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Mammalian Lignan Formation in Rats Fed a Wheat Bran Diet

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The dietary origin of lignan phytoestrogens is still poorly understood more than 20 years after their discovery in human urine. Their level in urine has been associated with the consumption of dietary fiber. This paper reports the study of the excretion of enterolactone, assayed by a time-resolved fluoroimmunoassay, in rats fed a diet supplemented with 15% wheat bran, one of the main sources of fiber in Western countries. Enterolactone excretion regularly increased during the two weeks of the diet to reach a value of 45 nmol/day. The level of excretion also increased upon preadaptation to ferulic acid, structurally related to secoisolariciresinol, an established precursor of enterolactone in flaxseeds, and decreased upon preadaptation to potato starch rich in fiber. These results show that the formation of lignans from wheat bran is influenced by the diet, possibly because of an adaptation of the colonic microflora.

KEYWORDS: Lignan phytoestrogens; enterolactone; enterodiol; wheat bran

INTRODUCTION

Lignans are phytoestrogens present in plant foods that have potential protective effects on human health (*1*). Enterolactone (Enl) and enterodiol (End) are the two main lignans identified in human urine and plasma (**Figure 1**). They are structurally related to synthetic estrogens, and they may function as weak estrogens or estrogen antagonists. As soy isoflavone phytoestrogens, lignans can bind to estrogenic receptors (*2*) and show estrogenic effects in cultured cells (*3*, *4*). Enl also inhibits the growth of MCF-7 human breast cancer cells induced by estradiol but stimulates the proliferation of these cells in the absence of estradiol (*5*).

As modulators of the estrogenic response, lignans may contribute to the prevention of hormone-dependent degenerative diseases. Supplementation of the diet with lignans was shown to influence the mammary gland structure (*6*) and to delay the progression of chemically induced mammary tumorigenesis in the rat (*7*). Epidemiologic studies have also stressed the potential protective role of lignan phytoestrogens. Inverse associations between the plasma concentration or urinary excretion of lignans and the risk of different degenerative diseases, most particularly breast and prostate cancers, have also been observed (*8*).

Despite the well-established and general occurrence of lignans in human plasma and urine, their dietary origin is still a matter of debate. Their dietary origin (*9*, *10*) and the role of the colonic microflora in their formation (*11*, *12*) were demonstrated soon after their discovery in human urine (*13*, *14*). Flaxseed was recognized as a rich source of lignans [secoisolariciresinol (SECO) and matairesinol (MAT)] (**Figure 1**) (*15*) and still appears today as the best dietary source of lignans (*16*). However, the low consumption of flaxseed in most human

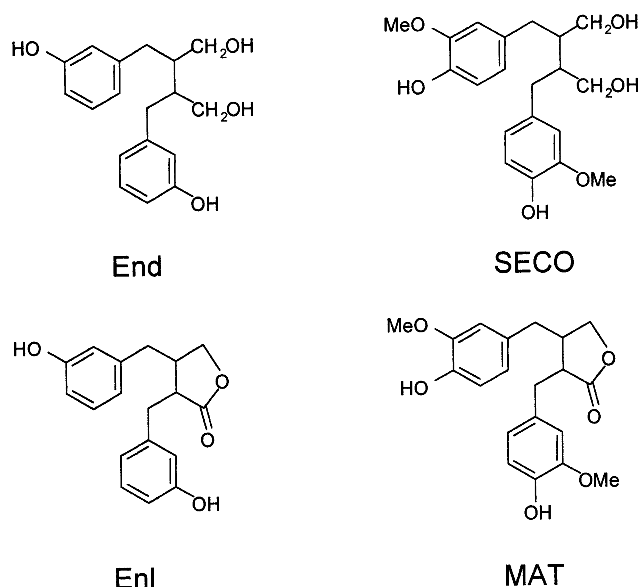

Figure 1. Chemical structures of mammalian and plant lignans: Enl, enterolactone; End, enterodiol; SECO, secoisolariciresinol; MAT, matairesinol.

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Table 1. Composition of the Experimental Diets

component (g/kg of diet)	basal diet	15% wheat bran	1.25% flaxseed	0.2% ferulic acid	15% potato starch	15% potato starch + 15% wheat bran	0.2% ferulic acid + 15% wheat bran
casein	150	150	150	150	150	150	150
wheat starch	755	605	742.5	753	605	455	603
mineral mixture[a]	35	35[b]	35	35	35	35[b]	35
vitamin mixture[c]	10	10	10	10	10	10	10
peanut oil	50	50	50	50	50	50	50
flaxseed			12.5				
ferulic acid				2			2
potato starch					150	150	
wheat bran		150				150	150

[a] Mineral mixture AIN-93M (per kg of diet): $CaHPO_4$, 15 g; K_2HPO_4, 2.5 g; KCl, 5 g; NaCl, 5 g; MgCl , 2.5 g; Fe_2O_3, 2.5 mg; $MnSO_4$, 125 mg; $CuSO_4$, 125 mg; $ZnSO_4{\cdot}7H_2O$, 100 mg; KI, 0.4 mg. Purchased from UAR (Villemoisson, Epinay-sur-Orge, France). [b] For the diets containing bran, the quantities of minerals added were corrected for the mineral content of wheat bran. [c] Vitamin mixture AIN-76A supplemented in choline (mg/kg of diet): thiamin, 15; riboflavin, 20; pyridoxine, 10; nicotinamide, 100; pantothenate, 70; folic acid, 5; biotin, 0.3; cyanocobalamin, 0.05; retinyl palmitate, 1.5; *dl*-α-tocopheryl acetate, 125; cholecalciferol, 0.15; menadion, 1.5; ascorbic acid, 50; *myo*-inositol, 100; choline, 1360. Purchased from UAR.

populations cannot explain the general occurrence of mammalian lignans in human tissues, and many authors have speculated on their dietary sources and the identities of their precursors.

A vegetarian diet rich in fiber is associated with a high lignan excretion (*9*, *17*), and various foods of plant origin such as cereals, vegetables, or fruits likely contribute to the mammalian lignan formation (*15*, *16*, *18*, *19*). A positive association was found between urinary lignan excretion and fiber intake in a group of 98 North Americans, the best correlation being observed for dietary fibers originating from grains (*20*). Rye bread, largely consumed in Scandinavian countries, is a good source of mammalian lignans (*21*, *22*). Precursors are essentially present in the bran (*16*, *23*), and whole cereals may thus significantly contribute to the lignan intake. Wheat bran is thus a potentially important dietary source of lignans in Western diets so far not examined.

In the present work, we have studied the formation of mammalian lignans from wheat bran in rats and compared the results to those obtained with a diet rich in flaxseed. We have also evaluated the influence of a previous adaptation to ferulic acid and potato starch, which may stimulate the production of enzymes or the growth of microorganisms involved in the formation of mammalian lignans from wheat bran. Ferulic acid was selected as it is structurally related to the SECO lignan. It shows the same 3-methoxy-4-hydroxy substitution pattern on the aromatic ring. Furthermore, it is known to be metabolized into 3-hydroxyphenylpropionic acid in rats (*24*), which has a ring substitution pattern identical to that of Enl and End metabolites. Supplementation of the diet with raw potato starch, largely resistant to digestion, provides large amounts of fermentable substrates to the large intestine, increases the microbial biomass in the cecum (*25*), and could thus also increase the Enl production.

MATERIALS AND METHODS

Rats, Diet, and Sampling Procedure. Male Wistar rats (Iffa-Credo, L'Arbresle, France) were housed two per cage in a room maintained at 22 °C with a 12-h light−dark cycle (light from 8:00 a.m. to 8:00 p.m.) and access to food from 4:00 p.m. to 8:00 a.m. Animals were maintained and handled according to the recommendations of the Institutional Ethics Committee (Institut National de la Recherche Agronomique), in accordance with decree 87-848. Rats were first fed a pelleted commercial diet for 1 week and then adapted for 1 week to a basal semipurified diet (**Table 1**). All rats were fed ad libitum during the whole experimental period.

In the first study (**Figure 2A**), 24 rats were randomized into three groups, and three different diets were given during 14 days to each

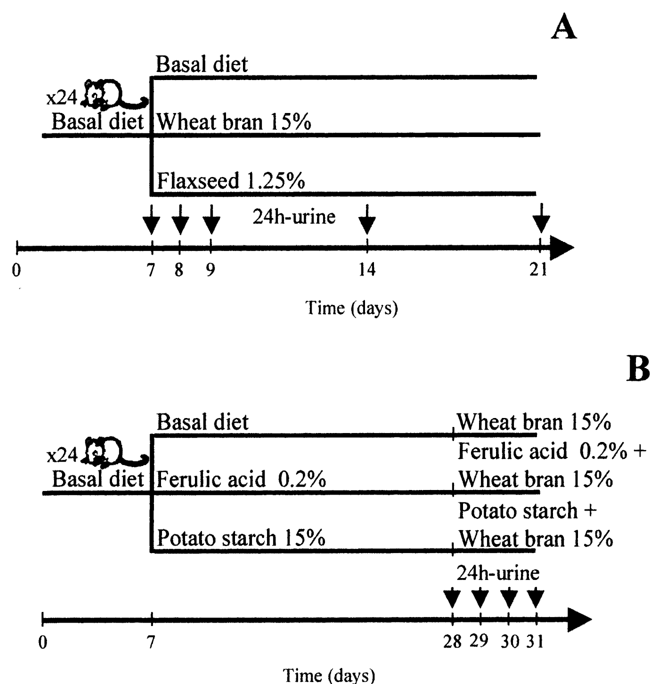

Figure 2. Study designs: (A) first study, rats were fed 1.25% flaxseed or 15% wheat bran diets; (B) second study, rats were adapted to ferulic acid diet or potato starch before they were fed a 15% wheat bran diet. Solid arrows indicate collection of 24-h urine.

group: a basal diet, the same diet supplemented with 15% wheat bran, and the same diet supplemented with 1.25% flaxseed (**Table 1**). Flaxseed and wheat bran were purchased from Celnat (St Germain Laprade, France).

In the second study (**Figure 2B**), 24 rats were randomized into three groups and first adapted for 14 days to the basal diet or to diet supplemented with either 0.2% ferulic acid (Sigma) or 15% raw potato starch (L. François, St Maur, France). During the four following days, each of these three diets was supplemented with 15% wheat bran.

The detailed composition of the diets and their contents in SECO and MAT were determined by GC-MS (see below) are given in **Tables 1** and **2**. The daily diet consumption and the body weight were measured throughout the experimental period for both studies. They did not significantly differ between groups (**Table 3**).

For 24-h urine collection, rats were transferred to metabolic cages 3 days before collection was begun. Urine was collected in plastic tubes containing ascorbic acid (Sigma; 60 mg) and then stored at −20 °C.

Time-Resolved Fluorescence Assay (TR-FIA) for Enterolactone in Urine Samples. This immunoassay allows one to specifically

Table 2. Content of Secoisolariciresinol and Matairesinol in the Flaxseed and Wheat Bran Diets

lignans (nmol/g of dry diet)	basal diet	15% wheat bran	1.25% flaxseed
SECO	0.15	0.79	87
MAT	0.02	0.08	nd[a]

[a] nd, not detected.

Table 3. Food Intake and Body Weight Gain during Wheat Bran and Flaxseed Feeding Periods

diet	food intake (g of dry matter/day)	body wt gain (g/day)
first study		
control diet	20.1 ± 0.5	5.2 ± 0.2
wheat bran, 15%	21.8 ± 0.2	5.2 ± 0.2
flaxseed, 1.25%	19.8 ± 0.2	5.3 ± 0.2
second study		
control + wheat bran, 15%	18.0 ± 0.4	4.4 ± 0.4
ferulic acid, 0.2% + wheat bran, 15%	19.9 ± 0.4	5.4 ± 0.5
potato starch, 15% + wheat bran, 15%	19.8 ± 0.5	4.8 ± 0.6

estimate Enl at low concentration with very low cross-reaction for End (0.28%) and no interference with other compounds (*26*). The assay was carried out as previously described. Briefly, urine samples (50 μL) were hydrolyzed by a sulfatase/glucuronidase mixture [450 μL of an 0.1 M acetate buffer, pH 5.0, containing 2 units/mL sulfatase (S-9626, Sigma Chemical Co., St. Louis, MO) and 0.2 unit/mL β-glucuronidase (Boehringer GmbH, Mannheim, Germany)]. The solutions were incubated at 37 °C overnight. Hydrolyzed samples were then treated with the DELFIA kit (Wallac Perkin-Elmer, Turku, Finland). The assay buffer was added, and aliquots (20 μL) were dispensed into prewashed microtitration 96 well plates coated with goat anti-rabbit IgG. Anti-Enl rabbit antiserum and europium-labeled Enl were added into each well. After gentle shaking for 90 min at room temperature, the excess of Enl, not complexed by the antisera, was washed with the plate washer. Enhancement solution was dispensed into each well, and the plate was shaken for 5 min. Europium fluorescence was measured with a Victor 1420 multilabel counter (excitation wavelength, 340 nm; emission wavelength, 613 nm). Analyses of standard solutions (0.4−300 nmol/L) and samples were carried out in duplicate (only immunoassay part of the method).

GC-MS Analysis of Lignans in Food Samples. The GC-MS method has been extensively described elsewhere (*27*). Briefly, freeze-dried diets (50 mg) were successively treated with an enzymatic extract of *Helix pomatia* and hot hydrochloric acid to hydrolyze the lignan glycosides. Deuterated SECO, anhydrosecoisolariciresinol, and MAT were added as internal standards. Lignans were extracted with diethyl ether, partially purified by ion-exchange chromatography, silylated, and analyzed by GC-MS (Hewlett-Packard HP 5995 quadrupole mass spectrometer). A capillary gas chromatography HP 5890 series II combined a flame ionization dectector (FID) was used, and the carrier gas was helium.

Statistical Analysis. Values are given as means ± SEM. Significant differences were determined by repeated-measures ANOVA with the Student−Newman−Keuls multiple-comparison test using Instat (San Diego, CA). Differences with $P < 0.05$ were considered to be significant.

RESULTS

Estimation of Enterolactone in Rat Urine. The wheat bran diet resulted in low levels of Enl urinary excretion, which made impossible their estimation by HPLC with coulometric detection. Enl was thus determined in urine by TR-FIA. The detection limit was ~0.4 nmol/L with TR-FIA against 15 nmol/L with the HPLC method. The values obtained by TR-FIA were compared to those obtained by HPLC with coulometric detection (*28*) on Enl-rich urines collected from rats fed a diet containing flaxseeds. A good correlation was observed between the values obtained by both methods (**Figure 3**).

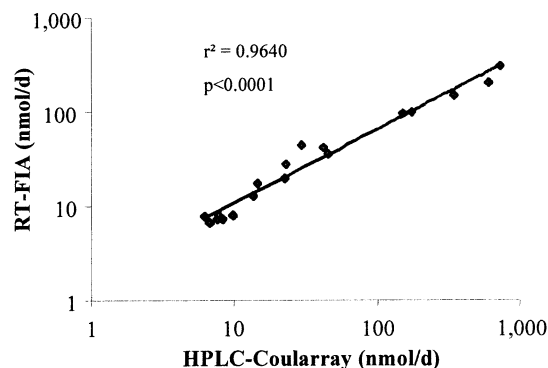

Figure 3. Correlation of levels of enterolactone urinary excretion measured by HPLC with coulometric detection and by TR-FIA. Samples are urine collected from rats fed wheat bran or flaxseed.

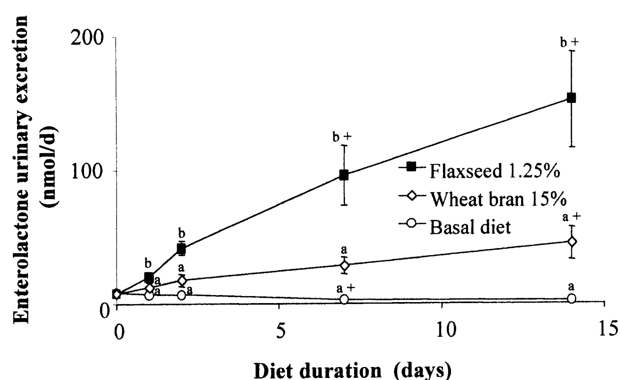

Figure 4. Urinary excretion of enterolactone in rats fed wheat bran and flaxseed. Enterolactone concentrations were measured by TR-FIA. For a given time of sampling, different letters indicate significant difference ($P < 0.05$). For different times of sampling, + indicates a mean value significantly different from the previous one ($P < 0.05$).

Mammalian Lignans Formed upon 2-Week Feeding with Wheat Bran. The Enl excretion regularly increased over the 14 days of wheat bran diet and reached 44 nmol/day (**Figure 4**). In contrast, the Enl excretion in the rats fed the semipurified control diet slowly decreased to a near-zero value during the same period. The decrease was marked from day 2 to the end of the experiment ($P < 0.01$). This slow increase of Enl excretion was also observed with flaxseed diets (**Figure 4**).

Effect of Adaptation to a Diet Containing Ferulic Acid or Potato Starch on Enterolactone Urinary Excretion. To study the influence of the diet on the formation of lignans from wheat bran, rats were first adapted to a diet supplemented with 0.2% ferulic acid or with 15% potato starch for 14 days and then fed the same diet supplemented with 15% wheat bran for 3 days. This 2-week adaptation period with potato starch had a major impact on the cecal microflora as shown by an increase of the cecum weight of 18%.

Basal levels of Enl urinary excretion after a 14-day period of adaptation to potato starch or ferulic acid diet were very low (2−3 nmol/day) and similar to those observed in the first experiment after a 14-day period of the semisynthetic diet. This shows that potato starch does not contain any Enl precursor and that ferulic acid itself is not metabolized (dimerized) into lignans. However, adaptation to a potato starch or ferulic acid diet affected the excretion level of Enl formed from wheat bran

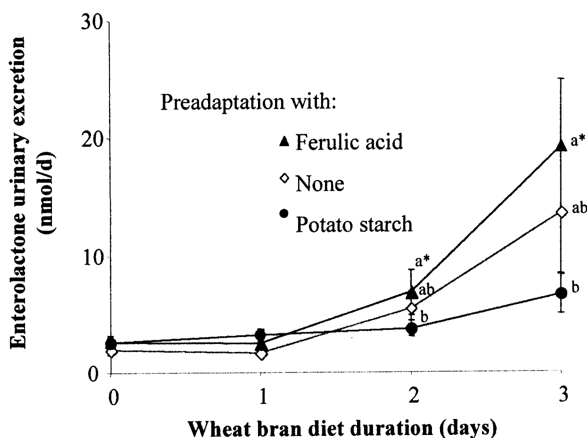

Figure 5. Enterolactone urinary excretion in rats fed wheat bran and previously adapted to a 14-day diet containing either ferulic acid (0.2% diet) or potato starch (15% diet). Urine enterolactone concentrations were determined by TR-FIA. For a given time of sampling, different letters indicate significant difference ($P < 0.05$). For different times of sampling, * indicates a mean value significantly different from the previous one ($P < 0.05$).

(**Figure 5**). Ferulic acid tends to increase Enl excretion, whereas potato starch tends to decrease it. These results clearly show that adaptation by the diet affects the capacity to produce Enl, as seen by the significant difference ($P < 0.05$) between ferulic acid and potato starch adapations.

DISCUSSION

The formation of lignans from wheat bran in rats is reported for the first time. About 45 nmol/day of Enl was excreted in urine after 2 weeks of a diet supplemented with 15% wheat bran. This level of excretion is of the same order as that previously reported for rye (*29*).

The formation of Enl from wheat bran was ∼3 times lower than that observed with flaxseed for an intake >10 times higher (15 versus 1.25%). The precursor of Enl in flaxseed is mainly the SECO diglucoside. SECO is converted to Enl in rats with a yield of ∼10% (*30*). In the present experiment, the urinary excretion of Enl observed with the flaxseed diet accounted for 9% (moles per mole) of the SECO ingested. Various authors have suggested that the lignans present in flaxseed could also be the precursors of Enl and End in other food sources (*19*). The same SECO diglucoside is present in cereal grains, as is MAT. SECO and MAT were estimated in the wheat bran diet used in the present work (**Table 2**) and the conversion yields calculated. The amount of Enl excreted in urine after 2 weeks of wheat bran feeding exceeded by a factor of 2.3 the amounts of SECO and MAT determined in the diet and thus exceeds by a factor of ∼25 the expected level of Enl excretion based on the SECO and MAT intake. This clearly indicates that wheat bran contains Enl precursors different from SECO and MAT, as has also been suggested for a rye bread diet consumed by Finnish subjects (*22*). These precursors could be other lignans such as pinoresinol, which was recently shown to be converted into Enl and End when it is incubated with a fecal microflora (*31*).

The kinetics of Enl excretion present some particular features: the level of Enl excretion was still increasing 2 weeks after the rats had been fed with wheat bran and flaxseeds (**Figure 4**). This is most probably explained by a slow modification of the microflora induced by the wheat bran diet. A similar observation was made on human subjects who needed a long adaptation to a high-fiber diet before an increase in Enl concentration could be observed in plasma (*32*). One may also note a slow decrease of Enl urinary excretion in the rats fed the control diet. This could be due to the retention of lignans within bacteria as has been suggested by the recent observation of the production of Enl and End from blank fecal human samples (*31*).

The slow acquisition of the capacity of the microflora to form lignans from their precursors was also observed by the progressive increase of the enterodiol/enterolactone ratio during the 2 weeks of a flaxseed diet fed to rats (C. Nicolle, C. Rémésy, and A. Scalbert, unpublished observations). Similar observations were made with women consuming flaxseeds during 8 days (*33*). This regular increase in Enl excretion is likely due to a progressive modification of the microflora and to the acquisition of the capability to oxidize End into Enl (*34*).

Attempts to modify the cecal microflora by a change in the diet were then made. Addition of ferulic acid to the semisynthetic diet tended to increase the production capacity of Enl from wheat bran (**Figure 5**). This could be due to an induction of the enzymes catalyzing demethylation and dehydroxylation of the Enl precursors or to a stimulation of the growth of some bacterial strains able to catalyze such reactions. No effect of a 10-day preadaptation to SECO diglucoside on the conversion of this same substrate could be observed in rats (*30*). This difference is probably explained by the low dose of SECO diglucoside used (1.5 mg/day) as compared to the dose of ferulic acid consumed by the rats in the present experiment (40 mg/day). The potato starch had an opposite effect. Although it increases very significantly the weight of the biomass in the cecum, it did not stimulate the formation of Enl. On the contrary, a lesser urinary excretion was observed, which could be due to a dilution effect in the cecum subsequent to the increase of the biomass volume (*25*) or to an accelerated transit and consequently reduced conversion of precursors into lignans and limited lignan absorption in the colon (*35*). These results suggest that the formation of lignans in the colon can be increased by a modification of the diet. It will be important in the future to better clarify the health effects of these metabolites and to identify the factors controlling their formation.

ABBREVIATIONS USED

End, enterodiol; Enl, enterolactone; MAT, matairesinol; SECO, secoisolariciresinol.

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LITERATURE CITED

(1) Adlercreutz, H.; Mazur, W. Phyto-oestrogens and Western diseases. *Ann. Med.* **1997**, *29*, 95−120.
(2) Adlercreutz, H.; Mousavi, Y.; Clark, J.; Höckerstedt, K.; Hämäläinen, E.; Wähälä, K.; Mäkelä, T.; Hase, T. Dietary phytoestrogens and cancer: *in vitro* and *in vivo* studies. *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 331−337.
(3) Jordan, V. C.; Mittal, S.; Gosden, B.; Koch, R.; Lieberman, M. E. Structure−activity relationships of estrogens. *Environ. Health Perspect.* **1985**, *61*, 97−110.

(4) Sathyamoorthy, N.; Wang, T. T.; Phang, J. M. Stimulation of pS2 expression by diet-derived compounds. *Cancer Res* **1994**, *54*, 957−961.

(5) 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*, 615−619.

(6) Ward, W. E.; Jiang, F. O.; Thompson, L. U. Exposure to flaxseed or purified lignan during lactation influences rat mammary gland structures. *Nutr. Cancer* **2000**, *37*, 187−192.

(7) Rickard, S. E.; Yuan, Y. V.; Chen, J.; Thompson, L. U. Dose effects of flaxseed and its lignan on *N*-methyl-*N*-nitrosourea-induced mammary tumorigenesis in rats. *Nutr. Cancer* **1999**, *35*, 50−57.

(8) Adlercreutz, H. Epidemiology of phytoestrogens. *Baillieres Clin. Endocrinol. Metab.* **1998**, *12*, 605−623.

(9) Adlercreutz, H.; Fotsis, T.; Heikkinen, R.; Dwyer, J. T.; Goldin, B. R.; Gorbach, S. L.; Lawson, A. M.; Setchell, K. D. Diet and urinary excretion of lignans in female subjects. *Med. Biol.* **1981**, *59*, 259−261.

(10) Coert, A.; Vonk Noordegraaf, C. A.; Groen, M. B.; van der Vies, J. The dietary origin of the urinary lignan HPMF. *Experientia* **1982**, *38*, 904−905.

(11) Setchell, K. D.; Lawson, A. M.; Borriello, S. P.; Harkness, R.; Gordon, H.; Morgan, D. M.; Kirk, D. N.; Adlercreutz, H.; Anderson, L. C.; Axelson, M. Lignan formation in man—Microbial involvement and possible roles in relation to cancer. *Lancet* **1981**, *2*, 4−7.

(12) Axelson, M.; Setchell, K. D. R. The excretion of lignans in rats—Evidence for an intestinal bacterial source for this new group of compounds. *FEBS Lett.* **1981**, *123*, 337−343.

(13) Stitch, S. R.; Toumba, J. K.; Groen, M. B.; Funke, C. W.; Leemhuis, J.; Vink, J.; Woods, G. F. Excretion, isolation and structure of a new phenolic constituent of female urine. *Nature* **1980**, *287*, 738−740.

(14) Setchell, K. D. R.; Lawson, A. M.; Mitchell, F. L.; Adlercreutz, H.; Kirk, D. N.; Axelson, M. Lignans in man and animal species. *Nature* **1980**, *287*, 740−742.

(15) Axelson, M.; Sjövall, J.; Gustafsson, B. E.; Setchell, K. D. R. Origin of lignans in mammals and identification of a precursor from plants. *Nature* **1982**, *298*, 659−660.

(16) Thompson, L. U.; Robb, P.; Serraino, M.; Cheung, F. Mammalian lignan production from various foods. *Nutr. Cancer* **1991**, *16*, 43−52.

(17) Adlercreutz, H.; Fotsis, T.; Bannwart, C.; Wähälä, K.; Mäkelä, T.; Brunow, G.; Hase, T. Determination of urinary lignans and phytoestrogens metabolites, potential antiestrogens and anticarcinogens, in urine of women on various habitual diets. *J. Steroid Biochem.* **1986**, *25*, 791−797.

(18) 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 Int. J.* **1995**, *24*, 1−12.

(19) Mazur, W. M.; Uehara, M.; Wahala, K.; Adlercreutz, H. Phyto-oestrogen content of berries, and plasma concentrations and urinary excretion of enterolactone after a single strawberry-meal in human subjects. *Br. J. Nutr.* **2000**, *83*, 381−387.

(20) Lampe, J. W.; Gustafson, D. R.; Hutchins, A. M.; Martini, M. C.; Li, S.; Wahala, K.; Grandits, G. A.; Potter, J. D.; Slavin, J. L. Urinary isoflavonoid and lignan excretion on a Western diet: relation to soy, vegetable, and fruit intake. *Cancer Epidemiol. Biomarkers Prev.* **1999**, *8*, 699−707.

(21) Hallmans, G.; Zhang, J. X.; Lundin, E.; Landstrom, M.; Aman, P.; Adlercreutz, H.; Harkonen, H.; Knudsen, K. E. B. Influence of rye bran on the formation of bile acids and bioavailability of lignans. *Cereal Foods World* **1997**, *42*, 696−701.

(22) Juntunen, K. S.; Mazur, W. M.; Liukkonen, K. H.; Uehara, M.; Poutanen, K. S.; Adlercreutz, H. C.; Mykkanen, H. M. Consumption of wholemeal rye bread increases serum concentrations and urinary excretion of enterolactone compared with consumption of white wheat bread in healthy Finnish men and women. *Br. J. Nutr.* **2000**, *84*, 839−846.

(23) Nilsson, M.; Aman, P.; Härkönen, H.; Hallmans, G.; Knudsen, K. E. B.; Mazur, W.; Adlercreutz, H. Content of nutrients and lignans in roller milled fractions of rye. *J. Sci. Food Agric.* **1997**, *73*, 143−148.

(24) Booth, A. N.; Emerson, O. H.; Jones, F. T.; DeEds, F. Urinary metabolites of caffeic and chlorogenic acids. *J. Biol. Chem.* **1957**, *229*, 51−59.

(25) Demigne, C.; Remesy, C. Influence of unrefined potato starch on cecal fermentations and volatile fatty acid absorption in rats. *J. Nutr.* **1982**, *112*, 2227−2234.

(26) Adlercreutz, H.; Wang, G. J.; Lapcik, O.; Hampl, R.; Wahala, K.; Makela, T.; Lusa, K.; Talme, M.; Mikola, H. Time-resolved fluoroimmunoassay for plasma enterolactone. *Anal. Biochem* **1998**, *265*, 208−215.

(27) Mazur, W.; Fotsis, T.; Wahala, K.; Ojala, S.; Salakka, A.; Adlercreutz, H. Isotope dilution gas chromatographic mass spectrometric method for the determination of isoflavonoids, coumestrol, and lignans in food samples. *Anal. Biochem.* **1996**, *35*, 169−180.

(28) Nurmi, T.; Adlercreutz, H. Sensitive high-performance liquid chromatographic method for profiling phytoestrogens using coulometric electrode array detection: application to plasma analysis. *Anal. Biochem* **1999**, *274*, 110−117.

(29) Landström, M.; Zhang, J. X.; Hallmans, G.; Aman, P.; Bergh, A.; Damber, J. E.; Mazur, W.; Wähälä, K.; Adlercreutz, H. Inhibitory effects of soy and rye diets on the development of Dunning R3327 prostate adenocarcinoma in rats. *Prostate* **1998**, *36*, 151−161.

(30) Rickard, S. E.; Thompson, L. U. Urinary composition and postprandial blood changes in ^{3}H-SECOisolariciresinol diglycoside (SDG) metabolites in rats do not differ between acute and chronic SDG treatments. *J. Nutr.* **2000**, *130*, 2299−2305.

(31) Heinonen, S.; Nurmi, T.; Liukkonen, K.; Poutanen, K.; Wahala, K.; Deyama, T.; Nishibe, S.; Adlercreutz, H. In vitro metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *J. Agric. Food Chem.* **2001**, *49*, 3178−3186.

(32) Pool-Zobel, B. L.; Adlercreutz, H.; Glei, M.; Liegibel, U. M.; Sittlingon, J.; Rowland, I.; Wahala, K.; Rechkemmer, G. Isoflavonoids and lignans have different potentials to modulate oxidative genetic damage in human colon cells. *Carcinogenesis* **2000**, *21*, 1247−1252.

(33) 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*, 549−555.

(34) Borriello, S. P.; Setchell, K. D. R.; Axelson, M.; Lawson, A. M. Production and metabolism of lignans by the human fecal flora. *J. Appl. Bacteriol.* **1985**, *58*, 37−43.

(35) Kilkkinen, A.; Stumpf, K.; Pietinen, P.; Valsta, L. M.; Tapanainen, H.; Adlercreutz, H. Determinants of serum enterolactone concentration. *Am. J. Clin. Nutr.* **2001**, *73*, 1094−1100.

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