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Erythritol Attenuates the Diabetic Oxidative Stress through Modulating Glucose Metabolism and Lipid Peroxidation in Streptozotocin-Induced Diabetic Rats

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We investigated the effects of erythritol on rats with streptozotocin- (STZ-) induced diabetes mellitus. Oral administration of erythritol [100, 200, or 400 mg (kg body weight)$^{-1}$ day^{-1} for 10 days] to rats with STZ-induced diabetes resulted in significant decreases in the glucose levels of serum, liver, and kidney. Erythritol also reduced the elevated serum 5-hydroxymethylfurfural level that is glycosylated with protein as an indicator of oxidative stress. In addition, thiobarbituric acid-reactive substance levels of serum and liver and kidney mitochondria were dose-dependently lower in the erythritol-treated groups than in the control diabetic group. Furthermore, the serum creatinine level was reduced by oral administration of erythritol in a dose-dependent manner. These results suggest that erythritol affects glucose metabolism and reduces lipid peroxidation, thereby improving the damage caused by oxidative stress involved in the pathogenesis of diabetes.

KEYWORDS: Erythritol; diabetes; glucose; 5-hydroxymethylfurfural; thiobarbituric acid-reactive substance; creatinine; rat

INTRODUCTION

Diabetes is a general term referring to disorders characterized by excessive urine excretion and a metabolic disorder induced by high blood glucose levels. Although numerous studies on the causes of diabetes have been carried out, the causes and mechanisms involved are still unclear. Recently, several workers suggested that oxygen free radicals and lipid peroxidation are implicated in the pathogenesis of diabetes and cause various complications of diabetes, such as nephropathy, retinopathy, and neuropathy (*1*−*3*). Excess reactive oxygen accelerates oxidative damage to DNA and other macromolecules, such as proteins and lipids (*4*). Oxidative modification of lipids has also been recognized to play a central role in atherogenesis and to contribute to the diverse vascular sequelae of diabetes and aging (*5*). Therefore, treatment with antioxidants may prevent or reverse abnormalities associated with diabetes mellitus and its complications.

Recently, various new sugars and sugar alcohols, including erythritol, have been developed for nutritional and therapeutic uses to manage the problems of overweight and diabetic complications. They are considered to be low-energy sweeteners, being less calorigenic than glucose or sucrose. Erythritol is a C4 polyol that occurs naturally in algae, wine, sake, beer, pears, grapes, watermelons, and mushrooms; its sweetness is 60−80% that of sucrose, and it has interesting physicochemical characteristics (*6*, *7*). More than 90% of ingested erythritol is not metabolized by the human body and is excreted unchanged in the urine, indicating that, in humans, erythritol is efficiently absorbed, not metabolized, and excreted by renal processes (*8*, *9*). The absence of systemic metabolism of erythritol means that it has limited potential to induce changes in plasma glucose and insulin levels. Consequently, this sweetener has potential value in the diets of patients with diabetes, who frequently crave sweets, which can adversely affect blood glucose levels. Therefore, erythritol may be able to be used advantageously in special foods for people with diabetes.

In this study, we used rats with streptozotocin- (STZ-) induced diabetes to investigate the effects of erythritol on glucose metabolism and oxidative stress. We expect to provide good evidence that use can be made of erythritol for diabetes as a therapeutic agent as well as a sweetener.

MATERIALS AND METHODS

Reagents. STZ was purchased from Sigma Chemical Co. (St. Louis, MO). Erythritol was supplied by Nikken Chemical Co., Ltd., Tokyo.

Animal Experiments. *(1) Animal Preparation.* Male Wistar rats (120−130 g) from Japan SLC, Inc. (Hamamatsu, Japan), were used. They were kept in wire-bottomed cages under a conventional lighting regimen with a dark night. The room temperature (about 25 °C) and humidity (about 60%) were controlled automatically. Laboratory pellet chow (CLEA Japan Inc., Tokyo, Japan; comprising 24.0% protein, 3.5% lipid, and 60.5% carbohydrate) and water were given ad libitum. After several days of adaptation, the rats were given STZ (50 mg/kg, injected intraperitoneally) dissolved in citrate buffer (pH 4.5) following overnight fasting. One week after injection, the glucose level of blood taken from each rat's tail vein was determined, and then the animals were divided

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Table 1. Body and Tissue Weights of Rats with Streptozotocin-Induced Diabetes after Oral Administration of Erythritol

group	dose [mg (kg body wt)$^{-1}$ day^{-1}]	body weight initial (g)	body weight final (g)	body weight gain (g/10 days)	liver weight (g/100 g body wt)	kidney weight (g/100 g body wt)
control		169.4 ± 4.3	203.1 ± 7.0	33.7 ± 2.8	4.08 ± 0.07	1.03 ± 0.02
erythritol	100	166.0 ± 2.7	199.7 ± 6.4	33.7 ± 4.3	4.05 ± 0.08	1.00 ± 0.02
erythritol	200	168.9 ± 4.9	193.4 ± 7.5	31.0 ± 2.5	4.11 ± 0.09	1.04 ± 0.03
erythritol	400	166.0 ± 3.7	196.6 ± 8.2	30.6 ± 5.4	4.14 ± 0.06	1.02 ± 0.02

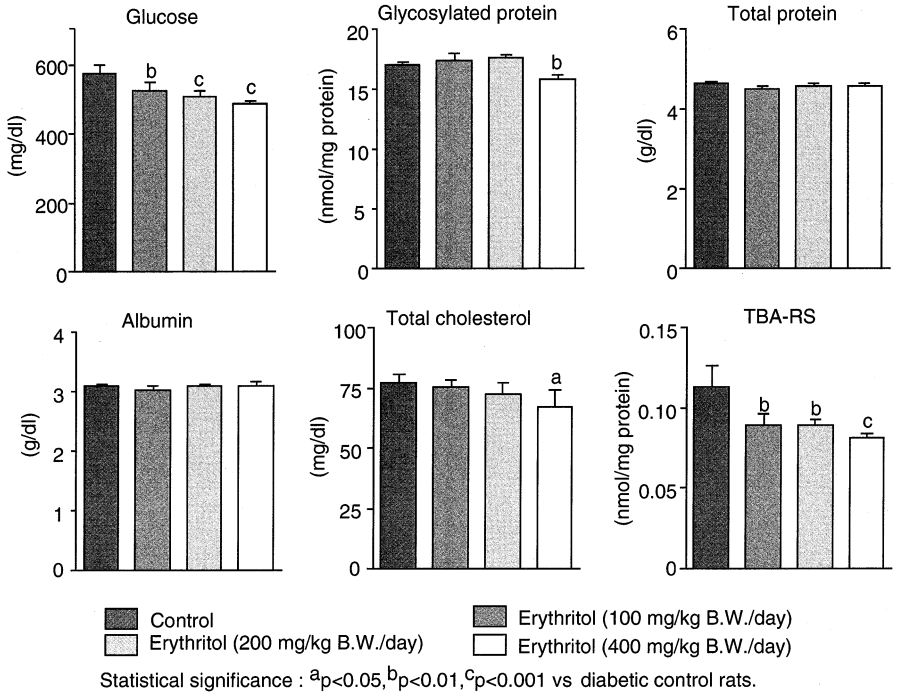

Figure 1. Serum constituents in streptozotocin-induced diabetic rats after oral administration of erythritol.

into four groups ($n = 7$ per group). The control group was given saline (vehicle) orally, while the other groups were given erythritol [at a dose of 100, 200, or 400 mg (kg body weight)$^{-1}$ day^{-1}] orally, for 10 consecutive days, after which the rats were killed by decapitation. Following the collection of blood samples, serum was separated immediately by centrifugation. The livers and kidneys were removed, rinsed with ice-cold saline, and frozen at −80 °C until assayed.

(2) Determination of Glucose. Blood glucose levels were measured using a commercial kit (Glucose-CII-Test Wako; Wako Pure Chemical Industries, Osaka, Japan). Each rat's liver and kidney were homogenized with 9-fold volumes of ice-cold physiological saline containing Ba-$(OH)_2$ (0.15 M) and $ZnSO_4$ (5%) and centrifuged (at 3000 rpm, 15 min), and then the glucose level of each supernatant (0.5 mL) was assayed using the kit described above.

(3) Determination of Glycosylated Serum Protein. Glycosylated serum protein levels were determined using a modified thiobarbituric acid (TBA) assay of Fluckiger and Winterhalter (*10*). Serum (0.1 mL) was diluted to 1.0 mL, mixed with 0.5 mL of oxalic acid (1 M), hydrolyzed for 4.5 h at 100 °C, and reacted with TBA, and then the glycosylated serum protein level was quantitated by measuring the absorbance at 443 nm.

(4) Determination of Protein, Albumin, and Total Cholesterol. Serum protein, albumin, and total cholesterol levels were quantified using commercial kits (A/G B-Test Wako and Cholesterol E-Test Wako obtained from Wako Pure Chemical Industries, Osaka, Japan), and tissue mitochondrial protein levels were evaluated by the method of Itzhaki and Gill (*11*) with bovine serum albumin as the standard.

(5) Determination of TBA-Reactive Substances. Serum TBA-reactive substance levels were determined using the method of Naito and Yamanaka (*12*).

(6) Preparation of Mitochondria and Measurement of Mitochondrial TBA-Reactive Substances. Mitochondria were prepared from the homogenates of liver and kidney by differential centrifugation (800*g* and 12000*g*) with a refrigerated centrifuge (4 °C), as described by Johnson and Jung with a slight modification (*13*, *14*). Each pellet was resuspended in preparation medium, and the TBA-reactive substance content was determined by the method of Uchiyama and Mihara (*15*).

(7) Determination of Glutamic Pyruvic Transaminase (GPT) and Creatinine (Cr). Serum GPT and Cr levels were determined using commercial kits (GPT-UV Test Wako obtained from Wako Pure Chemical Industries, Osaka, Japan, and CRE-EN Kainos obtained from Kainos Laboratories, Tokyo, Japan).

Statistical Analysis. The data were expressed as means ± SE. Differences among groups were analyzed by Dunnett's test and those at $p < 0.05$ were considered significant.

RESULTS

Body and Tissue Weights. Table 1 shows the changes in the body and tissue weights of rats with STZ-induced diabetes after the oral administration of erythritol. Oral administration of 100, 200, or 400 mg of erythritol (kg body weight)$^{-1}$ day^{-1} for 10 days did not result in any significant changes in body weight gain or liver and kidney weights compared with control rats given water instead of erythritol.

Serum Constituents. Figure 1 represents the effects of erythritol on serum constituents of rats with STZ-induced diabetes. The level of serum glucose was reduced by erythritol in a dose-dependent manner. The serum glucose levels of rats given 100, 200, and 400 mg of erythritol orally were 524, 506, and 488 mg/dL, respectively, while that of control rats was 576 mg/dL. We also measured 5-hydroxymethylfurfural (5-HMF)

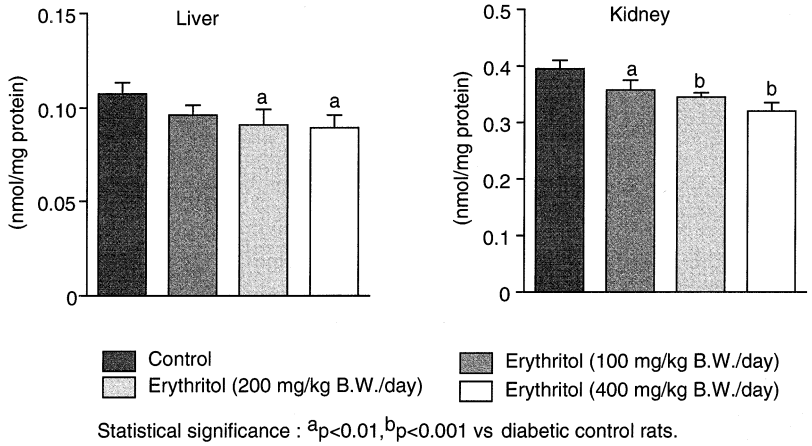

Figure 2. Thibarbituric acid-reactive substance levels in the hepatic and renal mitochondrial fractions in streptozotocin-induced diabetic rats after oral administration of erythritol.

Table 2. Hepatic and Renal Glucose Levels in Rats with Streptozotocin-Induced Diabetes after Oral Administration of Erythritol

group	dose[a] [mg (kg body wt)$^{-1}$ day^{-1}]	liver[a] [mg tissue^{-1} (100 g body wt)$^{-1}$]	kidney[a] [mg tissue^{-1} (100 g body wt)$^{-1}$]
control		32.61 ± 0.43	5.27 ± 0.16
erythritol	100	28.57 ± 0.87c	4.89 ± 0.19a
erythritol	200	28.03 ± 0.85c	4.76 ± 0.19b
erythritol	400	27.75 ± 1.14c	4.37 ± 0.14c

[a] Statistical significance: a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$ vs diabetic control rats.

levels to determine the extent of glycosylation of serum proteins, from which nonenzymatically bound glucose is released as 5-HMF. Oral administration of 400 mg of erythritol induced a significant reduction of the 5-HMF level ($p < 0.01$), but it did not change significantly the serum levels of total protein and albumin. The serum total cholesterol level was reduced by oral administration of 400 mg of erythritol (kg body weight)$^{-1}$ day^{-1} for 10 days in comparison with diabetic control rats. The serum TBA-reactive substance levels of diabetic rats given erythritol orally were significantly lower than those of the diabetic control group. After an oral dose of 100 and 400 mg, the serum TBA-reactive substance level decreased from 0.113 to 0.089 and 0.081 nmol/mg of protein, respectively.

Hepatic and Renal Glucose Levels. As shown in **Table 2**, erythritol administration reduced the hepatic and renal glucose levels of rats with STZ-induced diabetes. Even the lowest oral dose of erythritol, 100 mg (kg body weight)$^{-1}$ day^{-1} for 10 days, reduced the hepatic glucose level significantly. In addition, erythritol reduced the renal glucose level dose-dependently. The renal glucose levels of diabetic rats given 100, 200, and 400 mg/kg body weight erythritol orally were 4.89, 4.76, and 4.37 mg tissue^{-1} (100 g body weight)$^{-1}$, respectively, whereas that of diabetic control rats was 5.27 mg tissue^{-1} (100 g body weight)$^{-1}$.

Hepatic and Renal Mitochondrial TBA-Reactive Substance Levels. The TBA-reactive substance levels of hepatic and renal mitochondria from the groups treated orally with erythritol decreased significantly compared with those of the diabetic control rats (**Figure 2**). In the rats fed erythritol orally, 400 mg (kg body weight)$^{-1}$ day^{-1} for 10 days, the TBA-reactive substance level of hepatic mitochondria decreased from 0.107 to 0.089 nmol/mg of protein and that of kidney mitochondria from 0.397 to 0.320 nmol/mg of protein. In particular, the TBA-reactive substance level of renal mitochondria was reduced in a dose-dependent manner.

Table 3. Serum Glutamic Pyruvic Transaminase (GPT) and Creatinine (Cr) Levels in Rats with Streptozotocin-Induced Diabetes after Oral Administration of Erythritol

group	dose [mg (kg body wt)$^{-1}$ day^{-1}]	GPT (IU)	Cr[a] (mg/dL)
control		11.97 ± 1.15	0.37 ± 0.01
erythritol	100	10.37 ± 0.87	0.31 ± 0.01a
erythritol	200	11.77 ± 1.61	0.29 ± 0.01a
erythritol	400	11.89 ± 1.15	0.24 ± 0.01a

[a] Statistical significance: a, $p < 0.001$ vs diabetic control rats.

Serum GPT and Cr Levels. As shown in **Table 3**, oral administration of erythritol did not cause any significant changes in the serum GPT levels of rats with STZ-induced diabetes, whereas the serum Cr level was reduced significantly by erythritol in a dose-dependent manner. While the serum Cr level of the diabetic control rats was 0.37 mg/dL, those of diabetic rats given 100, 200, and 400 mg erythritol were 0.31, 0.29, and 0.24 mg/dL, respectively.

DISCUSSION

The link between diabetes and oxidative stress has been extensively discussed for years, but definite evidence confirming its importance is still awaited (*1*, *16*, *17*). Dandona et al. (*4*) demonstrated that the production of reactive oxygen species and lipid peroxidation are increased in diabetic patients, suggesting that oxidative stress is responsible for the pathophysiology of diabetes. The oxidative stress has frequently been proposed to be related to hyperglycemia (*1*−*3*). Other possible oxidative sources include elevated plasma lipid levels leading to increased lipid oxidation and reduced levels of the antioxidant defense systems (*17*−*19*). The oxidative stresses in diabetes include shifts in redox balance resulting from altered carbohydrate and lipid metabolism, increased generation of reactive oxygen species by glycation and lipid oxidation, and decreased antioxidant defenses. On the basis of these reports, diabetes can be considered to be a disease related to increased oxidative stress. Therefore, it has been suggested that a variety of antioxidants that scavenge reactive oxygen species may improve hyperglycemia and prevent diabetic complications from developing.

Recently, low-energy sweeteners, including erythritol, have been considered to be an attractive approach as low-calorie sugar substitutes for overweight and therapeutic use against diabetes. Erythritol has been subject to extensive investigation in metabolic, toxicological, and clinical studies as part of the

evaluation of its safety for use in foods. A number of metabolic studies and acute, subchronic, and chronic toxicity studies in rats, mice, and dogs and several clinical studies in humans have demonstrated that erythritol is safe to use as a food ingredient (*6*, *20*−*24*). In all of these studies, erythritol was well tolerated, even at very high doses, and was without adverse toxicological effects.

It is well established that STZ is toxic to β-cells, and it is widely used for the induction of experimental diabetes mellitus, resulting in the production of active oxygen species (*25*, *26*). Scavengers of oxygen radicals are effective in preventing diabetes in animal models. Therefore, we employed an STZ-induced diabetic animal model system to examine whether erythritol has antioxidant effects against the oxidative stress induced by diabetes.

The changes in body, liver, and kidney weights after oral administration of erythritol were evaluated (**Table 1**). The results showed that the weight gains and organ weights of diabetic animals given 100, 200, and 400 mg of erythritol (kg body weight)$^{-1}$ day^{-1} for 10 days did not differ from those of diabetic control rats, although the body weight gains in the groups given the two higher doses of erythritol were slightly, but not significantly, lower than the control value. Another study also demonstrated that only the high dose of erythritol caused physiological effects, not toxicological ones, such as slightly lower weight gains, transient laxative effects, increased water consumption, increased urine volume, increased caecal and kidney weights, and changes in urinalysis parameters compared with control animals (*24*). However, the oral doses of erythritol administered in this study did not significantly affect the physiological parameters measured.

Hyperglycemia, the primary clinical manifestation in diabetes, is associated with development of certain diabetic complications (*27*). In addition, impaired glucose metabolism leads to oxidative stress, and in particular, glycation of proteins produces free oxygen radicals (*1*). The oral administration of erythritol to diabetic rats resulted in dose-dependent reductions in the serum, liver, and kidney glucose levels (**Figure 1** and **Table 2**), indicating that the impaired glucose metabolism improved.

The extent of diabetic complications correlates with elevated blood glucose concentrations, and it is thus widely thought that excessive glucose is the major cause of tissue injury. Therefore, erythritol would be expected to prevent diabetic complications by controlling glucose level. People who have diabetes might also prefer erythritol, because it is rapidly absorbed in the small intestine, not metabolized, and then excreted, resulting in a lower glycemic response than most mono- and disaccharide sugars and complex carbohydrates (*28*). Although several reports demonstrated that erythritol on diabetes did not show any adverse effect of carbohydrate metabolism and other physiological parameters in contrast with other sugars and sugar alcohols (*20*, *21*, *24*), its improving effect on diabetes has not been investigated. In the present study, erythritol showed a beneficial effect through lowering glucose levels of serum and tissues rather than an adverse one against diabetes as most sweeteners show, suggesting that erythritol could be used in foods consumed in persons with diabetes. Furthermore, the study of erythritol on diabetes in connection with oxidative stress has not been conducted yet; thus we demonstrated in this study its effect, focusing on the improving effect against oxidative damage as well as glucose abnormalities impaired by diabetes.

Hyperglycemia causes nonenzymatic glycation of proteins through the Maillard reaction and alters energy metabolism, which may result in elevated levels of reactive oxygen species and in the development of diabetic complications (*29*). Glycation is facilitated at higher glucose levels and is detectable in many proteins from human diabetics. Furthermore, increased glycation of collagen and plasma proteins in subjects with diabetes may stimulate the oxidation of lipids, which in turn stimulate autoxidation reactions of sugars, enhancing damage to both lipids and proteins in the circulation and the vascular wall, continuing and reinforcing the cycle of oxidative stress and damage (*1*). 5-HMF is involved in the nonenzymatic browning process, and nonenzymatically bound glucose in serum is released as 5-HMF (*30*, *31*). Therefore, we evaluated serum 5-HMF levels to determine the extent of glycosylation of serum protein. After an oral dose of 400 mg of erythritol (kg body weight)$^{-1}$ day^{-1} for 10 days, the 5-HMF level reduced significantly (**Figure 1**). Hence, oral administration of erythritol may reduce the glycation of serum protein and, consequently, may prevent or slow the development of diabetes and its complications.

It has been reported that tissue and blood malondialdehyde levels of STZ-induced diabetic rats were increased by lipid peroxidation (*25*, *32*). Therefore, the concentration of lipid peroxidation products may reflect oxidative stress under diabetic conditions, so we evaluated TBA-reactive substance levels to determine the effects of erythritol on the lipid peroxidation of serum and tissues in rats with diabetes. The oral administration of erythritol to diabetic rats reduced significantly TBA-reactive substance levels of serum, liver, and kidney compared with those of diabetic control rats (**Figures 1** and **2**). These results suggest that erythritol may improve the pathological condition of diabetes by inhibiting lipid peroxidation. Erythritol also reduced the serum level of total cholesterol, suggesting that it may protect against diabetic complications. These results suggest that oral administration of erythritol may reduce the oxidative stress caused by diabetes and play a role in preventing the pathogenesis of diabetic complications caused by free radical-mediated oxidative stress.

As indicators of liver and renal functions, we measured serum GPT and Cr levels. As shown in **Table 3**, oral erythritol administration had no effect on the serum GPT level, whereas the serum Cr level was reduced dose-dependently, suggesting that erythritol ameliorated renal failure, one of the complications caused by poorly controlled diabetes. In light of these results, we suggest that erythritol has potential as a preventive and therapeutic agent for diabetes and its complications by controlling glucose metabolism and preventing lipid oxidation and kidney damage caused by diabetic complications.

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