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# Aronia juice suppresses the elevation of postprandial blood glucose levels in adult healthy Japanese

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SUMMARY

Aronia has various functions toward human health, including the beneficial effect on hypertension, hyperglycemia and hyperlipidemia. Recently, we identified cyanidin-3,5-O-diglucoside as DPP IV inhibitor from Aronia juice. We also found its beneficial effect on hyperglycemia in KKAy mice fed aronia juice. In this study, to examine the effect of aronia juice on postprandial blood glucose levels in Japanese, we performed an oral meal tolerance test (OMTT). We found that postprandial blood glucose levels were reduced in aronia juice-administered adult healthy Japanese. We also found that there was no difference of reduction levels of postprandial blood glucose between male and female. We also found that activities of dipeptidyl peptidase IV (DPP IV), α-glucosidase and angiotensin-converting enzyme (ACE) were reduced by aronia juice. These results suggest that aronia juice suppresses the elevation of postprandial blood glucose levels through inhibition

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of these enzyme activities and may be useful for prevention of metabolic diseases in adult healthy Japanese.



## 1. Introduction

Aronia berries contain many potential health effects through their antioxidant, hepatoprotective and cardioprotective activities [1], and have shown the beneficial effect on diabetes [2–4]. We have recently found that cyanidin-3,5-O-diglucoside, a component of aronia juice, inhibits activity of DPP IV that degrades glucose insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) [5]. The beneficial effect of aronia juice on improvement of diabetes is induced by inhibition of DPP IV and α-glucosidase activities in diabetic and obesity model KKAy mice [6].

In this study, to examine the effect of aronia juice on postprandial blood glucose levels in healthy adult Japanese who were administered aronia juice, we performed an oral meal tolerance test (OMTT).

## 2. Materials and methods

### 2.1. Subjects and study design

The subjects consisted of 37 healthy Japanese (male = 19 and female = 18) at more than 30 years old at the start of the study (Table 1). The study protocol was approved by the Research Ethics Committees of Tenshi College (permission No. 2012-29), and all of the subjects were provided as an informed consent to recipients. The present study was carried out in accordance with the Declaration of Helsinki. The study was an open-label, randomized, 2-period, 1-way crossover study (UMIN000024581). The randomization was done using simple randomization. OMTT was performed as follows: Adult healthy human first took 100 ml of aronia juice before a meal, and their blood glucose levels were then measured. At 30 min after the subjects took 100 ml of water or aronia juice, their blood glucose levels were measured. At 30, 45, 60, 75, 90, 120 and 150 min after the subjects took 200 g of rice (295 kcal), their blood glucose levels were then measured (Fig. 1). The blood glucose levels were measured using Glutest Every (Panasonic Healthcare, Tokyo, Japan). The area under the curve (AUC) of the postprandial blood glucose excursion for 150 min after the start of the meal (PPG $AUC_{0-150\ min}$) was calculated.

### 2.2. Material

Aronia juice was kindly provided by Nakagaki Consulting and Engineer (Osaka, Japan). Compositions of aronia juice and carbohydrates within aronia juice are shown in Tables 2 and 3, respectively. Gly-Pro-MCA and Lys-Met-MCA were purchased from Peptide Institute (Osaka, Japan). DPP IV and ACE were purified from porcine seminal plasma [7,8]. A Glutest sensor and α-glucosidase were obtained from Panasonic HealthCare (Tokyo, Japan) and Sigma–Aldrich (MO, USA), respectively. All other chemicals were of analytical grade and purchased from Wako Pure Chemicals (Osaka, Japan).

**Table 1**
The baseline characteristics in subjects.

| | Male | Female | All |
|---|---|---|---|
| n | 19 | 18 | 37 |
| Age | 45.1 ± 11.5 | 44.7 ± 10.6 | 44.9 ± 11.0 |
| BMI | 23.8 ± 2.5 | 20.4 ± 1.6 | 22.1 ± 2.0 |
| Fasting blood glucose (mg/dl) | 94.7 ± 9.0 | 85.0 ± 10.0 | 90.1 ± 10.8 |

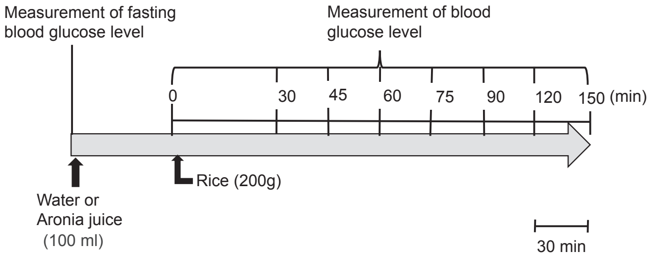


**Fig. 1.** Design of the OMTT for subjects. The fasting blood glucose levels of subjects were first measured as described in Materials and methods. At 30 min after the subjects took 100 ml of water or aronia juice, their blood glucose levels were measured. At 30, 45, 60, 75, 90, 120 and 150 min after the subjects took 200 g of rice (295 kcal), their blood glucose levels were then measured. The number of subjects was 37.

**Table 2**
The composition of aronia juice.

| Components | Aronia juice (g per 100 g) |
|---|---|
| Protein | 0.2 |
| Carbohydrate | 17.9 |
| Fat | <0.1 |
| Minerals | 0.5 |
| Fiber | 0.3 |
| Energy density (kcal per 100 g) | 73 |

**Table 3**
The carbohydrate composition of aronia juice.

| Components | Aronia juice (g per 100 g) |
|---|---|
| Glucose | 4.25 |
| Fructose | 3.87 |
| Sorbitol | 7.39 |

### *2.3. Proteolytic activity of DPP IV*

Enzyme activity was measured by fluorometrical determination (excitation, 380 nm; emission, 440 nm) of the liberation of AMC at 37 °C in a mixture containing 10 μl of 10 mM substrate, 100 μl of 0.5 M Tris–HCl (pH 9.0), 5 μl of enzyme solution and Milli Q water (18 mΩ) in a total volume of 1 ml. After incubation for 30 min, 2 ml of 0.2 M acetic acid was added to the mixture to terminate the reaction.

### *2.4. Proteolytic activity of angiotensin-converting enzyme (ACE)*

Enzyme activity of ACE was examined by measuring the fluorometrical number (excitation, 380 nm; emission, 440 nm) of liberation of AMC in a mixture containing 10 μl of 10 mM Z-Lys-Ala-Met-MCA, 100 μl of 0.5 M Tris–HCl buffer (pH 7.5), 5 μl of 1 M 2-mercaptoethanol, 20 μl of enzyme solution and water (18 mΩ) in a total volume of 1 ml. After incubation of the mixture at 37 °C for 30 min, 2 ml of 0.2 M acetic acid was added to the mixture to stop the reaction.

### *2.5. α-Glucosidase activity*

Enzyme activity was measured using p-nitrophenyl-α-D-glucopyranoside (Sigma–Aldrich, St. Louis, MO, USA) as a substrate. The substrate solution was prepared with dimethyl sulfoxide. The reaction mixture contained 10 μl of 20 mM substrate, 100 μl of 150 mM sodium phosphate (pH 7.0), 10 μl of enzyme solution and Milli Q water (18 mΩ) in a total volume of 300 μl. After incubation at 37 °C for 30 min, PNP-glycoside was quantified on a 96-microplate spectrophotometer at 405 nm.

### 2.6. Statistical analysis

The AUC of the postprandial blood glucose excursion was calculated using a method described previously [9]. Data are expressed as means ± S.E. Statistical analyses were performed using one way analysis of variance followed by unpaired Student's t test. For comparison of multiple samples, the Tukey–Kramer test was used.

## 3. Results

### 3.1. Baseline characteristics of subjects

Table 1 shows baseline characteristics of 37 subjects (19 males and 18 females) who participated in this study. An average of age and BMI (body mass index) was 44.9 ± 11.0 and 22.1 ± 2.0, respectively.

### 3.2. Reduction of blood glucose levels by aronia juice on OMTT

To examine the reduction of blood glucose levels of recipients who were administered aronia juice, we performed an oral meal tolerance test (OMTT) (Fig. 1). The result of OMTT showed that blood glucose levels after the meal were reduced in the aronia group (Fig. 2A) and that reduction levels of blood glucose were not changed between male and female in aronia group (Fig. 2C and E). PPG $AUC_{0-150\ min}$ of male, female and male/female was shown in Fig. 2B, D and F, respectively.

### 3.3. Reduction of DPP IV, α-glucosidase and ACE activities

DPP IV, α-glucosidase and ACE activities were significantly inhibited *in vitro* by aronia juice in a dose-dependent manner (Fig. 3A–C). The inhibition rate of DPP IV, α-glucosidase and ACE activity was 35, 75 and 95% by 40 μl of aronia juice, respectively.

## 4. Discussion

Reduction of a postprandial blood glucose level is very important for protection of the blood vessel and prevention of cardiovascular disease. Previous studies showed that blood glucose levels were reduced in aronia juice-administered patients with insulin-dependent and non-insulin dependent diabetes [4]. Our previous study also showed that blood glucose levels were reduced in aronia juice-administered KKAy mice of type 2 diabetes and obesity models [6], indicating that aronia juice has the beneficial effect on human and mice with diabetes. In this study, we found that the postprandial blood glucose level was reduced by administration of aronia juice in adult healthy Japanese. Furthermore, we showed that DPP IV, α-glucosidase and ACE activities were inhibited by aronia juice in a dose-dependent manner. We previously reported that DPP IV and α-glucosidase activities were inhibited in the small intestine from KKAy mice fed aronia juice [6]. Inhibition of these enzymes reduces blood glucose levels through differential processes: Incretin such as GIP and GLP-1 is not degraded by inhibition of DPP IV activity and induces insulin secretion from β-cells; glucose transportation into epithelial cells of the small intestine is, on the other hand, reduced by inhibition of α-glucosidase activity. Inhibition of these enzymes by aronia juice may lead to reduction of blood glucose levels in patients with insulin-dependent and non-insulin-dependent diabetes [4]. An extract of aronia melanocarpa reduces the blood pressure in patients with metabolic syndrome [10]. Since ACE activity was reduced by aronia juice in a dose-dependent manner, inhibition of ACE activity possesses ameliorative effects on insulin resistance and type 2 diabetes [11]. The mechanism of the improvement effect on type 2 diabetes might be linked to inhibition of ANG II-mediated lipogenesis [12,13]. ACE inhibitory activity by aronia juice may lead to prevention of type 2 diabetes mellitus through inhibition of the

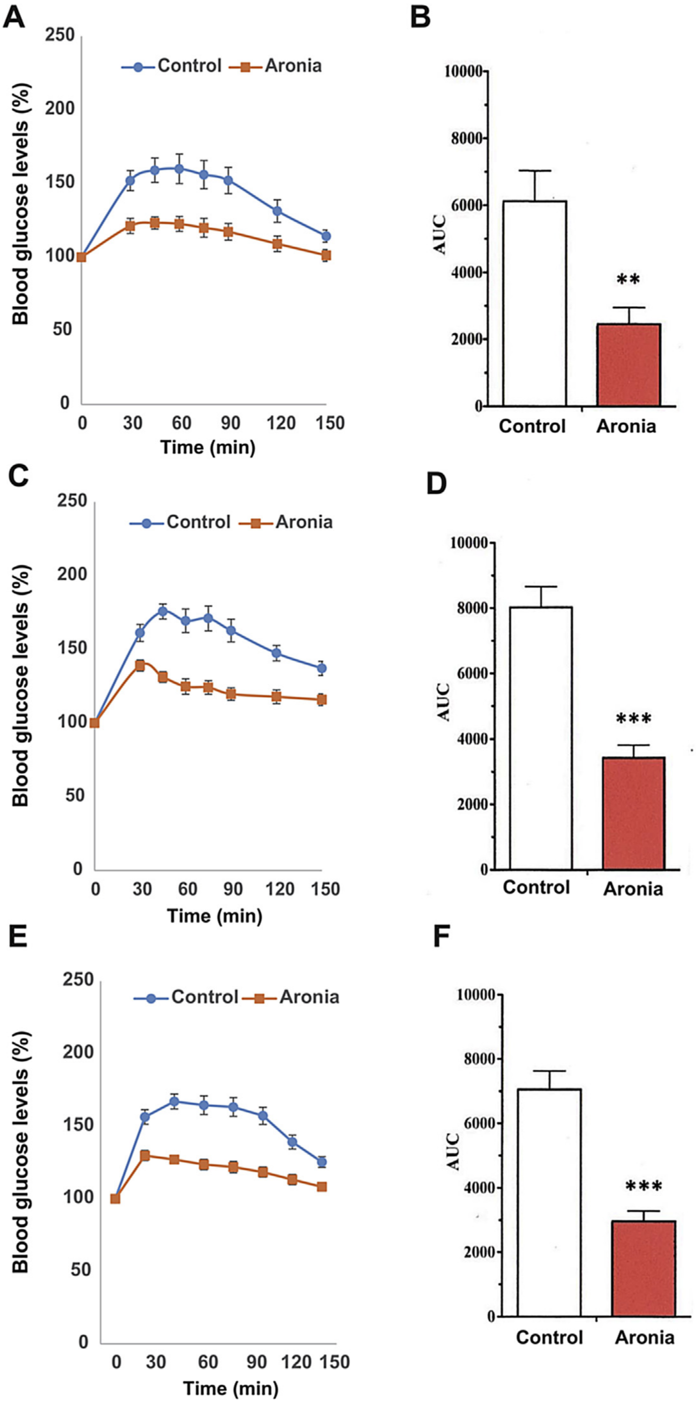


**Fig. 2.** Reduction of postprandial blood glucose levels of adult healthy Japanese by administration of aronia juice. Adult healthy human took 100 ml of aronia juice before a meal, and their blood glucose levels were then measured as described in Materials and methods. A. male (n = 19), C. female (n = 18), E. male and female (n = 37). AUC values were shown (B. male, D. female and F. male and female). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

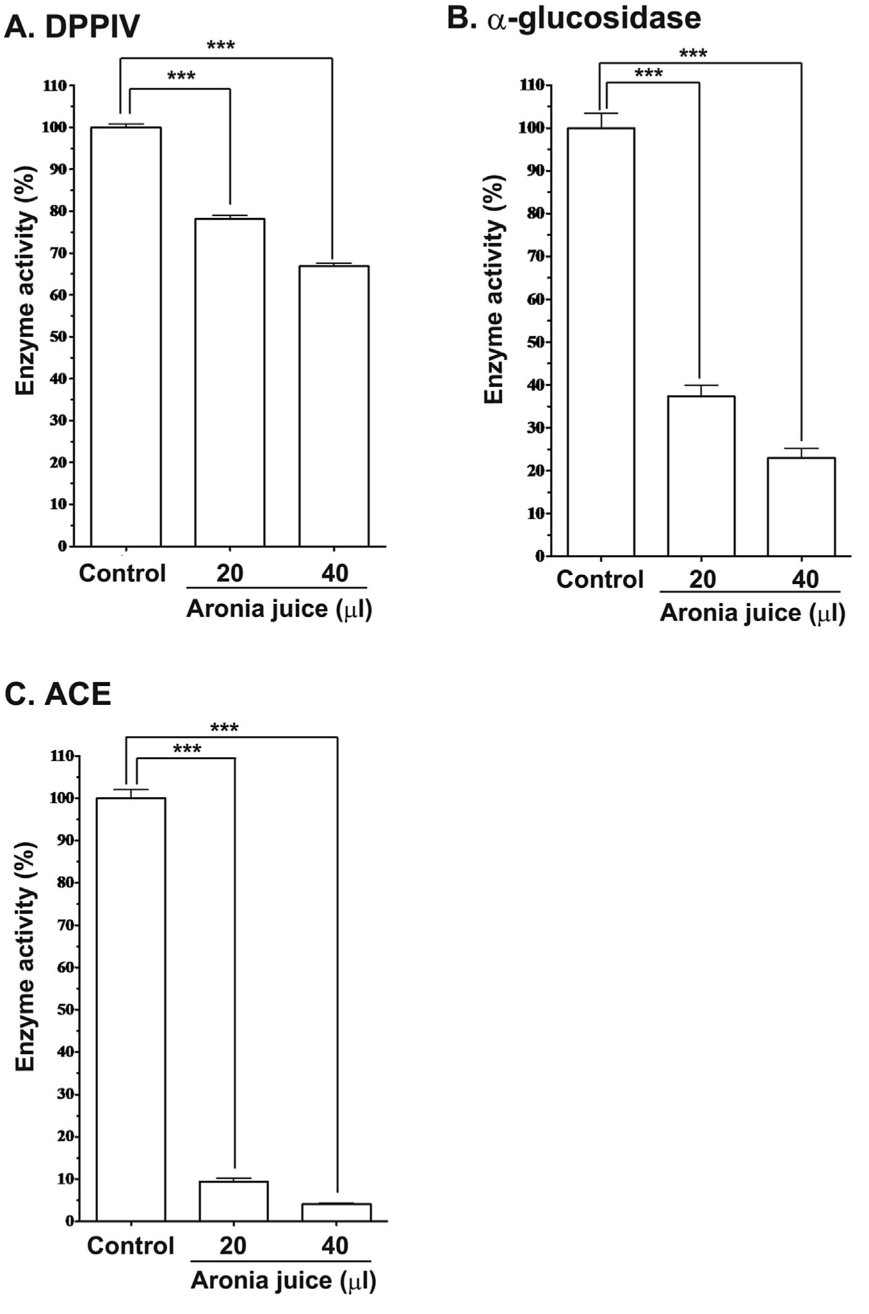


**Fig. 3.** Inhibition of three enzyme activities by aronia juice. DPP IV (A), α-glucosidase (B) and ACE (C) were reacted with various amounts of aronia juice and their activities were measured as described in Materials and methods. n = 5, $^{***}p < 0.001$.

renin-angiotensin system. These results indicate that inhibition of three enzymes by aronia juice is very important for the reduction of blood glucose levels and suggest that aronia juice may be useful for prevention of metabolic diseases such as insulin resistance, metabolic syndrome or type 2 diabetes in adult healthy Japanese.

## Conflict of interest

The authors declare that they have no conflict of interests.

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