Effects of Persimmon Fruit Polyphenols on Postprandial Plasma Glucose Elevation in Rats and Humans

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Summary Persimmon is a fruit rich in polyphenols (proanthocyanidins or condensed tannins). Using rats and humans, the effects of Kaki-tannin (Nara-type), persimmon polyphenols prepared using a new method, on postprandial plasma glucose levels were investigated in this study. Kaki-tannin (Nara-type) comprised mainly proanthocyanidins, composed of epicatechin : epicatechin gallate : epigallocatechin : epigallocatechin gallate in a ratio of 1 : 1 : 2 : 2 with a molecular weight of approximately 8,000 Da, with epicatechin gallate as a terminal unit. These polyphenols inhibited amylolytic enzymes, such as α-amylase, maltase, sucrase, and α-glucosidase in vitro, and sodium-dependent glucose transporter 1 in Caco-2 cells. These results suggested that the polyphenols suppressed digestion and absorption in the intestinal tract. The ingestion of 250 mg/kg body weight of the polyphenols significantly suppressed increased blood glucose levels after carbohydrate (2 g/kg body weight of glucose or maltose) loading in rats. In a human trial, 1.88 g of Kaki-tannin (Nara-type) significantly delayed increased plasma glucose levels after carbohydrate (150 kcal of maltooligosaccharides) loading. Thus, Kaki-tannin (Nara-type) holds promise to be developed as a food material that potentially improve blood glucose elevation after meals.

Key Words human trial, persimmon, polyphenols, postprandial plasma glucose, proanthocyanidin

Diabetes mellitus is a metabolic disorder with chronic hyperglycemia due to lack or deficiency of insulin action as the main symptom. Prolonged metabolic disorders increase the risk of developing characteristic chronic disorders, such as retinopathy, nephropathy, and neuropathy: Furthermore, in diabetes, arteriosclerosis progresses systemically, causing myocardial and cerebral infarction and arteriosclerosis obliterans of the extremities, leading to limb amputation (1).

In recent years, the number of patients with diabetes has increased in developed countries due to sattiety. According to the announcement of the International Diabetes Federation, the number of patients with diabetes in the world is currently estimated to be 537 million, and by 2045, is expected to increase to 783 million (2).

There is a strong correlation between diabetes and the risk of developing dementia, and there is increasing recognition that dementia is an important complication of diabetes (3, 4). Epidemiological studies have also shown that diabetes is a major risk factor for Alzheimer’s disease as well as cerebrovascular dementia (5).

Therefore, diabetes prevention has become a very important issue in the super-aging society. For this purpose, it is important to improve lifestyle habits, especially eating habits and the way of eating (6), and foods and their functional ingredients (7, 8) that suppress the rise in postprandial blood glucose levels have been studied and put into use.

Persimmon (Diospyros kaki Thunb.) belongs to the family Echinaceae (Ebenaceae). The scientific name Diospyros; dios refers to God, and Pyros refers to grain, meaning “God’s food.” Its origin is China and is cultivated mainly in East Asia, in China, Korea, and Japan; recently, the cultivation has increased in Spain. Production volume of the fruits is approximately 4.3 million tons (FAOSTAT 2019) worldwide, with China, Spain, Korea, and Japan accounting for approximately 90% of the production. In Japan, 193 thousand tons of persimmon fruits were produced in 2020 (Agriculture, Forestry and Fisheries Statistics, 2021).

Persimmon contains abundant polyphenols, mainly proanthocyanidins (so-called tannins), which are related to various physiological functions, such as improvement of hypercholesterolemia (9) and atherosclerosis (10), suppression of hyperlipidemia (11), and improvement of hypertension (12, 13). Proanthocyani-
Briefly, immature fruits of "Tonewase," a kind of persimmon fruits was performed as described previously (18).

Preparation of polyphenols from persimmon fruits (Nara-type). Preparation of polyphenols from persimmon fruits is mainly proanthocyanidins, which are also abundant in cacao, apples, and grape seeds (16). Compared with these, persimmon proanthocyanidins accumulate in vacuoles (so-called tannin cells) (17). Therefore, by separating the tannin cells and extracting them with hot water, high-purity proanthocyanidins could be extracted using a simple operation (18); polyphenols prepared by this method are called Kaki-tannin (Nara-type).

Polyphenols prepared using this method have a wide range of bioactivity that inactivate 12 types of viruses (19), have bacteriostatic and anti-inflammatory effects on Nontuberculous mycobacteria (20), and ameliorate the pathogenesis of ulcerative colitis (21). In addition, the polyphenols reportedly reduce the severity of infection and transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a Syrian hamster model (22).

In this study, the suppressive effects of these polyphenols on postprandial blood glucose elevation in humans, and the mechanism of the action were investigated.

**MATERIALS AND METHODS**

**Preparation of persimmon fruit polyphenols (Kaki-tannin (Nara-type)).** Preparation of polyphenols from persimmon fruits was performed as described previously (18). Briefly, immature fruits of "Tonewase," a kind of persimmon cultivated in Japan, were harvested and treated with ethanol to remove astringency and homogenized with a juice extractor. These homogenates were centrifuged at 1,630 g for 15 min to separate the homogenate into four layers. The second layer from the bottom, which contained the so-called tannin cells, was separated, removed from the tube, and then lyophilized. The dried powders were then extracted with 100 volumes of water at 121˚C for 15 min. Solubilized polyphenols were lyophilized again and were used in this study as a test material, so-called Kaki-tannin (Nara-type). It was dissolved in water and used in each experiment.

**Analyses of persimmon fruit polyphenols.** Total polyphenols were determined using the Folin-Ciocalteu method with (+)-catechin as a standard (23). Procyanidins were measured the vanillin-HCl method with (+)-catechin as a standard (24). Total carbohydrates were analyzed using the phenol-H\textsubscript{2}SO\textsubscript{4} method with glucose as a standard (25).

Thiolysis, high-performance liquid chromatography (HPLC), and liquid chromatography mass spectrometry (LC/MS) analysis. To analyze the structure of polyphenols, thiolysis was performed as described previously (26) with a few modifications. Persimmon polyphenols (25 mg/mL) were dissolved in ethanol containing 0.5% HCl and 1% 2-mercaptoethanol, and then incubated at 50˚C for 24 h. The mixture was analyzed using HPLC on an octyl silane column (Cosmosil 5C\textsubscript{8}-MS, 4.6 ID\times150 mm, Nacalai Tesque, Inc., Kyoto, Japan), eluting with a linear gradient of solvent B (100% methanol) on solvent A (2% acetic acid) at a flow rate of 1.0 mL/min at 40˚C. The gradient of 2% acetic acid (A) and 100% methanol (B) was as follows: 0–5 min, 0% B; 5–55 min, 0–100% B; 55–60 min, 100% B; 60–65 min, re-equilibration with A. The eluent was monitored at ultraviolet (UV) 280 nm.

Thiolytic products were also analyzed using a premier Waters 2695 LC/MS system with quadrupole time-flight on an octadeysil silane column (Atrantis T3 column, Waters, MA, USA), eluting with a linear gradient of solvent B (100% methanol) on solvent A (0.1% formic acid) at a flow rate of 0.2 mL/min at 25˚C. The gradient of 0.1% formic acid (A) and 100% methanol (B) was as follows: 0–5 min, 0% B; 5–55 min, 0–100% B; 55–60 min, 100% B; 60–65 min, re-equilibration with A. The sample was delivered to the ion source using a syringe pump at 5 μL/min. Detection was conducted using electrospray ionization ion trap mass spectrometry in positive mode.

The proportion of constituent catechins and the average degree of polymerization (DP) were calculated as described before (27, 28).

**Analyses of the inhibitory effects of persimmon polyphenols on amylolytic enzymes.** The activity of α-amylase from porcine pancreas (Sigma-Aldrich Corp., St. Louis, MO, USA) was determined by measuring the reducing power of oligosaccharides released from soluble starch using the Somogyi-Nelson method (29). Those of maltase and sucrase prepared from rat intestine acetone powder (Sigma-Aldrich Corp.) were determined by measuring glucose produced from maltose and sucrose as substrates, respectively, using Glucose C-II Test Wako Kit (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) based on the glucose oxidase method (30). The activity of α-glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich Corp.) was also determined by measuring glucose produced from maltose using the glucose oxidase method.

The rate of enzyme inhibition was calculated as a percentage of the control (without inhibitor) using the formula:

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\text{Enzyme inhibition (\%) = (Ac} - \text{Ai)/Ac} \times 100
\]

where Ac is control activity (activity without the inhibitor) and, Ai is activity with the inhibitor.

In addition, the IC\textsubscript{50}, the concentration of inhibitor required to inhibit 50% of its activity, was also calculated.

**Cell culture.** Caco-2 cells (American Type Culture Collection, Manassas, VA, USA) were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Sigma-Aldrich Corp.) containing 10% fetal bovine serum, 2% L-glutamine, 1% non-essential amino acids, 20 U/mL penicillin, and 20 μg/mL streptomycin in an atmosphere of 5% CO\textsubscript{2}–95% air at 37˚C. The cells were subcultured when confluent using trypsin before subsequent use in experiments. Caco-2 cells used in the transport experi-
ments were seeded at 1.0×10^5 cells/cm² and grown to confluence in 24-well plates, with medium changes every 4 d. All experiments were performed on cells of passage numbers 14–19.

For experiments, medium was discarded and cells were washed twice with phosphate buffered saline (pH 7.4; PBS). After washing, the medium containing 900 μM 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose (2-NBDG, Peptide Institute Inc., Osaka, Japan) (31), a fluorescent glucose analog, with or without persimmon polyphenols or phlorizin, was added to the well and incubated at 37°C for 4 h. Glucose uptake was stopped by washing the cells twice with ice-cold PBS (pH 7.4). Then, 200 μL lysis buffer consisting of 150 mM NaCl, 10% Triton X-100, 1% sodium dodecyl sulfate, 0.5% sodium deoxycholate, and 0.2% sodium azide in 100 mM PBS (pH 6.8) was added to lyse the cells, and aliquots were removed for fluorescence and protein measurement. Fluorescence spectra were recorded with an excitation wavelength of 475 nm and emission wavelength of 540 nm using a Hitachi FL-2700 spectrofluorometer (Hitachi High-Tech Science Co. Ltd., Tokyo, Japan). Fluorescent intensity, corrected for protein content (determined using the bicinchoninic acid method), was measured as a glucose transport values. The inhibitory effects of persimmon polyphenols (1 mg/mL) were examined, and phlorizin (0.5 mg/mL), an inhibitor of sodium-dependent glucose transporter 1 (SGLT1), was used as the positive control. When a sodium free buffer was required, NaCl and NaH₂PO₄ were replaced with equimolar amounts of KCl and KH₂PO₄, respectively.

Experimental animals. All experiments were performed in compliance with the guidelines of the experimental animal care committee of Kindai University Faculty of Agriculture (Approval No. KAAG-25-007), and the rats were managed in compliance with the guidelines for the care and use of laboratory animals. These guidelines are based on the Standards Relating to the Care and Management of Experimental Animals and the Method for Sacrificing Laboratory Animals.

Sprague Dawley (SD) rats were purchased from CLEA Japan, Inc. (Tokyo, Japan). The animals were housed in a temperature and humidity controlled room (temperature 23±1°C, humidity 55±5%) with a 12 h light-dark cycle and given free access to food (CE-2, CLEA Japan, Inc.) and tap water.

Oral carbohydrate (glucose or maltose) tolerance test in rats. After 1 wk of acclimation to the laboratory, an oral glucose tolerance test (2 g glucose/kg body weight) was performed on 9 wk-old male SD rats. These rats, after an overnight fasting, were randomly divided into three groups as follows: control group, 250 mg/kg group and 500 mg/kg group (orally ingested only 2 g/kg body weight glucose solution as a control. 250 mg/kg, and 500 mg/kg body weight persimmon polyphenols in 2 g/kg body weight glucose solution, respectively), each group consisting of 6–7 animals.

Blood was withdrawn from the tail vein at 0, 15, 30, 60, and 120 min after the administration of glucose. After centrifugation (820 × g for 10 min at 4°C), plasma samples were collected and stored at −80°C until analysis. Plasma glucose concentration was analyzed using the glucose oxidase method described above, and plasma insulin concentration was determined using the Rat Insulin ELASA Kit (Shibayagi Co. Ltd., Gunma, Japan). The oral maltose tolerance test (2 g maltose/kg body weight) was also performed on 11 wk-old male SD rats, similar to the oral glucose tolerance test.

Human trial. All procedures in the human trial were approved by the Ethics Committee of Kindai University, Faculty of Agriculture (Approval No. 2016-14) and that of Medical Corporation Kojinkai Housui Sogo Medical Clinic (Sapporo, Japan; Approval No. 17F009), and conducted in accordance with the ethical principles of the Declaration of Helsinki.

Participants were recruited at the Medical Corporation Kojinkai Housui Medical Clinic (Sapporo, Japan). Written informed consent was obtained from all participants at the beginning of the trial. All participants completed the study except for one who withdrew because of personal reasons.

This trial was a randomized double-blind placebo-controlled crossover trial, comprising 35 healthy participants. The participants included 17 males and 18 females, 38±1 y old, 164.4±8.3 cm in height, 63.0±8.9 kg in weight, and 23.3±2.8 in body mass index, and were healthy with no evidence of chronic diseases, according to diagnosis by the doctor and the results of a general health questionnaire. Exclusion criteria were as follows: use of medication, allergy for medicines or food, history of digestive tracts surgery, malfunction of the respiratory or circulatory system, a history of drug abuse, a heavy smoking habit (more than 20 cigarettes/d), irregular eating habits, pregnant or lactating, participating in any clinical trials within 12 wk on the screening day of the trial, or volunteers who were judged by a doctor not to be appropriate to participate in this trial.

Tablets consisting of 1.88 g of persimmon polyphenols and maltitol, cellulose, carboxymethylcellulose calcium and potassium stearate as excipients were prepared. In the placebo, persimmon polyphenols were replaced with the same amount of lactose.

All participants were randomly divided into two groups. After a 12-h overnight fasting and avoiding strenuous exercise, alcohol, and caffeinated beverages for the preceding 24 h, the participants came to the hospital for the trial. Immediately after tablets containing 1.88 g of persimmon polyphenols or 1.88 g of lactose as a placebo (visually identical to those containing polyphenols) were ingested, a resting blood sample (5 mL) was taken. After 30 min, the participants ingested 40 g of maltoligosaccharides (150 kcal, termed TK-16, Matsutani Chemical Industry Co., Ltd., Hyogo, Japan) made up with H₂O to a volume of 100 mL with 1 g of sucrose as a sweetener. Further blood samples (5 mL) were collected at 15, 30, 45, 60, 75, 90, and 120 min while the participants were seated. Plasma glucose and insulin concentrations were measured using the hexo-
kinase-glucose 6-phosphate dehydrogenase method (32) and chemiluminescent immunoassay method (33), respectively.

At the end of the above period, the participants entered a washout phase of 7 d duration and then were crossed over to the other condition.

**Statistical analysis.** Data are expressed as the mean ± standard deviation (SD). Data were tested using a one-way analysis of variance. The groups were statistically compared using Student’s t-test with values showing homogeneity of variance, or Welch’s t-test with values showing no homogeneity of variance or compared by Turkey-Kramer. SPSS (ver. 25, IBM Japan, Ltd.) was used as statistical software. All p values were 2-tailed, and differences were considered significant at $p<0.05$.

**RESULTS**

**Analyses of persimmon polyphenols**

Polyphenols from persimmon fruits (Kaki-tannin (Nara-type)) used in this study consisted of 70% polyphenol (93% of which were proanthocyanidins) and 20% carbohydrate, as analyzed using the Folin-Ciocalteu (using vanillin-HCl) and phenol-H$_2$SO$_4$ methods, respectively.

Thiolytic treatment (thiolysis) combined with LC/MS is the most useful tool for characterizing the structure of tannins (proanthocyanidins) (26). Thiolysis of the polyphenols revealed a mixture of degradation products with five components on an HPLC chromatogram compared to those before thiolysis (Fig. 1). The peak at retention time 25.251 min (Peak 4) was completely matched by epicatechin gallate (ECg) using co-chromatography of catechin standards, and with a $m/z=443.37$ [M+H]$^+$ using LC/MS (positive mode). This compound was considered to be a terminal unit of the polyphenols, because it is not a thioester, although it is a thiol breakdown product. Therefore, ECg was identified as the terminal unit. In addition, another four components, in the order of retention time [21.102 (Peak 1), 23.178 (Peak 2), 23.728 (Peak 3), and 28.371 min (Peak 5), respectively], had $m/z = 383.43$, 367.43, 535.53, and 519.53 [M+H]$^+$, indicating thiolytic derivatives (ethylthioesters) of epigallocatechin (EGC), epicatechin (EC), epigallocatechin gallate (EGCG), and ECg, respectively. In the chromatogram, A-type proanthocyanidin-derived products were not detected, suggesting
that persimmon polyphenols were B-type proanthocyanidins.

From the peak area in the HPLC chromatogram and the molar absorption coefficient of each compound (34), the molar ratio of catechin units in persimmon polyphenols was EC : ECg : EGC : EGCg = 1 : 1 : 2 : 2. The DP was calculated to be 20, estimating a molecular weight of approximately 8,000 Da, according to a previous report (28).

From these results, Kaki-tannin (Nara-type) was considered to consist mainly of proanthocyanidin, and its structure is shown in Fig. 2.

Fig. 2. Structure of persimmon polyphenols (Kaki-tannin (Nara-type)). Its structure consists of EC : ECg : EGC : EGCg = 1 : 1 : 2 : 2, with ECg as a terminal unit. n=20.

Oral carbohydrate tolerance test

To evaluate the effects of persimmon polyphenols on the suppression of postprandial blood glucose elevation, oral carbohydrate (glucose or maltose) tolerance tests were first conducted in rats.

Eleven-week-old male SD rats were orally administered maltose and their plasma glucose levels were measured over time (Fig. 3). Plasma glucose levels before the administration of maltose with or without polyphenols were 109.8–117.1 mg/dL, which were not significantly different between control and the 250 mg/kg or 500 mg/kg polyphenol group. At 15, 30, and 60 min after administration, plasma glucose levels in each polyphenol group were significantly (p<0.01) lower than those of controls (at 15 min after administration, the plasma glucose levels were 180.4, 155.5, and 145.9 mg/dL for controls, 250 mg/kg, and 500 mg/kg polyphenol groups, respectively). At 120 min after administration, plasma glucose levels of these three groups returned to normal levels (112.8–123.0 mg/dL). As shown in Fig. 4, before administration in all three groups, plasma insulin levels were 0.110–0.223 ng/mL, and there was no significant difference between the groups. At 15, 30, and 60 min after administration, plasma insulin levels in each polyphenol group were significantly (p<0.01) lower than those of controls (at 15 min after administration, plasma insulin levels in controls were 1.59 ng/mL, but were 0.653 and 0.471 ng/mL for the 250 mg/kg and 500 mg/kg polyphenol groups, respectively). At 120 min after administration, plasma insulin levels of
these three groups returned to normal levels (0.245–0.329 ng/mL).

When glucose was orally administered to 9-wk-old male SD rats with or without persimmon polyphenols, the changes in plasma glucose levels and plasma insulin levels were similar to those obtained when maltose was administered (data not shown).

These results indicated that persimmon polyphenols suppressed postprandial plasma glucose concentrations in rats, suggesting that they prevented both digestion by intestinal amylolytic enzymes and the resultant glucose absorption from the intestinal tract. *Mechanism of polyphenol on suppression of blood glucose elevation*

To elucidate the mechanism of the suppressive effects of persimmon polyphenols on blood glucose elevation after oral carbohydrate tolerance tests, the digestion of carbohydrates and absorption of glucose in the intestinal tract was examined. The effects of the polyphenols on digestive enzymes were examined in vitro using amylolytic enzymes, such as α-amylase, maltase, sucrase, and α-glucosidase, and the effects on glucose absorption were examined using Caco-2 cells.

In this study, α-amylase from porcine pancreas, maltase and sucrase from rat intestine, and α-glucosidase from S. cerevisiae were used. Since the former three enzymes were derived from mammalian cells, they were hypothesized to be a model for digestion in the small intestine. The IC₅₀ of the polyphenols to inhibit the amylolytic enzymes was 14 μg/mL for α-amylase, 1,840 μg/mL for maltase, 8,530 μg/mL for sucrase, and 49 μg/mL for α-glucosidase (Fig. 5). The inhibition mechanisms for these four enzymes were revealed to be non-competitive using Lineweaver-Burk plot. Persimmon polyphenols inhibited endo-type mammalian amylolytic enzymes more than exo-types.

Glucose produced by amylolytic enzymes in the small intestinal tracts is absorbed through the glucose transporter. Glucose is taken into the small intestine mainly through SGLT1, and in the presence of a large amount of glucose, glucose transporter 2 (GLUT2), not dependent on sodium ions, is also used. Caco-2 cells are an experimental system often used to study the absorption of substances as a model for the small intestine. In this study, a fluorescent glucose derivative, 2-NBDG (31), was used, which has been shown to be absorbed into mammalian cells via the glucose transporter, similar to glucose (35, 36). In addition, phlorizin, which is a polyphenol component of apples, suppresses glucose absorption by inhibiting SGLT1 in Caco-2 cells (37). Since phlorizin inhibits SGLT1 and suppresses glucose absorption in the presence of sodium, but does not suppress glucose absorption in the absence of sodium (37), it was used as a positive control for SGLT1 inhibition.

In the presence of sodium, both persimmon polyphenols and phlorizin reduced the 2-NBDG absorption rate to approximately 60% compared to that of controls, indicating that persimmon polyphenols may inhibit SGLT1, as well as phlorizin (Fig. 6). However, in the absence of sodium, both of them had the same 2-NBDG absorption rate as controls, indicating that they may not affect GLUT2.

These results suggested that persimmon polyphenols, Kaki-tannin (Nara-type), suppressed postprandial plas-
Persimmon Polyphenols Affect Postprandial Plasma Glucose Elevation

Persimmon polyphenols affect postprandial plasma glucose elevation in rats due to inhibition of amylolytic enzyme activities and suppression of SGLT1 in the digestive tract.

Human trial

As described above, in vivo and in vitro studies showed that persimmon polyphenols had a suppressive effect on plasma glucose elevation after carbohydrate loading. Therefore, a human trial to confirm the suppressive effects of polyphenols on the elevation of postprandial plasma glucose concentrations was conducted.

There were no significant differences in fasting plasma glucose (87 ± 10 and 88 ± 10 mg/dL) or plasma insulin (6.3 ± 2.1 and 6.0 ± 2.2 μM/L) levels between the placebo and the polyphenol groups. After administration of the sample, plasma glucose levels in the placebo increased more rapidly than those in the polyphenol groups. Between the placebo and the polyphenol group, there were significant differences (p < 0.01) at 15 and 30 min in plasma glucose concentrations (118 ± 14 mg/dL in the placebo and 107 ± 16 mg/dL in the polyphenol group at 15 min, and 140 ± 22 mg/dL in the placebo and 129 ± 27 mg/dL in the polyphenol group at 30 min; Fig. 7).

The time course of plasma insulin concentration was similar to that of plasma glucose. Fifteen minutes after administration of the sample, plasma insulin levels in the polyphenol group were significantly reduced compared to those of the placebo (p < 0.01; Fig. 8).

However, 1.88 g of Kaki-tannin (Nara-type) was presumed to delay plasma glucose and insulin elevation after the administration of 150 kcal of maltotriosaccharides, because peak points of plasma glucose elevation were almost same between the placebo and the polyphenol group.

**DISCUSSION**

Postprandial hyperglycemia is an important contributing factor to the development of atherosclerosis and cardiovascular diseases (38, 39). Control of postprandial plasma glucose levels is important, and therefore, various α-amylase or α-glucosidase inhibitors have been used to control blood glucose levels and to prevent or treat obesity and diabetes (40).

In this study, Kaki-tannin (Nara-type), made of persimmon polyphenols, was demonstrated to suppress the increase in blood glucose levels after carbohydrate loading in animal tests. Additionally, polyphenol structure and the mechanism of action on digestive enzymes using amylolytic enzymes and glucose absorption in Caco-2 cells were investigated. Finally, the suppressive effects of persimmon polyphenols on postprandial blood glucose elevation were examined in a human trial.

Using combination of thiolysis and LC/MS analysis, the structure of persimmon polyphenols from the variety “Tonewase,” as major compounds of Kaki-tannin (Nara-type), was found to consist of EC, ECg, EGC, and EGCg in a ratio of 1 : 1 : 2 : 2 (50% galloylated). The catechins were polymerized with a B-type linkage, and their average molecular weight was calculated to be approximately 8,000 Da. ECg was determined to be a terminal unit.

These data are similar to those for the structure of polyphenols from the variety “Hiratanenashi,” which comprises EC : ECg : EGC : EGCg = 1 : 2 : 2 (50% galloylated), with an average molecular weight of 13,000 Da (measured using gel-filtration chromatography (HPLC)), although the terminal unit has not been determined (41). As the variety “Tonewase” used in this study was identified as a bud sport, resulting from a nat-
ural mutation that occurs in a single branch in a tree of “Hiratanenashi,” these varieties were considered to be highly genetically similar, with a similar secondary metabolite pathway. Therefore, the structures of polyphenols from “Tonewase” and “Hiratanenashi” were thought to be similar to each other.

However, polyphenols obtained from Chinese persimmon, the variety “GongChengYueShi” have been polymerized with catechin units in a molar ratio of IC₅₀ of polyphenols from the variety “Tonewase”: 14 μg/mL for α-amylase from porcine pancreas, 1,840 μg/mL for maltase from rat intestine, 8,530 μg/mL for sucrase from rat intestine, and 49 μg/mL for α-glucosidase from S. cerevisiae, the inhibition for all was non-competitive. The molecular weight of Kaki-tannin (Nara-type) is approximately 8,000 Da, and these IC₅₀ values (expressed as μg/mL), especially those on maltase and sucrase, seemed to be higher than those of polyphenols, with molecular weights less than 1,000 (44), on amylolytic enzymes. Regarding the digestion of starch, it is considered that persimmon polyphenols exert a high inhibitory effect on α-amylase, due to the formation of complexes not only between persimmon polyphenols and amylolytic enzymes but also between persimmon polyphenols and starch (45). The IC₅₀ of polyphenols from the Japanese variety “Atago” have been reported as 1.7 μg/mL for α-amylase from porcine pancreas, 632 μg/mL for maltase from rat intestine, and 308 μg/mL for sucrase from rat intestine (46); the inhibition for these was also non-competitive. These results were similar to those of the current study, in which the IC₅₀ for α-amylase was relatively smaller than those for maltase and sucrase. In addition, the IC₅₀ of those from variety “GongChengYueShi” have been reported as 350 μg/mL for α-amylase from porcine pancreas and 240 μg/mL for α-glucosidase from S. cerevisiae; the inhibition type of the former was mixed (competitive and non-competitive) and that of the latter was competitive (42). In the case of amylolytic enzymes from mammalian cells, polyphenols from Japanese cultivars seemed to have stronger inhibitory activity for endo-type enzymes than for exo-type ones. However, polyphenols from Chinese cultivars seemed to have nearly the same activity for the amylolytic enzymes. As the polyphenols in the current study are B-type proanthocyanidin, and the variety “GongChengYueShi” contains the A-type one (42), their structures differed, and there are some genetic differences between cultivars (43); thus, secondary metabolites, such as polyphenols, between Japanese and Chinese cultivars, have been thought to also be different, suggesting different structures of the enzyme-inhibitor complex between the cultivars.

In glucose absorption experiments using Caco-2 cells, persimmon polyphenols in this study reduced glucose absorption to the same level as that of phlorizin. In contrast, while polyphenols from the Chinese variety “GongChengYueShi” also suppress glucose absorption (42), the degree of suppression is weaker than that of phlorizin. Most of the studies on glucose absorption have used monomer to trimer polyphenols (47–50), and therefore, studies on the relationship between polyphenols with a larger degree of polymerization and physiological properties are needed.

Since starch and polyphenols have been reported to interact (45), maltose and glucose were used in this carbohydrate tolerance test in rats to confirm the relationship between carbohydrates and digestive enzymes. Kaki-tannin (Nara-type) showed a significant decrease in plasma glucose levels and insulin secretion compared to those of the controls (Figs. 3 and 4). Although we intended to prepare a 500 mg/kg group due to the relatively high IC₅₀ value for maltase and the no blood glucose lowering effect of polyphenols from the cultivar “Atago” in the maltose tolerance test (46), administration of 250 mg/kg maltose in rats was considered to sufficiently inhibit the enzymes and suppressed post-prandial glucose elevation. These results are consistent with those from oral carbohydrate (maltose or glucose) tolerance tests in spontaneously hypertensive stroke prone rats using the same polyphenols (51).

In the case of the variety “GongChengYueShi,” oral administration of a polyphenol-starch complex (500 mg/kg body weight, polyphenol : starch = 3 : 17) to 9 wk-old male SD rats significantly suppresses increased blood glucose levels (14). Further study has demonstrated that persimmon polyphenols not only interact with starch directly, but also strongly inhibit α-amylase and α-glucosidase, with IC₅₀ values of 350 and 240 μg/mL, respectively (14). In addition, 20 μg/mL of persimmon polyphenols significantly decreased glucose uptake and transport in Caco-2 cells (14).

Overall, the current data suggested that persimmon polyphenols alleviated postprandial hyperglycemia by limiting the digestion of starch as well as inhibiting the uptake and transport of glucose. In addition, insulin secretion was also significantly decreased according to plasma glucose concentrations. However, there have been few studies on persimmon polyphenols, and thus it was thought that there was room for further research, including research on polyphenol structures and physiological properties in both animals and humans.

When converting the dosage in rats to humans, the conversion was performed using the body surface area as an index (52). A dose of 250 mg/kg rat was calculated to be 2,420 mg in human weighing 60 kg. Therefore, considering this value, 3 g (reference 49 and Supplemental Online Material, Fig. S1) or 1.88 g (in this human trial) of polyphenols was administered to participants in the trial.
When 150 kcal of maltooligosaccharides was orally administered to healthy participants aged 20–64 y, oral administration of 1.88 g of persimmon polyphenols significantly delayed the increase in plasma glucose levels and accordingly delayed insulin secretion. In general, 75 g of glucose (300 kcal) is used for oral glucose tolerance test, but in this study, 150 kcal of maltooligosaccharide was used because it could be taken orally without difficulty and it is the level consumed daily. As persimmons are known to contain 2–4% polyphenols (14), 2–3 g of polyphenols is taken up when 100 g of persimmon is ingested. Therefore, it is hypothesized that the amount of persimmon ingested in general may suppress the increase in blood glucose level after meals.

In a previous human trial using persimmon polyphenols, 10 healthy men and women in their 20 s were administered 3 g of Kaki-tannin (Nara-type) orally at the same time as 80 kcal of maltose solution (randomized placebo-controlled crossover trial). The increase in blood glucose levels was significantly \( p<0.05 \) suppressed 60 and 75 min after ingestion compared to ingestion of a placebo containing no persimmon polyphenols (51). In the same study using other participants, when 3 g of Kaki-tannin (Nara-type) was ingested 30 min before 80 kcal of maltose intake, blood glucose was significantly decreased 15 \( p<0.01 \) and 30 min \( p<0.05 \) after ingestion compared to that of the placebo group (Supplemental Online Material, Fig. S1). Therefore, it is possible that persimmon polyphenols suppress postprandial glucose elevation in a further human trial, although no trials on the suppression of blood glucose elevation in humans using persimmon polyphenols have been conducted as far as we know.

In conclusion, the Kaki-tannin (Nara-type), polyphenols from persimmon fruits, suppressed amylolytic enzymes, especially \( \alpha \)-amylase and SGLT1, suggesting that these polyphenols suppressed the digestion and absorption of carbohydrate in the intestine. An oral carbohydrate (maltose) tolerance test performed in humans as a preliminary trial (51) and Supplemental Online Material, Fig. S1) revealed suppressed postprandial plasma glucose elevation and reduced insulin secretion, similar to findings obtained in rats. In this study, a human trial clarified that blood glucose and insulin levels were delayed by the administration of persimmon polyphenols. Further study is required to explore the potential of these compounds to be used as a food material facilitating the reduction of postprandial glucose levels.

**Authorship**

The study was designed by KT and TK. The in vitro experiments were conducted and performed under the direction of TK, KA, MI, and MO. The in vivo experiments were conducted and performed under the direction of KT, KA, MI, and MO. The human trial was designed and directed by KT and TK. The results were discussed by all authors. The manuscript was written by KT and TK, and all authors commented on and revised the manuscript.

**Disclosure of state of COI**

No potential conflict of interest was reported by the authors.

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**Supporting information**

Supplemental online material is available on J-STAGE.

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