Dietary Cyanidin 3-*O*-β-D-Glucoside-Rich Purple Corn Color Prevents Obesity and Ameliorates Hyperglycemia in Mice

Takanori Tsuda,[1] Fumihiko Horio,* Koji Uchida,† Hiromitsu Aoki† and Toshihiko Osawa*

*Research Center for Biomarkers of Preventive Medicine, Doshisha University, Imadegawa-dori, Kamigyo-ku, Kyoto 602-8580, Japan; *Nagoya University Graduate School of Bioagricultural Sciences, Nagoya 464-8601, Japan; and †San-Ei Gen F.F.I., Incorporated, Toyonaka, 561-8588, Japan*

ABSTRACT Anthocyanins, which are used as a food coloring, are widely distributed in human diets, suggesting that we ingest large amounts of anthocyanins from plant-based foods. Mice were fed control, cyanidin 3-glucoside-rich purple corn color (PCC), high fat (HF) or HF + PCC diet for 12 wk. Dietary PCC significantly suppressed the HF diet–induced increase in body weight gain, and white and brown adipose tissue weights. Feeding the HF diet markedly induced hypertrophy of the adipocytes in the epididymal white adipose tissue compared with the control group. In contrast, the induction did not occur in the HF + PCC group. The HF diet induced hyperglycemia, hyperinsulinemia and hyperleptinemia. These perturbations were completely normalized in rats fed HF + PCC. An increase in the tumor necrosis factor (TNF)-α mRNA level occurred in the HF group and was normalized by dietary PCC. These results suggest that dietary PCC may ameliorate HF diet–induced insulin resistance in mice. PCC suppressed the mRNA levels of enzymes involved in fatty acid and triacylglycerol synthesis and lowered the sterol regulatory element binding protein-1 mRNA level in white adipose tissue. These down-regulations may contribute to triacylglycerol accumulation in white adipose tissue. Our findings provide a biochemical and nutritional basis for the use of PCC or anthocyanins as a functional food factor that may have benefits for the prevention of obesity and diabetes. J. Nutr. 133: 2125–2130, 2003.

KEY WORDS: • *anthocyanin* • *cyanidin 3-glucoside* • *obesity* • *hyperglycemia* • *body fat*

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. They are widely distributed in the human diet through crops, beans, fruits, vegetables and red wine (1), suggesting that we ingest considerable amounts of anthocyanins from plant-based daily diets. In general, anthocyanin pigments are stable under acidic conditions, but are unstable and rapidly broken down under neutral conditions (2). Therefore, anthocyanins have not been recognized as a physiological functional food factor (2). However, we demonstrated that anthocyanins have antioxidative and radical-scavenging activities against hydroxyl and superoxide radicals based on in vitro and in vivo studies (3–5). Among the anthocyanins tested, cyanidin 3-*O*-β-D-glucoside (C3G)[2] (**Fig. 1**) showed substantial antioxidative and anti-inflammatory activities in vivo (6–9). These findings suggest that C3G has beneficial effects beyond its antioxidant activity.

Obesity is defined as the accumulation of excess adipose tissue resulting from various metabolic disorders. It is a strong risk factor for hypertension, hyperlipidemia, heart disease and type2 diabetes mellitus (10,11). It is controlled by both genetic and environmental factors. Among the environmental factors, the chronic consumption of a high fat (HF) diet is strongly associated with the development of obesity. HF diet–induced obesity can lead to insulin resistance. Obesity is associated with a decreased capacity of insulin to regulate glucose and lipid metabolism in the peripheral tissues. The increase in adipose tissue is accompanied by elevations of circulating free fatty acids and tumor necrosis factor (TNF)-α levels (12,13). These can inhibit insulin signaling and pancreatic β-cell function (14).

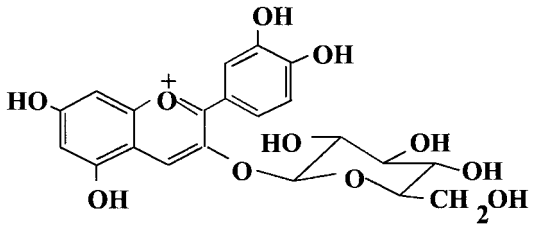
Recently, much attention has been focused on some food factors that may be beneficial in preventing HF diet–induced body fat accumulation and possibly reduce the risk of diabetes and heart disease. Ide et al. (15) showed that sesamin, found in sesame seed, decreases fatty acid synthesis and enhances β-oxidation in rat liver. Murase et al. (16) showed that dietary diacylglycerol suppresses HF diet–induced body fat accumulation in mice. However, there is little evidence that food factors themselves are beneficial for the prevention of obesity and the amelioration of insulin resistance.

The present study was designed to examine the preventive effect of anthocyanin-rich food color on the development of obesity and hyperglycemia induced by feeding a HF diet. No studies exist on a possible antiobesity effect of dietary anthocyanins. "Purple corn color" (PCC) is made from purple corn (*Zea mays* L.). In Japan, ~50,000 kg/y of PCC is used for the

[1] To whom correspondence should be addressed.
E-mail: tsudata@koto.kpu-m.ac.jp.

[2] Abbreviations used: ACC, acetylCoA carboxylase; ACS1, acyl-CoA synthase1; AMPK, AMP-activated protein kinase; BAT, brown adipose tissue; C3G, cyanidin 3-*O*-β-D-glucoside; dNTP, deoxynucleoside triphosphate; FAS, fatty acid synthase; GPAT, glycerol-3-phosphate acyltransferase; HF, high fat; IKKβ, IκB kinase β; NF-κB, nuclear factor-κB; PCC, purple corn color; SREBP-1, sterol regulatory element binding protein-1; TNF, tumor necrosis factor; WAT, white adipose tissue.

0022-3166/03 $3.00
Manuscript received 25 November 2002. Initial review completed 10 January 2003. Revision accepted 7 May 2003.



Cyanidin 3-*O*-β-D-glucoside

FIGURE 1 Chemical structure of cyanidin 3-*O*-β-D-glucoside (C3G).

coloring of foods, such as soft drinks and confections. PCC consists of anthocyanins and it contains a large amount of C3G. The C57BL/6J mice used in this study develop severe obesity, hyperglycemia and hyperinsulinemia when fed a HF diet (17). As one of the possible mechanisms of the antiobesity action of dietary PCC, we also examined its effect on the expression of genes involved in lipogenesis in the liver and white adipose tissue (WAT).

MATERIALS AND METHODS

Chemicals. PCC (containing ~70 g C3G/kg) was a gift from San-Ei Gen F.F.I., Osaka, Japan. Lard was purchased from Snow Brand Milk Products, Tokyo, Japan.

Animals and diets. Male C57BL/6J mice (n = 24), 4 wk of age (Japan SLC, Hamamatsu, Japan) were used and maintained at 23 ± 3°C under an automatic lighting schedule (0800–2000 h). The mice had free access to water and a laboratory nonpurified diet (CE-2, CLEA Japan, Tokyo, Japan) for 5 d. They were then randomly divided into 4 groups and fed the control, cyanidin 3-glucoside–rich purple 4 corn color (PCC), high fat (HF) or HF + PCC diet. The compositions of the diets and energy densities are listed in **Table 1**. PCC was added to diets at a C3G concentration of 2 g/kg diet. The diets were replaced once every 3 d to prevent oxidization of the fats and deterioration of the PCC. Body weight was measured weekly throughout the study. Food intake was measured once every 2 wk. This experimental design was approved by the Animal Experiment Committee, Nagoya University, and the mice were maintained in accordance with the guidelines.

Collection of serum, liver and adipose tissue. After 12 wk of consuming the diets, the mice were killed by decapitation and the blood was removed. The liver and adipose tissues [subcutaneous, epididymal, mesenteric and retroperitoneal WAT, and interscapular brown adipose tissue (BAT)] were removed according to defined anatomical landmarks. The weights of the tissues were measured. They were then immediately frozen using liquid nitrogen and kept at −80°C until use. The collected blood was kept at room temperature for 5 min for coagulation. After that, the serum was obtained from the coagulated blood by centrifugation at 1600 × g for 15 min at 4°C. The separation of the serum was finished within 30 min. The serum was immediately frozen at −80°C until use.

Measurement of serum lipid, glucose, insulin and leptin levels. The serum triacylglycerol, total cholesterol, free fatty acids and glucose concentrations were measured using commercial assay kits according to the manufacturer's directions (Triglyceride-E test, Cholesterol-E test, FFA-C test, Glucose B-test, Wako Pure Chemical, Osaka, Japan). Serum insulin and leptin levels were measured by ELISA using a commercial assay kit according to the manufacturer's directions (mouse insulin ELISA kit, Sibayagi, Gunma, Japan and mouse leptin ELISA kit, Morinaga, Yokohama, Japan).

Measurement of liver and fecal lipid concentrations. Total lipids in the liver and feces were extracted according to the method of Folch et al. (19). Aliquots of the extracted liver lipids were used for the measurement of the triacylglycerol and total cholesterol concentrations with the commercial assay kits used for serum lipids. Feces were collected for 48 h during the final week of feeding, and freeze-dried. The extracted total liver and fecal lipids were determined gravimetrically.

TABLE 1

Composition of the experimental diets

Ingredient	Control	PCC	HF	HF + PCC
		g/kg diet		
Casein	204	204	204	204
Mineral mixture[1]	35	35	35	35
Vitamin mixture[2]	10	10	10	10
Choline chloride	2	2	2	2
Corn oil	50	50	50	50
Lard	0	0	300	300
Cellulose powder	40	40	40	40
Sucrose	659	648	359	348
PCC[3]	0	11	0	11
		kJ/g diet		
Energy density	16.3	16.3	23.4	23.4

[1] AIN93G-MX (18).
[2] AIN93-VX (18).
[3] Purple corn color (PCC) was added to the control or high fat (HF) diet to give a final concentration of cyanidin 3-*O*-β-D-glucoside (C3G) of 2 g/kg diet.

Histological analysis of epididymal WAT. Small pieces of epididymal WAT were fixed with formalin (200 g/kg) neutral buffered solution and embedded in paraffin. Sections (8 μm) were cut and stained with hematoxylin and eosin. Images were captured using a CCD Camera (Olympus Optical, Tokyo, Japan) at a magnification of X200.

Isolation of total RNA in the liver and adipose tissue. Total RNA from the liver and epididymal WAT was isolated with Isogen reagent (Nippon Gene, Tokyo, Japan) according to the manufacturer's directions.

RT-PCR analysis. Total RNA concentration was measured by UV absorbance at 260 nm (absorbance of 1 mg/L of total RNA = 25.0). Total RNA (5 μg) was incubated with 9 μmol/L of the oligo dT primer at 65°C for 15 min; then it was reverse transcribed to cDNA in a reaction mixture containing buffer, 1.3 mmol/L deoxynucleoside triphosphates (dNTP, GIBCO BRL, Grand Island, NY), 10 mmol/L dithiothreitol (GIBCO), 20 U Rnase inhibitor (GIBCO), 200 U Moloney murine leukemia virus RT (GIBCO) at

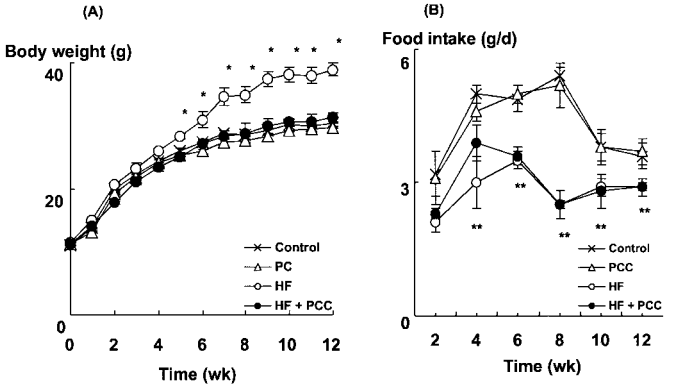


FIGURE 2 Body weight (*A*) and food intake (*B*) of mice fed the control, purple corn color (PCC), high fat (HF) or HF + PCC diets for 12 wk. Values are means ±SEM, n = 6. *The HF group differed ($P < 0.05$) from all other groups.**The HF group differed ($P < 0.05$) from the control and PCC groups.

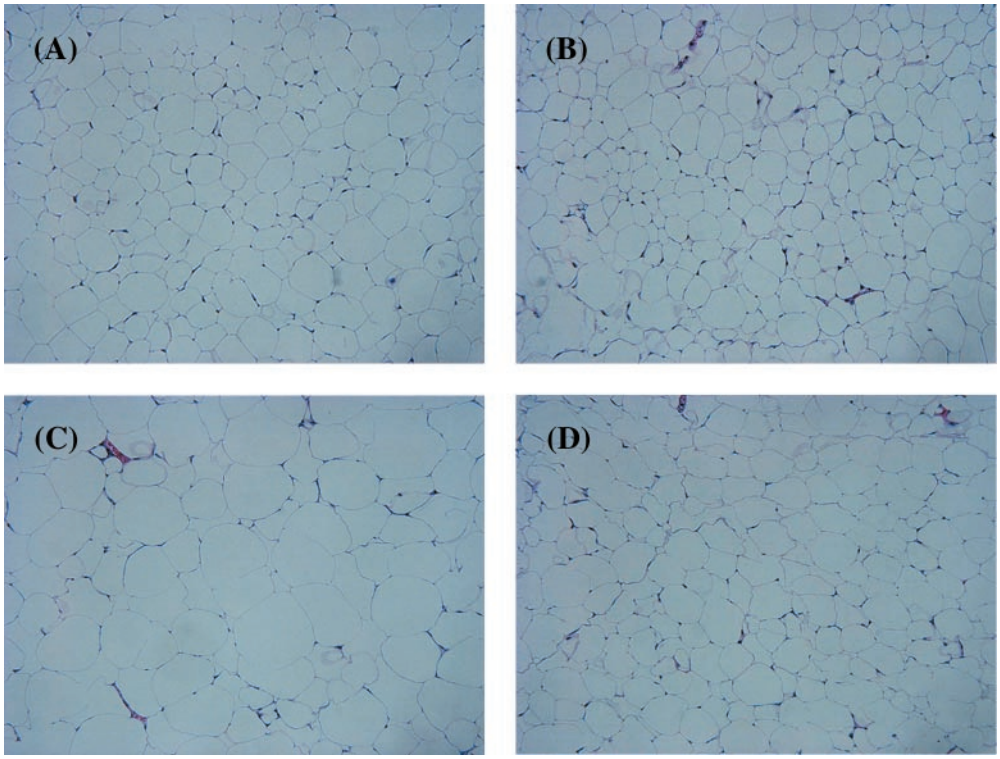


FIGURE 3 Histology of the epididymal white adipose tissue (WAT) of mice in the control (*A*), purple corn color (PCC) (*B*), high fat (HF) (*C*) or HF + PCC (*D*) groups. Each presented is typical and representative of 6 mice.

37°C for 3 h, then heated at 94°C for 2 min to terminate the reaction. The PCR were performed in a final 25 μL containing 0.5 μL of the RT first-strand cDNA product, 1 μmol/L of each forward (F) and reverse (R) primer, 75 mmol/L Tris-HCl (pH 8.8) containing 1 mg/L of Tween 20, 0.2 mmol/L of dNTP, 2 mmol/L of $MgCl_2$ and 1.5 U of *Taq* DNA polymerase (MBI Fermentas, Vilnius, Lithuania). Preliminary experiments were carried out with various cycles to determine the nonsaturating conditions of PCR amplification for all of the genes studied. The primers were: leptin (F) 5′-GTTTTGGAG-CAGTTTGGATC-3′, (R) 5′-GCATATGGGAAGTTTCACAA-3′, (522 bp); TNF-α, (F) 5′-GGGACAGTGACCTGGACTGT-3′, (R) 5′-GCAGAGGTTGAGTGATGTAG-3′, (520 bp); fatty acid synthase (FAS), (F) 5′-TACTTTGTGGCCTTCTCCTCTGTAA-3′, (R) 5′-CTTCCACACCCATGAGCGAGTCCAGGCCGA-3′, (447 bp); acyl-CoA synthase1 (ACS1), (F) 5′-TATCATGCT-TCACCTATGGC-3′, (R) 5′-CAAATAAGAGGAGCTCCAAC-3′, (492 bp); glycerol-3-phosphate acyltransferase (GPAT), (F) 5′-CAGTCCTGAATAAGAGGT-3′, (R) 5′-TGGACAAAGA-TGGCAGCAGA-3′, (444 bp); sterol regulatory element binding protein-1 (SREBP-1), (F) 5′-ACTTGATCAAGGCAGACTCA-CTG-3′, (R) 5′-CCTGCTTGTGCTTCTGGTGCTGT-3′, (425 bp); β-actin (F) 5′-CGTGGGCCGCCCTAGGCACCA-3′, (R) 5′-CTCTTTGATGTCACGCACGATTTC-3′, (541 bp).

The PCR cycle numbers were 20 cycles for leptin, 19 cycles for FAS and β-actin, 27 cycles for ACS1, 24 cycles for GPAT and SREBP-1, and 31 cycles for TNF-α. Each cycle consisted of denaturation at 94°C for 30 s, primer annealing at 52°C for GPAT, 56°C for leptin, FAS and ACS1, 57°C for SREBP-1 and 60°C for TNF-α, and primer extension at 72°C for 30 s. A final 10-min primer extension step at 72°C was performed on all of the samples. The products were electrophoresed on 10 g/L agarose gels and stained with ethidium bromide. The relative density of the bands was evaluated using an ATTO Lane Analyzer 10H Software Densitograph (Atto, Tokyo, Japan). All of the measured PCR products were normalized to the amount of cDNA of β-actin in each sample.

Statistical analysis. All data are expressed as means ± SEM. The differences among the means were analyzed by Fisher's protected least significant difference test after two-way ANOVA. Differences with *P*-values < 0.05 were considered significant. All of the statistical analyses were performed using the StatView version 5.0 software for Macintosh (SAS Institute, Cary, NC).

RESULTS

Body weight, adipose tissue weight and food and energy intakes. The body weight of the HF group was significantly

TABLE 2

Fat pad weights in mice fed control, purple corn color (PCC), high fat (HF) or HF + PCC diets for 12 wk[1]

	Control	PCC	HF	HF + PCC
		g		
Subcutaneous WAT[2]	0.53 ± 0.07^{b}	0.45 ± 0.08^{b}	1.24 ± 0.13^{a}	0.52 ± 0.08^{b}
Epididymal WAT	0.81 ± 0.08^{bc}	0.62 ± 0.07^{c}	2.16 ± 0.15^{a}	0.99 ± 0.12^{b}
Mesenteric WAT	0.34 ± 0.02^{b}	0.29 ± 0.16^{b}	0.74 ± 0.11^{a}	0.28 ± 0.02^{b}
Retroperitoneal WAT	0.25 ± 0.03^{bc}	0.20 ± 0.03^{c}	0.70 ± 0.06^{a}	0.35 ± 0.04^{b}
Interscapular BAT	0.17 ± 0.02^{b}	0.16 ± 0.02^{b}	0.24 ± 0.03^{a}	0.13 ± 0.01^{b}

[1] Values are means ± SEM, $n = 6$. Means in a row without a common letter differ, $P < 0.05$.
[2] WAT, white adipose tissue; BAT, brown adipose tissue.

TABLE 3

Liver and fecal lipids in mice fed purple corn color (PCC), high fat (HF) and HF + PCC diets for 12 wk[1]

	Control	PCC	HF	HF + PCC
Liver total lipids, *mg/g*	52.6 ± 1.6[b]	51.9 ± 4.1[b]	91.3 ± 14.4[a]	59.1 ± 4.3[b]
Liver triacylglycerol, *μmol/g*	26.7 ± 2.3[b]	32.9 ± 5.0[b]	71.3 ± 11.2[a]	34.9 ± 6.6[b]
Liver cholesterol, *μmol/g*	3.78 ± 0.18	3.80 ± 0.26	3.08 ± 0.28	3.80 ± 0.34
Fecal lipids, *mg/g dried feces*	2.3 ± 0.2	2.3 ± 0.4	4.6 ± 1.3	3.5 ± 0.6

[1] Values are means ± SEM, $n = 6$. Means in a row without a common letter differ, $P < 0.05$.

greater than that of the control, PCC, and HF + PCC groups from wk 5 to 12 (**Fig. 2**A). The control and HF + PCC groups did not differ throughout the experimental period. PCC itself did not affect food intake. Food intake was significantly lower in the HF and HF + PCC groups compared with the control group during wk 4 to 12 (Fig. 2*B*). However, food intake did not differ between the HF and HF + PCC groups throughout the experiment. Relative energy intakes did not differ among the groups. Intakes were 2006.6 ± 165.3, 2094.1 ± 215.9, 1777.8 ± 214.6 and 1999.1 ± 205.4 kJ/(kg body · d) in the control, PCC, HF and HF + PCC groups, respectively. All adipose tissue (subcutaneous, epididymal, mesenteric, retroperitoneal WAT and interscapular BAT) weights were markedly greater in the HF group than the control group (subcutaneous WAT; 130%, epididymal WAT; 170%, mesenteric WAT; 120%, retroperitoneal WAT; 180% and interscapular BAT; 40%) (**Table 2**). Dietary PCC suppressed the HF diet–induced increase in the tissue weight depots. Adipose tissue weights in the HF + PCC group did not differ from those in the control group.

Epididymal WAT histology. Feeding the HF diet induced hypertrophy of the adipocytes in the epididymal WAT (**Fig. 3**C) compared with that of the control and PCC groups (Fig. 3*A* and *B*). The hypertrophy did not occur in the HF + PCC group (Fig. 3*D*). The results obtained from the other mice were similar to those shown in Figure 3.

Serum, liver and fecal lipids. Serum triacylglycerol, total cholesterol and free fatty acid concentrations did not differ among the groups (data not shown). Liver total lipids and triacylglycerol concentrations were 60 and 170% greater, respectively, in the HF group than in the control group (**Table 3**). Dietary PCC significantly suppressed the HF diet–induced increase in the liver total lipids and triacylglycerol concentrations (Table 3). Fecal total lipids in the HF and HF + PCC groups tended to be greater than in the control and PCC groups ($P < 0.10$).

Serum glucose, insulin, and leptin concentrations and leptin and epididymal WAT TNF-α mRNA. Serum glucose and insulin concentrations were 70% greater in the HF group than in the control group. However, these were completely normalized in the HF + PCC group (**Table 4**). The serum leptin concentration was 6.7-fold greater in the HF group than in the control group and its mRNA level in the epididymal WAT was 50% greater in the HF group. Dietary PCC completely normalized the serum leptin concentration and mRNA level in the epididymal WAT (Table 4). The epididymal WAT TNFα mRNA level was 150% greater in the HF group than in the control group and dietary PCC normalized the level (Table 4).

Hepatic lipogenic enzyme and SREBP-1 mRNA levels. The FAS mRNA level was 67 and 33% lower than in controls in the HF and PCC groups, respectively (**Table 5**). The level in the HF + PCC group was 81% lower than in the control group and 57% lower than in the HF group. The ACS1 mRNA level was 24% lower in the PCC group than in the control group (Table 5). The GPAT mRNA level was lower in the HF + PCC group than in all other groups, which did not differ (Table 5). The SREBP-1 mRNA level was 38 and 41% lower in the HF and HF + PCC groups than in the control and PCC groups, which did not differ.

Epididymal WAT lipogenic enzyme and SREBP-1 mRNA levels. The FAS mRNA level was 45 and 40% lower than in the control group in the HF and PCC groups, respectively (**Table 6**). The level in the HF + PCC group was 74% lower than in the control group and 48% lower than in the HF group (Table 6). The ACS1 mRNA level was 60% greater in the HF group than in the control group. On the contrary, the level in the HF + PCC group was 40 and 75% lower than in the control and the HF + PCC groups, respectively (Table 6). The GPAT mRNA level was 32% lower in the HF + PCC group than in the HF group as well as that observed in the liver (Table 6). The SREBP-1 mRNA level in the PCC group was

TABLE 4

Serum glucose, insulin and leptin concentrations, and leptin and tumor necrosis factor (TNF)-α mRNA level in the epididymal white adipose tissue (WAT) of mice fed the control, purple corn color (PCC), high fat (HF) and HF + PCC diets for 12 wk[1]

	Control	PCC	HF	HF + PCC
Glucose, *mmol/L*	12.9 ± 0.3[b]	12.2 ± 0.8[b]	20.1 ± 0.6[a]	14.1 ± 0.8[b]
Insulin, *mmol/L*	0.31 ± 0.02[b]	0.31 ± 0.02[b]	0.52 ± 0.04[a]	0.29 ± 0.02[b]
Leptin, *mg/L*	15.3 ± 2.4[b]	15.1 ± 2.2[b]	118.1 ± 10.3[a]	20.2 ± 5.2[b]
		mRNA level,[2] *%*		
Leptin	100 ± 9[b]	88.9 ± 8.9[b]	177.8 ± 15.6[a]	102.2 ± 15.6[b]
TNF-α	100 ± 5[b]	110.1 ± 11.2[b]	253.5 ± 41.9[a]	109.3 ± 14.0[b]

[1] Values are means ± SEM, $n = 6$. Means in a row without a common letter differ, $P < 0.05$.
[2] The mRNA level was expressed by assigning 100 as the value in mice fed the control diet.

TABLE 5

mRNA level of lipogenic enzymes and SREBP-1 in the liver of mice fed control, purple corn color (PCC), high fat (HF) and HF + PCC diets for 12 wk[1]

	Control	PCC	HF	HF + PCC
		mRNA level,[2] *%*		
Fatty acid synthase (FAS)	100 ± 7[a]	66.7 ± 2.4[b]	33.3 ± 4.8[c]	19.0 ± 1.2[d]
Acyl-CoA synthase1 (ACS1)	100 ± 6[a]	76.5 ± 5.9[b]	100.0 ± 7.8[a]	80.4 ± 5.6[ab]
Glycerol-3-phosphate acyltransferase (GPAT)	100 ± 9[a]	86.3 ± 4.3[ab]	94.0 ± 12.8[a]	69.2 ± 3.4[b]
Sterol regulatory element binding protein (SREBP-1)	100 ± 8[a]	105.4 ± 12.4[a]	72.1 ± 4.4[b]	58.9 ± 2.7[b]

[1] Values are means ± SEM, $n = 6$. Means in a row without a common letter differ, $P < 0.05$.
[2] The mRNA level was expressed by assigning 100 as the value in mice fed the control diet.

20% lower than in the control group. The level in the HF + PCC group was 84% lower than in the control group and 63% lower than in the HF group.

DISCUSSION

Anthocyanins, which are phenolic phytochemicals, would not have any activity after ingestion in biological systems because they are unstable under neutral conditions. However, we demonstrated in in vitro and in vivo studies that C3G has antioxidant activity (3–9). The suppression of body fat accumulation was not due to inhibition of dietary fat digestion and reduction of energy intake. Therefore, dietary PCC may have potential in counteracting obesity.

Obesity is strongly associated with insulin resistance, and the improvement of insulin resistance is important in preventing the development of type2 diabetes. An increase in adipose tissue is accompanied by elevation of TNF-α expression in WAT (13). This can inhibit insulin signaling and pancreatic β-cell function (14). Our results suggest that dietary PCC can ameliorate HF diet–induced insulin resistance in mice. The normalization of hyperglycemia, hyperinsulinemia, hyperleptinemia and TNF-α mRNA level after PCC consumption is probably due to the prevention of the HF diet–induced accumulation of adipose tissue. Yuan et al. (20) showed that high doses of salicylate, one of the nonsteroidal anti-inflammatory drugs, improve hyperglycemia, hyperinsulinemia and dyslipidemia in Zucker fatty rats and *ob/ob* mice by sensitizing insulin signaling through inhibition of the IκB kinase β (IKKβ) activation. IKK activation initiates nuclear factor-κB (NF-κB)– mediated transcription, which in adipocytes would enhance the production of TNF-α. TNFα inhibits insulin signaling and disorders pancreatic β-cell function. Our previous studies clearly demonstrated that C3G functions as a potent antioxidant in vivo, and the administration of C3G suppresses proinflammatory cytokines (i.e., TNF-α) in the acute inflammation process (9). C3G-enriched PCC is expected to inhibit IKKβ activation and suppress TNFα expression, thereby ameliorating insulin resistance as efficiently as the salicylates. The effect of PCC on inhibition of IKKβ activation should be investigated.

To clarify the mechanism for the antiobesity activity of PCC, we focused on the expression of fatty acid and triacylglycerol metabolic enzymes. FAS is the key enzyme in fatty acid synthesis. ACS catalyzes the formation of acyl-CoA from fatty acids. Acyl-CoA is partitioned between β-oxidation and triacylglycerol synthesis. When adequate energy is present, acyl-CoA is utilized mainly for triacylglycerol synthesis in the liver and adipocytes. The enzyme GPAT is the first step in the pathway of triacylglycerol synthesis because it catalyzes acylation of the glycerol-3-phosphate. The gene expression of these lipogenic enzymes is affected by the nutritional state. Insulin up-regulates the enzymes, and SREBP-1 may be a major transcriptional factor involved in the insulin regulation of the lipogenic enzyme expression. Dietary PCC significantly decreased the FAS mRNA level even when it was added to the control diet. Although triacylglycerol accumulation increased in the HF diet–fed group, the FAS mRNA level was lower in the liver and epididymal WAT of the HF group than in the control group and there was a further significant decrease in that of the HF + PCC group compared with the HF group. This decrease may have contributed to the suppression of triacylglycerol accumulation in the HF + PCC group. ACS1 mRNA levels were found to be associated with triacylglycerol levels in mice (21). Marked increases in the ACS1 mRNA

TABLE 6

mRNA level of lipogenic enzymes and SREBP-1 in the epididymal white adipose tissue (WAT) of mice fed control, purple corn color (PCC), high fat (HF) and HF + PCC diets for 12 wk[1]

	Control	PCC	HF	HF + PCC
		mRNA level,[2] *%*		
Fatty acid synthase (FAS)	100 ± 12[a]	60.5 ± 9.3[b]	54.8 ± 2.4[b]	26.2 ± 2.4[c]
Acyl-CoA synthase1 (ACS1)	100 ± 10[b]	87.8 ± 8.8[b]	157.1 ± 12.2[a]	55.1 ± 0.1[c]
Glycerol-3-phosphate acyltransferase (GPAT)	100 ± 9[a]	97.6 ± 8.9[a]	111.9 ± 9.5[a]	76.2 ± 9.5[b]
Sterol regulatory element binding protein (SREBP-1)	100 ± 10[a]	80.1 ± 9.1[b]	43.6 ± 10.5[c]	16.0 ± 4.9[d]

[1] Values are means ± SEM, $n = 6$. Means in a row without a common letter differ, $P < 0.05$.
[2] The mRNA level was expressed by assigning 100 as the value in mice fed the control diet.

level in the liver and mesenteric WAT were observed in obese Zucker fatty rats and ventromedial hypothalamus-lesioned rats (22,23). The present study showed that the ACS1 mRNA level was markedly increased in the epididymal WAT of the HF group. In contrast, it was remarkably down-regulated by PCC feeding. The acylCoA synthesized by ACS1 can be used for triacylglycerol synthesis in the WAT through consumption of a HF diet. In addition, it is noteworthy that the GPAT mRNA level was also significantly lower in the HF + PCC group compared with the HF group. These results suggest that such changes also suppressed triacylglycerol accumulation in the WAT.

SREBP (SREBP-1a, -1c and -2) are synthesized as precursors bound to the endoplasmic reticulum; they regulate the gene expression of enzymes involved in lipogenesis and cholesterol biosynthesis (24). They are released from the membrane by a sequential two-step proteolytic cleavage. This released mature form enters the nucleus and promotes transcription of the target genes. SREBP-1 plays an important role in the response to activation of lipogenic enzyme expression. Ide et al. (15) clearly showed that dietary sesamin decreases lipogenic enzyme gene expression through the reduction of the SREBP-1 mRNA level and protein content of the precursor and its mature forms. Dietary PCC could have modulated the release of the mature form and resulted in the reduction of FAS mRNA level without the elevation of its mRNA level. Our present study demonstrated that PCC feeding significantly reduced the SREBP-1 mRNA level in the WAT, suggesting that dietary PCC would at least down-regulate the gene expression of these enzymes through a reduction in the SREBP-1 mRNA level.

Minokoshi et al. (25) demonstrated that leptin activates AMP-activated protein kinase (AMPK) and inactivated acetylCoA carboxylase (ACC) in the skeletal muscle. Inactivation of ACC decreases during malonylCoA synthesis; it shifts the balance toward fatty acid oxidation and suppresses triacylglycerol accumulation. The glucose-induced FAS mRNA level and GPAT activity were also down-regulated by AMPK (26,27), suggesting that PCC might accelerate direct or leptin-mediated AMPK activation. Zhou et al. (28) showed that metformin, which is a drug used for the therapy of type2 diabetes mellitus, down-regulated the SREBP-1 expression, thereby reducing the FAS mRNA level through AMPK activation. Therefore, there is a possibility that dietary PCC inactivated these enzymes and/or down-regulated gene expression through AMPK activation. Studies on the activation of AMPK and lipolysis by dietary PCC are now in progress.

In conclusion, dietary C3G-rich PCC significantly suppressed the development of obesity and ameliorated hyperglycemia induced by HF diet feeding in mice. Dietary PCC suppressed the mRNA levels of the enzymes included in the fatty acid and triacylglycerol synthesis accompanied by the reduction of the SREBP-1 mRNA level in the WAT. These down-regulations with PCC consumption may contribute to the suppression of triacylglycerol accumulation in the WAT. Our findings provide a biochemical and nutritional basis for the use of PCC or anthocyanins as a functional food factor, which may have important implications for preventing obesity and diabetes.

LITERATURE CITED

1. Harborne, J. B. & Grayer, R. J. (1988) The anthocyanins. In: The Flavonoids (Harborne J. B., ed.), pp. 1–20. Chapman and Hall, London, UK.

2. Brouillard, R. (1988) Flavonoids and flower color. In: The Flavonoids (Harborne, J. B., ed.), pp. 525–538. Chapman and Hall, London, UK.

3. Tsuda, T., Ohshima, K., Kawakishi, S. & Osawa, T. (1994) Antioxidative pigments isolated from the seeds of *Phaseolus vulgaris* L. J. Agric. Food Chem. 42: 248–251.

4. Tsuda, T., Watanabe, M., Ohshima, K., Norinobu, S., Choi, S. W., Kawakishi S. & Osawa, T. (1994) Antioxidative activity of the anthocyanin pigments cyanidin 3-*O*-β-D-glucoside and cyanidin. J. Agric. Food Chem. 42: 2407–2410.

5. Tsuda, T., Shiga, K., Ohshima, K., Kawakishi, S. & Osawa, T. (1996) Inhibition of lipid peroxidation and the active oxygen radical scavenging effect of anthocyanin pigments isolated from *Phaseolus vulgaris* L. Biochem. Pharmacol. 52: 1033–1039.

6. Tsuda, T., Horio, F. & Osawa, T. (1998) Dietary cyanidin 3-*O*-β-D-glucoside increases *ex vivo* oxidation resistance of serum in rats. Lipids 33: 583–588.

7. Tsuda, T., Horio, F., Kitoh, J. & Osawa, T. (1999) Protective effects of dietary cyanidin 3-*O*-β-D-glucoside on ischemia-reperfusion injury in rats. Arch. Biochem. Biophys. 368: 361–366.

8. Tsuda, T., Horio, F., Kato, Y. & Osawa, T. (2002) Cyanidin 3-*O*-β-D-glucoside attenuates the hepatic ischemia-reperfusion injury through a decrease in the neutrophil chemoattractant production in rats. J. Nutr. Sci. Vitaminol. 48: 134–141.

9. Tsuda, T., Horio, F. & Osawa, T. (2002) Cyanidin 3-*O*-β-D-glucoside suppresses nitric oxide production during a zymosan treatment in rats. J. Nutr. Sci. Vitaminol. 48: 305–310.

10. Lew, E. A. & Garfinkel, L. (1979) Variations in mortality by weight among 750,000 men and women. J. Chronic Dis. 32: 563–576.

11. Olefsky, J. M., Kolterman, O. G. & Scarlet, J. A. (1982) Insulin action and resistance in obesity and non-insulin dependent type II diabetes mellitus. Am. J. Physiol. 243: E15–E30.

12. Boden, G. (1997) Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46: 3–10.

13. Hotamisligil, G. S., Shargill, N. S. & Spiegelman, B. M. (1993) Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science (Washington, DC) 259: 87–91.

14. Hotamisligil, G. S., Peraldi, P., Budavari, A., Ellis, R., White, M. F. & Spiegelman, B. M. (1996) IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha and obesity-induced insulin resistance. Science (Washington, DC) 271: 665–668.

15. Ide, T., Ashakumary, L., Takahashi, Y., Kushiro, M., Fukuda, N. & Sugano, M. (2001) Sesamin, a sesame lignan, decreases fatty acid synthesis in rat liver accompanying the down-regulation of sterol regulatory element binding protein-1. Biochim. Biophys. Acta 1534: 1–13.

16. Murase, T., Mizuno, T., Omachi, T., Onikawa, K., Komine, Y., Kondo, H., Hase, T. & Tokimitsu, I. (2001) Dietary diacylglycerol suppresses high fat and high sucrose diet-induced body fat accumulation in C57BL/6J mice. J. Lipid Res. 42: 372–378.

17. Surwit, R. S., Kuhn, C. M., Cochrane, C., McCubbin, J. A. & Feinglos, M. N. (1988) Diet-induced type II diabetes in C57BL/6J mice. Diabetes 37: 1163–1167.

18. Reeves, P. G., Nielsen, F. H. & Fahey, G. C., Jr. (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123: 1939–1951.

19. Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226: 497–509.

20. Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z.-W., Karin, M. & Shoelson, S. E. (2001) Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of IKKβ. Science (Washington, DC) 293: 1673–1677.

21. Ikemoto, S., Takahashi, M., Tsunoda, N. Maruyama, K., Itakura, H., Kawanaka, K., Tabata, I., Higuchi, M., Tange, T., Yamamoto, T. T. & Ezaki, O. (1997) Cholate inhibits high-fat diet-induced hyperglycemia and obesity with acyl-CoA synthetase mRNA decrease. Am. J. Physiol. 273: E37–E45.

22. Shimomura, I., Tokunaga, K., Jiao, S. Funahashi, T, Keno, Y, Kobatake, T, Kotani, K, Suzuki, H, Yamamoto, T, Tarui, S & Matsuzawa, Y. (1992) Marked enhancement of acyl-CoA synthetase activity and mRNA, paralleled to lipoprotein lipase mRNA, in adipose tissues of Zucker obese rats (fa/fa). Biochim. Biophys. Acta 1124: 112–118.

23. Shimomura, I., Takahashi, M., Tokunaga, K., Keno, Y., Nakamura, T., Yamashita, S., Takemura, K., Yamamoto, T., Funahashi, T. & Matsuzawa, Y. (1996) Rapid enhancement of acyl-CoA synthetase, LPL, and Glut-4 mRNAs in adipose tissue of VMH rats. Am. J. Physiol. 270: E995–E1002.

24. Brown, M. S. & Goldstein, J. L. (1999) A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc. Natl. Acad. Sci. U.S.A. 96: 11041–11048.

25. Minokoshi, Y., Kim, Y.-B., Peroni, O. D., Fryer, L.G.D., Muller, C., Carling, D. & Kahn, B. B. (2002) Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature (Lond.) 415: 339–343.

26. Foretz, M., carling, D., Guichard, C., Ferre, P. & Foufelle, F. (1998) AMP-activated protein kinase inhibits the glucose–activated expression of fatty acid synthase gene in rat hepatocytes. J. Biol. Chem. 273: 14767–14771.

27. Muoio, D. M., Seefeld, K., Witters, L. A. & Coleman, R. A. (1999) AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that *sn*-glycerol-3-phosphate acyltransferase is a novel target. Biochem. J. 338: 783–791.

28. Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N., Musi, N., Hirshman, M. E., Goodyear, L. J. & Moller, D. E. (2001) Role of AMP-activated protein kinase in mechanism of metformin action. J. Clin. Investig. 108: 1167–1174.