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ORIGINAL CONTRIBUTION

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No effect on adenoma formation in Min mice after moderate amount of flaxseed

Summary *Background & aim* The mammalian lignan enterolactone (ENL) produced from plant lignans, e. g. secoisolariciresinol diglycoside (SDG), may protect against various cancers in humans. The present work aims to evaluate the effect of flaxseed on tumour formation in multiple intestinal neoplasia (Min) mice, a model for colon tumorigenesis. *Design* Male and female Min mice were fed either with a non-fibre control diet or the same diet supplemented with 0.5 % (w/w) defatted flaxseed meal. Conversion of SDG to the mammalian lignans enterodiol (END) and ENL in the gut, and plasma ENL, were measured by HPLC with coulometric electrode array detector (CEAD) and time-resolved fluoroimmunoassay, respectively. Wild-type mice were also fed with the experimental diets in order to see whether lignan metabolism is different in Min and wild-type mice. *Results* The total number of adenomas or their size in the small intestine was not different in the flaxseed and control groups. The flaxseed group had a tendency for a decreased number of colon adenomas in both genders. Gender and genotype based differences were found in the intestinal ENL levels. When compared to Min females, Min males in the flaxseed group had several fold higher ENL levels in the small intestine (Min males 125 ± 124.5 nmol/g vs. females 22.8 ± 16.0 nmol/g, $P = 0.048$) and caecum (47.6 ± 31.6 nmol/g vs. females 14.5 ± 6.6 nmol/g, $P = 0.001$). Presence of adenomas in the gut influences the intestinal lignan metabolism. Min mice had less intestinal END and ENL as compared with the wild-type mice ($P < 0.05$). Mean plasma ENL increased 7-fold during the flaxseed feeding (7 nmol/L in control vs. 50 nmol/L in flaxseed group) but no differences between gender and genotype were found. The plasma ENL level did not correlate with adenoma number in the small intestine and colon. *Conclusion* The number of intestinal adenomas in the Min mouse model is not related to ENL level in plasma nor is it associated with the levels of intestinal lignans. A gender difference in ENL lignan metabolism was found in the gut but not in the plasma.

Key words Min mice – flaxseed – enterolactone – enterodiol – plant lignans

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Introduction

Phytoestrogens, mainly isoflavonoids and lignans, have been proposed to prevent various cancers in humans. Potential mechanisms of anticancer action of phytoestrogens have been suggested to involve modulation of hormone metabolism and subsequent cancer risk [1, 2]. The main food sources of isoflavonoids in the Asian population are soy products [3]. Flaxseed is the richest source of plant lignans. However, it is a relatively minor dietary component, and plant foods such as whole-grain products, berries, fruits and vegetables are the main sources of lignans in the Western diet [4, 5]. Plant lignans secoisolariciresinol diglycoside (SDG) and matairesinol (MAT) are metabolised by the gut mi-

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crobes to mammalian lignans enterodiol (END) and enterolactone (ENL). Enterodiol may further be converted to enterolactone. Both ENL and END are absorbed and undergo enterohepatic circulation, eventually being excreted in the urine and faeces [6, 7].

Plasma ENL can be used as an indicator for plant lignan intake [8, 9]. Whether ENL as such is antitumorigenic, or it is just a biomarker of a healthy diet is still a matter of debate. However, a high dietary intake of plant lignans in women with particular cytochrome P450c17α genotype [10], or high plasma ENL level [11] was associated with a reduced risk of breast cancer. Furthermore, a low plasma ENL concentration was associated with increased risk of breast cancer [12]. Curiously, very high plasma ENL concentrations were also associated with an increased breast cancer risk in two out of three cohorts, whereas, in the third cohort the association was opposite. No association was found between plasma ENL level and the risk of prostate cancer in humans [13], nor between plasma ENL and an increase in tumour cell apoptosis in prostate cancer patients [14]. Both isoflavones and lignans have structural similarities with natural and synthetic estrogens and antiestrogens [7]. There is substantial evidence that hormone replacement therapy reduces the risk of colon cancer in women in their menopausal years [15, 16]. However, no direct evidence of a protective role of plant or mammalian lignans with regard to colon cancer in humans has been reported although studies in animals support this view [17]. Daily gavages of isolated SDG from flaxseed in carcinogen-treated male rats decreased the number of aberrant crypt foci (ACF) in the distal colon [18]. Also other plant lignans such as arctiin from burdock seeds or 7-hydroxymatairesinol (HMR)-containing crude extract isolated from coniferous trees have shown to be tumour preventive in other animal models used to study colon cancer [19, 20].

In the present experiment we studied the possible antitumour effect lignans provided by defatted flaxseed meal on intestinal adenoma formation in Min mice, a well-characterized animal model for human colon cancer [21]. Male and female Min mice were fed either with non-fibre control diet, or a diet supplemented with 0.5% (w/w) defatted flaxseed meal for seven weeks after which the intestinal adenomas were counted. To elucidate the relationship of lignans and intestinal tumour formation we also measured the conversion of the lignan SDG to END and ENL in the contents of both caecum and in the distal small intestine, and in the plasma of the experimental mice. Both genders were used to see if the intestinal adenoma number or the conversion of plant lignans differs between males and females. Wild-type mice were also fed with the experimental diets in order to determine if there is a genotype-based difference in the lignan metabolism between Min and wild-type mice.

Materials and methods

Lignan analysis of defatted flaxseed meal and diets

A GC-MS method was used for analysis of plant lignans, MAT, secoisolariciresinol (SECO, the aglycone of SDG), isolariciresinol (ISOL), lariciresinol (LAR), pinoresinol (PIN) and syringaresinol (SYR), in flaxseed raw material and diet samples. It was modified from previously published method for analysis of isoflavonoids and lignans in food matrixes [22]. All details of the method with complete evaluation will be published elsewhere. The method is briefly as follows. Duplicate analyses were carried out for each sample. Deuterated internal standards ($^2H_6$-matairesinol, $^2H_6$-secoisolariciresinol and $^2H_8$-anhydrosecoisolariciresinol), 0.5 ml of distilled water, and 0.5 ml of hydrolysis reagent (containing 5000 FU glucuronidase and 50000 RU sulphatase from *Helix pomatia* in 0.3 M acetate buffer, pH 5) were added to 50 mg of dry sample. Enzymatic hydrolysis was carried out at 37 °C overnight and then for 1 h at 60 °C. The hydrolysed sample was extracted twice with 6 ml diethyl ether. Combined organic phases were evaporated to dryness, dissolved in 0.5 ml methanol and stored at 4 °C. The water phase was subjected to acidic hydrolysis by adding 200 µl 6.0 M HCl followed by incubation of the sample for 2 h at 70 °C and twice extracted with 6 ml of diethyl ether:ethyl acetate, 1:1 (v:v). The organic phases were combined with organic phases obtained after the enzymatic hydrolysis. The sample was evaporated to dryness, dissolved in 0.5 ml of methanol and stored at 4 °C. The water phase was further hydrolysed with 1.5 M HCl 2h at 100 °C, followed by extraction with diethyl ether:ethyl acetate, 1:3 (v:v). The water phase was discarded. The organic phases were combined with the stored organic phases, obtained after enzymatic and mild acid hydrolyses. The sample was evaporated to dryness under nitrogen, dissolved in 200 µl of methanol, and applied in 2×200 µl of methanol on a Lipidex 5000 column (0.5×5.0 cm in MeOH:$CHCl_3$:H_2O, 4:1:1). Lignans were eluted with 4 ml of MeOH:$CHCl_3$:H_2O, 4:1:1 (v:v:v). The fraction was evaporated to dryness under nitrogen, and dissolved in 0.5 ml of MeOH. Further purification of the sample was carried out with chromatographies on DEAE-OH and QAE-Ac as described by Mazur et al. [22]. The sample was derivatised with 100 µl of QSM, (pyridine:HMDS:TMCS, 9:3:1) by incubating 30 min at room temperature, transferred to a micro vial and analysed by GC-MS. Deuterated internal standards were not available for the new plant lignans, so quantification was done using deuterated MAT for analysis of LAR, PIN and SYR, and deuterated SECO and anhydrosecoisolariciresinol (ANSE) for analyses of ISOL and anhydroisolariciresinol (ANIS), respectively.

Diets

Lignan analyses revealed that defatted flaxseed meal had a total lignan level of 14.6 μmol/g (5 mg/g). SECO was the main lignan (97.5 %) and the others existed only in trace amounts (0.1 % MAT, 1.7 % ISOL, 0.3 % LAR and 0.5 % PIN). The plant lignan concentration in the flaxseed diet was adjusted to be equivalent with a 2.5 % flaxseed containing diet [estimation based on ref 23]. Flaxseed diet had a total lignan concentration of 73-μmol (26 mg)/kg diet. We analysed also the non-fibre diet and found a small amount of SECO (0.23 μmol/kg diet), which probably originated from plant oils.

Otherwise the diets were AIN-93G-based [24] non-fibre high-fat diet (control) and a high-fat diet supplemented with 0.5 % (w/w) of defatted flaxseed meal (flaxseed) (Table 1). The oil (11.2 %), carbohydrate (41 %), and protein (34.5 %) contents of defatted flaxseed meal were compensated in rapeseed oil, dextrose and casein fractions. Both diets contained 20 % protein, 40 % carbohydrate and 40 % fat per energy. The intake of fat and fatty acid composition corresponded to that in a Western-type diet.

Animals

The Laboratory Animal Ethics Committee of the University of Helsinki approved the study protocol. The mice were bred at the Laboratory Animal Centre, University of Helsinki, from the mice originally obtained from the Jackson Laboratory (Bar Habor, ME). The Min pedigree was maintained by mating C57BL/6J- +/+ females with C57BL/6J-Min/ + (Min) males. Both breeding pairs and offspring were fed with 2014 Harlan Teklad diet (Harlan Teklad, Madison, WI), which does not contain any alfalfa or soy as a source of plant phytoestrogens. Offspring were genotyped after weaning by using the Promega's Wizard® Genomic DNA Purification Kit followed by allele-specific polymerase chain reaction [25]. All Min mice (12–17 Min mice/group/gender, n = 58) and wild-type mice (7–14 mice/group/gender, n = 42) were put on the experimental diets at the age of five weeks. Mice were housed in plastic cages in a temperature- and humidity-controlled animal facility, with 12-h light/dark cycle. They had a free access to the semisynthetic diets and tap water for the feeding period of seven weeks. The body weights of the animals were recorded weekly. At the end of the feeding period mice were killed by CO_2 asphyxiation. A blood sample was collected from the abdominal aorta, centrifuged at 6000 g for 1 min, after which plasma was stored at –70 °C for ENL analysis. The contents of the distal small intestine (five cm up from the caecum) and the caecum were collected and stored at –70 °C for lignan analysis. The scoring of adenomas was done as described in the previous study [26]. Briefly, the small intestine and colon + caecum were rinsed with ice-cold saline and spread flat on a microscope slide. The number, diameter and location of adenomas were determined with an inverse light microscope with a magnification of 2.5x by two observers blind to the dietary treatment.

Table 1 Nutrient composition (g/kg) of experimental diets[1]

Ingredient	Control	Flaxseed
Casein	236.2	234.5
Dextrose	479.0	477
Butter	148.9	148.9
Sunflower-seed oil	13.3	13.3
Rapeseed oil	62.2	61.6
Mineral mix	41.6	41.6
Vitamin mix	11.8	11.8
L-cystine	3.6	3.6
Choline chloride	3.6	3.6
Tertiary butylhydroxyquinone	0.014	0.014
Defatted flaxseed[2]	–	5.0

[1] Casein was obtained from Kainuun Osuusmeijeri (Sotkamo, Finland), dextrose from Six Oy (Helsinki, Finland), mineral and vitamin mix from Harlan Teklad (Madison, WI), L-cystine, choline chloride and tertiary butylhydroxyquinone from Yliopiston Apteekki (Helsinki, Finland), defatted flaxseed meal from Elixi Oil Oy (Somero, Finland) . Butter, sunflower oil, and rapeseed oil were from a local market.
[2] Defatted flaxseed meal had 34.5 % of protein, 40.7 % of carbohydrates, 11.2 % of fat, 8.8 % of moisture and 4.9 % of ash

ENL analysis of plasma samples

ENL was analysed from the plasma samples by time-resolved fluoroimmunoassay [27, 28]. Analysis was performed as described by Oikarinen et al. [29].

Lignan analysis of intestinal contents

The plant SECO and mammalian ENL and END lignan levels in the caecal and small intestinal contents were measured by HPLC with coulometric electrode array detector (CEAD). The sample was weighed and 0.5 ml of water and 0.5 ml of hydrolysis reagent (glucuronidase and sulphatase from H. pomatia in 0.3 M acetic buffer pH 5.0) were added. The enzymatic and acidic hydrolyses were carried out as described for lignan analysis of defatted flaxseed meal and diets. The hydrolysed and extracted samples were chromatographed on QAE chloride in acetate form as described by Mazur et al. [22]. Samples were diluted with the mobile phase and analysed by HPLC-CEAD applying the method described by Nurmi et al. [30] using the modified detection potentials. The original potentials 575, 600, 670 mV were changed to 520, 660 and 700 mV on channels 5, 6 and 7,

respectively and mammalian lignans END and ENL were quantified on channel 6. Due to the analysis procedure, the values of ENL and END represent the sum of all conjugated and unconjugated forms of these mammalian lignans.

Statistics

The results are expressed means ± SD. Statistical analyses were performed using SPSS 10 (SPSS Inc. Chicago, Illinois). Intestinal lignan data and adenoma data of the small intestine was analysed by non-parametric Mann-Whitney U test for two independent samples. Colon adenoma number was tested by χ^2 tests. Two-way ANOVA (general linear model) was used to test normally distributed plasma ENL data for diet × gender and diet × genotype interactions. Associations between variables were analysed with nonparametric Spearman's correlation test. Values of $P < 0.05$ were considered significant.

Results

Body weights

Weight gain during the experimental period was similar in both diet groups per gender and genotype (data not shown).

Adenoma number of Min mice

The total number of adenomas in the small intestine was not different between the control and flaxseed groups (Table 2). The mean adenoma size in the small intestine was 1.0 ± 0.2 mm for control males, 0.9 ± 0.1 mm for control females, and similarly, 1.0 ± 0.2 mm for flaxseed males and 0.9 ± 0.1 mm for flaxseed females.

There was no change in adenoma number, size, incidence or distribution in the colon between the diet groups, although the flaxseed groups had a tendency to have a fewer number of adenomas in the colon than the control groups (Table 2). When diet groups per gender are pooled together, Min males had twice as many adenomas in the colon (0.90 ± 0.94, $n = 29$) than Min females (0.45 ± 0.57, $n = 29$, $P = 0.048$). When males and females were compared within a diet group a similar but non-significant tendency could still be seen.

Table 2 The number of adenomas in the small intestine, colon and caecum, and tumour incidence in the colon in Min males and females fed with control non-fibre and 0.5 % flaxseed diets for seven weeks[1]

Adenoma number	Male	Female
Control		
n =	*12*	*13*
Small intestine	57.4 ± 29.7	56.7 ± 27.2
Colon and caecum	1.2 ± 1.1	0.7 ± 0.6
Tumour incidence (%) in the colon and caecum[2]	58 %	62 %
Flaxseed		
n =	*17*	*16*
Small intestine	45.8 ± 16.5	56.3 ± 29.3
Colon and caecum	0.8 ± 0.9	0.4 ± 0.6
Tumour incidence (%) in the colon and caecum[2]	53 %	31 %

[1] Values are mean ± SD; *n* number of animals
[2] The number of animals (%) bearing tumours in the colon and caecum

Intestinal lignan levels

Min mice and wild-type mice had several similarities in the intestinal lignan levels as well as plasma ENL concentrations. Flaxseed diet fed Min male and female mice, as well as their wild-type counterparts had significantly increased levels of intestinal ENL ($P \leq 0.004$), END ($P \leq 0.006$) and SECO ($P \leq 0.001$) as compared with the control group (Table 3). The ENL and END concentration found in Min and wild-type mice after the flaxseed diet were at the same level in the distal small intestine and caecum. There was a positive relationship between the small intestinal and caecal ENL ($r = 0.914$, $P = 0.001$, $n = 26$) and END levels ($r = 0.512$, $P = 0.008$, $n = 26$) in the flaxseed fed Min mice.

A difference between genders was found in the intestinal ENL levels. When compared to Min females, Min males in the flaxseed group had several fold higher ENL levels in the small intestine (Min males 125 ± 124.5 nmol/g vs. females 22.8 ± 16.0 nmol/g, $P = 0.048$) and caecum (Min males 47.6 ± 31.6 nmol/g vs. females 14.5 ± 6.6 nmol/g, $P = 0.001$) (Table 3). Although the control Min males had very low ENL level in the small intestine, it was still significantly higher than the ENL level found in Min females ($P = 0.014$) indicating a gender effect on lignan metabolism on a wide range of intake. Gender difference in the caecal ENL level was also found between wild-type males and females fed the flaxseed diet (wild-type males 69.1 ± 32.4 nmol/g vs. females 24.9 ± 17.6 nmol/g, $P = 0.027$). However, there were no differences between gender in the levels of intestinal SECO or END, which is a precursor of ENL. Therefore, we assume that possible differences between male and female mouse food intake does not explain the observed differences between gender in the intestinal ENL levels.

When comparing genotypes it was found that wild-type males on the flaxseed diet had a higher level of caecal ENL (69.1 ± 32.4 nmol/g) and END (15.7 ± 6.5 nmol/g) than corresponding flaxseed fed Min males (ENL 47.6 ± 31.6 nmol/g, $P = 0.045$; END 6.1 ± 5.6 nmol/g, $P = 0.001$). Similar tendency was also

Table 3 Concentrations of lignans in the distal small intestine and caecum of Min and wild-type male and female mice fed non-fibre control or 0.5 % flaxseed diets for seven weeks[a, b]

	Distal Small Intestine				Caecum			
	Min		Wild-type		Min		Wild-type	
Diet	Male	Female	Male	Female	Male	Female	Male	Female
Control								
n =	*8*	*8*	*7*	*2*	*12*	*13*	*9*	*3*
ENL, nmol/g	3.0±2.1[d]	1.0±0.7	6.1±4.1[e]	0.5±0.1	0.5±0.3	0.5±0.5	1.4±1.3	0.2±0.1
END, nmol/g	1.0±1.5	0.7±0.6	1.6±1.1	0.3±0.2	0.6±0.7	0.4±0.7	1.8±1.2[e]	0.1±0.0
SECO, nmol/g	4.9±2.1	5.6±3.3	3.5±2.3	3.9±1.9	0.6±0.7	0.5±0.7	0.7±0.6	0.9±0.1
Flaxseed								
n =	*12*	*14*	*9*	*3*	*17*	*16*	*10*	*5*
ENL, nmol/g	125.1±124.5[c, d]	22.8±16.0[c]	106.5±88.1[c]	38.4±36.6	47.6±31.6[c, d]	14.5±6.6[c]	69.1±32.4[c, d, e]	24.9±17.6
END, nmol/g	13.3±19.6[c]	9.8±8.4[c]	23.0±15.2[c]	17.2±13.3	6.1±5.6[c]	8.5±6.7[c]	15.7±6.5[c, e]	14.5±9.2
SECO, nmol/g	27.8±17.5[c]	38.8±38.9[c]	35.3±21.6[c]	21.5±10.9	26.4±16.9[c]	28.6±13.2[c]	18.5±6.2[c]	28.9±16.4

[a] Values are mean ± SD; *n* the number of analysed samples, which varies because of the amount of intestinal content in some animals was too small for lignan analysis. Statistical analysis was not carried out when $n < 5$
[b] *ENL* enterolactone; *END* enterodiol; *SECO* secoisolariciresinol
[c] Different from control, $P \leq 0.006$
[d] Gender difference between males and females fed the same diet, $P < 0.05$
[e] Genotype difference between wild-type and Min males fed the same diet, $P < 0.05$

found between wild-type and Min females in the caecal ENL (24.9 ± 17.6 nmol/g vs. 14.5 ± 6.6 nmol/g) and END levels (14.5 ± 9.2 nmol/g vs. 8.5 ± 6.7 nmol/g) although this was not significant.

Lignans were analysed in the distal small intestine, which had 17 % of the total adenoma number of the small intestine, and in the caecum where few adenomas were found. There was a weak significant negative association between levels of SECO and adenoma number of the distal small intestine ($r = -0.383$, $P = 0.026$, $n = 42$). No association was found between END or ENL and adenoma number of this area.

Table 4 Concentration of enterolactone (ENL, nmol/L) in plasma samples of Min and wild-type male and female mice fed non-fibre control or 0.5 % flaxseed diets for seven weeks[1, 2]

	Min		Wild-type	
	Male	Female	Male	Female
n =	*11*	*13*	*12*	*7*
Control	6.8±2.1	7.0±3.9	7.4±2.6	3.9±2.0
n =	*12*	*14*	*9*	*8*
Flaxseed	46.0±21.1*	54.6±17.5*	52.3±40.1*	44.5±13.2*

[1] Data are mean ± SD; *n* the number of analysed samples
* Different from control, $P = 0.001$
[2] General linear model Univariate analyses found non-significant diet x gender ($P = 0.806$) and diet x genotype ($P = 0.931$) interactions

Plasma enterolactone

The plasma ENL concentration increased significantly ($P = 0.001$) after the flaxseed diet in both genotypes when compared with the control groups (Table 4). However, no differences between genders ($P = 0.806$) or genotypes ($P = 0.931$) within diet groups were found. The plasma ENL level was not associated with adenoma number in the small intestine or colon.

Discussion

Flaxseed diet or isolated SDG have been shown to decrease ACF formation and mammary tumours [18, 31, 32] or reduce tumour growth [23, 33, 34]. We found no significant changes in intestinal adenoma formation in Min mice after the flaxseed diet, only a tendency of decreased number of colon adenomas in both genders. Furthermore, flaxseed diet fed Min females had a lower colon tumour incidence (31 %) than the control female group (62 %), although this was not significant. So far we cannot conclude that a 0.5 % defatted flaxseed meal containing diet (corresponding to a 2.5 % flaxseed diet), even though causing a considerable increase in plasma ENL and the intestinal plant and mammalian lignan levels, affects adenoma formation in Min mice. This result is in line with two other studies with Min mice [29, 35], that also showed that ENL production (up to plasma ENL concentration of 180 nmol/L in ref. 35) as a consequence of 5 % flaxseed or 10–30 % rye bran supplementation does not have any effect on adenoma formation in this animal model. On the contrary, other studies with carcinogen-induced rats have shown that flaxseed sup-

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plementation lowered ACF multiplicity and number in the gut of carcinogen-treated rats, and the protective effect was linked, in part, to pure SDG and associated with increased β-glucuronidase and reduced cell proliferation activities [18, 31]. Other types of plant lignans, 0.02 or 0.2 % arctiin and 0.02 % crude HMR extract in a diet [19, 20] have also been shown to be protective against chemical-induced colon carcinogenesis in rats and in Min mice, respectively. Metabolism of plant lignan SDG (or SECO) differs from that of arctiin or HMR. Arctigenin, a metabolic product of glycoside arctiin, was only to some extent converted to ENL and mainly to other metabolites [36]. HMR was metabolised to ENL and 7-hydroxyenterolactone [36, 37] whereas SDG/SECO was metabolised extensively mainly to ENL and END.

We found that Min male mice had higher numbers of colon adenomas than females. When Min mice were treated with azoxymethane a similar difference between genders in colon tumour number was also noticed [38]. This may indicate that female Min mice might be more protected against colon tumorigenesis than males. After ovariectomy, intestinal adenomas in Min mice has been shown to increase by 77 %, and estradiol replacement after ovariectomy had a tumour-reducing effect accomplished with changes in estrogen receptor (ER) α and β levels in the intestinal tissues [39]. Phytoestrogens might also have other ways to exert their effect on intestinal mucosal cells. In a recent study genistein was found to accumulate in the colon tissue and regulate vitamin D metabolism in such a way that the synthesis of antimitotic hormone 1.25-dihydroxyvitamin D3 was enhanced [40].

Our hypothesis was that the presence of adenomas in the gut might influence intestinal lignan metabolism in Min mice. Indeed, we found lower levels of intestinal END and ENL in Min mice when compared with the wild-type mice. However, the responses to diets was similar in both genotypes. Min mice usually become anaemic by 60 days of age due to the development of multiple adenomas, which cause bleeding into the intestinal lumen [41]. A rational explanation would be that bleeding changes levels or populations of microorganisms or bacterial enzyme activities, and in that way changes dietary lignan metabolism.

In the present study, the consumption of 0.5 % defatted flaxseed meal diet was sufficient to elevate plasma ENL level 7-fold in both Min and in wild-type mice. Two interesting findings were made: First, there were no differences in plasma ENL between genders or genotypes, which was quite surprising since clear gender differences in both genotypes in the intestinal ENL levels were found. Second, in flaxseed fed mice the ENL level in the plasma did not reflect ENL levels in the gut. It could be speculated that a saturated absorption process for ENL in the gut regulates the plasma ENL level. However, this hypothesis was rejected with an experiment (unpublished results of SO, S-MH, HA and MM) where 2.5 times higher flaxseed supplementation in Min mice roughly double ENL levels in the gut and plasma.

The metabolism of mammalian lignans involves an enterohepatic circulation similar to that of estrogens. It is estimated that 50–60 % of the conjugated estrogens in the liver are excreted into bile and re-enter the human intestine [42]. Estrogens and mammalian lignans ENL and END are excreted with the bile back into gut conjugated with glucuronic acid. We found substantial levels of mammalians lignans in the distal small intestine of flaxseed fed mice, and there was a significant positive correlation between the ENL and END values of the two intestinal segments. These results indicate that either luminal ENL/END originated from enterohepatic circulation or plant lignan conversion may already exist in the distal part of the small intestine in mice. Unfortunately, we did not separate the unconjugated and conjugated metabolites, nor bilinary lignans (glucuronides) from dietary lignans (glycosides). So far, we can ask if differences between genders in the luminal ENL could be due to the differences in bilinary secretion of ENL regulated by the liver or some hormonal control of gut bacterial enzymes that are involved in lignan metabolism.

In conclusion, this study showed that the number of intestinal adenomas in Min mouse is probably not related to ENL production or linked to the levels of plant lignan precursors in a diet. Lignan metabolism is complex, and is also partly affected by gender.

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References

1. Adlercreutz H (1998) Human health and phytoestrogens. In: Korach KS (ed) Reproductive and Developmental Toxicology. Mercel Dekker, Inc., New York, NY, pp 299–371
2. Adlercreutz H (2002) Phyto-oestrogens and cancer (review). Lancet Onc 3: 364–373
3. Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hämäläinen E, Hasegawa T, Okada H (1991) Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. Am J Clin Nutr 54:1093–1100
4. Mazur W (1998) Phytoestrogen content in foods. Baillière's Clin Endocrinol Metab 12:729–742
5. De Kleijn MJJ, van der Schouw YT, Wilson PWF, Adlercreutz H, Mazur W, Grobbee DE, Jacques PF (2001) Intake of dietary phytoestrogens is low in postmenopausal women in the United States: the Framingham study. J Nutr 131:1826–1832

6. Axelson M, Setchell KDR (1981) The excretion of lignans in rats – evidence for an intestinal bacterial source for this new group of compunds. FEBS Letters, 123:337–342
7. Setchell KDR, Adlercreutz H (1988) Mammalian lignans and phytoestrogens. Recent studies on their formation, metabolism, and biological role in health and disease. In: Rowland IR (ed) Role of the Gut Flora in Toxicity and Cancer. Academic Press New York, NY, pp 315–345
8. Jacobs Jr DR, Pereira MA, Stumpf K, Pins JJ, Adlercreutz H (2002) Whole grain food intake elevates serum enterolactone. Br J Nutr 88:111–116
9. Tarpila S, Aro A, Salminen I, Tarpila A, Kleemola P, Akkila J, Adlercreutz H (2002) The effect of flaxseed supplementation in processed foods on serum fatty acids and enterolactone. Eur J Clin Nutr 56:156–165
10. McCann SE, Moysich KB, Fredenheim JL, Ambrosone CB, Shields PG (2002) The risk of breast cancer associated with dietary lignans differs by CyP17 genotype in women. J Nutr 132: 3036–3041
11. Pietinen P, Stumpf K, Männistö S, Kataja V, Uusitupa M, Adlercreutz H (2001) Serum enterolactone and risk of breast cancer: A case-control study in Eastern Finland. Cancer Epidemiol, Biomarkers & Prevention 10:339–344
12. Hultén K, Winkvist A, Lenner P, Johansson R, Adlercreutz H, Hallmans G (2002) An incident case-referent study on plasma enterolactone and breast cancer risk. Eur J Nutr 41:168–176
13. Stattin P, Adlercreutz H, Tenkanen L, Jellum E, Lumme S, Hallmans G, Harvei S, Teppo L, Stumpf K, Luostarinen T, Lehtinen M, Dillner J, Hakama M (2002) Circulating enterolactone and prostate cancer risk: A nordic nested case-control study. Int J Cancer 99:124–129
14. Bylund A, Lundin E, Zhang JX, Nordin A, Kaaks R, Stenman U-H, Åman P, Adlercreutz H, Nilsson TK, Hallmans G, Bergh A, Stattin P (2003) Randomised controlled short-term intervention pilot study on rye bran bread in prostate cancer. E J Cancer Prev 12:407–415
15. Grodstein F, Newcomb PA, Stampfer MJ (1999) Postmenopausal hormone therapy and the risk of colorectal cancer; a review and meta-analysis. Am J Med 106:574–582
16. Writing Group for the Women's Health Initiative Investigators (2002) Risks and benefits of estrogen and progestin in healthy postmenopausal women. Principal results from the Women's Health Initiative Randomized Controlled Trial JAMA 288:321–333
17. Thompson LU (1998) Experimental studies on lignans and cancer. Baillière's Clin Endocrinol Metab 12: 691–705
18. Jenab M, Thompson LU (1996) The influence of flaxseed and lignans on colon carcinogenesis and β-glucuronidase activity. Carcinogenesis 17:1343–1348
19. Hirose M, Yamaguchi T, Lin C, Kimoto N, Futakuchi M, Kono T, Nishibe S, Shirai T (2000) Effects of arctiin on PhIP-induced mammary, colon and pancreatic carcinogenesis in female Sprague-Dawley rats and MeIQx-induced hepatocarcinogenesis in male F344 rats. Cancer Letters 155:79–88
20. Oikarinen SI, Pajari A-M, Mutanen M (2000) Chemopreventive activity of crude hydroxymatairesinol (HMR) extract in Apc^{Min} mice. Cancer Letters 161:253–258
21. Fodde R, Smits R, Clevers H (2001) APC, Signal transduction and genetic instability in colorectal cancer. Nature Reviews 1:55–67
22. Mazur W, Fotsis T, Wähälä K, Ojala S, Salakka A, Adlercreutz H (1996) Isotope dilution gas chromatographic-mass spectrometric method for the determination of isoflavonoids, coumesterol and lignans in food samples. Anal Biochem 223:169–180
23. Li D, Yee JA, Thompson LU, Yan L (1999) Dietary supplementation with secoisolariciresinol diglycoside (SDG) reduces experimental metastasis of melanoma cells in mice. Cancer Letters 142:91–96
24. Reeves PG, Nielsen FH, Fahey Jr GC (1993) AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J Nutr 123: 1939–1951
25. Dietrich WF, Lander ES, Smith JS, Moser AR, Gould KA, Luongo C, Borenstein N, Dove W (1993) Genetic identification of Mom-1, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse. Cell 75:631–639
26. Mutanen M, Pajari A-M, Oikarinen SI (2000) Beef induces and rye bran prevents the formation of intestinal polyps in ApcMin mice: relation to β-catenin and PKC-isozymes. Carcinogenesis 21: 1167–1173
27. Adlercreutz H, Wang GJ, Lapcík O, Hampl R, Wähälä K, Mäkelä T, Lusa K, Talme M, Mikola H (1998) Time-resolved fluoroimmunoassay for plasma enterolactone. Anal Biochem 265: 208–215
28. Stumpf K, Uehara M, Nurmi T, Adlercreutz H (2000) Changes in the time-resolved fluoroimmunoassay of plasma enterolactone. Anal Biochem 284: 153–157
29. Oikarinen S, Heinonen S, Karppinen S, Mättö J, Adlercreutz H, Poutanen K, Mutanen M (2003) Plasma enterolactone or intestinal Bifidobacterium levels do not explain adenoma formation in multiple intestinal neoplasia (Min) mice fed with two different types of rye bran fractions. Br J Nutr 90:119–125
30. Nurmi T, Adlercreutz H (1999) Sensitive high-performance liquid chromatographic method for profiling phytoestrogens using coulometric electrode array detection: application to plasma analysis. Anal Biochem 274:110–117
31. Serraino M, Thompson LU (1992) Flaxseed supplementation and early markers of colon carcinogenesis. Cancer Letters 63:159–165
32. Thompson LU, Seidl MM, Rickard SE, Orcheson LJ, Fong HHS (1996) Antitumorigenic effect of a mammalian lignan precursor from flaxseed. Nutr Cancer 26:159–165
33. Thompson LU, Rickard SE, Orcheson LJ, Seidl MM (1996) Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis. Carcinogenesis 17: 1373–1376
34. Yan L, Yee JA, Li D, McGuire MH, Thompson LU (1998) Dietary flaxseed supplementation and experimental metastasis of melanoma cells in mice. Cancer Letters 124:181–186
35. van Kranen HJ, Mortensen A, Sørensen IK, van den Berg-Wijnands J, Beems R, Nurmi T, Adlercreutz H, van Kreijl CF (2003) Lignan precursors from flaxseed or rye bran do not protect against the development of intestinal neoplasia in Apc^{Min} mice. Nutr Cancer 45:203–210
36. Heinonen S, Nurmi T, Liukkonen K, Poutanen K, Wähälä K, Deyama T, Nishibe S, Adlercreutz H (2001) In vitro metabolism of plant lignans: New precursors of mammalian lignans enterolactone and enterodiol. Journal of Agricultural and Food Chemistry 49: 3178–3186
37. Saarinen NM, Huovinen R, Wärri A, Mäkelä SI, Valentin-Blasini L, Needham L, Eckerman C, Collan YU, Santti R (2001) Uptake and metabolism of hydroxymatairesinol in relation to its anticarcinogenity in DMBA-induced rat mammary carcinoma model. Nutr Cancer 41:82–90
38. Paulsen JE, Steffensen I-L, Løberg EM, Husøy T, Namork E, Alexander J (2001) Qualitative and quantitative relationship between dysplastic aberrant crypt foci and tumorigenesis in the Min/+ mouse colon. Cancer Res 61:5010–5015

39. Weyant WJ, Carothers AM, Mahmoud NN, Bradlow HL, Remotti H, Bilinski RT, Bertagnolli MM (2001) Reciprocal expression of ERα and ERβ is associated with estrogen-mediated modulation of intestinal tumorigenesis. Cancer Res 61:2547–2551
40. Kállay E, Adlercreutz H, Farhan H, Lechner D, Bajna E, Gerdenitsch W, Campbell M, Cross HS (2002) Phytoestrogens regulate vitamin D metabolism in the mouse colon: relevance for colon tumor prevention and therapy. J Nutr 132:3490S–3493S
41. Moser AR, Pitot HC, Dove WF (1990) A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science 247:322–324
42. Adlercreutz H, Martin F (1980) Biliary excretion and intestinal metabolism of progesterone and estrogens in man. J Steroid Biochem 13:231–244