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Effects of Dietary Fiber on Digestive Enzyme Activity and Bile Acids in the Small Intestine[1] (42197)

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In humans and in a number of animal species consumption of high fiber diets has been associated with reductions in the digestibility and availability of protein, fats, and other nutrients such as minerals, vitamins, and carbohydrates. The ability of fiber to alter the rate of digestion and absorption in the gastrointestinal tract appears to be important in understanding its effects on metabolism, such as reducing plasma lipids and altering the glycemic response to a meal. The assimilation of nutrients from the diet requires the movement of digesta through the gastrointestinal tract, the enzymatic hydrolysis of complex compounds into simpler compounds which can be absorbed, the uptake of these compounds into the intestinal cell, and movement of nutrients from the intestinal cells to the portal circulation or lymph ducts. Dietary fibers influence these processes through several mechanisms which include altering the availability of bile acids and digestive enzyme activity, changing the characteristics of the intestinal contents where digestion occurs, altering the morphology of the small intestine so that structural changes are associated with functional changes in the gut, and causing adaptation in the synthesis of enzymes or compounds needed for nutrient absorption from the intestine. The objective of this paper is to consider the potential effects of dietary fiber on the rate of digestion in the small intestine. The effects of fiber on absorption from the intestine and subsequent metabolism of nutrients will be considered in other papers in this series.

One of the first questions to consider is the effect of various sources of dietary fiber on the activity of digestive enzymes *in vitro*. Several studies have reported changes in the activity of pancreatic enzymes following incubation with purified and nonpurified fiber sources (1–9). In Table I the results of *in vitro* incubation of fibers with human pancreatic enzymes are reported. The results are shown as percentage of the control activity without incubation with dietary fiber. In most cases enzyme activity was reduced below the control value after incubation with the fiber source. Pectin resulted in an increase in enzyme activity in one study and reductions in activity in another. This difference is undoubtedly due to differences in procedures and reporting of enzyme activity. Chymotrypsin is the enzyme that is most consistently reduced by the various fiber sources. Cellulose and xylan, an isolated hemicellulose, significantly reduced the activity of all four enzymes, with cellulose having a very marked effect on lipase activity. The reductions in enzyme activity can be due to nonspecific binding of the enzymes or, in the case of the nonpurified fiber sources, the presence of specific enzyme inhibitors (1, 2, 9, 10). The results in Table I are consistent with what has been reported in the literature using commercial enzymes from either bovine or porcine sources, that various sources of fiber are reported to reduce enzyme activity or have no effect. The interpretation of this type of *in vitro* data is limited and mainly demonstrates the potential for an interaction, but the physiological importance of this interaction must be examined in other experiments. The effects of dietary fiber sources on the rate of digestion in *in vitro* experiments have been evaluated by estimating the rate of substrate release during *in vitro* digestibility measurements with sources of fiber present. Gagne and Acton (11) have reported that the digestibility of casein by a mixture of trypsin, chymotrypsin, peptidase, and a bacterial protease can be reduced with the addition of fiber. The ability to decrease *in vitro* digestibility varied by the fiber source. In the presence of isolated fiber sources the reduction in casein digestibility was greatest in

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TABLE I. PERCENTAGE OF CONTROL ENZYME ACTIVITY *IN VITRO*

Fiber source	Amylase	Lipase	Trypsin	Chymotrypsin	Ref.
Alfalfa	87.3	72.8	29.0*	51.6*	(2)
Oat bran	72.6	83.8	94.9	71.3	
Wheat bran	66.9*	85.9	93.8	76.2	(2)
Xylan	32.7*	31.0*	11.2*	20.0*	(2)
Cellulose	20.4*	4.6*	55.3*	52.9*	(2)
Pectin	148.0*	123.0	100.0	129.0*	(2)
Pectin-HM[b]	50.0	20.0	40.0	20.0	(4)[a]
Guar gum	60.0	45.0	90.0	95.0	(4)[a]

[a] No statistics were reported.
[b] HM, high methoxy.
* A statistically significant difference from the control treatment without fiber was reported.

the presence of karaya gum, xylan, and pectin, followed by lignin and cellulose. Fibrous residues from foods also reduced *in vitro* casein digestibility. A 15% reduction in digestibility occurred in the presence of fiber residue from canned corn, a 7–9% reduction in the presence of residues from cooked blackeye peas, cooked broccoli, and brown rice, and wheat bran had very little effect. *In vitro* studies have also been used to examine the rate of starch hydrolyzed from various foods (12–14). Several factors appear to alter the rate of *in vitro* starch hydrolysis from cereals including the presence of fiber, the physical form of the food, and the viscosity of the mixture (14). The results from one study (12) suggested that the presence of fiber in whole wheat bread does not slow starch hydrolysis *in vitro* whereas it does slow starch hydrolysis for rice. Other cereals vary considerably in the rate of starch hydrolysis *in vitro* which may be affected by the presence of fiber in these products and by physical form.

Because of the evidence from *in vitro* studies that sources of fiber can interfere with digestive enzyme activity, its effects on the availability of digestive enzyme activity *in vivo* after a meal are of interest. Several studies have been conducted in which rats were adapted to a fiber-free diet or one containing the fiber source to be tested (15–21). Rats were killed approximately 2 hr after they had consumed a meal during a 15- to 20-min period of either the fiber-free diet or one containing either cellulose, wheat bran, pectin, or guar gum. The pancreatic enzymes, trypsin, chymotrypsin, amylase, and lipase, were determined in samples of the intestinal contents and mucosa and expressed as total units of enzyme activity in the small intestine. The units were adjusted based on the fiber-free control for each experiment. The results of these experiments indicate that the total amount of activity for these enzymes in the intestinal contents either does not differ from the control or, as seen with pectin and guar gum, is greater than the control (Table II). The elevation of activity following pectin consumption is consistent with the *in vitro* data and with results reported from

TABLE II. WEIGHT, PROTEIN CONTENT AND ENZYME ACTIVITY OF INTESTINAL CONTENTS OF FED RATS[a]

	Total units[a,b] × 10^2				Protein (mg)[b]	Dry weight (mg)[b]
	Amylase	Lipase	Trypsin	Chymotrypsin		
Fiber-free	17.6	9.5	2.07	2.14	31.5	208
20% Cellulose	25.0	8.5	2.36	1.49	44.8*	319*
20% Wheat bran	12.5	16.4*	2.09	1.72	53.4*	350*
5% Pectin	26.8*	20.6*	4.97*	2.97*		360*
10% Guar gum	24.8*	20.7*				332*

[a] Data from Ref. (10).
[b] Values were adjusted based on fiber-free control for each experiment.
* Value is significantly different from fiber-free control ($P < 0.05$).

a human study in which duodenal aspirates had a higher level of pancreatic enzyme activity after a pectin supplement was given (22). We have speculated that this elevation is most likely due to slower degradation of the digestive enzymes based on the presence of nondigestible material in the intestine which may help to stabilize digestive enzyme activity (10). With wheat bran and cellulose there does appear to be a discrepancy between the *in vitro* and *in vivo* data and the *in vivo* studies suggest that decreases in digestibility associated with fiber cannot be accounted for by a reduction in the total amount of pancreatic enzyme present in the intestinal contents. It is important to keep in mind that these values represent the potential amount of enzyme in the intestine and may not reflect the actual activity of enzymes under *in vivo* conditions within the contents. In this regard we observed that the amount of protein in the intestinal contents in two studies is significantly increased after fiber supplementation, suggesting that the actual rate of breakdown may be reduced (Table II). In addition, changes in the amount, volume, and viscosity of the intestinal contents may influence the ability of enzymes and substrates to interact. The dry weight of intestinal contents after a meal containing a fiber supplement is approximately 40% greater than after a fiber-free meal. Given the water-holding capacity of certain fiber sources, the wet weight and volume of the contents will also be increased. In rats fed a meal containing guar gum the wet weight of the intestine was approximately twofold greater than in rats fed a fiber-free meal (21). Data from the literature have indicated that the viscosity of the intestinal contents can be increased by addition of fiber (4).

The experimental evidence from these studies has indicated that fiber may interfere with digestive enzyme activity but mainly based on *in vitro* studies because the total amount of enzyme activity was not reduced *in vivo*. In addition increases in the bulk, volume, and viscosity of the intestinal contents could also interfere with the rate of digestion and slow diffusion in the intestinal contents.

To evaluate whether the rate of digestion can be altered in the intestinal contents by sources of fiber, the rate at which substrate disappears from the intestinal contents must be estimated. *In vitro* data suggest that cellulose substantially reduces the activity of lipase (1, 2), and if this effect is physiologically important then consumption of cellulose could slow the disappearance of triacylglycerol from the small intestine. To test this hypothesis rats were given, during a 20-min period, a 2-g meal that contained ^{14}C-labeled triolein plus ^{3}H-labeled cholesterol. The test meal was either fiber-free or contained 20% cellulose and was high in fat (30% by weight). If cellulose has a general effect on absorption, then the disappearance of both isotopes should be slowed, but if cellulose has a specific effect on lipase activity and triacylglycerol hydrolysis *in vivo*, then triacylglycerol disappearance should be specifically slowed since it is the substrate for lipase. Rats were killed at 1, 3, and 5 hr after the test meal, and the amount of isotope remaining in the stomach, small intestine, and cecum was measured. The rate of emptying from the stomach did not differ between the two diets. Within the small intestinal contents the disappearance of the tritium label from cholesterol did not differ between the two diets (Table III). Even when the distribution of the tritium label throughout each quarter of the intestine at each of the three time periods is examined there is no pattern of difference between the fiber-free and cellulose-supplemented test meal (23). This result indicates that cellulose does not interfere with the bulk phase movement of lipid as required for uptake of lipid into the intestinal cells. In contrast the cellulose did interfere with the disappearance of ^{14}C label from triolein from the intestinal contents (Table III). At each time period the total amount of label in the intestinal contents was significantly higher in the cellulose-fed rats. The distribution of isotope in each quarter of the small intestine at each time period was higher in the cellulose group. This elevation of ^{14}C in intestinal contents of the cellulose group was greatest at 3 hr after the meal in the lower half of the small intestine. Therefore cellulose delayed disappearance of the isotope from triacylglycerol but not of that from cholesterol. Consequently these results indicate that cellulose can interfere with lipase *in vivo* as suggested from *in vitro* studies (23). Very little of the isotope from triolein appeared in the cecum of either group and there were no differences in the cecal content of ^{14}C be-

TABLE III. LABEL FROM [^{14}C]TRIOLEIN AND [^{3}H]CHOLESTEROL REMAINING IN THE SMALL INTESTINE AFTER A HIGH FAT TEST MEAL[a]

Time (hr)	Radioactivity (μCi)			
	Fiber-free		20% Cellulose	
	^{3}H	^{14}C	^{3}H	^{14}C*
1	96.1 ± 11.58	29.0 ± 1.55	147.8 ± 10.83	34.7 ± 1.43
3	335.4 ± 11.43	23.0 ± 1.53	378.0 ± 12.35	33.2 ± 1.63
5	470.5 ± 12.38	23.4 ± 1.63	396.0 ± 11.4	28.6 ± 1.53

[a] Data from Ref. (23).
* Significantly different from the control at each time point.

tween the two groups at 5 hr after the meal, indicating that the triacylglycerol was almost completely absorbed in the small intestine (23). Because of this complete absorption in the small intestine, the interference with lipase activity resulted in shifting a greater proportion of fat absorption to the lower half of the small intestine. This shift in the site of lipid absorption may be important in understanding the metabolic effects of dietary fibers (10, 23–25). Currently we are investigating the effects of other fiber sources on the disappearance of cholesterol and triacylglycerol from the small intestine. Preliminary data suggest that guar gum may delay lipid disappearance but that it has a more general effect in slowing absorption as might be expected from its viscous properties. Imaizumi *et al.* (26) have reported that the appearance of triacylglycerol in the lymph and plasma is delayed in rats consuming a diet containing guar gum.

The studies discussed above have emphasized the potential effects of fiber on the rate of digestion by its potential effects on digestive enzyme activity. To summarize, some sources of fiber potentially interfere with digestive enzyme activity, as demonstrated in *in vitro* experiments and supported by *in vivo* data, fibers may interfere with diffusion and hence digestion and absorption by increasing the bulk, volume, and viscosity of intestinal contents, and sources of fiber can slow the rate of lipid absorption and shift absorption to more distal segments of the small intestine. In addition to enzymatic activity the digestion and absorption of fat in the small intestine require the presence of bile acids for enzyme activity and for diffusion of lipid through the unstirred layer. Dietary fibers have been shown to bind bile acids *in vitro* which could interfere with the diffusion and absorption of lipid from the small intestine (27–30). The ability of various sources of fiber to bind bile acids within the intestinal contents and the effect of that binding on the amount of lipid solubilized and hence available for absorption were determined in rats (31). Rats were given a high fat test diet which was fiber-free or contained a source of fiber (cellulose, wheat bran, oat bran, guar gum, or lignin) or cholestyramine which was used as a positive control due to its high bile acid binding capacity. Two hours after the meal the intestinal contents were collected, heated to inactivate lipase activity, and centrifuged to separate the aqueous phase of the contents which contains the bile acid micelles and hence lipid which has been solubilized and therefore available for absorption. The concentration of bile acids in the aqueous phase was 14 m*M* in the control group. This value was not significantly different from the bile acid concentration in the aqueous phase of the intestinal contents of any of the fiber-treated groups in which concentration ranged from 8.8 to 16.6 m*M*. Cholestyramine significantly reduced the concentration of bile acids in the aqueous phase by about half; however, none of the treatments reduced bile acid concentration below the critical micellar concentration. The degree of binding by bile acids is indicated by estimating the ratio of bile acids in the aqueous phase to that in the total intestinal contents. A ratio differing from the control fiber-free treatment indicates that binding of bile acids has occurred. Cellulose and wheat bran which have very low *in vitro* bile acid binding had ratios similar to the control indicating that bile acids are not bound

in vivo. The extent of bile acid binding by oat bran *in vitro* has not been published. Within the intestinal contents the ratio of soluble bile acids to the total bile acids present did not differ from that for the control group, indicating that it may not bind bile acids in the intestinal contents. Guar gum is a soluble polysaccharide and remains in large part in the aqueous phase; consequently the significant increase in the ratio, which was double the control value, indicates that bile acids have been sequestered by guar gum and, perhaps, are bound by it in the soluble phase. Lignin and cholestyramine significantly reduced the ratio by about half the control value, indicating that the bile acids have been bound and the proportion of bile acids in the aqueous phase reduced. Hence the three treatments known to bind bile acids *in vitro*, guar gum, lignin, and cholestyramine, appear to have a similar effect in the small intestine under physiological conditions. Phospholipid binding was also estimated since the phospholipids are important components of the micelle. Among the treatments, only cholestyramine significantly changed the ratio from the control treatment, and the ratio was about 50% lower. The ability of cholestyramine to bind phospholipids is supported by *in vitro* data (25). The only fiber treatment which indicated any trend toward binding phospholipids was oat bran; however, the difference from the control value was significant at $P < 0.10$ and not at the 5% level. The amount of lipid in the aqueous phase was determined gravimetrically to estimate if less lipid had been solubilized. The only treatment that significantly reduced the amount of lipid was cholestyramine. The ability of cholestyramine to reduce the amount of lipid available for absorption appears to be dependent on its ability to bind both phospholipids and bile acids in the small intestine. In the case of the fiber treatments this result suggests that the ability of fibers to bind bile acids may be more important in explaining the hypocholesterolemic effects of some fibers than a reduction in lipid solubilization. It also suggests that it may be more important to investigate the effects of fiber on the rate of lipid digestion and absorption rather than the total amount absorbed.

In conclusion, evidence exists that the nondigestible components of the diet may be important modulators of the rate at which foods are digested in the gastrointestinal tract and hence the availability of nutrients for absorption. This influence on the rate of digestion provides important insights into understanding the effects of fiber on metabolism.

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