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Effects of barley variety, dietary fiber and β-glucan content on bile acid composition in cecum of rats fed low- and high-fat diets☆

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Abstract

Diet-induced obesity and insulin resistance have been linked to changes in bile acid (BA) profiles, which in turn are highly dependent on the dietary composition and activity of the gut microbiota. The objective of the present study was to investigate whether the type and level of fiber had an effect on cecal BA composition when included in low- and high-fat diets. Groups of rats were fed two barley varieties, which resulted in three test diets containing three levels of β-glucans and two levels of dietary fiber. BAs were preconcentrated using hollow fiber liquid-phase microextraction and quantified by gas chromatography. The amount of the secondary BAs, lithocholic-, deoxycholic- and hyodexycholic acids was generally higher in groups fed high-fat diets compared with corresponding acids in groups fed low-fat diets ($P<.05$). In contrast, most of the primary and the secondary BAs, ursodeoxycholic acid and β- and ω-muricholic acids, were two to five times higher ($P<.05$) in groups fed low-fat diets than in groups fed high-fat diets. This was particularly true for groups fed the highest level of β-glucans and in some cases also the medium level. The BA profile in the gut was strongly dependent on the amount and type of dietary fiber in the diet, which may be useful in the prevention/treatment of diseases associated with changes in BA profiles.

Keywords: Bile acids; Barley; β-Glucans; Cellulose; Dietary fiber; Gas chromatography

1. Introduction

Dietary fiber (DF) is considered as nondigestible in the upper part of the gastrointestinal tract and reaches colon were it can be degraded by the microbiota [1,2]. A regular consumption of DF provides protective effects against the development of the metabolic syndrome, which is a cluster of metabolic disorders that increase the incidence of cardiovascular disease and type 2 diabetes [3,4]. The mechanism behind this is not clearly elucidated but is increasingly suggested to be associated with the degradation of DF in the colon, which in turn is related to the solubility of the fiber. The β-glucans, highly soluble DF found in oats and barley, are known to be extensively fermented in the colon producing short-chain fatty acids (SCFAs), where some (especially butyric and propionic acid) are known to be more beneficial to the human body than others [5]. In contrast, cellulose, also a polysaccharide consisting of glucose but more or less insoluble, is rather resistant to colonic fermentation (not more than 20% is fermented) and forms low amounts of SCFAs both in vitro and in vivo in rats and humans [6–8].

One mechanism behind the lowered blood cholesterol levels associated to soluble fiber is their binding capacity to bile acids (BAs), which is claimed to increase with increased viscosity [9–11]. With a high viscosity, there is a decreased absorption of BAs in the intestinal lumen [12], and the BAs are transported to the colon and out of the body [13]. However, soluble fiber, including the viscous ones, is extensively degraded by the colonic microbiota, forming SCFAs, and bound BAs may therefore be liberated. A high production of SCFAs is known to decrease the colonic pH [14], and as a consequence, the activity of the enzyme 7 α-dehydroxylase, which catalyzes the conversion of primary to secondary BAs, is reduced [15].

The primary BAs, cholic acid (CA) and chenodeoxycholic acid (CDCA), formed from cholesterol, are dehydroxylated and transformed by the colonic microbiota to the secondary BAs, deoxycholic acid (DCA) and lithocholic acid (LCA), respectively [16]. CDCA can also be converted by the microbiota to ursodeoxycholic acid (UDCA), which in turn can be transformed to LCA by bacterial dehydroxylation [17]. Hyodeoxycholic acid (HDCA) is then formed from LCA by hydroxylation [18]. Rats form a different BA profile than humans. Thus, α-muricholic acid (α-MCA) is a primary BA only present in rodents as well as the secondary BAs, β-muricholic acid (β-MCA) and ω-muricholic acid (ω-MCA) [19]. β-MCA is the predominant

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secondary BA in rats, and it can be synthesized from α-MCA but also from UDCA [20]. Studies have shown that β-MCA can dissolve gallstones [21] and inhibit cholesterol cholelithiasis in hamsters [22]. Similarly, CDCA and UDCA have been reported to dissolve cholesterol gallstones in humans [23]. UDCA is also used as a therapeutic drug for treating cholestasis liver disease and primary biliary cirrhosis [24,25]. Furthermore, UDCA can decrease the mucosal permeability for toxic and inflammatory compounds [26].

Some secondary BAs are associated with negative health effects. For instance, DCA has been reported to be a promoter of colonic cancer in an animal model experiment [27], while LCA may inhibit the DNA repairing mechanism [28]. Interestingly, the BA profile may be of importance for metabolic features, and a high ratio between 12α-hydroxylated (CA and DCA) and non-12α-hydroxylated BAs has been reported to be related to the characteristics of insulin resistance in humans, healthy as well as people with type 2 diabetes [29]. Notably, patients with type 2 diabetes show a twofold elevation of total plasma BAs compared with healthy subjects [30]. The formation of secondary BAs is affected by the gut microbiota composition and consequently the type of DF consumed. In this context, barley is an interesting cereal, since it contains high amounts of β-glucans that have been shown to decrease cholesterol levels [31], at an intake of at least 3 g per day [32].

The aim of the present work was to evaluate whether different levels of DF and β-glucans could affect the BA profile in the cecum of rats. The two barley varieties (Hadm and SW) were included in low-fat and high-fat diets. The design of the experiment resulted in six test diets, three with low-fat content and three with high-fat content. To keep the DF content at constant levels (50 and 80 g/kg) in the three low-/high-fat diets, the barley varieties were included at two levels (299 or 481 g/kg), which resulted in three levels of β-glucans (13, 20 and 25 g/kg). Furthermore, low- and high-fat controls containing cellulose, an insoluble DF containing no β-glucans and more or less resistant to degradation by the microbiota, were also included as control (80 g/kg). A recently developed method, hollow fiber liquid-phase microextraction (HF-LPME), followed by gas chromatography (GC), was used for quantitative analysis of BAs [19].

2. Materials and methods

2.1. Animals and diets

Male Wistar rats (Taconic, Lille Skensved, Denmark) with an initial weight of 110± 1 g were randomly divided into eight groups of seven. The rats were housed at room temperature of 22°C with a 12-h light and 12-h dark cycle. Three test diets were prepared from two barley varieties (SW and Hadm, Lantmännen SW Seed, Svalöv, Sweden) having different contents of β-glucans [8.2 and 4.3 g/100 dry weight basis (dwb), respectively]. The barley flour was included at two levels in the diet [299 g/kg (50 Hadm and SW) or 481 g/kg (Hadm)], resulting in three levels of β-glucans [low (13 g/kg, dwb), medium (20 g/kg, dwb) and high (25 g/kg dwb)] and two levels of DF (50 g/kg and 80 g/kg, dwb). The barley was milled to a particle size less than 0.5 mm before being included in the diets (CT193 Cyclotec™, FOSS, Denmark). As a control, a cellulose (MCC, FMC BioPolymer, Cork, Ireland) diet (80 g/kg, dwb) was used. The control did not contain any β-glucans. All groups were studied at a low-fat (50 g/kg, dwb) and high-fat content (240 g fat/kg dwb). In the high-fat diets, butter (180 g/kg, dwb) and 10 g cholesterol/kg (dwb) were included. The design of the study resulted in eight groups of rats (four fed low-fat and four fed high-fat diets), of which six were test diets and two control diets. The experiment lasted for 25 days, and the composition of the low- and high-fat diets is presented in Table 1.

Based on previous studies, the daily food intake for each rat was restricted to 12 g (dwb) for the first 11 days, which increased to 15 g (dwb) per day the following week and to 20 g (dwb) per day in the last week [33]. There was free access to water during the whole experiment. Feed residues were collected daily and weighed. The ethics committee for animal studies at Lund University approved the experiment (M56-12).

2.2. Chemicals and reagents

BAs (CA, CDCA, DCA, LCA, UDCA, HDCA) and 5β-cholanic acid (internal standard, IS) were purchased from Sigma-Aldrich Chemicals Co. (Steinheim, Germany), while α-MCA, β-MCA and ω-MCA were obtained from Steraloids, Inc. (Newport, RI, USA). Methanol HPLC grade, hydrochloric acid (HCl) and sodium hydroxide (NaOH) were from Merck (Darmstadt, Germany). Di-n-hexylether (DHE), N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA, derivatization grade), Tri-n-octylphosphine oxide (TOPO), ammonium iodide (NH_4I) and dithioerythritol (DTE) were supplied by Sigma. Ultra-pure reagent water purified by a Milli-Q gradient system (Millipore, Bedford, MA, USA) was used.

Table 1
Composition of the test diets (g/kg, dwb)

Diet ingredient	Control (80 g/kg[b]) 0 g/kg[a]	50 Hadm (50 g/kg[b]) 13 g/kg[a]	Hadm (80 g/kg[b]) 20 g/kg[a]	SW (80 g/kg[b]) 25 g/kg[a]
	Low fat/ high fat	Low fat/ high fat	Low fat/ high fat	Low fat/ high fat
Casein[c]	120	120	120	120
Maize oil[d]	50	50	50	50
DL-Methionine[c]	1.2	1.2	1.2	1.2
Sucrose[e]	100	100	100	100
Mineral mixture[f]	48	48	48	48
Vitamin mixture[g]	8	8	8	8
Choline chloride[c]	2	2	2	2
Butter[h]	0/180	0/180	0/180	0/180
Cholesterol[c]	0/10	0/10	0/10	0/10
MCC	80	0	0	0
Hadm	0	299	481	0
SW	0	0	0	299
Wheat starch[i]	591/401	372/182	190/0	372/182

MCC, microcrystalline cellulose (FMC BioPolymer, Philadelphia, USA).
[a] Beta-glucan content in the diet.
[b] Total dietary fiber content in the diet.
[c] Sigma Aldrich, St. Louis, MO, USA.
[d] ICA AB, Solna, Sweden.
[e] Nordic Sugar, Copenhagen, Denmark.
[f] Containing (g/kg): 0.37 $CuSO_4.5H_2O$, 1.4 $ZnSO_4.7H_2O$, 332.1 KH_2PO_4, 171.8 $NaH_2PO_4.2H_2O$, 324.4 $CaCO_3$, 0.068KI, 57.2 $MgSO_4$, 7.7 $FeSO_4$. $7H_2O$, 3.4 $MnSO_4.H_2O$, 0.02 $CoCL.6H_2O$, 101.7 NaCl, 0.019 chromium(III)chloride and 0.011 sodium selenite.
[g] Containing (g/kg): 0.62 menadione, 2.5 thiamin hydrochloride, 2.5 riboflavin, 1.25 pyridoxine hydrochloride, 6.25 calcium pantothenate, 6.25 nicotinic acid, 0.25 folic acid, 12.5 inositol, 1.25 p-aminobenzoic acid, 0.05 biotin, 0.00375 cyanocobalamin, 0.187 retinol palmitate, 0.00613 calciferol, 25 d-α-tocopheryl acetate, 941.25 maize starch (Lantmännen, Stockholm, Sweden).
[h] Skånemejerier, Malmö, Sweden.
[i] Nordfoods Sweden AB, Malmö, Sweden, varied according to the content of fat, MCC or barley flour in the diets.

2.3. Analytical methods

BAs in the cecum were quantified by HF-LPME followed by GC, showing satisfactory linearity for all BAs [19]. A mixture of BA standard solution was prepared in water at a concentration of 100 μg/ml and used for standard addition in the extraction process [19]. A solution of IS was prepared in methanol at a concentration of 100 μg/ml. The 250 μl of this solution was evaporated in a Mivac concentrator (Kovalent, Sweden), and the evaporated leftover was dissolved in 100 μl of the derivatization mixture [19].

Freeze-dried cecum contents were used for the extraction, and to avoid matrix effect from cecum, the standard addition method was used [19]. In brief, five samples of 20 mg freeze-dried cecum content were incubated in NaOH (5 ml 0.01 M) at 80°C for 1 h. Standard was added and the cecal sample solutions were adjusted to pH 6 with HCl (0.01 M), and the volume was adjusted to 20 ml by Millipore water. Polypropylene hollow fiber membranes (HF) (200 μm wall thickness, 600 μm inner diameter, 0.2 μm pore size, model Q3/2 Accurel PP, Membrana, Wuppertal, Germany) were cut to approximately 8.5-cm-long pieces, and the two ends of each piece were sealed using hot surface (soldering, iron). Prior to extraction, the HFs were filled with the organic solvent [DHE with 10% (w/v) TOPO] by sonication for 1 h.

Each HF was immersed into the cecal sample solution for 2 h to extract the BAs. To allow the maximum contact area, the HF was bent as a U shape inside the analysis tube. After extraction, it was cut one by one at one end to allow a microsyringe to fit in and pull out about 18 μl of acceptor phase. Ten microliters of this solvent was transferred into a GC vial, and 8 μl of a derivatization mixture (MSTFA:NH4I:DTE at a ratio of 500:4:2 v/w/w) and 2 μl of the IS were added and then incubated in a water bath at 60°C for 30 min. The derivatized extract (1 μl) was injected onto a fused-silica capillary column (HP-ULTRA 1, J&W Scientific, Agilent) coated with cross-linked methyl silicone (Ultra-1, 25 m×0.2 mm inner diameter, 0.33 μm film thickness) [19].

2.4. Calculation and statistical analysis

Minitab statistical software (release 16.0) was used for statistical evaluation. All analyses were performed at least in duplicate. All results were preanalyzed with two-way ANOVA to detect the effects of barley, fat and their interactions. Once the