Increased Luminal Mucin Does Not Disturb Glucose or Ovalbumin Absorption in Rats Fed Insoluble Dietary Fiber

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Abstract

We tested whether increased mucin secretion due to ingestion of insoluble dietary fiber (IDF) affects small intestinal nutrient absorption in rats. Polystyrene foam (PSF) with a true expansion ratio of 54.9 was used as a model for IDF with high bulk-forming properties. In Expt. 1, rats were fed a control diet or diet containing 50 g PSF/kg for 1, 3, 5, or 7 d. Small intestinal mucin fractions were isolated, and O-linked oligosaccharide chains were measured. The luminal mucin content reached a maximum within 5 d after PSF ingestion. In Expt. 2, rats were fed a control diet or diet containing 50 g PSF/kg for 7 d, and then all rats were switched to the control diet for 1, 3, or 5 d. The increased capacity for luminal mucin secretion disappeared within 5 d after ceasing PSF ingestion. In Expt. 3, rats were fed a control diet or diet containing 70 g PSF/kg for 7 d. Glucose (1g/kg) was administered orally after 12 h of food deprivation. The blood glucose concentrations did not differ between the groups. In Expt. 4, rats were fed a control diet or diet containing 90 g PSF/kg for 14 d. At d 7, portal cannulae were installed. A mixed solution of glucose (1g/kg) and ovalbumin (OVA, 250 mg/kg) was orally administered after 12 h of food deprivation, and responses of portal glucose and OVA concentrations were monitored for 120 min. Although luminal mucin contents were almost doubled in the 9% PSF group compared with the control group, neither portal glucose nor OVA concentration differed at any time point. The results suggest that the short-term ingestion of IDF significantly increases the luminal mucin content, but that this does not disturb nutrient absorption.


Introduction

Certain dietary fibers, particularly water-soluble, gel-forming fibers (SDF)4, such as guar and pectin, improve glucose tolerance in humans and rats when fed concurrently with an oral glucose load (1–4). These acute effects on glucose tolerance or glucose absorption rate are considered closely related to delayed gastric emptying (1,4) and delayed diffusion of intraluminal glucose (5,6) due to an augmented viscosity of the fiber-added solution. In contrast, water-insoluble dietary fibers (IDF) have little, if any, acute effect on glucose tolerance (5). However, Brodribb and Humphreys (7) showed improved oral glucose tolerance in patients with diverticular disease despite the fact that they chronically received IDF (25 g of wheat bran daily for 6 mo), suggesting that there may be a different mechanism for the effect on glucose tolerance rather than viscosity. These subjects had not ingested fiber for at least 12 h before the glucose tolerance test was performed. These findings have been substantiated in normal subjects (8). These chronic effects were also supported in some animal experiments where rats were fed a 10% cellulose- or a 30% coarse- but not fine-bran–supplemented diet for 5–8 wk (9,10). They suggested that the mechanism of chronic effect was probably different from that responsible for the effect of SDF on the acute feeding studies and might involve histological and functional adaptive changes of the mucosal surface of the small intestine. However, it should be noted that in contrast to the human experiments, whether such a chronic effect occurs in rats has not been resolved (5).

In general, consumption of IDF appears to enhance the total capacity of mucin secretion in the small intestinal lumen, although the stimulatory effect on mucin secretion depends not only on the quantity but also the quality of the ingested IDF (11–15). In recent experiments with rats, we found that small intestinal mucins were secreted in proportion to the settling volume in water (SV, a numerical representation for bulk-forming properties) of the indigestible components of diets, and that a prolonged ingestion of IDF was required for enhanced mucin secretion to appear (16,17). Before certain nutrients in the small intestinal lumen can interact with digestive and transport sites, it must pass a diffusion barrier that is considered to be mainly composed of glycocalyx and mucins (18,19). Accordingly, and

* Abbreviations used: OVA, ovalbumin; IDF, insoluble dietary fiber; PAS, periodic acid-Schiff reagent; PSF, polystyrene foam; SDF, soluble dietary fiber; SV, settling volume in water.
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as suggested by the results of previous studies, alterations in the amount and composition of mucin secreted into the lumen might modify nutrient absorption (9,10,14). However, further information assessing the relation among the augmented luminal mucins due to IDF ingestion, and the rate of nutrient absorption in the small intestine is scarce.

In the present study, we tested whether ingestion of IDF affects small intestinal nutrient absorption, and we focused especially on how it relates to the change in mucin secretion. For this purpose, we used polystyrene foam (PSF) with high SV value as an IDF preparation that has been shown to stimulate small intestinal mucin secretion (16). In preliminary experiments, we found that mucin secretion reaches its maximum within 5 d after starting PSF ingestion, and that this effect disappears within the same period after ceasing PSF ingestion. Based on these observations, the effect of IDF ingestion on the improvement of glucose tolerance was examined in rats fed a PSF-supplemented diet for 7 d. Glucose absorption rate in the small intestine was also assessed by monitoring portal blood glucose concentrations under unrestrained conditions. The short-term ingestion of PSF in the present study may minimize metabolic effects, such as body fat content and the related difference in insulin sensitivity (20), and may facilitate an understanding for the effect of IDF on glucose absorption solely from the perspective of luminal mucin content. Further, we examined the effect of PSF ingestion on the absorption of ovalbumin (OVA). Because OVA has a higher molecular weight than glucose, we expected that the luminal diffusion of OVA might be more affected by increased mucin secretion.

Materials and Methods

Materials. Polystyrene foam, with an experimentally determined expansion ratio of 54.9 (defined ratio = 60) was provided from JSP Co., Tokyo. The expansion ratio is the value obtained by dividing 1,000 g/L as the density of original polystyrene particle by the density (g/mL) of the formed product. Polystyrene foam was powdered to the mesh size of 30–50 using a Wiley mill. The SV measure was useful for determining the bulk-forming property of IDF in the lumen (16). SV is defined as the volume (mL) occupied by 1 g of IDF after sedimentation equilibrium is attained in water. The SV in water of PSF was determined to be 15.0 mL/g by the method described by Takeda and Kiriyama (21). Ovalbumin from chicken eggs was purchased from Wako Pure Chemicals.

Care of animals. Male rats of the Wistar or Sprague-Dawley strain (purchased from Shizuoka Laboratory Animal Center) 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 0800–2000). For adaptation, rats were fed a control diet for at least 5 d. This diet (16) 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 consisted of vitamins and minerals. Rats were then divided into groups on the basis of body weight and allowed free access to experimental diets and water. Body weight and food intakes were recorded every morning before replenishing the diet. The study was approved by the Animal Use Committee of Shizuoka University, Faculty of Agriculture, and animals were maintained according to the guidelines for the care and use of laboratory animals, Shizuoka University, Faculty of Agriculture.

Expt. 1. After being acclimated, 48 rats of the Wistar strain, weighing 127–144 g, were divided into 2 groups of 24 and allowed free access to the control diet or diet containing 50 g of PSF-60/kg. Polystyrene foam was added to diet at the expense of an equal amount of cornstarch. Rats were fed the respective diets for 1–7 d. At the end of experiment, diets were withdrawn from 1030 to 1830 to empty the stomach and small intestine. Rats (6/dietary group) were then refed 3 g of the respective diets from 1830 to 2000. Normally, rats consumed the diets completely within 90 min. Rats were killed with diethyl ether and the small intestine was excised. Luminal contents were gathered by flushing with 15 mL of ice-cold phosphate buffer saline (pH 7.4) containing 0.02 mol sodium azide/L and then by the same volume of air. The contents were freeze-dried and stored for luminal mucin analysis. For histologic evaluation, the upper half of the small intestine was defined as the jejunum, and the distal portion of the jejunum from each of the 6 rats fed the control and PSF diets for 7 d was removed and placed in 10% buffered formalin.

Expt. 2. After acclimation, 36 rats of the Wistar strain weighing 129–145 g were divided into 2 groups of 18 and were allowed free access to the control diet or diet containing 50 g of PSF-60/kg. After rats were fed the respective diets for 7 d, they were all switched to consume the same control diet for a successive 1–5 d. At the end of experiment, diets were withdrawn from 1030 to 1830. The rats (6/dietary group) were then refed 3 g of the control or PSF diet, as in the previous 7–d dietary history, during 1830–2000. The gathering and preparing of luminal contents were conducted in a similar manner as for Expt. 1. For histologic evaluation, the distal jejunum from each of 6 rats fed the control diet for the successive 5 d were removed and placed in 10% buffered formalin.

Expt. 3. After acclimation, 18 rats of the Wistar strain weighing 133–155 g were divided into 2 groups of 9 and were allowed free access to the control diet or diet containing 70 g of PSF-60/kg for 7 d. After diets were withdrawn from 0800 to 2000, rats were intubated with glucose solution to provide 1 g of glucose/kg body weight (0.47 mL/100 g body weight). Blood samples (20 μL) were collected from the tail vein at time intervals of 0, 15, 30, 60, and 90 min. Plasma glucose concentrations were determined with a commercially available kit (Glucose B test Wako, Wako Pure Chemical Industries). After blood sampling, the gathering and preparation of luminal contents were conducted in the same manner as for Expt. 1.

Expt. 4. Eighteen rats of the Sprague-Dawley strain weighing 270–282 g (2 groups of 9 rats) were maintained in the same manner as for Expt. 3, except the dietary level of PSF was at 90 g/kg diet. After rats were fed the respective diets for 7 d, rats were subjected to the portal cannulation at 0800–1000. Cannulation into the portal vein was performed by the method of Matsuura et al. (22) with slight modification. Under pentobarbital anesthesia (0.24 mmol/kg Nembutil, Abbott Laboratories), a midline incision was made in the abdominal cavity and the portal vein was exposed. The tip (2 mm length) of portal cannula was inserted directly into the portal vein near the junction of the splenic vein and was fixed in situ with a surgical bond (Aron Alpha A, Sankyo). The cannula was composed of 2-mm long polyethylene tube (0.28 mm i.d., 0.61 mm o.d., Intramedic PE 10, Clay Adams) successively connected with 8-cm long silicone tube (0.5 mm i.d., 1.0 mm o.d., Ficon Tube, 00, Fuji Systems), 5-cm long polyethylene tube and 15-cm long silicone tube. The cannula was fastened to the abdominal wall, and then the end of the cannula was passed subcutaneously to the dorsal base of the neck and was stopped with −1 cm protruding from the skin. The cannula was filled with heparinized saline solution (30 KU of heparin/L) except at sampling times. After the rats were further fed the respective diets for 6 d, diets were withdrawn from 0800 to 2000. Rats were then orally administered a mixed solution to provide 1 g of glucose and 250 mg of OVA/kg body weight. Before blood sampling, an adequate volume of saline was injected through the cannula, and then portal blood samples (50 μL) were collected at time intervals of 0, 10, 20, 30, 45, 60, 90, and 120 min. After blood sampling, luminal contents were gathered and prepared in the same manner as for Expt. 1.

Preparation of mucin fraction. Mucin fraction was isolated by the method of Lien et al. (23) with some modification (24). Briefly, total freeze-dried samples were suspended in sodium chloride solution (0.15 mol/L) containing 0.02 mol sodium azide/L at 4°C. Samples were homogenized for 1 min and immediately centrifuged at 10,000 × g for 30 min to obtain supernatant. Mucins were recovered as a 60% ethyl alcohol precipitate of the supernatant and were finally dissolved in 3.0 mL distilled water for SDS/PAGE analysis and for determinations of sialic acid and O-linked oligosaccharide chains as markers of mucin content.

SDS/PAGE analysis of PAS-reactive substances. The mucin fraction prepared from the small intestinal contents was analyzed by SDS/PAGE (3% stacking gel/4% running gel) according to the method of Tytgat et al.
Part of the mucin fraction (0.1 mL) was hydrolyzed with 6 N HCl at 100°C clearly showed that the mucin fraction (Expt. 1) obtained from rats fed the control diet or PSF diet for 1 day (Expt. 1) contained significantly more mucin markers than those obtained from rats fed the control diet or PSF diet for 3 days (Expt. 1)

**Statistical analyses.** Data were analyzed by 1- or 2-way ANOVA or 2-way repeated measures ANOVA. Differences between the groups were analyzed by Student’s t-test. Results were expressed as means ± SEM. Differences were considered significant at P < 0.05. Statistical calculations were carried out using Stat View 5.0 computer software (SAS Institute).

**Results**

**Stimulatory effect of PSF on mucin secretion (Expt. 1).** Except on d 3, food intakes did not differ between the groups, and body weight gain did not differ at any time (Table 1). The amounts of PAS-reactive substances, sialic acid, and O-linked oligosaccharide chains in mucin fractions from the small intestinal contents were determined as mucin markers. At d 1 and d 3, these mucin markers did not differ between the groups, but at d 5 and d 7, levels were higher in PSF-fed rats than in controls (Table 1). Histologic evaluation showed that there were more goblet cells (densely stained particles) in rats fed the PSF diet than in those fed the control diet. At d 3, mucin markers in the PSF group were greater only in the amount of O-linked oligosaccharide chains than the control group. On d 5, mucin markers did not differ between control and PSF groups.

**Effects of ceasing PSF ingestion on mucin secretion (Expt. 2).** There were no significant differences in food intake and body weight gain between the groups at any experimental periods (Table 2). At d 1, where rats in both groups were fed the control diet for 1 day after the 7-d feeding of the respective diets, all mucin markers continued to be greater in rats previously fed the PSF diet than in those previously fed the control diet. But at d 3, mucin markers in the PSF group were greater only in the amount of O-linked oligosaccharide chains than the control group. On d 5, mucin markers did not differ between control and PSF groups.

**Histologic evaluation.** For each rat, 6 sections of distal jejunum, 5 μm thick, were prepared from paraffin-embedded samples stained with periodic acid-Schiff reagent (PAS) for sugars (glycoproteins) according to Konad et al. (26). The density of the PAS stained area was scanned and analyzed using NIH image as described previously (16).

**O-linked oligosaccharide chains.** After an appropriate dilution of mucin fraction, O-linked oligosaccharide chains were measured using a fluorimetric assay (27) that discriminated O-linked glycoproteins (mucin) from N-linked glycoproteins as described by Bovee-Oudenhoven et al. (28). Standard solutions of N-acetylgalactosamine (Sigma) were used to calculate the amount of oligosaccharide chains liberated from mucins during the procedure.

**Plasma OVA analysis.** The plasma OVA concentration was measured by sandwich ELISA according to the method of Saito et al. (29).

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**Figure 1** Light micrographs of distal jejunums from rats fed control (A, C) or 50 g/kg PSF (B, D) diets for 7 days (Expt. 1; A, B) or 5 days after switching to the control diet (Expt. 2; C, D). Micrographs are stained with PAS reagent and hematoxylin. Magnification = 200×.
TABLE 2  Cumulative food intake, total body weight gain, and the amount of small intestinal mucins in rats fed the control or 50 g/kg PSF diet for 7 d and then fed the control diet for the next 1, 3, or 5 d (Expt. 2)\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>d 1(^2)</th>
<th>d 3</th>
<th>d 5</th>
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<td>PSF</td>
<td>Control</td>
<td>PSF</td>
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<tr>
<td>Cumulative food intake, g</td>
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<td>115 ± 3</td>
<td>143 ± 4</td>
<td>143 ± 5</td>
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<td>Total body weight gain, g</td>
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<td>42 ± 1</td>
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<td>PAS-reactive substance, AU(^4)</td>
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<td>922 ± 28*</td>
<td>672 ± 80</td>
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<td>Glycemic acid, (\mu)mol</td>
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<td>1.1 ± 0.03**</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>(O)-linked oligosaccharide chain, (\mu)mol</td>
<td>2.3 ± 0.2</td>
<td>3.1 ± 0.1**</td>
<td>2.0 ± 0.2</td>
<td>2.6 ± 0.1*</td>
</tr>
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\(^1\) Values are means ± SEM, \(n = 6\). Asterisks indicate different from control, *\(P < 0.05\), **\(P < 0.01\).
\(^2\) Day of autopsy. The rats previously consumed the control or PSF diet for 7 d, were killed at d 1, d 3, and d 5 after switching to the same control diet. Therefore, food intake and body weight gain represent values for 8, 10, or 12 d in total.
\(^3\) Significant effects (\(P < 0.05\) of diet \(d\), time \(t\) and their interaction \(d \times t\)).
\(^4\) Arbitrary unit.

**PSF ingestion and glucose tolerance (Expt. 3).** Food intake (control, 105 ± 2 g; PSF, 113 ± 3 g) and body weight gain (control, 183 ± 3 g; PSF, 180 ± 3 g) for 7 d did not differ between the groups. Although the amount of \(O\)-linked oligosaccharide chains in the small intestinal contents was significantly greater in rats fed the PSF diet (3.0 ± 0.3 \(\mu\)mol) than in those fed the control diet (1.6 ± 0.2 \(\mu\)mol), the glycemic response after oral loading of glucose did not differ between the groups (data not shown).

**PSF ingestion and the small intestinal absorption of glucose and OVA (Expt. 4).** Except on the day of portal cannulation, body weight gain and food intake were constant (Fig. 3). Total food intake for 14 d was greater in rats fed the PSF diet (272 ± 8 g) than in those fed the control diet (250 ± 8 g), but total body weight gain did not differ between the control (54 ± 3 g) and PSF groups (50 ± 2 g). Although the amount of \(O\)-linked oligosaccharide chains in the small intestinal contents significantly differed between the control (1.8 ± 0.3 \(\mu\)mol) and 9% PSF (3.8 ± 0.3 \(\mu\)mol) groups, neither portal glucose nor OVA concentration differed significantly at any time point (Fig. 4).

**Discussion**

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 (18,19), leading to a general consideration that an increase in luminal mucin secretion may modify the rate of nutrient absorption (30). Schwartz and Levine (9) have reported that after the ingestion of a 10% cellulose diet for 5 wk, glucose tolerance was slightly but significantly improved in rats. Also, Dryden et al. (10) showed that the ingestion of 30% coarse bran diet for 8 wk decreased glucose absorption during subsequent perfusion of the small intestine in the absence of bran. Using a scanning electron microscope, Cassidy et al. (31) showed that the ingestion of wheat bran stimulated goblet cell activity, resulting in a greater quantity of mucins being produced at the surface of the jejunal. Consequently, these observations support their hypothesis that chronic ingestion of IDF may slow nutrient absorption by the small intestine via the thickening of the unstirred water layer as a result of increased luminal mucin secretion (9,10,14,30).

The present study examined whether an ingestion of IDF affects small intestinal nutrient absorption solely from increased mucin secretion. We first examined the duration required for an increased luminal mucin secretion when rats were fed PSF, a model substance for high bulk-forming IDF (16). Judged from the amounts of PAS-reactive substances, sialic acid, and \(O\)-linked oligosaccharide chains in the small intestinal mucin fractions, we found that mucin secretion reached its maximum within 5 d of PSF feeding and that this effect disappeared within the same time after ceasing PSF ingestion (Fig. 2). These changes in capacity for mucin secretion were likely to be associated with the number of goblet cells migrated on the villi (Fig. 1). Kinetic analysis of goblet cell dynamics in the small intestine showed that oxidative, cells migrated from the baseline of the crypt to the villus tip, where they are sloughed into the lumen (32). This process is accomplished by 3–5 d cycle in the rat (33). Therefore, it is possible to assume that the increased capacity for luminal mucin secretion by IDF ingestion is connected with goblet cell turnover, as Vahouny et al. (34) suggested. At present, the precise mechanism by which IDF ingestion stimulates proliferation of goblet cells and increases luminal mucin secretion is not fully understood. But the fact that small intestinal mucins are secreted in proportion to the SV of ingested IDF (16) or water-holding capacity of whole diet (35) may suggest that more bulky chyme and more abrasion of the mucus might be counter-balanced by enhanced synthesis and secretion by goblet cells (30). Nevertheless, it is clear that a long-term ingestion of IDF is not necessary for the maximum induction of mucin secretory activity.

Second, we performed a glucose tolerance test in rats after short-term ingestion (7 d) of the 7% PSF diet. In these rats, the amount of small intestinal mucins, as assessed by \(O\)-linked oligosaccharide chains, increased significantly by 80% compared with that in the control group, but responses of the peripheral blood glucose concentrations in both groups were virtually the same (Expt. 3). This simply means that the increased luminal mucins did not work in the present experimental condition as a diffusion barrier against glucose transport. Thus, we were unable to confirm that the improved glucose tolerance observed in previous studies by Schwartz and Levine (9) and Dryden et al. (10) was associated with an increased capacity of the luminal mucin secretion. Accordingly, it is possible to assume that such a chronic effect of IDF on glucose tolerance might be partially due to a change in glucose metabolism through a reduced body-fat accretion and subsequent changes in insulin sensitivity (20). Additionally, Ebihara and Kiriyama (5) reported that they could not confirm such a chronic effect on glucose tolerance after rats were fed 10% cellulose diet for 9 wk, suggesting that a chronic effect of IDF per se is still controversial in rats.
Nimmerfall and Rosenthaler (36) reported that the mucus layer acts like a molecular sieve; absorption of molecules is directly proportional to the molecule’s diffusion rate through the mucus layer, and diffusion rate is indirectly proportional to molecular weight. Part of the orally administered OVA is absorbed from the intestine intact (MW; 45,000), without being digested, even in healthy rats, and is mainly detected in portal blood (37). Finally, we examined the effects of IDF ingestion on the intestinal absorption rate of glucose and OVA in rats fed the 9% PSF diet for 14 d. Portal glucose and OVA concentrations were monitored under unrestrained conditions using portal-cannulated rats. We expected that OVA absorption in the intestine would be depressed because it is a large molecule and its absorption rate is considerably slower than that of glucose; OVA is reported to be mainly absorbed in the distal part of the intestine where stored mucin on the villi is predominantly distributed (38). However, the portal OVA and glucose concentrations between the groups did not differ at any time interval, although the luminal mucin content in rats fed the 9% PSF diet increased by 100% compared with those fed the control diet (Fig. 4). Therefore, we assume that the increased mucin secretion by IDF ingestion does not affect nutrient absorption in the small intestine. Previous studies reported that glucose absorption from the intestine was reduced when the intestine was perfused with SDF-added glucose solution (6,39). Indeed, the presence of gel-forming fibers’ films surrounding the villi may greatly increase the inhibitory effect on nutrient diffusion and can become a rate-limiting factor to glucose absorption. However, this is not the case for the usual dietary levels of IDF that have no gel-forming properties in solution.

Taking these findings into consideration, we conclude that the increased capacity for luminal mucin secretion by IDF ingestion is connected with goblet cell turnover and reaches maximum within 5 d, but the increased luminal mucin secretion per se does not disturb the small intestinal nutrient absorption in rats under physiological conditions.

**Literature Cited**


![Figure 2](https://academic.oup.com/jn/article-abstract/136/10/2486/4746684/figure-2)

**Figure 2** Relative amounts of PAS-reactive substances (A), sialic acids (B), and O-linked oligosaccharide chains (C) in small intestinal contents from rats fed the control diet or diet containing 50 g/kg PSF (Expts. 1 and 2). Values are means ± SEM, n = 6. Different from control, P < 0.05.

![Figure 3](https://academic.oup.com/jn/article-abstract/136/10/2486/4746684/figure-3)

**Figure 3** Changes in body weight (A) and cumulative food intake (B) in rats fed the control diet or diet containing 90 g/kg PSF for 14 d (Expt. 4). On d 8, portal cannulation was performed, and rats were fed the diets for 6 d. Values are means ± SEM, n = 8. *Different from control at that time, P < 0.05.

![Figure 4](https://academic.oup.com/jn/article-abstract/136/10/2486/4746684/figure-4)

**Figure 4** Change in plasma glucose (Glu) and ovalbumin (OVA) concentrations after oral loading in rats fed the control diet or diet containing for 14 d (Expt. 4). Values are means ± SEM, n = 8. Data were analyzed by 2-way repeated measures ANOVA.

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