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IN VITRO BINDING OF ESTROGENS BY DIETARY FIBER AND THE *IN VIVO* APPARENT DIGESTIBILITY TESTED IN PIGS

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Summary—Within the framework of experiments related to the association between dietary fiber and breast cancer an *in vitro* test system was used to study the binding of estrogens to various fibers (e.g. cholestyramin, lignin and cellulose) and fiber sources (e.g. wheat bran, cereals, seeds and legumes). Furthermore, the *in vivo* apparent digestibility of the different fiber sources was tested using a mobile nylon bag technique in intestine-cannulated pigs. Estradiol-17β (E_2) bound more strongly to the various fibers than did estrone (E_1), estriol or estrone-3-glucuronide. At increasing pH (>7) binding of both E_1 and E_2 to wheat bran decreased significantly. Cholestyramine and lignin bound almost all estrogens present in the medium. Linseed (91%), oats (83%), barley chaff (88%) and wheat bran (82%) are other excellent binders of E_2. Corn, rye and white wheat flour showed lower binding capacity with a relatively low affinity. Cereals with the highest percentage of lignin in the fiber (>3%) were also the fiber sources with the lowest apparent digestibility. Estrogens bound with the highest affinity (relative to bovine serum albumin) to these fiber sources. Together with wheat bran and lignin, oats, linseed and soybean seem to be products with good perspectives for *in vivo* evaluation of the lowering effect of dietary fiber on estrogen exposure of estrogen-sensitive tissues.

INTRODUCTION

It has been well documented that estrogens are involved in breast cancer development [1]. Estrogens play an important role in cell proliferation [2–6] and hence in tumor initiation and/or promotion [7]. It has been hypothesized that fiber affects breast cancer risk [8, 9]. One of the mechanisms in which fiber might be involved is its influence on the enterohepatic circulation (EHC) of estrogens [10].

The estrogens, synthesized by the gonads and to a very small extent by the adrenals, are metabolized mainly in the liver, but also in the peripheral tissues [11]. The estrogen(s) (metabolites) are readily conjugated with glucuronic acid and/or sulfuric acid. About one-third to one-half of the circulating estrogens are secreted in the bile, and 80% of this fraction is reabsorbed after hydrolysis in the intestinal canal [12]. When the hydrolysis reaction is blocked [13] or the reabsorption of estrogens by the intestine is lowered as a result of binding to fiber, fecal estrogen excretion increases, urinary estrogen excretion decreases and plasma estrogen levels might be decreased as well.

Vegetarians on a high-fiber diet excrete significantly more estrogens in their feces, resulting in lower plasma estrogen levels, than omnivores on a low-fiber diet [14, 15]. Comparable results were described for rats receiving a high-fiber diet on the basis of wheat bran and a low-fiber diet on the basis of wheat flour [16]. It seems that reabsorbtion of estrogens, which is usually around 80%, can be diminished by dietary fiber. In this context, the binding of estrogens by dietary fiber plays an important role [17, 18], which might result in an interrupted EHC of estrogens.

An *in vitro* method for assessment of estrogen binding can be useful in selecting appropriate

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Abbreviations: E_1 = estrone; E_2 = estradiol-17β; E_3 = estriol; E_1-gluc = estrone-3-glucuronide; BSA = bovine serum albumin; MNBT = mobile nylon bag technique; NDF = neutral detergent fiber; ADF = acid detergent fiber; EHC = enterohepatic circulation.

fiber sources for further *in vivo* studies on fiber–hormone interactions, i.e. the effect of fiber on hormone-sensitive tumors. Shultz and Howie [17] reported an *in vitro* study of estrogen binding to fibers. In this paper we extend these data to various cereals, seeds and legumes and fiber compounds. Relative binding affinity was measured by adding bovine serum albumin (BSA) to the incubation medium. As *in vivo* binding of estrogens to dietary fiber might be affected by the degree of fermentation we also measured the *in vivo* apparent digestibility of the various fiber types and/or their components in intestine-cannulated pigs. The fermentation grade of various wheat varieties was tested *in vivo* as well.

MATERIALS AND METHODS

Chemicals

All chemicals used were Analar grade and obtained from Merck, (Darmstadt, Germany) unless stated otherwise. The tritium-labeled hormones [2,4,6,7-^{3}H(N)]estradiol (sp. act. 115 Ci/mmol), [6,9-^{3}H(N)]estrone-3β-D-glucuronide (sp. act. 12.6 Ci/mmol), [2,4,6,7-^{3}H(N)]estrone (sp. act. 105 Ci/mmol), [2,4,6,7-^{3}H(N)]estriol (sp. act. 105 Ci/mmol) and [1,2,6,7,-^{3}H(N)]progesterone (sp. act. 90.1 Ci/mmol) were purchased from New England Nuclear, Dupont ('s-Hertogenbosch, The Netherlands). The labeled compounds were purified before use by HPLC as described previously [19]. The steroids 17β-estradiol (art. E 8875; E_2), estrone (art. E 9750; E_1), estriol (art. E 1253; E_3), progesterone (art. P 0130) and estrone-3-glucuronide (art. E 1252; E_1-gluc), as well as α-cellulose (art. C 8002), cholestyramine (art C 4650), pectin (from apple; art. P 2157), cellulose (Sigmacell, Type 100; art. S 3755) and BSA (art. A 7030) were obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A.). Pepsin (porcine gastric mucosa) was obtained from Boehringer Mannheim (Germany). Wheat, wheat bran, white wheat flour, soybean, rye, oat, barley, linseed, buckwheat and corn were obtained from a local miller (Van Rooy, Wageningen, The Netherlands). Lignin was kindly donated by Westvaco (Charleston Heights, S.C., U.S.A.). Lignin sulfonate and lignin extracted from rye chaff were kindly donated by Dr M. van Oort (IGMB-TNO, Wageningen, The Netherlands) and pea bran by Dr J. Huisman of the same institute. The wheat varieties: Soft Red Winter (SRW), Soft White Winter (SWW), Durum, Hard Red Winter (HRW) and Hard Red Spring (HRS) were kindly donated by Dr J. de Waart (TNO-CIVO Institutes, Zeist, The Netherlands).

Methods

Before performing the *in vitro* test the brans, cereals, seeds and legumes were grinded using an ultracentrifugal grinder with ring-sieves of 0.5 or 1 mm (Retsch B.V., Ochten, The Netherlands). The *in vitro* binding method was a modification of Shultz and Howie's method [17]. In brief, 50 mg of each grinded fiber source or fiber component was weighed in duplicate into tubes (16 × 100 mm) and suspended in 400 μl of phosphate-buffered saline (PBS, pH 7.0). After incubation (30 min, 37°C) the pH was adjusted to 2.0 and to each tube 0.2 U of pepsin was added in 200 μl of PBS pH 2.0. After incubation (60 min, 37°C) the pH was adjusted to 7.0 and ^{3}H-labeled steroids were added. After another incubation (60 min, 37°C) tubes were centrifuged and an aliquot of 400 μl of supernatant and 4 ml of scintillation liquid (Safefluor; Lumac B.V., Olen, Belgium) were added in counting vials. Tubes for total counts (0% binding; containing no test binding substance) were treated similarly. The quantity of steroid bound was calculated as the difference between the amount of labeled steroid added (total counts) and that recovered in the supernatant. For testing relative binding affinity, incubation was performed with and without BSA for each fiber type. A scheme of the assay is presented in Table 1.

Intra-assay variance, which was calculated from the duplicate values of the percentages of

Table 1. Schematic presentation of the method

	Total	− BSA	+ BSA
Binder (mg)	—	50	50
PBS[a] pH 7.0 (μl)	400	400	400
	Incubation, 30 min, 37°C, shaking		
PBS[a] pH 2.0 + pepsin (μl)	200	200	200
	Incubation, 60 min, 37°C, shaking		
NaOH 0.67 M (μl)	100	100	100
Tracer[b] (μl)	50	50	50
BSA[c]	—	—	250
PBS pH 7.0 (μl)	250	250	—
	Incubation, 60 min, 37°C, shaking Centrifugation, 20 min, 3200 *g*, 4°C in small tubes (12 × 75 mm)		

[a]0.1 M sodium phosphate in saline pH 7.0 or by adding HCl pH 2.0.
[b]Each tracer is diluted to 25,000 dpm/2.5 μl ethanol. One of the following solvents is added to the various binders: (1) 2.5 μl of [^{3}H]E_2, evaporated and soluted in 5 μl ethanol and 45 μl PBS; (2) 2.5 μl of [^{3}H]E_2, 0.625 μl (= 500 ng) to 2.5 μl (= 2000 ng) unlabeled E_2, evaporated and soluted in 5 μl ethanol and 45 μl PBS; (3) 2.5 μl of [^{3}H]E_1, [^{3}H]E_3 or [^{3}H]E_1-gluc, evaporated and dissolved in 5 μl ethanol and 45 μl PBS.
[c]2% BSA in 250 μl PBS.

estrogen binding to the various fiber types, was found to be lower than 5% for binding of E_1 ($n = 67$) and E_2 ($n = 106$), 6.5% for E_3 ($n = 78$) and 11% for E_1-gluc ($n = 66$). Interassay variance, which was calculated from the binding of E_2 to wheat bran (particle size = 0.5 mm), white wheat flour and cellulose, was found to be 2.9% (wheat bran; $n = 27$), 3.6% (white wheat flour; $n = 12$) and 7.0% (cellulose; $n = 8$).

The effect of pH on binding was statistically analyzed with ANOVA techniques using pH as the factor and white wheat flour and bran as variates for both E_1 and E_2.

In vivo apparent digestibility tests were performed using the mobile nylon bag technique (MNBT) as described by Sauer *et al.* [20], and as modified recently [21]. In brief, pigs fed a standard-type pig grower diet were fitted with a simple T-cannula in the duodenum. Of each fiber type tested (particle size = 1 mm) 1 g aliquots were put into nylon bags (25 × 40 mm; pore size 48 μm) in quadruplicate. The nylon bags were pre-incubated in 1 l. 0.01 M HCl, containing 4000 U pepsine (Merck, art. 7189) for 5 h at 37°C under shaking. Each of the nylon bags was inserted into the duodenal cannula of 4 pigs. The bags passed with the feces. Next, the contents of the nylon bags were lyophilized and weighed. The difference in weight of the contents of the nylon bags before and after *in vivo* MNBT is a measure for the apparent digestibility of the fibers.

The dietary fiber composition of cereals was analyzed using the AOAC method [22] for total dietary fiber, whereas neutral detergent fiber (NDF) and acid detergent fiber (ADF), analyzed according to a modification of the method of Van Soest [23], were used to analyze the amounts of hemicellulose, cellulose and lignin.

RESULTS

Binding of estrogens

The data obtained for the relative binding of steroids to the various fiber sources are summarized in Table 2. Of all steroids tested, E_2 showed the highest binding to the various fiber sources, followed by E_1 and E_3. Binding of E_1-gluc to the fibers was lowest. Cholestyramine and lignin (from rice) bound almost all estrogens present in the medium. Linseed, oats, barley chaff and wheat bran are other good binders of E_2 and E_1.

Table 2. Binding of estrogens to various fibers and fiber sources[a] without and in the presence of 2% (w/v) BSA ($n = 2$)

	E_1			E_2			E_3			E_1-gluc		
Binding	−BSA	+BSA	Diff.[b]	−BSA	+BSA	Diff.	−BSA	+BSA	Diff.	−BSA	+BSA	Diff.
1. Wheat bran	68.4	66.8	1.6	82.0	68.1	13.9	46.5	46.0	0.5	30.7	29.1	1.6
2. White wheat flour	50.9	36.4	14.5	71.4	40.0	31.4	40.5	31.5	9.0	28.2	22.2	6.0
3. Wheat	65.2	42.4	22.8	78.6	42.7	35.9	49.8	36.9	12.9	27.7	18.5	9.2
4. Soybean	59.3	56.6	2.8	78.2	64.8	13.4	43.3	37.1	6.2	21.0	12.4	8.6
5. Buckwheat meal	65.2	38.8	26.4	79.4	41.3	38.1	46.3	28.7	17.6	21.5	11.9	9.6
6. Barley	65.9	46.2	19.7	80.8	45.9	34.9	55.6	39.8	15.8	32.2	21.5	10.7
7. Barley chaff	—	—	—	88.5	—	—	—	—	—	—	—	—
8. Oats	66.3	55.8	10.5	83.2	60.8	22.4	54.8	44.6	10.2	18.6	17.6	1.0
9. Corn	59.7	22.5	37.2	73.4	22.9	50.5	45.6	28.2	17.4	15.6	6.2	9.4
10. Rye	62.4	34.6	27.8	70.0	36.8	33.2	48.2	28.6	19.6	22.7	6.6	16.1
11. Linseed	82.7	72.0	10.7	90.6	70.6	20	48.0	38.1	9.9	24.9	25.7	0.4
12. Pea bran	59.5	—	—	73.5	—	—	—	—	—	—	—	—
13. Rice (unpolished)	58.0	—	—	72.0	—	—	—	—	—	—	—	—
14. Lignin from rice	95.1	92.9	2.2	98.9	97.8	1.1	97.8	95.3	2.5	85.7	85.1	0.6
15. Lignin-sulfonate	83.0	81.8	1.2	82.9	80.4	2.5	82.9	83.6	−0.7	85.2	83.2	2.0
16. Lignin (Westvaco)	—	—	—	96.0	90.2	5.8	—	—	—	—	—	—
17. Cholestyramine	95.8	94.9	0.9	99.1	98.6	0.5	98.9	98.3	0.6	97.8	97.0	0.8
18. α-Cellulose	26.9	2.9	24	45.9	7.5	38.4	23.6	9.5	14.1	0	0	—
19. Sigmacell	49.8	—	—	63.4	20.8	42.6	28.4	—	—	6.5	—	—

—, Analysis not performed.
[a]The binding of steroids to grains with a particle size of 0.5 mm are presented.
[b]Diff. = difference in binding of estrogens to the various sources with and without the presence of BSA.

When BSA was added to the incubation mixture binding was lowered for almost all fiber types tested. The extent of the decrease in binding capacity in the presence of BSA was interpreted as indicative of binding affinity. As the affinity contants of E_1, E_2 and E_3 with regard to albumin are not equal (5×10^4, 1.8×10^5 and 1×10^5 mol·l, respectively [11], the relative affinity can only be compared for each of the estrogens separately. A relatively small difference between the percentage of binding without, or in the presence of BSA means a high affinity for binding or the estrogen to the fiber, whereas a larger difference suggests a lower affinity. As shown in Table 2, cholestyramine, lignin sulfonate, lignin (from rice), wheat bran, soybean, linseed and oats showed the highest relative affinity for estrogens.

Apparent digestibility of fiber(s) sources

The results of the MNBT test are presented in Table 3. Wheat and pea bran, soybean, oats, linseed and lignin showed the lowest apparent digestibility in the MNBT test, suggesting a relatively low fermentability. In contrast, the apparent digestibility was 97% for white wheat flour and 80, 82 and 86% for corn, rye and wheat, respectively. After lyophilization, the residues in the nylon bags were also used to assess the *in vitro* binding of E_2. Residual binding of E_2 was clearly lower for digested white wheat flour than for undigested flour, for this *in vitro* binding test was performed with only a small amount of binder (16 mg in 320 μl of volume) as most of the white wheat flour was digested. Binding of E_2 to the residues of soybean, corn and rye were higher than without the *in vivo* MNBT test. Binding of E_2 to the other residues of the fiber sources was similar as compared with binding to the undigested sources.

Table 3. Apparent digestibility of various dietary fiber sources (particle size = 1 mm) tested with the MNBT in cannulated pigs and residual *in vitro* binding with E_2

Binder	Apparent digest. (%; $n = 4$)	Binding[a] (%; $n = 8$)
1. Wheat bran, 0.5 mm	44 ± 1.0	78 ± 2.6
2. Wheat bran, 1.0 mm	42 ± 2.3	79 ± 2.4
3. White wheat flour	97 ± 0.4	50 ± 4.9[b]
4. Soybean	57 ± 4.8	87 ± 2.6
5. Barley	73 ± 0.8	81 ± 1.5
6. Oats	59 ± 3.7	82 ± 0.9
7. Corn	80 ± 2.9	81 ± 2.2
8. Rye	82 ± 1.5	79 ± 1.9
9. Wheat	86 ± 2.0	80 ± 2.1
10. Linseed	54 ± 2.5	86 ± 2.9
11. Pea bran	44 ± 4.3	74 ± 2.2
12. Lignin (Westvaco)	54 ± 5.4	96 ± 0.5

[a] *In vitro* binding of tritiated E_2 with the residues of the fermented products.
[b] Binding performed with 16 mg, $n = 4$.

The apparent digestibility of various wheat varieties (SRW, SWW, Durum, HRW, HRS) were found to be 86, 86, 85, 85 and 83%, respectively. Before the *in vivo* fermentation tritiated E_2 was bound to the flours of these varieties for 60–68% without BSA in the incubation medium and for 31–35% in the presence of BSA. Tritiated E_2 was bound with the residues of the fermented products for about 88% without BSA and for about 80% in the presence of BSA, indicating an increased capacity and affinity after fermentation.

Dietary fiber composition of the binders

The highest percentage of dietary fiber was measured in wheat bran (40%) followed by oats (26.6%) and linseed (22.3%). In these products the highest percentage of lignin are found (>3%). In soybean the percentage of lignin is 2.3 while in the other cereals tested a percentage of lignin <1.2 is found (Table 4).

Table 4. Dietary fiber composition (% w/v) of the various binders analyzed with the AOAC method (total fiber) and a modified method of Van Soest [23]

Binder	Total fiber	NDF	ADF	Hemicell.	Cell.	Lignin
1. Wheat bran	40.4	39.5	11.8	27.8	8.3	3.5
2. White wheat flour	3.7	2.2	0.2	2.0	0.2	<1
3. Soybean	16.8	11.9	7.8	4.1	5.6	2.3
4. Buckwheat meal	2.6	2.1	0.6	1.5	0.5	<1
5. Barley	17.2	16.4	5.9	10.6	4.7	1.2
6. Oats	26.6	26.5	12.4	14.1	9.4	3.1
7. Corn	9.3	9.8	2.5	7.3	2.1	<1
8. Rye	14.7	12.5	2.6	9.9	1.6	1.0
9. Wheat	9.7	9.9	2.7	6.2	1.9	<1
10. Linseed	22.3	19.9	13.0	6.9	9.9	3.1
11. Wheat, SRW[a]	9.4	9.2	2.4	6.9	1.8	<1
12. Wheat, SWW	10.8	9.3	2.0	7.3	1.8	<1
13. Wheat, Durum	10.4	9.2	2.4	6.7	2.0	<1
14. Wheat, HRW	11.2	10.0	2.3	7.7	2.0	<1
15. Wheat, HRS	12.0	10.4	2.7	7.7	2.2	<1
16. Wheat, European	10.7	8.8	2.0	6.8	1.7	<1

[a] SRW = Soft Red Winter; SWW = Soft White Winter; HRW = Hard Red Winter; HRS = Hard Red Spring.

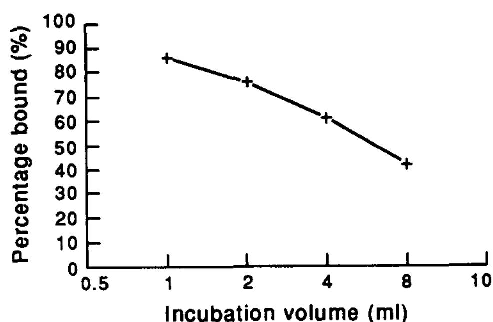

Fig. 1. Relative binding of E_2 with wheat bran as function of incubation volume.

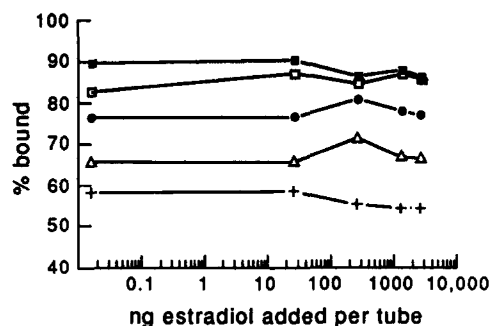

Fig. 2. Relative binding of E_2 with wheat bran estimated at various levels of both unlabeled E_2 and wheat bran: +, 25 mg; △, 50 mg; ●, 100 mg; □, 250 mg; ■, 500 mg bran/tube.

The dietary fiber composition of the various wheat varieties is similar. The amount of fiber and hemicellulose of the HRW and HRS varieties is 0.5–1% higher than in the others.

Binding properties

In Fig. 1 the percentage of binding of tritiated E_2 to 50 mg of wheat bran suspended in various incubation volumes is shown. The percentage of E_2 bound to wheat bran decreases when concentration of the binder is lower, i.e. the incubation volume is increased. The solubility of tritiated E_2 was found to be 100% in the various incubation volumes.

As shown in Fig. 2 a distribution of E_2 between the binder (wheat bran) and the liquid phase is found which seems to be independent of the amount of estradiol present, but dependent on the amount of binder in the medium.

It was found that the pH of the incubation mixture affects binding of both E_1 and E_2 to wheat bran while binding to white wheat flour was not influenced by pH. At higher pH, binding of E_1 and E_2 to wheat bran was significantly lower (Table 5).

DISCUSSION

The breast cancer hypothesis of Gorbach [10] is based on interruption of the EHC of estrogens by a change in intestinal microflora composition resulting in a lower β-glucuronidase activity [24]. The conjugated estrogens excreted by bile in the intestine cannot be reabsorbed since only free estrogens can pass the intestinal mucosa [12]. Other possibilities for interrupting the EHC of estrogens are an accelerated intestinal transit and a bulking effect of dietary fiber, resulting in lower intestinal estrogen concentration making them less available for reabsorption.

A more effective way of interrupting the EHC of estrogens and other compounds such as bile acids [25] seems to be the binding to dietary fiber [17]. As binding capacity depends on fiber composition, i.e. the origin of dietary fiber, various cereals and fiber components were tested in an *in vitro* system.

Calculation of the affinity constant using Scatchard analysis [26] was not possible as, independent of the amount of estrogens, a constant percentage of the estrogens was bound to a particular amount of binder (Fig. 2). As the affinity constants for binding of estrogens to albumin are known [11], the affinity with which the estrogens are bound to the various fiber types was expressed relative to BSA. This protein does not bind to fiber: addition of fiber compounds to a 2% BSA solution did not affect the BSA concentration as measured with the method of Lowry *et al.* [27]. Although we realize that our *in vitro* experimental conditions represent a complex physicochemical system, we assume that measuring the binding relative to BSA gives an impression of the affinity of the individual steroids bound to the various binders.

Table 5. Percentage of binding of tritiated E_1 and E_2 to wheat bran and white wheat flour (n = 2) depending on pH

	pH						
	4.6	5.0	6.0	7.0	8.0	SED	P
E_1 to bran	70.9	73.2	71.6	66.9	61.2	0.953	<0.001
E_1 to flour	55.9	57.7	55.9	57.5	56.1	1.298	0.52
E_2 to bran	84.8	83.7	84.2	81.6	79.8	1.151	<0.05
E_2 to flour	72.6	72.7	73.4	71.4	73.3	1.035	0.407

Contrary to Shultz and Howie [17], we used 50 mg of binder substance in a total volume of 1.0 ml instead of the 5 ml they used. This may explain the lower percentage of binding of the steroids to bran and cellulose in the previous study [17], which is supported by our findings that an increased incubation volume decreases estrogen binding (Fig. 1).

In our *in vitro* binding assay E_2 was the steroid bound with the highest percentage to the various fiber types tested. In contrast, Shultz and Howie [17] found that the percentage of binding of E_2 to oat bran and corn bran was similar as that of E_1; to wheat bran and oat hulls the percentage of binding of E_2 was lower and to cellulose higher than binding of E_1. We cannot explain these different findings.

Cholestyramine bound more of each of the estrogens tested than any of the natural types of fiber. This ion exchange resin is specifically designed to bind bile salts. The lignin used in our experiments exhibited substantial estrogen binding properties, as was found previously [17], making it an excellent candidate for further tests concerning its dietary effects on estrogen balance *in vivo*.

The various wheat varieties are used for different purposes. HRW and HRS cultivars are used for bread and hard rolls. Durum wheat is used for pasta. SRW and SWW are used for flat loaves, cakes, pastry and crackers (information from U.S. Wheat Associates). The varieties used for bread and hard rolls were found to have the highest content of fiber and hemicellulose. In our *in vitro* and *in vivo* tests no differences were found between the various wheat types.

A relatively high binding of an estrogen or a carcinogenic compound to a fiber source is only meaningful if the fiber source is not or only poorly fermented *in vivo*. Fiber sources showing the highest relative binding of estrogens also showed the lowest apparent digestibility in the MNBT tests as well as a high binding affinity relative to BSA. A relatively low apparent digestibility was measured for wheat bran (42%), linseed (54%), oats (59%) and soybean (57%). These products also showed the highest amounts of the unfermentable lignin and of the poorly fermentable hemicellulose (Table 4). The chemically inert lignin binds almost all estrogens with a relatively high affinity (Table 2). The relatively large amount of hemicellulose in oats has a high content of the polysaccharide (1->3) (1->4)-β-D-glucan. Experiments performed previously in our institute have shown that oats diets significantly lowered rat serum cholesterol levels, in contrast to barley, wheat and rice [28]. The hypocholesterolemic effect of oats has been confirmed recently [29]. This effect may be ascribed to β-D-glucan [30].

Concerning the influence of pH on binding properties, our findings suggest that pH affects binding depending on the type of fiber involved. Binding of E_1 and E_2 to wheat bran was lower at higher pH, while binding of these estrogens to white wheat flour was not influenced by pH (Table 5). Opposite effects of pH on the binding of (lipophilic) carcinogenic compounds, such as dimethylbenz(*a*)anthracene (DMBA [31]) and 1,2-dimethylhydrazine (DMH [32]), to various dietary fibers have been reported previously. As fiber consists of a matrix of polysaccharides and lignin and as fiber has cation exchange properties, adsorption may depend upon charges [33]. It might be concluded that pH affects binding depending on both the types of fiber and the compound to be bound.

When particular fiber components or fiber sources are evaluated *in vivo* it has to be considered that not only the binding of estrogens to these fibers can exert an effect on estrogen exposure of estrogen-sensitive tissues, but also other effects might be introduced. When a pure dietary fiber component like lignin is administered to a diet, it might be expected that the EHC of estrogens is interrupted by binding of estrogens to lignin followed by fecal excretion. When a dietary fiber source like wheat bran, oats or linseed is administered to a diet, various effects might be introduced: the dietary fiber will interrupt the EHC by binding the estrogens [17], the intestinal microflora will change [34], which results in changed activities of enzymes in the intestine, such as lower β-glucuronidase, azoreductase and nitroreductase activities [35]. The lower β-glucuronidase activity may diminish estrogen reabsorption. The different fiber components in the products mentioned each have their own characteristics: pectin, cellulose and hemicellulose have a water-absorbing capacity; while pectin and cellulose are fermented (partly) to short-chain fatty acids resulting in lower fecal pH [36]. A lower pH may increase the binding of estrogens to the products remaining after fermentation as shown. A very important effect of administering wheat bran, oats or linseed is the introduction of lignins, such as precursors of enterolacton and enterodiol, and phytoestrogens present in these cereals. These compounds have antiestrogenic

properties and might counteract estrogen exposure of estrogen-sensitive tissues [37].

This *in vitro* test is much cheaper and less time consuming than *in vivo* experiments. However, the *in vitro* binding procedure described has certain drawbacks (only insoluble dietary fibers can be tested; binding of estrogens to other products of the cereals, like proteins and fat, might influence the estrogen binding; the applicability to the *in vivo* situation is hard to interpret). It still can be contended that this *in vitro* binding study, in combination with the *in vivo* apparent digestibility tests, will contribute to an improved selection of specific dietary fibers for *in vivo* evaluation of hormonal balance. In conclusion, wheat bran, soybean, oats and linseed seem to be promising compounds for further *in vivo* experiments in which estrogen exposure should be lowered.

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