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Effects of Whole Wheat Flour and Mill-Fractions on Lipid Metabolism in Rats (40395)

MEI LING W. CHANG, MARGARET A. JOHNSON AND DORIS BAKER

Nutrition Institute, Science and Education Administration, U.S. Department of Agriculture, Beltsville, Maryland 20705

Despite the fact that bran has some capacity for binding bile acids and bile salts in vitro (1), many studies of humans (2–6) and rats (7, 8) failed to show that wheat fiber lowered cholesterol in serum or increased fecal sterol excretion (3–6). The substitution of white flour for whole wheat flour in the diet even elevated the serum cholesterol level in a community of Cistercian monks who were all lactovegetarians (9). A similar incident was also observed in rats fed the atherogenic diet (10, 11). Rats that received a bran diet as compared with a no-bran diet showed increased serum cholesterol concentration.[1] Ranhotra (12) reported on the effect of wheat mill-fractions in cholesterol-diets on plasma and liver cholesterol levels in rats. Both farina and patent flour prevented elevation of plasma cholesterol, but bran and shorts elevated plasma cholesterol. The red-dog fraction caused the highest elevation of plasma cholesterol level among all fractions. In that study, it was not clear that the elevation of plasma cholesterol was from dietary or endogenous cholesterol. Furthermore, the quantity of mill-fraction he used was calculated to make 10% protein in each diet and was not equivalent to the quantity of the fraction found in the whole wheat. Therefore, those results could not differentiate the effect of mill-fractions on serum cholesterol levels as they were in the whole wheat. We undertook the present study with rats to compare the effects between commercial whole wheat and white flours which are commonly used, and to evaluate the effects of hard-red winter wheat and its mill-fractions in cholesterol-free diets on lipid metabolism. Amounts of mill-fractions in the diet were kept in the proportion found in the whole wheat.

Materials and methods. Experiment 1 was designed to test the effects of commercial[2] whole wheat flour (WW) and white flour (WF) in cholesterol-free diets on lipid metabolism in rats. The composition of diet was (g/100 g): casein, 11.8; methionine, 0.2; corn oil, 10; salt mix,[3] 4; vitamin mix,[4] 2; and 72 either WW or WF to make up 100. Wheat flours contain 10 to 13% protein, so the total protein in the diet was about 20%.

Male Wistar rats[5] weighing 210 ± 4 g were randomly assigned to two diet groups of 20 each, and were fed ad libitum for 4 or 6 weeks with water available at all times. Rats were weighed once a week and food intake was recorded. On the last 4 days of the 4-week feeding period, feces were collected from four rats of each group. Fecal wet weight, and size and number of pellets were recorded. Fecal bile acid was extracted by chloroform/methanol (2:1) and was determined by the method of Mosbach *et al.* (13).

Rats were decapitated at the end of the feeding period. Abdominal fat pads (epididymal and perirenal) were excised, blotted and weighed. Levels of serum and liver in cholesterol and total lipid were measured (14). Protein in liver was determined by the method of Lowry *et al.* (15). Activities were measured

[1] Forsythe, W. A., Chenoweth, W. L., and Bennink, M. R., Fed. Proc. 36, 119 (1977).

[2] Pillsbury's Best, The Pillsbury Company, Minneapolis, Minnesota 55402. Pillsbury's Best white flour contains: wheat flour, malted barley flour, niacin, iron, thiamin and riboflavin.

[3] Jones, J. H., and Foster, C., J. Nutr. 24, 245 (1942).

[4] Vitamin fortification mixture (#40060, Teklad Test Diets, Madison, Wisconsin) contains (g/kg mix): *p*-aminobenzoic acid, 11.013; ascorbic acid, 101.66; biotin, 0.044; vitamin B-12 (0.1% trituration in mannitol), 2.974; calcium pantothenate, 6.608; choline chloride dihydrogen citrate, 349.692; folic acid, 0.198; inositol, 11.013; menadione, 4.956; niacin, 9.912; pyridoxine·HCl, 2.203; riboflavin, 2.203; thiamin·HCl, 2.203; retinyl palmitate concentrate (500,000 IU/g), 3.96; ergocalciferol (500,000 IU/g), 0.441; tocopheryl acetate (500 IU/g), 24.229; cornstarch, 466.688.

[5] Hilltop Laboratory Animals, Inc., Scottdale, Pennsylvania 15683. (Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.)

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TABLE I. ANALYSIS OF MILL-FRACTIONS OF HARD-RED WINTER WHEAT.

Fractions[a]	Percentage fraction of whole[b]	Moisture	Protein	Ash	Fat	Crude fiber	Acid detergent fiber[c]	Lignin	Neutral detergent fiber
					Percent				
Whole wheat	100	12.07	12.01	1.96	1.47	2.43	2.89	0.79	9.81
Bran	14.5	12.72	15.83	6.59	5.08	9.11	11.56	3.20	33.50
Shorts	9.0	12.81	17.50	4.88	5.91	5.26	7.43	2.63	20.45
Germ	0.5	14.79	36.62	6.77	14.58	2.34	2.11	0.53	8.90
Low-grade flour	9.75	10.03	11.12	0.94	1.90	0.37	0.41	0.24	1.43
Patent flour	66.25	14.89	10.02	0.31	0.84	0.16	0	0	0.72

[a] All fractions were fine flour; except bran and germ were flake-like.

[b] Percentages of fractions were supplied by Dr. Dale Eustace of Kansas State University; other analyses were performed in our laboratory.

[c] Acid detergent fiber equivalent to neutral detergent fiber minus hemicellulose.

(14) of hepatic glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44) and malic enzyme (ME, EC 1.1.1.40). Enzyme activities were defined as the amount of enzyme that produced 1 μmole of measured product per minute under the respective assay condition.

In Experiment 2, we evaluated the effect of mill-fractions of hard-red winter wheat[6] (HRW) on lipid metabolism. Compositional data for the mill-fractions (bran, shorts, germ, low-grade and patent) are presented in Table I. The reference diet contained (g/100 g): casein, 18; methionine, 0.2; corn oil, 5; salt mix, 4; vitamin mix, 2; cellulose (alphacel), 2.89; and cornstarch to make up 100. In the test diets, whole wheat or mill-fractions replaced cornstarch in the reference diet according to the percentage of each fraction in the whole wheat. Because of its low percentage, germ was combined with shorts as the shorts-germ fraction. Cellulose was added to the diets in amounts required to make the total fiber equal to that of acid detergent fiber in the whole wheat diet. The difference between neutral detergent fiber and acid detergent fiber is an estimate of hemicellulose (16); therefore, the difference in dietary fiber among diets is hemicellulose content. The methods for analyses were the same as aforementioned. Fecal neutral sterol was determined from a portion of a 2:1 ($CHCl_3$:methanol) extract by the same method as for liver cholesterol.

[6] All hard-red winter wheat mill-fractions were generously supplied by Dr. Dale Eustace of Kansas State University.

Results and discussion. Experiment 1. Over 4-week feeding period, food intake and weight gain of rats fed either WW or WF diet were about the same, but the size of abdominal fat pads (g/100 g body wt) tended to be greater (13%) in WW-fed than in WF-fed rats (Table II).

For the 4-week feeding period, the activities of liver G6PD and 6PGD were significantly higher in WW- than in WF-fed rats (Table III) and ME showed a similar trend. The elevated activities of those enzymes were associated with the heavy abdominal fat pads but were not related to levels of liver or serum total lipid. The discrepancy of the relationship of hepatic lipogenic enzyme activities and level of lipid in the liver (17) could be due to homeostatic action on lipid in liver at normal condition, and, thus, the excess fat would be deposited in fat depots such as abdominal fat pads.

This presumption was supported by the positive correlation between liver G6PD activity/100 g body wt and g of fat pads/100 g body wt (Table IV). Levels of cholesterol in serum and liver did not significantly differ between the two diet groups. This agrees with the previous report (18) on the effect of bran versus cellulose in rats fed cholesterol-free diet. In general, dietary wheat fiber fed to either humans (19) or rats (7, 8) showed no effect on lowering cholesterol in serum; however, there were few positive results reported. The findings dealt either with advanced age in humans (20) or differences in the composition of diets fed rats (21, 22).

Amount and frequency of fecal excretion and amount of bile acid excretion increased from rats fed WW diet (Table V). The high

TABLE II. EFFECT OF DIET ON FOOD INTAKE, WEIGHT GAIN, AND SIZE OF ABDOMINAL FAT PADS (EPIDIDYMAL + PERIRENAL, E + P) IN RATS.

Diet	Food intake	Body weight gain	E + P
	g/week		g/100 g body wt
White flour (WF)			
0–3 weeks	153 ± 3.8[a] (20)[b]	49.4 ± 2.2 (20)	—
3–4 weeks	141 ± 6.9 (20)	30.7 ± 2.3 (20)	2.36 ± .20 (20)
4–6 weeks	141 ± 3.8 (10)	21.3 ± 1.3 (10)	3.63 ± .36 (10)
Whole wheat (WW)			
0–3 weeks	158 ± 2.7 (20)	46.9 ± 1.3 (20)	—
3–4 weeks	146 ± 5.6 (20)	30.8 ± 1.7 (20)	2.66 ± .20 (10)
4–6 weeks	130 ± 2.9* (10)	15.4 ± 0.2* (10)	3.31 ± .22 (10)

[a] Mean ± SEM.
[b] Numbers in parentheses represent number of rats.
* Significantly different between two corresponding diet groups ($P < 0.05$).

TABLE III. EFFECT OF DIET ON RELATIVE LIVER SIZE, PROTEIN, ENZYME ACTIVITIES, TOTAL LIPIDS AND CHOLESTEROL, AND SERUM TOTAL LIPIDS AND CHOLESTEROL LEVELS AT TWO PERIODS OF FEEDING.

	White flour (WF)		Whole wheat (WW)	
	4-wk feeding	6-wk feeding	4-wk feeding	6-wk feeding
Relative liver size g/100 g body wt	3.43 ± .10[a]	3.06 ± .06	3.51 ± .05	3.07 ± .08
Liver protein mg/100 g body wt	821 ± 21	778 ± 16	851 ± 24	765 ± 15
Enzyme activity				
G6PD	12.5 ± 1.5	16.5 ± 2.0	21.7 ± 3.3*	19.1 ± 3.7
6PGD	8.8 ± 0.5	9.1 ± 0.5	11.7 ± 0.8*	11.1 ± 1.1
ME	9.2 ± 1.1	11.9 ± 0.5	15.0 ± 2.8	12.8 ± 1.5
Total lipid mg/100 g body wt	225 ± 12	193 ± 8	206 ± 10	213 ± 11
Cholesterol mg/100 g body wt	12.3 ± 1.2	11.2 ± 0.5	13.8 ± 1.4	11.7 ± 0.5
Serum: total lipids mg/100 ml	815 ± 72	547 ± 91	690 ± 47	1032 ± 122*
Cholesterol mg/100 ml	131 ± 8.5	115 ± 4.7	146 ± 5.3	110 ± 4.4

[a] Mean ± SEM of 10 rats.
* Significantly different between two corresponding diet groups ($P < 0.05$).

TABLE IV. RELATIONSHIP BETWEEN LIVER G6PD ACTIVITY (X) AND SIZE OF ABDOMINAL FAT PADS (Y), AS EXAMINED IN TWO WAYS.

	Method 1[a]	Method 2[b]
Regression slope = $\frac{\text{g of fat E + P pads/100 g body wt}}{\text{units of G6PD activity/100 g body wt}}$	0.052	0.060
Correlation coefficient	0.721[a]	0.733[b]

[a] Deviations in X and Y were calculated against a single mean for all 20 rats in Method 1 and against the appropriate mean for 10 rats of the same diet group in Method 2. The regression slopes were calculated by the method of least squares.
[b] Differs from 0 ($P < 0.01$).

fiber content of the WW diet explained fecal bulk. The high excretion of bile acids, however, did not agree with observations on humans who ate dietary fiber (5), but agreed with the report on rats that received bran diet as compared to cellulose diet (17).

For the 6-week feeding period, food intake (4- to 6-week) by WW-fed rats tended to decrease (Table II). It resulted in less body weight gain and smaller abdominal fat pads, regardless of the fact that enzyme levels in the liver tended to be higher than levels for WF-fed rats (Table III). However, serum total lipid level of WW-fed rats was signifi-

TABLE V. FECAL WEIGHT AND FREQUENCY, PELLET SIZE AND BILE ACID CONTENTS OF RATS FED TWO DIETS AT 4-WEEK FEEDING PERIOD.

	Diets	
Fecal excretion	White flour	Whole wheat flour
Fecal wet wt, g/day	1.01 ± 0.11[a]	3.30 ± 0.22*
H_2O content, %	30.8 ± 5.0	32.2 ± 1.0
Frequency, pellets/day	6.8 ± 0.5	18.8 ± 0.8*
Pellet size, mm		
C[b]	3.9 ± 0.1	4.8 ± 0*
L[b]	7.8 ± 0.4	10.5 ± 0.3*
Bile acids, mg/day	11.2 ± 1.9	28.3 ± 1.6*

[a] Mean ± SEM of 4 rats with 4 consecutive day collection.
[b] C = cross section; L = length.
* Significantly different between two diet groups ($P < 0.05$).

cantly higher at 6 than 4 weeks, suggesting that there was a shift of lipids between tissues (serum versus liver and adipose).

Experiment 2. Of the criteria examined, only food intake, serum cholesterol (Table VI) and fecal excretions (Table VII) differed between groups fed the reference and HRW whole wheat diets. The differences in food intake and fecal excretion probably were due to the relative amounts of dietary fiber in the two diets. The HRW whole wheat diet contained more dietary fiber than any of the other diets. High food intake could compensate for the nonnutritive fiber in the diet. Serum cholesterol level was lower for the wheat-fraction diets than for the reference diet.

For weight gain, size of abdominal fat pads, relative liver size (g/100 g body wt), and fat and protein content of liver, values were significantly lower in rats fed low-grade flour than in rats fed any other mill-fraction diet. Food intake was also somewhat lower for that group, but differed significantly only from the group fed whole-wheat flour. The activities of hepatic enzymes in response to diets varied and the differences were not significant. This result did not agree with that of Experiment 1 in which the activity of hepatic enzymes was significantly higher in WW-fed than in WF-fed rats. That discrepancy could be due to the difference in quality of the two flours, since the commercial white flour contained additives (footnote #3). Lev-

TABLE VI. EFFECTS OF DIETS ON FOOD INTAKE, BODY WT GAIN, ABDOMINAL FAT PADS (EPIDIDYMAL AND PERIRENAL), RELATIVE LIVER SIZE (g/100 g BODY WT), HEPATIC ENZYME ACTIVITIES, AND LIVER AND SERUM LIPIDS IN RATS.

		Diets				
	Reference (12)*	Whole mill (8)	Bran (12)	Shorts-germ (12)	Low-grade (12)	Patent (12)
Total food intake, g	472 ± 10**	551 ± 11[a]	482 ± 12	485 ± 10	454 ± 15	477 ± 8
Body weight gain, g	181 ± 5	187 ± 6	187 ± 5	174 ± 9	164 ± 5[a]	180 ± 5
Abdominal fat pads, g	9.4 ± .6	9.5 ± .9	9.1 ± .7	7.4 ± .7	6.8 ± .5[a]	8.4 ± .6
Relative liver size	3.8 ± .04	3.9 ± .05	3.9 ± .06	3.8 ± .11	3.5 ± .11[a]	3.8 ± .14
Liver protein mg/100 g body wt	846 ± 18[a,b,c]	912 ± 19[c]	876 ± 19[a,c]	895 ± 31[a,c]	796 ± 27[b]	850 ± 19[b]
Liver enzyme activity						
G6PD	24.9 ± 3.9	18.0 ± 2.5	26.0 ± 2.9	18.5 ± 1.3	20.7 ± 1.8	22.2 ± 1.7
6PGD	12.5 ± 1.4	12.0 ± 0.5	12.5 ± 0.8	11.3 ± 0.6	11.5 ± 1.2	12.4 ± 0.6
ME	21.1 ± 2.3	19.1 ± 2.6	21.7 ± 2.5	17.0 ± 0.7	15.6 ± 1.5	16.8 ± 1.4
Liver total fat mg/100 g body wt	195 ± 6[a]	189 ± 4[a]	190 ± 4[a]	196 ± 6[a]	173 ± 3[b]	215 ± 7[c]
Cholesterol mg/100 g body wt	12.0 ± .4[a]	12.0 ± .2[a]	12.4 ± .3[a]	10.4 ± .5[b]	14.1 ± .4[c]	8.3 ± .4[d]
Serum total fat mg/100 ml	858 ± 39[b,c]	779 ± 43[a,b,c]	693 ± 41[a]	717 ± 46[a]	756 ± 33[a,b]	817 ± 22[a,b,c]
Cholesterol mg/100 ml	146 ± 8[a]	128 ± 3[b]	102 ± 5[c]	107 ± 5[c]	124 ± 5[b]	101 ± 7[c]

* Numbers in parentheses represent number of rats.
** Mean ± SEM; data in the table not sharing a common superscript letter or no letter in the same row are significantly different ($P < 0.05$).

TABLE VII. FECAL EXCRETIONS.

		Diets				
Fecal excretions	Reference	Whole wheat	Bran	Shorts-germ	Low-grade	Patent
Fecal weight (dry) g/day	0.73 ± 0*	2.03 ± 0.1[a]	0.93 ± 0.04	0.80 ± 0.05	0.64 ± 0.07	0.77 ± 0.05
Neutral sterol mg/day	4.8 ± 0.1[a]	12.7 ± 0.7[c]	5.5 ± 0.4[a, b]	6.4 ± 0.5[b]	6.4 ± 0.6[b]	5.6 ± 0.6[a]
Bile acids mg/day	3.9 ± 0.4	13.8 ± 3.6[a]	4.2 ± 0.1	4.5 ± 0.1	3.7 ± 0.2	4.3 ± 0.55

* Mean ± SEM of 4 rats; data in the table not sharing a common superscript letter or no letter are significantly different ($P < 0.05$).

els of cholesterol in serum and liver were, however, significantly higher in rats fed HRW whole wheat and low-grade flour than in rats fed shorts-germ and patent flours. For bran-fed rats, the level of cholesterol was higher in liver but similar in the serum as compared with rats fed the shorts-germ and patent flour. The present data indicate that cholesterol levels in serum and liver appear to have no relationship to total food intake, dietary fiber content of diet or to steroid excretion. The data rather indicate that some unidentified dietary factor might stimulate cholesterol metabolism. This factor in wheat seemed to remain mainly in the low-grade flour fraction of HRW wheat. This phenomenon is similar to the previous report (12) on rats fed red-dog fraction-cholesterol diet.

Summary. Effects on lipid metabolism in rats were studied of (1) commercially available whole wheat flour (WW) and (2) hard-red winter (HRW) whole wheat and mill-fractions in cholesterol-free diets. For similar food intake, dietary WW as compared with white flour (WF) increased bile acid excretion, fecal dry weight, pellet number and size. The activities of hepatic dehydrogenases in the hexose–monophosphate shunt pathway were significantly higher in WW-fed than in WF-fed rats, and malic enzyme activity showed a similar trend. These higher enzyme activities are associated with a trend of higher levels of cholesterol in serum and liver and bigger size of abdominal fat pads. The effects on lipid metabolism varied among fractions of HRW whole wheat and mill-fractions. Cholesterol contents of both serum and liver were higher in rats fed whole wheat and low-grade flour diets than those fed shorts-germ and patent diets. Rats fed bran diet, however, had low serum cholesterol content. The levels of serum and liver total lipid also varied among groups and showed no consistent pattern in relation to cholesterol levels. The excretion of neutral steroids was significantly higher in rats fed whole wheat than in rats fed any other diets. The results suggest that a factor in wheat affected the levels of cholesterol in serum and liver and was concentrated in the low-grade flour fraction and also that dietary fiber from wheat did not alter cholesterol levels in serum and liver.

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