consent. Six men and 6 women participated in this study. The participants ranged from 18 to 25 y old. None of the subjects had diarrhea or had used antibiotics or other medication in the past 3 months, except for oral contraceptives or painkillers. All subjects were generally healthy (self-reported). The weight of the men was 73.0 ± 6.8 kg (mean ± SD), and the weight of the women was 67.6 ± 4.5 kg. The BMI in men was 21.5 ± 1.3 kg/m² and 23.5 ± 1.6 kg/m² in women. Subjects were excluded if they were pregnant or lactating. Women.

**Diet.** To avoid interference from other dietary sources of lignans, the participants started a diet poor in lignans 7 d prior to the study and followed it throughout the experiment. The participants were given a list of lignan-containing foods and beverages and were asked to avoid them. They avoided dried fruits, berries, several vegetables (e.g., asparagus, broccoli, and zucchini), legumes, seeds and nuts (e.g., flax, sesame, and peanut), breakfast cereals, cereal and muesli bars, whole-grain products (e.g., rye bread, whole grain bread, and brown rice), olives, virgin olive oil, herbal tea, grape juice, and orange juice. Furthermore, they limited their consumption of black tea and coffee to a maximum of 2 cups (500 mL) a day. Consumption of selected wheat products (white bread, pasta), white rice, milk products (milk, yogurt, cheese), meat and fish, several fruits (e.g., apple, pear), and vegetables (e.g., cucumber, tomato, paprika, cabbage) was allowed so that, in principle, the intake of micro- and macronutrients was adequate. To ensure an adequate fiber intake, wheat bread with a low lignan content (370 nmol lignans/100 g bread), was supplied daily. Bread is an important source of fiber in the Netherlands. Every day a standard breakfast (low lignans) was provided at the Division of Human Nutrition. Lunch and dinner were also provided on the first 2 d of the study.

**Lignan supplement.** On d 1 of the study after a 12-h overnight fast, the subjects consumed 1.31 μmol SDG/kg body wt (0.9 mg SDG/kg body wt) in water, just before having their breakfast at around 0800 h. SDG was obtained from the Institute of Food Chemistry, Technical University of Braunschweig. SDG was isolated from a natural source, i.e., flax (Linum usitatissimum L.) (41). For isolation, extraction, and purification of SDG only p.a. quality solvents (food grade) were used. In order to remove remaining traces of solvents the SDG extract was freeze-dried. The purity was above 93%. One day before consumption, the supplement was weighed, dissolved in 50 mL water, and then kept at −20°C. The supplement was thawed 1 h before consumption.

**Collection of samples.** Venous blood samples were taken into vacuum tubes containing EDTA immediately before the intake of SDG (0 h), every 3 h over the next 36 h, and at time points 48, 72, and 96 h. Samples were centrifuged within 30 min at 1187 × g for 10 min at 4°C, and plasma was stored at −80°C until analysis.

**Logistics.** During the study, blood samples t = 0–12 h and t = 24–96 h were taken at the Division of Human Nutrition at Wageningen University. Samples t = 15–21 h, which were collected during the night, were drawn at the hospital Gelderse Vallei in Ede, where the subjects stayed overnight. Volunteers were transported between the 2 sites under supervision of a research nurse.

**Analytical methods.** Total enterodiol and enterolactone concentrations were measured in plasma and urine after hydrolysis of conjugates using a freshly prepared enzyme mixture of β-glucuronidase; AUC, area under the curve; SDG, secoisolariciresinol diglucoside; f_onset, onset of the plasma curve.
detection of enterodiol and enterolactone was 3 nmol/L. The recovery of 10 nmol/L enterodiol and enterolactone aglycone was 98 ± 16% (mean ± SD, n = 6). The within-run CV was 6% for enterodiol and 3% for enterolactone (n = 6), and the between-run CV was 16–18% for both enterolignans (n = 12).

**Pharmacokinetic analysis.** A 1-compartmental pharmacokinetic model was used to describe the absorption and disposition of lignans (MW/Pharm, Mediware) (43). The area under the curve (AUC) for plasma was calculated using the trapezoidal rule. When participants followed a diet low in lignans for 7 d, total enterolignan concentrations in plasma were reduced by half (data not shown). However, due to the abundance of lignans in foods, plasma concentrations of enterodiol were not zero at the start of the study. Baseline plasma concentrations of enterodiol fluctuated between 0.3 and 12 nmol/L (mean 3.4 nmol/L), and concentrations of enterolactone fluctuated between 3.3 and 15 nmol/L (mean 7.2 nmol/L). To calculate the AUC and maximum concentration, baseline values for each person were subtracted from the crude pharmacokinetic parameters.

**Statistical analysis.** An independent t test was used to study sex differences. Two-sided Pearson correlation coefficients were calculated. In all tests, differences were considered significant at P ≤ 0.05. Differences between enterodiol and enterolactone were not tested because the pharmacokinetic parameters are not independent. All statistical analyses were performed using the SPSS statistical software package (version 10.0). Data are means ± SD, unless stated differently.

**RESULTS**

Pharmacokinetic analysis of the plasma curves showed that it took ~8–10 h (Table 1) before both enterolignans appeared in plasma. Although the maximum plasma concentration of enterodiol (73 ± 40 nmol/L), corrected for baseline, exceeded the maximum plasma concentration of enterolactone (56 ± 30 nmol/L), the AUC of enterolactone (1762 nmol L·h) was approximately twice that of enterodiol (966 nmol L·h). As expected, we found clear differences in the plasma concentration-time course for enterodiol and enterolactone. The maximum concentration of enterodiol was reached 14.8 ± 5.1 h after consumption of SDG, while the maximum concentration of enterolactone was reached 19.7 ± 6.2 h postdose. In addition, the elimination half-life of enterodiol (4.4 ± 1.3 h) was much shorter than that of enterolactone (12.6 ± 5.6 h). The residence time of enterodiol was ~21 h and that of enterolactone was ~36 h.

Most of the enterolignans were excreted in urine within the first 2 d (Fig. 2). Within 3 d, up to 40% of the ingested SDG was excreted via urine, with the majority excreted (58%) as enterolactone (Fig. 3). The urinary excretion of enterolactone correlated very well with the amount of enterolactone in plasma, based on the AUCs (r = 0.673, P = 0.016). The correlation between the urinary excretion of enterodiol and the amount of enterodiol in plasma was not significant, but tended to be positive (r = 0.432, P = 0.16).

Although all subjects ingested the same dose of SDG per kilogram of body weight, there was a substantial variation among subjects in plasma concentration and urinary excretion of enterodiol and enterolactone (Figs. 2 and 4). In 5 subjects the AUC of enterolactone was more than twice that of enterodiol (Fig. 4A and B). In 5 other subjects the AUC of enterolactone was only 1–2 times the AUC of enterodiol (Fig. 4C). In 2 subjects the AUC of enterodiol exceeded the AUC of enterolactone. In 1 of those subjects enterolactone concentrations hardly increased at all (Fig. 4D).

When data for men and women were analyzed separately, some pharmacokinetic parameters differed (Table 1). The onset of the plasma curve (t½) of both enterolignans tended to
be earlier in women than in men (enterodiol, \( P = 0.06 \); enterolactone, \( P = 0.42 \)). This was in agreement with the time to reach the maximum concentration, which was earlier in women. Furthermore, the maximum concentrations of both enterolignans tended to be higher in women than in men (enterodiol, \( P = 0.52 \); enterolactone, \( P = 0.11 \)). Additionally, the residence time of enterodiol and enterolactone was shorter in women than in men. The AUC and elimination half-life of both enterolignans did not differ between men and women.

**DISCUSSION**

Our study is the first pharmacokinetic study on enterodiol and enterolactone in humans consuming a single dose of purified SDG. A substantial part (at least 40%) of the metabolites of SDG, enterodiol and enterolactone, becomes available in the blood circulation and is subsequently excreted. Enterodiol and enterolactone are absorbed 8–10 h after consumption of SDG and eliminated slowly. The systemic exposure to enterolactone, as computed from the mean AUC, was approximately 2 times the exposure to enterodiol. This difference in systemic exposure might be explained by enterohepatic circulation. This causes enterodiol to reach the colon for a second time, where it is available for oxidation into enterolactone. As a result, predominantly enterolactone is absorbed. This is evidenced by the second peak of enterolactone, which was seen in 5 out of 12 subjects in this study. We did not observe a clear second peak of enterodiol in plasma in any of the subjects. Evidence for enterohepatic circulation of enterolignans has been demonstrated in rats (44) and pigs (45). Alternatively, the difference in systemic exposure between enterodiol and enterolactone might be explained by an efficient enterolactone production from enterodiol immediately after it is formed from SDG. A third option could be that enterolactone is more efficiently absorbed. However, the absorption half-life of enterolactone is longer than that of enterodiol, thus suggesting the opposite.

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Enterodiol</th>
<th>Enterolactone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>( n )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( t_{lag}, \text{h} )</td>
<td>10.1 ± 4.3</td>
<td>12.5 ± 4.4</td>
</tr>
<tr>
<td>Absorption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_{\text{max}}, \text{nmol/L} )</td>
<td>73 ± 20</td>
<td>75 ± 33</td>
</tr>
<tr>
<td>( t_{\text{max}}, \text{h} )</td>
<td>14.8 ± 5.1</td>
<td>17.8 ± 4.1</td>
</tr>
<tr>
<td>Elimination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{AUC}, \text{nmol/L \cdot h} )</td>
<td>966 ± 639</td>
<td>1019 ± 794</td>
</tr>
<tr>
<td>Mean residence time, ( \text{h} )</td>
<td>20.6 ± 5.9</td>
<td>23.9 ± 4.7</td>
</tr>
</tbody>
</table>

1 All values are means ± SD. * Different from males, \( P \leq 0.05 \).
2 \( \text{AUC and } C_{\text{max}} \) are corrected for baseline values. \( t_{\text{lag}} \) is time to reach first appearance in plasma; \( t_{1/2 \text{abs}} \) is absorption half-life; \( C_{\text{max}} \) is maximum plasma concentration; \( t_{\text{max}} \) is time to reach \( C_{\text{max}} \); \( t_{1/2} \) is elimination half-life.
The delayed appearance of enterodiol and enterolactone in plasma indicates that absorption of lignans occurs in the colon. Other studies observed the same delayed appearance of 8–9 h with lignan-rich products (24,40), suggesting that the food matrix did not play an important role in the release of enterolignans.

The difference in time to reach the maximum plasma concentration between enterodiol and enterolactone might be overestimated. Data points from the enterohepatic circulation (second peak) were used in the 1-compartmental model, and thus the time to reach the maximum plasma concentration might be overestimated, especially for enterolactone. A similar problem may have influenced the absorption and elimination half-lives. A specific kinetic model, which takes into account enterohepatic circulation, may lead to more precise kinetic parameters. However, this is only feasible when there are enough data points to calculate the enterohepatic contribution. This kind of experiment would impose a considerable burden to the volunteers involved. The order in which SDG is converted, SDG → enterodiol → enterolactone, is consistent with the difference in absorption half-life between enterodiol and enterolactone. In a number of subjects the absorption and elimination half-lives were identical for both compounds. This means that the absorption governs the elimination, that the intrinsic elimination of the compound is faster than measured here, and that the observed elimination half-life is apparent.

The difference between men and women in the time to reach the maximum plasma concentration might be explained by the smaller blood volume in women, even when adjusted for body weight (46), because the enterolignans are confined to the blood compartment. When this volume is smaller, enterolignans will reach maximum concentrations earlier, and maximum plasma concentrations will be higher.

In our study the percentage of enterolignans excreted via urine is higher than that in animal studies. In a study with rats 28–32% of the ingested [3H]SDG was excreted in urine within 48 h (47). Knudsen et al. (45) found that 24% of the ingested lignans were excreted as enterolignans via urine when pigs were fed a low-lignan wheat bread diet. However, only 14% of the ingested lignans were excreted via urine in rats fed a high-lignan diet. In humans, the excretion of enterolignans via urine was 47.3 μmol/d after consumption of flaxseed powder (10 g/d) (37). Unfortunately, the percentage of the ingested dose excreted via urine could not be calculated because the authors did not report the amount of lignan in the flaxseed powder.

We did not measure metabolites of SDG other than enterodiol and enterolactone or the plant lignan itself. Jacobs et al. (48) detected 9 hydroxylated metabolites of enterodiol and enterolactone in the urine of 4 humans ingesting flax for 5 d. These metabolites accounted for <5% of the total urinary lignan excretion. Additionally, enterodiol and enterolactone accounted for 82% of the total amount of lignans excreted in the urine of humans consuming their habitual diet (unpublished results, Tarja Nurmi, University of Kuopio, Finland). Thus, we expect enterodiol and enterolactone to be the main metabolites.

In 1 subject plasma concentrations of enterolactone did not increase after consumption of SDG, while plasma enterodiol concentrations did increase. The habitual concentrations of enterolactone, measured before the lignan-poor diet was begun, were also exceptionally low in this subject (3 nmol/L) compared to others (29 ± 7 nmol/L). The urinary enterolactone excretion after consumption of SDG was also low, only 4% of the ingested dose, whereas the total amount of enterolignans excreted was approximately the same as in other subjects. This suggests that this subject was not able to convert enterodiol to enterolactone, likely due to the absence of specific bacteria in the colon that are responsible for the oxidation of enterodiol. The enterolactone present was likely formed from other lignan precursors in the diet, such as matairesinol, which can be directly converted to enterolactone. A similar observation was made by Nesbitt et al. (40), who
found that 2 of 9 subjects produced little or no enterolactone during flaxseed supplementation for 7 d.

As demonstrated in other studies (37,40,49), we observed a wide variation in both urinary excretion and plasma concentrations of enterolignans among subjects. The variation is most likely due to differences in microflora between subjects. Other factors that could explain variation, such as background diet and age, were controlled for in our study. SDG was consumed purified; therefore, the food matrix could not have contributed to the variation either.

The health implications of the higher systemic exposure to enterolactone than to enterodiol are not clear. Thus far, most studies investigated only the effect of enterolactone. A few studies compared the effects of enterolactone and enterodiol and showed that they have similar antioxidant activities (1,2). However, enterolactone had a greater ability than enterodiol to inhibit the binding of estradiol and testosterone to sex steroid binding protein (50) or to inhibit human aromatase in vitro (51). Further studies are necessary to determine whether the physiological effects of enterodiol and enterolactone are different. Therefore, investigators must quantify concentrations of enterodiol and enterolactone in experimental and epidemiological studies in order to understand the metabolism and effect of both compounds. Furthermore, bioavailability studies for other important dietary enterolignan precursors, such as pinoresinol and lariciresinol, are needed. Whether the absorption, distribution, and elimination are influenced by other factors, such as food matrix, is also of interest.

Our data show that at least 40% of the ingested SDG is available for the body. The measured residence time and elimination half-life indicate that enterolignans will accumulate in plasma when consumed 2–3 times a day. Thus steady-state plasma concentrations of enterodiol and enterolactone are likely to be achieved because plant lignans are present in many foods and beverages (21–26). As a result, plasma enterolignan concentrations are expected to be suitable biomarkers of lignan exposure and may be used to evaluate the effects of lignans.

ACKNOWLEDGMENTS

The authors thank Michel Buijsman for his excellent technical assistance and Lucy Okma for blood sampling.

LITERATURE CITED


