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Long-Term Ingestion of Insoluble Dietary Fiber Increases Luminal Mucin Content, but Has No Effect on Nutrient Absorption in Rats

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We reexamined the hypothesis that increased mucin secretion by the ingestion of insoluble dietary fiber (IDF) could affect small intestinal nutrient absorption. Polystyrene foam (PSF) was used as IDF. Rats were fed a diet with or without 90 g of PSF/kg for 1, 2 and 4 wk. At the end of each period, a glucose and ovalbumin (OVA) solution was intubated after 12 h of food depletion, and the changes in serum concentrations of these components were monitored. Luminal mucin was measured as *O*-linked oligosaccharide chains and also determined by ELISA. In all periods, the luminal mucin content was greater in the PSF-fed group than in the fiber-free control. However, the changes in serum glucose and OVA concentrations were comparable between the groups at any time during any period. These results show that the enhancement of luminal mucin secretion lasted even after chronic ingestion of IDF, but that the increased luminal mucin content had no effect on the rate of luminal nutrient absorption.

Key words: dietary fiber; luminal mucin; glucose and ovalbumin absorption; rat

As nutrient molecules are actively or passively absorbed from the intestinal lumen into the body, they must pass a major diffusion barrier, *i.e.*, the unstirred water layer composed mainly of hydrated mucins,[1,2] leading to the general consideration that an increase in luminal mucin secretion may modify the rate of nutrient absorption.[3] Schwartz and Levine[4] have reported that, after the ingestion of a 10% cellulose diet for 5 wk, glucose tolerance was a little but significantly improved in rats. Dryden *et al.*[5] have also shown that the ingestion of a 30% coarse bran diet for 8 wk decreased the glucose absorption during subsequent perfusion of the small intestine in the absence of the bran. There is evidence that the consumption of some types of dietary fiber results in a quantitative alteration in gastrointestinal mucin.[6,7] Therefore, it has been proposed that these observations support the long-standing hypothesis that chronic ingestion of water-insoluble dietary fiber (IDF) with a bulk-forming property may slow nutrient absorption from the small intestine by thickening the unstirred water layer due to increased luminal mucin secretion.[3–5,8] Indeed, the presence of a gel-forming fiber film surrounding the villi may greatly increase the inhibitory effect on nutrient diffusion, and could become a rate-limiting factor to glucose absorption. However, such an effect from water-soluble dietary fiber (SDF) is still controversial at the usual dietary level of IDF that has no gel-forming property.

We have previously shown that the luminal mucin secretion reached a maximum within 5 d after starting IDF ingestion with rats, but that such a short-term ingestion of IDF did not disturb the glucose absorption.[9,10] This was also the case for ovalbumin (OVA) that has an overwhelmingly larger molecule than glucose and can be expected to be more disturbed by the mucin layer. Thus, we could not prove that the improved glucose tolerance observed in the previous studies was associated with an increased capacity for luminal mucin secretion. However, previous observations by Schwartz and Levine[4] and Dryden *et al.*[5] have been based on long-term experiments, and one might expect that there may be an overproduction of mucin and/or a qualitative change in mucin composition that would only take over after more chronic feeding. Cassidy *et al.*[8] and Satchithanandam *et al.*[11] have observed an

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Abbreviations: OVA, ovalbumin; IDF, insoluble dietary fiber; PSF, powdered polystyrene foam; SDF, soluble dietary fiber; SV, settling volume in water

increase in the proportion of sulphomucin as well as in the amount of total mucin in the small intestine of rats fed for 4 wk with several types of dietary fiber. Acidic mucins such as sulpho- and sialo-mucin confer different physicochemical properties from those of neutral mucins, resulting in higher viscosity in the lumen.[12] Higher viscosity of the mucus overlying the epithelial surface might have more effect on nutrient absorption in the intestine.

In the present study, therefore, we conducted a long-term feeding study of IDF for up to 4 wk in each defined period and examined the weekly rates of glucose and OVA absorption from the small intestine. Changed in the luminal mucin contents during the feeding period were measured by the usual method (*O*-linked oligosaccharide chains) and a newly prepared enzyme-linked immunosorbent assay (ELISA) method. Based on the present results, we discuss the validity of the long-standing hypothesis[3,8] that increased luminal mucin could delay luminal nutrient absorption.

Materials and Methods

Materials. We used powdered polystyrene foam (PSF) as a model IDF substance, because PSF with a higher value for the settling volume in water (SV) has shown a much more potent effect on luminal mucin secretion than other IDFs such as cellulose and wheat bran.[9] PSF with an experimentally determined expansion ratio of 54.9 (defined ratio = 60) was provided by JSP Co. (Tokyo, Japan). This expansion ratio is the value obtained by dividing 1,000 g/l as the density of the original polystyrene particle by the density (g/l) of the formed product. Polystyrene foam was powdered to adjust the mesh size to 30–50 by using a Wiley mill. SV is defined as the volume (ml) occupied by one gram of IDF after sedimentation equilibrium has been attained in water.[13] SV of PSF was determined to be 15.0 ml/g by the method described previously.[9]

Care of animals. Male rats of the Wistar strain (purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were housed in individual stainless steel cages with wire-screen bottoms in a room with controlled temperature (23 ± 2 °C) and lighting (light on from 08:00–20:00). The rats were fed on a control diet for at least 5 d for adaptation. This diet[10] was formulated from 250 g/kg of casein, 652.25 g/kg of cornstarch and 50 g/kg of corn oil. The remainder of the diet was vitamins and minerals.[14] The rats were divided into groups on the basis of body weight and allowed free access to the experimental diets and water. The body weight and food intake were recorded every morning before replenishing the diet. The study was approved by the Animal Use Committee of Shizuoka University, and the animals were maintained in accordance with the Shizuoka University guidelines for the care and use of laboratory animals.

Animal experiment. Thirty-six rats of the Wistar strain weighing 150–170 g were divided into 2 groups of 18 rats each after acclimatization and were allowed free access to the fiber-free control diet or diet containing 90 g of PSF/kg. Polystyrene foam was added to the diet at the expense of an equal amount of cornstarch. The rats were fed on the respective diets for 1, 2 and 4 wk. At the end of each experimental period, the diet was withdrawn from 08:00 to 20:00, and then the rats (6 per group in each period) were intubated with a mixed solution to provide 1 g of glucose and 250 mg of OVA per kg of body weight (a 0.47 ml solution/100 g of body weight). Blood samples (40 µl) were collected from the tail vein after various times (0, 15, 30, 60, 90 and 120 min). The plasma glucose concentration was determined with a commercially available kit (Glucose B test Wako, Wako Pure Chemical Industries, Tokyo, Japan). The plasma OVA concentration was measured by sandwich ELISA according to the method of Saito *et al.*[15] After blood sampling, the luminal contents were gathered by flushing with 15 ml of ice-cold phosphate-buffered saline (pH 7.4) containing 0.02 mol/l of sodium azide and then by the same volume of air. The contents were freeze-dried and stored for a luminal mucin analysis. The mucin fraction was isolated by the method of Lien *et al.*[16] with some modifications.[9] Mucin was recovered as a 60% ethanol precipitate of the supernatant and was finally dissolved in 5.0 ml of distilled water for subsequent analyses.[17]

O-Linked oligosaccharide chains. After an appropriate dilution of the mucin fraction, the *O*-linked oligosaccharide chains were measured by a fluorometric assay[18] that discriminated *O*-linked glycoproteins (mucin) from *N*-linked glycoproteins as described by Bovee-Oudenhoven *et al.*[19] Standard solutions of *N*-acetylgalactosamine (Sigma, MO, USA) were used to calculate the amount of oligosaccharide chains liberated from the mucins during the procedure.

Enzyme-linked immunosorbent assay of mucin. Mucin ELISA was performed as previously described.[20] Briefly, small intestinal mucins were purified according to the method of Satchithanandam *et al.*,[21] and the antiserum against rat mucin was prepared by the method of Vaitukaitis *et al.*, using rabbits.[22] The ELISA procedure was basically that of Satchithanandam *et al.*[21] except for the dilution rate of anti-mucin antiserum (1:12,000).

Statistical analyses. Data were analyzed by one-way ANOVA. Differences between the groups were analyzed by Student's *t*-test. The results are each expressed as the mean ± SE, and all statements of significant differences show the 5% level of probability. The statistical calculations were carried out by using Stat View 5.0 computer software (SAS Institute).

Table 1. Food Intake and Body Weight Gain of Rats Fed on the Fiber-Free Control and 9% PSF Diets for 1, 2 and 4 wk

	1 wk		2 wk		4 wk	
	Control	PSF	Control	PSF	Control	PSF
Food intake, g	103 ± 2	115 ± 4*	210 ± 7	226 ± 9	416 ± 13	458 ± 12*
Initial body weight, g	173 ± 4	173 ± 4	173 ± 3	174 ± 2	171 ± 4	173 ± 2
Final body weight, g	205 ± 3	201 ± 1	230 ± 3	233 ± 4	271 ± 5	268 ± 2
Body weight gain, g	32 ± 2	28 ± 5	57 ± 4	59 ± 3	100 ± 5	94 ± 4

Data are expressed as the mean ± SE (n = 6).
*P < 0.05 *vs.* control when analyzed by Student's *t*-test.

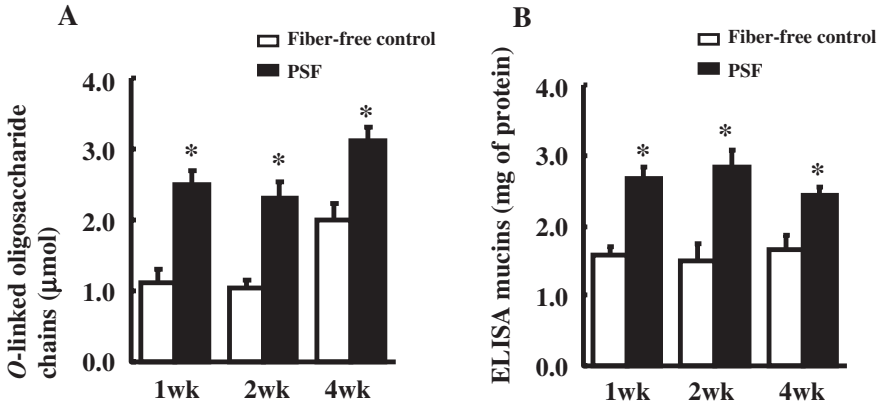

Fig. 1. Amounts of *O*-Linked Oligosaccharide Chains (A) and Mucins (B) in the Small Intestinal Contents of Rats Fed on the Respective Diets. Each value is the mean ± SE (n = 6). *P < 0.05 when analyzed by Student's *t*-test.

Results

At 1 and 4 wk, the food intake by the rats fed on the PSF diet were significantly higher than by those fed on the control diet, although the body weight gain was comparable between the groups in all experimental periods, suggesting that the animals had balanced their calorie yield by increasing the food consumption (Table 1). Both the amounts of *O*-linked oligosaccharide chains (Fig. 1A) and ELISA-determined mucin (Fig. 1B) in the small intestinal contents were significantly greater in the rats fed on the PSF diet than in those fed on the control diet in all experimental periods. This was particularly manifest in wk 1 and 2, the mucin content in the rats fed on the PSF diet being 100% more than that in the rats fed on the control diet. This enhanced mucin secretory effect by PSF ingestion lasted until at wk 4 (about 50% increase *vs.* the control). A significant correlation was observed between the amounts of *O*-linked oligosaccharide chains and ELISA-determined mucin ($y = 0.54 + 1.05$, $r = 0.694$, $P < 0.01$) (Fig. 2). On the other hand, the changes in serum concentrations of glucose (Fig. 3A) and OVA (Fig. 3B) did not differ between the dietary groups at any time during any experimental period.

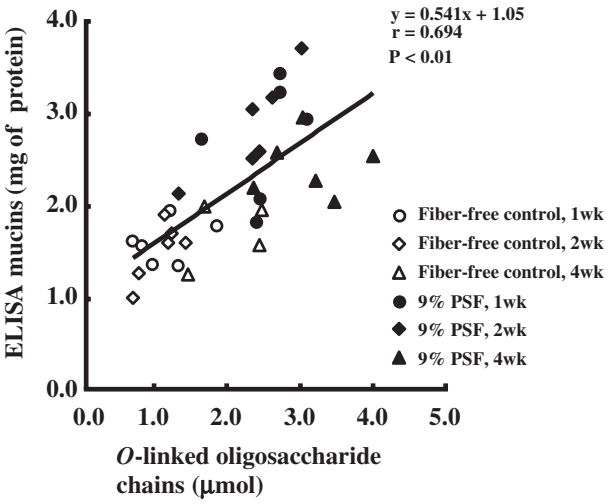

Fig. 2. Correlation between the Amounts of *O*-Linked Oligosaccharide Chains and Mucins.

Discussion

Many lines of evidence have suggested that the consumption of IDF resulted in a quantitative alteration in gastrointestinal mucin.[8,23–25] An increase in the relative number of mucin-secreting goblet cells was noted after feeding a fiber-containing diet.[26] Reduced rates of glucose[4,5] and lipid absorption[27] were observed after chronic feeding of wheat bran or cellulose, even after over-night fasting (in complete absence of fiber in the lumen). It has therefore been considered that

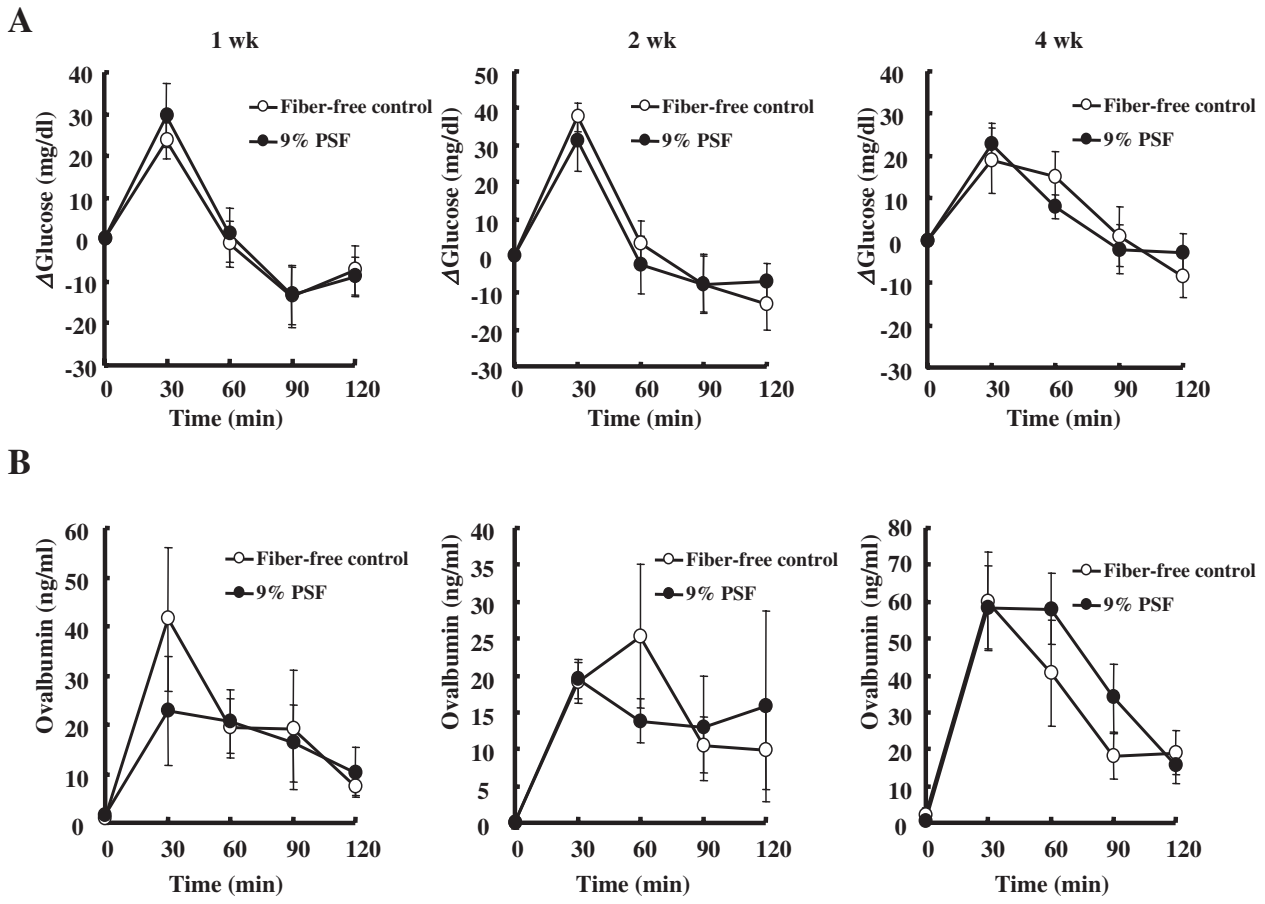

Fig. 3. Changes in Serum Glucose (A) and Ovalbumin (B) Concentrations after Oral Administration. Each point represents the mean ± SE (n = 6).

these chronic-feeding effects might have been due to the increased resistance of the unstirred water layer, or to a thickened mucin layer associated with the mucosal surface.[3)]

Although we did not distinguish between the acidic and neutral mucin, the present results show that the enhancement of total mucin secretion in the lumen lasted even after the chronic ingestion of IDF, and that the degree of stimulated mucin secretion was unchanged over an extended period of time (Fig. 1A, B). These results for the luminal mucin contents were confirmed by the usual analytical method (measurement of *O*-linked oligosaccharide chains) and mucin-specific method of ELISA. The highly significant correlation between the results of the two methods indicates an appropriateness of measurement of the luminal mucin contents (Fig. 2). Since mucin is highly viscous in nature and partially adheres to the surface of the epithelium, complete recovery of the mucin by flushing the lumen with a buffer might be difficult. However, Satchithanandam *et al.* have reported that when they measured the luminal mucin as the sum of "buffer-flushed," "surface-vacuumed" plus "tissue-scraped" mucin, "buffer-flushed" was found to contain the highest amount of mucin,[11)] suggesting that "buffer-flushed" in the present study could substantially reflect the total luminal mucin. Thus, it is plausible to conclude that luminal mucin continued to be secreted at the initially attained level even after a long-term period of ingestion of IDF.

The present results also clearly show that the increased luminal mucin content had no effect on the rates of luminal glucose and OVA absorption, even after 4 wk of feeding IDF (Fig. 3A, B). We recovered the luminal mucin by flushing and did not provide a detailed image of the actual location of mucin in the intestines. One might expect that if PSF promotes the abrasion of secreted mucin, it is conceivable that mucin will not effectively adhere to the mucosal surface, but rather be dragged in the lumen and therefore exert little effect on nutrient absorption, in spite of its abundance in the intestinal segments. However, when glucose and OVA were orally loaded, the diets were withdrawn from 08:00 to 20:00. This withdrawal may have left the stomach and small intestine free from PSF, but the luminal mucin contents were still greater in the PSF diet groups (Fig. 1A, B), indicating that stimulated mucin secretion continued to last after food depletion. It has also been observed that the mucin secretory effect from PSF ingestion lasted for 3–5 d even after ceasing PSF

ingestion.[10] It is therefore reasonable to assume that part of the mucin was adhering to the mucosal surface when the glucose/OVA absorption test was performed. Nevertheless, luminal glucose and OVA absorption was not disturbed in the rats chronically fed with the PSF diet. Hadler has shown that, in a 2.5% matrix of hyaluronate dissolved in buffered saline (pH 7.4), glucose diffusivity was enhanced 3-fold.[28] This fact suggests that, in a solution where a biopolymer network or matrix has been established, the fluid molecules are not necessarily stationary, but rather that the transport function is enhanced with regard to the random motion of nutrients. A recent study has shown that a lack of intestinal Muc1-mucin impaired the cholesterol uptake in mice, implicating that a physiological level of membrane-associated mucin would be necessary for normal intestinal uptake and absorption of cholesterol in mice.[29]

Taking these findings into consideration, we conclude that the long-standing hypothesis that the chronic ingestion of IDF with a bulk-forming property may slow nutrient absorption from the small intestine by thickening the unstirred water layer due to increased luminal mucin secretion[3–5,8] is invalid. The present results also cast doubt on the chronic effect of IDF ingestion on glucose tolerance described by Schwartz and Levine[3] and Dryden *et al.*[4] Ebihara and Kiriyama have also negated such a chronic effect on glucose tolerance or on the jejunal glucose absorption in rats after 8 to 9 wk ingestion of 10% cellulose and 5% pectin diets.[30]

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