In Vitro Study of Possible Role of Dietary Fiber in Lowering Postprandial Serum Glucose

Shiyi Ou,*,† Kin-chor Kwok,‡ Yan Li,† and Liang Fu†

Research Center of Food Science and Technology, Jinan University, Guangzhou 510632, People's Republic of China, and Department of Applied Biology and Chemical Technology, Hong Kong Polytechnic University, Hong Kong

There have been many reports concerning the role of dietary fiber in lowering postprandial serum glucose, and the main mechanism was regarded as the viscosity of different dietary fibers in hampering diffusion of glucose and postponing absorption and digestion of carbohydrates. In this paper, two kinds of water-insoluble dietary fibers, water-insoluble dietary fiber of wheat bran and enzyme-resistant starch of maize amylose, and four kinds of water-soluble dietary fibers, water-soluble dietary fiber of wheat bran, carboxymethyl cellulose, guar gum, and xanthan gum, were used to investigate their postprandial serum glucose lowering mechanism in vitro. The results showed that these dietary fibers lowered postprandial serum glucose levels at least by three mechanisms. First, dietary fibers increase the viscosity of small intestine juice and hinder diffusion of glucose; second, they bind glucose and decrease the concentration of available glucose in the small intestine; and, third, they retard α-amylase action through capsuling starch and the enzyme and might directly inhibit the enzyme. All of these decreased the absorption rate of glucose and the concentration of postprandial serum glucose.

Keywords: *Dietary fibers; serum glucose; lowering mechanisms*

INTRODUCTION

The dietary factors that can mitigate the symptoms of diabetes have been described by many researchers (*1*−*7*). Of these, dietary fibers and resistant starch have been reported to have beneficial effects in lowering the glycemic level in serum by (1) increasing the growth and function of the upper gastrointestinal tract as well as the plasma levels of the intestinotrophic factor, glucagons-like peptide 2 (*8*), (2) lowering the insulin response (*9*), and (3) slowing glucose absorption through an effect on gastric emptying and/or entrapment of materials in the viscous digesta (*10*).

Viscous water-soluble dietary fibers have the effects of hampering the diffusion of glucose and postponing the absorption and digestion of carbohydrates, thus resulting in lowered postprandial blood glucose (*11*). However, the viscosity increase by dietary fiber does not seem to give an adequate explanation of the extent of the decrease in blood glucose. Liljeberg et al. (*12*) reported that the blood glucose was decreased more than 50% in healthy men with high dietary fiber bread intake within 30−70 min as compared to those who ingested barley or oat porridge. Schweizer et al. (*13*) compared serum glucose response of a study group with low enzyme-resistant starch (RS) intake and a group with high RS intake and found that the glucose index of the former was 100% higher than that of the latter, although the former only took 16% more starch than the latter. RS is a water-insoluble dietary fiber, which would not greatly increase the viscosity of the small intestine's digesta. These results suggested that there could be some other mechanisms for dietary fibers to lower postprandial serum glucose. The objective of this work was to investigate the possible roles of dietary fibers in this process.

* Author to whom correspondence should be addressed (e-mail tosy@jnu.edu.cn).

† Jinan University.

‡ Hong Kong Polytechnic University.

MATERIALS AND METHODS

Dietary Fibers. Water-soluble dietary fiber (WSDF) and water-insoluble dietary fiber (WIDF) were prepared according to the methods of Ou et al. (*14*). High-amylose maize starch was used to prepare enzyme-resistant starch (RS) according to the method described by Sivert and Pomeranz (*15*): 100 g of deproteinated high-amylose maize starch (70%) was mixed with 400 g of water and heated in an autoclave at 121 °C for 1 h. The gelatinized starch was kept at 4 °C for 12 h. After four cycles of heating (121 °C) and cooling (4 °C), thermally stable α-amylase (pH 6.0, 100 °C, 30 min) and glucoamylase (pH 4.5, 60 °C, 30 min) were used to hydrolyze the non-RS starch. After filtration, the residue was washed with alcohol and deionized water, dried, and ground. Xanthan, carboxymethyl cellulose (CMC), and guar gum were purchased from Sigma Chemical Co., St Louis, MO. All of the dietary fibers were passed through a 100-mesh sieve before use.

Effect of Dietary Fiber on Diffusion of Glucose in a Glucose—Dietary Fiber System. The glucose−dietary fiber system comprised 100 mmol/L of glucose and one of the following dietary fibers: WSDF, CMC, xanthan gum, guar gum, WIDF, and RS. The concentrations of the dietary fibers used in the system were 0.8% (w/v) for WSDF, CMC, xanthan gum, and guar gum and 2.0% (w/v) for WIDF and RS. A total of 21 samples (3 replicates of 6 different dietary fibers and a blank), 25 mL each, were dialyzed in dialysis bags with a cutoff molecular weight of 12000 against 200 mL of deionized water at pH 7.0 and 37 °C. The glucose content in 2.0 mL of the dialysate was determined after 10, 20, 30, 60, 90, 120, 150, 180, and 300 min according to the hexokinase method of Yokoyama et al. (*11*).

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Table 1. Effect of Dietary Fiber on Dialysis of Glucose

	glucose in dialysate (μmol)								
dietary fiber	10 min	20 min	30 min	60 min	90 min	120 min	150 min	180 min	300 min
control	183.6 ± 1.4[a]	352.4 ± 1.8	504.8 ± 2.0	909.2 ± 2.6	1287.4 ± 3.4	1593.6 ± 3.7	1885.9 ± 3.5	2203.8 ± 4.1	2456.0 ± 3.9
WIDF[b]	148.3 ± 1.1	312.7 ± 1.6	441.9 ± 2.1	763.4 ± 2.2	1065.8 ± 2.3	1323.9 ± 3.1	1578.4 ± 2.9	1845.6 ± 3.7	2078.8 ± 3.4
RS[c]	153.2 ± 1.7	314.3 ± 1.9	458.9 ± 1.8	814.2 ± 2.4	1186.4 ± 2.7	1479.5 ± 3.4	1783.6 ± 3.4	2024.9 ± 3.1	2083.5 ± 3.6
WSDF[d]	135.6 ± 1.5	282.7 ± 2.2	403.4 ± 2.3	752.7 ± 2.5	1048.0 ± 2.5	1350.1 ± 3.5	1626.4 ± 3.2	1830.1 ± 3.3	2363.8 ± 3.7
CMC[e]	143.0 ± 1.8	294.5 ± 2.3	408.6 ± 1.7	774.5 ± 1.8	1101.3 ± 2.7	1379.4 ± 2.9	1647.8 ± 3.0	1883.5 ± 3.6	2368.4 ± 4.2
guar gum	128.6 ± 1.3	267.0 ± 1.6	384.2 ± 1.9	722.4 ± 2.3	1014.5 ± 2.3	1304.8 ± 3.2	1566.2 ± 2.7	1763.4 ± 3.1	2343.5 ± 4.1
xanthan gum	131.8 ± 0.8	278.4 ± 1.5	392.6 ± 2.3	728.0 ± 1.9	1034.8 ± 3.1	1324.4 ± 2.6	1612.8 ± 3.2	1859.3 ± 3.7	2369.4 ± 3.8

[a] Mean values ± standard deviation. [b] WIDF, water-insoluble dietary fiber from wheat bran. [c] RS, enzyme-resistant starch from maize. [d] WSDF, water-soluble dietary fiber from wheat bran. [e] CMC, carboxymethyl cellulose.

Table 2. Maximum Velocity of Glucose Diffusion in Glucose−Dietary Fiber System

	dietary fiber						
	control	WIDF[e]	RS[f]	WSDF[g]	CMC[h]	guar gum	xanthan gum
V_{max} (μmol/min)	15.79 ± 0.13[a]	13.04 ± 0.11[cd]	14.79 ± 0.12[b]	13.15 ± 0.10[cd]	13.59 ± 0.10[c]	12.74 ± 0.09[d]	12.41 ± 0.11[d]

[a−d] Mean values (mean ± SD, determined in three replicates) with the same superscript are not significantly different at the 5% level. [e] WIDF, water-insoluble dietary fiber from wheat bran. [f] RS, enzyme-resistant starch from maize [g] WSDF, water-soluble dietary fiber from wheat bran. [h] CMC, carboxymethyl cellulose.

The maximum diffusion velocity of glucose (V_{max}) was calculated as follows. The experimental data were fitted with an equation of parabola: $Y = ax^2 + bx + c$, where Y is the glucose content (μmol); x is time (min); and a, b, and c are coefficients. The equation to calculate the diffusion rate (Y') at any time is $Y' = 2ax + b$. When x is close to 0, $Y' = V_{max} = b$.

Effect of Dietary Fiber on Diffusion of Glucose in a Starch—α-Amylase—Dietary Fiber System. Forty grams of potato starch was added to ~900 mL of 0.05 M phosphate buffer (pH 6.5). The solution, after stirring at 65 °C for 30 min, was made up to a final volume of 1000 mL to give a 4% (w/v) starch solution. The starch−α-amylase−dietary fiber system comprised the above starch solution, 0.4% (w/v) α-amylase, and one of the following dietary fibers: WSDF, CMC, xanthan gum, guar gum, WIDF, and RS. The concentrations of the dietary fibers used in the system were 0.8% (w/v) for WSDF, CMC, xanthan gum, and guar gum and 2.0% (w/v) for WIDF and RS. Twenty-five milliliters of each of these solutions was dialyzed against 200 mL of deionized water at 37 °C. The glucose content in the dialysate was measured at 20, 30, 60, 90, 120, 150, and 180 min.

Effect of Dietary Fiber on Adsorption of Glucose. One gram of RS or WIDF was added to 100 mL of glucose solution (concentration ranging from 0.5 to 100 mmol/L). The mixture was stirred, held in a water bath at 37 °C for 6 h, and then centrifuged at 4000*g* for 20 min. The glucose content in the supernatant was determined.

WSDF, CMC, xanthan gum, or guar gum [0.8% (w/v)] was added to 25 mL of glucose solution (concentration ranging from 0.5 to 100 mmol/L). The solution mixtures were dialyzed against 200 mL of deionized water at 37 °C. The glucose content in the dialysate was determined after 6 h.

Effect of Dietary Fiber on the Activity of α-Amylase. One gram of the dietary fiber was mixed, with stirring, with 100 mL of potato starch solution in a 200 mL beaker. One gram of α-amylase was then added to this solution mixture and stirred vigorously at 37 °C. After 30 min, 0.1 mol/L NaOH was added to terminate the α-amylase activity. The glucose content of the solution mixture was determined.

RESULTS AND DISCUSSION

Effects of Dietary Fiber on Diffusion of Glucose. Diffusion rates of glucose were affected by dietary fibers (Table 1). Diffused glucose for water-soluble dietary fibers (WSDF, xanthan gum, CMC, and guar gum) treatments was 70−77.9 and 78.2−82.8% of control at 10 and 60 min, respectively. Compared with water-soluble dietary fibers, diffused glucose was less affected by water-insoluble dietary fibers, with 80.8−83.4 and 84.0−89.8% of control at 10 and 60 min, respectively (Table 1). Maximum diffusion velocity decreased in the order control > RS > CMC > WIDF ~ WSDF > guar gum ~ xanthan gum (Table 2). Obviously, the decreased extent of glucose across the membrane was much less than that reported by Liljeberg et al. (*12*) in vivo. Although the situation for absorption in the small intestine could not be completely judged by the results from Table 1 because the former is an active process, it is difficult to explain the results of our experiments only by the hypothesis that the effect of dietary fibers on the diffusion was mainly due to their viscosity. First, the diffusion rate of glucose would decrease as time increased (see control in Table 1), but glucose diffusion of dietary fibers treatment did not slow until 60 min, especially for WSDF, CMC, and xanthan. Second, the diffusion rate of glucose was decreased by water-insoluble dietary fibers even if they contributed little to the viscosity of the solution. Third, there was a difference in diffused glucose between dialysate from dietary fibers and control when dialysis reached equilibrium (300 min; the results at 12 h proved 300 min was enough for equilibrium). These phenomena can be explained by adsorption of dietary fibers for glucose. At the beginning of dialysis, diffusion of glucose was affected by adsorption and viscosity of dietary fibers; thus, the diffusion rate of glucose was slow, although the concentration in the dialysis bag was high. As the adsorption saturated, the diffusion of glucose was affected only by the viscosity of dietary fibers, and the diffusion rate was not significantly decreased even when the concentration of glucose in the dialysis bag decreased. As dialysis reached equilibrium, the difference of glucose in the dialysate between control and treatment can be regarded as the amount of glucose adsorbed.

In the system of α-amylase, starch, and dietary fibers, the glucose in the dialysate at 180 min was 32.9−45.8% of the control, suggesting that glucose diffusion in the system of α-amylase, starch, and dietary fibers was much more affected by dietary fibers than that in the glucose−dietary fibers system (Tables 1 and 3).

Effect of Dietary Fiber on the Adsorption of Glucose. A series of different concentrations of glucose were used to prove our above speculation and to

Table 3. Effect of Dietary Fiber on Dialysis of Glucose Produced by α-Amylase-Catalyzed Potato Starch

	glucose in dialysate (μmol)						
dietary fiber	20 min	30 min	60 min	90 min	120 min	150 min	180 min
control	70.2 ± 1.8[a]	113.5 ± 1.9	268.4 ± 2.3	403.6 ± 3.1	548.3 ± 3.5	702.5 ± 4.5	845.5 ± 4.9
WIDF[b]	58.5 ± 1.5	89.3 ± 2.1	183.6 ± 1.8	312.7 ± 2.9	424.8 ± 3.2	483.4 ± 3.4	545.5 ± 3.8
RS[c]	45.4 ± 1.7	90.5 ± 1.7	211.6 ± 2.1	347.2 ± 3.1	448.4 ± 3.7	514.6 ± 3.7	567.2 ± 3.5
WSDF[d]	32.8 ± 1.3	58.6 ± 1.3	123.8 ± 1.3	212.4 ± 2.7	306.4 ± 2.7	388.5 ± 3.1	476.8 ± 3.4
CMC[e]	33.6 ± 1.3	59.8 ± 1.3	128.2 ± 1.6	218.3 ± 2.3	304.8 ± 3.1	376.7 ± 2.9	464.2 ± 3.2
guar gum	31.7 ± 1.4	54.5 ± 1.5	116.3 ± 1.4	204.2 ± 2.4	296.2 ± 3.3	372.6 ± 3.4	458.1 ± 3.7
xanthan gum	34.5 ± 1.7	58.4 ± 1.1	125.2 ± 1.6	208.6 ± 2.5	307.5 ± 2.6	383.8 ± 3.6	475.2 ± 3.8

[a] Mean values ± standard deviation. [b] WIDF, water-insoluble dietary fiber from wheat bran. [c] RS, enzyme-resistant starch from maize. [d] WSDF, water-soluble dietary fiber from wheat bran. [e] CMC, carboxymethyl cellulose.

Table 4. Glucose Bound by Dietary Fiber in Different Concentrations of Glucose

	glucose bound[g] (μmol/g)					
dietary fiber	100 mmol/L	50 mmol/L	10 mmol/L	5 mmol/L	1 mmol/L	0.5 mmol/L
WIDF[b]	555.6 ± 3.4[a]	478.3 ± 3.6	205.6 ± 2.6	98.7 ± 0.9	27.3 ± 0.5	ND
RS[c]	413.5 ± 3.3	324.3 ± 2.7	186.7 ± 2.3	48.5 ± 1.1	24.5 ± 0.5	ND
WSDF[d]	454.2 ± 3.5	386.8 ± 3.4	91.5 ± 1.9	26.4 ± 0.7	ND[f]	ND
CMC[e]	418.3 ± 2.9	352.4 ± 2.7	72.8 ± 2.3	28.3 ± 0.6	ND	ND
guar gum	538.4 ± 3.3	462.3 ± 3.5	138.9 ± 1.7	49.4 ± 0.7	ND	ND
xanthan gum	431.5 ± 3.1	362.8 ± 2.4	94.5 ± 0.9	25.8 ± 0.5	ND	ND

[a] Mean values ± standard deviation. [b] WIDF, water-insoluble dietary fiber from wheat bran. [c] RS, enzyme-resistant starch from maize. [d] WSDF, water-soluble dietary fiber from wheat bran. [e] CMC, carboxymethyl cellulose. [f] ND, no determination. [g] For WIDF and RS, glucose bound = (glucose concentration of original solution − glucose concentration when the adsorption reached equilibrium) × volume of solution ÷ weight of dietary fiber. For water-soluble dietary fiber, glucose bound = [glucose concentration (μmol/mL) in retentate before start of diffusion × 25 (volume of retenate, mL) − glucose concentration (μmol/mL) in dialysate after 6 h of diffusion × 225 (total volume of retenate and dialysate)] ÷ weight of dietary fiber.

Table 5. Effect of Dietary Fiber on the Activity of α-Amylase

	dietary fiber						
	control	WIDF[f]	RS[g]	WSDF[h]	CMC[i]	guar gum	xanthan gum
glucose produced (μmol/h/g)	1036.2 ± 4.9[a]	923.4 ± 4.3[b]	826.6 ± 4.2[e]	850.7 ± 4.5[d]	829.8 ± 4.3[e]	855.7 ± 4.2[d]	878.0 ± 4.1[c]

[a−e] Mean values (mean ± SD, determined in three replicates) with the same superscript are not significantly different at 5% level. [f] WIDF, water-insoluble dietary fiber from wheat bran. [g] RS, enzyme-resistant starch from maize. [h] WSDF, water-soluble dietary fiber from wheat bran. [i] CMC, carboxymethyl cellulose.

investigate adsorption capacity of dietary fibers for glucose. The results showed that all of the dietary fibers can bind glucose (Table 4), but they showed no adsorption capacity for glucose when the concentration of glucose decreased to 0.5 mmol/L, which may suggest that glucose in the small intestine could be kept at low concentration by dietary fibers.

Effect of Dietary Fiber on Activity of Amylase. The results showed that the ratio of diffused glucose treated by dietary fibers to that of control in Table 3 was much higher than that in Table 1 at any time, suggesting that glucose diffusion at lower concentration was more affected by the viscosity and adsorption of dietary fibers, but the effect of dietary fibers on the activity of amylase could not be excluded. The results in Table 5 proved that the activity of α-amylase was directly influenced by dietary fibers.

According to the view of Annison and Topping (*16*), dietary fibers can be adsorbed to starch and thus hinder hydrolysis of starch by α-amylase. In our experiment, 0.1 g of water-insoluble dietary fibers or 1.0 mL of 1% water-soluble dietary fibers was mixed with 1.0 mL of 1% α-amylase solution for 0.5 h, and then the mixture was put into 100 mL of starch−phosphate buffer; the results showed that the activity of α-amylase was influenced (data not shown) to some extent according to the kind of dietary fiber. Further studies are needed to investigate whether the dietary fibers are competent inhibitors of α-amylase or simply act as a barrier between the enzyme and starch.

Conclusion. The results of the above showed that postprandial serum glucose was lowered by dietary fiber at least through three pathways: The first is to increase the viscosity of the small intestinal content and retard the diffusion of glucose; the second is to adsorb glucose and prevent its diffusion; and the third is to inhibit the activity of α-amylase and postpone the release of glucose from starch.

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