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Cyanidin 3-glucoside ameliorates hyperglycemia and insulin sensitivity due to downregulation of retinol binding protein 4 expression in diabetic mice

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ABSTRACT

Adipocyte dysfunction is strongly associated with the development of obesity and insulin resistance. It is accepted that the regulation of adipocytokine expression is one of the most important targets for the prevention of obesity and improvement of insulin sensitivity. In this study, we have demonstrated that anthocyanin (cyanidin 3-glucoside; C3G) which is a pigment widespread in the plant kingdom, ameliorates hyperglycemia and insulin sensitivity due to the reduction of retinol binding protein 4 (RBP4) expression in type 2 diabetic mice. KK-A^{y} mice were fed control or control +0.2% of a C3G diet for 5 weeks. Dietary C3G significantly reduced blood glucose concentration and enhanced insulin sensitivity. The adiponectin and its receptors expression were not responsible for this amelioration. C3G significantly upregulated the glucose transporter 4 (Glut4) and downregulated RBP4 in the white adipose tissue, which is accompanied by downregulation of the inflammatory adipocytokines (monocyte chemoattractant protein-1 and tumor necrosis factor-α) in the white adipose tissue of the C3G group. These findings indicate that C3G has significant potency in an anti-diabetic effect through the regulation of Glut4-RBP4 system and the related inflammatory adipocytokines.

1. Introduction

Adipocyte dysfunction plays an important role in the development of insulin resistance. Adipocyte synthesizes and secretes biologically active molecules called adipocytokines [1]. Obesity is associated with macrophage infiltration into adipose tissue and the activation of inflammatory pathway causing the development of insulin resistance

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Abbreviations: AMPK, AMP-activated protein kinase; BAT, brown adipose tissue; CCR2, CC chemokine receptor2; C3G, cyanidin 3-glucoside; Cy, cyanidin; G6Pase, glucose-6-phasphatase; Glut4, glucose transporter 4; MCP-1, monocyte chemoattractant protein-1; PPAR, peroxisome proliferator-activated receptor; RBP4, retinol binding protein 4; ROS, reactive oxygen species; TZD, thiazolidinediones; TTBS, Tris–HCl-buffered saline containing 0.05% Tween 20; WAT, white adipose tissues.

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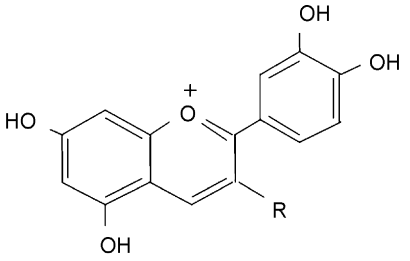
[2,3]. Inflammatory molecules including monocyte chemoattractant protein-1 (MCP-1), TNF-α, IL-6 and plasminogen activator inhibitor-1 are expressed and upregulated in adipose tissue of the obese state including type 2 diabetes [4–10]. Among the inflammatory adipocytokines, MCP-1 is a member of the CC chemokine family and recruit monocytes from the blood into atherosclerotic lesions. Recent studies have clearly demonstrated that an increase in MCP-1 expression in adipose tissue contributes to the development of insulin resistance and is a significant signal that triggers inflammation by the macrophage infiltration into the tissue [4,11,12].

Another recent important study provided by Yang et al. showed that retinol binding protein 4 (RBP4) is a new adipocytokine [13]. They identified that the expression and secretion of RBP4 in adipose tissue closely link glucose uptake and insulin sensitivity. In an obese or diabetic state, the expression of glucose transporter 4 (Glut4) is reduced in adipocytes, and the reduction is accompanied by an increase in RBP4 expression and secretion into the blood. The increase causes impairment of insulin signaling in skeletal muscle and stimulates glucose production in the liver. These changes lead to high glucose concentration in the blood. Therefore, dysregulation of adipocyte Glut4-RBP4 system is strongly associated with type 2 diabetes involving the metabolic syndrome, and lowering RBP4 is a new potentially important target molecule for prevention and therapy of type 2 diabetes. More recent studies also demonstrate that RBP4 is associated with insulin resistance, and that single nucleotide polymorphism in the RBP4 gene is also associated with type 2 diabetes in human subjects [14–18], although some reports have revealed that the RBP4 level is not correlated with obesity [19,20].

There are some drugs, which are the target for regulation of the adipocyte function, that improve insulin sensitivity or glucose homeostasis [21,22]. Thiazolidinediones (TZD) are one of the synthetic peroxisome proliferator-activated receptor (PPAR) ligands and are used as antidiabetic drugs through their effects on the adipocyte differentiation and activation of adipocyte genes [23,24]. However, the administration of TZD can produce undesirable side effects (obesity and edema), suggesting that development of selective PPARγ modulators is needed [24].

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.

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 [25], 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 [26]. Therefore, anthocyanins have not been recognized as a physiologically functional food factor [26]. However, we demonstrated that cyanidin 3-glucoside (C3G) (Fig. 1), which is a typical anthocyanin, had antioxidative activity based on in vitro and in vivo studies [27–30].



R = -*o*-β-D-glucose; cyanidin 3-glucoside (C3G)
R = OH; cyanidin (Cy)

Fig. 1 – Chemical structures of cyanidin 3-glucoside (C3G) and cyanidin (Cy).

Moreover, we demonstrated that dietary C3G-rich crude extract significantly suppressed the development of obesity and normalized the hypertrophy of the adipocytes in the epididymal white adipose tissues (WAT) induced by the high-fat diet feeding of C57BL/6 mice [31]. Our prior studies also showed that anthocyanins enhanced the gene expression of adiponectin in isolated rat adipocytes and human adipocytes [32,33], and enhanced PPARγ target adipocyte-specific genes expressions in isolated rat adipocytes without stimulation of the PPARγ ligand activity [32]. This will promote an increased understanding of how C3G influences adipocytokine expression and regulates those responsible for the amelioration of insulin sensitivity in type 2 diabetes. However, the molecular action of the C3G responsible for the enhancement of insulin sensitivity through regulation of the adipocyte function is not fully understood in vivo. Therefore, the present study was designed to examine whether the administration of C3G reduces blood glucose level and improves insulin sensitivity in type 2 diabetic mice. Concerning the molecular action and mechanism, the present study demonstrates that C3G modulates the Glut4-RBP4 system including inflammatory adipocytokines. These changes resulted in improving hyperglycemia and insulin sensitivity in type 2 diabetes.

2. Materials and methods

2.1. Chemicals

C3G (the purity, greater than 96%) was purified from purple corn color pigment using HPLC and provided from San-Ei Gen F.F.I., Inc., Japan [27].

2.2. Animals and diets

Male KK-A^y mice, 4 weeks of age (CLEA, Japan) were used and maintained at 23 ± 3 °C under an automatic lighting schedule (08:00 h to 20:00 h). The mice were allowed free access to water and a laboratory diet (CE-2, CLEA Japan, Tokyo, Japan) for 1 week. They were then divided into two groups and assigned to the control (CE-2) or the experimental diet (CE-2 + C3G). C3G was added to the control diet at the concentration of 2 g/kg

diet. The C3G supplemented diet was replaced every day to avoid destruction of the C3G. The dose of C3G was based on our prior study [31] and the preliminary experiment that the used supplemented level did not affected the food intake. This experimental design was approved by the Animal Experiment Committee, Nagoya University, and the mice were maintained in accordance with the guidelines.

2.3. Collection of serum, liver, skeletal muscle and adipose tissue

After 5 weeks of feeding the diets, the mice were killed by decapitation, and the blood was removed. The liver, skeletal muscle and adipose tissues (subcutaneous, epididymal, mesenteric and retroperitoneal WAT, and interscapular brown adipose tissue (BAT) were removed. 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 kept at −80 °C prior to use.

2.4. Measurement of serum lipid, glucose, insulin, adiponectin and RBP4 levels

The serum triacylglycerol, total cholesterol and glucose concentrations were measured using commercial assay kits according to the manufacturer's directions (Triglyceride-E test, Cholesterol-E test, Glucose CII-test, Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum insulin, adiponectin and RBP4 levels were measured by ELISA using a commercial assay kit according to the manufacturer's directions (mouse insulin ELISA kit, Morinaga, Yokohama, Japan, and mouse/rat adiponectin ELISA kit, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan, and Dual mouse/rat RBP4 ELISA kit, AdipoGen, Inc., Korea).

2.5. Insulin tolerance test

After 4 weeks of feeding the diets, the mice were fasted for 14 h, then intraperitoneally injected with 0.67 U/kg of human insulin (Humulin R; Eli Lilly, Japan). Blood samples were collected from the tail vein at the times indicated, and glucose was measured with an automatic blood glucose meter (GR-102, TERUMO, Japan).

2.6. Isolation of total RNA and measurement of gene expression level

Total RNA from the tissues was isolated with QIAzol™ reagent (QIAGEN, Tokyo, Japan) according to the manufacturer's directions. Total RNA (1.0 μg) was reverse transcribed to cDNA in a reaction mixture in a final 20 μL using a Takara RNA PCR kit (AMV) (Takara Bio, Inc., Shiga, Japan) according to the manufacturer's directions. Quantification of gene expression in the adipocytes was measured using the real-time PCR system (ABI PRISM 7300 Sequence Detection System, Applied Biosystems, Tokyo, Japan). Amplification was performed in a final 25 μL containing 50 ng of cDNA, optimized specific primers and probes (TaqMan Gene Expression Assays, Applied Biosystems) and Takara Premix Ex Taq™ (Takara Bio Inc.) according to the manufacturer's directions. The assay ID No. of the TaqMan Gene Expression Assays were as follows; adiponectin: Mm00456425_m1, RBP4: Mm00803266_m1, TNF-α: Mm00443258_m1, MCP-1: Mm00441242_m1, CC chemokine receptor 2 (CCR2): Mm99999051_gH, AdipoR$_1$; Mm01291334_mH, AdipoR$_2$; Mm01184030_m1, Glut4: Mm00436615_m1, glucose-6-phosphatase (G6Pase): Mm00839363_m1, β-2 microglobulin: Mm00437762_m1, TATA box binding protein; Mm00446973_m1, ribosomal protein large P2: Mm00782638_s1. Results were expressed as fold increase relative to the controls after normalization using β-2 microglobulin (WAT), TATA box binding protein (skeletal muscle) or ribosomal protein large P2 (liver) gene expression level.

2.7. Immunoblot analysis of Glut4 protein in the WAT

The tissue was homogenized and fractioned plasma membrane as previously described [34]. The protein concentrations were determined by the method of Lowry et al. with bovine serum albumin employed as a standard [35]. Aliquots of the supernatant were treated with Laemmli sample buffer for 5 min at 100 °C [36]. The samples (30 μg protein) were then loaded into the SDS-PAGE system. The resulting gel was transblotted on a polyvinylidene difluoride (PVDF) membrane (Pall Corporation, NY). It was next blocked by 5% skim milk for 1 h at room temperature. After washing with 20 mM Tris–HCl-buffered saline containing 0.05% Tween 20, the membrane sheets were then reacted with anti-Glut4 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, 1:1500 dilutions) or anti-β-actin antibody (Sigma, St. Louis, MO, 1:5000 dilutions) containing 5% Blocking One (Nacalai Tesque, Kyoto, Japan) for 1 h at room temperature. After washing with TTBS, it was reacted with horseradish peroxidase (HRP)-conjugated anti-goat IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA, 1:20,000 dilutions) or HRP-conjugated anti-mouse IgG antibody (Santa Cruz, 1:30,000 dilutions) containing 5% Blocking One for 1 h at room temperature. After washing, the immunoreactivity was visualized using the ECL plus reagent (GE Health Care UK, Ltd., Buckinghamshire, England).

2.8. Statistical analysis

The differences among the means were analyzed by the Student's *t*-test if data in the two groups were normally distributed with equal variance. In other cases, the nonparametric Mann-Whitney test was used. Differences in the $P < 0.05$ were considered significant. All the statistical analyses were performed using the StatView version 5.0 software (SAS Institute, Inc., Cary, NC, USA).

3. Results

3.1. Serum glucose level and insulin sensitivity

Table 1 shows the effects of the C3G on body weight, serum glucose, triglyceride, total cholesterol and insulin

Table 1 – Body weights, food intake, serum glucose, triglyceride, total cholesterol and insulin concentrations in KK-A^y mice fed the control or C3G diet

	Period	Control	C3G
Body weight (g)	0 week	19.2 ± 1.0	18.8 ± 1.2
	5 weeks	37.4 ± 0.8	37.0 ± 1.2
Serum glucose (mg/dL)	0 week	244.6 ± 20.6	231.9 ± 18.4
	3 weeks	393.9 ± 15.3	300.1 ± 14.0*
	5 weeks	454.2 ± 26.1	356.5 ± 28.0*
Serum triglyceride (mg/dL)	5 weeks	409.3 ± 39.0	336.7 ± 31.9
Serum total cholesterol (mg/dL)	5 weeks	96.4 ± 6.5	89.3 ± 12.7
Serum insulin (ng/mL)	5 weeks	17.4 ± 1.2	14.4 ± 1.2

The values are the means ± S.E. ($n = 6$).
* Significantly different at $P < 0.05$ compared to the control group.

concentration. The body weight gain between the control and C3G groups did not differ, accompanied by no significant difference in food intake during the experimental period. The blood glucose concentration was significantly suppressed in the C3G group compared to that in the control group during weeks 3 and 5. Serum insulin, triglyceride and total cholesterol concentrations were not significantly changed between the control and C3G groups. Adipose tissue weight (subcutaneous, epididymal, mesenteric and retroperitoneal WAT, and interscapular BAT) did not significantly differ between the control and C3G groups (data not shown).

The result of the insulin tolerance test clearly showed that dietary C3G ameliorates insulin resistance. The glucose-lowering effect was significantly elevated in the C3G group at 30, 60 and 120 min after insulin injection (30 min, 0.83-fold; 60 min, 0.67-fold; 120 min, 0.81-fold, compared to the control group) (Fig. 2).

3.2. *Adiponectin and adiponectin receptors expression*

The gene expression level of adiponectin in the mesenteric WAT and the serum concentration of the two groups are shown in Fig. 3. There was no significant difference in the gene expression level of adiponectin between the groups (Fig. 3A). The serum adiponectin concentration was also not affected by the administration of C3G (Fig. 3B). No significant difference was observed in the gene expression level of adiponectin receptors (AdipoR$_1$ and R$_2$) in both skeletal muscle and liver between the control and C3G groups (Fig. 3C and D).

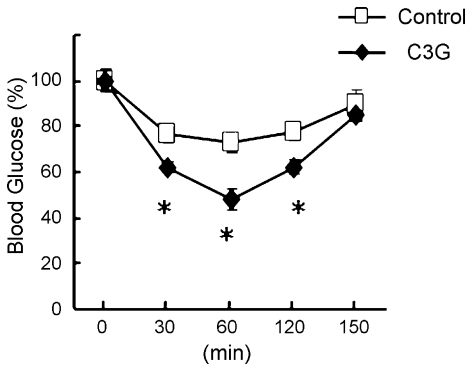


Fig. 2 – Insulin tolerance test in KK-A^y mice fed the control or C3G diet for 4 weeks. Values are the means ± S.E. ($n = 6$). (*) Significantly different at $P < 0.05$ compared to the control in each period.

3.3. *Inflammatory adipocytokines expression*

Inflammatory molecules including MCP-1 and TNF-α are expressed and upregulated in adipose tissue of the obese state including type 2 diabetes, suggesting that adipose tissue in obesity is characterized by a chronic low-grade inflammation that might contribute to the insulin resistance.

The gene expression level of TNF-α and MCP-1 in the mesenteric WAT was significantly decreased in the C3G group compared to the control group (Fig. 4A and B, TNFα, decreased by 76%; MCP-1, decreased by 47%). However, no significant difference was observed in the gene expression of CCR2 (receptor for MCP-1) in the WAT in both the control and C3G groups (data not shown).

3.4. *Glut4 expression*

Glut4 is expressed in adipocytes, and the transport of glucose by Glut4 is the rate-limiting step for glucose use by adipose tissue and skeletal muscle. The expression of Glut4 is decreased in adipose tissue, but not in skeletal muscle, in obesity and type 2 diabetes [13,37,38]. Yang et al. demonstrated that the expression of Glut4 changes the glucose influx, and the change regulates the expression and secretion of RBP4 from adipocytes using mice with the adipocyte-specific ablation of Glut4 [13]. The gene expression level of Glut4 in the mesenteric WAT was significantly higher in the C3G group than that in the control group (2.4-fold) (Fig. 5A). Also, the marked and significant increase in Glut4 protein at the whole cell lysate and plasma membrane was observed in the WAT of the C3G group compared to that of the control group (Fig. 5B). The protein expression level in the C3G group was 2.6-fold (whole lysate) and 3.1-fold (plasma membrane) higher than that in the control group.

3.5. *RBP4 expression*

Recent studies provided a new link between RBP4, which is recognized as one of the adipocytokines, and insulin resistance. RBP4 contributes to the pathogenesis of type 2 diabetes, and lowering of the RBP4 level can be a new target for preventing and treating type 2 diabetes. The gene

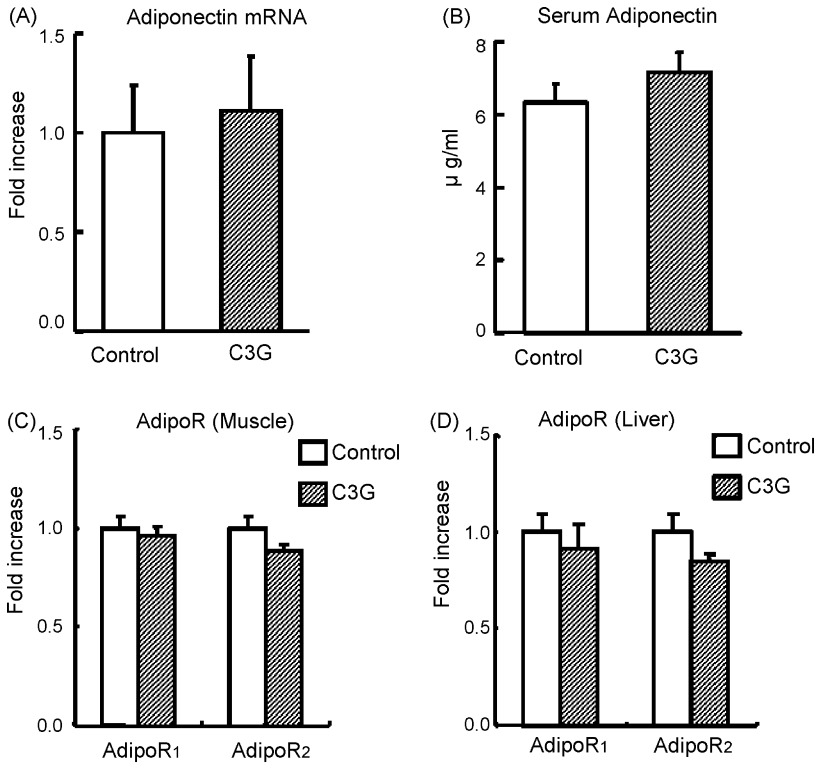


Fig. 3 – Gene expression level of adiponectin in the mesenteric WAT (A), serum adiponectin concentration (B) and the gene expression level of adiponectin receptors (Adipo R_1 and R_2) (C) in KK-A^y mice fed the control or C3G diet for 5 weeks. The gene expression level was expressed as fold increase relative to the control (=1.0) after normalization using the β-2 microglobulin (WAT), TATA box binding protein (skeletal muscle) or ribosomal protein large P2 (liver) gene expression level. The statistics was made in fold increase level. Values are the means ± S.E. (n = 6).

expression level of RBP4 in the mesenteric WAT was significantly suppressed in the C3G group compared to the control group (decreased by 53%) (Fig. 6A). On the other hand, the gene expression level of RBP4 in the liver did not differ between the control and C3G groups (Fig. 6A). The serum RBP4 concentration was also significantly reduced in the C3G group compared to the control group (decreased by 47%) (Fig. 6B).

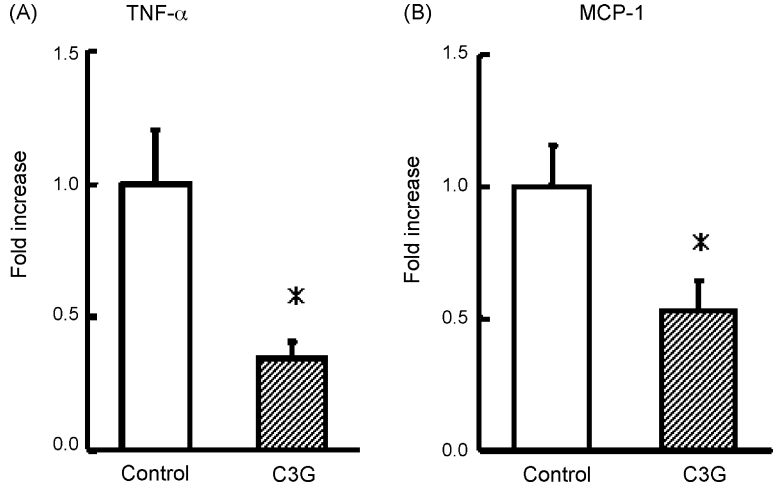


Fig. 4 – Gene expression level of TNF-α (A), MCP-1 (B) and CCR2 (C) in the mesenteric WAT in KK-A^y mice fed the control or C3G diet for 5 weeks. The gene expression level was expressed as fold increase relative to the control (=1.0) after normalization using the β-2 microglobulin gene expression level. The statistics was made in fold increase level. Values are the means ± S.E. (n = 6). (*) Significantly different at $P < 0.05$ compared to the control.

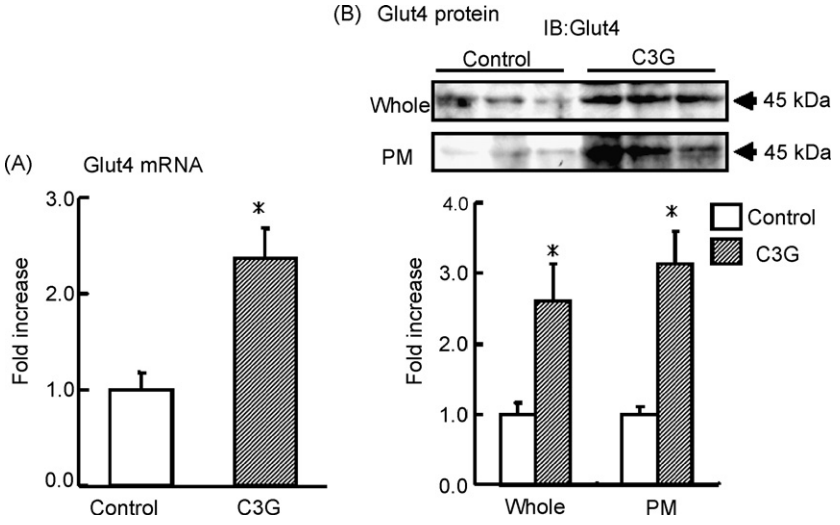


Fig. 5 – Gene expression level (A) and protein level (B) of Glut4 in the mesenteric WAT of KK-A^y mice fed the control or C3G diet for 5 weeks. The gene expression level was expressed as fold increase relative to the control group after normalization using the β-2 microglobulin gene expression level. The protein level of Glut4 was expressed as fold increase relative to the control group. The protein level of Glut4 in the whole lysate was normalized using the β-actin protein level. The statistics was made in fold increase level. Values are the means ± S.E. (n = 5–7). (*) Significantly different at $P < 0.05$ compared to the control.

3.6. *G6Pase expression in the liver*

Based on the results for significant RBP4 reduction in the C3G group, we examined whether the gene expression level of gluconeogenic enzymes was suppressed by the administration of C3G. G6Pase is one of the rate-limiting gluconeogenic enzymes, and the expression is increased in the diabetic state. The upregulation of RBP4 through lowering the glucose influx by a decrease in Glut4 expression causes upregulation of the gluconeogenic enzymes expression and resulted in hyperglycemia. The gene expression level of G6Pase was significantly lower in the C3G group than that in the control group (decreased by 62%) (Fig. 6C).

4. Discussion

Some drugs are used for the therapy of obese-related metabolic diseases. However, there has been little evidence that food factors themselves are directly beneficial for the improvement of insulin resistance responsible for adipocytokine expression. Recent studies suggested that RBP4, a novel adipocytokine is an important target molecule for prevention and therapy of type 2 diabetes [13–18]. The present study has demonstrated that C3G has significant potency in anti-diabetic effects due to the increase in Glut4 expression and decrease in RBP4 expression.

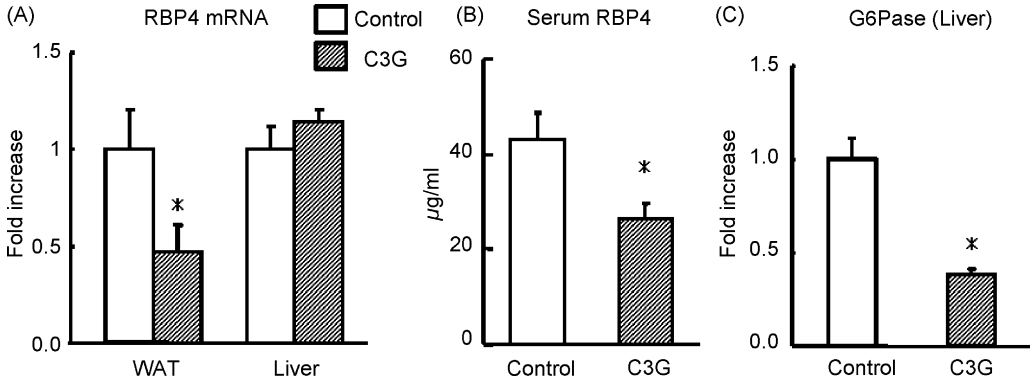


Fig. 6 – Gene expression level of RBP4 in the mesenteric WAT or liver (A), serum RBP4 concentration (B) and gene expression level of G6Pase (C) in the liver in KK-A^y mice fed the control or C3G diet for 5 weeks. The gene expression level was expressed as fold increase relative to the control (=1.0) after normalization using the β-2 microglobulin (WAT) or ribosomal protein large P2 (liver) gene expression level. The statistics was made in fold increase level. Values are the means ± S.E. (n = 6). (*) Significantly different at $P < 0.05$ compared to the control.

Dietary C3G significantly reduced the serum glucose concentration and increased in insulin sensitivity in type 2 diabetic mice. Our prior study has already shown that the administration of C3G resulted in significant elevation of adiponectin gene expression in adipocytes [33]. In this study, C3G did not significantly affect the expression level of both adiponectin and its receptors in type 2 diabetic mice. These results suggest that the effect of C3G on adiponectin expression in type 2 diabetic mice is different from that in vitro and it has another molecular mechanism for the anti-diabetic effect. Several observations suggest that some polyphenols have inhibitory effects on α-glucosidase activity. However, the inhibitory effect on α-glucosidase activity by both C3G and cyanidin (Cy), which is an aglycon of C3G, was at an extremely low level and the amelioration of insulin resistance by dietary C3G is not due to inhibition of α-glucosidase activity [39,40].

Inflammatory molecules including MCP-1 and TNF-α are expressed and upregulated in adipose tissue of the obese state including type 2 diabetes. Recent studies demonstrated that an increase in MCP-1 expression in adipose tissue contributes to the development of insulin resistance and induces macrophage infiltration into the tissue [11,12]. Some reports showed that anthocyanins have a potency of the anti-inflammatory activity [41,42], and the downregulation of TNF-α and MCP-1 expression can contribute to amelioration of diabetic state. Generation of reactive oxygen species (ROS) also plays an important role in the development of insulin resistance [43,44]. TNF-α promotes ROS production and it causes impairment of insulin signaling [43,44]. Our previous study demonstrated that C3G has the potency of an antioxidant in vivo. As one of the possible mechanisms, scavenging ROS and/or downregulation of TNF-α by C3G may contribute to the anti-diabetic function of C3G. Furukawa et al. reported that treatment of 3T3-L1 adipocytes with H_2O_2 caused downregulation of adiponectin and that the administration of an antioxidant (*N*-acetyl cysteine) canceled this downregulation [45]. Also, the impairment of insulin signaling by TNF-α was significantly improved by scavenging ROS using antioxidants [44]. The C3G can contribute to the anti-diabetic effect due to its antioxidant activity and/or down-regulation of TNF-α. However, at least, the antioxidant capacity of C3G cannot lead to upregulation of adiponectin expression in the type 2 diabetic model.

Dysregulation of the adipocyte Glut4-RBP4 system is strongly associated with type 2 diabetes, and lowering RBP4 is a new important target molecule for prevention and therapy of type 2 diabetes [13]. Glut4 expression is decreased selectively in adipose tissue of type 2 diabetes [13,37,38]. The gene expression level of Glut4 in the WAT was markedly higher in the C3G group than that in the control group. The protein expression level in both the whole lysate and the plasma membrane of the WAT was also significantly higher in the C3G group. This increase induces a marked decrease in the gene expression level of the RBP4 in the WAT and the serum concentration in the C3G group, despite no significance of that in the liver. These changes lead to downregulation the G6Pase involving a decrease in the glucose output into the blood and can result in anti-hyperglycemia (Fig. 7).

There is one question about how anthocyanins upregulate Glut4 in the WAT. Sartipy and Loskutoff demonstrated that the administration of MCP-1 strongly reduced the expression of Glut4 and the ability for insulin-stimulated glucose uptake in 3T3-L1 adipocytes [4]. A decrease in the MCP-1 expression by C3G can contribute to the inhibition of the downregulation of Glut4 expression. There is another possibility for the regulation of Glut4 expression. AMPK activation stimulates glucose transport through increased Glut4 translocation in adipocytes [46]. Our prior study demonstrated that C3G activates AMPK in adipocytes [32]. Based on these reports and our findings, it is speculated that downregulation of MCP-1 by C3G blocked the downregulation of Glut4 gene expression and activation of AMPK by C3G may contribute to increase in the protein expression of Glut4 in the plasma membrane. These significant changes may modulate the RBP4 expression (Fig. 7).

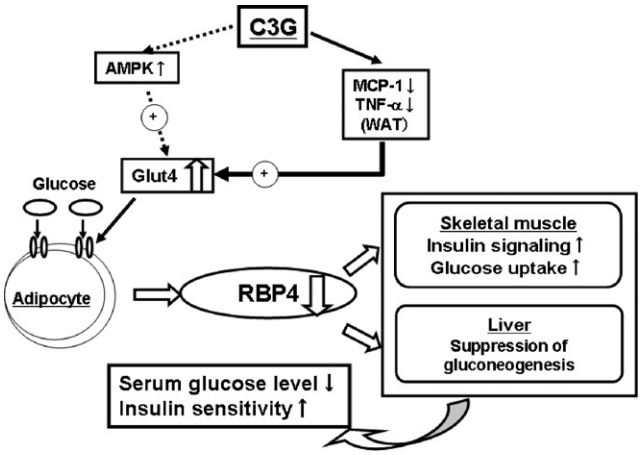


Fig. 7 – Proposed scheme for amelioration of hyperglycemia and insulin sensitivity by the C3G.

In conclusion, the present study demonstrates that C3G, which is one of the most typical anthocyanins, reduce blood glucose level and enhance insulin sensitivity in type 2 diabetic mice. The C3G regulates the new network for adipocytokines (Glut4-RBP4 system) and the related inflammatory adipocytokines. Our findings provide a biochemical basis for the use of anthcyanins, which can also have important implications for preventing and treating type 2 diabetes.

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