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Effect of guar gum on glucose metabolism in cecectomized rats

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We hypothesized that short chain fatty acid (SCFA) fermented in the cecum of rats fed a guar gum diet is responsible for the hypoglycemic effect. Thus, to evaluate the role of SCFA in glucose metabolim, rats with or without cecum were fed a guar gum-containing diet for 4 weeks, and parameters including blood glucose and SCFA in portal blood were determined. The level of insulin-responsive glucose transporter (GLUT4) was also measured to evaluate the effect of SCFA on glucose metabolism at the cellular level.

Blood glucose levels in both cecectomized and normal rats that were fed a cellulose-based diet significantly decreased after their diet was changed to one containing guar gum at the level of 5% (w/w). However, no subsequent difference was found between the two groups. When an oral sucrose load was performed after 3 weeks, untreated and cecectomized rats responded similarly. After feeding a guar gum diet for 4 weeks, the rats were killed, and blood from the portal vein and aorta was taken to measure SCFA and plasma biochemical parameters.

Cecectomy did not have any influence on glucose, lipids, and insulin levels in the blood. Portal plasma acetic and propionic acids in cecectomized rats dramatically decreased by one-half and one-tenth those of the control rats, respectively. There was no difference in the amount and translocation rate of GLUT4 isolated from adipose tissues between the two groups when the animals were killed after 4 weeks.

From these results, it is suggested that, at least in normal rats, SCFA fermented in the digestive tract, if any, is not a primary cause of glucose-lowering action by guar gum. Physicochemical characteristics of guar gum in the upper intestine (e.g., gel-forming capacity) may be important in determining blood glucose level. (J. Nutr. Biochem. 7:303–308, 1996.)

Keywords: guar gum; glucose; cecectomy; short-chain fatty acid

Introduction

It is known that undigestable polysaccharides such as dietary fiber influence lipid and glucose metabolism,[1,2] although the exact mechanism still remains unclear. Dietary fibers are thought to physically delay gastric emptying and interfere with diffusion of nutrients, resulting in a decreased absorption of nutrients.[3] Furthermore, it is possible that the prolonged use of dietary fiber affects functions of the gastrointestinal tract, altering the rate and amount of absorption of nutrients. It is also plausible that because of their ion exchange characteristic, some dietary fibers bind to bile acids, and lead to excretion of these acids from the body, thereby decreasing serum cholesterol.[4]

Dietary fiber that are not absorbed in the small intestine eventually reach the lower digestive tract where they are subjected to fermentation by intestinal microflora. Although the kind and amount of fermented products depend on the animal species used and the diet ingested, acetic, propionic, and butyric acids are yielded at an approximate ratio of 60:20:15.[5] These short-chain fatty acids (SCFAs) have divergent physiological functions in addition to being a fuel source.[6] Among these, SCFAs, especially propionic acid, is known to inhibit cholesterol and glucose production in isolated hepatocytes.[7,8] Hypocholesterolemic action by propionate has also been reported in vivo.[9,10] However, some in-

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Received September 5, 1995; accepted February 21, 1996.

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0955-2863/96/$15.00
SSDI 0955-2863(96)00031-7

Table 1 Compositions of experimental diets

	5% Cellulose basal diet	5% Guar gum diet
	(%)	
Casein	20.0	20.0
DL-methionine	0.3	0.3
Corn oil	5.0	5.0
Corn starch	15.0	15.0
Sucrose	50.2	50.2
Mineral mixture[1]	3.5	3.5
Vitamin mixture[2]	1.0	1.0
Cellulose	5.0	0
Guar gum	0	5.0

[1]AIN-76 mineral mixture.
[2]AIN-76 vitamin mixture.

vestigators are skeptical about the physiological significance of SCFAs in terms of controlling cholesterol metabolism, because the concentration required to reduce cholesterogenesis is relatively higher than that found under physiological conditions.[11]

On the other hand, although the role of dietary fiber in glucose metabolism has been extensively reviewed by Anderson et al.,[12] the specific mechanisms by which dietary fibers regulate blood glucose and insulin remain unclear. In general, soluble dietary fibers such as guar gum and pectin have been shown to lower blood glucose rise and insulin level when given to humans simultaneously with a carbohydrate-containing meal.[13] We previously showed that viscosity of dietary fiber was related to the absorption and diffusion of glucose in vitro and in situ.[14] Furthermore, in a fasted state, rats given dietary fibers for 5 weeks showed a decreased rise of glucose level after ingestion of glucose alone,[15] indicating that fiber itself or a metabolite of fiber indirectly affect glucose metabolism through, for example, altered intestinal cell function and gut hormone secretion. This has also been suggested by Dubois et al., who showed that chronic bran intake might modify postmeal metabolic response.[16]

Furthermore, Hjøllund and co-workers reported that monocytes derived from insulin-dependent and non-insulin-dependent diabetic patients who were fed dietary fibers had increased insulin binding.[17,18] Oral intake of propionate has been shown to decrease insulin secretion after an oral glucose tolerance test in humans, implying enhanced insulin sensitivity.[19] Cummings and co-workers showed an important contribution of the large intestine to blood acetate and suggested a possible influence of fermentation on the metabolic process.[20] Based on these findings, it is suggested that dietary fibers not only directly affect glucose absorption in the upper digestive tract but also alter glucose metabolism through an increased insulin sensitivity by SCFAs fermented in the lower bowel.

In the present study, to elucidate the role of SCFAs in glucose metabolism, rats were cecectomized and given fermentable guar gum, and parameters such as blood glucose and insulin were measured. A glucose transporter (GLUT4) in adipocyte was also prepared from untreated and cecectomized rats to clarify the effect of SCFA on insulin sensitivity at the cellular level.

Methods and materials

Animals and experimental design

In the experiment, male 5-week-old Sprague Dawley rats weighing 120 to 140 g (Charles River Co., Shiga) were housed individually in wire-bottomed cages. Rats were fed a commercial chow diet (MF, Oriental Yeast Co., Tokyo, Japan) and acclimated for 3 days. After overnight fasting, rats were then divided into two groups. One group of rats were cecectomized according to the method of Lambert.[21] Cecectomized rats were not provided with food or water overnight after surgery. Similarly, the control rats were not given food or water overnight. Rats in both groups were then given a cellulose-based diet for 7 days. During this period, rats that grew slowly were eliminated from the study. Food intake and body weight were recorded daily. Throughout the experiment, the animals were kept in a temperature-controlled room (24°C ± 0.5°C) with a 12-hr dark (0700 to 1900) and 12-h light period. Composition of the basal and experimental diets is shown in *Table 1*.

As illustrated in *Figure 1*, parameters were determined weekly: blood was taken from the tail vein in non-fasted condition at the beginning of the experiment. After 1 and 2 weeks, blood glucose level was measured. After 3 weeks, the rats were fasted overnight, and were given a 30% sucrose solution (2 g/kg BW) and blood sugar was measured at 0, 15, 30, and 60 min. After 4 weeks, portal and aortic blood was taken from rats that had inhaled carbon dioxide, and concentrations of SCFA and other serum biochemical parameters were then measured. After collecting blood, adipose tissue was excised according to Rodbell.[22] Isolated adipocytes were incubated in the presence or absence of insulin at a final concentration of 10^{-7} M. After incubation for 30 min, cells were homogenized, and plasma membranes (PM) and low-density microsome (LDM) fractions were prepared by ultracentrifugation.[23]

Blood glucose from the tail vein and aorta vein was enzymatically measured using Glucose oxidase (Sankyo-Miles Co., Tokyo, Japan and Wako Pure Chemical Co., Tokyo, Japan). Triglyceride and cholesterol in serum were enzymatically determined (Kyowa Medex Co., Tokyo, Japan). Serum insulin was analyzed by radioimmunoassay (Novo Nordisk, Denmark). To analyze GLUT4 in adipose tissue, 5 μ g protein was electrophoresed according to Laemmli[24] and transferred to polyvinylidene difluoride membrane (GVHP304F0, Japan Millipore Limited Co., Tokyo, Japan). The transferred proteins were incubated in the presence of rabbit anti-GLUT4 antibody (kindly provided by Dr. Y. Oka, Yamaguchi University, Yamaguchi, Japan). GLUT4 was detected using ECL Kit according to the manufacturer's instructions (Amersham International Plc, Buckinghamshire, England).

SCFAs were extracted with ethyl ether and measured by gas chromatography on a Chromatopack C-R3A with a slight modification of the method of Murase et al.[25] Briefly, one ml of plasma was added to acrylic acid (Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) as an internal standard and mixed with 0.2 ml sulfosalicylic

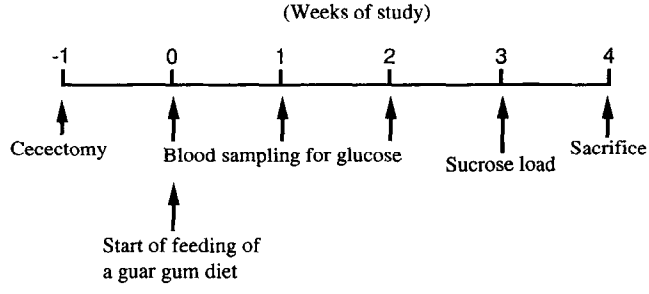

Figure 1 Experimental design and sampling schedule.

acid to precipitate proteins, followed by extraction with ethyl ether. After vigorously mixing on a vortex mixer, samples were centrifuged at 1,500 rpm for 10 min. Then, the supernatant was prepared as described previously.[25]

Statistics

The analysis of average between the two groups was done by Student's *t*-test. Blood glucose at day 0, and weeks 1 and 2 was analyzed by Paired *t*-test

Results

No differences were found in growth and food intake between cecectomized and untreated rats for one week after surgery (during the basal diet period) and throughout the experiment, as shown in *Figure 2.*

Changes in blood glucose levels at the beginning of the experiment and after 1 and 2 weeks are illustrated in *Figure 3.* Although, after 1 week, blood glucose in both groups decreased significantly compared to the level at the beginning of the experiment (8.36 ± 0.75 to 7.32 ± 0.63 mmol/L, $P < 0.001$, in control rats, and 8.20 ± 0.86 to 7.08 ± 0.35 mmol/L, $P < 0.001$, in cecectomized rats), there were no differences between the two groups throughout the experiment. In each group, subsequent blood glucose levels did not decrease further.

Sucrose challenge was carried out after 3 weeks. As shown in *Figure 4,* cecectomy did not affect the response of blood glucose to sucrose load (2 g/kg BW) as well as fasting blood glucose.

Table 2 shows blood glucose, triglyceride, cholesterol, and insulin levels in non-fasted rats after 4 weeks. Glucose and insulin levels were not affected by cecectomy. There was also no difference in blood lipids between cecectomized and untreated animals.

The effect of cecectomy on the concentration of SCFAs in portal blood is shown in *Table 3.* Because blood was taken 6 hours after the start of the dark period, and because animals in both groups were fed, SCFAs in portal blood should reflect the diet the rats ingested. Acetic acid was reduced from 843 μmol/l in the control rats to 460 μmol/l in the cecectomized animals, and the difference became significant. Propionic acid, the second abundant SCFA, in treated rats was about one-tenth that in the untreated animals (14 μmol/l in cecectomized rats versus 131 μmol/l in the untreated rats, $P < 0.001$). However, no influence of cecectomy was noticed on the concentration of butyric acid. Both groups gave about 40 μmol/l.

Figure 5 shows GLUT4 prepared from adipocytes. Intracellular (LDM) and cell surface (PM) pools of GLUT4 were not affected by cecectomy in the presence of insulin, indicating no difference of translocation from LDM to PM. No difference was also detected in the absence of insulin (data not shown).

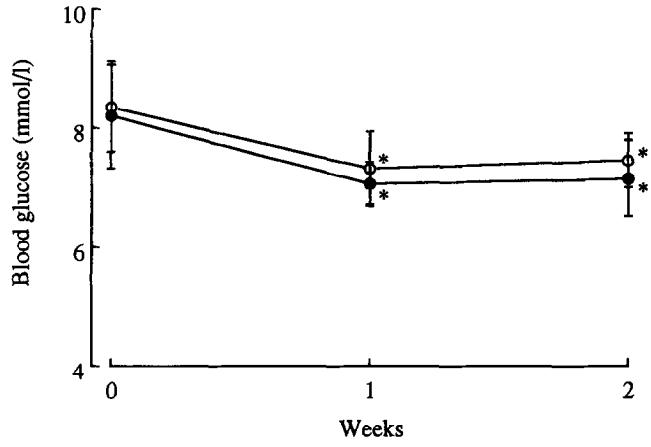

Figure 3 Change in blood glucose. Symbols are the same as in Figure 2. Values are expressed as mean ± SD. * significantly different from the value at day 0 in each group.

Discussion

The effect of fibers on glucose metabolism, especially decreased levels of blood glucose and insulin, has been attributed to direct interaction between fibers and carbohydrate in

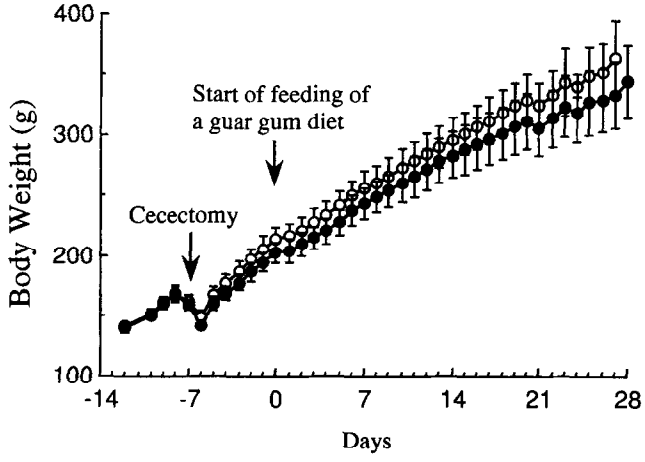

Figure 2 Body weight gain. ○: control rats; ●: cecectomized rats. Values are expressed as mean ± SD.

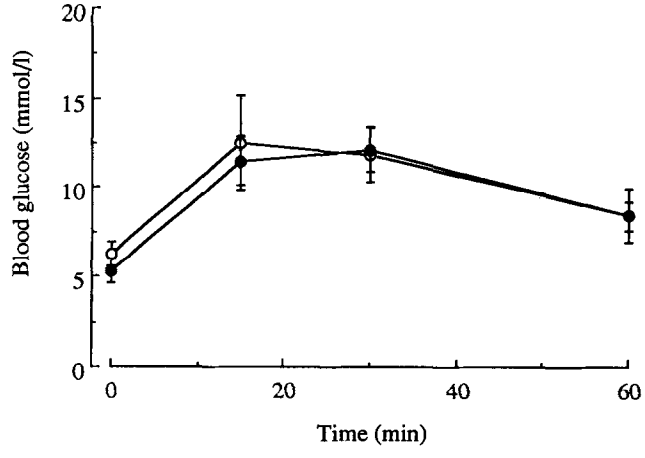

Figure 4 Effect of cecectomy on blood glucose after sucrose loading. Symbols are the same as in Figure 2. Values are expressed as mean ± SD.

Table 2 Effect of cecectomy on biochemical parameters

	Glucose	TG	TC	Insulin
		(mmol/l)		(ng/ml)
Control (10)[a]	10.64 ± 2.77	1.00 ± 0.27	1.60 ± 0.21	3.52 ± 2.00
Cecectomized (20)[a]	9.12 ± 1.11	0.97 ± 0.24	1.77 ± 0.25	3.45 ± 2.24

Data are expressed as means ± SD.
[a]Value in parentheses indicates the number of animals used.

the intestine through a gel-forming capacity and decreased gastric emptying.[3,14,26] In addition, in experimental animals, long-term feeding of dietary fibers decreased fasting blood glucose and slowed an increase in blood glucose and insulin levels after an oral dose of glucose solution without fibers, indicating that dietary fibers have an indirect effect on carbohydrate metabolism.[15] Hjøllund et al. measured insulin receptor activity in erythrocytes and monocytes derived from subjects fed dietary fibers for 3 to 4 weeks and found increased binding of insulin to monocytes.[18] Venter et al. administered propionate, one of the major products fermented by intestinal microflora, to humans for 3 weeks and observed that the levels of fasting serum glucose and maximum insulin increment during oral glucose tolerance test were reduced.[19] On the contrary, it has been reported that rectal infusion of SCFAs such as propionate does not lower blood glucose compared to a saline infusion, but leads to a fall in serum-free fatty acids.[27] This indicates that because of a fatty acid glucose cycle,[28] the decreased level of free fatty acids improves glucose metabolism over an extended period through greater uptake by insulin-sensitive tissues. Taken together, SCFAs fermented from dietary fibers in the large bowel may be considered one of the mechanisms by which dietary fibers such as guar gum regulate carbohydrate metabolism. Thus, to investigate the role of SCFAs in carbohydrate metabolism, guar gum, a readily-fermentable fiber, was fed to cecectomized rats, and the changes in blood glucose and insulin were followed under conditions in which SCFA production was diminished.

As illustrated in *Figure 2,* cecectomy did not have an effect on body weight gain.

Food intake also was not influenced by cecectomy (data not shown). Under this condition, guar gum showed lowered blood glucose levels compared with that of cellulose, irrespective of cecectomy. This supports the previous report that guar gum has a higher gel-forming capacity than other fibers, and thus leads to improved glucose tolerance in rats.[3,14] Furthermore, it has been reported that in non-insulin-dependent diabetics, the addition of guar gum to diets has decreased the levels of fasting blood glucose, insulin, and HbA_1, and has enhanced the binding of insulin to moncoytes.[29] In the present study, animals were fed cellulose before a guar gum-containing diet was given. Because cellulose is water-insoluble and is not a gel-forming fiber, glucose absorption may not have been suppressed by cellulose, thus maintaining a higher blood glucose level. However, no tendency to lower blood glucose after 1 week was observed in the cecectomized and untreated groups. The lack of further decrease of blood glucose by guar gum may be due to guar gum directly interfering with gastric emptying and glucose diffusion only in the upper intestine, but not affecting intestinal cell function.

In rats without cecectomy, the concentration of acetic acid in portal blood was about 800 μmol/l, which is similar to that reported previously.[30] The levels of acetic acid and propionic acid in cecectomized rats were reduced by half and one-tenth those found in the control animals, respectively. Because cecum is a major site of fermentation in rats[6] and because the levels of SCFA in treated rats were the same as those in rats fed a fiber-free diet (Murase et al., unpublished data), it is unlikely that the lack of difference of blood glucose levels between the two groups is due to an unappropriate removal of cecum.

Although dietary fibers have been reported to enhance insulin sensitivity,[17,18] the intracellular and cell surface pool of insulin-responsive GLUT4 in adipose tissues was not influenced by cecectomy, as shown in *Figure 5,* indicating that fermentation products do not alter insulin sensitivity in rats fed guar gum. In contrast to our hypothesis, the follow-

Table 3 Effect of cecectomy on SCFAs in portal plasma

	Acetic acid	Propionic acid (μmol/l)	*n*-Butyric acid
Control (10)[a]	843 ± 353	131 ± 86	48 ± 62
Cecectomized (17)[a]	460 ± 100*	14 ± 15*	41 ± 46

Data are expressed as means ± SD.
*Significant difference from control rats at $P < 0.05$.
[a]Value in parentheses indicates the number of animals used.

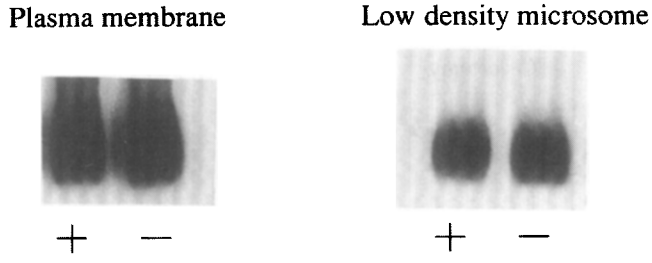

Figure 5 Immunoblotting of GLUT4 in adipocyte. In each line, 5 μg plasma membrane or low-density microsome were loaded. Immunoblotting and developing as described in "Methods and materials." +: control rats; −: cecectomized rats.

ing reasons are given to explain why no significant relation between fermentability of dietary fibers and blood glucose was found: firstly, even in control rats fed highly-fermentable fiber, the levels of SCFAs might not be high enough to alter glucose metabolism as found in cholesterol metabolism.[11] Secondly, because the rats used in the present study had normal glucose metabolism even in the cecectomized rats, the effect of SCFA may not be evident because of homeostasis. Lastly, rats may not be a proper experimental model because the effect of infused SCFAs on plasma insulin was dependent on the animal species used.[31] Thorburn et al. also showed that fermentation of undigested carbohydrates and dietary fibers might play a role in regulating postprandial glycemia.[32] On the other hand, gut-derived acetate was shown to have no effect on glucose turnover in man.[33] Very recently, it was suggested that oral propionate supplementation was not a determinant for metabolism of carbohydrates and lipids in nondiabetic and STZ-diabetic rats.[34] Thus, to clarify the detailed relation between glucose metabolim and SCFA fermented from dietary fiber, methodology and dietary fibers used in the studies must be considered. If animals with impaired glucose tolerance or with a low-insulin sensitivity are used, clear effects of SCFA on glucose metabolism may be observed. A higher level of guar gum in the diet may have been effective in altering glucose metabolism.

Kiriyama et al. used normal, ileorectostomized and cecectomized rats and found that the levels of total SCFAs and individual SCFA such as propionic acid in cecum were negatively related to serum cholesterol level.[35] On the contrary, although propionate decreased cholesterol synthesis in vitro and in vivo,[7,9] the concentration of propionate required is quite outside the physiological range.[11] It is likely that the negative correlation between serum cholesterol and cecum SCFA content may result from the fact that dietary fibers bind steroids and interfere its absorption in the upper intestine, and that such undigested fibers can reach the lower bowel and can be consequently fermented in the lower intestine. A similar relationship could be seen between the direct effect of dietary fiber on the digestion and absorption of carbohydrates in the upper intestine and fermentability in the lower intestine. Thus, the role of guar gum in carbohydrate metabolism in the upper intestine, but not in the lower intestine, is thought to be plausible.

From the results mentioned, at least in normal rats, it is conceivable that the glucose-lowering action of guar gum is not primarily due to SCFAs derived from a microbial fermentation in the digestive tract. Physicochemical characteristics of guar gum in the upper intestine may be important in determining blood glucose level.

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