Dietary Anthocyanin-rich Haskap Phytochemicals Inhibit Postprandial Hyperlipidemia and Hyperglycemia in Rats

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Abstract: Haskap (Lonicera caerulea L.) fruit contains some bioactive phenolic phytochemicals, mainly cyanidin-3-glucoside (cy3-glc) and chlorogenic acid. The purpose of this study was to investigate the effects of anthocyanin-rich phenolic phytochemical (containing 13.2% anthocyanin) purified from a Haskap fruit (named Haskap phytochemical) on postprandial serum triglyceride and blood glucose levels. The Haskap phytochemical (containing cy3-glc at 300 mg/kg of body weight) was administered orally to rats fasted for 24 h and 30 min later, a corn oil emulsion was administered to these rats. After the administration, serum triglyceride concentration was measured. An increase in serum triglyceride concentration and the AUC significantly lowered in the Haskap phytochemical-administered group than in the saline-administered group. To evaluate the effect of serum glucose levels, the Haskap phytochemical was orally administered to rats fasted for 24 h and sucrose solution (2 g/kg of body weight) was administered to these rats after 30 min. After the administration, blood glucose level was measured. The Haskap phytochemical significantly reduced the increase in blood glucose levels and AUC in the Haskap phytochemical-administered group than in the saline-administered group. Furthermore, to investigate the long-term effects of Haskap phytochemical intake, high-fat diet (HF diet) with 1.5% or 3.0% Haskap phytochemical was administered to rats for four weeks. The investigation of chronological changes in the serum components of the rats fed HF diets in addition to the administration of Haskap phytochemical showed that the increase in serum triglyceride concentrations, total cholesterol concentrations and blood glucose were significantly suppressed compared to the HF diet-fed control (HF-control). These results suggest that the decrease in postprandial blood lipids and blood glucose by short or long-term Haskap phytochemical ingestion is due to anthocyanin and other polyphenols contained in the Haskap phytochemical.

Key words: Haskap, phytochemicals, anthocyanin, polyphenol, postprandial hyperlipidemia

1 Introduction

According to a 2011 WHO report, arteriosclerotic diseases such as myocardial infarction and cerebral infarction are the number one cause of death worldwide. This cause is revealed to be strongly related to a combined metabolic syndrome involving abnormal lipid metabolism, high blood pressure, obesity, and diabetes.

The plasma triglyceride response to the ingestion of fat by normal and hypertriacylglycerolemic rats in the postabsorptive state is important because of the association between the increase in postprandial triglyceride concentration and coronary heart disease.11

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The oxidative modification of low-density lipoproteins is involved in aging and in various diseases, including atherosclerosis, cataract, rheumatoid arthritis, and neurodegenerative disorders. It has a special influence in cardiovascular diseases, in which polyunsaturated fatty acids are particularly susceptible to oxidation mediated by free radicals. Therefore, medical treatment and the improvement of dietary life are very important. The preventive effects of anthocyanin and other flavonoids abundantly contained in fruits have been reported for metabolic syndrome, lipid peroxidation, and cardiovascular disease.

We have studied the antioxidant activity of phytochemicals from plants, including berries and herbs, which grow in the northern areas of Japan (Hokkaido Island), using rats with ethanol-induced gastric injury; we have also studied the absorption kinetics and lipid metabolism. It has been discovered that this fruit contains antioxidant polyphenols such as cy 3-glc and chlorogenic acid. Concerning other studies on the Haskap fruit, protective effects against oxidative damages at cellular level and anti-microbial activity have been studied in addition to component analyses. However, the effects on hyperlipidemia and hyperglycemia with regard to the components in the Haskap fruit have not yet been clearly demonstrated.

Therefore, in this study, to reveal the functionality of the anthocyanin-rich phenolic phytochemical that is purified from the Haskap fruit, longitudinal changes in the post-prandial blood lipid concentration after a lipid load and in the blood glucose level after a sucrose load were investigated. Furthermore, daily changes in the serum lipid concentrations and the blood glucose levels of the rats fed HP diets in addition to the Haskap phytochemical were measured.

2 Materials and methods

2.1 Preparation of the Haskap phytochemical and high performance liquid chromatography (HPLC) analysis

Haskap fruits were extracted with 50% ethanol in water, and the concentrate was applied to a column of Dia-ion HP-20, and the anthocyanin-rich phytochemical fractions were eluted with 50% ethanol in water.

The analysis of nutritional components of the sample was conducted by Japan Food Research Laboratories, Inc. Anthocyanin and polyphenols contained in the Haskap phytochemical were analyzed using HPLC (CLASS-VP made by Shimadzu Corp.). A photodiode array detector scanned at 280 nm. ODS (4.6 × 150 mm) was used for elution column chromatography, and 0.05% phosphoric acid solution was used for the mobile phase A, whereas methanol: 5% phosphoric acid (99:1, v/v) solution was used for the mobile phase B. The solution was set at the flow rate of 1 mL/min. Measurements were obtained with the column oven temperature set at 40°C and a UV detector at 280 nm during the 80 min run time.

2.2 Animals

A total of 26 and 18 rats were used in Experiments 1 and 2, respectively. Six-week-old male SD/SPF rats were obtained from Sankyo Labo. Co Ltd (Sapporo, Japan). All rats were kept in clean room conditions of temperature (23 ± 1°C), humidity (55 ± 10%), and lighting (12-h dark-light cycle). The rats were allowed free access to a commercial diet (CE-2, CLEA Japan, Tokyo, Japan) and water for one week.

This experimental design was approved by the Animal Experiment Committee, Fuji Women’s University, and the rats were maintained in accordance with the guidelines.

2.3 Effects of the Haskap phytochemical on changes in the serum triglyceride concentration of rats after a corn oil emulsion load (Experiment 1-1)

Twelve male SD/SPF rats, which were 6 weeks old with an average body weight of 200 g, were divided into control group and Haskap phytochemical-administered (containing anthocyanin 300 mg/kg body weight) group (six rats for each group). They were not fed for 24 h when the corn oil emulsion load experiment was conducted. The experiment was conducted according to Han’s method. The corn oil emulsion (3 mL corn oil, 50 mg sodium cholate, and 3 mL water) was orally administered (12.5 mL/kg of body weight) without an anesthetic, and a blood sample was obtained from the tail vein without the anesthetic at 0, 30, 60, 120, 180, and 240 min after the administration. The concentration of serum triglycerides was measured using a Fuji Dri-Chem 3500V (Fujifilm Corp. Tokyo, Japan). In addition, lipase activity was measured using a porcine pancreatic lipase (Sigma-Aldrich, St Louis, MO, USA) according to the method detailed by Kawasaki et al.

2.4 Effects of the Haskap phytochemical on changes in the blood glucose level of rats after a sucrose load (Experiment 1-2)

Fourteen 6-week-old male SD/SPF rats were randomly divided into two groups. Each rat (n = 7, 184 ± 2.3 g) was made to fast for 24 h before a single oral administration of the Haskap phytochemical using a gastric tube. At 30 min after the Haskap phytochemical administration, 10 mL of sucrose solution (2 g/kg body weight) was administered to each rat. The control rats were administered with the same volume of 0.9% sodium chloride (NaCl) solution. At each
time point up to 120 min, a 5-μL blood sample was collected from the tail vein. Blood samples were immediately subjected to a blood glucose level measurement using a disposable glucose sensor (Glutest Pro, Sanwa Chemical Research, Co., Tokyo, Japan).

2.5 Effects of the Haskap phytochemical on the lipid and carbohydrate metabolism in rats after a long-term intake of the HF diet (Experiment 2)

Eighteen male SD/SPF rats, which were 6 weeks old with an average body weight of 160-180 g, were habituated to the diet for one week, the rats were then divided into three groups (six rats in each group) so that the average body weight, total serum cholesterol concentration, serum triglyceride concentration and blood glucose level were the same between the groups. Each group was fed the HF diet containing 20% lard and 5% corn oil (HF-control group) or the HF diet with either 1.5% or 3.0% of the Haskap phytochemical (equivalent to 0.2% or 0.4% cy 3-gluc) for four weeks (Table 1). The weight of the Haskap phytochemical-added diet was adjusted by modulating the sucrose content. Micronutrients, like a vitamin mixture and a mineral mixture (Hokudo Co., Ltd., Sapporo, Japan), were added to the diet according to AIN-93G (26). The diet and water were consumed ad libitum, and body weight and food intake were measured every day. Blood samples were obtained from the tail vein every week, and the serum triglycerides and total cholesterol concentrations were measured using the Fuji Dri-Chem 3500V (Fujifilm Corp., Tokyo, Japan). Blood glucose levels were measured using a Glutest Pro (Sanwa Chemical Research, Co., Tokyo, Japan). Under pentobarbital (45 mg/kg body weight) anesthesia, cecum and adipose tissue were removed and weighed. The cecum was frozen under liquid N2 and stored at −20°C for further analyses.

2.6 Statistical Analysis

All results were expressed as the mean ± SE. Data were analyzed by Tukey-Kramer multiple comparison tests after one-way ANOVA. Differences with p-values < 0.05 were considered significant. All statistical analyses were performed using the Stat View 5.0 (SAS Institute, Cary, NC).

3 Results and discussion

3.1 Analysis of the Haskap phytochemical components

The components of the Haskap phytochemical were analyzed using HPLC. As shown in Table 2, the main anthocyanin pigment was cy 3-glucoside and contained 13.2%. The other polyphenols present in the phytochemical were chlorogenic acid (4.6%), (+) catechin (0.7%), procatechuic acid (0.1%), and ferulic acid (0.1%). In addition, traces of an unknown polyphenol were detected in the Haskap phytochemicals. Also the total amount of polyphenol in the Haskap phytochemical was 70.9%; therefore, it is presumed that a high-molecular-weight polyphenol is contained in the pigment.

3.2 Effects of the Haskap phytochemical on the short-term responses of the serum triglyceride concentration of rats after corn oil emulsion loading (Experiment 1-1)

We investigated the changes in the serum triglyceride concentrations caused by the Haskap phytochemical after loading with a lipid. The results showed that the serum triglyceride concentrations of the Haskap phytochemical group significantly lowered at 30–240 min after the lipid load compared with those of the control group (0.9% NaCl) (Fig. 1). The area under the blood concentration-time curve (AUC) at these times was 10,937 ± 1,055 mg/dL.

<table>
<thead>
<tr>
<th>Table 1 Composition of diets used in experiment 2.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF-control</td>
</tr>
<tr>
<td>Casein</td>
</tr>
<tr>
<td>Mineral mix*</td>
</tr>
<tr>
<td>Vitamin mix*</td>
</tr>
<tr>
<td>Choline chloride</td>
</tr>
<tr>
<td>Corn oil</td>
</tr>
<tr>
<td>Lard</td>
</tr>
<tr>
<td>Haskap phytochemicals</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
</tbody>
</table>

*Mineral mixture and vitamin mixture prepared according to AIN 93-G (26).

<table>
<thead>
<tr>
<th>Table 2 Composition of haskap phytochemicals.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Components</td>
</tr>
<tr>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Fat</td>
</tr>
<tr>
<td>Carbohydrate, by difference</td>
</tr>
<tr>
<td>Ash</td>
</tr>
<tr>
<td>Moisture</td>
</tr>
<tr>
<td>Pytochemicals</td>
</tr>
<tr>
<td>Cyanidin-3-glucoside</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
</tr>
<tr>
<td>(+) catechin</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
</tr>
<tr>
<td>Ferulic acid</td>
</tr>
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</table>

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Fig. 1  Effects of haskap phytochemicals on serum triglyceride level after a single oral administration of 12.5 mL/kg corn oil emulsion in rats. ▲, Five milliliter of a haskap pigment (300 mg/kg B.W. anthocyanin) was dosed in male 7 week-old SD rats. After 30 minutes, corn oil emulsion (12.5 mL/kg B.W.) was administered to each rat. ●. Control was administered with the same volume of 0.9% NaCl solution. Each value is the mean ±SE, n=6. Those not sharing a letter differ, p < 0.05.

Table 3  Effects of anthocyanin-rich fruits extract and phenolic components on pancreatic lipase activity.

<table>
<thead>
<tr>
<th></th>
<th>Pancreatic lipase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haskap phytochemicals</td>
<td>1.54±0.01</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>2.64±0.04</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>2.89±0.08</td>
</tr>
<tr>
<td>Tea catechin</td>
<td>1.86±0.02</td>
</tr>
</tbody>
</table>

Each value is the mean ±SD, n=3.

Fig. 2  Effects of haskap phytochemicals on blood glucose level after a single oral administration of 2 g/kg sucrose in rats. ▲. Five milliliter of a haskap pigment (300 mg/kg B.W. anthocyanin) was dosed in male 7 week-old SD rats. After 30 minutes, 10 mL of a 2 g/kg sucrose solution was administered to each rat. ●. Control was administered with the same volume of 0.9% NaCl solution. Each value is the mean ±SE, n=6. Those not sharing a letter differ, p < 0.05.

3.3 Effects of the Haskap phytochemical on the short-term responses of the blood glucose level of rats after sucrose loading (Experiment 1-2)

We investigated the short-term responses caused by the Haskap phytochemical on the blood glucose levels of rats after sucrose loading. The results showed that blood glucose levels at 30 min after the sucrose load were significantly lower in the Haskap phytochemical group than in the control group (Fig. 2). AUC at this time was calculated, and the area for the Haskap phytochemical group was significantly lower (11,522±308 mg/dL·min) compared with that for the control group (13,596±645 mg/dL·min). These results suggest that the Haskap phytochemical inhibits sucrose. It has been reported that anthocyanin inhibits α-glucosidase. Furthermore, Matsui et al. reported that peonidin glucoside in the sweet potato inhibits glucose and maltose, but not sucrose. However, we have discovered...
that the Haskap phytochemical reduced the increase in blood glucose level by inhibiting sucrase. Thus, it is suggested that different anthocyanins have different effects on \(\alpha\)-glucosidase activity. In addition, the Haskap phytochemical contains chlorogenic acid, which has been reported to reduce the increase in blood glucose levels\(^{30}\). From these reports and the results of this experiment, it is presumed that anthocyanin and chlorogenic acid contained in the Haskap phytochemical reduced the increase in blood glucose level by inhibiting the synergically enzymatic degradation of sucrose in the intestine.

3.4 Effects of long-term intake of the Haskap phytochemical on changes of rats’ weights

The rats were fed the HF diet (HF-control group) or the HF diet either with 1.5% or 3.0% of the Haskap phytochemical for four weeks. Rats of each group grew steadily, and there was no significant difference in the amount of dietary intake between the three groups. The final weight and weight gain of the rats fed the Phytochemical diet tended to be lower than those of the HF-control group (Table 4).

3.5 Chronological changes in the serum components (serum triglyceride concentration, cholesterol concentration, and blood glucose level) when the Haskap phytochemical is administered for a long time

3.5.1 Serum triglyceride concentration

The rats were fed the HF diet with 1.5% or 3.0% of the Haskap phytochemical; a blood sample was collected from their tail vein every week to investigate the chronological changes of the serum triglyceride concentration. The results showed that the triglyceride concentrations of the group fed 3.0% diet tended to be lower in the first week; in the second and third weeks, the increase in the triglyceride concentrations in this group significantly reduced than in the HF-control group (Fig. 3). Moreover, the triglyceride concentrations of the group fed the diet with 1.5% of the

![Fig. 3](image-url) The longitudinal change in serum triglyceride on consumption of haskap phytochemicals in rats. ●, HF-control group; △, 1.5% haskap phytochemicals group; ▲, 3.0% haskap phytochemicals group. Each value is the mean ± SE, n=6. Those not sharing a letter differ, \(p < 0.05\).

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Effects of consuming dietary haskap phytochemicals on body weight, food intake and adipose tissue weight in rats for 4 weeks.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF-control</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>346.32±10.85</td>
</tr>
<tr>
<td>Body weight gain (g/ 4 weeks)</td>
<td>277.28±9.31</td>
</tr>
<tr>
<td>Food intake (g/ 4 weeks)</td>
<td>519.99±16.43</td>
</tr>
<tr>
<td>Feed efficiency (body weight gain g/g diet)</td>
<td>0.44±0.01(^{a})</td>
</tr>
<tr>
<td>g/100g Body weight</td>
<td></td>
</tr>
<tr>
<td>Mesenteric adipose tissue</td>
<td>1.48±0.08</td>
</tr>
<tr>
<td>Perirenal adipose tissue</td>
<td>2.69±0.11</td>
</tr>
<tr>
<td>Epididymal adipose tissue</td>
<td>2.18±0.15</td>
</tr>
<tr>
<td>Total adipose tissue</td>
<td>6.35±0.29</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE, n=6. Data were analyzed with ANOVA (\(p < 0.05\)).
Haskap phytochemical tended to be lower during the second and third weeks. These results suggest that the Haskap phytochemical has a dose-dependent effect in the reduction of the increase in serum triglyceride concentrations.

There have been reports that the serum triglyceride levels of guinea pigs fed the HF diet with grape polyphenol for 12 weeks were significantly lowered\(^{31}\), and that the increase of serum triglyceride concentrations in rats fed the HF diet with boysenberries lowered because components of boysenberries control or slow fat absorption in the intestine\(^{32}\). An antiobesity effect of chlorogenic acid, which is also present in the Haskap phytochemical, has also been reported\(^{33,34}\). It can be presumed that the same effect was shown in Haskap, which also contained various polyphenols such as anthocyanin. Moreover, in the experiment evaluating the short-term responses in blood lipids, we have discovered that the Haskap phytochemical controls the post-prandial increase in the serum triglyceride concentration and inhibits pancreatic lipase activity. Because of this, it is suggested that the reduction in serum triglyceride concentration after the long-term intake of the Haskap phytochemical is also related to pancreatic lipase inhibition. From these results, it is possible that the consumption of the Haskap phytochemical may have lowered the postprandial increase of the serum triglyceride concentrations and decreased the adipose tissue due to the synergic effects of anthocyanin and other polyphenols.

3.5.2 Total serum cholesterol concentration

We investigated the chronological changes in the total serum cholesterol concentrations of the rats that had been fed diet with pigment. With the rats fed the diet without cholesterol, the total cholesterol concentration in the HF-control group gradually rose during the second week and rapidly rose during the third week. However, the concentrations in the group fed the diet with the Haskap phytochemical stayed almost the same as the starting values throughout the examination period; this indicated that the increase of serum cholesterol decreased (Fig. 4). Moreover, in this experiment, the rebound phenomenon seen with pectin was not observed\(^{35}\). It has been reported that tea catechin may lower cholesterol levels by inhibiting the micelle formation by bile acid\(^{36}\). It has also been reported that the total serum cholesterol of the rats fed a high-cholesterol diet with apple polyphenol was lowered; the mechanism involves the control of cholesterol absorption by polyphenol\(^{37}\). This suggests that anthocyanin and other polyphenols in the Haskap phytochemical have similar effects to tea catechin and apple polyphenol.

Lactobacilli growing in the cecum absorb bile acid and discharge it with their excretion out of the body\(^{38}\). The weight of the cecum and the contents of the Haskap phytochemical significantly increased compared with the HF-control group. This result is consistent with the report that anthocyanin accumulates in the cecum increasing the weight of the contents in the cecum and then is excreted in feces\(^{39}\). Recently, it has also been reported that there is a relationship between the microbial flora in the contents of the cecum and obesity\(^{40,41}\). We analyzed the microbial flora in the contents of the cecum of the rats fed the HF diet with the Haskap phytochemical for four weeks and discovered that the intestinal flora had been improved compared with that of the rats fed only the HF diet (No data).

Moreover, the pH of the contents of the cecum of the high-fat diet group significantly decreased compared with that of the HF-control group (Table 5). It has been reported that propionic acid in the rat cecum significantly reduced the increase in serum cholesterol\(^{42}\). This experiment suggests the possibility that the intake of the Haskap phytochemical enlarged the rat cecum, formed short-chain fatty acids by promoting intestinal fermentation, and lowered serum cholesterol. There may also be a relationship between the improvement of the intestinal flora and the antiobesity effect. Further study is necessary to examine the effect of anthocyanin on the contents of the cecum and microbial flora in feces.

3.5.3 Blood glucose level

To determine the effect of the Haskap phytochemical on hyperglycemia, rats were fed the HF diet with 1.5% or 3.0% Haskap phytochemical, and a blood sample was obtained from their tail veins every week to investigate the chronological changes in blood glucose levels. The results showed that the rapid increase of blood glucose level sig-

Fig. 4 The longitudinal change in serum total cholesterol on consumption of haskap phytochemicals in rats. ◆, HF-control group; △, 1.5% haskap phytochemicals group; ▲, 3.0% haskap phytochemicals group. Each value is the mean ±SE, n=6. Those not sharing a letter differ, p < 0.05.
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No statistically significant difference was observed in the body weight of rats fed different diets from the first to the last week when compared with the HF-control group (Fig. 5). Furthermore, the increase in blood glucose levels in the group fed the diet with 1.5% Haskap phytochemicals gradually decreased compared with those in the HF-control group (Fig. 1). With regard to the relationship between anthocyanin and blood glucose level, Tsuda et al. has reported that the pigment of purple corn, which contains high level of cy 3-glc, improves hyperglycemia[43]. In addition, this research revealed that the Haskap phytochemical controls the rapid increase of blood glucose levels in the rats, which were administered sucrose one time, and also controls the accumulation of visceral adipose tissues. These results suggest that the Haskap phytochemical may be able to control the increase of blood glucose levels by inhibiting α-glucosidase in the intestine. It is necessary to investigate the effects of the Haskap phytochemical on insulin response and to determine its mechanism to improve hyperglycemia.

In conclusion, this research showed that consumption of the Haskap phytochemical significantly reduced the postprandial serum triglyceride concentrations and the increase in blood glucose levels. These actions are synergistic because anthocyanin and other polyphenols contained in the Haskap phytochemical compositely inhibit the absorption of lipid and glucose in the intestine.

From the results of these three experiments, we suggest that the decrease of postprandial blood lipid and blood glucose by short or long-term Haskap phytochemical ingestion is due to anthocyanin and other polyphenols contained in the Haskap phytochemical.

Some parts of this study were conducted with the grant of the Grant-in-Aid for Scientific Research B (Grant number 25292017).

Table 5 Effect of haskap phytochemicals on the cecum with contents, wall of cecum and pH of cecal contents in rats.

<table>
<thead>
<tr>
<th></th>
<th>HF-control</th>
<th>1.5% haskap phytochemicals</th>
<th>3.0% haskap phytochemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/100g Body weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecum</td>
<td>0.80±0.05</td>
<td>0.99±0.06</td>
<td>1.14±0.09</td>
</tr>
<tr>
<td>Wall of cecal</td>
<td>0.22±0.02</td>
<td>0.25±0.01</td>
<td>20.25±0.0</td>
</tr>
<tr>
<td>Cecal contents</td>
<td>0.57±0.03</td>
<td>0.68±0.04</td>
<td>0.77±0.06</td>
</tr>
<tr>
<td>pH of cecal contents</td>
<td>7.49±0.05</td>
<td>7.24±0.07</td>
<td>7.27±0.08</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE, n=6. Data were analyzed with ANOVA (p<0.05).

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