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Improvement of blood glucose levels and obesity in mice given aronia juice by inhibition of dipeptidyl peptidase IV and α-glucosidase☆

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Abstract

Aronia berries have many potential effects on health. Previous human studies have shown that aronia juice may be useful for treatment of obesity disorders. Recently, we have reported that aronia juice has an inhibitory effect on dipeptidyl peptidase (DPP IV) activity and that the DPP IV inhibitor in aronia juice was identified as cyanidin 3,5-diglucoside. In this study, we found that body weights and blood glucose levels were reduced in diabetes model KK-Ay mice given aronia juice. We also found that weights of white adipose tissues were reduced in KK-Ay mice given aronia juice. Furthermore, levels of DPP IV activity in the serum and liver from KK-Ay mice were lower than those in the serum and liver from C57BL/6JmsSlc mice. Interestingly, although levels of DPP IV activity were not changed in the serum and liver from aronia-juice-administered KK-Ay mice, levels of DPP IV activity were increased in those from aronia-juice-administered C57BL/6JmsSlc mice. Furthermore, α-glucosidase activity was inhibited in the upper region of the small intestine from aronia-juice-administered KK-Ay mice but not in the lower region. Inhibition of α-glucosidase activity in the upper portion of the small intestine induced a reduction of glucose-dependent insulinotropic polypeptide (GIP) level. The results suggest that DPP IV activity in diabetic mice is inhibited by aronia juice, that the GIP level in the upper region of the small intestine is reduced by inhibition of α-glucosidase activity and that weights of adipose tissues are reduced by aronia juice.

Keywords: Aronia juice; DPP IV inhibitor; KK-Ay mice; Antidiabetic effect; α-Glucosidase inhibition

1. Introduction

Aronia berries have been used in traditional medicine to treat atherosclerosis and hypertension in Russia and Eastern European countries [1]. Aronia berries have high contents of phenolic phytochemicals. The concentrations of active compounds in aronia berries, including anthocyanins, procyanidins and flavonoids, are over fivefold higher than those in cranberries [2,3]. Aronia berries also have various potential health effects [4], and aronia juice has been shown to have beneficial effects on plasma glucose levels in diabetic humans [5] and rats [6] and on total cholesterol and lipid levels in diabetic humans [7]. Furthermore, results of human studies have suggested that aronia juice is useful for treatment of obesity disorders [8]. Recently, we have reported that aronia juice has a dipeptidyl peptidase IV (DPP IV) inhibitory effect and that the DPP IV inhibitor in aronia juice was identified as cyanidin 3,5-diglucoside [9]. DPP IV (EC 3.4.14.5) is a serine peptidase [10] that cleaves the N-terminal region of incretins such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), and reduction of insulin secretion is induced by inactivation of incretin by DPP IV [11–14]. DPP IV inhibitors have beneficial effects on plasma glucose level in diabetic patients [15]. DPP IV inhibitors have also been found in several plants [16]. In this study, we found that body weight, weight of white adipose tissues and serum levels of blood glucose were reduced in diabetes model KK-Ay mice given aronia juice. DPP IV and α-glucosidase activities were inhibited in KK-Ay mice given aronia juice. These results suggest that aronia juice has beneficial effects on diabetes and obesity through inhibition of DPP IV and α-glucosidase activities under a diabetic condition.

2. Materials and methods

2.1. Materials

Aronia juice was kindly provided by Nakagaki Consulting and Engineer (Osaka, Japan). The composition of the aronia juice is shown in Table 1. The carbohydrate composition of the aronia juice is also shown in Table 2. Gly-Pro-MCA was purchased from Peptide Institute (Osaka, Japan). DPP IV was purified from porcine seminal plasma [4]. A

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Table 1
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 diet)	73

Glutest Neo sensor and α-glucosidase were obtained from Panasonic Health Care (Tokyo, Japan) and Sigma-Aldrich (St. Louis, MO, USA), respectively. An insulin assay kit was purchased from MIoBS (Yokohama, Japan). RNA*later* and RNeasy Mini were purchased from Qiagen (Venlo, Netherlands). PrimeScript RT Master Mix and SYBR Premix Ex Taq II were obtained from Takara (Shiga, Japan). All other chemicals were of analytical grade and were purchased from Wako Pure Chemicals (Osaka, Japan).

2.2. Animals

C57BL/6JmsSlc and KK-Ay male mice were obtained at 4 weeks of age from Japan SLC (Tokyo, Japan). All mice were fed a normal diet (Mouse Diet Auto/YS, LabDiet, St. Louis, MO, USA). After 2 weeks, C57BL/6JmsSlc and KK-Ay mice were divided into two groups of five mice in each group: one group was given water (control group), and the other group was given aronia juice (aronia group). Aronia juice was given by free intake. At 28 days after starting the diets, serum was obtained from veins of mice, and mice were sacrificed by isoflurane anesthesia. Liver and adipose tissues were isolated and weighed.

2.3. Blood glucose levels

Blood glucose levels were measured using a small blood glucose measurement apparatus, Glutest Neo alpha (Panasonic Health Care, Tokyo, Japan).

2.4. Proteolytic activity

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.

2.5. 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. Insulin level

Blood levels of insulin were measured using a mouse insulin measurement kit (MIoBS, Yokohama, Japan).

2.7. Reverse transcription quantitative polymerase chain reaction (PCR)

Total RNAs were prepared from intestines from mice using an RNeasy mini kit. Reverse transcription was carried out in a mixture containing 500 ng of total RNAs and specific primers under the conditions of 95°C for 30 s, 40 cycles of 95°C for 10 s and 60°C for 30 s by using SYBR *Premix Ex Taq* II (Takara) and a real-time PCR system (MiniOpticon, Bio-Rad, Hercules, CA, USA). β-Actin (ACTB) mRNA was also amplified as an internal control. Nucleotide sequences of oligonucleotides used for primers of real-time PCR are shown in Table 3.

Table 2
The carbohydrate composition of aronia juice

Components	Aronia juice (g per 100 g)
Glucose	4.25
Fructose	3.87
Sucrose	ND
Sorbitol	7.39

ND: not detected.

Table 3
Nucleotide sequences of primers and PCR conditions used for real-time PCR

Gene	Nucleotide sequence		PCR condition
mGIP	Sense	5′-AGGGCAACATCTTTGTCACC-3′	95°C 3 min, 95°C 10 s,
	Antisense	5′-CCACGTCAAAGTGTCCAATG-3′	60°C 30 s × 50 cycles
mproGCG	Sense	5′-GGCCACTCCAACACAGAAAT-3′	95°C 3 min, 95°C 10 s,
	Antisense	5′-CTCTGCCAGGAGGACAACTC-3′	60°C 30 s × 50 cycles
mACTB	Sense	5′-GTCGCTCTTTGTCTCGTTCC-3′	95°C 3 min, 95°C 10 s,
	Antisense	5′ -TGCAGGCATTTCTTGTTCAG-3′	60°C 30 s × 50 cycles

2.8. Statistical analysis

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.

2.9. Ethics statement

All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the protocols were approved by the Committee for Animal Research at Hokkaido University (permit number: 15-0009).

3. Results

3.1. Beneficial effects of aronia juice on body weight and blood glucose levels

To examine the effects of aronia juice on body weight and blood glucose levels, aronia juice and water were administered orally to mice in the aronia group and control group, respectively. Body weight and blood glucose levels were measured every 3 or 4 days. As shown in Fig. 1A, body weight of KK-Ay mice, but not that of C57BL/6JmsSlc mice, in the aronia group was significantly reduced compared to that of mice in the control group. Body weight of KK-Ay mice given aronia juice was about 12% of that in the control group at 28 days after the start of administration. Blood glucose level of KK-Ay mice, but not that of C57BL/6JmsSlc mice, in the aronia group was also significantly reduced compared to that of mice in the control group. Blood glucose level of KK-Ay mice given aronia juice was about 45% of that in the control group at 28 days after the start of administration (Fig. 1B).

3.2. Beneficial effects of aronia juice on adipose tissues

Twenty-eight days after the start of administration, adipose tissues were extracted from the mice and weighed. The weights of white adipose tissues in KK-Ay mice, but not those in C57BL/6JmsSlc mice, in the aronia group were significantly reduced compared to those in the control group. The weights of epididymal, mesenteric, retroperitoneal and subcutaneous white adipose tissues in KK-Ay mice given aronia juice were about 27%, 26%, 38% and 48% of those in the control group, respectively (Fig. 2A–D).

3.3. Inhibitory effect of aronia juice on DPP IV activity in the serum, liver and intestine

Twenty-eight days after the start of administration, serum and livers were extracted from the mice, and their DPP IV activities were measured. As shown in Fig. 3A, serum DPP IV activities in KK-Ay mice were lower than those in C57BL/6JmsSlc mice. Serum DPP IV activities in the control group of C57BL/6JmsSlc mice were significantly increased compared to those in the aronia group of C57BL/6JmsSlc mice. On the other hand, serum DPP IV activities in the control group of KK-Ay mice were not significantly different from those in the aronia

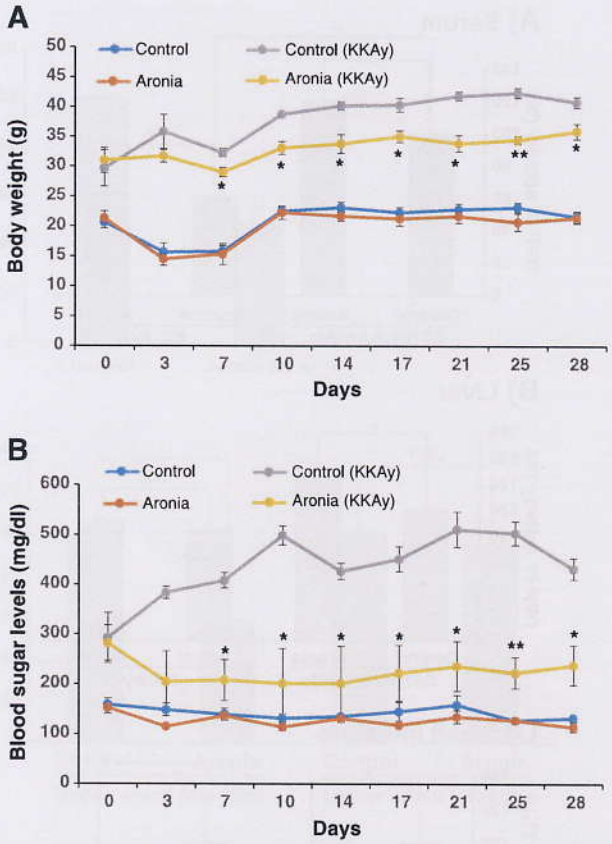

Fig. 1. Differences in body weight and blood glucose levels between control and aronia-juice-administered mice. Body weight (A) and blood glucose level (B) in mice were measured every 3 or 4 days for 28 days. There were significant differences between KK-Ay control mice and KK-Ay mice administered aronia juice. $^{*}P<.05$, $^{**}P<.01$, $n=5$.

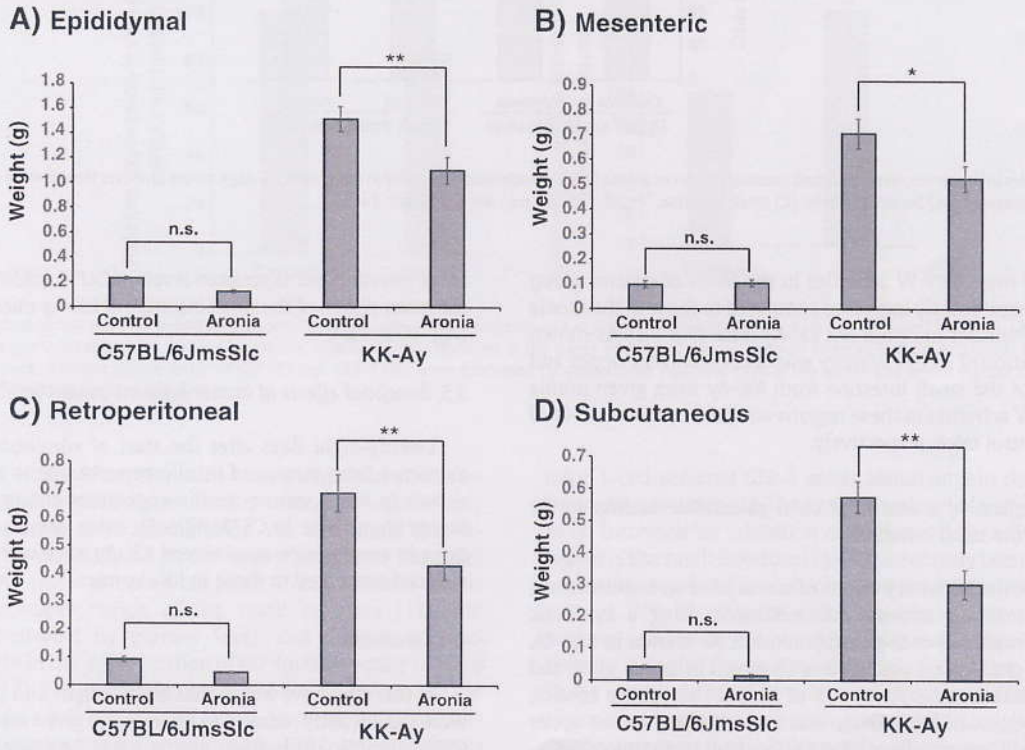

Fig. 2. Reductions of adipose tissue weights in KK-Ay mice administered aronia juice. Water or aronia juice was administered orally to mice. After 28 days, white adipose tissues were obtained and weighed. (A) Epididymal white adipose tissue, (B) mesenteric white adipose tissue, (C) retroperitoneal white adipose tissue, (D) subcutaneous white adipose tissue. $^{*}P<.05$, n.s.: not significant. $n=5$.

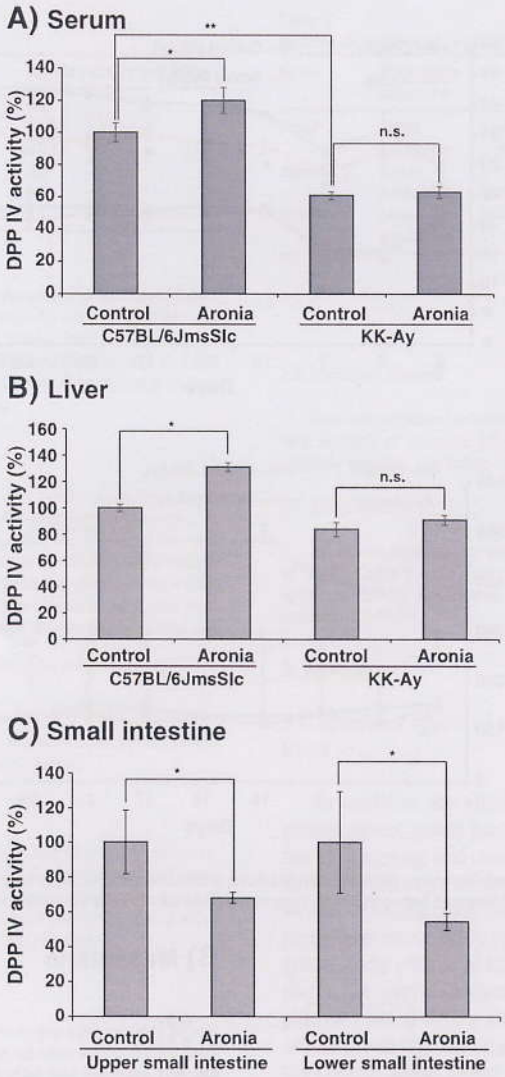

Fig. 3. DPP IV activities in the serum, liver and small intestine. Water or aronia juice was administered orally to mice. After 28 days, serum and liver tissues were obtained, and their DPP IV activities were measured. (A) Serum, (B) liver, (C) small intestine. $^{*}P<.05$, $^{**}P<.01$, n.s.: not significant. $n=5$.

group of KK-Ay mice. DPP IV activities in the livers of control group mice were also significantly increased compared to those in the aronia group of C57BL/6JmsSlc mice but not KK-Ay mice (Fig. 3B). As shown in Fig. 3C, inhibition of DPP IV activity was observed in the upper and lower regions of the small intestine from KK-Ay mice given aronia juice, and DPP IV activities in these regions were about 65% and 54% of those in the control mice, respectively.

3.4. Inhibitory effects of aronia juice on α-glucosidase activity in the upper region of the small intestine

To determine the inhibitory effects of aronia juice on α-glucosidase activity, α-glucosidase activity was measured using a synthetic substrate, *p*-nitrophenyl-α-D-glucopyranoside. As shown in Fig. 4A, inhibition of α-glucosidase activity was observed in aronia juice, and α-glucosidase activity was about 51% of that in the vehicle control. Inhibition of α-glucosidase activity was also observed in the upper region of the small intestine from KK-Ay mice given aronia juice, and α-glucosidase activity was about 42% of that in the control mice (Fig. 4B). Expression levels of GIP mRNA were reduced in the upper region of small intestine and expression levels of GLP-1 mRNA were increased in the lower region of the small intestine of KK-Ay mice given aronia juice (Fig. 4C and D).

3.5. Beneficial effects of aronia juice on insulin levels

Twenty-eight days after the start of administration, serum was extracted from mice, and insulin concentrations were measured. As shown in Fig. 5, serum insulin concentrations in KK-Ay mice were higher than those in C57BL/6JmsSlc mice. Serum insulin concentrations in aronia-juice-administerd KK-Ay mice were also significantly reduced compared to those in KK-Ay mice.

4. Discussion

In this study, we found that body weight and blood glucose level were significantly reduced in KK-Ay mice given aronia juice but not in C57BL/6JmsSlc mice given aronia juice. Recently, we reported that aronia juice has an inhibitory effect on DPP IV [9]. The results of this study and previous studies suggest that blood glucose level is reduced

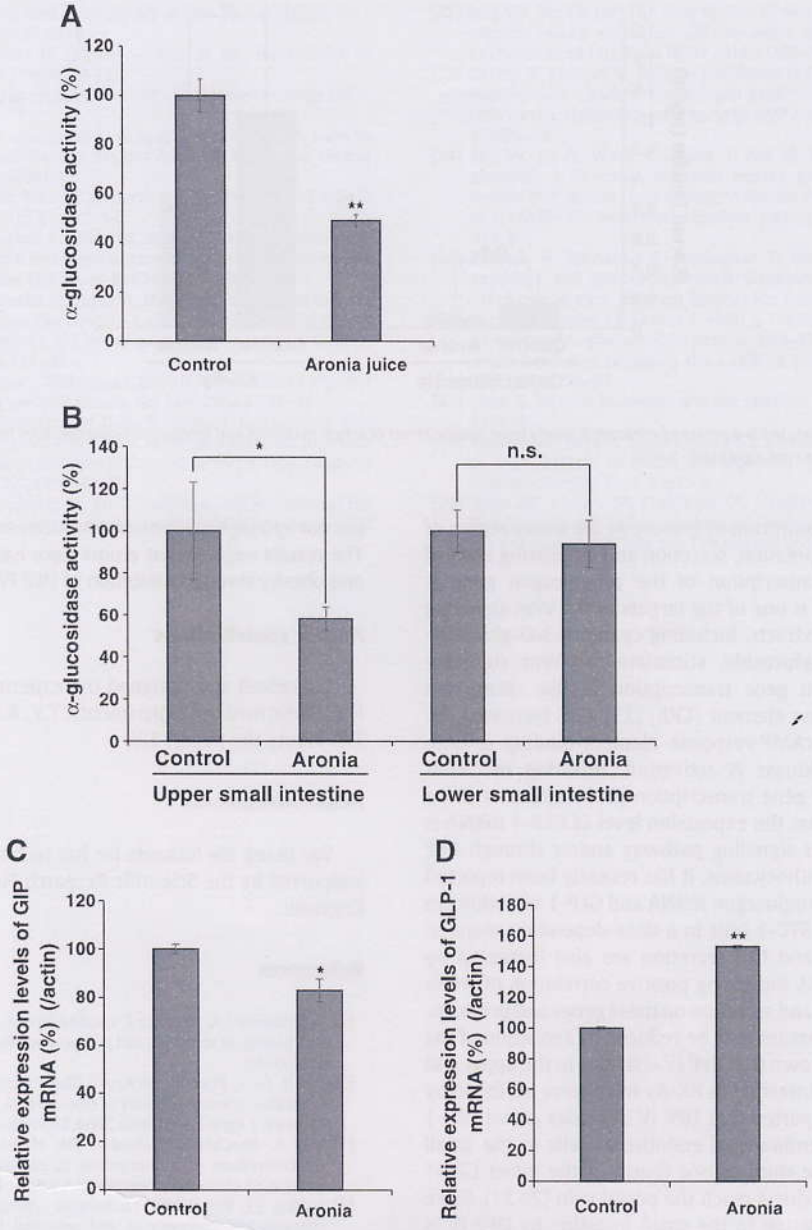

Fig. 4. Inhibitory effect of aronia juice on α-glucosidase activity in the upper small intestine and expression levels of GIP and GLP-1 in the small intestine. (A) Aronia juice has an inhibitory effect on α-glucosidase activity. (B) α-Glucosidase activities were measured in the upper and lower regions of small intestines of KK-Ay control mice and KK-Ay mice administered aronia juice. Relative mRNA levels of GIP (C) and GLP-1 (D) were examined by quantitative real-time PCR in the upper and lower regions of the small intestine, respectively. $^{*}P<.05$, $^{**}P<.01$, n.s.: not significant. $n=5$.

though inhibition of DPP IV activity, thus having beneficial effects on diabetes. Weights of white adipose tissues were also significantly reduced in KK-Ay mice given aronia juice. We also found that α-glucosidase activity was reduced in the upper portion of the small intestine of KK-Ay mice given aronia juice. K-cell-secreted GIP is abundant in the upper region of the small intestine [17]. GIP expression is controlled by glucose level, and inhibition of α-glucosidase activity in the upper portion of the small intestine reduces the level of GIP [18]. The expression level of GIP was reduced in the upper region of the small intestine of aronia-juice-administered KK-Ay mice. Accumulation of fat is also induced by GIP in adipose tissues [19]. On the other hand, α-glucosidase activity was not inhibited in the lower region of the small intestine of aronia-juice-administered KK-Ay mice. L-cell-secreted GLP-1 exists abundantly in the lower region of the small intestine [17]. The expression level of GLP-1 was also shown to be increased by inhibition of α-glucosidase activity in the upper region of the small intestine [18]. It has recently been reported that the circulating level of GLP-1 is elevated more by combination therapy using a DPP IV inhibitor and an α-glucosidase inhibitor than by therapy using a DPP IV inhibitor alone and that this combination therapy is useful for treatment of type 2 diabetes and obesity [20]. These previous studies and our findings indicate that the α-glucosidase inhibitor in aronia juice inhibits α-glucosidase activity in the upper region of the small intestine and that expression, secretion and circulating level of GIP were reduced by a decrease in absorption of glucose in the upper region of the small intestine,

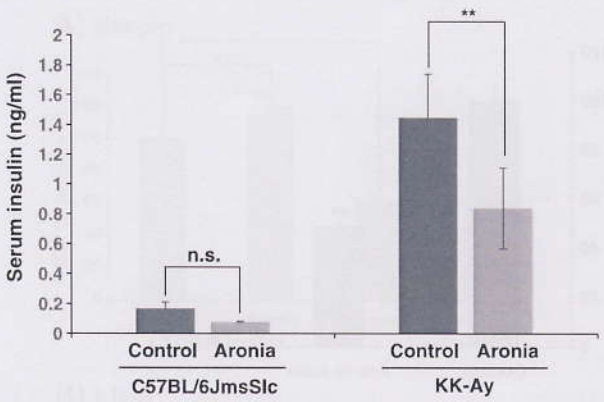

Fig. 5. Changes in serum insulin levels in KK-Ay mice administered aronia juice. Insulin levels of serum in control and aronia-juice-administered mice were measured by enzyme-linked immunosorbent assay. $^{**}P<.01$, n.s.: not significant. $n=5$.

leading to an increase in absorption of glucose in the lower region of the small intestine, and expression, secretion and circulating level of GLP-1 were increased. Transcription of the proglucagon gene is activated by β-catenin and is one of the targets of the Wnt signaling pathway [21]. Black rice extracts, including cyanidin-3-O-glucoside (C3G) and peonidin-3-O-glucoside, stimulate the Wnt signaling pathway [22]. Proglucagon gene transcription is also stimulated through cyclic AMP response element (CRE) [23]. C3G increased the phosphorylation level of cAMP-response element-binding protein (CREB) through protein kinase A activation, resulting in CREB-mediated up-regulation of gene transcription [24]. Results of those previous studies suggest that the expression level of GLP-1 mRNA is increased through the Wnt signaling pathway and/or through CRE targeted by aronia berry anthocyanins. It has recently been reported that the expression of preproglucagon mRNA and GLP-1 secretion are increased by nesfatin-1 in STC-1 cells in a dose-dependent manner. Expression of GIP mRNA and GIP secretion are also increased by nefatin-1 in STC-1 cells [25], indicating positive correlation of levels between mRNA expression and secretion on these genes and products. Weights of white adipose tissues may be reduced by reduction of the GIP level. It has also been shown that DPP IV activities in the upper and lower regions of the small intestine in KK-Ay mice were inhibited by aronia juice. It has been reported that DPP IV degrades active GLP-1 peptide in the luminal membrane of endothelial cells in the small intestine and that only one third to one fourth of the intact GLP-1 peptide is left once the products reach the portal vein [26,27]. Since inhibition of GLP-1 degradation in the small intestine by DPP IV is increased and increased active GLP-1 levels affect glucose levels, our findings in this study and previous studies indicate that glucose level in KK-Ay mice given aronia juice is reduced through inhibition of both DPP IV and α-glucosidase activities in the small intestine. On the other hand, increased serum DPP IV activity in the aronia group of C57BL/6JmsSlc mice may be necessary for prevention of hypoglycemia due to the increased GLP-1 level induced by DPP IV and α-glucosidase inhibitors. Furthermore, serum DPP IV activity in KK-Ay mice is lower than that in C57BL/6JmsSlc mice. These results indicate that DPP IV activity is not required for using cleaved incretin peptides in KK-Ay mice because incretin peptides are required for reduction of the blood glucose level in KK-Ay mice. Furthermore, serum insulin levels were decreased in aronia-juice-administered KK-Ay mice. Since weights of white adipose tissues were also significantly reduced in KK-Ay mice given aronia juice, hyperinsulinemia and insulin resistance were reduced in aronia-juice-administered KK-Ay mice. Combination therapy using DPP IV and α-glucosidase inhibitors reduces serum insulin concentration [28,29]. After reduction of the insulin resistance level, absorption of blood glucose is induced by a lower insulin concentration but not by a high insulin concentration such as that in hyperinsulinemia. The results suggest that aronia juice has beneficial effects on diabetes and obesity through inhibition of DPP IV and α-glucosidase activities.

Author contributions

Conceived and designed the experiments: T.Y., Y.N., T.N., I.O. and H.A. Performed the experiments: T.Y., K.M. and D.K. Analyzed the data: T.Y. Wrote the paper: T.Y.

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