Bioavailability of Vitamins A and E as Influenced by Wheat Bran and Bran Particle Size

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ABSTRACT

The effect of wheat bran and bran particle size on the bioavailability of vitamins A and E was investigated in diets containing 5.7–10.7% dietary fiber. Fifty-six male weanling Sprague-Dawley rats were randomly assigned to four diets containing 5% cellulose (C5), 10.5% cellulose (C10), 21.5% coarse (2-mm) wheat bran (CB), or 22.2% fine (0.5-mm) wheat bran (FB) in a six-week study. At the end of six weeks, plasma retinol values were significantly higher in rats fed C10 than those fed C5 and CB diets (0.38 vs. 0.31 and 0.32 μg/ml, respectively). Also after six weeks, liver retinyl palmitate was significantly higher in rats consuming high-fiber diets. These data indicate that dietary fiber did not adversely influence liver storage of vitamin A. Plasma values did not consistently reflect liver status for either vitamins A or E. Liver α-tocopherol values were significantly lower in rats fed CB than those fed FB and C10 diets (39.24 vs. 44.04 and 44.03 μg/g, respectively), suggesting that CB significantly decreased bioavailability of α-tocopherol as compared to FB or C10 diets when fed similar levels of dietary fiber. Similar liver vitamin E values with fine bran and high-cellulose diets indicate that the vitamin E in wheat bran was not readily available. Data further suggest that coarse bran also reduced availability of the vitamin E in the diet. The significant impairment in bioavailability of vitamin E by coarse bran compared to fine bran indicated that this impairment may not be a function of adsorption of vitamin E on the surface of bran particles. The effect of CB may be partly caused by its interaction and effect on the morphology and physiological processes of the intestinal lumen and its absorption sites.

Wheat bran is a common source of dietary fiber in the western diet. The influence of wheat bran fiber on plasma cholesterol and lipids as well as in preventing digestive tract disorders has received significant attention in the scientific literature (Ranhotra et al 1977, 1978; Liebman et al 1983; Moore et al 1985), as well as in the popular press. Studies on the influence of increased wheat bran consumption on the bioavailability of fat-soluble vitamins have produced conflicting results. Omaye and Chow (1984a) reported significantly lower vitamin E in rat plasma when wheat bran was increased from 5 to 20% in a five-week study, but no effect was observed on rat plasma vitamin A and E levels after an eight-week study (Omaye and Chow 1984b). Mongeau et al (1986) observed no detrimental effect on vitamin E status in the rat as reflected in plasma and liver values in a six-week study when diets contained 4–20% wheat bran.

The present study was conducted to evaluate the influence of wheat bran and bran particle size on the bioavailability of vitamins A and E, as determined by plasma and liver levels of these vitamins.

MATERIALS AND METHODS

Fifty-six male weanling Sprague-Dawley rats (Bantin and Kingman, Fremont, CA) were housed in individual stainless steel wire-bottom cages at 21 ± 1°C, 65% relative humidity, in a room illuminated automatically for 12 hr daily from 0600 to 1800 hours. The cages were equipped with an automatic watering system for drinking and cleaning. All animals had continuous access to water and food. After being fed AIN-76 diet (AIN 1977) for seven days, animals were randomly assigned to four treatment groups of 14 animals each.

Based on an initial determination of dietary fiber content of bran and cellulose, diets were formulated to contain 5 or 10% total dietary fiber. Diets contained 5% cellulose (C5), 10.5% cellulose (C10), 21.5% coarse (milled through 2-mm screen) AACC-hard red (HR) wheat bran (CB), or 22.2% fine (milled through 0.5-mm screen) AACC-HR wheat bran (FB). Both samples consisted of the entire bran milled through the respective screens. No wheat bran was discarded; CB and FB differed only in particle size and not in product composition. Particle size distribution of the milled bran was determined by passing it through U.S. standard sieves (Table I).

Analyzed dietary fiber values for C5, C10, CB, and FB diets were 5.7, 10.7, 10.7, and 10.0%, respectively, as determined by a procedure described elsewhere (Prosky et al 1984). Composition of the treatment diets is given in Table I. All diets were stored at 4°C prior to feeding.

In order to avoid taking excessive blood from the animals, each treatment group was further subdivided into two groups, A and B, of seven animals each. Blood samples were taken from the tail vein of group A animals on days 8, 22, and 36 and from group B on days 15 and 29. Food was removed for 16 hr before blood collection. Animals were anesthetized with methoxyflurane. Blood was collected by inserting a needle rinsed in ethylenediaminetetraacetic acid (EDTA)-dipotassium salt (15% solution) and letting blood drip into a plastic tube containing EDTA (10 μl/cc of blood). Plasma was obtained by centrifuging the samples for 20 min at 4°C, 1,500 × *g*. Plastic tubes prevented hemolysis of blood cells. Plasma samples were extracted immediately for retinol and α-tocopherol and stored at −17°C until they were analyzed by high-performance liquid chromatography (HPLC).

After 42 days, animals were fasted for 16 hr and anesthetized with methoxyflurane; the thorax was opened and blood was collected by cardiac puncture. Livers were removed, blotted with

TABLE I
Composition of Diets

Ingredient (%)	5% Cellulose	10.5% Cellulose	21.5% HR Bran, 2 mm[a]	22.2% HR Bran, 0.5 mm[a]
HR wheat bran	0.0	0.0	21.5	22.2
Corn starch	30.0	24.5	13.5	12.8
Cellulose	5.0	10.5	0.0	0.0
Sucrose	35.0	35.0	35.0	35.0
Casein	20.0	20.0	20.0	20.0
Corn oil	5.0	5.0	5.0	5.0
AIN mineral mix[b]	3.5	3.5	3.5	3.5
AIN vitamin mix[b]	1.0	1.0	1.0	1.0
DL-Methionine	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2
Dietary fiber, %	5.7	10.7	10.7	10.0

[a] AACC hard red (HR) reference wheat bran milled through 2-mm and 0.5-mm screen, respectively. Mean particle size distribution expressed as bran remaining on a given screen mesh size (mm) as % of total bran sample. Distribution for the 2-mm bran (mean 0.684 mm) was: 1.4, 2%; 1.0, 26%; 0.72, 34%; 0.51, 23%; 0.36, 8%; and 0.19, 7%; for the 0.5-mm bran (mean 0.341 mm): 0.46, 1%; 0.39, 58%; 0.33, 22%; 0.24, 10%; and 0.15, 9%.
[b] American Institute of Nutrition (1977). (Vitamin A = 4,000 IU/kg and vitamin E = 50 IU/kg of diet.)

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cheesecloth, and stored on dry ice. They were subsequently transferred to a −70° C freezer.

Plasma Extraction and Analysis

Plasma samples were extracted in duplicate using the procedure of Bieri et al (1979) for extraction, except that 0.125% butylated hydroxytoluene (BHT) was used as an antioxidant. Extracted material was evaporated to dryness in a vacuum sample concentrator (Savant Instruments, Inc., Hicksville, NY) for 30 min. Tubes were wrapped in aluminum foil to protect samples from light and stored at −17° C for analysis. Evaporated samples were dissolved in 100 μl of methanol, and injections were made using the WISP autosampler (Waters Associates, Milford, MA), onto a 0.46 × 15 cm stainless steel column packed with C-18 reverse-phase, 5 μm spherical particles (Rainin Instrument Co. Inc., Woburn, MA). Chromatographic conditions consisted of a step gradient, beginning with 93% methanol in water (v/v) at 1.0 ml/min, changing to 100% methanol, and increasing the flow rate to 1.5 ml/min at time 7.5 min (Waters 590 solvent delivery system). Vitamin peaks were detected by ultraviolet absorption at 292 nm (Spectromonitor III, LDC Milton Roy, Riviera Beach, FL). Retention times for retinol and α-tocopherol were 5.3 and 12.2 min, respectively. Vitamins were quantitated by a standard curve determined under the same chromatographic conditions. A standard mixture of vitamins A and E containing amounts in the range of plasma and liver levels was prepared and stored at −70° C. Daily standards were run to ascertain and correct for any procedural variations.

Liver Extraction and Analysis

In order to minimize interlobular variability in vitamin A distribution, 0.5–1.0 g of liver sample was taken from the right lobe. Frozen samples were minced with a razor blade on a glass plate and transferred to an 18 × 150 mm glass tube containing 10 ml of ethanol (0.1% BHT) and homogenized in an ice bath for 15 sec with a PT10ST probe (Polytron, Brinkman Instruments, Westbury, NY). Homogenates were transferred to a 16 × 125 mm glass tube with Teflon-lined screw-cap, centrifuged for 15 min at 4° C, 1,300 × g, and decanted into a 40-ml round-bottom glass centrifuge tube. The liver pellet was extracted twice with 10 ml of chloroform and methanol (2:1), containing 0.2% BHT, by vortexing for 1 min and centrifuging 15 min at 4° C, 1,300 × g. Supernatants were combined with the ethanol layer for evaporation in a vacuum sample concentrator. The residue after evaporation was redissolved in 2 ml of methanol, filtered, and an aliquot injected for vitamin A and E analysis by HPLC. Samples were analyzed on the same column used for plasma analysis; in addition, a 0.46 × 3 cm guard column containing 10 μm C-18 reversed-phase sorbent (Brownlee Labs, Santa Clara, CA) was installed above the analytical column. Retinol was eluted with 90% methanol in water in 9.1 min at 1.0 ml/min. The mobile phase was then switched to a solution of methanol, tetrahydrofuran, and water (65:30:5), and the flow rate was increased to 1.5 ml/min to elute α-tocopherol and retinyl palmitate at 21.2 and 28.5 min, respectively. Vitamins A and E were quantitated the same way as they were in plasma samples.

RESULTS AND DISCUSSION

Mean food dry matter intakes (mean ± SEM) for rats fed C5, C10, CB, and FB diets were 919.3 ± 19.8, 927.2 ± 22.6, 869.7 ± 17.1, and 896.2 ± 18.9 g, respectively. Animals gained 284.7 ± 9.6, 273.9 ± 7.8, 270.7 ± 7.9, and 287.3 ± 6.9 g on C5, C10, CB, and FB diets, respectively. There were no significant differences in food intakes or weight gains in rats fed treatment diets over the 42-day period. Based on diet composition, vitamin A levels were similar for all diets. Bran increased vitamin E content of the diets by 4–5%.

Fasting plasma retinol values (μg/ml) were determined weekly with seven animals per treatment and at the termination of the six-week study with all 14 animals per treatment (Table II). After six weeks, plasma retinol values in rats fed high-cellulose (C10) were significantly higher ($P < 0.05$) than those fed low-cellulose (C5) and coarse bran (CB) diets. There were no significant differences in plasma retinol values in group A animals after one, three, or five weeks of treatment. In group B, after two weeks, plasma retinol values ranged from 0.27 to 0.33 μg/ml among the four treatments; differences in values among treatments were nonsignificant, although the highest level was associated with fine bran (FB). This trend changed after four weeks in group B animals, in which plasma retinol values were then significantly higher ($P < 0.05$) in rats on the CB diet than on the FB diet (0.37 vs. 0.29 μg/ml). These data indicate that wheat bran did not have a consistent effect on plasma retinol values.

Plasma α-tocopherol values over the six-week period of the study are presented in Table III. At the termination of the study, there were no significant differences in α-tocopherol values among treatments; however, CB treatment resulted in lower plasma α-tocopherol values than did FB or C10 treatments. In group A animals after five weeks, plasma α-tocopherol in rats fed FB was significantly higher ($P < 0.05$) than in those fed the other three diets. In group B animals, plasma α-tocopherol values after two weeks were significantly lower ($P < 0.05$) for all high-fiber treatments (C10, CB, and FB) than for the low-fiber diet (C5). These data may represent a short-term lowering of plasma values within this group with high-fiber diets. Large weekly variability in plasma α-tocopherol values on high-fiber diets within randomized groups may be caused, in part, by the small number of animals per group; it may also relate to the difficulty involved in obtaining plasma from tail veins. The difficulty we experienced in tail bleeding was that, with use of methoxyflurane, if a vein was not

TABLE II
Weekly Plasma Retinol Levels (μg/ml) in Rats Fed Wheat Bran or Cellulose

	Weeks on Diet[a]					
Variable	1	2	3	4	5	6
Group sampled (*n*)	A (7)	B (7)	A (7)	B (7)	A (7)	A + B (14)
Diet						
5% Cellulose	0.24	0.31	0.32	0.33 ab[b]	0.34	0.31 b
10.5% Cellulose	0.29	0.27**[c]	0.32**	0.33 ab	0.32	0.38 a
21.5% HR bran (2 mm)[c]	0.24	0.29	0.32	0.37 a	0.34	0.32 b***
22.2% HR bran (0.5 mm)[c]	0.27	0.33*	0.34	0.29 b	0.35	0.34 ab
SEM	0.01	0.02	0.02	0.02	0.01	0.01

[a] Blood was sampled by tail bleeding weeks 1–5 and by cardiac puncture at week 6. * and ** $n = 5$ and 6, respectively (differences in *n* reflect difficulty in tail bleeding); *** $n = 13$, one animal died.
[b] Means within columns with different letters are significantly different ($P < 0.05$).
[c] HR = hard red.

TABLE III
Weekly Plasma α-Tocopherol Levels (μg/ml) in Rats Fed Wheat Bran or Cellulose

	Weeks on Diet[a]					
Variable	1	2	3	4	5	6
Group (*n*)	A (7)	B (7)	A (7)	B (7)	A (7)	A + B (14)
Treatment						
5% Cellulose	2.87	3.27 a[b]	3.16	2.91	2.67 b	2.81
10.5% Cellulose	2.98	2.34 b**	3.16**	2.93	2.86 b	3.20
21.5% HR[c] bran (2 mm)	2.86	2.03 b	2.62	2.62	2.66 b	2.54***
22.2% HR[c] bran (0.5 mm)	3.33	2.19 b*	3.38	2.23	3.49 a	3.14
SEM	0.21	0.24	0.25	0.25	0.21	0.15

[a] Blood was sampled by tail bleeding weeks 1–5 and by cardiac puncture at week 6. * and ** $n = 5$ and 6, respectively (differences in *n* reflect difficulty in tail bleeding); *** $n = 13$, one animal died.
[b] Means within columns with different letters are significantly different ($P < 0.05$).
[c] HR = hard red.

TABLE IV
Liver Values for Retinol, Retinyl Palmitate, Retinol Equivalents, and α-Tocopherol in Rats Fed Wheat Bran or Cellulose for Six Weeks

Treatment	n	Vitamin (μg/g of liver)			
		Retinol	Retinyl Palmitate	Retinol Equivalents	α-Tocopherol
5% Cellulose	14	1.88	91.46 b[a]	51.74 b	43.32 ab
10.5% Cellulose	14	2.23	119.48 a	67.36 a	44.03 a
21.5% HR bran (2 mm)[c]	13*[b]	1.92	111.60 a	62.75 a	39.24 b
22.2% HR bran (0.5 mm)[c]	14	2.56	112.01 a	63.62 a	44.04 a
SEM		0.26	6.49	3.59	1.44

[a] Means within columns with different letters are significantly different ($P < 0.05$).
[b] * One animal died.
[c] HR = hard red.

punctured in the first few attempts, the blood flow to the tail was significantly reduced if not totally shut off for a period of up to 20 min. Later, blood was observed to be dripping out of the several previous punctures. As a result, comparisons are made only within groups A or B.

Plasma retinol and α-tocopherol values obtained in this experiment were consistently lower than those reported by Alam and Alam (1983) or Omaye and Chow (1984a,b). Part of the variability between studies may be explained by the use of external standard curves in our study, along with the running of daily standards for further corrections. Slopes for retinol and α-tocopherol standards have significantly different regression ($Y = a + b X$) parameters. Thus, using an internal standard and assuming extreme linearity, as reported in the literature, assumes a slope of 1 and an intercept of 0, which may not be a valid assumption.

Liver values for retinol, retinyl palmitate, retinol equivalents, and α-tocopherol at the end of the study are shown in Table IV. There were no significant differences in liver retinol values among any of the treatments. Liver retinyl palmitate values reveal that high-fiber diets (C10, CB, and FB) resulted in significantly higher ($P < 0.05$) liver storage of retinyl palmitate than did the low-cellulose diet (C5). Liver vitamin A values, when expressed as retinol equivalents (retinol equivalents = retinol + retinyl palmitate/1.8345), showed the same pattern of significant differences as that observed for retinyl palmitate values. Dietary fiber did not adversely influence liver storage of vitamin A; on the contrary, all high-fiber diets resulted in significantly higher liver storage of vitamin A after six weeks.

In plasma, retinol is the circulating form of vitamin A. Liver retinol may be controlled or regulated by conversion of retinyl palmitate to retinol in liver by a homeostatic mechanism depending on circulating levels of retinol in plasma. The major storage form of vitamin A in liver is retinyl palmitate. Gray et al (1940) reported 90–95% of liver vitamin A is present as an ester form, possibly of one kind. In contrast, Goodman et al (1965) reported that liver retinyl palmitate in rat accounts for 70–75% of liver stores of vitamin A ester, whereas Bhat and Lacroix (1983) observed retinyl palmitate to be 79% of the ester form of vitamin A in rat liver. In the present study, one major vitamin A ester peak was observed that was designated as retinyl palmitate. Our liver retinol data suggest that retinyl palmitate may account for over 90% of the ester form of vitamin A stored in rat liver, with only 3% of liver vitamin A being present as retinol (calculated from retinol equivalents and retinol values, Table IV). Values reported by Goodman et al and Bhat and Lacroix may be lower because they were obtained by short-term isotope equilibration experiments.

Liver α-tocopherol values in rats fed CB were significantly lower ($P < 0.05$) than those fed C10 and FB diets (Table IV). These data indicate that coarse bran significantly lowered the bioavailability of α-tocopherol as compared to fine-bran or high-cellulose fiber diets at the same dietary fiber level. A similar, but nonsignificant, influence of coarse wheat bran on plasma α-tocopherol values was observed in these animals at the termination of study. On the basis of plasma values, studies by Omaye and Chow (1984b) and Mongeau et al (1986) also did not show a significant decrease in the bioavailability of vitamin E with diets containing 10% dietary fiber supplied by wheat bran. In addition, Mongeau et al (1986) did not observe any differences in liver storage of vitamin E among rats fed diets containing 4–20% wheat bran. Our data indicated that plasma circulating levels of vitamin A and E did not consistently reflect the liver status of these vitamins.

Liver vitamin A and E values are reported per gram of right lobe. Liver tissue weights (mean ± SEM) at the conclusion of the study for rats fed C5, C10, CB, and FB diets were 10.96 ± 0.39, 10.08 ± 0.47, 10.12 ± 0.35, and 10.78 ± 0.25 g, respectively. There were no significant differences in liver weights of rats fed the various treatment diets. High variability in vitamin A content among various lobes of liver, with the right lobe containing the highest level, is reported by Olson et al (1979). Thus, extrapolation to total liver vitamin (liver weight in grams × vitamin per gram of liver) would not change the relative significance of the results but could result in erroneously higher values.

Although calculated vitamin E content of bran increased total vitamin E in the diet by 4–5%, similar liver vitamin E values obtained in rats fed fine-bran and high-cellulose diets indicate that vitamin E in the wheat bran was not readily available. Lower liver levels of vitamin E with the coarse-bran diet further suggest that not only was the vitamin E in the bran unavailable, but coarse bran also interfered with availability of some of the vitamin E in the diet. Significantly greater impairment in the bioavailability of vitamin E by coarse bran compared to fine bran further suggests that this impairment is not caused by adsorption of vitamin E by bran, because the fine bran particles offered nearly twice the surface area as did the coarse bran particles in the diet. The effect of coarse bran may partly result from its interaction and effect on the morphology and physiological processes of the intestinal lumen and its absorption sites. Increased muscle thickness in the colon, deeper colonic crypts, and mucosal cell hyperplasia have been reported by Jacobs and Schneeman (1981) in rats fed a 20% bran diet.

In conclusion, coarse but not fine wheat bran significantly lowered the bioavailability of dietary α-tocopherol as reflected in liver values. Bioavailability of retinol was not impaired by wheat bran or cellulose fiber in this study.

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