Whole Wheat and Triticale Flours with Differing Viscosities Stimulate Cecal Fermentations and Lower Plasma and Hepatic Lipids in Rats[1]

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ABSTRACT Whole flours from oat, rye or barley effectively modify digestive fermentation and lipid metabolism, whereas the effectiveness of whole wheat flour has not been established. To address this question, cecal digestion, short-chain fatty acid (SCFA) metabolism and cholesterol metabolism were investigated in four groups of rats fed the following semipurified diets differing in their carbohydrate source: a control diet (purified wheat starch) and three whole cereal flour diets [Valoris wheat (Wv), Soissons wheat (Ws), or Carnac triticale (Tc)]. Wv is particularly viscous and rich in arabinoxylans, and Tc is richer in hemicellulose than wheat. Compared with controls, rats fed the whole-flour diets had enlarged ceca and a moderate acidification of the bulk pH (~6.4). In these rats, the cecal SCFA pool size was enhanced ($P < 0.05$), and the SCFA molar ratio reflected propionic/butyric acid–rich fermentations, especially in those fed Tc. The portal SCFA concentrations reflected the rise of the acetic and propionic acid pools in the cecum, whereas portal butyric acid remained relatively low, probably reflecting extensive metabolism by the cecal wall. The fecal excretion of total steroids (bile acids + sterols) was markedly enhanced by all of the whole-flour diets, with Wv (+78%) > Tc (+64%) > Ws (+47%). In parallel, there was a significant plasma cholesterol–lowering effect for rats fed Wv (−27%) and Tc (−32%) and a plasma triglyceride–lowering effect (approximately −40%) in all rats fed whole-flour diets ($P < 0.05$). This effect was observed mainly for triglyceride-rich lipoprotein-cholesterol, whereas HDL cholesterol was unaffected. These results indicate that whole wheat flours can strikingly affect cecal SCFA, especially butyrate, and are effective plasma cholesterol–lowering agents. J. Nutr. 131: 1770–1776, 2001.

KEY WORDS: • *rats* • *dietary fiber* • *wheat flour* • *cholesterol* • *butyric acid*

In most Western countries, cereals (e.g., wheat, oat or rye) represent a major source of dietary fiber because they contain 9–13% total fiber, but the soluble fiber percentage varies from ~20% for wheat to 50% for oats. Cereal and grain consumption, however, has decreased dramatically over the last century (1). In addition, cereals are consumed as more refined products than in the past, which lowers the daily fiber intake. In parallel, cardiovascular diseases, diabetes, obesity, hypertension and cancers have emerged as major health problems. It has been suggested that the decrease in fiber intake contributes to the development of these problems (2–5); thus, diet modifications involving a substantial increase in fiber intake have been recommended. Cereal fibers are chiefly insoluble, which explains why they may help prevent constipation and diverticular disease (6,7). Nevertheless, they have also been considered to be lipid-lowering agents although this effect is generally ascribed to soluble fibers (8,9).

Oat, rye and barley products have generally been found to be effective cholesterol-lowering agents (10–15), whereas the effectiveness of wheat products remains unresolved (16,17). In fact, in the 1970–1980s, a series of investigations, later compiled into a review (18), did not provide clear conclusions about the effects of wheat fiber, although there were some works showing a significant effect of these products on lipids (19–21). Thereafter, studies on wheat fiber have not been frequent; rather, they have focused on purified fractions such as bran or germ (22–26). Thus, little is known about the effectiveness of whole wheat flour on digestive fermentations and lipid metabolism. However, this point is nutritionally relevant because whole cereal flour represents a good way in which to improve the daily supply of fiber, minerals and other micronutrients. Whole flour is relatively rich in fiber (~13%) but it contains other constituents also liable to affect lipid metabolism, i.e., specific proteins such as gluten (27) or phytosterols (28).

To examine the effects of whole wheat flour on digestive fermentation and lipid metabolism, we compared in rats the effects of two varieties of wheat (Soissons, Ws and Valoris, Wv)[3] having different fiber levels with one variety of triticale

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[3] Abbreviations used: SCFA, short-chain fatty acids; TGRLP, triglyceride-rich lipoprotein; Tc, Carnac triticale; Ws, Soissons wheat; Wv, Valoris wheat.

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(Carnac, Tc), which is naturally more rich in hemicellulose than wheat. The two varieties of wheat that were chosen have very different viscosities.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats (IFFA-CREDO, l'Arbresle, France) weighing ~140 g were fed semipurified diets distributed as moistened powder for 21 d. The control diet is described in **Table 1**. The two varieties of wheat (Ws and Wv) and the variety of triticale (Tc) were provided by ITCF (Institut Technique des Céréales et des Fourrages, Paris, France). The flours were obtained by grinding with a 2-mm grating. Specific viscosity was measured by the Biochemistry Laboratory of ITCF (Boigneville, France) according to Saulnier et al. (29). Values were 1.44, 5.15 and 8.07 mL/g, for Ws, Wv and Tc, respectively. Rats were housed two per cage and maintained in temperature-controlled rooms (22°C), with the dark period from 2000 to 0800 h. They were maintained and handled according to the recommendations of the Institutional Ethics Committee (Clermond-Ferrand University). The body weight of rats was recorded twice per week during the experimental period. During the last 7 d of the experimental period, rats were transferred to metabolic cages and food intake and fecal excretion were recorded over the last 4 d of the experiment.

Sampling procedures. Rats were killed at the end of the dark period, when cecal fermentations are still very active (30). They were first anesthetized with sodium pentobarbital (40 mg/kg) and maintained at 37°C. An abdominal incision was made and blood was withdrawn from the portal vein (2 mL) and the abdominal aorta (5 mL) into heparinized tubes. After centrifugation at 10,000 × *g* for 5 min, the plasma was collected and stored at 4°C for lipid and lipoprotein analysis. After blood sampling, the cecum with its contents was removed and weighed, and two samples of cecal contents were transferred to microfuge tubes and immediately frozen at −20°C. A portion of liver was freeze-clamped and stored at −80°C for the measurement of liver lipids.

Analytical procedure. Short-chain-fatty acid (SCFA) concentrations were measured by gas-liquid chromatography after ethanolic extraction of plasma samples as described by Rémésy and Demigné (31) and on supernatants (8000 × *g*, 5 min at 4°C), after acidification by 0.2 vol H_3PO_4 10%. Bile acids and sterols were extracted from cecal contents and feces by 40 volumes ethanolic KOH (4mol/L) and quantified using the reaction catalyzed by 3 α-hydroxysteroid dehydrogenase (EC 1.1.1.50; Sigma, L'Isle D'abeau Chesnes, France) (32). Neutral steroids were extracted three times with 1 mL hexane from a 100-μL aliquot of the alkaline ethanolic extract, after addition of 5 α-cholestane as an internal standard. The solvent was evaporated under N_2 and the residue dissolved in hexane. Extract (200 μL) was injected into the gas chromatograph (Danieducational, Paris, France) fitted with a 12 m × 0.25 mm (i.d.) fused silica capillary column (BP10; SGE, Villeneuve-St-Georges, France) and a flame-ionization detector. Helium was used as the carrier gas, and the sterols were isothermally separated at 260°C. Sterol concentrations were calculated from the peak areas relative to the area of the internal standard. Triglycerides and total cholesterol were determined in plasma by enzymatic procedures using commercial kits (Biotrol, Paris, France and BioMerieux, Charbonnières-les-bains, France, respectively). Liver triglyceride and cholesterol were extracted and analyzed as described by Mazur et al. (33), and a control serum (Biotrol-33 Plus, Biotrol, Paris, France) was treated in parallel to check the accuracy of the analyses.

Plasma lipoproteins were separated on a density gradient by preparative ultracentrifugation as described (34) in a TST 41.14 swinging-bucket rotor (Kontron, Zürich, Switzerland) at 100,000 × *g* for 24 h (15°C). The gradient was then fractionated into 500-μL fractions, and the cholesterol and triglyceride contents of each fraction were determined by the method described for plasma samples. Because of the low level of plasma LDL and the partial overlapping of the HDL1 and HDL2 fractions in rats, only two fractions were considered, i.e., the fraction with $d < 1.040$ kg/L (chiefly triglyceride-rich lipoprotein, TGRLP, together with some LDL) and the fraction with $d > 1.040$ kg/L (HDL).

The total dietary fiber of each variety of cereal (Ws, Wv, Tc) was analyzed by the method approved by the AOAC (35).

Calculation and data analysis. The cecal pool was calculated as cecal concentration (μmol/L) × cecal contents volume (L). Values are given as the means ± SEM and, where appropriate, significance of differences ($P < 0.05$) between mean values was determined by ANOVA coupled with the Student-Newman-Keuls test.

RESULTS

Effects of dietary fibers on food intake and weight gain and digestive fermentations. The total fiber contents were 12.0, 15.2 and 15.4 g/100 g whole flour for Ws, Wv and Tc,

TABLE 1

Composition of the diets[1]

	Control diet	Ws	Wv	Tc
		g/kg		
Digestible wheat starch	752.5	210	210	210
Casein	75	75	75	75
Wheat Gluten	75	—	—	—
Mineral mix[2]	35	10	10	10
Vitamin mix[3]	10	3	3	3
Peanut oil	50	35	35	35
Cholesterol	2.5	2.5	2.5	2.5
Whole flour	—	700	700	700
Energy, *kJ/g*	15.44	13.72	13.72	14.56

[1] There were three experimental diets with two different cereals. Two varieties of wheat were used (Ws, Soissons and Wv, Valoris). The second cereal was triticale, variety, Carnac (Tc). For each diet, 700 g/kg of whole flour was used. All of the diets were equal in carbohydrates (~75%); only the fiber content varied. For the control, Ws, Wv and Tc diets, the starch content was 73.7, 62.7, 62.7, and 65.6%, respectively. The protein content was 13.9, 15, 14.4, and 14%, respectively, and the lipid content was 5, 4.8, 4.8 and 5.2%, respectively; total dietary fiber levels (not reported) were 84, 106 and 108 g/kg, respectively.

[2] All diets contained (per kg diet): 6 g Ca, 0.7 g Mg, 5 mg Cu, 40 mg Fe, 40 mg Zn. Mineral contents of all diets were checked before the beginning of the experiment.

[3] Vitamin supplied (mg/kg control diet): thiamin, 15; riboflavin, 20; pyridoxine, 10; nicotinamide, 100; pantothenate, 70; folic acid, 5; biotin, 0.3; cyanocobalamin, 0.05; retinyl palmitate, 1.5; *dl*-α-tocopheryl acetate, 125; cholecalciferol, 0.15; menadione, 1.5; ascorbic acid, 50; myo-inositol, 100; choline, 1.36 g. Provided by UAR (Villemoisson, Epinay-sur-Orge, France). The vitamin content of the whole flours was taken into account; therefore, the vitamin mix supply was reduced to 3 g/kg in the four cereal diets instead of 10 g/kg in the control diet.

TABLE 2

Effects of wheat (Ws, Wv) and triticale (Tc) whole flours on growth and cecal variables in rats[1,2]

Diet	Food intake[3]	Weight gain[3]	Cecum			Feces
			Total weight[4]	Wall weight[4]	pH[4]	Daily excretion[3]
	g/d		*g*			*g dry matter/d*
Control	19.6 ± 1.0	4.4 ± 0.4	1.86 ± 0.21[b]	0.61 ± 0.03[b]	7.19 ± 0.06[a]	0.8 ± 0.1[b]
Ws	20.2 ± 0.6	4.7 ± 0.2	3.01 ± 0.18[a]	0.82 ± 0.02[a]	6.45 ± 0.08[b]	1.5 ± 0.1[a]
Wv	20.9 ± 0.5	4.7 ± 0.3	3.03 ± 0.15[a]	0.87 ± 0.03[a]	6.48 ± 0.08[b]	1.7 ± 0.1[a]
Tc	21.2 ± 0.9	3.5 ± 0.3	2.99 ± 0.24[a]	0.91 ± 0.04[a]	6.51 ± 0.07[b]	1.7 ± 0.1[a]

[1] Values are means ± SEM; $n = 8$.
[2] Values in a column with superscripts not sharing a letter are different, $P < 0.05$.
[3] Variables measured during the study.
[4] Variables measured postmortem.

respectively, leading to fiber percentages between 8.4 and 10.8% (Table 1).

The presence of 70% whole flour in the diet did not affect daily food intake or weight gain (**Table 2**). Rats fed cereal diets had significantly greater fecal excretions than controls ($P < 0.001$) and enlarged ceca (+58, +68 and + 61% in rats fed the Ws, Wv and Tc diets, respectively, $P < 0.01$), which was due principally to an increase in the weight of the cecal contents. The cecal wall was significantly heavier ($P < 0.001$) in rats fed cereal diets than in controls. The cecal enlargement was accompanied by a significant acidification ($P < 0.001$) of the cecal contents.

In parallel, there were striking alterations in the concentrations and molar ratios of cecal (**Table 3**) and portal vein SCFA (**Table 4**). Compared with the controls, the Ws group had a 41% higher cecal SCFA concentration, which was due to a higher concentration of butyric acid ($P < 0.001$). In the Wv and Tc groups, the cecal total SCFA concentration was particularly high, reflecting high concentrations of propionic ($P < 0.001$) and butyric acids ($P < 0.001$) (Table 3). In rats fed cereal diets, the molar proportion of butyric acid in the cecum was quite high (21–25%) (Table 4).

Effects of cereals on arterial and portal vein SCFA. Only acetic acid was present in measurable amounts in arterial blood. Propionic and butyric acids are quantitatively taken up by the liver and are almost undetectable in systemic blood, but there was significantly more acetic acid in the aorta of rats fed the whole-flour diets than in controls, which reflected the development of cecal fermentations (Table 4). Portal vein concentrations (reflecting digestive absorption) were greater than artery concentrations in all of the groups and they were generally proportional to the cecal pools of acetic and propionic acids. In contrast, the quantities of butyric acid appearing in the portal vein were relatively low and not proportional to the cecal pools, suggesting extensive metabolism by the cecal wall. The molar ratio of acetic/propionic/butyric for the SCFA in the cecum (51:28:21) and the portal vein (72:19:9) of control rats illustrates this discrepancy.

Effects of cereals on fecal steroids. Daily cholesterol intake was not significantly different among the groups (**Table 5**). Rats fed the Wv and Tc diets had significantly greater fecal excretions of bile acids than controls (+100 and +70%, respectively), whereas coprostanol excretion was significantly enhanced in all of the cereal-fed groups to the same extent (~105%). Cholesterol excretion did not differ among groups. Total steroid excretion was significantly higher than controls in rats fed the cereal diets but it did not differ among the three cereal-fed groups. Estimated cholesterol absorption was 6763 μmol/d in controls and it was significantly depressed in the Wv and Tc diet groups (−42 and −37%, respectively). In rats fed the Ws diet, total steroid excretion was significantly different from controls, but due to the slight, nonsignificant difference in cholesterol intake, no significant difference was found for cholesterol absorption. However, the percentage of cholesterol absorbed relative to that consumed was lower in the cereal groups than in controls ($P < 0.05$).

Effects of cereals on lipid metabolism. Plasma cholesterol was lower than controls in rats fed Wv and Tc diets (−27 and

TABLE 3

Effects of wheat (Ws, Wv) and triticale (Tc) whole flours on cecal short-chain fatty acids (SCFA) in rats[1,2]

Diet	Acetic acid	Propionic acid	Butyric acid	Total SCFA	Cecal SCFA pool
	mmol/L				*μmol*
Control	60 ± 7[b]	27 ± 4[b]	8 ± 1[c]	95 ± 11[c]	117 ± 20[b]
Ws	68 ± 5[a,b]	38 ± 4[a,b]	28 ± 3[b]	134 ± 9[b]	281 ± 26[a]
Wv	85 ± 4[a]	51 ± 6[a]	34 ± 3[b]	170 ± 10[a]	359 ± 23[a]
Tc	80 ± 3[a]	42 ± 3[a,b]	41 ± 2[a]	163 ± 6[a]	354 ± 20[a]

[1] Values are means ± SEM; $n = 8$.
[2] Values in a column not sharing a superscript are different, $P < 0.05$.

TABLE 4

Effects of wheat (Ws, Wv) and triticale (Tc) whole flours on portal vein short-chain fatty acids (SCFA) in rats[1,2]

Diet	SCFA in the aorta	SCFA on the portal vein			SCFA molar ratio	
	Acetic (Ac) acid	Acetic acid	Propionic (Pr) acid	Butyric (Bu) acid	Cecum	Portal vein
	mmol/L				*Ac/Pr/Bu*	
Control	0.11 ± 0.01[b]	0.27 ± 0.03[b]	0.08 ± 0.02	0.03 ± 0.001[c]	63/28/9	71/21/8
Ws	0.27 ± 0.02[a]	0.46 ± 0.03[a]	0.12 ± 0.01	0.06 ± 0.002[b,c]	51/28/21	72/19/9
Wv	0.22 ± 0.03[a]	0.57 ± 0.05[a]	0.19 ± 0.06	0.07 ± 0.01[b]	50/30/20	69/23/8
Tc	0.27 ± 0.02[a]	0.52 ± 0.02[a]	0.20 ± 0.03	0.10 ± 0.01[a]	49/26/25	63/25/12

[1] Values are means ± SEM; $n = 8$.
[2] Values in a column with superscripts not sharing a letter are different, $P < 0.05$.

−32%, respectively), and triglycerides were lower in rats fed cereal diets (from −36 to −43%) (**Table 6**). Hepatic cholesterol was significantly lower in rats fed cereal diets compared with the control group, whereas hepatic triglycerides were not affected by the diets.

Plasma lipoproteins were fractionated by gradient ultracentrifugation and a pool was further analyzed for lipid content. The cholesterol concentration was lower in the TGRLP ($d < 1.040$ kg/L) of cereal-fed rats (**Fig. 1**A), whereas it was unaffected in the HDL fraction. The TGRLP fraction in Ws-, Wv- and Tc-fed groups had 28, 42 and 49% lower cholesterol than controls. Triglyceride concentration was also markedly lower in TGRLP (~23%) in rats fed the cereal diets (Fig. 1*B*).

DISCUSSION

In spite of their different specific viscosities, the cereals studied elicited very similar fermentations in the cecum because the total weight was close to 3 g and the pH ~6.5 in all groups. This limited enlargement of the cecum suggests that only a minor fraction of dietary fiber was readily fermentable, presumably the soluble part (chiefly arabinoxylans). It has been reported that viscosity of a wheat extract is due principally to the arabinoxylans, which have an intrinsic viscosity comparable to that of guar gum or low methylated pectin (36). This may be explained by the arabinoxylans' helical structure, which depends on the arabinose/xylose branching (37).

High propionic acid fermentations in the cecum have generally been reported with diets containing soluble substrates (guar gum, resistant starch, β-cyclodextrin) (38–41) and for luminal pH close to 6.0. In contrast, butyric acid–rich fermentations have been observed either with cellulose-rich diets or with some types of starch that escape digestion in the small intestine (42). Under the present conditions, fermentation of relatively limited quantities of soluble fibers in the presence of an excess of insoluble fibers seems favorable to the simultaneous production of large quantities of propionic and butyric acids. It was shown recently that an arabinoxylan-rich fiber extract from wheat flour was readily fermented at acidic pH, with high acetic acid fermentations, in contrast to the corresponding wheat bran, which yielded high propionic/butyric acid fermentations (43). Butyrate may play an important role in vivo in the physiology of the colon and inhibit the growth of neoplastic colonic cells (44,45).

Most of the insoluble moiety of the fiber fraction is composed of cellulose, considered in rats to be an inert diluent of the diet, especially crystalline cellulose. However, it is conceivable that when cellulose fibers are dispersed into the complex matrix of the flour, they are more exposed to microbial degradation. Dry matter fecal excretion (1.75 g/d) was in the same range as total fiber intake (2 g/d), even though bacteria represent a substantial percentage of fecal dry matter (up to 40–50 g/100g). Measurement of the portal SCFA concentrations showed that the portal vein-artery difference was ~0.5 mmol/L in rats fed the whole-flour diets. This supply

TABLE 5

Effects of wheat (Ws, Wv) and triticale (Tc) whole flours on cholesterol fecal excretion and absorption in rats[1,2]

Diet	Cholesterol daily intake	Fecal excretion				Cholesterol absorption	
		Bile acids	Cholesterol	Coprostanol	Total steroids		
	μmol/d						*% of intake*
Control	127 ± 6	23 ± 3[c]	16 ± 1	18 ± 1[b]	55 ± 4[b]	67 ± 9[a]	54 ± 5[a]
Ws	131 ± 4	33 ± 3[b,c]	15 ± 3	37 ± 3[a]	81 ± 6[a]	48 ± 7[a,b]	37 ± 5[b]
Wv	135 ± 3	46 ± 4[a]	17 ± 2	38 ± 2[a]	101 ± 5[a]	39 ± 6[b]	28 ± 4[b]
Tc	137 ± 6	39 ± 4[a,b]	18 ± 2	37 ± 3[a]	94 ± 7[a]	42 ± 3[b]	32 ± 4[b]

[1] Values are means ± SEM; $n = 8$.
[2] Values in a column with superscripts not sharing a letter are different, $P < 0.05$.

TABLE 6

Effects of wheat (Ws, Wv) and triticale (Tc) whole flours on plasma and hepatic lipid concentration in rats[1,2]

	Plasma		Liver	
Diet	Cholesterol	Triacylglycerols	Cholesterol	Triacylglycerols
	mmol/L		*nmol/g*	
Control	2.70 ± 0.30[a]	1.74 ± 0.14[a]	28.5 ± 3.2[a]	18.8 ± 1.2
Ws	2.31 ± 0.10[a,b]	1.11 ± 0.09[b]	13.1 ± 3.2[b]	14.7 ± 1.4
Wv	1.96 ± 0.05[b]	1.01 ± 0.09[b]	11.1 ± 2.9[b]	16.0 ± 1.3
Tc	1.82 ± 0.17[b]	0.99 ± 0.12[b]	9.8 ± 1.7[b]	14.3 ± 1.2

[1] Values are means ± SEM; $n = 8$.
[2] Values in a column with superscripts not sharing a letter are different, $P < 0.05$.

consists mainly in acetic acid and to a lesser extent in propionic acid. From available data (46), we infer that the hepatic uptake of acetic and propionic acids was probably of similar magnitude. It is noteworthy that butyric acid appeared in very small concentration in the portal vein, even in rats fed the cereal diets and exhibiting high butyric acid fermentations. This suggests that most of the butyric acid transferred across the cecal wall was metabolized in situ even when butyric acid is ~40 mmol/L in the cecum.

Inclusion of highly viscous whole flours in the diet (Wv and Tc) induced a reduction in both hepatic and plasma cholesterol levels, compared with the control group fed a fiber-free diet. Ws, which is the less viscous whole flour, was the least effective in modifying cholesterol metabolism because it did not significantly lower plasma cholesterol although it did significantly reduce liver cholesterol. Plasma triglyceride levels were markedly lowered in rats fed the whole-flour diets. Only TGRLP fraction was affected by the whole-flour diets, which is consistent with previous experiments on cholesterol-fed rats fed a fiber diet (47). Several mechanisms that might explain the hypocholesterolemic effect of dietary fiber, whether working alone or in combination, have been proposed (48), i.e., slowing down the rate of gastric emptying, modification of bile acid absorption and metabolism, interference with lipid absorption and metabolism, production of SCFA from fermentation of fiber in the colon, up-regulation of the hepatic LDL receptor (49) and alterations in the plasma concentration or tissue sensitivity to insulin or other hormones.

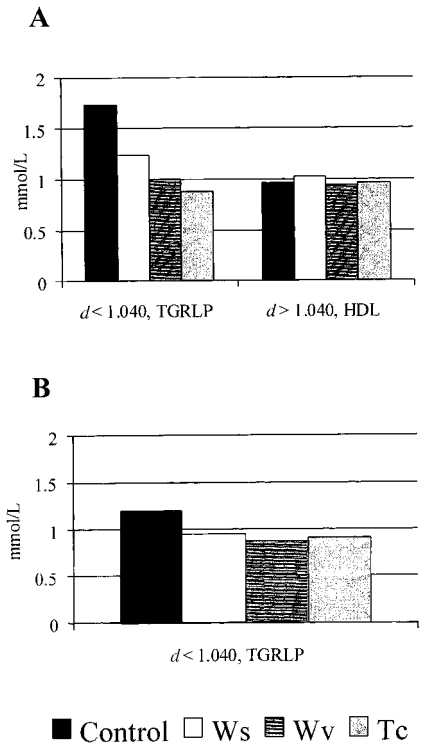

FIGURE 1 Differences in the repartition of cholesterol (*panel A*) and triglycerides (*panel B*) in plasma lipoprotein fractions of rats fed the control, Ws (wheat Soissons), Wv (wheat Valoris) or Tc (triticale Carnac) diet. Each value is a mean of a triplicate analysis of a pool of eight plasma samples. The fractions with $d < 1.040$ kg/L corresponded chiefly to triglyceride-rich lipoproteins (TGRLP) with a lower contribution of LDL. The fractions with $d > 1.040$ kg/L corresponded essentially to HDL.

Chronic consumption of wheat bran has been shown to increase the total lipase activity present in the small intestine of rats (50) and in humans receiving 20 g/d of a corn-wheat-pectin mixture (51). Some binding or entrapment of pancreatic lipase may occur in the presence of fibers, as shown in in vitro conditions mimicking the physiologic conditions (52) with wheat bran and to a lesser extent with pectin. Vahouny (53) also demonstrated that cellulose reduces lymphatic appearance of intestinally infused fatty acid and cholesterol. A shift of the site of intestinal lipid absorption toward a more distal part of the intestine could contribute to this effect (54).

Neutral sterol output was increased, and notably that of coprostanol. In the feces, the ratio of coprostanol to cholesterol increased from ~1:1 in controls to 1:2 in cereal-fed groups. This likely reflects a more active metabolism of sterols by the microflora in moderately acidic pH conditions. The present results indicate that the percentage of cholesterol absorption was also markedly altered by the various whole flours, from 54% (controls) to 28% in rats fed Wv wheat flour. The mechanisms of inhibition of cholesterol absorption, in which viscosity is an important contributor, have been well documented; they include disturbance of micelle formation, slowing of cholesterol transfer to the brush border across the unstirred layer and inhibition of ileal bile acid reabsorption (55–57). In rats fed diets containing fiber, the intestinal bile acid pool is increased (47,58). This could reflect an entrapment of bile acids within the viscous medium (59), as well as an accelerated biliary influx. It is noteworthy that in the present experiment, whole flours generally induced a greater elimination of bile acids. It has been shown with another viscous fiber (guar gum) that the bile acid pools in the small intestine and in the cecum may be enlarged, and there may be

a more effective reabsorption in the portal vein (60). Nevertheless, this reabsorption seems insufficient to prevent the rise in fecal excretion of the bile acids. This contrasts with previous results in which wheat bran did not alter the bile acid enterohepatic cycle or the bile acid pool size (61,62).

Wheat germ accounts for 2–3 g/100 g of the kernel weight and it contains 8–12 g/100 g total dietary fiber. The present diet contained ~2% wheat germ, and it is conceivable that this fraction played a significant role in the hypocholesterolemic and hypotriglyceridemic effects. In previous short-term studies in rats and humans, the ingestion of raw wheat germ lowered plasma triglycerides and cholesterol (63). Studies in hypercholesterolemic and hypertriglyceridemic humans have shown that wheat germ intake decreases VLDL cholesterol and triglycerides dramatically (64). The mechanism at work may be an inhibition of pancreatic lipase by wheat germ proteins, which could interact with the emulsified substrate and hinder the adsorption of the enzyme on the interface (24). The wheat germ also provides phytosterols (~0.05% in the diet vs. 0.25% cholesterol), tocopherols (10 mg/kg diet) and tocotrienols (19 mg/kg diet), which may lower cholesterol (65,66), but it remains to be established whether they play an important role in the cholesterol-lowering effect of the whole flours.

Another mechanism described in the literature is the potential effect of SCFA, notably propionate, on cholesterol metabolism. From early studies of isolated hepatocytes, it was postulated that propionate has an inhibitory effect on cholesterol synthesis (67–69). This hypothesis received some support from observations that propionate (at concentrations <1 mmol/L) can significantly inhibit cholesterol biosynthesis from acetate in hepatocytes (70,71). Nevertheless, cholesterol synthesis has repeatedly been shown to increase and not to decrease in experimental animals fed fermentable soluble fiber or propionate (72,73). Moreover, by-passing the small intestine by administering pectin or propionate into ceca of pigs (74) or perfusing propionate in the colon in humans (75) did not lower serum cholesterol. This does not mean that SCFA reaching the liver do not have any capacity to modulate lipid and carbohydrate synthesis (76). In the present study, an increase in propionate absorption was consistently found when rats were fed cereal diets (~0.20 mmol/L propionate in the portal vein of rats fed Wv and Tc). However, the question of an effect of propionate on the β-hydroxyl-β-methyl glutarate-CoA reductase remains open.

To differing degrees, the cereal flours studied in this work were as effective as lipid-lowering agents. A substantial cholesterol-lowering effect was obtained with a limited supply of soluble fiber, and it would be interesting to examine whether the arabinoxylan fraction is specifically responsible for these effects or whether the association between soluble and insoluble fibers is more important. The present results indicate that the replacement of refined flour by whole flour in various starchy foods could be an effective means to protect against the cardiovascular risks through the hypocholesterolemic effects of fibers and also through the improved daily supply of minerals and micronutrients.

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LITERATURE CITED

1. Burkitt, D. P. & Spiller, G. A. (1986) Dietary fiber: from early hunter-gatherers to the 1980s. In: Handbook of Dietary Fiber in Human Nutrition (Spiller, G. A., ed.), pp. 3–6. CRC Press, Boca Raton, FL.

2. Eastwood, M. A. & Passmore, R. (1983) Dietary fibre. Lancet 2: 202–206.

3. Jenkins, D.J.A., Kendall, C.W.C. & Ransom, T.P.P. (1998) Dietary fiber, the evolution of the human diet and coronary heart disease. Nutr. Res. 18: 633–652.

4. Burkitt, D. P. & Trowell, H. C. (1975) Refined Carbohydrate Foods and Disease: The Implications of Dietary Fiber. Academic Press, London, UK.

5. Anderson, J. W., Hanna, T. J., Peng, X. & Kryscio, R. J. (2000) Whole grain foods and heart disease risk. J. Am. Coll. Nutr. 19: 291S–299S.

6. Smith, A. N., Drummond, E. & Eastwood, M. A. (1981) The effect of coarse and fine Canadian Red Spring Wheat and French Soft Wheat bran on colonic motility in patients with diverticular disease. Am. J. Clin. Nutr. 34: 2460–2463.

7. Trowell, H. & Burkitt, D. (1986) Physiological role of dietary fiber: a ten-year review. ASDC J. Dent. Child. 53: 444–447.

8. Arjmandi, B. H., Ahn, J., Nathani, S. & Reeves, R. D. (1992) Dietary soluble fiber and cholesterol affect serum cholesterol concentration, hepatic portal venous short-chain fatty acid concentrations and fecal sterol excretion in rats. J. Nutr. 122: 246–253.

9. Kris-Etherton, P. M., Krummel, D., Russell, M. E., Dreon, D., Mackey, S., Borchers, J. & Wood, P. D. (1988) The effect of diet on plasma lipids, lipoproteins, and coronary heart disease. J. Am. Diet. Assoc. 88: 1373–1400.

10. Davidson, M. H., Dugan, L. D., Burns, J. H., Bova, J., Story, K. & Drennan, K. B. (1991) The hypocholesterolemic effects of beta-glucan in oatmeal and oat bran. A dose-controlled study [see comments]. J. Am. Med. Assoc. 265: 1833–1839.

11. Ney, D. M., Lasekan, J. B. & Shinnick, F. L. (1988) Soluble oat fiber tends to normalize lipoprotein composition in cholesterol-fed rats. J. Nutr. 118: 1455–1462.

12. Bourdon, I., Yokoyama, W., Davis, P., Hudson, C., Backus, R., Richter, D., Knuckles, B. & Schneeman, B. O. (1999) Postprandial lipid, glucose, insulin, and cholecystokinin responses in men fed barley pasta enriched with beta-glucan. Am. J. Clin. Nutr. 69: 55–63.

13. Anderson, J. W., Gustafson, N. J., Bryant, C. A. & Tietyen-Clark, J. (1987) Dietary fiber and diabetes: a comprehensive review and practical application. J. Am. Diet. Assoc. 87: 1189–1197.

14. Leinonen, K., Liukkonen, K., Poutanen, K., Uusitupa, M. & Mykkanen, H. (1999) Rye bread decreases postprandial insulin response but does not alter glucose response in healthy Finnish subjects. Eur. J. Clin. Nutr. 53: 262–267.

15. Bartram, P., Gerlach, S., Scheppach, W., Keller, F. & Kasper, H. (1992) Effect of a single oat bran cereal breakfast on serum cholesterol, lipoproteins, and apolipoproteins in patients with hyperlipoproteinemia type IIa. J. Parent. Enteral Nutr. 16: 533–537.

16. Choct, M., Illman, R. J., Biebrick, D. A. & Topping, D. L. (1998) White and wholemeal flours from wheats of low and higher apparent metabolizable energy differ in their nutritional effects in rats. J. Nutr. 128: 234–238.

17. Illman, R. J., Storer, G. B. & Topping, D. L. (1993) White wheat flour lowers plasma cholesterol and increases cecal steroids relative to whole wheat flour, wheat bran and wheat pollard in rats. J. Nutr. 123: 1094–1100.

18. Anderson, J. W. (1985) Health implications of wheat fiber. Am. J. Clin. Nutr. 41: 1103–1112.

19. Anderson, J. W. & Chen, W. J. (1979) Plant fiber. Carbohydrate and lipid metabolism. Am. J. Clin. Nutr. 32: 346–363.

20. Liu, K., Stamler, J., Trevisan, M. & Moss, D. (1982) Dietary lipids, sugar, fiber and mortality from coronary heart disease. Bivariate analysis of international data. Arteriosclerosis 2: 221–227.

21. Sacks, F. M., Castelli, W. P., Donner, A. & Kass, E. H. (1975) Plasma lipids and lipoproteins in vegetarians and controls. N. Engl. J. Med. 292: 1148–1151.

22. McIntosh, G. H., Whyte, J., McArthur, R. & Nestel, P. J. (1991) Barley and wheat foods: influence on plasma cholesterol concentrations in hypercholesterolemic men. Am. J. Clin. Nutr. 53: 1205–1209.

23. Anderson, J. W., Gilinsky, N. H., Deakins, D. A., Smith, S. F., O'Neal, D. S., Dillon, D. W. & Oeltgen, P. R. (1991) Lipid responses of hypercholesterolemic men to oat-bran and wheat-bran intake. Am. J. Clin. Nutr. 54: 678–683.

24. Borel, P., Lairon, D., Termine, E., Grataroli, R. & Lafont, H. (1989) Isolation and properties of lipolysis inhibitory proteins from wheat germ and wheat bran. Plant Foods Hum. Nutr. 39: 339–348.

25. Lairon, D., Lacombe, C., Borel, P., Corraze, G., Nibbelink, M., Chautan, M., Chanussot, F. & Lafont, H. (1987) Beneficial effect of wheat germ on circulating lipoproteins and tissue lipids in rats fed a high fat, cholesterol-containing diet. J. Nutr. 117: 838–845.

26. Borel, P., Lairon, D., Senft, M., Chautan, M. & Lafont, H. (1989) Wheat bran and wheat germ: effect on digestion and intestinal absorption of dietary lipids in the rat. Am. J. Clin. Nutr. 49: 1192–1202.

27. Jenkins, D. J., Kendall, C. W., Vuksan, V., Augustin, L. S., Mehling, C., Parker, T., Vidgen, E., Lee, B., Faulkner, D., Seyler, H., Josse, R., Leiter, L. A., Connelly, P. W. & Fulgoni, V., 3rd (1999) Effect of wheat bran on serum lipids: influence of particle size and wheat protein. J. Am. Coll. Nutr. 18: 159–165.

28. Jones, P. J., MacDougall, D. E., Ntanios, F. & Vanstone, C. A. (1997)

Dietary phytosterols as cholesterol-lowering agents in humans. Can. J. Physiol. Pharmacol. 75: 217–227.

29. Saulnier, L., Peneau, N. & Thibault, J. F. (1995) Variability in grain extract viscosity and water-soluble arabinoxylan content in wheat. J. Cereal Sci. 22: 259–264.

30. Illman, R. J., Trimble, R. P., Snoswell, A. M. & Topping, D. L. (1982) Daily variations in the concentrations of volatile fatty acids in the blood vessels of rats fed diets high in pectin and bran. Nutr. Rep. Int. 26: 439–446.

31. Rémésy, C. & Demigné, C. (1974) Determination of volatile fatty acids in plasma after ethanolic extraction. Biochem. J. 141: 85–91.

32. Turley, S. D. & Dietschy, J. M. (1978) Re-evaluation of the 3 alpha-hydroxysteroid dehydrogenase assay for total bile acids in bile. J. Lipid Res. 19: 924–928.

33. Mazur, A., Remesy, C., Gueux, E., Levrat, M. A. & Demigne, C. (1990) Effects of diets rich in fermentable carbohydrates on plasma lipoprotein levels and on lipoprotein catabolism in rats. J. Nutr. 120: 1037–1045.

34. Serougne, C., Ferezou, J. & Rukaj, A. (1987) A new relationship between cholesterolemia and cholesterol synthesis determined in rats fed an excess of cystine. Biochim. Biophys. Acta 921: 522–530.

35. Prosky, L., Asp, N.-G., Furda, I., Devries, J. W., Schweizer, T. F. & Harland, B. F. (1984) Determination of total dietary fiber in foods, food products, and total diets: interlaboratory study. J. Assoc. Off. Anal. Chem. 67: 1044–1052.

36. Udy, D. C. (1956) The intrinsic viscosity of the water soluble components of wheat flour. Cereal Chem. 33: 67–74.

37. Izydorczyk, M. S., Biliaderis, C. G. & Bushuk, W. (1991) Comparison of the structure and the composition of water soluble pentosans from wheat. Cereal Chem. 68: 139–144.

38. Younes, H., Levrat, M. A., Demigné, C. & Rémésy, C. (1995) Resistant starch is more effective than cholestyramine as a lipid-lowering agent in the rat. Lipids 30: 847–853.

39. Levrat, M. A., Moundras, C., Younes, H., Morand, C., Demigné, C. & Rémésy, C. (1996) Effectiveness of resistant starch, compared to guar gum, in depressing plasma cholesterol and enhancing fecal steroid excretion. Lipids 31: 1069–1075.

40. Favier, M. L., Rémésy, C., Moundras, C. & Demigné, C. (1995) Effect of cyclodextrin on plasma lipids and cholesterol metabolism in the rat. Metabolism 44: 200–206.

41. Favier, M. L., Bost, P. E., Guittard, C., Demigné, C. & Rémésy, C. (1997) Reciprocal influence of fermentations and bile acid excretion on cholesterol-lowering effect of fermentable carbohydrate. Nutr. Biochem. 8: 127–132.

42. Bernalier, A., Dore, J. & Durand, M. (1999) Biochemistry of fermentations In: Colonic Microbiota, Nutrition and Health (Gibson, G. R. & Roberfroid, M. B., eds.), pp. 37–53. Kluwer Academic Publishers, Dordrecht, The Netherlands.

43. Lu, Z. X., Gibson, P. R., Muir, J. G., Fielding, M. & O'Dea, K. (2000) Arabinoxylan fiber from a by-product of wheat flour processing behaves physiologically like a soluble, fermentable fiber in the large bowel of rats. J. Nutr. 130: 1984–1990.

44. Velasquez, O. C., Lederer, H. M. & Rombeau, J. L. (1996) Butyrate and the colonocytes. Implications for neoplasia. Dig. Dis. Sci. 41: 727–739.

45. Scheppach, W., Bartram, H. P. & Richter, F. (1995) Role of short-chain fatty acids in the prevention of colorectal cancer. Eur. J. Cancer 31A:1077–1080.

46. Demigné, C., Rémésy, C. & Morand, C. (1999) Short chain fatty acids. In: Colonic Microbiota, Nutrition and Health (Gibson, G. R. & Roberfroid, M. B., eds.) pp. 55–69. Kluwer Academic Publishers, Dordrecht, The Netherlands.

47. Moundras, C., Behr, S. R., Rémésy, C. & Demigné, C. (1997) Fecal losses of sterols and bile acids induced by feeding rats guar gum are due to greater pool size and liver bile acid secretion. J. Nutr. 127: 1068–1076.

48. Anderson, J. W., Deakins, D. A., Floore, T. L., Smith, B. M. & Whitis, S. E. (1990) Dietary fiber and coronary heart disease. Crit. Rev. Food Sci. Nutr. 29: 95–147.

49. Jackson, K. A., Suter, D. A. & Topping, D. L. (1994) Oat bran, barley and malted barley lower plasma cholesterol relative to wheat bran but differ in their effects on liver cholesterol in rats fed diets with and without cholesterol. J. Nutr. 124: 1678–1684.

50. Schneeman, B. O. & Gallaher, D. (1993) Effects of dietary fiber on digestive enzymes. In: Handbook of Dietary Fiber in Human Nutrition (Spiller, G., ed.), pp. 377–386. Eagan Press, Saint Paul, MN.

51. Dukehart, M. R., Dutta, S. K. & Vaeth, J. (1989) Dietary fiber supplementation: effect on exocrine pancreatic secretion in man. Am. J. Clin. Nutr. 50: 1023–1028.

52. Lairon, D., Lafont, H., Vigne, J. L., Nalbone, G., Leonardi, J. & Hauton, J. C. (1985) Effects of dietary fibers and cholestyramine on the activity of pancreatic lipase in vitro. Am. J. Clin. Nutr. 42: 629–638.

53. Vahouny, G. V. (1982) Dietary fibers and intestinal absorption of lipids. In: Dietary Fibers in Health and Disease (Vahouny, G. V. & Kritchevsky, D., eds.), pp. 203–227. Plenum Press, New York, NY.

54. Gallaher, D. & Schneeman, B. O. (1985) Effect of dietary cellulose on site of lipid absorption. Am. J. Physiol. 249: G184–G191.

55. Gee, J. M., Blackburn, N. A. & Johnson, I. T. (1983) The influence of guar gum on intestinal cholesterol transport in the rat. Br. J. Nutr. 50: 215–224.

56. Poksay, K. S. & Schneeman, B. O. (1983) Pancreatic and intestinal response to dietary guar gum in rats. J. Nutr. 113: 1544–1549.

57. Stedronsky, E. R. (1994) Interaction of bile acids and cholesterol with non-systemic agents having hypocholesterolemic properties. Biochim. Biophys. Acta 1210: 255–287.

58. Ebihara, K. & Schneeman, B. O. (1989) Interaction of bile acids, phospholipids, cholesterol and triglyceride with dietary fibers in the small intestine of rats. J. Nutr. 119: 1100–1106.

59. Turley, S. D., Daggy, B. P. & Dietschy, J. M. (1991) Cholesterol-lowering action of psyllium mucilloid in the hamster: sites and possible mechanisms of action. Metabolism 40: 1063–1073.

60. Moriceau, S., Besson, C., Levrat, M. A., Moundras, C., Rémésy, C., Morand, C. & Demigné, C. (2000) Cholesterol-lowering effects of guar gum: changes in bile acid pools and intestinal reabsorption. Lipids 35: 437–444.

61. Payne, D., Juste, C., Corring, T. & Fevrier, C. (1989) Effects of wheat bran on bile secretion in the pig. Nutr. Rep. Int. 40: 761–771.

62. Ide, T., Horii, M., Yamamoto, T. & Kawashima, K. (1990) Contrasting effects of water-soluble and water-insoluble dietary fibers on bile acid conjugation and taurine metabolism in the rat. Lipids 25: 335–340.

63. Cara, L., Armand, M., Borel, P., Senft, M., Portugal, H., Pauli, A. M., Lafont, H. & Lairon, D. (1992) Long-term wheat germ intake beneficially affects plasma lipids and lipoproteins in hypercholesterolemic human subjects. J. Nutr. 122: 317–326.

64. Cara, L., Borel, P., Armand, M., Senft, M., Lafont, H., Portugal, H., Pauli, A. M., Boulze, D., Lacombe, C. & Lairon, D. (1991) Plasma lipid lowering effects of wheat germ in hypercholesterolemic subjects. Plant Foods Hum. Nutr. 41: 135–150.

65. Watkins, T., Lenz, P., Gapor, A., Struck, M., Tomeo, A. & Bierenbaum, M. (1993) γ-Tocotrienol as a hypocholesterolemic and antioxidant agent in rats fed atherogenic diets. Lipids 28: 1113–1118.

66. Ling, W. H. & Jones, P. J. (1995) Dietary phytosterols: a review of metabolism, benefits and side effects. Life Sci. 57: 195–206.

67. Nishina, P. M. & Freedland, R. A. (1990) Effects of propionate on lipid biosynthesis in isolated rat hepatocytes. J. Nutr. 120: 668–673.

68. Anderson, J. W. & Bridges, S. R. (1984) Short-chain fatty acid fermentation products of plant fiber affect glucose metabolism of isolated rat hepatocytes. Proc. Soc. Exp. Biol. Med. 177: 372–376.

69. Chen, W. J., Anderson, J. W. & Jennings, D. (1984) Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats. Proc. Soc. Exp. Biol. Med. 175: 215–218.

70. Demigné, C., Morand, C., Levrat, M. A., Besson, C., Moundras, C. & Rémésy, C. (1995) Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. Br. J. Nutr. 74: 209–219.

71. Wright, R. S., Anderson, J. W. & Bridges, S. R. (1990) Propionate inhibits hepatocyte lipid synthesis. Proc. Soc. Exp. Biol. Med. 195: 26–29.

72. Topping, D. L., Illman, R. J., Taylor, M. N. & McIntosh, G. H. (1985) Effects of wheat bran and porridge oats on hepatic portal venous volatile fatty acids in the pig. Ann. Nutr. Metab. 29: 325–331.

73. Levrat, M. A., Favier, M. L., Moundras, C., Rémésy, C., Demigné, C. & Morand, C. (1994) Role of dietary propionic acid and bile acid excretion in the hypocholesterolemic effects of oligosaccharides in rats. J. Nutr. 124: 531–538.

74. Beaulieu, K. E. & McBurney, M. I. (1992) Changes in pig serum lipids, nutrient digestibility and sterol excretion during cecal infusion of propionate. J. Nutr. 122: 241–245.

75. Wolever, T. M., Spadafora, P. & Eshuis, H. (1991) Interaction between colonic acetate and propionate in humans. Am. J. Clin. Nutr. 53: 681–687.

76. Rémésy, C., Demigné, C. & Morand, C. (1992) Metabolism and utilisation of short chain fatty acids produced by colonic fermentation. In: Dietary Fiber-Component of Food (Schweitzer, T. F. & Edwards, C.A., eds.), pp. 137–150. Springer, London, UK.