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Dietary Chinese Quince Polyphenols Suppress Generation of α-Dicarbonyl Compounds in Diabetic KK-A^y Mice

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ABSTRACT: Many dietary polyphenols can provide health benefits, such as antioxidant and antidiabetic effects, and can down-regulate the progression of glycation (one cause of diabetic complications). Chinese quince (CQ) is rich in polyphenols, especially procyanidins. A few studies have indicated that CQ has an effect on diabetes. In this study, a procyanidin-rich extract was prepared from Chinese quince fruit (CQE), and its effects were investigated and compared with those of green tea extract (GTE) in type 2 diabetes model KK-A^y mice. Mice were provided one of two high-fat (HF) diets for 4 weeks: a HF diet containing 0.5% CQE or a HF diet containing 0.5% GTE. Blood glucose was suppressed in mice fed CQE and GTE during the experimental period ($p < 0.05$), although the effect of CQE was weaker than that of GTE. Intake of CQE had no effect on the blood insulin level, whereas GTE decreased the insulin level. Body weight gain was suppressed in mice fed CQE similarly to mice fed GTE ($p < 0.05$). Hepatic lipid content and α-dicarbonyl compounds in the kidney were reduced in mice fed CQE and GTE ($p < 0.05$). These results suggest that intake of CQE could moderate type 2 diabetes and diabetic complications.

KEYWORDS: *antidiabetic effect, green tea extract, phenols, procyanidin, Chinese quince, glycation, α-dicarbonyl compounds*

INTRODUCTION

About 350 million people worldwide suffer from diabetes, and type 2 diabetes (T2D) affects 90% of people with diabetes.[1] T2D is a lifestyle-related disease that can be induced by multiple factors such as overfeeding, excess body weight, physical inactivity, and genetic factors. Hyperglycemia is the primary symptom of T2D. Sustained hyperglycemia induces insulin hypersecretion from the pancreatic islets and leads to insulin resistance. The pathogenesis of T2D is aggravated by obesity, a condition in which abnormal lipid accumulation disrupts secretion of adipocytokines and leads to lipid peroxidation.[2] Adipocytokines secreted from adipose tissue affect insulin sensitivity.[3] For example, a decrease in blood adiponectin decreases insulin sensitivity in adipose tissue and muscle and increases systemic inflammation.[3]

Hyperglycemia also accelerates nonenzymatic glycation, leading to accumulation of advanced glycation endproducts (AGEs), which are associated with the development of diabetic complications such as diabetic nephropathy and retinopathy.[4,5] As such complications decrease patients' quality of life, it is important to suppress production of AGEs. α-Dicarbonyl compounds, 3-deoxyglucosone (3-DG), glyoxal (GO), and methylglyoxal (MG), are known to be intermediate products of glycation. They are generated through the polyol pathway including enzymes such as aldose reductase and degradation of glucose.[6,7] It has been shown that α-dicarbonyl compounds such as 3-DG, GO, and MG are increased in the plasma of diabetic rats and humans.[8,9] These compounds have higher reactivity with proteins than the reactivity of reducing sugars, and they damage proteins (the products are AGEs) and lipids (the products are advanced lipoxidation endproducts; ALEs). Therefore, the reaction is called carbonyl stress,[10] which has a vital role in the pathogenesis of diabetic nephropathy and the progression of renal failure.[5] Moreover, oxidative stress increases in diabetes (autoxidation through AGE formation and low-grade inflammation-induced oxidative stress). Proteins, lipids, and nucleic acids are damaged by oxidative stress and lose normal structure and function.[11]

Polyphenols exist in many plants and have various health effects that are thought to be partially due to their antioxidant activity. One of the more well-known polyphenols is epigallocatechin gallate (EGCG), which is known to be present mainly in green tea extract. Green tea extract has many beneficial effects such as strong antioxidant activity, anti-inflammatory effect, and cardiovascular protective effect.[12] Green tea extract and EGCG have been shown to have antidiabetic activity caused by the protection of β cells the improvement of insulin sensitivity.[13,14] Procyanidins are a class of polyphenols and consist of catechin or epicatechin polymers. Similarly to EGCG, procyanidins have been reported to show antioxidant activity,[15,16] anti-inflammatory activity,[17] beneficial effects on lipid metabolism,[18] antidiabetic effects,[19] inhibition of AGE formation, and inhibition of methylglyoxal-induced damage to enzymes.[20] Therefore, it is expected that procyanidin-rich food materials could be therapeutic agents for treatment of diabetes owing to their documented antidiabetic effects.

Chinese quince (*Chaenomeles sinensis*; CQ) is a popular tree in Japan and China. Its fruit has been used in liquor and traditional medicines. CQ contains organic acids, vitamin C, polyphenols such as phenolic acids, flavonoids, and abundant procyanidin polymers.[21] Extracts of CQ have been reported to show beneficial activities, such as antiulcer,[22] antipruritic,[23] anti-

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influenza virus,[24] and antioxidant effects.[24] Sancheti et al. demonstrated that CQ extract had antidiabetic effects on streptozotocin-induced diabetes[25] and antihyperlipidemic effects.[26] These activities have been considered to be responsible for procyanidins, which are the main components of polyphenols in CQE. However, no studies have mentioned the antidiabetic effects of CQE on T2D, particularly from the point of view of antiglycation. Therefore, the aims of the present study were to investigate whether CQE can improve hyperglycemia in type 2 diabetic model animals and if it can influence obesity and glycation (which are profoundly associated with the progress of diabetes and diabetic complications). Additionally, the strength of the antidiabetic effects of CQE was compared with that of GTE. To evaluate the effects of the extracts, type 2 diabetic model animals (KK-A^y mice) were used, and their blood sugar and lipid parameters were measured; to investigate the progression of glycation, α-dicarbonyl compounds in the kidney were measured.

MATERIALS AND METHODS

Materials. Green tea extract (GTE) was provided from Taiyo Kagaku Co., Ltd. (Mie, Japan) and contained 48.8% EGCG, 17.8% epigallocatechin, 7.6% epicatechin, 7.6% epicatechin gallete, 4.0% gallocatechin, 1.8% catechin, 1.5% gallocatechin gallate, and 1.4% gallic acid (data from maker). Fresh fruit of the Chinese quince (CQ) was obtained from a farmer in Iwate, Japan. 3-Deoxyglucosone detection reagents including 3-deoxyglucosone standard and 2,3-diaminonaphthalene were purchased from Dojin Laboratories (Kumamoto, Japan). Methylglyoxal was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of the highest quality available and purchased from Wako Pure Chemical Industries. (Osaka, Japan).

Preparation of Chinese Quince Extracts (CQE). Two hundred fifty grams of fresh Chinese quince fruit was washed and cut in 5 mm squares, and the seeds were removed. The fruit was extracted in 10-fold by weight ethanol with stirring overnight. The extract was filtered with the filter paper, and then the ethanol was removed using a vacuum evaporator. The concentrated extracts were applied to a Sephadex LH-20 column (GE Healthcare UK Ltd., Buckinghamshire, UK) (5 × 18 cm) to remove sugars. The column was washed with 10 L of deionized water, and then the polyphenols were eluted with 1 L of 80% (v/v) acetone. The eluted sample was evaporated to dryness, resolved in water, and then lyophilized (FPU-830, Eyela, Tokyo, Japan) and stored in a freezer (−20 °C) until use. This series of processes was repeated to obtain a sufficient quantity of Chinese quince extracts (CQE). The content of polyphenols in CQE and GTE was determined according to the Folin−Denis method using gallic acid as the standard.[27] CQE contained 0.35 g of polyphenol as gallic acid equivalent per 1 g dry weight, and GTE contained 1.1 g of polyphenol as gallic acid equivalent per 1 g dry weight. In our preliminary experiment, we confirmed that >90% of total polyphenols were procyanidins (data not shown). A total of 1.94 ± 0.16 mg (gallic acid equivalent) phenolic content was extracted from 1 g of fresh Chinese quince fruit.

Animal Experiments. Male KK-A^y mice 5 weeks of age were obtained from CLEA Japan Inc. (Tokyo, Japan) and housed individually in stainless steel cages. They were maintained in an air-conditioned room at 22 ± 1 °C at 55% relative humidity with a 12 h light/dark cycle (lights on from 6:00 a.m. to 6:00 p.m.). The mice were allowed free access to water and a standard chow (rodent diet CE-2, CLEA, Tokyo, Japan) for a week and were subsequently fed an AIN93-G diet for a week. Then, the mice were randomly divided into three groups (n = 4−5) and then fed the experimental diet ad libitum: a high-fat diet (diabetic control group; DC), a high-fat diet containing 0.5% CQE (diabetic Chinese quince group; DQ), or a high-fat diet containing 0.5% GTE (diabetic green tea group; DG) as the concentration of total phenolic content (5 g gallic acid equiv/kg diet weight). The details of each experimental diet are shown in Table 1.

Table 1. Composition of the Test Diets (Grams per Kilogram Diet)

	high-fat diet (HFD)	HFD + CQE	HFD + GTE
α-corn starch[a]	329.5	315.2	325.3
casein, vitamin free[a]	20.0	20.0	20.0
sucrose[b]	100.0	100.0	100.0
powdered cellulose[a]	50.0	50.0	50.0
soybean oil[c]	70.0	70.0	70.0
lard oil[f]	200.0	200.0	200.0
mineral mix[a,e]	35.0	35.0	35.0
vitamin mix[a,e]	10.0	10.0	10.0
choline bitartrate[c]	2.5	2.5	2.5
L-cystine[d]	3.0	3.0	3.0
CQE[g] (powder)		14.3	
GTE[h] (powder)			4.2

[a]Oriental Yeast Co., Ltd., Tokyo, Japan. [b]Toyo Sugar Refining Co., Ltd., Tokyo. [c]Wako Pure Chemical Industries, Ltd., Osaka. [d]Ajinomoto Co., Ltd., Tokyo. [e]AIN93-G diet composition. [f]Sigma Aldrich, St. Louis, MO, USA. [g]CQE, Chinese quince extract. [h]GTE, green tea extract, Taiyo Kagaku Co., Ltd., Mie.

Blood glucose was checked weekly during the experimental period for 4 weeks using Ascensia Breeze-2 (Bayer AG, Leverkusen, Germany). The oral glucose tolerance test (OGTT) was performed on 12 h fasted mice on day 27. Blood was collected from the tail vain at 0, 15, 30, 60, and 120 min after administration of 2 g of glucose/kg body weight and was measured with an Ascensia Breeze-2. On day 28, HbA1c was determined by a DCA 2000 System with a DCA 2000 HbA1c cartridge (Siemens AG, Munich, Germany) using blood from the tail vain. Feces were collected for 2 days using a metabolic cage from day 28 to day 30. On day 30, after 1 h of fasting, each mouse was sacrificed under anesthesia with diethyl ether, and blood was obtained from the inferior vena cava. The liver was perfused with cold saline to remove the remaining blood. The liver, kidney, perirenal, epididymal, and abdominal adipose tissues were collected, and the serum was immediately prepared from blood. All samples were frozen in liquid N_2 and stored at −80 °C until analysis. The animal care protocol in this study was approved by the Iwate University Animal Research Committee (A201117) under Guidelines for Animal Experiments in Iwate University.

Biochemical Analysis of Serum and Liver. Serum glucose, triglyceride (TG), total cholesterol (TC), and HDL-cholesterol levels were measured enzymatically with commercial kits (Glucose CII-Test, Triglyceride E-Test and Cholesterol E-Test, Wako Pure Chemical Industries, Osaka, Japan, respectively). Serum insulin and adiponectin concentrations were measured using ELISA kits (Mouse Insulin Kit, Morinaga Institute of Biological Science, Yokohama, Japan; and Mouse and Rat Adiponectin ELISA Kit, Otsuka Pharmaceutical, Tokyo, Japan, respectively). Liver lipids were extracted according to the method of Folch et al.,[28] and the cholesterol and TG contents were measured with the above kits.

Fecal Component Analysis. Feces collected were lyophilized and weighed (dry weight). Powdered dry feces were extracted and purified using the method described by Folch et al.[28] The cholesterol and TG levels in feces were measured using the same enzymatic kit used in the plasma analysis.

Determination of α-Dicarbonyl Compounds in Kidney. α-Dicarbonyl compounds were analyzed for 2,3-diaminonaphthalene (DAN) adducts identified as fluorescing derivatives according to the method of Yamada et al. using reverse-phase HPLC.[8] The tissue sample (100 mg) was homogenized with 1 mL of ice-cold 10 mM sodium phosphate buffer at pH 7.4 and was centrifuged at 14000g for 15 min. The supernatant was transferred into a new tube, and proteins were precipitated by adding 50 μL of 0.005% 2,3-pentanedione and 1 mL of 6% perchloric acid, and the sample was centrifuged at 3350g at 4 °C for 20 min. The supernatant was neutralized with 2 mL of saturated sodium bicarbonate solution and was reacted with 0.1% (w/v) of 2,3-

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Table 2. General Characteristics and Serum Analyses after Treatment for 4 Weeks with Chinese Quince Extract (CQE) and Green Tea Extract (GTE) in KK-A^y Mice[a]

	DC	DQ	DG
body weight gain (g)	12.5 ± 0.5 a	9.4 ± 0.9 b	7.4 ± 0.7 b
food intake (g/day)	4.31 ± 0.18	3.98 ± 0.08	4.12 ± 0.09
tissue weight (g)			
liver	2.86 ± 0.09 a	2.12 ± 0.13 b	1.84 ± 0.12 b
kidney	0.58 ± 0.01 a	0.53 ± 0.03 a	0.49 ± 0.02 b
perirenal adipose tissue	0.87 ± 0.05	0.84 ± 0.05	0.89 ± 0.04
epididymal adipose tissue	1.91 ± 0.08	1.89 ± 0.06	1.74 ± 0.06
mesenteric adipose tissue	1.78 ± 0.08 a	1.57 ± 0.11 a	1.37 ± 0.07 b
component of serum			
glucose (mg/dL)	560 ± 5 a	438 ± 55 a	362 ± 43 b
triglyceride (mg/dL)	283 ± 53	226 ± 46	209 ± 35
total cholesterol (mg/dL)	147 ± 8	133 ± 8	145 ± 3
HDL-cholesterol (mg/dL)	107 ± 7	100 ± 11	110 ± 3
insulin (ng/mL)	43.7 ± 5.0	43.6 ± 10.5	21.7 ± 8.6
adiponectin (μg/mL)	9.7 ± 0.2	11.6 ± 1.7	15.1 ± 1.9

[a]DC, diabetic control mice fed a high fat diet; DQ, diabetic mice fed a 0.5% CQE supplemented high-fat diet; DG, diabetic mice fed a 0.5% GTE supplemented high-fat diet. Data are expressed as the mean ± SEM (n = 4−5). Significant difference (p < 0.05) among the groups was obtained with ANOVA and is indicated with different letters (by Tukey test).

diaminonaphthalene overnight at 4 °C. The derivatized compounds were extracted with 4 mL of ethyl acetate three times, and the solvent was dried under reduced pressure. The dried extract was dissolved with 200 μL of methanol for HPLC analysis. Standards (3-DG, GO, and MG) were derived with 2,3-diaminonaphthalene as described above. HPLC analysis was carried out on a Lichrosphere 100RP-18e column (4.6 × 250 mm, Merck KGaA, Darmstadt, Germany) at 40 °C using a PU-2089 Plus pump (JASCO Co., Tokyo, Japan). Chromatographic separation was performed with isocratic elution using 10 mM sodium phosphate/acetonitrile (70:30, v/v). The flow rate was 1 mL/min, and the injected volume was 5 μL. The wavelength used for detection was 503 nm (excitation, 271 nm) on an FP-920 fluorescence spectrophotometer (JASCO Co.).

Statistical Analysis. Data are presented as the mean ± SE for each group. A computer program (Graphpad InStat Software, version 2.03, 1995, San Diego, CA, USA) was used for statistical analysis. Analysis of variance (ANOVA) was performed to determine whether there were significant (p < 0.05) differences between the groups. Significant differences between means identified by ANOVA were further analyzed by the Tukey multiple-comparison test.

RESULTS

General Characteristics. Body weight gain of mice fed the high-fat diets containing CQE (DQ) and GTE (DG) was significantly less than that of mice fed the DC high-fat diet (Table 2). Daily food intake of the DQ group was slightly decreased compared to the mice in the other two groups, but the difference was not significant. Dietary CQE and GTE resulted in lower liver weight than in mice fed a high-fat diet. Moreover, intake of GTE reduced kidney weight and mesenteric adipose tissue weight compared to mice fed the other experimental diets.

Serum Analyses. Hyperglycemia observed in DC mice was lowered by ingestion of a diet supplemented with GTE and tended to improve with ingestion of a diet supplemented with CQE (Table 2). Serum lipids, TG, total cholesterol (TC), and HDL-cholesterol levels were unchanged after consumption of diets containing CQE and GTE. There was a trend toward reduced insulin in DG mice compared with DC (p = 0.079), but the variation in serum insulin between animals precluded obtaining significant differences. Although no significant differences were observed for serum adiponectin levels in DQ and DG compared to DC, the serum adiponectin level tended to be proportionate to the degree of suppression of serum glucose level.

Blood Glucose and HbA1c Level and Blood Glucose Level in OGTT. Blood glucose level in DC increased gradually during the experimental period (Figure 1A). The blood glucose level in DQ mice was kept significantly lower than in DC mice, whereas it was kept around 200 mg/dL in DG (lower than the level at the beginning of the experiment) (Figure 1A). HbA1c was reduced by consumption of CQE and GTE (Figure 1B). The OGTT showed that blood glucose at 15 min in DG was also significantly lower (Figure 1C) and that the area under the curve (AUC) in DG mice tended to be smaller than that in DC

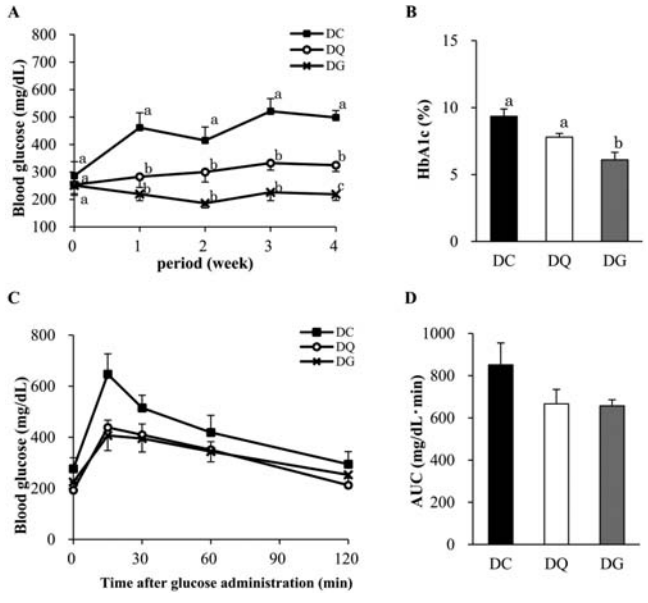

Figure 1. Effect of dietary CQE and GTE supplementation on blood glucose (A) for 4 weeks, HbA1c (B), blood glucose in OGTT (C), and AUC (D) in KK-A^y mice. DC, high-fat diet; DQ, 0.5% CQE−high-fat diet; DG, 0.5% GTE−high-fat diet. Values are means ± SEM (n = 4−5). Different letters indicate significant differences (p < 0.05).

mice. Although a slight decrease in blood glucose and the AUC value was observed in DQ mice, this difference was not significant (Figure 1D).

Hepatic Lipids. Hepatic TG and cholesterol contents in DG mice were significantly lower than those in DC mice, which was 25−30% of DC (Figure 2). In DQ mice, hepatic TG levels

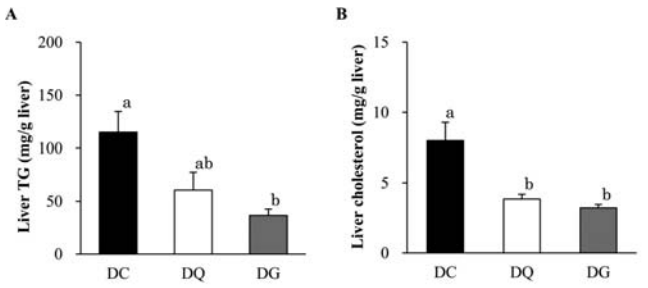

Figure 2. Effect of dietary CQE and GTE supplementation on triglyceride (TG) (A) and cholesterol (B) levels in the liver of KK-A^y mice. DC, high-fat diet; DQ, 0.5% CQE−high-fat diet; DG, 0.5% GTE−high-fat diet. Values are means ± SEM (n = 4−5). Different letters indicate significant differences (p < 0.05).

were reduced by approximately 50% of that of DC, but the difference was not significant, and hepatic cholesterol levels were significantly reduced compared to that of DC mice (Figure 2).

Fecal Lipids. CQE and GTE had no effect on the weight of dried feces (Figure 3A). The excretion of TG in feces was not increased by the consumption of CQE and GTE (Figure 3B). Meanwhile, the excretion of cholesterol in feces in DQ and DG mice was significantly greater than that of DC, and fecal total bile acid levels of DQ and DG were approximately one-fourth of DC (Figure 3C,D).

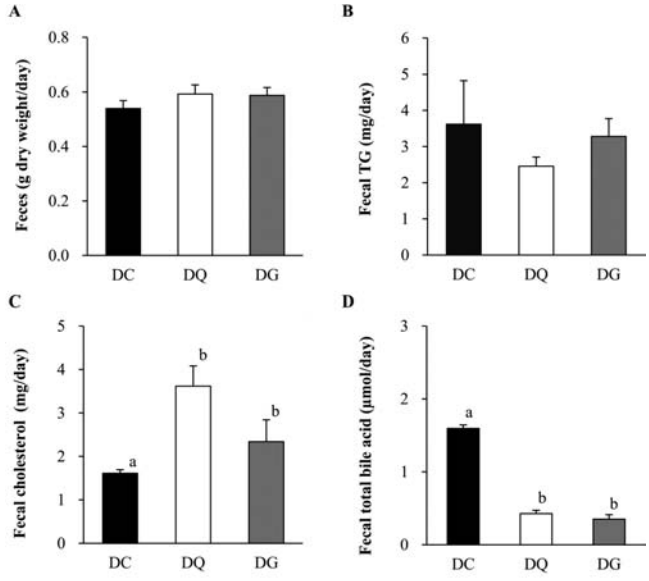

Figure 3. Effect of dietary CQE and GTE supplementation on fecal dry weight (A), triglyceride (TG) (B), cholesterol (C), and total bile acid (TBA) (D) levels in feces of KK-A^y mice. DC, high-fat diet; DQ, 0.5% CQE−high-fat diet; DG, 0.5% GTE−high-fat diet. Values are means ± SEM (n = 4−5). Different letters indicate significant differences (p < 0.05).

α-Dicarbonyl Compounds in Kidney. The content of 3-DG in the kidney was not significantly altered among the groups (Figure 4A). GO and MG in kidneys of mice fed the

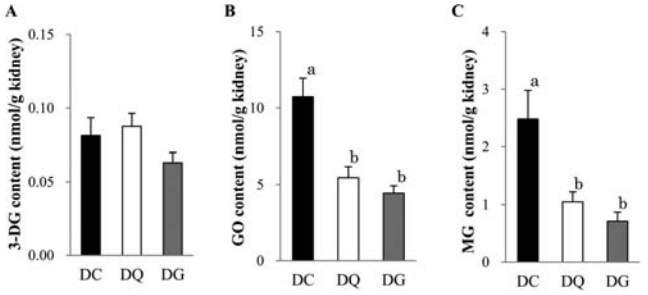

Figure 4. Effect of dietary CQE and GTE supplementation on 3-deoxyglucosone (3-DG) (A), glyoxal (GO) (B), and methylglyoxal (MG) (C) levels in kidney of KK-A^y mice. DC, high-fat diet (diabetic); DQ, 0.5% CQE−high-fat diet (diabetic); DG, 0.5% GTE−high-fat diet (diabetic). Values are means ± SEM (n = 4−5). Different letters indicate significant differences (p < 0.05).

high-fat diet containing each polyphenol compound were lower than that of mice fed the high-fat diet (Figure 4B,C), suggesting that the polyphenol compounds used in the present study reduced carbonyl stress.

DISCUSSION

Polyphenols are natural products in many plants and are ingested as vegetables, fruits, juices, and supplements in our diets. Procyanidins, a kind of polyphenol, have been observed to exert diverse protective activities against diabetes and obesity.[16,17,29] The Chinese quince extract (CQE) used in this study is rich in procyanidins;[30] however, it is not yet clear whether CQE has antidiabetic and antiobese effects on T2D. In the present study, the effect of CQE on T2D was investigated. It was revealed that ingestion of CQE is a valuable treatment for T2D. The diet containing 0.5% CQE did not appear to cause hepatic damage in this preliminary study (data not shown).

In this study, body weight gain in KK-A^y mice was suppressed by ingestion of CQE as well as GTE, and the suppression in KK-A^y mice fed the CQE and GTE extracts was mainly attributed to decrease of mesenteric adipose tissue and liver weight (Table 2). Several studies on the antiobesity effect of GTE have been carried out in experimental animals and humans.[31,32] Rains et al. indicated that GTE reduced nutrient absorption and appetite and elevated fatty oxidation and energy expenditure,[33] resulting in the suppression of body weight gain. Yokozawa et al. demonstrated in the rat that the administration of grape seed extract containing oligomer and polymer procyanidins resulted in the suppression of body weight gain.[34] Procyanidins in CQE might act as an inhibitor of nutrient absorption and an accelerator of fatty metabolism, as it has been reported that procyanidins inhibit lipase, α-amylase, and α-glucosidase activity[35,36] and enhance fatty acid oxidation.[18]

Ingestion of CQE was found to reduce blood glucose during the experimental period, and GTE further decreased blood glucose (Figure 1A). It was considered that absorption of carbohydrate was depressed by continuous administration of CQE and GTE, because procyanidins and catechins have been shown to inhibit both α-glucosidase and α-amylase.[36,37] On the

other hand, OGTT showed that the blood glucose level in KK-A^y mice tends to be suppressed by CQE similarly to how it is suppressed by GTE after glucose loading (Figure 1C,D). Montagut et al. reported that grape seed procyanidins enhanced insulin sensitivity by activating the insulin receptor and key proteins of the insulin signaling pathway via a mitogen-activated protein kinase (MAPK) pathway.[38] Wu et al. have shown that green tea increased glucose uptake in rat adipocyte because it directly enhanced the binding of insulin to the insulin receptor.[14] Thus, it is possible that procyanidins in CQE might play insulin-like roles in diabetic mice, and the mechanisms might differ from GTE.

Adiponectin is closely related to insulin sensitivity and stimulates glucose uptake in adipocyte and muscle, and excess fatty accumulation in adipose tissues reduces the secretion of adiponectin.[2] Décordé et al. indicated that intake of grape seed procyanidin extract increased plasma adiponectin level in obese hamsters induced by a high-fat diet.[39] However, in the present study, the serum adiponectin level was not significantly increased by intake of CQE and GTE, but there was a tendency of serum adiponectin to increase along with the hypoglycemic effect of CQE and GTE. It was considered that the increase of serum adiponectin level by intake of GTE was induced by down-regulation of mesenteric adipose tissue, whereas intake of CQE had no apparent effect on the suppression of mesenteric adipose tissue compared to that of GTE, resulting in a slight increase in serum adiponectin level by intake of CQE (Table 2). Consequently, adiponectin was not observed to have a conclusive involvement in the glucose-lowering effect of CQE in this experiment.

In KK-A^y mice fed CQE, hepatic TG and cholesterol decreased by half, which was similar to the decrease seen in mice fed GTE (Figure 2A,B). Intake of CQE and GTE resulted in increased excretion of cholesterol in the feces, but did not change fecal TG (Figure 3A,B), implying that the extracts inhibited absorption of cholesterol and affected lipid metabolism in the liver. Oxidation and synthesis of fatty acid are regulated by many factors (including sterol regulatory element binding protein-1 (SREBP-1)), and cholesterol synthesis in liver is partly regulated by SREBP-2.[40] It has been reported that procyanidins derived from grape seed and persimmon peel suppressed SREBP-1 and SREBP-2 proteins in the liver, resulting in improvement of hepatic steatosis.[29,34] However, details about the regulation of hepatic lipid metabolism by CQE remains uncertain, and further studies are required.

Kidney disease caused by diabetes is called diabetic nephropathy and is a major complication of diabetes. It is known that AGEs accumulate in diabetic nephropathy;[5] therefore, AGEs are key metabolites that can be used to evaluate the degree of complications of diabetic nephropathy. α-Dicarbonyl compounds are precursors of AGEs and are involved in the pathogenic mechanism of diabetic nephropathy. Thus, it is important to inhibit generation of α-dicarbonyl compounds for prevention and suppression of diabetic complications. As shown in Figure 4, the content of 3-DG in the kidney was unchanged, but the contents of GO and MG were considerably reduced after the intake of CQE and GTE. The decrease is considered to be relevant to control of blood glucose by polyphenols, because it has been known that formation of α-dicarbonyl compounds increases with hyperglycemia.[41] Although the control of the blood glucose level with CQE was not as large as compared to that with GTE, the formation of GO and MG in the kidney was equally suppressed by consumption of diets supplemented with both extracts. Peng et al. showed that cinnamon bark procyanidins (containing catechin, epicatechin, procyanidin B_2, etc.) scavenged reactive carbonyl compounds (such as MG)[42] and inhibited formation of AGEs.[20] Therefore, monomeric catechins or procyanidins in CQE and GTE might prevent generation of GO and MG and result in suppression of accumulation of AGE in the kidney. It is known that generation of α-dicarbonyl compounds is related to ROS,[43] which are elevated under chronic inflammatory diseases such as diabetes.[44] Many studies have demonstrated that catechin monomers and procyanidins attenuate inflammation and enhance endogenous antioxidant capacity.[16,17,45,46] Moreover, green tea and Chinese quince have antioxidant activities,[25,47] and the extracts used in the present study might have a protective effect against ROS in vitro (data not shown). Therefore, it is possible that antioxidant components in CQE and GTE could abolish ROS both indirectly and directly if the responsible components could be absorbed from the digestive tract. Taken together, these results could suggest that CQE might down-regulate the abundance of GO and MG in the kidney because of attenuation of oxidative stress, resulting in decreased the accumulation of AGEs.

Monomeric catechins such as EGCG were reported to be absorbed from the intestine, and the metabolites were seen in the plasma at a quite high level (C_{max} = 1500 μM).[48] It is difficult to absorb polymer procyanidins compared to tetramer procyanidins.[49] Dimer and trimer procyanidins were detected in rat plasma after ingestion of procyanidin-rich compounds, but the concentration was considerably less than the catechin monomers (C_{max} = 1.4 μM).[48] However, metabolites degraded by intestinal bacterial flora could be absorbed and in that way might exert health effects.[48] Gonthier et al. showed that procyanidins B_3 and C_2 were degraded to 3-hydroxyphenylpropionic acid, 3-hydroxyphenylacetic acid, protocatechuic acid, and 4-hydroxybenzoic acid by intestinal bacterial flora, and they were detected in rat urine.[50] Thus, it is conceivable that the difference in absorption of CQE (containing monomers and polymers) and GTE (containing all monomers: catechin, epicatechin, and EGCG) affected the variation of effects in KK-A^y mice.[51]

In conclusion, the present study indicates that intake of CQE in KK-A^y mice attenuated both increases of blood glucose and accumulation of hepatic lipids. These effects may be due to procyanidins in CQE, which regulate the absorption and metabolism of sugar and lipids. Furthermore, this study demonstrated for the first time that CQE could suppress the generation of α-dicarbonyl compounds in the kidney. Therefore, the present study suggests that CQE may be capable of attenuation of the pathology of type 2 diabetes and inhibition of the progress of diabetic complications.

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Notes

The authors declare no competing financial interest.

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