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Impact of Whole Wheat Flour and Its Milling Fractions on the Cecal Fermentations and the Plasma and Liver Lipids in Rats

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The aim of the present work was to evaluate the cholesterol-lowering potency of the different milling fractions of whole wheat flour, by investigating the effects of these wheat fractions (white flour, whole flour, and bran) on digestive fermentations and lipid metabolism in Wistar rats. Compared to the control, which was fiber-free, the different cereal fractions did not affect the daily food intake or weight gain. The white flour and whole flour diets markedly enlarged the cecum and elicited acidic fermentations (pH $\approx$ 6.2), whereas bran was less effective. It appears that white flour rather promoted propionate-rich fermentations (+62%), whereas bran favored butyrate-rich fermentations (+178%). White flour or bran did not significantly affect total steroid excretion, but whole flour was effective (+41%). Both white flour and whole flour decreased cholesterol in the $d < 1.040$ fraction, but only whole flour significantly lowered cholesterolemia. However, all the cereal diets significantly decreased liver lipids, whole flour being the most potent (−54%). In conclusion, the totality of the wheat grain is important for cholesterol- and triglyceride-lowering effects, and the splitting up of the grain alters its health effects.

KEYWORDS: Wheat; bran; white flour; whole flour; rat; cholesterol; digestive fermentations

INTRODUCTION

Because many studies have shown the limited metabolic effects of wheat bran (*1*, *2*), it is important to be able to distinguish the effects of wheat bran extracted of its matrix or in combination with endosperm (white flour). The most important effects of cereal products concern the carbohydrate (*3*) or lipid (*4*, *5*) homeostasis and colon physiology (*6*). However, the quantity and quality of dietary nonstarch polysaccharides (NSP) in cereal products have a substantial influence on the nutritional properties of those products. It has been recently shown that whole wheat flour has hypocholesterolemic effects, chiefly dependent on the soluble fiber content, in rats (*7*). White flour has also been reported to lower plasma cholesterol in humans (*8*) and rats (*9*). In contrast, wheat products that are high in insoluble NSP appear to have little or no cholesterol-lowering activity (*8*, *9*). In studies comparing the specific effects of various cereal fractions, wheat bran has frequently been considered as an inert control, which is questionable because this fraction is not devoid of effects, and this probably explains why conflicting data continue to appear. Wheat bran has been found to be able to affect lipid metabolism (*10*) and to provide the most favorable ratio of butyrate to propionate and acetate (*11*), which is interesting because recent research has focused on the ability of butyrate to protect against the development of colon cancer by inhibiting cancer cell growth (*12*, *13*). Studies in which hypocholesterolemic effects were detected suggest that the variety of wheat and the coarseness of the bran might modulate this effect (*14*, *15*). To further assess the effects of whole wheat flour and of its constitutive fractions on lipid levels and cecal short-chain fatty acids (SCFA), groups of male Wistar rats were adapted to semipurified diets containing whole wheat flour, wheat bran, or white flour.

MATERIALS AND METHODS

Whole Wheat Flour and Milling Fractions. Because of its high content in soluble fiber (2%) compared to most other wheat varieties, the variety Valoris was chosen. Valoris was provided by ITCF (Institut Technique des Céréales et des Fourrages, Paris, France). The milling fractions were obtained after processing of 8 kg of wheat whole grain in a Bühler mill: 30.5% bran + remilling, 69.5% white flour (0.55 g/100 g of ash). The first experimental diet (whole flour) was composed of 70% whole wheat flour, which was reconstituted with 48.6% white flour and 21.4% bran + remilling. The second experimental diet (white flour) was constituted of 48.6% white flour. The third experimental diet (bran) was made up of 21.4% bran + remilling.

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Table 1. Composition of the Diets[a]

	control (g/kg)	whole flour (g/kg)	white flour (g/kg)	bran (g/kg)
digestible wheat starch	760	160	336	597
casein	75	75	75	75
wheat gluten	75		27	43
mineral mix[b]	26.4	17.6	25.4	20.5
vitamin mix	10	8	10	8
peanut oil	50	35	45	40
cholesterol	2.5	2.5	2.5	2.5
whole cereal		700		
white flour			486	
bran				214
insoluble fiber		68.5	11.7	56.7
soluble fiber		12	9.5	2.5
energy (kJ/g)	16.9	16.1	16.4	16.5

[a] There were three experimental diets consisting of whole wheat flour and different milling fractions of wheat (white flour or bran). The whole flour diet brought the same quantity of bran as the bran diet and the same quantity of white flour as the white flour diet. All the control and experimental diets were balanced and equal in proteins (15%), lipids (5%), and carbohydrates (around 75%); only the fiber content varied. [b] All diets contained (per kg diet) 4 g of Ca, 1.2 g of Mg, 5 mg of Cu, 36 mg of Fe, and 38 mg of Zn. The mineral content of all diets was checked before the beginning of the experiment.

Animals and Diets. Male Wistar rats weighing approximately 140 g were used. They were from the colony of laboratory animals of the National Institute of Agronomic Research (INRA; Clermont-Ferrand/Theix, France). They were fed one of the experimental semipurified diets distributed as moistened powder for 21 days. All the diets were balanced in nutriments, vitamins (provided by UAR, Villemoisson, Epinay-sur-Orge, France), and minerals taking into account the endogenous supply of cereal fractions (**Table 1**). The animals were allowed free access to fresh food and tap water. Rats were housed two per cage and maintained in temperature-controlled rooms (22 °C), with the dark period from 20.00 to 08.00 h. They were maintained and handled according to the recommendations of the Institutional Ethics Committee (Clermont-Ferrand University). The body weight of rats was recorded twice per week during the experimental period. During the last 7 days of the experiment period, rats were transferred to metabolic cages and food intake and fecal excretion were recorded over the last 4 days of the experiment.

Sampling Procedures. Rats were killed at the end of the dark period, when cecal fermentations are still very active. 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), respectively, 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 content 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 freezed−clamped and stored at −80 °C for the measurement of liver lipids.

Analytical Procedure. SCFA concentrations were measured by gas−liquid chromatography on the supernatants (8.000*g*, 5 min at 4 °C) of cecal contents as described by Rémésy and Demigné (*16*). Bile acids and sterols were extracted from feces by 40 volumes ethanolic KOH (4 mol/L), and bile acids were quantified using the reaction catalyzed by the 3-α-hydroxysteroid dehydrogenase (E.C. 1.1.1.50; Sigma, L'Isle D'abeau Chesnes, France) (*17*). Neutral sterols were extracted three times with 1 mL of 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 was dissolved in hexane. Extract (200 μL) was injected into a gas chromatograph (Danieducational, Paris, France) fitted with a 12 m × 0.25 mm (inner diameter) 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. (*18*), and a control serum (Biotrol-33 Plus, Biotrol) was treated in parallel to check for the accuracy of the analyses.

Plasma lipoproteins were separated on a density gradient by preparative ultracentrifugation as described in the literature (*19*), 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 in 500 μL fractions, and the cholesterol and triglyceride contents of each fraction were determined by the method described for plasma samples. Because of a low level of plasma low-density lipoprotein (LDL) and the partial overlapping of HDL1 and HDL2 (HDL, high-density lipoprotein) fractions in rats, only two fractions were considered: the $d < 1.040$ kg/L fraction (chiefly triglyceride-rich lipoprotein, TGRLP, together with some LDL) and the $d > 1.040$ kg/L fraction (HDL).

The soluble, insoluble, and total dietary fibers of each milling fraction and of whole wheat flour (whole flour, bran, white flour) were analyzed by the method approved by the Association of Official Analytical Chemists (*20*).

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

RESULTS

Effects of Whole Wheat Flour and Its Milling Fractions on Food Intake, Weight Gain, and Digestive Fermentations. The calorie content of the diets varied from 16.1 to 16.9 kJ/g. The diets were designed to provide similar lipid, protein, and carbohydrate content, but the proportion of available carbohydrates varied according to the fiber content of the diets. The presence of different cereal fibers in the diet did not affect daily food intake or weight gain (**Table 2**). Rats fed white flour, whole flour, and bran diets had significantly higher fecal excretions of dry matter as compared to the control (+83%, +262%, +279%, respectively, $P < 0.001$).

The total weight of the cecum was apparently correlated to the soluble fiber content of the diets ($R^2 = 0.95$). The rats fed whole flour and white flour diets showed a significant increase of the cecum weight (+70% and +59%, respectively, $P < 0.001$) compared to controls. Rats fed the bran diet showed only a slight increase of the cecum weight (+30%, $P < 0.05$). The cecal enlargement was accompanied by a significant acidification ($P < 0.001$ for control vs white flour and whole flour groups, $P < 0.01$ for controls vs bran group) of the cecal content. In parallel, there were striking alterations in the concentrations of cecal SCFA (**Table 3**). There was a direct correlation between the cecal SCFA pool and the soluble fiber content in the diets ($R^2 = 0.95$). However, the molar ratio of cecal SCFA was dependent on the type of fiber in the diet. Compared to controls, white flour fed rats had a higher propionic acid concentration (+62%, $P < 0.001$), whereas butyric acid concentration was unchanged. The bran-fed rats had a higher butyric acid concentration than did the controls (+178%, $P < 0.01$), whereas propionic acid concentration did not vary. Compared to the controls, the whole flour group had a higher cecal SCFA concentration (+125%, $P < 0.001$), which was due to a higher concentration of both butyric acid (+255%, $P < 0.001$) and propionic acid (+69%, $P < 0.001$). This was in agreement with the cecal pH, which was particularly acidic in the whole flour fed rats. Basal fermentations exist in rats fed a fiber-free diet.

Table 2. Effects of Whole Wheat Flour and Its Fractions on Growth and Cecal Variables in Rats[a,b]

			cecum			
diet	food intake[c] (g/day)	weight gain[c] (g/day)	total weight[d] (g)	wall weight[d] (g)	pH[d]	feces, daily excretion[c] (g dry matter/day)
control	19.5 ± 1.1	6.1 ± 0.5	2.10 ± 0.09[g]	0.73 ± 0.02[f]	7.07 ± 0.05[e]	0.53 ± 0.04[g]
white flour	19.4 ± 1.0	6.3 ± 0.5	3.35 ± 0.20[e]	0.94 ± 0.04[e]	6.32 ± 0.08[f]	0.97 ± 0.06[f]
whole flour	21.6 ± 0.9	6.4 ± 0.4	3.57 ± 0.26[e]	1.02 ± 0.04[e]	6.20 ± 0.08[f]	1.92 ± 0.06[e]
bran	20.8 ± 0.8	5.8 ± 0.3	2.73 ± 0.11[f]	0.90 ± 0.04[e]	6.72 ± 0.07[g]	2.01 ± 0.11[e]

[a] Values are means ± SEM; $n = 8$. [b] Values in a column with superscripts not sharing a letter are different, $P < 0.05$. [c] Variables measured during study. [d] Variables measured postmortem.

Table 3. Effects of Whole Wheat Flour and Its Fractions on Cecal SCFA[a,b]

diet	dietary fiber intake (g/day)	acetic acid (mmol/L)	propionic acid (mmol/L)	butyric acid (mmol/L)	total (mmol/L)	SCFA molar ratio	cecal SCFA pool (μmol/cecum)	increase of SCFA pool (μmol/cecum)	SCFA/ DF intake (μmol/g)
control		81 ± 5[c]	29 ± 2[d]	9 ± 1[d]	119 ± 7[d]	68/24/8	181 ± 12[e]		
white flour	0.41 ± 0.02	75 ± 4[d]	47 ± 4[c]	10 ± 2[d]	133 ± 8[d]	56/35/7	398 ± 27[c]	217	542
whole flour	1.74 ± 0.07	83 ± 5[c]	49 ± 3[c]	32 ± 5[c]	164 ± 8[c]	50/30/20	408 ± 16[c]	227	130
bran	1.23 ± 0.05	85 ± 4[c]	32 ± 2[d]	25 ± 2[c]	142 ± 6[d]	60/23/17	272 ± 19[d]	91	74

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

Table 4. Effects of Whole Wheat Flour and Its Milling Fractions on Plasma and Hepatic Lipid Concentration in Rats[a,b]

	plasma		liver	
diet	cholesterol (mmol/L)	triacylglycerols (mmol/L)	cholesterol (mg/g)	triacylglycerols (mg/g)
control	2.38 ± 0.22[c]	1.67 ± 0.17[c]	9.4 ± 0.6[c]	26.5 ± 1.3[c]
white flour	2.09 ± 010[c,d]	1.45 ± 0.17[c,d]	6.6 ± 0.4[d]	18.1 ± 1.1[d]
whole flour	1.71 ± 0.13[d]	1.13 ± 0.09[d]	4.3 ± 0.4[e]	14.6 ± 0.9[d]
bran	2.27 ± 0.16[c]	1.30 ± 0.10[c,d]	7.2 ± 0.7[d]	16.0 ± 1.4[d]

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

The SCFA cecal pool increase is a good model to evaluate the fermentability of fiber (*21*). It appears that the fermentability of white flour fibers seems 7 times more important than fermentability of bran fibers.

Effects of Whole Wheat Flour and Its Milling Fractions on Lipid Metabolism. Whole flour fed rats had a lower plasma cholesterol as compared to controls (−28%, $P < 0.05$) and bran-fed rats (−25%, $P < 0.05$). Plasma triglycerides were reduced in whole flour fed rats (−32%, $P < 0.05$) compared to controls (**Table 4**). Hepatic cholesterol and triglycerides exhibited changes parallel to those observed in plasma. Compared to controls, all the cereal-fed rats had a significant decrease in hepatic cholesterol (−30% for white flour, −54% for whole flour, and −23% for bran groups) and triglyceride concentrations (around −40%, $P < 0.001$).

Plasma lipoproteins from a plasma pool were fractionated by gradient ultracentrifugation, and the separated fractions were further analyzed for lipid content. The cholesterol concentration was lower in the TGRLP ($d < 1.040$ kg/L) of cereal-fed rats than in controls (**Figure 1A**), whereas cholesterol concentration in the HDL was unaffected. The cholesterol concentration in the TGRLP fraction in whole flour fed groups was markedly lower than in the control (−48%). Triglyceride concentration in TGRLP was also markedly lower (∼21%) in rats fed the whole cereal or cereal fraction than in controls (**Figure 1B**).

Effects of Whole Wheat Flour and Its Milling Fractions on Fecal Steroids. Cholesterol daily intake was not significantly different among the groups (**Table 5**). Rats fed the whole flour diet had the greatest fecal excretion of total steroids compared to controls (+46%) or bran-fed and white flour fed rats (+47%

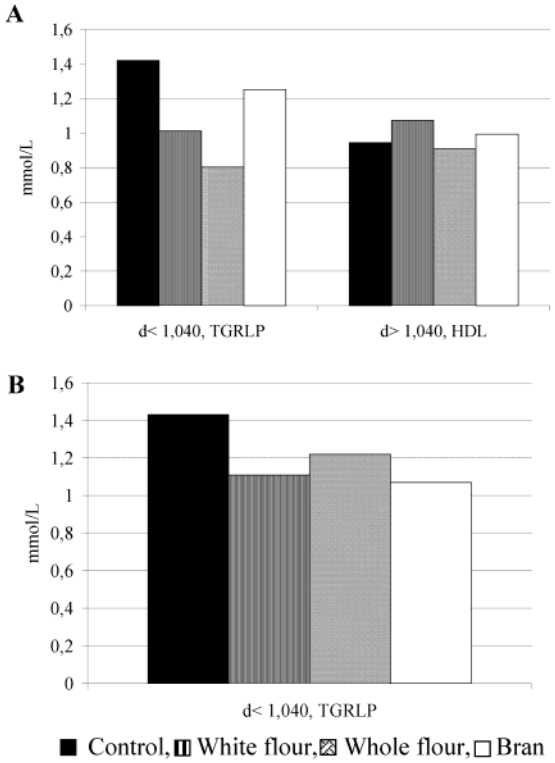

Figure 1. Differences in the repartition of cholesterol (panel A) and triglycerides (panel B) in plasma lipoprotein fractions of rats fed the control, wholeWF (whole wheat flour), whiteWF (white wheat flour), and WB (wheat bran) diets. Each value is a mean of a triplicate analysis of a pool of eight plasma samples. The fractions with $d < 1.040$ kg/L corresponded chiefly to TGRLP with a lower contribution of LDL. The fractions with $d > 1.040$ kg/L corresponded essentially to HDL.

and +28%, respectively). Compared to the white flour fed rats, whole flour fed rats had a higher bile acids fecal excretion (+287%, $P < 0.05$) but a similar neutral sterol excretion. However, the ratio coprostanol/cholesterol was dependent on the diets: compared to white flour fed rats, whole flour fed rats had a lower fecal cholesterol excretion (−52%, $P < 0.001$) but a greater fecal coprostanol excretion (+73%, $P < 0.001$). Whole flour fed rats had a greater neutral sterols fecal excretion (+49%, $P < 0.05$) than bran-fed rats, but their bile acids fecal

Table 5. Effects of Whole Wheat Flour and Its Fractions on Cholesterol Fecal Excretion and Absorption[a,b]

		fecal excretion					cholesterol absorption	
diet	cholesterol daily intake (μmol/day)	bile acids (μmol/day)	cholesterol (μmol/day)	coprostanol (μmol/day)	total neutral sterols (μmol/day)	total steroids (μmol/day)	μmol/ day	% of intake
control	126 ± 7	$10.1 \pm 4.4^{c,d}$	29.9 ± 1.6^{c}	22.3 ± 1.9^{d}	$52.8 \pm 4.9^{c,d}$	62.1 ± 9.1^{d}	$63 \pm 7^{c,d}$	51 ± 1^{c}
white flour	125 ± 6	6.1 ± 3.5^{c}	35.6 ± 3.7^{c}	28.7 ± 5.9^{d}	64.2 ± 5.4^{c}	70.4 ± 6.1^{d}	$55 \pm 7^{c,d}$	44 ± 4^{c}
whole flour	135 ± 4	23.6 ± 6.3^{d}	17.1 ± 1.4^{d}	49.8 ± 4.5^{c}	66.8 ± 6.2^{c}	90.4 ± 3.1^{c}	43 ± 6^{d}	32 ± 4^{d}
bran	135 ± 5	$16.7 \pm 3.4^{c,d}$	16.7 ± 1.1^{d}	28.1 ± 1.9^{d}	44.8 ± 2.1^{d}	61.5 ± 3.9^{d}	74 ± 7^{c}	54 ± 3^{c}

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

excretion was not significantly different. In comparison to bran-fed rats, white flour fed rats also had a higher neutral sterols fecal excretion. The percentage of cholesterol absorbed relative to that consumed was lower in the whole flour group than in controls (32%), followed by the white flour group (44%). Cholesterol absorption in the bran-fed group was not significantly different from that of controls.

DISCUSSION

Considering the effects of the extraction rates on the nutritional quality of flours, it is interesting to know the impact of the whole wheat flour and its constitutive fractions on SCFA production and lipid metabolism. Our results show that arabinoxylans (AX) (88% of NSP, of whom 1/3 are soluble) in primary unlignified cell walls from endosperm were more extensively broken down by the cecal microflora than NSP in the secondary lignified cell walls from pericarp/testa, which is in keeping with the results of Bach Knudsen et al. (*22*). Indeed, the bran diet elicited lesser fermentations in the cecum than the white flour diet, accompanied by a moderate acidification (pH = 6.72) and a limited enlargement of the cecum (about 2.7). This suggests that only a minor fraction of dietary fiber was readily fermentable, presumably the soluble part, brought essentially by the aleurone layer (chiefly arabinoxylan). Bran is composed of 50% pericarp/testa and 50% aleurone layer. The pericarp/testa is constituted of lignified cell walls, where cellulose microfibrils are dispersed in acidic arabinoxylans which are mainly insoluble in water (*23*−*26*). The aleurone cells are mainly composed of AX and mixed linked $\beta(1\rightarrow3;1\rightarrow4)$-D-glucan (*27*), which are more easily broken down than NSP from pericarp cells (*22*). The organization of the polysaccharides and their cross-linkages to other macromolecules makes the polysaccharides of bran less accessible to the enzymatic breakdown than AX of the white flour. These structural features could explain why white flour fed rats had a higher SCFA cecal concentration than bran-fed rats.

Butyric acid rich fermentations are generally observed either with cellulose-rich diets or with some types of starch that escape digestion in the small intestine (*28*). Compared to the white flour diet, the bran diet markedly enhanced the concentration of butyric acid in the cecum ($P < 0.001$), whereas high propionic acid fermentations were observed in rats fed the white flour diet. This last type of fermentation has generally been reported with diets containing soluble substrates (guar gum, resistant starch, β-cyclodextrin) (*29*−*31*). It seems that with whole flour, fermentation of relatively limited quantities of soluble fibers in the presence of an excess of insoluble fibers favors the simultaneous production of large quantities of propionic and butyric acids. This is interesting for wheat whole grain or bran, because butyrate may play an important role in vivo in the physiology of the colon (energetic fuel, control of mucosa integrity) and has been suggested to inhibit the growth of neoplastic colonic cells (*32*).

It has been previously shown that inclusion of highly viscous whole flour in the diet induces a reduction in both hepatic and plasma cholesterol levels, compared with the control group fed a fiber-free diet (*7*). The question as to whether the observed cholesterol-lowering properties of whole wheat flour are due to its bran or its white flour fractions, or both, is still open. In accordance with previous studies in man or animals, a bran diet did not affect plasma cholesterol and triglycerides (*33*, *34*). On the other hand, Illman et al. and Anderson et al. have observed that white flour lowers plasma cholesterol in rats and in humans (*8*, *9*). These data are in discrepancy with the present results, since neither the bran diet nor the white flour diet lowered plasma cholesterol. But the simultaneous presence of bran and white flour fractions (whole wheat flour) was effective to lower plasma cholesterol and triglycerides. White flour and bran diets showed similar effects on liver cholesterol, and as reported above, the mix of both fractions reinforced the cholesterol-lowering properties. Several mechanisms to explain the hypocholesterolemic effects of soluble fibers, whether working alone or in combination, have been proposed (*35*): reduction of the rate of gastric emptying, modification of bile acids 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 (*36*), and alterations in the plasma concentration or tissue sensitivity to insulin or other hormones (*37*, *38*). The hypocholesterolemic effects observed in rats fed whole flour were likely due to white flour which provided around 80% of the soluble fiber in the diet. Indeed, neutral sterols output was significantly enhanced with the white flour diet compared to the bran diet. Here again, the combination of bran and white flour was particularly effective to significantly raise fecal excretion of total steroids and to lower cholesterol absorption (32%, vs 44% for white flour fed rats). Fibers are effective lipid-lowering components, but the synergistic action of bran and white flour could also be a consequence of the presence of phytosterols (≈0.05%), tocopherols (≈10 mg/Kg), and tocotrienols (≈19 mg/Kg) in the germ, which may lower cholesterol (*39*, *40*). It is conceivable that bran and wheat germ (contained in bran) might play a complementary role in the hypocholesterolemic and hypotriglyceridemic effects. In previous short-term studies in rats and humans, the ingestion of raw wheat germ and bran lowered plasma triglycerides and cholesterol (*10*, *41*−*43*) by altering fat digestion.

In conclusion, the totality of the cereal grain is necessary to exert health effects and splitting up of whole grains alters their hypocholesterolemic and hypotriglyceridemic properties. The present study supports the view that the presence of various components in a whole flour affords a notable lipid-lowering effect through synergistic mechanisms.

ABBREVIATIONS USED

AX, arabinoxylan; C, cholesterol; DF, dietary fiber; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NSP, nonstarch polysaccharides; SCFA, short-chain fatty acids; TGRLP, triglyceride-rich lipoproteins−cholesterol; VLDL, very low-density lipoprotein.

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