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Tissue Distribution of Lignans in Rats in Response to Diet, Dose−Response, and Competition with Isoflavones

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This paper investigates the occurrence and distribution of the lignan metabolites enterodiol (END) and enterolactone (ENL) and the isoflavone daidzein (DAID) in rat tissues by use of liquid chromatography−electrospray ionization mass spectrometry (LC−ESI/MS^n) following a variety of dietary regimes. Furthermore, we examined the dose−response and distribution of END and ENL in liver, testes, prostate, and lung, and we investigated the effects of competition between lignans and isoflavones on metabolite distribution. In liver, testes, prostate, and lung tissue, dose-related increases in END concentration were observed. In the testes, coadministration of 60 mg/kg secoisolariciresinol diglycoside (SDG) with 60 mg/kg isoflavones produced alterations in the resulting metabolite profile, causing increased END concentration and decreased DAID concentration. Results indicate lignan accumulation in tissues occurs, and coadministration of lignans with isoflavones affects the metabolite profile, with effects dependent on tissue type.

KEYWORDS: Lignan; isoflavone; extraction; tissue; distribution; LC−ESI/MS

INTRODUCTION

Phytoestrogens, compounds with estrogenlike properties produced by plants, have been implicated in having beneficial effects in a wide range of disease conditions (*1*, *2*). Lignans are an important class of phytoestrogens as, unlike the more studied isoflavones, they are found in many grains and grain products, which constitute the basis of most diets worldwide (*3*, *4*). The "mammalian" lignan metabolites enterodiol (END) and enterolactone (ENL) were first discovered in human urine and plasma (*5*); they have shown estrogenic effects in cultured cells and can modulate the response to endogenous estrogens (*6*, *7*). The primary precursors of END and ENL, secoisolariciresinol diglycoside (SDG) and matairesinol (MAT), are found abundantly in flaxseed (*8*).

Animal studies present evidence that lignans may have a preventative role with respect to cancers. Supplementation of the diet with plant lignans has been shown to delay the onset and reduce the number and progression of chemically induced mammary tumorigenesis in rats (*9*). Similarly, dietary supplementation with flaxseed significantly reduced the total number of aberrant crypts and crypt foci in the colon of azoxymethane-treated rats, suggesting a reduction in the risk of development of colon cancer (*10*). An inverse relationship between plasma concentration and urinary excretion of lignans and the incidence of breast cancer has also been observed in a number of studies (*11*−*13*), and high concentrations of ENL significantly inhibited growth of MCF-7 breast cancer cells, possibly indicating a toxic effect on the cancer (*7*). The effect of mammalian lignans on multiple prostate cancer cell lines has also been investigated, and it was demonstrated that END and ENL were able to inhibit the growth of human prostate cancer cell lines in vitro (*14*). Furthermore, administration of SDG to nursing rat dams improved bone strength of young female offspring (*15*), suggesting that lignans can influence bone metabolism. In addition, lignan compounds were found to possess significant antioxidant activity (*16*), which may be another critical mechanism through which they confer health benefits.

Despite the potential importance of lignans in the reduction of disease and cancer risks, little is known concerning their metabolic fate and distribution in the body, as well as the concentrations of the metabolites in the tissues resulting from ingestion of the precursor compounds (*17*). This information is essential for an assessment of pharmacological activity of lignans and for a complete understanding of their potential mechanisms of action as a dietary supplement (*18*).

This paper describes measurement of the occurrence and distribution of the lignans END and ENL in four tissues from rats consuming diets fortified with linola meal for 7 days. The strength of our approach was the controlled administration of the compounds on a milligrams per kilogram of body weight basis at two dose levels and robust, sensitive, and precise analytical determinations consequent of using mass spectrometry (MS^n) with deuterium-labeled genistein as an internal standard.

MATERIALS AND METHODS

This study was performed in three phases. An initial development phase was carried out with eight animals, to establish dosages required

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and to allow optimization of the extraction procedure. In the second phase the dose−response at two doses for 18 animals was investigated, while in the third phase we examined the effect of dietary competition between lignans and isoflavones for 24 animals.

Animals. Male Sprague-Dawley rats [Animal Resources Centre (ARC), WA Australia] were used throughout the study. All procedures involving care and handling of the animals were approved by the University Animal Ethics Committee (AE 05/03). Upon arrival at the University of Wollongong Animal House, the rats, aged 3−5 weeks, were housed in pairs in standard rat cages with sawdust bedding, and were given phytoestrogen-free food and water ad libitum. During the dietary intervention, the animals were housed in individual cages to ensure that each rat had consumed its entire dose. Rats were weighed and handled daily.

Rat Diet Treatments. The rats were given a phytoestrogen-free AIN-93 feed washout diet, replacing soy protein with casein, for at least 1 week before initiation of experimental diets (*19*). This washout period ensured that the majority of phytoestrogens consumed prior to arrival were eliminated from the tissues. Phytoestrogen-free food was prepared in-house as required. The administration of the lignans was achieved through fortification of the AIN-93 phytoestrogen-free feed. The lignan (secoisolariciresinol diglycoside, SDG) source was linola meal (Specialty Cereals Pty Ltd., NSW), which provided 4130 ± 80 μg/g SDG, determined by the manufacturer, and was substituted into the feed in place of an equal weight of corn flour. For the dose−response and dietary competition phases, control animals were maintained on the phytoestrogen-free diet throughout the period of dietary intervention. Lignan fortification was carried out as stated above. Isoflavone enrichment of the diet was achieved through substitution of corn flour with soy germ flour (Isolife, Netherlands). One gram of this flour yielded approximately 22.5 mg of isoflavones, consisting of 12.2 mg/g daidzin, 3.3 mg/g genistin, and 7.0 mg/g glycitin, as determined by the manufacturer.

Choice of Dose and Tissue. Results from preliminary experiments revealed that a minimum dose of 15 mg/kg was required to elicit a quantifiable tissue concentration of the analytes. Further, the 60 mg/kg dose appeared to result in modulation of absorption, metabolism, and tissue uptake of the compounds compared to lower doses and was selected as the high dose level. Results from a similar study by Chang et al. (*18*) showed higher concentrations of the isoflavone genistein in endocrine-responsive organs expressing proportionally higher levels of estrogen receptor (ER) β than ER α, such as prostate, and organs with metabolic function, such as liver. This suggested that accumulation in these tissues may be a result of their ER subtype expression, metabolic function, and/or adiposity. Four tissues were selected for analysis. Liver was selected due to its role in the metabolism and conjugation of the compounds (*20*), and its balanced expression of ER β and ER α (*21*). Prostate was selected due to its high adiposity and the predominant expression of ER β over ER α (*21*). Testes were chosen due to their endocrine-responsive nature and approximately equal expression of ER α and ER β (*21*). Finally, lung was chosen due to its ER expression pattern (*21*) and nonendocrine nature.

Dosing Regime. Animals were fed twice a day at 9 a.m. and 5 p.m. (±1 h); with 8 h between the first and second dose to maintain a high bioavailability of lignan metabolites in the blood and tissues (*22*−*24*). As there was no literature available on tissue levels of the lignan metabolites END and ENL in relation to dietary fortification with SDG, dose ranges were derived from literature on similar experiments using isoflavone genistein (*18*). The daily doses of the animals were close to 0.3, 6, and 30 mg of isoflavone kg^{-1} day^{-1}, respectively. The doses of SDG tested were 0.3, 15, 30, and 60 mg/kg. The feed was fortified (where linola meal or soy flour is added to basal diet) at three different levels, 5, 500, and 1000 μg/g, to achieve the required dose levels. Doses (milligrams per kilogram) were calculated for individuals on the basis of their body mass at the start of the 7-day dietary intervention. The size of the fortified food block was adjusted to provide each animal with the correct dose, while providing sufficient food intake. The food block weights ranged from approximately 5 g for the smaller animals, up to approximately 14 g for the larger animals for each dose. The 0.3 mg/kg food blocks were made from phytoestrogen-free food fortified at 5 μg/g; the 15 and 30 mg/kg food blocks were made with food fortified at 500 μg/g; and the food blocks providing 60 mg/kg were made from food fortified at 1000 μg/g. To ensure animals were not deprived of access to food, phytoestrogen-free food was also provided each day in addition to the fortified food. As the rats preferentially consumed the fortified food rather than the washout diet, the fortified food blocks and phytoestrogen-free food were provided together, without compromising the total consumption of fortified diet.

Sacrifice and Tissue Collection. Rats were sacrificed after 7 days consumption of the SDG-enriched diet by an intraperitoneal (ip) overdose of sodium pentobarbitol (60 mg/kg). The consumption of the final dose was 19 ± 2 h prior to sacrifice. Sacrifice was followed by whole body perfusion with approximately 200 mL of 0.9% saline delivered by a perfusion pump through the left ventricle. Perfusion was considered complete when exsanguinated fluid was clear, which generally was around 5 min. Perfusion ensured removal of lignans and isoflavones from tissue capillary beds. Selected tissues were dissected, rapidly frozen in foil by freeze-clamping in liquid nitrogen, and stored at −80 °C until processed. The following organs were collected: liver, testes, prostate, and lung.

Chemicals. Enterodiol, enterolactone, and daidzein were purchased from Sigma−Aldrich (NSW, Australia). Deuterated genistein (6,8,3′,5′-d_4, 98% purity, 95% isotopic enrichment) was purchased from Cambridge Isotope Laboratories (Andover, MA). β-Glucuronidase enzyme (G-0751) and citric acid were obtained from Sigma−Aldrich (St Louis, MO). Trisodium citrate was purchased from Ajax Chemicals (NSW, Australia). Sodium pentobarbitol was obtained from Virbac (NSW, Australia). Solvents were HPLC−grade, and Milli Q water was used throughout the study.

Tissue Preparation. A portion of the tissue was excised and weighed (78−300 mg) and then finely homogenized at 24 000 rpm for 60 s at room temperature by use of an Ultra Turrax Dispenser (T25 Basic, IKA Labortechnik) in citrate buffer (25 mM, pH 5.0) added at 1 mL/100 mg of tissue. Aliquots (200 μL) of the homogenate containing 20 mg of tissue were transferred to glass tubes, and a crude extraction and isolation of the compounds was achieved through the addition of 800 μL of methanol followed by sonication for 10 min. To establish the optimal quantity of enzyme required for maximal deconjugation, two concentrations were tried. Initially, following the method of Chang et al. (*18*), 45 units of enzymatic activity was added to each sample, but the addition of 90 units slightly increased deconjugation. The enzyme mixture was made by dissolving 20 mg of β-glucuronidase enzyme (492 000 units/g solid (Sigma−Aldrich, St Louis, MO) in citrate buffer to yield a solution with 9000 units/mL enzymatic activity. An aliquot (10 μL) of this solution providing 90 units of enzymatic activity was added to the samples in addition to 1990 μL of citrate buffer prior to vortex mixing, and the tissue extracts were incubated in a water bath at for 1 h at 37 ± 2 °C. Lipid removal was achieved with 3 × 1 mL washes of *n*-hexane, and the remaining fraction was further separated by centrifugation, eliminating any remaining solids and lipids from the sample. Samples were centrifuged at 3800 rpm (1652*g*) for 30 min at 4 °C to ensure removal of any solids and remnant lipids.

Solid-Phase extraction. Specific isolation of the lignan compounds from the resultant supernatant was facilitated through solid-phase extraction (SPE). Extraction was achieved by use of 6 cm^3 (200 mg) Oasis HLB SPE cartridges (Waters, Milford, MA). The cartridges were preconditioned by sequential elution of 1 mL of methanol, 1 mL of water, and 1 mL of citrate buffer through the cartridge prior to use. For the dietary competition part of the study, the SPE procedure was optimized for the recovery of the two lignans and for daidzein. Following centrifugation, the supernatant was applied to the preconditioned SPE cartridge and the cartridge was washed free of contaminants with 1 mL of Milli Q water. The bound compounds were then eluted with 2 mL of methanol. The eluted sample was concentrated by complete evaporation of the solvent under a nitrogen gas stream in a water bath at 37 ± 2 °C. The residue was then reconstituted with 50 μL of the loading solvent and acetonitrile/water (1:3) and vortex-mixed for 30 s before transfer to the total recovery injection vials. At this point, 50 μL (concentration 100 ng/mL) of the internal standard

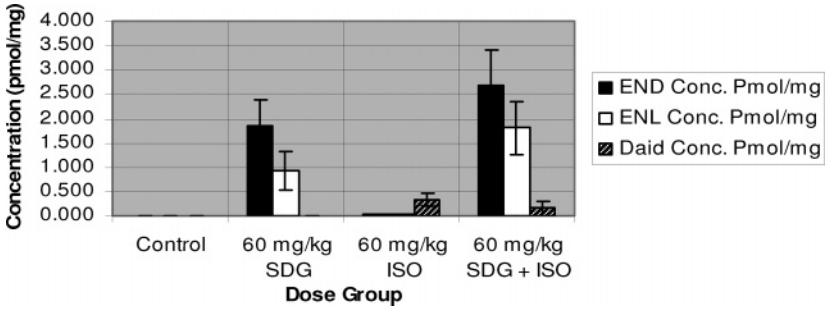

Figure 1. Concentration of enterodiol (END), enterolactone (ENL), and daidzein (Daid) in liver tissue of rats exposed to 60 mg/kg SDG, 60 mg/kg isoflavone, or 60 mg/kg SDG + 60 mg/kg isoflavone in the dietary competition phase. Values are means ($n = 6$) ± SEM.

Table 1. Instrument Conditions for HPLC–ESI-MS^n Analysis of the Selected Lignans and Isoflavones

compd	retention time (min)	precursor ion (*m/z*)	SRM transitions (*m/z*)	normalized collision energy (%)
enterodiol	10.7	301.0	301.0 → 253.0	40
			301.0 → 271.0	
			301.0 → 241.0	
enterolactone	13.0	296.9	296.9 → 252.9 → 194.9	30, 45
			296.9 → 252.9 → 182.9	
			296.9 → 252.9 → 106.9	
daidzein	10.1	252.9	252.9 → 224.9	46
			252.9 → 223.9	
			252.9 → 208.8	
genistein-d_4	12.5	272.9	272.9 → 184.9	49
			272.9 → 204.9	
			272.9 → 244.9	

deuterated (d_4) genistein (Sigma−Aldrich, St Louis, MO) was added to the samples.

Analysis. Quantitation of the lignans END and ENL and the isoflavone DAID in the extracted tissue samples was performed on a LTQ linear ion trap mass spectrometer (ThermoFinnigan, San Jose, CA) equipped with an electrospray ionization (ESI) source. A ThermoElectron Surveyor HPLC system was interfaced to the mass spectrometer for automated LC/MS^n analyses. The method used was previously established and developed within our laboratory (*25*) and was adapted for the present study. The mass spectrometer was operated in negative ion electrospray mode; the mass spectrometric conditions for the analysis were optimized by direct infusion of standards into the mass spectrometer. Data acquisitions used selected reaction monitoring (SRM) with either MS^2 or MS^3 scan mode. The selection of MS^2 or MS^3 transitions was carried out by consideration of both the selectivity and sensitivity of each analyte. Three characteristic SRM transitions were selected for each analyte; one SRM transition in conjunction with matrix-matched calibration curves was used for quantitation and the other two SRM transitions for confirming the identity of analyte (*25*). The optimized instrument conditions for the analysis are shown in **Table 1**. The limit of quantitation (LOQ) was 0.008 pmol/mg of tissue for END and ENL and 0.01 pmol/mg of tissue for DAID. The recovery of known amounts of analytes spiked into control tissue samples ranged from 33% to 87%, depending on tissue matrix. No corrections were made for the recoveries to the data presented in **Table 2**. The precision of the individual measurements, determined from repeatability of the recoveries ($n = 3$), was found to be less than 5% (which was considerably less than the interindividual variation within groups). More details about the general method have been published previously (*25*).

The HPLC column was an XTerra MS C18, 3.5 μM, 2.1 × 100 mm column with a 2.1 × 20 mm guard column. The mobile phase consisted of acetonitrile and water with a constant concentration of 0.1% formic acid. A gradient program was employed with a variable proportion of acetonitrile (20% to 90% v/v); 90% acetonitrile was maintained for 8 min to wash all organics and fat from the column; and then acetonitrile was decreased back to 20% to re-equilibrate the column for the next run. The total running time was 26 min. Trials of different loading solvents and ratios were carried out, with optimal performance achieved with sample solvent ratio of acetonitrile/water (1:3) and an injection volume of 5 μL. The mobile phase flow rate was 200 μL/min.

Statistical Analysis. Statistical analyses were performed with the software package JMP (version 5.1, SAS Institute Inc.). Effects of treatment were evaluated with one-way analysis of variance (ANOVA), followed by Student's *t*-test and Tukey−Kramer HSD post hoc analysis to determine differences between groups. Data are expressed as means ± SEM, unless specified. Values were considered statistically significant at a level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Dose−Response. The liver exhibited a dose-dependent increase in END concentration with increased dose. Administration of the 60 mg/kg dose resulted in significant increases in tissue concentration, compared to controls ($p < 0.01$) (**Figure 1**, **Table 2**). With the 60 mg/kg dose, the tissue END concentration significantly increased 4.7-fold compared to the 15 mg/kg dose ($p < 0.01$) (**Table 2**). The uptake of END and ENL into tissues may be a function of the chemical properties of the compounds, as it was found that the binding affinities of phytoestrogens to human sex hormone binding globulin (SHBG) were influenced by the substitution pattern of the aromatic rings (*26*). This may indicate a selective uptake of END into tissues due to its dihydroxy moiety or decreased conversion of END to ENL in the gastrointestinal tract of the animals at the higher dose level, supported by findings of another study (*27*). Blood levels of both metabolites would help determine whether END is more bioavailable than ENL. Furthermore, binding to transport proteins, metabolism, and renal clearance of ENL may influence tissue concentrations, resulting in disproportional changes in tissue levels.

The testes exhibited a significant increase in END level compared to controls with the administration of the 60 mg/kg dose ($p < 0.02$) (**Figure 2**). This seems to indicate that accumulation of lignans in reproductive tissues occurs in a dose-dependent manner, and it has been suggested that accumulation of the chemically similar isoflavone genistein in endocrine responsive tissues is predominately dependent on the adiposity and the estrogen receptor density and subtype (*18*).

The prostate achieved the highest concentration of END (1.899 pmol/mg) of all tissues examined at the 60 mg/kg dose, a significant increase over the levels achieved in the controls ($p < 0.02$) and the 15 mg/kg dose group ($p < 0.05$), (**Table 2**). This may indicate greater bioavailability of END or an increased clearance of ENL. The substantial rise in END concentration in prostate with increased dose suggests that the prostate is highly sensitive to increases in levels of dietary SDG and has a relatively high capacity for accumulation of lignans. This finding is biologically significant, as lignan consumption has

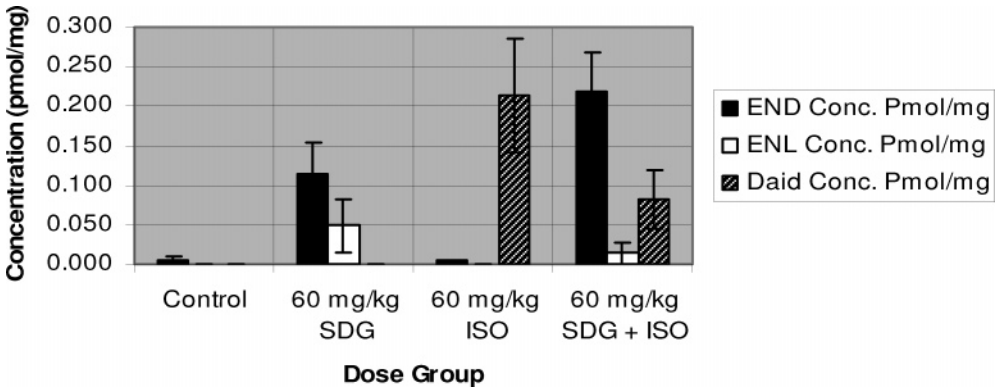

Figure 2. Concentration of enterodiol (END), enterolactone (ENL), and daidzein (Daid) in testes of rats exposed to 60 mg/kg SDG, 60 mg/kg isoflavone, or 60 mg/kg SDG + 60 mg/kg isoflavone in the dietary competition phase. Values are means ($n = 6$) ± SEM.

Table 2. Tissue Concentrations of Enterodiol, Enterolactone, and Daidzein in Rats[a]

	enterodiol (pmol/mg)		enterolactone (pmol/mg)		daidzein (pmol/mg)	
tissue	mean	SEM	mean	SEM	mean	SEM
			Control			
liver	0.002 A,I	0.002	0.008 J	0.007	0.000 K	0.000
testes	0.005 C,L	0.004	0.000	0.000	0.000 N	0.000
prostate	0.003 D	0.002	0.000	0.000	0.000 P	0.000
lung	0.000 F	0.000	0.001 G	0.001	0.000	0.000
			15 mg/kg SDG			
liver	0.386 B	0.147	0.458	0.208		
testes	0.058	0.029	0.034	0.029		
prostate	0.258 E	0.118	0.038	0.038		
lung	0.014	0.004	0.008 H	0.003		
			60 mg/kg SDG			
liver	1.844 A,B	0.528	0.923	0.392		
testes	0.114 C,M	0.040	0.049	0.034		
prostate	1.899 D,E	0.768	0.288	0.261		
lung	0.054 F	0.028	0.022 G,H	0.006		
			60 mg/kg Isoflavones			
liver					0.331 K	0.134
testes					0.213 N,O	0.072
prostate					0.471 P	0.243
lung					0.000	0.000
			60 mg/kg SDG + 60 mg/kg Isoflavones			
liver	2.689 I	0.717	1.802 J	0.554	0.159	0.144
testes	0.217 L,M	0.050	0.014	0.014	0.082 O	0.038
prostate	0.960	0.852	0.096	0.096	0.121	0.055
lung	0.023	0.011	0.020	0.011	0.000	0.000

[a] Rats were dosed with either 15 mg/kg SDG, 60 mg/kg SDG, 60 mg/kg isoflavones, or 60 mg/kg SDG + 60 mg/kg isoflavones. Values linked by the same letter are significantly different from one another at $p < 0.05$.

been reported to inhibit development and increase apoptosis in prostatic tumor cell lines (*14*). Whether this effect is due to the actions of lignans directly on the ER, effects on other enzymes such as 5α-reductase, antioxidant action, or effects on hormone binding proteins has yet to be established. Apropos to this line of reasoning is the recent study demonstrating the capability of ENL to displace dihydrotestosterone (DHT) from SHBG (*28*). Supporting the hypothesis of increased clearance of ENL, Nesbitt et al. (*22*), investigating dose−response in humans of dietary flaxseed supplements for 7 days, showed that urinary ENL excretion increased over the duration of the study. In addition, possible gender differences in lignan excretion may exist, as it was demonstrated that men excrete more ENL, but less END, than women (*29*). This may be an important factor to consider in future research, as our study used male rats throughout all phases, and it is possible that ENL concentration in tissues and plasma will be higher or different in females.

Finally, lung exhibited a significant 2.8-fold increase in ENL concentration with the dose increase from 15 to 60 mg/kg ($p < 0.02$) (**Table 2**). END concentrations in the 60 mg/kg dose group were also significantly different from controls ($p < 0.05$). Although lung tissue is less sensitive to increases in dose, and despite its nonendocrine nature, the results demonstrate that accumulation in lung tissue occurs in a dose-dependent manner. These results may suggest that accumulation in lung tissue is mediated by ER subtype, distribution, and/or adiposity.

Dietary Competition. In the liver, prostate, and lung tissue analyzed, the coadministration of SDG and isoflavones did not result in any significant alteration of the metabolite profile, compared to the isolated administration of each compound (**Figure 1**, **Table 2**). However, in the testes, coadministration of 60 mg/kg SDG and 60 mg/kg isoflavones resulted in a significant 1.9-fold increase in END concentration ($p < 0.05$), compared to the administration of 60 mg/kg SDG alone. In addition, a significant 29% decrease in DAID concentration was observed, compared to the administration of 60 mg/kg isoflavones alone ($p < 0.05$) (**Figure 2**, **Table 2**). This is a surprising result, as a recent in vitro study investigating binding affinities of a range of phytoestrogens to uterine extract relative to estradiol and DHT (*28*) found DAID to have substantially higher binding affinity than ENL, while END did not bind to the uterine tissue extract at all.

The altered concentrations of END and DAID in the testes resulting from the coadministration of SDG and isoflavones, compared to the administration of each compound alone, demonstrated that some level of competition was evident between the compounds. One can only speculate on the underlying mechanisms, although bioavailability, differential binding affinities, metabolism, and clearance are all likely to be involved.

Overall, our results suggest that competition for uptake into tissues can occur between the compounds when they are concomitantly administered in the diet. Another possibility is that the compounds are competing for metabolic enzyme systems, such as cytochrome P450, or the hepatic phase II enzymes UDP-glucuronyl transferase and sulfotransferase, which are implicated in the conjugation of isoflavones and lignans to glucuronic acid and sulfate, respectively (*30*). As this process facilitates transport from the bloodstream into the urine and is essential for renal clearance of these compounds, the preferential conjugation of the isoflavones compared to END and ENL could result in a greater proportion of END and ENL being bioavailable for uptake into the tissues, resulting in higher tissue concentrations being achieved.

In liver, testes, prostate, and lung tissue, dose-related increases in END concentration were observed. In the testes, coadministration of 60 mg/kg SDG with 60 mg/kg isoflavones produced alterations in the resulting metabolite profile, causing increased END concentration and decreased DAID concentration, compared to the administration of each compound alone. On an individual basis, for all tissues and dose groups analyzed with the exception of liver at the dose of 15 mg/kg SDG, the majority of rats showed a higher uptake of END than ENL. This predominance was increased with the higher dose level of 60 mg/kg SDG compared to 15 mg/kg SDG. However, due to the large variation between individuals within and between groups, the uptake of END for any one group or overall was not determined to be statistically significantly different from the uptake of ENL. The results from this study indicate there is potential for increasing the effectiveness of lignan compounds in conferring beneficial health effects against specific pathological conditions such as chronic disease and certain cancers prevalent in Western culture, through coadministration of lignans with isoflavones. Coadministration appears to increase the bioavailability of lignan compounds for certain tissues, and thus may increase their efficacy in relation to these and other hormone-dependent pathologies. The benefits of concomitant intake may extend to the dual administration of current anticancer therapeutic agents, such as tamoxifen, in conjunction with lignans, to increase the beneficial effects of the compounds in certain tissues.

ABBREVIATIONS USED

LC−ESI/MS^n, liquid chromatography−electrospray ionization mass spectrometry; END, enterodiol; ENL, enterolactone; DAID, daidzein; GEN, genistein; SDG, secoisolariciresinol diglycoside; MAT, matairesinol; d_4, deuterated; HPLC, high-performance liquid chromatography; ip, intraperitoneal; ^{3}H, tritiated; DHT, dihydrotestosterone; ANOVA, analysis of variance; SPE, solid-phase extraction; ER, estrogen receptor; SHBG, sex hormone binding globulin.

LITERATURE CITED

(1) Adlercreutz, H.; Mazur, W. Phyto-oestrogens and Western diseases. *Ann. Med.* **1997**, *29*, 95−120.

(2) Cornwell, T.; Cohick, W.; Raskin, I. Dietary phytoestrogens and health. *Phytochemistry* **2004**, *65*, 995−1016.

(3) Mazur, W. Phytoestrogen content in foods. *Bailliere's Clin. Endocrinol. Metab.* **1998**, *12*, 729−742.

(4) Penalvo, J. L.; Heinonen, S. M.; Nurmi, T.; Deyama, T.; Nishibe, S.; Adlercreutz, H. Plant lignans in soy-based health supplements. *J. Agric. Food Chem.* **2004**, *52*, 4133−4138.

(5) Setchell, K. D.; Lawson, A. M.; Mitchell, F. L.; Adlercreutz, H.; Kirk, D. N.; Axelson, M. Lignans in man and in animal species. *Nature* **1980**, *287*, 740−2.

(6) Adlercreutz, H.; Mousavi, Y.; Clark, J.; Hockerstedt, K.; Hamalainen, E.; Wahala, K.; Makela, T.; Hase, T. Dietary Phytoestrogens and Cancer—Invitro and Invivo Studies. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 331−337.

(7) Mousavi, Y.; Adlercreutz, H. Enterolactone and estradiol inhibit each other's proliferative effect on MCF-7 breast cancer cells in culture. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 615−9.

(8) Rickard, S. E.; Orcheson, L. J.; Seidl, M. M.; Luyengi, L.; Fong, H. H.; Thompson, L. U. Dose-dependent production of mammalian lignans in rats and in vitro from the purified precursor secoisolariciresinol diglycoside in flaxseed. *J. Nutr.* **1996**, *126*, 2012−9.

(9) Saarinen, N. M.; Huovinen, R.; Warri, A.; Makela, S. I.; Valentin-Blasini, L.; Sjoholm, R.; Ammala, J.; Lehtila, R.; Eckerman, C.; Collan, Y. U.; Santti, R. S. Enterolactone inhibits the growth of 7,12-dimethylbenz(*a*)anthracene-induced mammary carcinomas in the rat. *Mol. Cancer Ther.* **2002**, *1*, 869−76.

(10) Serraino, M.; Thompson, L. U. Flaxseed supplementation and early markers of colon carcinogenesis. *Cancer Lett.* **1992**, *63*, 159−65.

(11) Dai, Q.; Franke, A. A.; Jin, F.; Shu, X. O.; Hebert, J. R.; Custer, L. J.; Cheng, J. R.; Gan, Y. T.; Zheng, W. Urinary excretion of phytoestrogens and risk of breast cancer among Chinese women in Shanghai. *Cancer Epidemiol. Biomarkers Prev.* **2002**, *11*, 815−821.

(12) Adlercreutz, H.; Heikkinen, R.; Woods, M.; Fotsis, T.; Dwyer, J. T.; Goldin, B. R. Excretion of the Lignans Enterolactone and Enterodiol and of Equol in Omnivorous and Vegetarian Post-Menopausal Women and in Women with Breast-Cancer. *Lancet* **1982**, *2*, 1295−1299.

(13) Pietinen, P.; Stumpf, K.; Mannisto, S.; Kataja, V.; Uusitupa, M.; Adlercreutz, H. Serum enterolactone and risk of breast cancer: A case-control study in eastern Finland. *Cancer Epidemiol. Biomarkers Prev.* **2001**, *10*, 339−344.

(14) Lin, X.; Switzer, B. R.; Demark-Wahnefried, W. Effect of mammalian lignans on the growth of prostate cancer cell lines. *Anticancer Res.* **2001**, *21*, 3995−3999.

(15) Ward, W. E.; Yuan, Y. V.; Cheung, A. M.; Thompson, L. U. Exposure to purified lignan from flaxseed (*Linum usitatissimum*) alters bone development in female rats. *Br. J. Nutr.* **2001**, *86*, 499−505.

(16) Kitts, D. D.; Yuan, Y. V.; Wijewickreme, A. N.; Thompson, L. U. Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. *Mol. Cell Biol.* **1999**, *202*, 91−100.

(17) Setchell, K. D. R.; Cassidy, A. Dietary isoflavones: Biological effects and relevance to human health. *J. Nutr.* **1999**, *129*, 758S−767S.

(18) Chang, H. C.; Churchwell, M. I.; Delclos, K. B.; Newbold, R. R.; Doerge, D. R. Mass spectrometric determination of genistein tissue distribution in diet-exposed Sprague-Dawley rats. *J. Nutr.* **2000**, *130*, 1963−1970.

(19) Thigpen, J. E.; Setchell, K. D. R.; Saunders, H. E.; Haseman, J. K.; Grant, M. G.; Forsythe, D. B. Selecting the appropriate rodent diet for endocrine disruptor research and testing studies. *Inst. Lab. Anim. Res. J.* **2004**, *45*, 401−416.

(20) Cassidy, A.; Hanley, B.; Lamuela-Raventos, R. M. Isoflavones, lignans and stilbenes—origins, metabolism and potential importance to human health. *J. Sci. Food Agric.* **2000**, *80*, 1044−1062.

(21) Kuiper, G.; Carlsson, B.; Grandien, K.; Enmark, E.; Haggblad, J.; Nilsson, S.; Gustafsson, J. A. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* **1997**, *138*, 863−870.

(22) Nesbitt, P. D.; Lam, Y.; Thompson, L. U. Human metabolism of mammalian lignan precursors in raw and processed flaxseed. *Am. J. Clin. Nutr.* **1999**, *69*, 549−55.

(23) Rickard, S. E.; Thompson, L. U. Chronic exposure to secoisolariciresinol diglycoside alters lignan disposition in rats. *J. Nutr.* **1998**, *128*, 615−23.

(24) Nesbitt, P. D.; Thompson, L. U. Lignans in homemade and commercial products containing flaxseed. *Nutr. Cancer* **1997**, *29*, 222−7.

(25) Kang, J.; Price, W. E.; Hick, L. A. Simultaneous determination of isoflavones and lignans at trace levels in natural waters and wastewater samples using liquid chromatography/electrospray ionization ion trap mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2411−2418.

(26) Schottner, M.; Gansser, D.; Spiteller, G. Lignans from the roots of *Urtica dioica* and their metabolites bind to human sex hormone binding globulin (SHBG). *Planta Med.* **1997**, *63*, 529−32.

(27) Lampe, J. W. Isoflavonoid and lignan phytoestrogens as dietary biomarkers. *J. Nutr* **2003**, *133*, 956S-964S.

(28) Hillerns, Pablo, I.; Wink, M. Binding of flavonoids from *Sophora flavescens* to the rat uterine estrogen receptor. *Planta Med.* **2005**, *71*, 1065−8.

(29) Kirkman, L. M.; Lampe, J. W.; Campbell, D. R.; Martini, M. C.; Slavin, J. L. Urinary lignan and isoflavonoid excretion in men and women consuming vegetable and soy diets. *Nutr. Cancer* **1995**, *24*, 1−12.

(30) Holder, C. L.; Churchwell, M. I.; Doerge, D. R. Quantification of Soy Isoflavones, Genistein and Daidzein, and Conjugates in Rat Blood Using LC/ES-MS. *J. Agric. Food Chem.* **1999**, *47*, 3764−3770.

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