

Quantitative aspects of the metabolism of lignans in pigs fed fibre-enriched rye and wheat bread

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A diet rich in lignans has been suggested to be protective against a range of chronic diseases. The distribution and metabolic fate of lignans is, however, very poorly understood. We fed high-fibre wheat breads low in lignans (*n* 8) or high-fibre rye breads (*n* 9) rich in plant lignans to pigs for 58–67 d, and analysed the content of plant lignans and their metabolites in the diet, blood, bile, faeces, urine and selected tissues. Apparent faecal digestibility of dietary precursors was higher than of total (plant- and entero-) lignans due to conversion to enterolactone and enterodiol. The digestibility of lariciresinol and matairesinol was lower than that of the sum of plant lignans. This suggests that interconversion of plant lignans during digestion and enterohepatic circulation occur without complete conversion to enterolignans. The majority of lignans present in plasma and urine was in the form of enterolignans, but up to 23 % in the plasma, and 11 % in the urine of the rye-fed pigs were in the form of plant lignans. There was a very high concentration of lignans in bile from the rye-fed pigs with as much as 77 % in the form of plant lignans. Lignans were detected in the tissue of colon, liver, breast and brain at a much higher level with rye than with wheat, but only in the form of enterolactone. The importance and implications of systemic exposure to plant lignans remain to be elucidated.

Lignans: Whole grains: Metabolism: Plasma: Tissue: Bile

Several epidemiological, intervention and animal studies point to a protective effect of a lignan-rich diet towards a range of lifestyle-related diseases such as certain cancers, CVD, and possible involvement in glycaemic control^(1–3). Intervention studies with lignan-rich food such as flaxseed⁽⁴⁾ and rye bread⁽⁵⁾ reduce the cholesterol level in hypercholesterolaemic subjects. The high content of plant lignans in the diet may contribute to this hypocholesterolaemic effect. Increased enterolactone (ENL) concentrations in serum have been associated with a lower risk of CVD⁽⁶⁾, and a high lignan intake to lower aortic stiffness⁽⁷⁾ and TAG level⁽⁸⁾. Mechanisms suggested to be involved in the protective effect of some lignans against CVD include antioxidative effects⁽⁹⁾, stimulation of sex hormone-binding globulin synthesis⁽¹⁰⁾, up-regulation of LDL receptor activity⁽¹¹⁾, inhibition of 7 α -hydroxylase and acyl-CoA-cholesterol acyltransferase activity⁽¹²⁾ and increased nitrogen oxide production⁽¹³⁾.

Originally only secoisolariciresinol and matairesinol were identified as precursors of the enterolignans ENL and enterodiol (END). Improved methods of analysis led to the discovery of more precursors such as pinoresinol, syringaresinol,

lariciresinol and medioresinol^(14,15), and recently further eighteen plant lignans were identified in food items including four found in rye and wheat⁽¹⁶⁾. These plant lignans account for 30–45 % of the total content of lignans in rye and 1–18 % of the total content in wheat⁽¹⁶⁾. The discovery of new enterolignan precursors and the recent development of methods to quantify them in food matrices, excreta and body fluids and tissues made possible the estimation of dietary lignan bio-availability and metabolism. We have recently reported that a diet based on wholegrain rye enriched with rye bran compared with wheat flour enriched with purified wheat fibre at iso-dietary fibre levels had a hypocholesterolaemic effect in hypercholesterolaemic pigs⁽¹⁷⁾. From the present study, it was concluded that the ability of dietary fibre from rye to interfere with digestion and absorption was more important for whole-body cholesterol homeostasis than regulation in the liver at gene level. Here we report new results on the quantitative metabolism and concentrations in biofluids and tissues of lignans in a pig model using high-fat cholesterol-enriched bread-based diets based on either wheat flour and refined wheat fibre, or wholegrain rye with added rye fibre.

Abbreviations: CEAD, coulometric electrode array detector; END, enterodiol; ENL, enterolactone; MeOH, methanol.

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Materials and methods

Diets

The study included two experimental diets: a high-fibre rye bread-based diet and a high-fibre wheat bread-based diet. Before administration of the high-fibre diets the pigs were fed an atherogenic diet for 2 weeks, followed by a wash-out period consisting of a low-fat wheat flour-based diet for 2–3 weeks as previously described⁽¹⁷⁾. The diets during the experimental period consisted of breads high in dietary fibre and enriched with fat and cholesterol (Table 1). The rye bread (produced at Holstebro Technical College, Denmark) contained finely ground wholegrain rye (Valsemøllen, Esbjerg, Denmark) and ground rye bran (Nordmills, Uppsala, Sweden), while the wheat bread (produced at Lantmännen Unibake, Karup, Denmark) contained white wheat flour supplemented with refined cellulose-rich wheat fibre (Vitacel WF 600; J. Rettenmaier & Söhne GmbH, Rosenberg, Germany) in order to achieve the same dietary fibre level. Whey protein concentrate (Lacprodan 87; Arla Foods Amba, Viby, Denmark) was used to adjust the protein content, and

Table 1. Ingredients (g/100 g as-is)*, DM content (g/100 g as-is) and content of macronutrients and plant lignans (per 100 g DM) in the experimental breads

	Wheat	Rye
Ingredients (g/100 g as-is)		
White wheat flour	52.8	0.0
Wholemeal rye flour	0.0	31.0
Vitacel WF 600†	15.7	0.0
Rye bran	0.0	40.0
Whey protein concentrate	2.5	0.0
Yeast	2.0	2.0
Sugar	1.5	1.5
Egg powder	15.0	15.0
Rapeseed oil	2.0	2.0
Lard	5.0	5.0
Cholesterol	0.5	0.5
Vitamin–mineral mixture‡	3.0	3.0
Composition		
DM (g/100 g fresh weight)	66.1	68.0
Energy (MJ)	21.10	21.16
Ash (g)	3.9	5.0
Protein (g)	18.2	17.2
HCl fat (g)	15.3	15.9
Starch and sugar (g)	43.7	38.4
Dietary fibre§ (g)	19.4	20.3
NSP (g)	17.3	16.3
Klason lignin (g)	2.2	4.0
Plant lignans (µg)	124.0	4152.7
Secoisolariciresinol	7.0	77.6
Matairesinol	–	79.3
Lariciresinol	19.9	508.4
Pinoresinol	8.5	572.8
Syringaresinol	77.9	2628.6
Medioresinol	10.6	286.0

–, Not detectable.

* Before water addition and baking.

† J. Rettenmaier & Söhne GmbH (Rosenberg, Germany).

‡ Provided (mg/kg diet): Ca(H₂PO₄)₂, 6642; NaCl, 4122; CaCO₃, 16580; FeSO₄·7H₂O, 286; ZnO, 114; Mn₂O₃, 41; CuSO₄·5H₂O, 92; KI, 0.3; Na₂SeO₃·5H₂O, 0.8; retinoacetate, 2.1; cholecalciferol, 0.03; α-tocopherol, 69; menadione, 2.52; riboflavin, 4.58; D-pantothenic acid, 12.59; cyanocobalamin (vitamin B₁₂), 0.025; thiamin (vitamin B₁), 2.52; niacin, 25.2; pyridoxine (vitamin B₆), 3.78; biotin, 0.063.

§ Dietary fibre was calculated as the sum of NSP and Klason lignin.

lard, egg powder (Danæg Products A/S, Roskilde, Denmark) and cholesterol (Sigma-Aldrich Co., St Louis, MO, USA) to increase the fat and cholesterol content. The diets were stored at –20°C until consumption, and chromic oxide (Cr₂O₃) was added to ground experimental diets during the final 2.5 weeks of the study.

Experimental design and animals

A total of seventeen Duroc × Danish Landrace × Yorkshire gilts, aged 4 months and obtained from Aarhus University, Faculty of Agricultural Sciences (Foulum, Denmark), were used for the study. The seventeen pigs, responding to *ad libitum* feeding of the atherogenic diet for 2 weeks by having elevated plasma cholesterol levels (7.2 (SEM 0.51) mmol/l), were subsequently fed the low-fat wash-out diet for 2–3 weeks, by which the cholesterol level fell again to 2.5 (SEM 0.08) mmol/l⁽¹⁷⁾. Following this, the pigs were fed the experimental diets for 6–7 weeks, followed by about 2.5 weeks where the breads were ground and Cr₂O₃ (2 g/kg on an as-is basis) was added. In week 2 after chromium addition to the diets the pigs were transferred to metabolism cages for 7 d, where faeces and urine were collected quantitatively on a daily basis and pooled per pig. Urine was collected over 4 g ascorbic acid per bottle, and pH of the collected urine was measured on a daily basis. The pigs were subsequently returned to their pens and euthanised 2–4 d after the end of the balance period. When not placed in metabolism cages, the pigs were housed individually in 4 m² smooth-walled pens with a concrete floor. The feed intake during the period, where the pigs ate the experimental bread-based diets, was restricted to 2 kg/d increasing to 3 kg/d for about the last 2.5 weeks of the study.

Postprandial blood samples from the jugular vein, lateral auricular artery, hepatic vein and portal vein were taken into heparinised vacutainers 3 h after the morning meal from anaesthetised pigs. The pigs were subsequently euthanised with an overdose of sodium pentobarbital followed by exsanguination⁽¹⁷⁾. Samples were taken directly from the gall bladder and the urine bladder and stored frozen. Biopsies taken from the left lobe of the liver, tissue of the mid colon, breast and the brain were quick-frozen in liquid N₂, and stored at –80°C until analysis.

The animal experiment was conducted according to protocols approved by the Danish Animal Experiments Inspectorate and complied with the guidelines of the Danish Ministry and Justice concerning animal experimentation and care of animals under study.

Analytical methods

All analyses were performed in duplicate. Freeze-dried samples of diets and faeces were ground to a particle size of less than 0.5 mm before analysis. The DM content of feed and freeze-dried faeces was determined by drying to constant weight at 105°C for 20 h. Cr₂O₃ concentrations in faeces and diets were analysed as described by Schürch *et al.*⁽¹⁸⁾. Macro-nutrient composition of the diets was analysed as previously described⁽¹⁷⁾.

A total of six plant lignans, namely secoisolariciresinol,atairesinol, lariciresinol, pinoresinol, medioresinol and

syringaresinol, and two enterolignans, END and ENL, were profiled in the different samples. The content of plant lignans in the diets was analysed by isotope dilution GC-MS in the selected ion-monitoring mode (ID-GC-MS-SIM) as previously described⁽¹⁹⁾. The same instrumental technique was used for the determination of plant lignans and enterolignans in faeces, using the corresponding extraction protocol⁽¹⁴⁾. The lignan profile in urine⁽²⁰⁾, plasma and bile⁽²¹⁾ was analysed by HPLC coupled with a coulometric electrode array detector (HPLC-CEAD).

The procedure for determination of lignans in tissues was as follows. Freeze-dried liver and brain tissue samples were ground to a fine powder, whereas colon and breast tissues were homogenised by crushing frozen with an Ultra Turrax T8 (IKA Werke GmbH & Co. KG, Staufen, Germany). To 50 mg of the samples and controls 300 µl distilled water were added and the samples were sonicated for 10 min. ³H-labelled oestradiol-17β-glucuronide (18 000 counts per min, NEN[®]; Perkin-Elmer, Shelton, CT, USA) in methanol (MeOH)–water (9:1, v/v) was added to serve as an internal standard for recovery correction. Thereafter 700 µl MeOH were added and the sample kept overnight at –20°C in order to precipitate proteins and lipophilic material. The samples were centrifuged (3000 rpm) for 10 min below 0°C, and the supernatant fraction collected. The precipitate was washed once with 1 ml ice-cold (–20°C) 70% MeOH, centrifuged. The two supernatant fractions were combined, and evaporated under N₂ at 50°C until only water remained (500 µl), and subsequently still extracted twice with 2.5 ml n-hexane by vortex mixing for 4 min. The water phase was frozen in a dry-ice ethanol bath, and the n-hexane was discarded. The remaining traces of n-hexane were evaporated from the sample, and the water phase (500 µl) was hydrolysed by adding 500 µl of hydrolysis reagent containing β-glucuronidase (0.2 U/ml), sulfatase (2 U/ml) and 0.1 M-acetate buffer (pH 5.0) and incubated overnight at 37°C. The samples were extracted twice with 3 ml diethyl ether, mixing for 4 min, then evaporated to dryness and reconstituted in 500 µl MeOH. A 3 cm column of ion exchanger QAE-Ac in MeOH was prepared in a disposable Pasteur pipette and the sample in MeOH was applied to the column. The sample vial was rinsed once with 300 µl MeOH and applied to the column. The lignans were further eluted with 4 ml MeOH, and evaporated to dryness. For the HPLC analysis, the dry sample was reconstituted with 100 µl MeOH, 100 µl HPLC mobile phase (20% phase B/A; see below) was added, and 10 µl immediately subjected to HPLC-CEAD. A subsample of 20 µl was taken for determination of [³H]oestradiol-17β-glucuronide recovery to correct for losses during the procedure. The mean recovery of [³H]oestradiol-17β-glucuronide for liver and colon tissues was 62.4% (CV% 14.7) and for brain and breast tissues 84.2% (CV% 10.4).

We used an HPLC system (ESA Biosciences, Inc., Chelmsford, MA, USA) equipped with a model 540 autosampler, two model 580 solvent pumps, and a model 5600 CEAD with eight electrode pairs. The analytical column was an Inertsil ODS-3 (GL Sciences Inc., Tokyo, Japan), 3 × 150 mm with particle size 3 µm, connected to a Quick Release RP-18 (Upchurch Scientific Inc., WA, USA) 3 × 10 mm guard column. The mobile phases used in HPLC-CEAD were: (A) 50 mM-sodium acetate buffer–MeOH (80:20, v/v); (B) 50 mM-sodium acetate

buffer (pH 5.0)–MeOH–acetonitrile (40:40:20, by vol.). The chromatographic conditions were: detection potential, 660 mV; flow rate, 0.3 ml/min. The separation of compounds was carried out using gradient elution. All standards were dissolved in MeOH–20% B/A (1:1, v/v). The seven-point standard curve ranged from 3.5 to 1393 ng/ml for ENL. No other lignans were detected in the tissue samples.

All lignan analyses included hydrolysis of any conjugate (glucosides in the diets, glucuronides and sulfates in the plasma and bile) and therefore the results are expressed as aglycones.

Calculations and statistical analysis

The apparent digestibility of the individual lignans (*X*) was calculated relative to the concentration of Cr₂O₃ as:

$$\text{Digestibility of } X = 1 - \frac{Cr_2O_{3(d)} \times X_{(f)}}{Cr_2O_{3(f)} \times X_{(d)}}$$

where *X*_(d) and *X*_(f) denote the concentrations of the lignans (*X*) in diet and faeces, respectively, and Cr₂O_{3(d)} and Cr₂O_{3(f)} are the corresponding concentrations of Cr₂O₃.

The estimated production of enterolignans (EL_{prod}) was calculated as:

$$EL_{\text{prod}} = PL_{\text{intake}} - (PL_{\text{faeces}} + PL_{\text{urine}}),$$

where PL_{intake}, PL_{faeces} and PL_{urine} are the quantitative intake and excretion of plant lignans in faeces and urine.

The excretion of enterolignans (EL_{excretion}) was calculated as:

$$EL_{\text{excretion}} = EL_{\text{urine}} + EL_{\text{faeces}},$$

where EL_{urine} and EL_{faeces} are the sum of excreted ENL and END in faeces and urine during the 7 d collection period.

All statistical analyses were carried out using SAS for Windows (version 8.2; SAS Institute Inc., Cary, NC, USA). The level of significance was *P* < 0.05 in all analyses. Variables meeting the criteria of normal distribution are reported as least squares means (LSMeans) with their standard errors (SEM). When variables were not normally distributed, statistical evaluation was performed on logarithmic transformations of the values, and results are reported as geometric means with 95% CI.

The effect of diet and site of blood collection on plasma concentrations of lignans was examined by PROC MIXED, taking repeated measurements made on the same individuals into account⁽²²⁾.

The effect of diet on faecal and urine concentrations, digestibility, and excretion in urine and faeces were subjected to one-way analysis using PROC MIXED with GROUP option to account for variance heterogeneity.

Results

The markedly higher content of plant lignans in the rye diet compared with the wheat diet enriched with purified wheat dietary fibre (Table 1) resulted in a thirty-four times higher intake of lignans (Table 2). Syringaresinol was by far the dominating component of the rye diet, accounting for 60% of the total plant lignan content, while secoisolaricresinol

Table 2. Intake, faecal excretion and digestibility of lignans from pigs fed the experimental wheat or rye diets (Least squares means (LSMeans) with their standard errors)

	Intake ($\mu\text{mol/d}$)		Excretion ($\mu\text{mol/d}$)				Digestibility†			
			Wheat		Rye		Wheat		Rye	
	Wheat	Rye	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM
Total lignans	6.2	213.6	1.7	0.067	74.13***	1.901	0.70	0.013	0.61***	0.007
Enterolignans	–	–	0.58	0.039	42.34***	1.210				
Enterolactone	–	–	0.56	0.038	41.93***	1.179				
Enterodiols	–	–	0.02	0.002	0.41***	0.042				
Plant lignans	6.2	213.6	1.12	0.058	31.8***	0.979	0.80	0.013	0.83	0.004
Secoisolariciresinol	0.4	4.4	0	0.002	0.49***	0.048	0.99	0.005	0.87***	0.013
Matairesinol	–	4.5	0.02	0.002	1.41***	0.070	–	–	0.65	0.016
Lariciresinol‡	1.1	28.8	0.32	0.014	7.12***	0.330	0.69	0.016	0.72	0.011
Pinoresinol	0.5	32.6	0	0.003	1.27***	0.089	0.99	0.008	0.96**	0.003
Syringaresinol	3.7	128.3	0.74	0.054	20.31***	0.723	0.78	0.019	0.82	0.005
Medioresinol	0.5	15	0.04	0.004	1.2***	0.050	0.93	0.008	0.91	0.005

–, Not detectable.

LSMean was significantly different from that for wheat: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For digestibility calculation, see Calculations and statistical analysis section.

‡ Including isolariciresinol in faeces.

and matairesinol accounted for only 4–6% of the total dietary intake of lignans.

The higher intake of plant lignans with the rye diet was reflected in a twenty-eight times higher total faecal excretion of plant lignans and enterolignans compared with the wheat diet (Table 2). Enterolignans accounted for 34–57% of the total faecal excretion of lignans, while 43–66% was in the form of plant lignans.

The apparent faecal digestibility of the plant lignans was, in decreasing order: pinoresinol > medioresinol > secoisolariciresinol > syringaresinol > lariciresinol > matairesinol (Table 2).

As could be expected from the higher intake, there was a much higher concentration of enterolignans in plasma at all sites (portal vein, hepatic vein, lateral auricular artery and jugular vein) of pigs fed the rye compared with the wheat diet (Table 3). ENL was the primary circulating lignan accounting for 58–85% of the total lignan content in plasma obtained from all four sites of collection. END, on the other hand, accounted for only approximately 1% of the circulating lignans. Overall, the concentration of lignans was highest in the portal and hepatic vein, while the concentration was significantly reduced in peripheral venous and arterial blood.

The bile of the wheat-fed pigs contained no measurable lignans, whereas the bile from the rye-fed pigs contained 6.2 (SEM 0.9) $\mu\text{mol/l}$. Plant lignans accounted for 77% of the measured lignans, and was dominated by matairesinol followed by lariciresinol, and secoisolariciresinol (Fig. 1). Enterolignans in the form of ENL accounted, on the other hand, only for 23% of the total lignan content in bile from the rye-fed pigs.

Both concentrations of lignans in bladder urine and in urine collected over a 7 d collection period were higher in pigs fed the rye compared with the wheat-fed pigs (Table 4). The lower concentration in urine from the 7 d collection compared with bladder urine may in part be explained by dilution with drinking water spillage collected from the cages together with urine. However, for calculation of quantitative excretion,

this lower concentration is counteracted by the collection of larger volumes. Based on the 7 d quantitative collection of urine, and the concentration of lignans herein, the total urinary excretion of plant lignans and enterolignans was calculated to be 1.4 (SEM 0.02) $\mu\text{mol/d}$ for pigs fed the wheat diet, whereas the urinary excretion was 71.0 (SEM 5.79) $\mu\text{mol/d}$ for pigs on the rye diet ($P < 0.0001$). While plant lignans could not be detected in urine of the wheat-fed pigs, they contributed with 7.6 (SEM 0.72) $\mu\text{mol/d}$ for pigs on the rye diet.

Significant concentrations of enterolignans in the form of ENL were found in the colon, liver and breast tissue, and minor concentrations in the brain of the rye-fed pigs (Table 5). ENL was also found in lower concentrations in the colon, liver and breast tissue of the wheat-fed pigs, while no measurable lignan was found in brain tissue. Thus, the highest concentrations are found in tissues that are in close proximity to the production site, the gut, and the organ for re-conjugation, the liver.

The estimated production of enterolignans calculated from the intake and excretion of plant lignans was 174 (SEM 1.4) $\mu\text{mol/d}$ for the rye compared with 5 (SEM 0.1) $\mu\text{mol/d}$ for the wheat diet (Fig. 2). Thus, the estimated production of enterolignans was 1.5–2.5 times higher than the 2 (SEM 0.2) and 106 (SEM 4.9) $\mu\text{mol/d}$ actually recovered in faeces and urine from the wheat- and the rye-fed pigs, respectively.

Discussion

The diets were formulated to be similar in macronutrients but different in dietary fibre characteristics and content of plant lignans⁽¹⁷⁾. As the plant lignans are concentrated in the aleurone and pericarp/testa layer of the rye kernel^(23,24) the experimental rye bread enriched with rye bran had a lignan content that was about seven times higher than in ordinary rye bread (594 $\mu\text{g}/100\text{g DM}$)⁽²⁵⁾. Lignan analysis of the diets confirmed other studies showing that secoisolariciresinol and matairesinol contribute only marginally to the total intake of plant lignans^(19,25,26).

Table 3. Concentration (nmol/l) of lignans in plasma collected 3h postprandially from the portal vein, hepatic vein, a peripheral artery (lateral auricular artery) and jugular vein of pigs fed fibre-enriched wheat or rye diets*

(Geometric means and 95 % confidence intervals or least squares means (LSMeans) with their standard errors)

			Total lignans	Enterolignans	ENL	END	Plant lignans	SECO	MAT	IsoLar	LAR	PIN	SYR	MED
Portal vein	Wheat	Geometric mean	5.7	5.7	3.9									
		95 % CI	4.4, 7.5	4.2, 7.7	2.9, 5.3									
		LSMean SEM				1.8 0.5	-	-	-	-	-	-	-	-
	Rye	Geometric mean	193.5	149.0	147.3									
		95 % CI	150.1, 249.4	112.6, 197.1	110.3, 196.9									
		LSMean SEM				2.0 0.5	44.1 6.5	3.9 1.1	6.7 1.9	1.7 0.3	13.1 1.5	7.7 1.4	9.7 1.2	1.3 0.2
Hepatic vein	Wheat	Geometric mean	5.0	5.0	3.8									
		95 % CI	4.2, 6.0	4.1, 6.1	3.1, 4.7									
		LSMean SEM				1.2 0.3	-	-	-	-	-	-	-	-
	Rye	Geometric mean	178.4	147.0	145.8									
		95 % CI	149.8, 212.5	120.5, 179.2	118.1, 180.0									
		LSMean SEM				1.4 0.3	31.2 2.1	3.4 0.7	3.6 0.9	1.9 1.0	11.2 1.0	4.9 0.7	3.8 0.5	0.8 0.1
Lateral auricular artery	Wheat	Geometric mean	6.0	6.0	3.6									
		95 % CI	4.4, 8.2	4.4, 8.2	2.5, 5.2									
		LSMean SEM				2.2 0.3	-	-	-	-	-	-	-	-
	Rye	Geometric mean	110.8	95.0	94.1									
		95 % CI	82.8, 148.3	70.5, 128.1	66.1, 133.9									
		LSMean SEM				1.1 0.3	16.5 2.5	1.9 0.5	1.4 0.3	0.8 0.2	7.2 1.0	2.1 0.5	2.2 0.6	0.8 0.3
Jugular vein	Wheat	Geometric mean	4.0	4.0	2.3									
		95 % CI	2.3, 6.7	2.3, 6.9	1.4, 3.7									
		LSMean SEM				2.2 0.5	-	-	-	-	-	-	-	-
	Rye	Geometric mean	82.7	69.0	68.3									
		95 % CI	50.2, 136.5	41.0, 116.1	43.6, 107.1									
		LSMean SEM				0.9 0.5	14.7 2.0	1.6 0.4	1.3 0.2	0.4 0.2	6.3 0.9	2.1 0.3	2.6 0.4	0.4 0.1
<i>P</i>	Diet	<0.0001	<0.0001	<0.0001	NS									
	Site	0.0015	0.0041	<0.0001	0.0018	0.0002	0.0053	<0.0001	<0.0001	0.0015	0.017	0.0004	0.0051	
	Diet × site	0.0015	0.0024	0.19	0.0007									

Metabolism of lignans

ENL, enterolactone; END, enterodiol; SECO, secoisolariciresinol; MAT, matairesinol; IsoLar, isolariciresinol; LAR, lariciresinol; PIN, pinoresinol; SYR, syringaresinol; MED, medioresinol; -, below detection limit.

* For portal vein, lateral auricular artery and jugular vein: *n* 8 for wheat and *n* 9 for rye. For hepatic vein: *n* 8 for wheat and *n* 7 for rye.

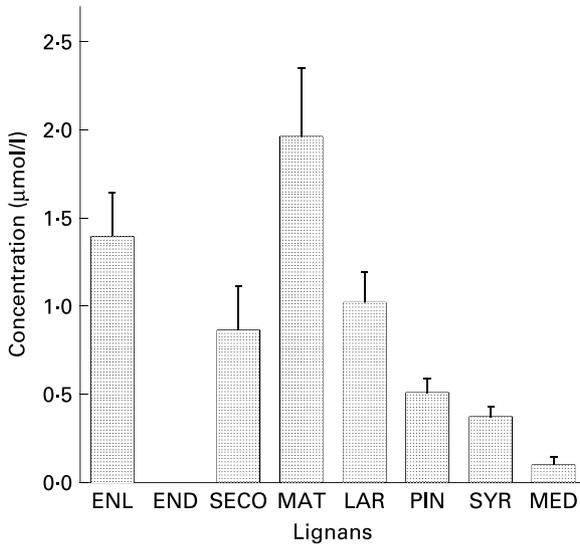


Fig. 1. Concentration of lignans ($\mu\text{mol/l}$) in bile taken from the gall bladder 3 h postprandial of pigs fed fibre-enriched rye diets. Values are least squares means, with standard errors represented by vertical bars. ENL, enterolactone; END, enterodiol; SECO, secoisolariciresinol; MAT, matairesinol; LAR, lariciresinol; PIN, pinoresinol; SYR, syringaresinol; MED, medioresinol.

The proportion of the ingested plant lignans that were excreted in faeces in the form of enterolignans was lower in the present study than in a similar study with pigs fed wheat and rye diets (50–76%)⁽²⁷⁾. The main reason is that fewer lignans were identified previously, and only matairesinol and secoisolariciresinol were analysed in faecal material⁽²⁷⁾. In the faeces of human subjects on a habitual diet, 34–35% lignans were excreted as enterolignans^(14,28,29). It has previously been shown that the amount of excreted lignans was higher in pigs than in human subjects, while the excretion per g ingested dietary fibre was identical⁽²⁴⁾.

The apparent digestibility of the individual plant lignans was generally higher than of the sum of plant lignans and enterolignans, reflecting that some lignans were converted to enterolignans before faecal excretion. The difference in digestibility

among the plant lignans may partly be caused by conversion into intermediate plant lignans as previously shown *in vitro*⁽¹⁴⁾, and subsequent excretion without further conversion to enterolignans. The higher digestibility of lariciresinol, pinoresinol and syringaresinol in the present study than the estimated 10–19% in a previous study⁽²⁷⁾ is most probably a consequence of differences in the method of determination (direct or indirect), and a higher microbial activity due to a longer period of adaptation to the diet in the present study. Metabolites of fermentation of syringaresinol have been detected *in vitro* but not identified⁽¹⁴⁾. To what extent these metabolites, directly or indirectly, contribute to the faecal excretion of plant lignans in modified form is presently not known. However, based on the *in vitro* conversion their contribution to the excretion of enterolignans is considered to be low.

The increased levels of enterolignans at all sites of blood collection confirm several intervention studies^(30–33), cross-sectional^(34–36) and animal studies⁽²⁷⁾, where positive associations between intake of rye and wholegrain product and the ENL concentration in plasma or serum have been found. The higher concentrations of plant lignans and enterolignans in the portal vein than in the hepatic vein confirm previous findings that a substantial part of the lignans participate in the enterohepatic circulation or are converted to other metabolites. The significantly higher concentration of lignans in portal than in arterial blood is considered a consequence of the enrichment of the portal blood due to absorption from the gut⁽²⁷⁾. Generally, enterolignans are considered to be absorbed more efficiently than plant lignans⁽³⁷⁾. Nevertheless, in the present study plant lignans accounted for 15–23% of the total lignan concentration in plasma. Some enterolignans may be reconverted to plant lignans in the liver before excretion to the intestine by the biliary route⁽²⁴⁾ as indicated by the remethylation of the lignans arctiin and tracheloside in the liver of rats⁽³⁸⁾. *In vitro* it has also been shown that ENL and END can undergo oxidative biotransformation by hepatic microsomes from rats and humans, which has been confirmed by the *in vivo* finding of several hydroxylated and methylated metabolites of ENL and END in the urine and bile of flaxseed-fed rats⁽³⁹⁾.

Table 4. Concentration (nmol/l) of lignans in urine collected during a 7 d balance period or taken directly from the bladder at slaughter of pigs fed fibre-enriched wheat or rye diets (Least squares means (LSMeans) with their standard errors)

	7 d balance				Bladder			
	Wheat		Rye		Wheat		Rye	
	LSMean	SEM	LSMean	SEM	LSMean	SEM	LSMean	SEM
Total lignans	375	61	18 552***	1628	2013	468	36 252***	7795
Enterolignans	375	61	16 567***	1478	1447	342	32 884***	7216
Enterolactone	375	61	16 269***	1461	1412	335	32 514**	7118
Enterodiol	–	–	297	35	35	11	370*	110
Plant lignans	–	–	1986	192	566	134	3368***	666
Secoisolariciresinol	–	–	504	41	59	20	352*	95
Matairesinol	–	–	241	30	24	6	361**	78
Isolariciresinol	–	–	132	20	84	21	224*	51
Lariciresinol	–	–	655	65	124	29	860**	189
Pinoresinol	–	–	95	19	168	25	624**	120
Syringaresinol	–	–	309	41	87	38	791**	203
Medioresinol	–	–	49	10	19	9	157**	38

LSMean was significantly different from that for wheat: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 5. Concentration of enterolactone (pmol/g fresh weight) in various tissues of pigs fed fibre-enriched wheat or rye diets

(Least squares means (LSMeans) with their standard errors)

	Wheat		Rye	
	LSMean	SEM	LSMean	SEM
Colon	16.7	1.79	691.7***	93.63
Liver	2.6	0.50	125.0***	19.63
Breast	0.3	0.21	25.8***	4.12
Brain	–	–	2.3	0.41

–, Below detection limit.

*** LSMean was significantly different from that for wheat ($P < 0.001$).

In the present study, the very high proportion of plant lignans found in bile and a higher proportion in portal blood compared with the other blood vessels suggest that plant lignans are diverted toward systemic elimination via the bile. It has previously been shown that simple monobenzene derivatives with molecular weight below 300 kDa were poorly excreted in the bile, while compounds containing two or more rings and a highly polar anionic group with a minimum molecular weight of 325 kDa were effectively excreted⁽⁴⁰⁾. The authors suggested that foreign compounds resembling the endogenous compounds (bile acids) would be effectively excreted in the bile.

Whether the excretion of the plant lignans by the biliary route is a protective mechanism only occurring after a systemic overload at very high intakes of plant lignans is not known. Because the plant lignan values are higher than the ENL values it seems that most of the ENL formed may pass the liver like oestriol-3-glucuronide for immediate excretion in urine⁽⁴¹⁾, while the plant lignans may be selectively taken up by the liver cells, conjugated and excreted in the bile.

Before conjugation the plant lignans are certainly biologically active in the liver cells or in any other cell in the body. However, in conjugated form as in the bile, the lignans will not be biologically active again before they reach the intestine, are deconjugated by the microflora, and continue in a second enterohepatic circulation.



Fig. 2. Estimated production (□) and calculated excretion (■) of enterolignans (µmol/d) of pigs fed fibre-enriched wheat or rye diets. Values are least squares means, with standard errors represented by vertical bars.

To what extent the plant lignans have any potential protective effect against diseases such as cancer and CVD if not converted to enterolignans is not yet fully elucidated. The conversions to END and ENL are not necessary steps for biological action, but the relative importance of plant lignans respective to enterolignans is currently not known. We know very little about the biological effects of plant lignans, but high intake of matairesinol has been related to lower breast cancer risk^(1,42). Different *in vitro* assays suggest that both plant lignans and enterolignans have antioxidative capacity^(9,43–45) with the antioxidative activities of secoisolariciresinol and matairesinol being significantly higher than that of ENL and END using a ferric-reducing/antioxidant power assay (FRAP)⁽⁴³⁾. The additional aromatic methoxy group in the plant lignans was suggested to be responsible for the higher antioxidant activities⁽⁴³⁾. However, the antioxidative effect has not been confirmed in human intervention studies^(45–48).

Ingestion of foods rich in plant lignans leads to higher urinary ENL concentrations^(49,50). The present results confirm previous results obtained after rye and wheat consumption both with pigs⁽²⁷⁾ and human subjects⁽³⁰⁾. Enterolignans were the dominating components of the lignans in urine, but plant lignans accounted for 11% of the lignans found in urine excreted from the rye-fed pigs. This suggests that approximately one-tenth of the lignans taken up from the intestine escapes microbial conversion and are absorbed directly as plant lignans, as also suggested in studies with rats^(37,51). A large variation in the pattern of plant lignans and enterolignans has been observed in the urine of human subjects⁽²⁰⁾, where the excretion of plant lignans was higher than for those of enterolignans in two of ten subjects. Ingestion of secoisolariciresinol led to an increased excretion of lariciresinol in the urine of rats⁽⁵¹⁾, where lariciresinol was the main component of the excreted plant lignan also seen in the present study. This demonstrates that plant lignans can be converted to other plant lignans *in vivo*. The high excretion of lariciresinol may at least in part be caused by the high intake of pinoresinol as suggested by *in vitro* microbial conversion results obtained in a previous study⁽¹⁴⁾.

In the present study there was a slightly lower conversion from plant lignans to enterolignans than in the study of Bach Knudsen *et al.*⁽²⁷⁾ that used comparable diets. In that study, the conversion was estimated to be 48 and 60% for pigs on the wheat- and rye-based diets, respectively, and the enterolignans were primarily excreted via the faeces (46% of ingested plant lignans). In the present study only 9 and 20% of ingested plant lignans from the wheat and rye breads, respectively, were recovered as enterolignans in the faeces, while 27 and 35% of ingested plant lignans were recovered in the urine as plant lignans or enterolignans. This is in the same range as previously observed in rats given radio-labelled secoisolariciresinol diglucoside⁽⁵²⁾, but slightly lower than in human studies with flaxseeds, where approximately 40% of the ingested secoisolariciresinol diglucoside was excreted as enterolignans in the urine⁽⁵³⁾. Based on the assumption that the colonic microflora would efficiently deconjugate the majority of lignans, only unconjugated lignans were determined in the faeces^(24,54). If present at higher levels than anticipated, conjugated lignans may have contributed to the discrepancy between estimated production and

measured excretion of enterolignans in the present study. They would also result in lower true faecal digestibility of the plant lignans than the calculated apparent digestibilities presented here. Another minor contribution may be caused by oxidation to other metabolites^(43,55,56). Finally, quantitative losses of urine and/or further microbial activity in the collection bottle may contribute to the gap between estimated production and the actual recovery of enterolignans. The lower concentration of lignans in urine collected quantitatively than in the urine collected directly from the bladder is believed mainly to be caused by dilution with drinking water, but may also reflect losses as suggested above.

In contrast to the present study, previous studies have recovered two to ten times more enterolignan in faeces and urine than was expected from the intake of precursors^(24,30), the main reason being the incomplete knowledge of the amounts of enterolignan precursors in the diet. Conversion of lignin to lignans is another possible contributor to enterolignan excretion both in the present and previous studies. Previously it was found that lignin accounted for 26–32% of the ENL production in rats fed rye and wheat bran⁽⁵⁷⁾.

Body retention could potentially also contribute to the difference between estimated production and excretion, as enterolignans in the form of ENL were detected in various tissues. However, in the present study accumulation is expected only to parallel the growth of tissue, as a 'steady-state' condition can be expected after continuous ingestion for 9–10 weeks. Peroral exposure to ³H-labelled secoisolariciresinol diglycoside from flaxseed in rats has shown that the majority of radioactivity was recovered in the faeces and urine, while of twelve tissues analysed the highest concentrations were found in the liver, kidney and uterus⁽⁵²⁾. The high concentrations in these tissues after chronic exposure (10 d) was argued to be a consequence of the liver being the main site of conjugation with glucuronide, a high concentration of anti-oestrogenic binding sites in the liver, significant renal excretion of lignan metabolites, and the antagonising oestrogen action of enterolignans in the uterus⁽⁵²⁾. A study in rats fed diets enriched with linseed meal showed dose-related increases of END and ENL in the liver, testes, prostate and lung tissue⁽⁵⁸⁾. The present study is to our knowledge the first to demonstrate ENL in various tissues after intake of high amounts of rye. The moderate levels of ENL found in breast tissue and high levels found in plasma could definitely have biological activity. Previously it was shown that ENL accumulates in breast cysts, and that it modulates the risk related to high intracystic levels of epidermal growth factor⁽⁵⁹⁾, and it has recently been shown that higher blood concentrations of ENL reduce the risk of breast cancer. While Olsen *et al.*⁽⁶⁰⁾ found an incidence rate ratio of 0.71 per 20 nmol/l higher plasma ENL associated with oestrogen receptor- α -negative tumours, Sonestedt *et al.*⁽⁶¹⁾ showed that blood concentrations above 16 nmol/l diminished the risk for breast cancer in oestrogen receptor α -positive (OR 0.75) and oestrogen receptor β -negative (OR 0.60) tumours. Hence, ENL appears to modulate oestrogen receptor function, but the protective effect against breast cancer is probably mediated through several other mechanisms and factors related to the food consumed, particularly cereal fibre intake^(1,62).

We have previously demonstrated an attenuating effect on plasma total and LDL-cholesterol of the diets in the

hypercholesterolaemic pigs used in the present study. Lignans have been proposed to exert a cholesterol-lowering effect by stimulation of the LDL receptor activity⁽¹¹⁾, although this could not be confirmed by the expression of mRNA in the liver of the pigs from the present study⁽¹⁷⁾. Currently, it is not known to what extent the lignans contribute to the hypocholesterolaemic effect obtained. The protective effect of whole grain against cancers and CVD as seen in some but not all epidemiological studies may be brought about by many mechanisms as discussed in a recent review⁽¹⁾. To what extent the lignans play an essential role by themselves, or are merely biomarkers, is still debated.

Conclusion

The present study shows that the lignans of rye are interconverted in the intestine and taken up to the systemic circulation to a variable extent. Some lignans are deposited in different tissues, but only ENL at a concentration high enough to be well quantified. At high intake levels, plant lignans are also taken up to the bloodstream and excreted via the urine and particularly via bile. This implies that the enterolignans and their precursors undergo enterohepatic circulation to a variable extent. The present study is to our knowledge the first to show that plant lignans are found in high concentrations in bile. Whether this is a mechanism of systemic elimination awaits further investigation.

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