JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Regulation of Adipocyte Function by Anthocyanins; Possibility of Preventing the Metabolic Syndrome

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Obesity is defined as the accumulation of excess adipose tissue resulting from various metabolic disorders. Adipocyte dysfunction is strongly associated with the development of obesity and insulin resistance. Metabolic syndrome is characterized by a group of metabolic risk factors in one person. Abdominal obesity and adipocyte dysfunction play an important role in the development of this syndrome. Anthocyanins are used as a food coloring, and they are widely distributed in human diets including berries, suggesting that large amounts of anthocyanins are ingested from plant-based foods. This study shows that anthocyanins have a significant potency of antiobesity and ameliorate adipocyte function in in vitro and in vivo systems and also that they have important implications for preventing metabolic syndrome.

KEYWORDS: Anthocyanins; cyanidin 3-glucoside; cyanidin; obesity; diabetes; metabolic syndrome; adipocyte

INTRODUCTION

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 significant 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 cyanidin 3-*O*-*β*-D-glucoside (C3G) (**Figure 1**), which is a typical anthocyanin, had antioxidative and anti-inflammatory activities based on in vitro and in vivo studies (*3–6*). These findings suggest that C3G has more beneficial effects beyond its antioxidant activity.

The "metabolic syndrome" is characterized by a group of metabolic risk factors in one person. Abdominal obesity, especially an increase in the visceral adipose tissue, is the central causal component in metabolic syndrome. The component can lead to complications including hardening of the arteries and an increased risk for cardiovascular disease.

Adipocyte is the primary site of energy storage and accumulates triacylglycerol during nutritional excess. Recent studies showed that adipocyte dysfunction plays an important role in the development of obesity and insulin resistance. Adipocyte synthesizes and secretes biologically active molecules called adipocytokines (*7*). For example, leptin is the product of the ob gene and is secreted from adipocytes; it reduces food intake and increases energy expenditure (*8*). Adiponectin is one of the most important adipocytokines and is specifically and highly expressed in adipocytes (*9*). The plasma adiponectin concentration and mRNA expression level are decreased in the obese and insulin-resistant state (*10, 11*). The administration of adiponectin improves insulin action accompanied by increases in fatty acid oxidation and a decreased triacylglycerol level in muscle (*12, 13*).

More recent studies demonstrated that obesity is associated with macrophage infiltration into adipose tissue and the activation of inflammatory pathway caused by the development of insulin resistance (*14, 15*). Inflammatory molecules including monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), IL-6, and plasminogen activator inhibitor-1 are expressed and up-regulated in adipose tissue of the obese state including type 2 diabetes. Among the inflammatory adipocytokines, MCP-1 is a member of the CC chemokine family and recruits monocytes from the blood into atherosclerotic lesions. Recent studies have clearly demonstrated that an increase in MCP-1 expression in adipose contributes to development of insulin resistance and is a significant signal that triggers inflammation by the macrophage infiltration into the tissue (*16–18*).

Recently, much attention has been focused on some food factors that may be beneficial for reducing the risk of metabolic syndrome. Although some drugs are used for the therapy of obese-related metabolic diseases, there has been little evidence that food factors themselves are directly beneficial for the improvement of the dysfunction of the adipocyte responsible for adipocytokine expression and insulin sensitivity.

In this paper, the preventive effect of anthocyanins on the development of obesity and hyperglycemia induced by feeding a high-fat (HF) diet and regulation of adipocyte

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10.1021/jf073113b CCC: $40.75
Published on Web 01/23/2008

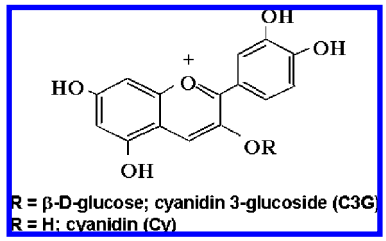

Figure 1. Chemical structure of anthocyanins.

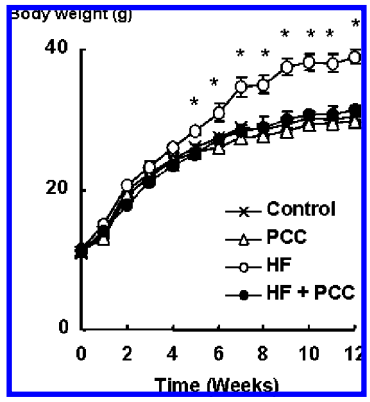

Figure 2. Body weight changes of mice fed the control, PCC, HF, or HF + PCC diets during 12 weeks. Values are means ± SEM, $n = 6$. ∗, the HF group differed ($P < 0.05$) from all other groups. Reprinted with permission from ref *19*. Copyright 2003 American Society for Nutrition.

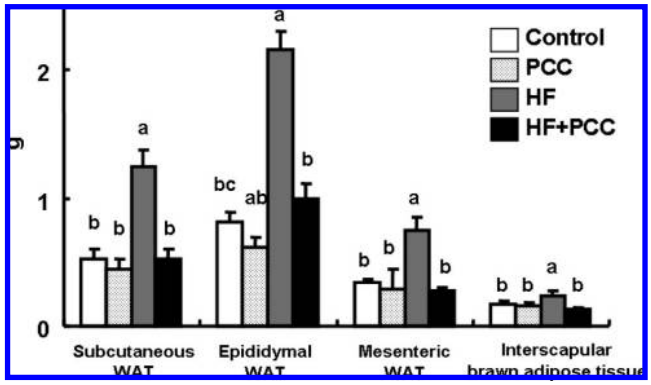

Figure 3. Adipose tissue weight of mice fed the control, PCC, HF, or HF + PCC diets during 12 weeks. Values are means ± SEM, $n = 6$. Means without a common letter differ, $P < 0.05$.

function including adipocytokine expression by anthocyanins are examined.

ANTHOCYANINS PREVENT HIGH-FAT-DIET-INDUCED OBESITY IN MICE (*19*)

C3G-rich purple corn color (PCC) made from purple corn (*Zea mays* L.) was a gift from San-Ei Gen F.F.I., Inc., Osaka, Japan. Male C57BL/6 mice at 4 weeks old were used. The mice were divided into four groups ($n = 6$; I, control diet; II, control + PCC; III, HF diet; and IV, HF + PCC group) for 12 weeks. The HF diet contained 30% lard. The control + PCC and HF + PCC diets contained PCC at a C3G concentration of 0.2%.

The body weight of the HF group was significantly higher than that of the control, PCC, and HF + PCC groups during the 5−12 weeks. On the other hand, the control and HF + PCC groups did not differ throughout the experimental period (**Figure 2**). PCC itself did not affect the food intake. Also, the energy

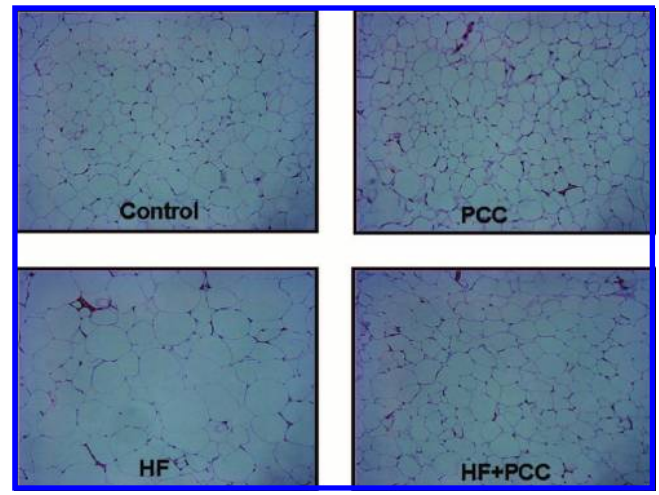

Figure 4. Histology of the epididymal WAT of mice in the control (**A**), PCC (**B**), HF (**C**), and HF + PCC (**D**) groups. Each presented is typical and representative of six mice. Reprinted with permission from ref *19*. Copyright 2003 American Society for Nutrition.

intake and fecal lipid content did not differ among the groups. These data suggest that the suppression of the body weight gain was not due to the inhibition of the dietary fat digestion and reduction of energy intake.

All of the adipose tissue weights were significantly greater in the HF group compared to the control group. However, the dietary PCC clearly suppressed the HF-diet-induced increase in the tissue weight deposits. The data indicate that the dietary PCC has a significant potency for antiobesity (**Figure 3**).

Figure 4 shows the histology of the epididymal white adipose tissue (WAT) of mice using the HE stain. Feeding the HF diet induced hypertrophy of the adipocytes in the adipose tissue. The hypertrophy did not occur in the HF + PCC group and completely normalized.

ANTHOCYANINS REGULATE THE ADIPOCYTE FUNCTION INCLUDING THE ADIOPOCYTOKINE EXPRESSION: APPROACH FROM DNA MICROARRAY ANALYSIS (*20, 21*)

Obesity is the key player in the metabolic syndrome. Also, amelioration of the adipocyte dysfunction is one of the crucial targets for prevention of this syndrome. Nutrigenomics is the application of high-throughput genomic tools in nutrition research. DNA microarray technology has significantly advanced. It will promote an increased understanding of how anthocyanins regulate the gene expression responsible for the prevention of obesity and the amelioration of insulin sensitivity. Therefore, microarray profiling of the gene expression in human adipocytes in response to the anthocyanins was observed.

Human preadipocytes obtained from subcutaneous adipose tissue were cultured and differentiated into adipocytes. At 13 days after the differentiation, the adipocytes were treated with anthocyanins for 24 h, and the total RNA was obtained. The obtained fragmented cRNA was hybridized to the Human Genome Focus Array (Affymetrix, Santa Clara, CA). Hierarchical clustering display of the array data based on the significant genes showed that the genes were grouped into nine clusters, each containing from 19 to 234 genes. These profiles indicate 32% of the genes did not have the same response in human adipocytes between the C3G and cyanidin (Cy) treatment.

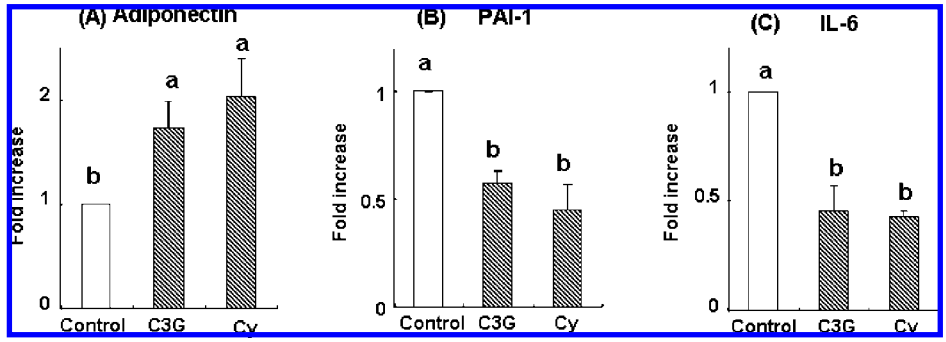

Figure 5. Gene expression level of adiponectin (**A**), PAI-1 (**B**), and IL-6 (**C**) in human adipocytes treated with C3G or Cy determined by real-time PCR analyses. The gene expression level was expressed as fold increase relative to the control group after normalization using the β-actin gene expression level. Values are means ± SEM, $n = 3$. Means without a common letter differ, $P < 0.05$. Reprinted with permission from *21*. Copyright 2006 Elsevier.

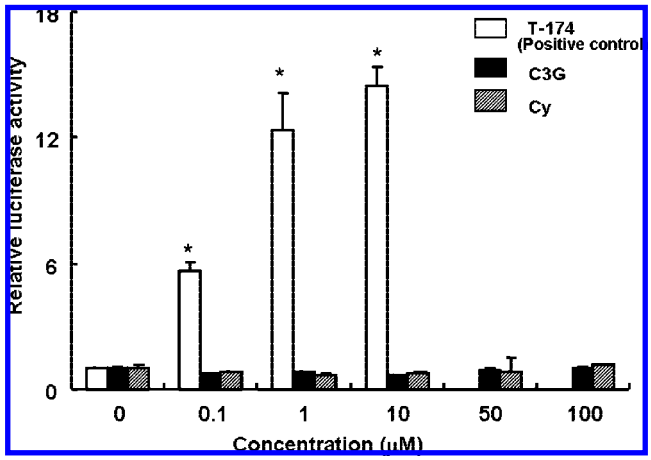

Figure 6. PPARγ transcriptional activity of C3G, Cy, or T-174. The activity of a vehicle control (0 μM) was set at 1.0, and the relative luciferase activities were presented as fold induction to that of the vehicle control. Values are means ± SEM, $n = 4$. ∗, significantly different at $P < 0.05$ compared to 0 μM T-174.

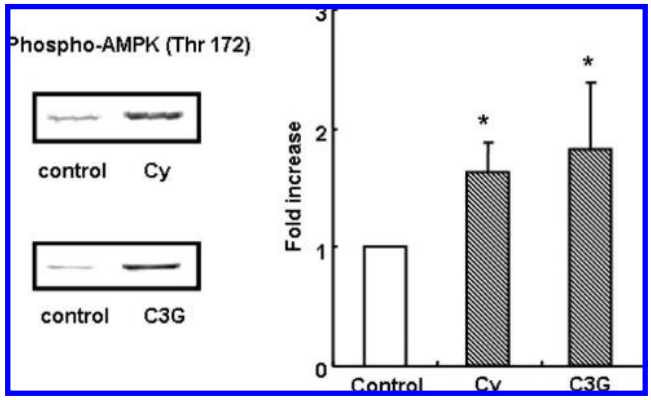

Figure 7. Immunoblot analysis of the phosho-AMP-activated protein kinase (AMPK) protein (Thr 172) in adipocytes treated with anthocyanins for 24 h. Values are means ± SEM from two or three independent experiments. ∗, significantly different at $P < 0.05$ compared to the control. Reprinted with permission from ref *22*. Copyright 2004 Elsevier.

The microarray data demonstrated that adiponectin, which is one of the most important adipocytokines, was up-regulated. On the contrary, plasminogen activator inhibitor-1 (PAI-1) and IL-6 were down-regulated. Elevation of adipose PAI-1 expression is associated with both obesity and type 2 diabetes, suggesting that regulation of PAI-1 expression is one of the important therapeutic targets for the metabolic syndrome. IL-6 is one of the cytokines and an important regulator of the acute phase response. The plasma IL-6 is elevated in obese subjects and type 2 diabetes patients. The administration of the anthocyanins caused down-regulation of the expression of these adipocytokines. The gene expression level of these genes using the quantitative real-time PCR system was consistent with that obtained from microarray analysis (**Figure 5**).

Table 1. AMP/ATP Ratio and ATP, ADP, and AMP Concentrations in Rat Adipocytes Treated with Anthocyanins[a]

		nmol/dish		
	AMP/ATP ratio	ATP	ADP	AMP
		7 Hours		
control	0.416 ± 0.021 a	1.054 ± 0.086 b	0.440 ± 0.048 a	0.438 ± 0.041 a
Cy	0.119 ± 0.018 b	1.685 ± 0.060 a	0.410 ± 0.038 a	0.202 ± 0.034 b
C3G	0.105 ± 0.014 b	1.696 ± 0.240 a	0.446 ± 0.103 a	0.183 ± 0.047 b
		24 Hours		
control	0.335 ± 0.036 a	0.830 ± 0.037 a	0.437 ± 0.065 a	0.281 ± 0.041 a
Cy	0.238 ± 0.014 b	0.696 ± 0.041 b	0.327 ± 0.029 a	0.165 ± 0.003 b
C3G	0.195 ± 0.012 b	0.724 ± 0.012 a	0.327 ± 0.029 a	0.141 ± 0.008 b

[a] Data were obtained from three experiments and represent the means ± SEM. Values with different letters are significantly different ($P < 0.05$). Adapted from ref *22* with permission of Elsevier.

ARE ANTHOCYANINS AGONISTS FOR PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ (PPARγ) (*22*)?

The gene expression of adiponectin is regulated by PPARγ. PPARγ is a ligand-activated transcription factor and a member of the nuclear hormone receptor superfamily that functions as a heterodimer with a retinoid X receptor. Agonists, such as thiazolidinediones, induced activation of PPARγ. It causes adipocyte differentiation and plays an important role for insulin sensitivity. The liver receptor homologue-1 enhances the PPARγ-mediated transactivation of the adiponectin gene in adipocyte (*23*). As the one of the possible mechanisms of the regulation of the adiponectin gene expression, anthocyanins act as a PPARγ ligand, resulting in an increased adiponectin gene expression. To confirm their mechanism, the PPARγ ligand activity was assayed.

Figure 6 shows the PPARγ ligand activity of C3G or Cy using a GAL4−PPARγ chimera assay system. These anthocyanins did not induce luciferase activity even if their concentration was increased to 100 μM. These data indicate that anthocyanins induce the adiponectin gene expression, and it is

not due to stimulation of the PPARγ ligand activity, but is due to a PPARγ-independent mechanism.

ANTHOCYANINS ACTIVATE AMP-ACTIVATED PROTEIN KINASE (AMPK) WITHOUT ELEVATION OF THE AMP/ATP RATIO IN ADIPOCYTES (*22*)

AMPK has been implicated as a potential target of type 2 diabetes mellitus and obesity (*24*). It is understood that AMPK functions as a fuel gauge to monitor the cellular energy status, and it is activated by AMP allosterically and by phosphorylation mediated by LKB1 (*25*). Leptin enhances AMPK phosphorylation, which resulted in fatty acid oxidation (*26*). This activation is also performed in adipocytes to prevent excess lipid accumulation in them (*27*). Wu et al. demonstrated that the treatment of rat adipocytes with the globular domain of adiponectin increased in glucose uptake and AMPK activation without stimulating the tyrosine phosphorylation of the insulin receptor or insulin receptor substrate-1 and Akt (*28*). Adiponectin may modulate the adipocyte function as well as leptin by the autocrine system. We examined AMPK activation in adipocytes treated with anthocyanins. **Figure 7** shows the detection of phosphorylated AMPK (Thr-172) using a phosphospecific antibody in adipocytes treated with Cy or C3G for 24 h. Significant elevation of the phospho-AMPK (Thr-172) protein level was observed in both the Cy- and C3G-treated groups compared to the control groups.

An increase in the AMP/ATP ratio activates AMPK. Also, AMPK can be stimulated through the AMP/ATP-ratio-independent pathway (*29*). Therefore, we examined whether or not phosphorylation of AMPK in adipocytes treated with anthocyanins is due to an increase in the AMP/ATP ratio. **Table 1** shows the AMP/ATP ratio and ATP, ADP, and AMP concentrations in adipocytes treated with Cy or C3G. The AMP/ATP ratio significantly decreased in both of the anthocyanin-treated groups compared to the control group throughout the experimental period (Cy, decreased by 71.5% in 7 h and 39.0% in 24 h; C3G, decreased by 74.8% in 7 h and 41.8% in 24 h). The AMP concentrations were also significantly decreased in both of the anthocyanin-treated groups compared to the control group. On the contrary, the ATP concentrations were significantly increased in both of the anthocyanin-treated groups within 7 h compared to the control group (**Table 1**). Anthocyanins activate AMPK regardless of the decrease in the AMP/ATP ratio, indicating that its mechanism for the activation by anthocyanins is independent of its ratio and may be due to another activating mechanism for AMPK.

CONCLUSION

Anthocyanins are widely distributed in the human diet, and we ingest significant amounts of anthocyanins from plant-based daily diets including "berries". Anthocyanins have a great potency of health-promoting effects and can be used as a functional food factor. These studies indicate anthocyanins have a unique therapeutic advantage responsible for the regulation of the adipocyte function. These findings provide a biochemical basis for the use of anthocyanins, which can also have important implications for preventing metabolic syndrome.

ABBREVIATIONS USED

AMPK, activated protein kinase; C3G, cyanidin 3-*O*-β-D-glucoside; Cy, cyandin; HF, high fat; MCP-1, monocyte chemoattractant protein-1; PCC, purple corn color; PAI-1, plasminogen activator inhibitor-1; PPARγ, peroxisome proliferator-activated receptor; TNF-α, tumor necrosis factor-α; WAT, white adipose tissue.

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Received for review October 23, 2007. Accepted November 27, 2007. This study was supported in part by Grant-in-Aid for Scientific Research 18580133 from the Japanese Ministry of Education, Culture, Sports, Science and Technology, the Nutrition and Food Science Fund of the Japanese Society of Nutrition and Food Science, the Elizabeth Arnold Foundation, and the Kao Research Council for the Study of Health Science.

JF073113B