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Interactions between dietary fibre-rich preparations and glycoconjugated bile acids *in vitro*

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Abstract

Binding in small intestine and excretion of bile acids constitute a major hypocholesterolemic pathway. Interactions between different types of commercial and laboratory-made dietary fibres and glycoconjugated bile acids were investigated *in vitro* at pH 5.0 and 6.5. The interactions were greater at the lower pH and with dihydroxy-bile acids. Digested cereal products (barley, oat, rye and wheat flour; oat bran), alcohol-insoluble substances from apples, strawberries, rowan berries, carrots, white cabbage, red beets and sugar beet pulp, as well as arabinoxylan, bound 1.21–1.77 μmol bile acids/100 mg of preparation at pH 5.0. Novelose bound approximately 0.65 μmol bile acids/100 mg. Carob fibre had the highest binding capacity (1.83–1.96 μmol bile acids/100 mg) whereas cellulose had no effect. Besides the source and chemical composition, the bile acid binding correlated especially well with the presence of three-dimensional cell wall structures of the tested preparations but less well with the proportions of soluble and insoluble dietary fibre.

Keywords: Dietary fibre; Cereals; Fruits; Vegetables; Bile acid binding; *In vitro*

1. Introduction

Dietary fibres consist of a large group of substances (mainly of plant origin) that are not hydrolysed by enzymes of the human small intestine. The main sources of dietary fibre in human nutrition are cereals, fruits and vegetables. Dietary fibres have several preventive medical and nutritional effects in the intestinal tract, depending on their structure and molecular weight, as well as on their solubility and on their physicochemical properties (water binding, viscosity). They occur in isolated, more or less soluble form (e.g., pectin, β-glucan, carrageenan, guaran) in the diet or as a part of the more or less intact complex cell wall architecture in plant materials. Food processing may also influence the properties of dietary fibres (van der Kamp, Asp, Miller Jones, & Schaafsma, 2004). Therefore, it is often difficult to find the mechanisms behind the physiological dietary fibre effects.

It has been shown, in several studies, that dietary fibre may influence bile acid and cholesterol metabolism. Bile acids are necessary for the digestion of lipids in the small intestine. Normally, they are practically reabsorbed completely in the ileum and then transported to the liver via the enterohepatic circulation by different mechanisms (Hofmann, 1994). Several dietary fibres are able to interact with bile acids in the small intestine, resulting in a lower reabsorption, in an increased transport toward the large intestine and, finally, in a higher excretion of bile acids (Dongowski, Huth, & Gebhardt, 2003; Marlett et al., 1994). Because the bile acid pool is limited, a higher excretion of bile acids requires an increased hepatic synthesis of bile acids from blood cholesterol. This is probably the

Abbreviations: AIS, alcohol-insoluble substance; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; TCA, taurocholic acid.

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major hypocholesterolemic pathway, occurring especially in hypercholesterolemic individuals or animals (Braaten et al., 1994; Garcia-Diez, Garcia-Mediavilla, Bayon, & Gonzalez-Gallego, 1996).

In most studies, only one type of dietary fibre has been investigated *in vitro* in relation to bile acid-binding properties, for example isolated macromolecular dietary fibre components, such as pectin, β-glucan, galactomannans and psyllium (Dongowski, 1995; Kritchevsky, 1996; Lee et al., 2003), preparations with more or less intact botanically-grown cell wall structures (Dongowski & Ehwald, 1999; Kotcharian, Kunzek, & Dongowski, 2004; Pickard, Dongowski, & Kunzek, 2004) or complex dietary fibre-containing food products (Camire & Dougherty, 2003; Camire, Zhao, & Violette, 1993; Goel et al., 1998; Górecka, Korczak, Konieczny, Hęś, & Flaczyk, 2005; Hoagland & Pfeffer, 1987; Huth, Dongowski, Gebhardt, & Flamme, 2000; Kahlon & Woodruff, 2003a, 2003b; Sayar, Jannink, & White, 2005). Furthermore, proteins or protein-rich foods were also able to interact with bile acids (Kahlon & Woodruff, 2002; Yoshie-Stark & Wasche, 2004).

In this study, different types of commercial and laboratory-made dietary fibre preparations, with different structures and from different sources, were allowed to interact with glycoconjugated bile acids *in vitro* under the pH conditions of the small intestine.

2. Materials and methods

2.1. Dietary fibre products

Wheat fibre Vitacel® type WF 101 (Rettenmaier & Söhne, Holzmühle, Germany) was prepared from straw by pre-grinding, mechanical removing of fines and foreign materials, aqueous extraction of carbohydrates, disintegration of cellulose and hemicelluloses (NaOH, >100 °C; overpressure), filtration and washing, followed by drying, grinding and sieving. The microcrystalline cellulose preparation, Vivapur® type 101 (Rettenmaier & Söhne), was prepared from cellulose pulp by acidic hydrolysis, followed by filtration, drying and classifying. The arabinoxylan was prepared, at the Technical University Berlin, from process water of a wheat starch plant in a pilot scale process consisting of enzymatic, fermentative and mechanical treatments, ultrafiltration and spray-drying (Zunft, Lueder, Koebnick, Imhof, & Meuser, 2004). The carob fibre preparation Caromax® (Nutrinova GmbH, Frankfurt/M., Germany) was prepared from carob pulp by water extraction of deseeded locust bean husk. The commercial res7istant starch preparation, Novelose 330®, was obtained from National Starch & Chemical (Bridgewater, HJ, USA).

Commercially-available barley, rye and wheat flours were used. Oat flour and bran were obtained from Peter Kölln Köllnflockenwerke (Elmshorn, Germany).

To prepare the alcohol-insoluble substances (AIS), ripe fruits and vegetables, as well as fresh sugar beet pulp, were cut into small pieces in ethanol (end concentration 65%; w/v), using a mixer, and then boiled under reflux, first for 30 min and then twice for 15 min. The liquid phase was removed after each heating step. The AIS was intensively washed with 65% and 96% (v/v) ethanol, as well as with acetone, and dried in air and in vacuum.

2.2. Analytical methods

Insoluble and soluble dietary fibre were analysed by the enzymatic-gravimetric AOAC method (Prosky et al., 1988). Pectin (galacturonan) was determined by the *m*-hydroxydiphenyl method and the degree of esterification of pectin with methanol was analysed by the chromotropic acid method (Dongowski, 1995). Resistant starch was measured by a modified Englyst method (Englyst, Klingman, & Cummings, 1992). First, the digestible starch was hydrolysed by incubation with pancreatin (Merck, Darmstadt, Germany) and amyloglucosidase (Sigma, St. Louis, MO, USA) in acetate buffer (pH 5.2) for 2 h at 37 °C, simulating starch hydrolysis in the small intestine. After addition of the fourfold amount of 96% EtOH and centrifugation (10 min at 4 °C and 2800*g*), the hydrolysed starch products were extracted twice (with 80% EtOH). The freeze-dried resistant starch-containing residue was dissolved in 1 M NaOH. The diluted solution was hydrolysed with amyloglucosidase at pH 4.6, and the released glucose was determined enzymatically using a hexokinase and glucose-6-phosphate dehydrogenase kit (Boehringer, Mannheim, Germany). β-Glucan was determined as described previously (Drzikova, Dongowski, Gebhardt, & Habel, 2005).

2.3. In vitro digestion of the cereal products

The cereal flours and oat bran were digested enzymatically to remove digestible starch, proteins and lipids, simulating physiological conditions by treatment with a mixture of pancreatin (Merck) and amyloglucosidase (Sigma), at pH 5.2 and 37 °C for 2 h. Then, a fourfold amount of 96% EtOH was added and the mixture was centrifuged (10 min at 4 °C and 2800*g*). The residue was extracted twice (with 80% EtOH), heated under reflux in 96% EtOH for 15 min (enzyme inactivation), centrifuged and then dried.

2.4. Binding of bile acids by the cereal products and dietary fibre preparations

The binding experiments were performed using the conjugated bile acids, glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA) and glycodeoxycholic acid (GDCA), as sodium salts, from Sigma. These bile acids are predominant in human bile.

In most of the experiments, the dietary fibre-containing samples (100 mg dry matter) were suspended in 4 ml of Sörensen buffer (pH 5.0 or 6.5) containing 0.5 mM bile acid and treated for 2 h at 37 °C under shaking. After centrifugation (10 min at 4 °C and 3000*g*), 0.5 ml of the supernatant (containing the un-bound bile acids) was purified by

solid-phase extraction on Bakerbond spe C_{18}-columns in the BAKER spe-12 G system (J.T. Baker, Gross Gerau, Germany).

The proportion of unbound bile acids was estimated on a non-polar stationary phase (Nucleosil 100 Å; C_{18}; 5 μm; 250 × 4.6 mm) at 40 °C by HPLC (Gynkotek, Germering, Germany), after pre-column derivatization with 4-bromomethyl-7-methoxycoumarin, using 18-Crown-6 as a catalyst and fluorescence detection (excitation λ 320 nm; emission λ 385 nm). Linear gradients, consisting of acetonitrile (30–100%), methanol (40–0%) and water (30–0%) were applied (Dongowski et al., 2003).

2.5. Statistical analysis

Results are expressed as mean values ± SD. The statistical significance was determined using one-way analysis of variance (ANOVA). $P < 0.05$ was taken to indicate a statistically significant difference.

3. Results

3.1. Composition of the dietary fibre preparations

The *in vitro* digested cereal preparations had total dietary fibre contents between 32% and 50%. The composition of the dietary fibre components and their solubility may be changed partly during the simulated digestion. The preparations from rye and wheat flour were relatively rich in soluble dietary fibre. Small amounts of resistant starch were present in the flour preparations. The digested samples from oat and barley contained distinctly high amounts of β-glucan (Table 1).

The AIS preparations from fruits, vegetables and sugar beets were rich in dietary fibre. Their total dietary fibre concentration was greater than 70%. The insoluble dietary fibre fraction dominated in all AIS. The preparations contained 13–32% pectin (Table 1). The degrees of methoxylation of the pectin component were: apple AIS, 85.4%; strawberry AIS, 79.6%; rowan berry AIS, 70.5%; carrot AIS, 46.4%; white cabbage AIS, 55.1%; red beet AIS, 63.4%; sugar beet AIS, 44.5%.

The wheat fibre consisted almost completely of insoluble dietary fibre with a cellulose–hemicellulose ratio of approximately 3:1. The microcrystalline cellulose consisted exclusively of insoluble dietary fibre (cellulose). The arabinoxylan preparation consisted of more than 70% of soluble dietary fibre. The content of insoluble dietary fibre was 5% (Table 1) and that of pentosan was approximately 63%. The carob fibre consisted mainly of insoluble dietary fibre. The total dietary fibre concentration was more than 80% (Table 1). Zunft et al. (2001) reported that carob fibres contained 50–65% lignin and polyphenols, 15–25% cellulose, 15–25% hemicelluloses and 0.5–2% pectin. Furthermore, 3–7% tannins were present. Novelose 330 consisted of approximately 40% resistant starch type III (non-granular, retrograded starch) (Jacobasch, Dongowski, Schmiedl, & Müller-Schmehl, 2006) (Table 1).

3.2. Interactions between digested cereal products and bile acids

All tested digested cereal flours were able to interact with the three applied glycoconjugated bile acids to a high degree. The preparations from rye and wheat flour were

Table 1
Composition of the dietary fibre preparations (in %)

	Insoluble dietary fibre	Soluble dietary fibre	Resistant starch	Total dietary fibre	β-Glucan	Pectin
Digested cereal products						
Barley flour	21.9	10.3	1.11	32.2	12.26	0
Oat flour	17.9	15.6	0.33	43.8	16.41	0
Rye flour	21.4	27.2	1.82	50.4	0	0
Wheat flour	16.5	24.1	5.18	45.4	0	0
Oat bran	36.5	4.77	0	40.3	8.92	0
Alcohol-insoluble substances						
Apples	72.1	16.3	0	98.3	0	21.2
Strawberries	36.5	33.7	0	70.2	0	24.2
Rowan berries	60.2	18.4	0	79.0	0	12.7
Carrots	51.3	34.8	0	86.1	0	32.2
White cabbage	73.1	17.6	0	80.5	0	16.6
Red beets	52.4	26.5	0	79.0	0	13.5
Sugar beet pulp	52.1	24.8	0	76.9	0	17.2
Commercial dietary fibre preparations						
Wheat fibre[a]	94.5	2.61	0	96.1	0	0
Novelose 330[a]	0	0	40.4	40.4	0	0
Arabinoxylan[b]	5.12	72.2	0	77.4	0	0
Microcrystalline cellulose[a]	96.5	0	0	96.5	0	0
Carob fibre[a]	74.7	8.60	0	83.3	0	0

Values are means ($n = 3$–6).
[a] Commercial products.
[b] Prepared at the Technical University Berlin.

Table 2
Interactions between glycoconjugated bile acids and digested cereal products

Bile acid	pH	Binding of bile acids (μmol/100 mg cereal product)				
		Barley flour	Oat flour	Rye flour	Wheat flour	Oat bran
GCA	5.0	1.36 ± 0.009a	1.26 ± 0.008a	1.58 ± 0.029a	1.44 ± 0.013a	1.22 ± 0.021a
GCDCA	5.0	1.55 ± 0.012b	1.43 ± 0.014c	1.73 ± 0.019b	1.65 ± 0.010c	1.47 ± 0.035c
GDCA	5.0	1.54 ± 0.007b	1.41 ± 0.013b	1.72 ± 0.015b	1.57 ± 0.016b	1.31 ± 0.016b
GCA	6.5	1.18 ± 0.011a*	0.94 ± 0.008a*	1.36 ± 0.014a*	1.26 ± 0.014a*	0.91 ± 0.018a*
GCDCA	6.5	1.30 ± 0.013c*	1.10 ± 0.020c*	1.48 ± 0.031b*	1.41 ± 0.015c*	1.27 ± 0.034c*
GDCA	6.5	1.28 ± 0.008b*	1.07 ± 0.005b*	1.45 ± 0.042b*	1.34 ± 0.011b*	1.10 ± 0.038b*

GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid. Values are means ± SD ($n = 6$).
Values within a column and within the same pH value in the medium, followed by a different letter (a–c), are significantly different ($P \leqslant 0.05$).
* $P \leqslant 0.001$ (compared to pH 5.0).

Table 3
Interactions between glycoconjugated bile acids and alcohol-insoluble substances (AIS) from fruits or vegetables

Bile acid	pH	Binding of bile acids (μmol/100 mg AIS)						
		Apples	Strawberries	Rowan berries	Carrots	White cabbage	Red beets	Sugar beet pulp
GCA	5.0	1.65 ± 0.013a	1.28 ± 0.009a	1.60 ± 0.026a	1.51 ± 0.011a	1.46 ± 0.004a	1.09 ± 0.008a	1.38 ± 0.007a
GCDCA	5.0	1.80 ± 0.010c	1.42 ± 0.007c	1.77 ± 0.018b	1.62 ± 0.011b	1.58 ± 0.006c	1.23 ± 0.011c	1.50 ± 0.009b
GDCA	5.0	1.74 ± 0.008b	1.40 ± 0.007b	1.76 ± 0.010b	1.64 ± 0.012c	1.56 ± 0.007b	1.18 ± 0.013b	1.45 ± 0.011c
GCA	6.5	1.44 ± 0.012a*	1.04 ± 0.010a*	1.41 ± 0.015a*	1.35 ± 0.012a*	1.27 ± 0.006a*	0.92 ± 0.018a*	1.21 ± 0.014a*
GCDCA	6.5	1.61 ± 0.035c*	1.21 ± 0.008c*	1.56 ± 0.017c*	1.44 ± 0.012c*	1.38 ± 0.005c*	1.04 ± 0.009c*	1.29 ± 0.016c*
GDCA	6.5	1.51 ± 0.023b*	1.19 ± 0.011b*	1.52 ± 0.010b*	1.40 ± 0.009b*	1.35 ± 0.009b*	1.02 ± 0.011b*	1.26 ± 0.013b*

GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid. Values are means ± SD ($n = 6$).
Values within a column and within the same pH value in the medium, followed by a different letter (a–c), are significantly different ($P \leqslant 0.05$).
* $P \leqslant 0.001$ (compared to pH 5.0).

most effective. The lowest binding to bile acids was found with the digested oat flour. The bile acids were bound by oat bran and oat flour to a similar degree (Table 2). The binding of the bile acids was lower at pH 6.5 than at pH 5.0 ($P < 0.001$). GCDCA interacted in each case to the highest degree. The differences in proportion of bound GCDCA and GDCA were relatively low. These bile acids are dihydroxy-bile acids. However, the trihydroxy-bile acid GCA was bound to a significantly smaller extent by the cereal products (Table 2).

3.3. Interactions between alcohol-insoluble substances and bile acids

In contrast to the cereal products, the AIS prepared from fruit and vegetable materials were not digested prior to the binding experiment. The reason was the high concentration of total dietary fibre in these preparations (Table 1) and the absence of starch and digestible proteins.

Within this group of preparations, the AIS from apples interacted most strongly with the three bile acids. The AIS from red beets and sugar beets were less effective (Table 3). In most cases, more GCDCA was bound than was GCDA; however, differences were small between these bile acids. The lowest binding rate was measured for GCA. The binding of the bile acid was significantly reduced at a higher pH of the medium (Table 3).

3.4. Interactions between commercial dietary fibre preparations and bile acids

The degree of interactions with bile acids differed strongly in dependence on the source, pre-treatment and structural composition of the applied commercial dietary fibre preparations. Wheat fibre and microcrystalline cellulose practically did not interact with the bile acids. The resistant starch preparation, Novelose 330, bound between 0.5 and 0.7 μmol bile acid/100 mg under the used conditions. The interactions between the arabinoxylan preparation and bile acids were in same range as those of the beet AIS. The carob fibre preparation was able to bind between 1.83 and 1.98 μmol of the glycoconjugated bile acids per 100 mg at pH 5.0 (Table 4). This was the highest measured binding rate among all tested dietary fibre-rich preparations.

These results were confirmed in an additional experiment with 0.25 mM bile acids and 100 mg carob fibre. The following binding rates were found at pH 5.0 (in μmol/100 mg): GCA, 0.94 ± 0.001; GCDCA, 0.99 ± 0.001; GDCA, 0.98 ± 0.002. At pH 6.5, the binding rates were (in μmol/100 mg): GCA, 0.89 ± 0.004; GCDCA, 0.93 ± 0.003; GDCA, 0.93 ± 0.002.

The binding of GCDCA to carob fibre increased, at a constant fibre content, with increasing bile acid concentration. Furthermore, the amount of bound bile acid

Table 4
Interactions between glycoconjugated bile acids and commercial dietary fibre preparations

Bile acid	pH	Binding of bile acids (μmol/100 mg dietary fibre preparation)				
		Wheat fibre	Novelose 330	Arabinoxylan	Microcrystalline cellulose	Carob fibre
GCA	5.0	0.02 ± 0.002a	0.62 ± 0.011a	1.21 ± 0.035a	0.01 ± 0.004	1.83 ± 0.010a
GCDCA	5.0	0.07 ± 0.011c	0.67 ± 0.017b	1.40 ± 0.026b	0.02 ± 0.008	1.98 ± 0.012c
GDCA	5.0	0.05 ± 0.010b	0.63 ± 0.019ab	1.41 ± 0.031b	0.02 ± 0.007	1.96 ± 0.003b
GCA	6.5	0.00 ± 0.007a*	0.48 ± 0.016a*	0.96 ± 0.046a*	0.00 ± 0.004	1.72 ± 0.008a*
GCDCA	6.5	0.02 ± 0.005a*	0.54 ± 0.011b*	1.14 ± 0.015c*	0.01 ± 0.004	1.80 ± 0.006c*
GDCA	6.5	0.01 ± 0.005b*	0.52 ± 0.017b*	1.08 ± 0.032b*	0.01 ± 0.007	1.78 ± 0.006b*

GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid. Values are means ± SD ($n = 6$).
Values within a column and within the same pH value in the medium, followed by a different letter (a–c), are significantly different ($P \leqslant 0.05$).
* $P \leqslant 0.001$ (compared to pH 5.0).

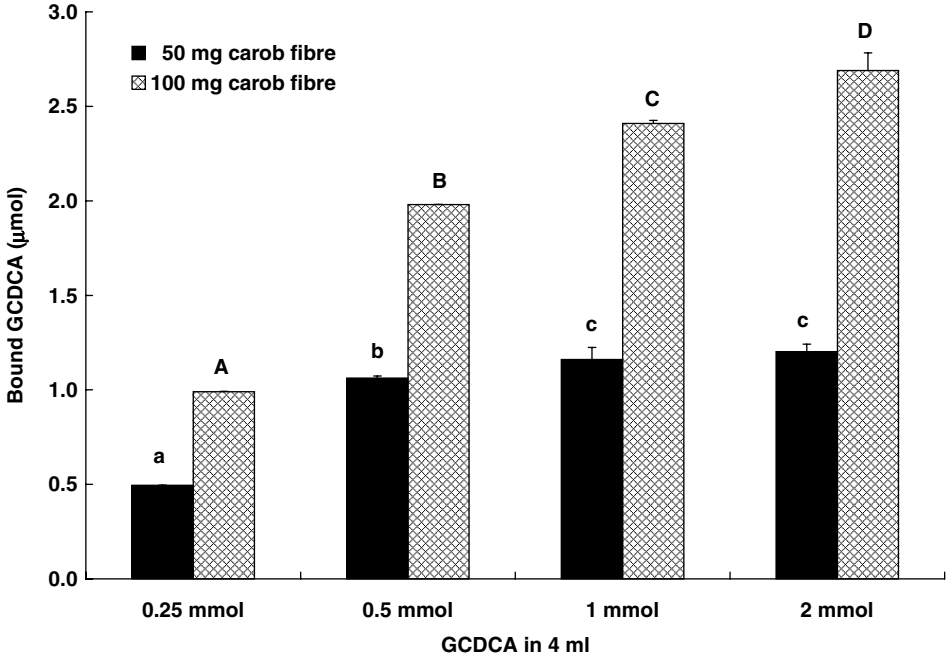

Fig. 1. Effect of concentrations of glycochenodeoxycholic aid (GCDCA) and carob fibre on the binding of the bile acid at pH 5.0. Values within the same carob fibre concentration followed by a different letter (a–d; A–D) are significantly different ($P \leqslant 0.01$).

increased in a non-linear manner with increased carob fibre concentration (Fig. 1).

As in the case of the tested cereal fibre and AIS group, the interactions were greater with the dihydroxy-bile acids and better at the lower pH value (Table 4).

4. Discussion

Brown, Rosner, Willett, and Sacks (1999) found, in a meta-analysis of 67 controlled clinical trials, that various soluble dietary fibres reduced the total and LDL cholesterol concentrations in blood. Soluble fibres are those components that appear in the soluble fraction during dietary fibre determination using the AOAC method (Prosky et al., 1988). Therefore, pectin and β-glucan are soluble dietary fibre components, independent of their real solubilization status in plant materials. Some of the soluble dietary fibres may form viscous systems, even in the intestinal tract (Dongowski et al., 2005). However, Zunft et al. (2001) recently showed that also the insoluble carob fibre reduced both total and LDL cholesterol in serum.

Several mechanisms by which dietary fibre lowers blood cholesterol are discussed in the literature. Because of the partial interruption of the enterohepatic circulation (diminished reabsorption and higher excretion) of bile acids in the presence of dietary fibre-rich diets, more bile acids must be synthesized in the liver from the free cholesterol pool (Braaten et al., 1994; Garcia-Diez et al., 1996). The binding mechanisms between dietary fibres and bile acids are not completely understood. Besides hydrophobic interactions or hydrogen-bonding gel-forming and viscosity effects or inclusion in three-dimensional cell wall matrices are important (Dongowski, 1995; Lairon, 1996; Topping, 1991). Key enzymes of hepatic bile acid or cholesterol synthesis may by indirectly affected by dietary fibre. Thus, the activity of 7α-hydroxylase, the rate limiting enzyme in hepatic bile

acid synthesis, was stimulated by dietary fibre-rich diets (Buhman, Furumoto, Donkin, & Story, 1998). The hypothesis that propionate, a bacterial fermentation product of dietary fibre, inhibits hepatic cholesterol synthesis has not yet been supported by the evidence (Topping, 1995).

Total effects of interaction between dietary fibres and bile acids may be investigated in animal experiments and human studies. To determine the mechanisms behind the effects, however, *in vitro* and cell culture studies are needed. The results of *in vitro* determinations of bile acid binding to dietary fibre, given in the literature, are not comparable in each case, because several modifications are used. Some authors have preferred to digest food samples in the presence of bile acid, simulating the conditions in the stomach and small intestine (Camire & Dougherty, 2003). Other authors have used pre-digested starch- and protein-rich dietary fibre samples (Drzikova et al., 2005). For removal of the un-bound proportion of bile acid, different techniques were applied, e.g., filtration (Górecka et al., 2005), pressure filtration (Dongowski, 1995; Hoagland & Pfeffer, 1987) or centrifugation (Drzikova et al., 2005; Kahlon & Chow, 2000). Most authors have preferred to test only one bile acid per experiment (Goel et al., 1998; Huth et al., 2000); other authors used bile acid mixtures (Kahlon & Chow, 2000). Furthermore, the concentrations of bile acid and dietary fibre differed strongly between the studies. Finally, different methods of bile acid analysis were applied, including HPLC (Drzikova et al., 2005), colorimetry (Górecka et al., 2005), determination of radioactivity (Goel et al., 1998) or enzymatic methods (Camire & Dougherty, 2003).

The concentrations of bound bile acids in the present study are in accordance with the results of most of the other binding studies. Thus, Goel et al. (1998) found a binding rate of 2.3–3.5 μmol taurocholic acid (TCA)/100 mg cereal bran, whereas rhubarb stalk fibre and cellulose bound 6.2 and 1.0 μmol TCA/100 mg. The binding increased linearly with the concentration of rhubarb fibre. Kahlon and Chow (2000) and Kahlon and Woodruff (2003a, 2003b) found, in a series of experiments, that different cereal brans, de-hulled barley, β-glucan-enriched barley and ready-to-eat breakfast cereals bound 0.3–1.8 μmol bile acid/100 mg. Hoagland and Pfeffer (1987) showed that 100 g carrot fibre bound 1–2 g of bile acid. Later, Hoagland (1989) reported that 100 mg of AIS from broccoli, cabbage, carrots and onions were able to interact with 1.6–5.1 μmol chenodeoxycholic acid.

There are also effects of bile acid structure on the extent of the interactions with dietary fibre. Dihydroxy-bile acids (such as GCDCA and GDCA) were more strongly bound to dietary fibre preparations than were trihydroxy-bile acids (such as GCA) (Dongowski & Ehwald, 1999; Drzikova et al., 2005; Huth et al., 2000). This effect was also found in the present study. Górecka et al. (2005) reported that dihydroxy-bile acid deoxycholic acid was bound more strongly by cereal products than were monohydroxy-bile acid lithocholic acid or trihydroxy-bile acid cholic acid.

The interactions between bile acids and dietary fibres were greater at pH 5.0 than at pH 6.5 (Dongowski & Ehwald, 1999; Drzikova et al., 2005; Huth et al., 2000; Pickard et al., 2004). The lower pH value is typical of the conditions in the upper small intestine, where bile acids and dietary fibre can first interact during lipid digestion. The higher pH occurs in the ileum where the digestion and absorption processes are practically completed. Finally, there are also effects of pre-treatment of the dietary fibre preparations on their ability to interact with bile acids. Thus, Huth et al. (2000) found that autoclaved and extruded barley products bound more GCA and GDA than did untreated meal.

The present study showed that the applied carob fibre preparation bound very high amounts of bile acids. Likewise, Würsch (1979) found that carob fibre and carob fibre-containing diets (used in feeding experiments with rats) were able to interact with GCA and TCA *in vitro*. It was interesting that a diet containing 10% carob fibre absorbed as much GCA as did 50 mg carob fibre alone. It has been supposed that also other components of the diet (e.g., starch and casein) may be involved in the stabilisation of the dietary fibre-bile acid-complex. However, starch and proteins are digested in the small intestine. On the other hand, removal of tannins from the carob fibre resulted in a sharp decrease of the bile acid binding. It is important to note that the applied carob fibre preparation contained relatively high amounts of lignin and polyphenols (Zunft et al., 2001). The extractable polyphenols were gallic acid and its derivatives, tannins, flavonol glucosides and isoflavonoids (Owen et al., 2003; Papagiannopoulos, Wollseifen, Mellenthin, Haber, & Galensa, 2004).

The used cereal products, the AIS and the carob fibre contain (at least partly) the more or less intact three-dimensional plant cell wall architecture. This complex over-structure is released during digestion of starch, proteins and lipids in the small intestine. Bile acids may interact with the active surfaces of these systems (Dongowski & Ehwald, 1999). However, some isolated macromolecular dietary fibre components can also interact with bile acids, as was shown for pectin (Dongowski, 1995) or β-glucan (Bowles, Morgan, Furneau, & Coles, 1996). The tested arabinoxylan preparation belongs to the same group. These three dietary fibre preparations may also form viscous solutions in the intestinal tract. On the other hand, resistant starch, which is not digested in the human gastrointestinal tract, and does not form viscous solutions, was able to interact with bile acids only to a relatively low extent. This was shown in feeding experiments with animals where the concentration of excreted bile acids was low if resistant starch was given (Drzikova, Dongowski, & Gebhardt, 2005; Jacobasch et al., 2006). Extremely low bile acid-binding rates were measured in the present study if isolated cellulose preparations (wheat fibre, microcrystalline cellulose) were used. These preparations are completely insoluble. Cellulose seems only to play a certain role in bile acid-binding when it stabilizes the cell wall architecture after the digestion process.

Recently Drzikova et al. (2005) found that the *in vitro* binding of bile acids increased with increasing proportions of oat bran, total and insoluble dietary fibre, as well as β-glucan, in pre-digested oat-based extrudates. The results of the study show, that the source, the chemical composition and the pre-treatment of dietary fibre preparations strongly influence their ability to interact with bile acids. Besides several isolated viscous dietary fibre preparations, a large group of preparations from cereals, fruits and vegetables containing the more or less intact cell wall were able to bind bile acids *in vitro*. However, it was not possible to find a distinct correlation between the contents or proportions of soluble and insoluble dietary fibre components, or the contents of pectin and β-glucan, and the extent of bile acid-binding.

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