Urinary Composition and Postprandial Blood Changes in 3H-Secoisolariciresinol Diglycoside (SDG) Metabolites in Rats Do Not Differ between Acute and Chronic SDG Treatments

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ABSTRACT Although chronic exposure to secoisolariciresinol diglycoside (SDG) was shown to alter 3H-SDG metabolite disposition in rats, the proportion of measured radioactivity attributed to known or unknown SDG metabolites was not determined. Using HPLC and GC-MS, two experiments were conducted to determine the effect of acute (1 d) vs. chronic (10 d) SDG treatment on major urinary metabolites of 3H-SDG in female, Sprague-Dawley rats (70–72-d-old) over a 48-h period and if new urinary metabolites were detectable in rats fed nonradioactive flaxseed or SDG. A third experiment was conducted to determine changes in postprandial blood levels of 3H-SDG metabolites over a 24-h period with acute or chronic SDG treatment. Regardless of treatment, enterodiol, enterolactone and secoisolariciresinol accounted for 75–80% of urine radioactivity. Four potential new lignan metabolites, two of which were detected in the urine of rats fed nonradioactive flaxseed or SDG, were found. Type of treatment had no effect on levels of individual urinary metabolites of 3H-SDG. As observed for plasma lignans in women fed flaxseed, blood radioactivity peaked at 9 h and remained high until 24 h in both treatment groups, suggesting that blood lignan kinetics might be similar with flaxseed or SDG consumption and that they were comparable between humans and rats. In conclusion, the main urinary lignan metabolites were enterodiol, enterolactone and secoisolariciresinol. Urinary composition or blood levels of radioactive lignans were not affected by the duration of SDG exposure. Thus, while chronic SDG exposure alters lignan disposition in rats, it does not change the metabolite profile.


KEY WORDS: lignans • urine • blood • secoisolariciresinol diglycoside • rats

In the last two decades, the mammalian lignans enterolactone (EL) and enterodiol (ED) have attracted much attention in light of their association with reduced cancer risk in epidemiological studies (Adlercreutz 1998). Mammalian lignans are found in a wide variety of plant products, but the richest source (by 75–800-fold) is flaxseed (Thompson et al. 1991). The major lignan precursor in flaxseed is secoisolariciresinol diglycoside (SDG), but other precursors such as matairesinol exist (Adlercreutz and Mazur 1997). Many studies in vitro using ED and EL and in vivo using flaxseed or SDG have demonstrated the inhibitory effect of lignans at the initiation, promotion, progression and metastatic stages of cancer (Rickard and Thompson 2000, Thompson 1998).

Despite advances in the area of lignans and cancer, fewer studies have examined the metabolic fate of lignans in vivo. Having a better understanding of the identity and quantity of lignan metabolites produced is necessary for the assessment of the biological activity of lignans and their potential mechanisms of action in animals and humans. In addition, knowledge of the blood kinetics of lignans is important for the determination of the timing of doses to maintain peak concentrations. The impact of acute vs. chronic intakes of lignans on lignan metabolism and thus potentially lignan bioactivity had not been explored.

In the most controlled human study done to date, Nesbitt and colleagues (1999) found that after a single 25-g dose of flaxseed, plasma lignan levels peak at 9 h and are maintained to 24-h postconsumption. They also observed that mean 24-h plasma and urinary lignans are significantly higher with chronic (7 d) vs. acute (1 d) flaxseed consumption, suggesting that chronic intake enhances absorption and increases tissue exposure. Because fecal lignan measurements were not performed in this study, the effect of acute vs. chronic exposure to lignan precursors on body retention of lignans cannot be determined.

Using 3H-SDG, we previously determined that chronic (10 d, 1.5 mg/d) vs. acute (1 d) exposure to SDG can increase lignan levels in target tissues such as the cecum, liver, kidney, and uterus up to threefold and delay fecal lignan excretion in rats (Rickard and Thompson 1998). However, because only total radioactivity was measured, the relative amounts of different metabolites present in the tissues, blood or excretory products are unknown (Rickard and Thompson 1998). This
was due to the lack of a suitable tissue extraction procedure for lignans and insufficient quantity of blood collected which would allow detailed analysis of radioactive lignan metabolites. However, the urine collected in this previous study (Rickard and Thompson 1998) was considered to be appropriate for the examination of known and potentially unknown 3H-SDG metabolites because the method of urinary lignan analysis is well-established in our laboratory, sufficient urine quantities were collected, and a large proportion of the radioactive dose was detected in the urine. In previous studies, the urinary lignan metabolites measured with SDG feeding were only ED, EL and secoisolariciresinol (SECO, the aglycone of SDG) where usually ED > SECO > EL (Rickard et al. 1996). Recent studies have shown that other lignan metabolites can be produced from ED and EL in vitro from liver microsomes (Jacobs et al. 1999). It would be of interest to identify and quantify the metabolites for the major lignan precursor SDG.

Therefore, the objectives of our first experiment were to determine using HPLC and GC-MS analysis of urine (i) whether the major metabolites of 3H-SDG were ED, EL and SECO and (ii) if the levels of individual 3H-SDG metabolites differed with acute vs. chronic treatment with SDG. If ED, EL and SECO accounted for the majority of urinary radioactivity, this would minimize the possibility that hydrogen exchange took place and would suggest that these metabolites were mainly responsible for the biological activity of SDG. In addition, it was expected that chronic administration of SDG would increase the level of ED and EL (considered to be the more active metabolites) vs. SECO in the urine.

A second experiment was conducted to determine whether new metabolites, if found, could be detected in the urine of rats fed nonradioactive flaxseed or SDG using routine GC-MS analysis. This would provide further evidence that the potentially new metabolites were derived from lignans. However, it is possible that they may be present in quantities that are too small for adequate detection.

A third experiment was conducted to determine the changes in postprandial blood levels of 3H-SDG metabolites over a 24-h period with acute and chronic SDG treatment to assess whether the blood kinetics of lignans in rats was similar to that determined in humans consuming flaxseed (Nesbitt et al. 1999). A similarity in blood lignan kinetics would suggest that the results of metabolic studies in rats might be directly applicable to humans.

### MATERIALS AND METHODS

#### Experiment 1: Urinary Metabolites of 3H-SDG in Rats

**Preparation of 3H-SDG radioisotope.** The benzyl methylenes of the purified SDG were labeled with tritium by Amersham International (Little Chalfont, Buckinghamshire, United Kingdom) using a gas exchange method resulting in a specific radioactivity of 999 GBq/mmol as previously described (Rickard and Thompson, 1998). The radiochemical purity was 98.5%. The benzyl methylene groups were chosen for labeling because (i) they are present in the known metabolites ED and EL and (ii) tritium is more stable at these sites in comparison to the benzene ring.

**Animals, diets and urine collection.** Details of the experimental design and diet are described in Rickard and Thompson (1998). Briefly, after a 5–6 d acclimation to a high fat (20% soybean oil) AIN-93G basal diet (Reeves et al. 1993) and the environment, female, Sprague-Dawley rats (Charles River, Montreal, Quebec, Canada) in the acute group (n = 12) were food-deprived overnight and then given a single gavage of 3H-SDG (3.7 kBq/g body). Rats in the chronic group (n = 12) were similarly treated after a 10 d period of daily gavaging with 1.5 mg unlabeled SDG dissolved in 1 mL of distilled water. At the time of administration of the radioisotope, rats in both acute and chronic groups were 70–72 d of age and 200–250 g in weight. The average total dose given per rat was 835 ± 9 kBq. The rats had been placed in metabolic cages for urine collections at 0–12, 0–24, 0–36 and 0–48 h (n = 3 rats per time point) after administration of the 3H-SDG (Rickard and Thompson 1998).

**Extraction of radioactive urinary lignan metabolites.** Urine (5.0 mL) samples were centrifuged (Beckman, Mississauga, Ontario, Canada) at 1000 × g for 15 min to remove any precipitate. An aliquot (100 μL) was set aside for radioactivity counting. The remaining urine (4 mL) was mixed with 1.2 mL sodium acetate buffer (0.1 mol/L, pH 4.5) and processed using a modification of the method described by Rickard et al. (1996). Reverse-phase octadecylsilane-bonded columns (C-18; 14% C; 200 mg solid-phase packed in 3-mL capacity column, 6.2-cm length and 1-cm diameter; Scientific Products and Equipment, Concord, Ontario, Canada) were conditioned with 3 mL methanol followed by 3 mL sodium acetate buffer (0.1 mol/L, pH 4.5). The urine mixture was then added to the column and allowed to run through followed by rinsing with 3 mL sodium acetate buffer. The lignans adsorbed onto the column were eluted with 2 mL methanol into small glass vials. The eluates were dried under nitrogen, resuspended in sodium acetate buffer and incubated overnight in a 37°C water bath with 500 μL of a β-glucuronidase solution (passed through a conditioned C-18 column and diluted with sodium acetate buffer in a 1:1 ratio) from Helix pomatia (110 U/L; Sigma Chemical, St. Louis, MO) to deglucuronidate the metabolites. The enzyme-treated sample was then transferred to Eppendorf tubes and centrifuged in a microfuge (Biofuge A, Canlab, Mississauga, Ontario, Canada) at 8,800 × g for 5 min. The supernatant was extracted using a C-18 column activated as described above. The unconjugated lignans were collected with 2 mL methanol in small glass vials and then dried under nitrogen. The residues were redissolved in the HPLC mobile phase (5.67 mol/L acetonitrile in 0.035 mol/L glacial acetic acid) and 100 μL set aside for radioactivity counting. The remainder was stored at −20°C until HPLC analysis.

**HPLC analysis of radioactive urinary lignans.** The HPLC (Shimadzu SPD-M6A, Columbia, MD) analysis was carried out using an Alltech (Deerfield, IL) C18 Econosil column (250 mm × 4.6 mm i.d., 5 μm) with guard column (7.5 mm × 3.2 mm; Alltech), a sample injection volume of 100 μL, an isotropic mobile phase of 30% acetonitrile in 0.2% acetic acid, a total run time of 40 min and a flow rate at 1.2 mL/min. The absorbance was monitored at 280 nm with an ultraviolet photodiode array detector (Shimadzu Model SPD-M6A, Columbia, MD). Fractions were collected every 30 s (total volume = 0.4 mL) using the Advantec SF-2120 Super Fraction Collector (Shimadzu). The ED and EL standards were synthesized in Dr. Mark Lautens’ laboratory in the Department of Chemistry at the University of Toronto. The SECO standard was provided by Dr. Alister Muir at the Saskatoon Research Center of Agriculture and Agrifood Canada.

**Liquid scintillation counting of HPLC fractions, urine and urine extracts.** Radioactivity in the collected fractions (0.4 mL), urine (100 μL) and urine extracts dissolved in the HPLC mobile phase (100 μL) was measured with the LKB Wallac 1217 Rackbeta liquid scintillation counter (Fisher Scientific, Ottawa, ON), which had a mean counting efficiency of 63% for tritium (based on unquenched standards). After adjusting the volumes to 1 mL with distilled water for consistency, 15 mL of scintillation cocktail (Cytoscin ES; ICN Biomedicals, Aurora, OH) was added to each vial followed by vigorous shaking. Vials were counted for 10 min. Distilled water (1 mL) and 1H-SDG (10 μL 1H-SDG + 990 μL distilled water = 370 Bq) were used as counting controls to adjust for background radioactivity levels and to evaluate the counting consistency between runs.

**GC-MS analysis of radioactive HPLC fractions.** After determining which fractions contained radioactivity, another series of fractions were collected for GC-MS analysis. The fractions were dried using a rotary evaporator at 70°C, resuspended in 1 mL methanol and the metabolites converted to trimethylsilyl (TMS) ethers for GC-MS analysis as described previously (Rickard et al. 1996). The mass spectra were generated by electron impact ionization at 70 eV.
URINARY METABOLITES OF SDG IN RATS

Experiment 2: Identification of Unknown Urinary Lignan Metabolites in Non-Radioactive Rats Fed Flaxseed or SDG

Animals and diets. The urine used for the identification of potential new lignan metabolites had been collected from rats used in a previous study. Briefly, female Sprague-Dawley rats (n = 12; Charles River) were given free access to either a semisynthetic high-fat basal diet alone (n = 4; Dyets, Bethlehem, PA) or supplemented with 5 g/100 g ground, full-fat flaxseed (n = 4; Linott variety; Omega Products, Melfort, SK) or 1.5 mg/g SDG (n = 4) for 20 wk. The basal diet was based on the AIN-93G formulation (Reeves et al. 1993) except that a higher fat concentration (200 g/kg soybean oil) was used at the expense of cornstarch and dextrose. Dyets prepared the flaxseed-free base so that the ground flaxseed could be added prior to feeding (in batches of 5 kg) with corrections having been made for flaxseed’s contribution to the fat, fiber, available carbohydrate and protein components. Rats were placed in metabolic cages and their urine collected for 3 d before killing. Animal care and use conformed to the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care 1994), and the experimental protocol was approved by the University of Toronto Animal Care Committee.

GC-MS analysis of the nonradioactive urine. Three-day urine samples (10 mL) were analyzed for lignan metabolites by GC-MS as described in Rickard et al. (1996).

Experiment 3: Postprandial Levels of 3H-SDG Metabolites in Whole Blood over a 24-h Period

Animals, diets and blood collection. Rats assigned to the acute (n = 3) or chronic (n = 3) treatment groups were treated as described in exppt. 1 and detailed in Rickard and Thompson (1998), except that blood was collected via the tail vein at 0, 3, 6, 9, 12 and 24 h in each rat after administration of the 3H-SDG dose (3.7 kBq/g body). Rats were killed by CO2 asphyxiation after the last blood sample was collected.

Liquid scintillation counting of radioactive blood. Whole blood samples (50 μL) were processed for radioactivity measurements as described in Rickard and Thompson (1998). Blood radioactivity determined at each time point in each rat was adjusted for the radioactivity determined at 0 h.

Statistical Analyses

Data were analyzed using SigmaStat 2.0 (Jandel Scientific, San Rafael, CA). Within a treatment group, comparisons of the percentage recovery over time for a particular urinary metabolite or of the adjusted blood radioactivity over time were analyzed by one-way ANOVA. Two-way ANOVA was used to analyze treatment multiplied by time interactions. Comparisons between acute and chronic groups at particular time points for each urinary metabolite or blood sample were done using unpaired Student’s t test. Data not normally distributed were analyzed nonparametrically using either the Kruskal-Wallis one-way ANOVA on ranks or the Mann-Whitney Rank Sum Test. Post-hoc multiple comparison tests included Student-Neuman-Keul’s Test (parametric) or Dunn’s Method (nonparametric) (Rosner 1990). Relationships between different urinary metabolites were analyzed by linear regression. For all analyses, the acceptable level of significance was P ≤ 0.05. Results were expressed as means ± SEM.

RESULTS

Experiment 1

HPLC analysis of radioactive urinary lignan metabolites. For both treatment groups, >98% of the urinary radioactivity was detected in the lignan extract analyzed by HPLC, suggesting very small losses in the clean-up procedure. Similarly, nearly the entire amount of radioactivity injected into the HPLC was recovered during the fraction collection. The total recovery ranged from 97.4 to 98.6% in the acute group and 95.9 to 97.2% in the chronic group over the 48-h period.

A typical trace of the radioactivity of timed urine fractions collected using HPLC is shown in Figure 1. Identification of the peaks was done by comparison with the retention time of authentic standards. The retention times of ED, EL and SECO were determined to be about 11–12 min, 25–27 min and 8–9 min, respectively, and their identities were confirmed with GC-MS analysis (data not shown).

Three unknown peaks in the HPLC chromatogram were consistently seen in all the urine samples analyzed (Fig. 1). Two of these peaks overlapped each other and occurred at 4–6 min. These peaks did not correspond to glucose since glucose appears at 2–3 min (data not shown) where no radioactivity was detected (Fig. 1). The third unknown peak seen on the HPLC trace occurred between the SECO and ED peak at 9–10 min.

The proportion of urinary radioactivity attributable to the known lignan metabolites and the unknown peaks over the 48-h experimental period is shown in Figure 2. In the acute group, the %SECO decreased from 12 to 48 h (P < 0.05), but the %ED and %EL did not significantly increase. In contrast, the %SECO in the urine of the chronic group was stable.

There was a trend for a treatment multiplied by time interaction for ED (P = 0.078). For unknown peak 2, there appeared to be opposite trends in percentage recovery with treatment (P = 0.1), but this did not reach significance.

By 48 h, the known metabolites ED, EL and SECO accounted for 79.7 ± 4.0% and 73.8 ± 7.7% of the total urinary radioactivity recovered in the acute and chronic treatment groups, respectively. There was no difference in the proportion of urinary ED + EL between the acute and chronic treatment groups, which accounted for 65.7 ± 4.8% and 57.5 ± 5.9%, respectively, by 48 h.

Relationships between radioactive urinary lignan metabolites. In the acute group, the expected inverse relationship was observed between ED and SECO (r² = 0.669, P = 0.001) and ED + EL and SECO (r² = 0.821, P < 0.001) (Fig. 3). A significant, although not as strong, inverse relationship was also observed between unknown peak 2 and ED (r² = 0.484, P = 0.012). Despite an inverse relationship between ED + EL and SECO (P = 0.006), no relationship existed between urinary ED and SECO in the chronic group (r² = 0.111, P = 0.316, not shown). As seen in the acute group, there was an inverse relationship between ED and the unknown peak 2 with chronic SDG treatment (r² = 0.398, P = 0.037). In contrast, a significant and strong positive relationship was observed between EL and unknown peak 2 (r² = 0.722, P < 0.001) with chronic treatment. Despite these observations, the actual relationship between ED, EL and unknown peak 2 is still uncertain.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Typical trace of radioactivity of urine fractions collected using HPLC. Identification of the enterodiol (ED), enterolactone (EL) and secoisolariciresinol (SECO) peaks was done by comparing the peak retention times with that of the pure compounds.
The HPLC fractions corresponding to the three unknowns were analyzed by GC-MS. The mass spectra of compounds corresponding to unknown peaks 1, 2 and 3 are shown in Figure 4. Unknown peak 1 may be an EL metabolite since it has a similar molecular weight (476 vs. 442 g/mol) and mass spectra breakdown pattern to the TMS ether derivative of EL (Setchell et al. 1981). For unknown peak 2, the mass spectra indicated that although this potential metabolite was not diphenolic and hence not a lignan, the molecule did contain a hydroxy group based on the difference between the suspected molecular ion peak (m/z 580) and the peak at m/z 490. This difference is equivalent to the loss of a TMSOH group from a phenolic ring (90 g/mol). No other similar losses were determined. A monophenolic metabolite of lignans would not be unusual since a metabolite of the soybean isoflavone genistein, p-ethylphenol, is also monophenolic (Mazur et al. 1996). The mass spectra for unknown peak 3 contained an ion peak (m/z 179) that would be indicative of a hydroxy-substituted benzene ring (Setchell et al. 1983).

Analysis of the HPLC fractions containing ED by GC-MS revealed the presence of another unknown, which was called unknown peak 4 (Fig. 5). Its highest abundance occurred in the 12 min HPLC fraction and therefore coeluted with ED (Fig. 1). As mentioned for unknown peak 3, the intense ion peak at m/z 179 (base peak) suggested the presence of a hydroxyl group on a benzene ring (Setchell et al. 1983). The similarities of the other ion peaks to that observed for the TMS ether derivative of ED suggested that this compound might be an ED metabolite. Because the compound was present in a fraction that also contained ED, it was possible that part of the radioactivity attributed to ED was due to this compound.

**Experiment 2**

**GC-MS analysis of unknown urinary lignan metabolites in nonradioactive rats fed flaxseed or SDG.** In all flaxseed and SDG urine samples examined, unknown peak 4 was detected toward the end of the peak previously considered to be only SECO on the gas chromatogram (data not shown). Upon further examination of flaxseed urine samples, a peak with very similar mass spectra to unknown peak 3 was found (data not shown), but this was not consistently observed in all samples. This peak may not have been detected in the SDG urine samples due to interference by the EL peak. The potential lignan metabolites corresponding to unknown peaks 1 and 2 were not found. When a larger volume of urine (10 mL) was analyzed, one of the ortho-substituted metabolites of ED (3,4-catechol) detected by Jacobs et al. (1999) was found in the urine of rats fed flaxseed but not SDG. No other metabolites that were detected by Jacobs and colleagues (1999) in humans were found.

**Experiment 3**

**Postprandial levels of 3H-SDG metabolites in whole blood over a 24-h period.** In both acute and chronic treatment groups, whole blood radioactivity levels (adjusted for baseline radioactivity at 0 h) peaked at 9 h and were still higher than
baseline at 24 h (Fig. 6). The blood radioactivity tended to be higher in the chronic group than the acute group at 24 h (P = 0.078).

**DISCUSSION**

This study suggested that ED, EL and SECO constituted a majority of the urinary lignans detected in rats with acute or chronic treatment of SDG (75–80%). In addition to the known lignan metabolites, at least four others might exist. Two of these metabolites, one of which coelutes with ED on the HPLC but has the same retention time as SECO on the GC, have been detected in the urine of rats fed SDG or flaxseed. The level of individual lignan metabolites did not differ with acute vs. chronic treatment of SDG. However, time trends in urinary levels of SECO, ED and unknown peak 2 appeared to be the opposite for the two treatment groups, suggesting that there could be a difference in the metabolic conversion of lignan metabolites with chronic SDG exposure.

Based on the urinary lignan excretion data in the study by Rickard et al. (1996), the average daily proportion of ED, EL and SECO detected in the urine of rats fed 1.5 mg/d SDG for 2 wk was 60, 8 and 32%, respectively. Using the 24-h time point as a comparison, the proportion of radioactivity detected in the chronic group for ED, EL and SECO in this study was 55, 10 and 13%, respectively. Therefore, except for SECO, the proportion of known lignan metabolites determined in this study was consistent with our previous observations (Rickard et al. 1996). The higher proportion of SECO previously determined (Rickard et al. 1996) might be due to the coelution of unknown peak 4 and SECO as a single peak on the GC. In addition, it should be noted that the proportions calculated from the data in Rickard et al. (1996) were based on the assumption that ED, EL and SECO were the only lignan metabolites present in the urine. The data from this study and other studies (Jacobs et al. 1999) suggest that other urinary lignan metabolites exist.

Although urinary SECO levels were constant throughout the 48-h period in the chronic group, SECO levels in the acute group were significantly higher at 12 h vs. all other time points. Thus urinary SECO levels in rats not previously exposed to SDG required 24 h to reach a steady state.
In both the acute and chronic treatment groups, the known SDG metabolites ED, EL and SECO accounted for a majority of the urine radioactivity. However, other potential SDG metabolites in the urine appeared to be present at radioactivity levels similar to that of EL. The unknown peaks 1 and 2 must be more polar than the known lignan metabolites because of their earlier elution on the HPLC. From the HPLC retention time of unknown peak 3, this compound would be less polar than SECO but more polar than ED. Unknown peak 4 would be slightly less polar than ED since its highest radioactivity levels were detected in 12 min HPLC fraction, whereas the majority of the radioactivity for ED was found in the 11-min fraction.

The definitive identification of the four potential lignan metabolites would require more investigation. Nuclear magnetic resonance (NMR) spectrometry (proton and $^{13}$C) would be an asset in the delineation of the unknown structures. However, relatively pure samples of at least 1 mg are required for each analysis. The radioactivity data suggest that these compounds occur in the urine at concentrations close to that of EL, which is typically < 50 µg/d in animals treated with 1.5 mg/d SDG (Rickard et al. 1996). The typical daily urinary volume of a rat is 7–10 mL. Therefore, relatively large volumes of urine would be needed to isolate and purify sufficient amounts of these compounds for analysis.

Nevertheless, the mass spectra generated for each unknown does provide some clues on their molecular structure. The similarity in the molecular weight of the TMS ether derivative of EL (442 g/mol) to the suspected molecular weight of unknown peak 1 (476 g/mol) coupled with similarities in the breakdown pattern of the molecule (Setchell et al. 1981) suggested that unknown peak 1 was a derivative of EL. For unknown peak 4, many of the ion peaks found were identical to or close to ($\pm 1$) the ion peaks observed in the mass spectra for ED.

The derivation of the identity of unknown peak 2 or 3 from their mass spectra was not as clear. Patterns in their mass spectra suggested that they both were hydroxylated compounds, and the ion peak at $m/z$ 179 for unknown peak 3 was indicative of a hydroxyl substitution of a benzene ring that occurs in the mammalian lignans. The positive linear relationship between urinary levels of unknown peak 2 and ED suggests that unknown peak 2 may be a metabolite of ED.

The detection of unknown peaks 3 and 4 in the urine of rats fed flaxseed or SDG using routine GC-MS analysis provided further evidence that they were lignan metabolites. However, in contrast to unknown peak 4, unknown peak 3 was not consistently observed in the urine samples tested, suggesting that its production in different rats was variable.

The mass spectra obtained for the four unknown compounds did not match that of the hydroxylated ED or EL metabolites detected in the urine of humans (Jacobs et al. 1999) fed flaxseed. This might be related to the relatively higher levels of these metabolites in the study samples of Jacobs et al. (1999) because they used flaxseed. Based on the human study, these metabolites accounted for <5% of the total urinary lignans (Jacobs et al. 1999). Therefore, they may not have been easily detected with the SDG dose used in the rats treated with $^3$H-SDG. Previous studies in rats have shown that urinary lignans produced from the SDG isolated from flaxseed is less than that produced from flaxseed itself (Rickard et al. 1996). Interestingly, only one of the hydroxylated ED metabolites found by Jacobs and colleagues (1999) was detected in the urine of flaxseed-fed, but not SDG-fed, rats once a larger urine volume was analyzed. The inability to detect the other metabolites may be partly attributed to the use of different clean-up procedures for the urine. Nevertheless, using a large volume of urine to detect these minor lignan metabolites decreased the sensitivity of detection for the major lignan metabolites, particularly ED and EL.

Hydrogen exchange was not thought to contribute significantly to the results observed for three reasons. First, care was taken to label the benzyl methylene hydrogens as opposed to the phenolic hydrogens with tritium to reduce the risk of tritium dissociation from the molecule. If there were significant exchange of radioactive tritium in vivo, a radioactive water peak would be expected at the solvent front of the HPLC analysis. From the radioactive tracing shown in Figure 1, this was not the case. Second, the HPLC traces of radioactive activity of the urine extracts at 12, 24, 36 and 48 h were consistent in that radioactivity was detected at specific times during each HPLC run. If tritium dissociation were significant during analysis, one would expect the radioactivity to be detected throughout the HPLC run. In addition, over 98% of the urine radioactivity was present in the samples injected on the HPLC, and almost the entire amount of radioactivity injected (96–99%) was recovered during the fraction collection. Radioactivity losses were probably due to adherence of some of the tritiated metabolites to the column and to differences in the radioactive counts determined for the fractions, for the original urine sample and for the urines extracts. Third, a majority of the radioactivity was detected at the retention times of the known lignan metabolites ED, EL and SECO, and the proportion of radioactivity determined for each metabolite was consistent with urinary levels found in a previous study (Rickard et al. 1996).

Because blood radioactivity levels were found to be highest at 12 h, the first time point, in our previous study (Rickard and Thompson 1998), the third experiment was conducted to determine whether blood radioactivity peaked before the 12-h time point. We observed that levels peaked at 9 h in both the acute and chronic treatment groups. They remained steady until 24 h in the chronic SDG group. In the acute group, blood radioactivity started to drop at 24 h but still was not significantly different compared to the 12-h time point. Similar profiles were observed in premenopausal women given 25 g dose of flaxseed where plasma lignan levels peaked at 9 h post-consumption and remained at high level until 24 h post-consumption (Nesbitt et al. 1999). This suggests that the blood lignan kinetics are similar with flaxseed or SDG consumption and comparable between humans and rats.

In conclusion, the major urinary lignan metabolites of rats fed SDG were determined to be ED, EL and SECO. However, there may be at least four other urinary metabolites that are possible derivatives of ED or EL. Two of these potential lignan metabolites were detected in the urine of rats fed flaxseed or SDG. The levels of ED + EL vs. SECO at each time point did not change with acute vs. chronic treatment with SDG, but time trends in individual urinary lignan metabolites might be influenced by chronic exposure to SDG. This study suggests that although chronic SDG exposure alters lignan disposition in rats, it does not affect the metabolite profile in comparison to acute exposure. The large proportion of ED, EL and SECO present in the urine suggests that these metabolites are largely responsible for the biological effects observed for lignans in other studies.

As observed in premenopausal women consuming flaxseed, blood radioactivity in rats levels peaked at 9 h and remained high until 24 h regardless of treatment. This suggests that the blood lignan kinetics may be similar with flaxseed or SDG consumption and that they are comparable between humans and rats. Once-daily doses of lignans in rats, as observed
previously in humans, appear to be sufficient to maintain peak blood lignan concentrations.

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LITERATURE CITED


