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# Effect of Dietary Cyanidin-3-Glucoside on Fat Accumulation and Expression of Glycerol 3 Phosphate Acyltransferase 1 in Mice Fed a High Fat Diet and Control Diet

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Cyanidin-3-glucoside (C3G) is the most common anthocyanin and abundant in purple corn. Inhibitory effects on cancer, insulin resistance and obesity of C3G have been emphasized for a decade. To investigate the effect of dietary cyanidin-3-glucoside on fat accumulation and expression of glycerol 3 phosphate acyltransferase 1 (GPAT1) in mice fed a high fat diet and control diet, the experimental study was carried out on twelve male mice (*Mus musculus*, ddy strain). Four groups of mice were randomly allocated and fed control diet, high fat diet with or without 0.2% purple corn color containing 20% C3G for 12 weeks. Then, mice were sacrificed and pieces of liver, retroperitoneal and epididymal fat pads were taken. Fat accumulations (triacylglycerol levels of liver and adipose tissue) were determined. For detection of hepatic and adipose GPAT1 expression, mRNA levels of them were measured by real-time RT-PCR. Significant differences were not found in hepatic triacylglycerol levels and in adipose triacylglycerol levels of mice with control diet (Group 1) and control diet with C3G (Group 2) (0.2±0.1 *vs.* 0.23±0.06 mg/g and 1.58±0.9 *vs.* 1.51±0.6 mg/g, respectively). Also not significantly different in mice with high fat diet (Group 3) and mice with high fat diet with C3G (Group 4) (0.8±0.4 *vs.* 0.61±0.1 mg/g *vs.* 3.29±0.8 *vs.* 3.02±0.6 mg/g, respectively). The relative expressions of hepatic and adipose GPAT1 in mice of Group 1, 2, 3 and 4 were 1: 1.3: 0.5: 0.4 and 1: 1.1: 0.6:0.7, respectively. Therefore, regardless of C3G addition, fat accumulation and expression of hepatic and adipose GPAT1 were not significantly different between mice of control diet and high fat diet. Twelve-week dietary supplementation of cyanidin-3-glucoside had no preventive effect on fat accumulation despite suppression of glycerol 3 phosphate acyltransferase 1 only in mice with high fat diet.

## INTRODUCTION

Many researchers have focused on the properties of flavonoids, abundant in berries, red wine, tea, chocolate, grapes and soybeans. The most prominent flavonoids are the anthocyanins, universal plant colorants responsible for the red, purple, and blue colors in many fruits, vegetables, cereal grains, and flowers. Anthocyanins were incorporated into the human diet many centuries ago. They were components of the traditional health medicines used by North American Indians, Europeans and Chinese, and were habitually derived from dried leaves, fruits, storage roots or seeds. Because of their diverse actions on physiological processes, the consumption of anthocyanins may play a significant role in preventing life-style related diseases.[1]

Among over 200 anthocyanins in nature, cyanidin-3-glucoside (C3G) is found in a wide variety of plants and is actually the most abundant in some foods, such as purple corn (*Zea mays* L.), black rice extract, the juice of ruby oranges (*Citrus sinensis* L.)

and blackberry extract (*Rubus alleghe-niensis* L.).[2] Inhibitory effects on cancer, insulin resistance and obesity of C3G have been emphasized for a decade. In 2003, it was stated that cyanidin 3-O-β-glucoside rich purple corn color provide a nutritional and biochemical basis for the use of the pigment as a "functional food factor".[3] Recently, much attention has been focused on some active ingredients of food that may be beneficial in preventing high fat diet-induced body fat accumulation.

The enzymes which comprise triacyl-glycerol (TAG) synthesis are controlled via allosteric interaction, by covalent modi-fication and via in gene expression. Among the enzymes, glycerol 3 phosphate acyl-transferase (EC 2.3.1.15) operates the first committed, rate-limiting step of de novo triacylglycerol synthesis. Mammalian tissues contain two glycerol 3 phosphate acyltransferase (GPAT) isoforms [mito-chondrial (GPAT1) and microsomal (GPAT2)] and their functions have not been known whether same or different.

Synthesis of triacylglycerol is a fundamental metabolic pathway important for energy storage. Therefore, the question has been raised whether triacylglycerol synthesis could be prevented by dietary cyanidin-3-glucoside (C3G), through decreased expression of glycerol 3 phosphate acyl-transferase enzyme. The aim of the study was to investigate the effect of dietary cyanidin-3-glucoside on fat accumulation (liver and adipose tissues triacylglycerol levels) and mRNA expression of glycerol 3 phosphate acyltransferase 1 in mice fed a high fat diet and control diet.

## MATERIALS AND METHODS

The study was a randomized controlled trial. A total 12 male mice (Genus: *Mus musculus*, ddy strain), 8 weeks of age, were obtained from Laboratory Animal Services Division, Department of Medical Research (Lower Myanmar). The following formula was employed to compute sample size for comparing two groups means.[4]

$$N = 1 + 2C\,(s/d)^2$$

N=Sample size
C=10.51 [Constant which depends on α and β (In this case, α =0.05, 1-β=0.9)]
*s* =Standard deviation of control group
*d*=Effect size (Difference between means of control and test group)

One study showed that liver triacylglycerol of control group and test group were 71.3±11.2 μmol/g and 34.9±6.6 μmol/g, respectively.[3] Therefore, 3 in each group or a total of 12 mice for the whole study were used.

*Randomization of mice*

Numbers (1, 2, 3, 4) were given to four cages and randomly allocated three mice were kept into each cage. Before giving numbers to the cages, Control diet was named as number 1, control diet with C3G as number 2, high fat diet as 3 and high fat diet with C3G as 4. They meant that the mice in cage no.1, cage no. 2, cage no. 3 and cage no. 4 were fed the control diet (Group 1), control diet with C3G (Group 2), high fat diet (Group 3) and high fat diet with C3G (Group 4), respectively. Each of three mice was randomly allocated in case no.1, no. 2, no. 3 and no. 4.

*Preparation of different types of animal diet*

"Chow diets" for animals of Laboratory Animal Services Division, Department of Medical Research (Lower Myanmar) was used as the control diet (Group 1). Powder of control diet was mixed with water (1:14 w/w) and lard (30%) to form the high fat diet (Group 3).

Each five grams of purple corn color (PCC) containing 20% cynindin-3-glucoside was mixed with two kilogram powder of control diet and high fat diet to make control diet with C3G and high fat diet with C3G. The concentration of C3G was 0.5 gm/kilogram diet. After mixing, each diet was made pallets and baked in an oven for 2½ hours. Cynindin-3-glucoside (20%) rich purple corn color was purchased from Daxing-

anling Koralle Bioengineering Co., Ltd, China. All diets were prepared monthly and protein, fat and carbohydrate content of them were estimated after preparation for this particular study. The compositions of four types of diet are shown in Table 1.

Table 1. Compositions of four types of diet

| Ingredients | Group 1 (gm) | Group 2 (gm) | Group 3 (gm) | Group 4 (gm) |
|---|---|---|---|---|
| Wheat bran | 400 | 400 | 400 | 400 |
| Rice bran | 300 | 300 | 300 | 300 |
| Rice flour | 300 | 300 | 300 | 300 |
| Corn flour | 250 | 250 | 250 | 250 |
| Peanut flour | 200 | 200 | 200 | 200 |
| Sesame flour | 50 | 50 | 50 | 50 |
| Dry fish flour | 250 | 250 | 250 | 250 |
| Chickpea flour | 250 | 250 | 250 | 250 |
| Lard (30%) | - | - | 600 | 600 |
| PCC (0.25%) | - | 5 | - | 5 |
| Total weight | 2000 | 2005 | 2600 | 2605 |

*Supplementation of cyanidin-3-glucoside rich purple corn color*

Mice were kept in the labeled cages on a cycle of 12 hours light and relatively stable room temperature (about 23°C). The four groups of mice were fed one of the four diets with free access to food and water for 12 weeks. Basal body weights and four weekly body weights were measured with the animal balance. Daily food intake of each mouse was estimated by differences between fed food weight and left-over food weight.

*Collection of liver and adipose tissue*

After 12-week intervention, mice were sacrificed and the liver and retroperitoneal adipose tissue (the largest fat mass of the mice's body) and epididymal fat were exposed according to defined anatomical landmarks. Two pieces of liver tissue and en bloc dissection of retroperitoneal and epididymal fat pads were excised. The weights of retroperitoneal and epididymal fat pads were measured. Two pieces of liver and adipose tissues samples were kept separately for tissue triacylglycerol determination and real-time RT-PCR. They were then immediately frozen at liquid nitrogen and stored at -80°C until analysis.

*Determination of liver and adipose tissue triacylglycerol levels*

Total lipids from the homogenized liver and adipose tissues were extracted with 2:1 chloroform: methanol (v/v) mixture and after evaporation of organic solvent, the extracted lipid residues were used for the measurement of the triacylglycerol concentrations with Triacylglycerol GPO liquicolor Mono, complete test kit (Catalog no. 10724, Human Diagnostic Kit Germany).

*Measurement of mRNA level of liver and adipose tissue glycerol 3 phosphate acyltranferase 1 by quantitative RT-PCR analysis*

Total RNA from liver and adipose tissue was purified with the RNeasy Mini Kit. Determination of total RNA concentration was carried out by a UV absorbance at 260 nm wavelength. First strand cDNA synthesis was carried out with QuantiTech Reverse Transcriptase Kits. One microgram of total RNA was used for synthesis of cDNA with the activity of RNA-dependent DNA-polymerase and mixture of Oligo (dT) and random hexamers. The primers for mice glycerol 3 phosphate acyltransferase 1 (GPAT1) gene and β-actin for control gene were ordered according to the gene sequences contained in NCBI References Sequence Database.[5]

The primers are: mitochondrial glycerol 3 phosphate acyltransferase 1 (GPAT1), (F) 5′-CAGTCCTGAATAAGAGGT-3′, (R)5′-TGGACAAAGATGGCAGCAGA-3′ (444 bp); and β-actin (F) 5′- CGTGGGCCG CCC TAGGCACCA-3′, (R) 5′-CTCTT TGATGTCACGCACGATTTC-3′, (541 bp).

The quantifications of RNA for GPAT1 and β-actin expression in liver and adipose tissues were carried out by using SYBR Green-based PCR Kit on the Rotor-Gene Cycler (Rotor Gene 6000 Series, Model: Artus 3000). According to the reaction setup of the PCR Kit, Rotor-Gene SYBR Green PCR Master Mix, cDNA and primers for GPAT1 and β-actin were mixed and then the cycling program was started. Cycling conditions on Rotor-Gene Cycler were:

PCR activation at 95°C for 5 minutes, denaturation at 95°C for 5 seconds, combined annealing/extention at 60°C for 10 seconds and 40 numbers of cycle. The required primers and reagent test kits were purchased from QIAGEN, Samples & Assay Technologies. The transcription of cDNA template for GPAT1 and β-actin and products renaturation compete with specific primers binding develop fluorescent signals as quantitative data.

*Normalization of expression of glycerol 3 phosphate acyltranferase 1*

After performing real-time RT-PCR, the $C_T$ values for glycerol 3 phosphate acyltransferase 1 (target) and β-actin (reference) were determined. The standard curves for target and reference by plotting $C_T$ values (Y-axis) against the dilution of template amount (X-axis) were constructed. The amount of target and reference in samples of hepatic and adipose tissues of mice were calculated with the build-in software of the Rotor-Gene Cycler. The normalized amount of target in each mouse was calculated by division of the amount of target by the amount of reference. The normalized amount of glycerol 3 phosphate acyltransferase 1 (GPAT1) in mice with control diet (Group 1) was set as 1. The relative expression of GPAT1 in mice fed the control diet with C3G (Group 2), high fat diet (Group 3) and high fat diet with C3G (Group 4) were shown by division of the normalized amounts of GPAT1 in mice (Group 1).

*Statistical analysis*

Data were analyzed with SPSS 11 software. All data were expressed as mean±SD. Comparisons among body weights, dietary intakes, weights of adipose tissue, triacylglycerol levels of liver and adipose tissues and normalized mRNA amount of mitochondrial glycerol 3 phosphate acyltransferase 1 of mice fed four different dietary groups were analyzed with One-way Anova. Differences with p values <0.05 were considered as significant.

## RESULTS

A total of 12 mice (3 mice in each group) were fed with either the control diet, control diet with C3G, high fat diet or high fat diet with C3G for 12 weeks. The mean values with standard deviation of calories intake of the 1st, 4th, 8th, and 12th are shown in Table 2.

Table 2. Mean calories intake of mice fed with four different diets

| Diet groups | Calories intake of mice | | | |
|---|---|---|---|---|
| | 1st week | 4th week | 8th week | 12th week |
| Control diet | 48.9±6.8[a] | 57.6±8.9 | 61.9±7.4 | 60.7±8.5 |
| Control diet with C3G | 47.8±5.9[a] | 58.1±4.9 | 59.1±5.8 | 60.5±6.4 |
| High fat diet | 54.1±9.6[b] | 55.9±6.5 | 58.8±9.1 | 61.5±7.2 |
| High fat diet with C3G | 58.8±10.1[b] | 57.6±7.5 | 61.7±8.2 | 62.3±6.7 |

Values are mean±SD, n=12, One-way Anova

a=Calories intake of mice fed control diet with and without C3G, b=Calories intake of mice fed high fat diet with and without C3G. They are significantly different ($p<0.05$).

During the first week of intervention, calories intakes of mice fed with high fat diet were significantly higher than those of mice with control diet. However, in the 4th, 8th, and 12th weeks of intervention, there were no significant differences in calories intakes of mice with all four dietary groups. The overall trends of calories intake in each mouse were increased according to the time because they were age dependent.

Table 3. Mean body weights of mice fed with four different diets

| Diet groups | Mean body weights (g) | | | |
|---|---|---|---|---|
| | Before | 4th week | 8th week | 12th week |
| Control diet | 30.0±2.6 | 33.3±2.2 | 35.7±2.3 | 37.2±2.3 |
| Control diet with C3G | 27.3±2.7 | 30.3±4.5 | 32.7±2.6 | 34.0±3.4 |
| High fat diet | 28.5±1.6 | 32.3±2.8 | 34.8±3.6 | 36.8±4.1 |
| High fat diet with C3G | 27.7±2.3 | 33.1±2.4 | 34.7±2.5 | 35.5±2.5 |

Values are mean±SD, n=12, One way ANOVA

The body weights of mice with four dietary groups were measured before, at the 4th week, 8th week and 12th week of intervention. The mean body weight and standard deviation of mice are shown in Table 3. They were not statistically different

($p>0.5$), before intervention, in the fourth week, eighth week and twelfth week of intervention. However, the body weights of mice with each dietary group were gradually increased along with the time.

After 12-week feeding, all mice were sacrificed to study the fat accumulation by measuring the weights of retroperitoneal and epididymal fat pads and tissue triacyglycerol levels. The weight of retroperitoneal fat pad in mice with high fat diet was larger than those of the remaining dietary groups but there were no significant differences (data not shown). Comparisons of mean fat pad weight and tissue triacylglycerol levels are shown in Table 4.

Table 4. Fat pad weight (g) and triacylglycerol concentrations (mg/g) of mice with four diets

| | Control diet | Control diet with C3G | High fat diet | High fat diet with C3G |
|---|---|---|---|---|
| Epididymal fat pad (g) | 0.26± 0.12[a] | 0.37± 0.08[a] | 0.72± 0.16[b] | 0.76± 0.2[b] |
| Liver triacylglycerol (mg/g wet tissue) | 0.2± 0.1[a] | 0.23± 0.06[a] | 0.81± 0.4[b] | 0.61± 0.1[b] |
| Adipose tissues-triacylglycerol (mg/g wet tissue) | 1.58± 0.9[a] | 1.51± 0.6[a] | 3.29± 0.8[b] | 3.02± 0.6[b] |

a=Fat pad weight, liver and adipose TAG concentration of mice fed control diet with and without C3G, b=Fat pad weight, liver and adipose TAG concenration of mice fed high fat diet with and without C3G. They are significantly different ($p<0.05$).

Regardless of addition of C3G, epididymal fat pad weight of mice with two high fat diets were significantly larger than those of two control diets ($p<0.001$ for high fat diet and control diet, and $p<0.01$ for high fat diet with C3G and control diet with C3G).

Liver triacylglycerol levels of mice with high fat diet were significantly higher than those of mice with other diets ($p<0.0001$ for each). When the C3G was added to the high fat diet, lower but not significant liver triglyceride levels were found in mice fed high fat diet with C3G ($p>0.05$) than those with high fat diet only. However, liver triglycerol levels of mice with C3G enriched control diet and those of control diet were not statistically different ($p>0.05$). Adipose tissue triacylglycerol levels of mice with high fat diets with and without C3G were higher than those of mice with control diet ($p=0.012$ for high fat diet group, $p=0.032$ for high fat diet with C3G group and control diet group, respectively). Although, adipose tissue triacylglycerol levels of mice fed control diet with C3G were higher than those of mice with control diet only, a significant difference was not found ($p=0.06$).

Table 5. Relative expression of hepatic and adipose tissue glycerol 3 phosphate acyltransferase 1 (GPAT1) of mice with four diets

| Dietary groups | Relative expression of Liver GPAT1 A | B | C | D | Adipose GPAT1 A | B | C | D |
|---|---|---|---|---|---|---|---|---|
| Group 1 | 2.9x $10^5$ | 1.7x $10^5$ | 1.68[a] | 1 | 7.8x $10^5$ | 6.1x $10^5$ | 1.27[a] | 1 |
| Group 2 | 2.9x $10^5$ | 1.3x $10^5$ | 2.16[a] | 1.3 | 4.1x $10^5$ | 3.0x $10^5$ | 1.36[a] | 1.1 |
| Group 3 | 1.3x $10^5$ | 1.6x $10^5$ | 0.77[b] | 0.5 | 1.3x $10^5$ | 1.7x $10^5$ | 0.78[b] | 0.6 |
| Group 4 | 1.4x $10^5$ | 2.0x $10^5$ | 0.69[b] | 0.4 | 2.0x $10^5$ | 2.2x $10^5$ | 0.9[b] | 0.7 |

A=Mean GPAT1RNA (pg)
B=Mean β-actin RNA (pg)
C=Mean normalized amount of GPAT1
D=Ratio
Group 1=Control diet, Group 2=Control diet with C3G, Group 3=High fat diet, Group 4= High fat diet with C3G, a=Normalized amount of hepatic and adipose GPAT1 in mice of control diet with or without C3G, b=Normalized amount of hepatic and adipose GPAT1 in mice of high fat diet with or without C3G. They are significantly different ($p<0.05$).

After 12-week feeding of different food, hepatic and adipose GPAT1 expressions of mice were compared. Quantitative RT-PCR was done to determine the expression of GPAT1 and β-actin (reference gene). mRNA level of GPAT1 of each mouse in each group was normalized with that of β-actin and the normalized amount was expressed in the Table 5. The normalized amount of mice fed control diet was set as 1.

The relative expressions of hepatic GPAT1 in mice of Group 3 and that of Group 4 were significantly lower than those in mice of Group 1, (0.4: 0.5: 1). Similarly, the relative expression of adipose GPAT1 in mice of

Group 1, 3 and 4 were 1: 0.6: 0.7. However, expressions of hepatic GPAT1 and adipose GPAT1 were not different by addition of C3G in both high fat and control diet (1: 1.3 and 1: 1.1, respectively).

## DISCUSSION

Diet induced weight gain in mice was found in many studies and this study also found weight gain in mice fed high fat diet than control diet. However, the body weight gain in high fat diet mice in this study was low to compare with other studies. One study found the effect of 100 days feeding of different dietary fat on metabolic syndrome, obesity, central adiposity, dyslipidemia, and insulin resistance in C57BL/6NCrlBR mice.[6] They used various test diets from different fat sources: porcine lard, hydrogenated vegetable oil, coconut oil and butterfat. Fat contributes 60% of the total energy and calories content were 5.21 calories/gm in lard containing diet whereas 12% fat and 3.9 calories/gm contained in the low fat diet. The daily consumption of the high fat diet was 5.2 calories/gm/day. In contrast, the animals on the low fat control diet ate 3.9 calories/gm/day. There were 54% and 38% weight gain in lard containing diet group of mice and low fat diet of mice, respectively, on day 98 or week 14. They also found the apparent significant differences in fat distribution in the epididymal fat deposits, i.e., 5.1% body weight in lard diet group and 4.39% body weight in low fat control diet group on day 85 (week 12). In this study, the epididymal fat pad weights were 2% and 0.7% of body weight in mice with high fat diet and control diet, respectively.

The ingredients of the control diet used in this study were wheat bran, rice bran, rice flour, corn flour, sesames flour, dry fish flour, and chickpea flour. The high fat diet was made up of the control diet with addition of lard (30%). However, one study used purified ingredients formulations which were produced by Research Diet Incorporation.[6] Two kinds of high fat diet (D12451 and D12492) for animal diet induced obesity (DIO) were consisted of corn starch, maltodextrin, sucrose, cellulose, casein, lard, soybean oil, minerals mix, vitamins mix and food dyes. Therefore, the high fat diet used in this study was different from that of the study in diet induced metabolic syndrome for mice.[6]

In comparison with calories intake and weight gain[7], low calories intakes and low weight gains in all studied mice were found because of different animal diets and different strains (ddy strain *vs.* C57BL/6J strain). *Mus musculus*, ddy strain used in this study may not be the model strain for diet-induced obesity (genetically not predisposed to obesity). In 1999, a study established a new mouse model of spontaneous diabetes derived from male obese mice of ddy strain by the selective breeding.[8] However, inability to use of specific strain for diet induced obesity is one limitation of this study.

A study reviewed that flavonoids could induce lipolysis both in liver and adipose tissue, likely through competitive inhibition of phosphodiesterase and antagonism of cAMP degradation. Flavonoid-mediated cellular cAMP elevation activates hormone sensitive lipase and the specialized proteins to initiate the lipid hydrolysis.[9]

Based on this mechanism, a study investigated the effect of dietary cyanidin-3-glucoside (C3G) rich purple corn color (C3G concentration of 2 gm/kg diet) on fat pad weights (epidydimal and retroperitoneal), triacylglycerol levels of liver and adipose tissue, and mRNA level of glycerol 3 phosphate acyltransferase enzymes in male C57BL/6J mice fed high fat diet (addition of lard 30%) and control diet for 12 weeks.[3] They found that all adipose tissue (epididymal and retroperitoneal) weights were markedly greater in the high fat diet group than in the control diet group (2.16±0.15 gm *vs.* 0.81±0.08 gm for epididymal fat pad and 0.7±0.06 gm *vs.* 0.25±0.03 gm for retroperitoneal fat, $p<0.05$). The weight of epidydimal fat pad

was lower significantly in mice fed high fat diet with C3G than those without C3G (0.35±0.04 gm *vs.* 0.7±0.06 gm, $p<0.05$). Therefore, dietary C3G rich purple corn color suppressed the weights of fat pad which were induced by high dose of fat.[3]

However, in this study, epidydimal fat pad weight, triacylglycerol levels of liver and adipose tissue of mice fed control diet with C3G and those of mice with control diet only were not significantly different ($p>0.05$). When these parameters of mice fed high fat diet with C3G and without C3G were compared, they were not significantly decreased (Table 3). The significant differences were found only in hepatic and adipose triacylglycerol concentrations between the high fat diet mice and control diet mice (0.81±0.4 *vs.* 0.2±0.1 mg/gm hepatic tissue and 3.29±0.8 *vs.* 1.58±0.9 mg/gm adipose tissue, $p<0.05$) (Table 3). Therefore, dietary C3G rich purple corn color could not reduce the fat pad weights as well as tissue triacylglyerol accumulation both in mice fed high fat diet and control diet.

The dose of C3G (2 gm/kg diet) used in a study[3] was higher than that used in this study (0.5 gm/kg) because such high dose of C3G could not be accepted by the studied mice. Animal diet pellets are usually baked at 100°C for two and half hours to reduce moisture preventing from fungus infection. This is one of the restrictions to achieve full activity of anthocyanins like C3G. Moreover, in 2002, a study found that the bioavailability of blackberry anthocyanins was very low compared with other flavonoids in rats.[10] Therefore, low hepatic and adipose TAG contents in HFD+C3G group of mice by the effect of C3G were not found in this study.

GPAT1 overexpression *in vivo* specially increased hepatic TAG and diacylglycerol (DAG) levels and decreased the availability of fatty acids for β-oxidation.[11] Compared with lean littermates, total hepatic GPAT1 specific activity in ob/ob mice was 33% higher and both GPAT1 activity and mRNA expression was 2.2 folds higher. However, in this study, after normalization with hepatic GPAT1 expression in mice with control diet, those in mice with high fat diet without C3G and with C3G became half, (1: 0.5 and 0.4). Nearly half expressions of adipose GPAT1 in mice of high fat diet without C3G and with C3G (1: 0.6 for each) were also found. However, one study showed that hepatic and adipose GPAT1 mRNA levels were 31% and 32% lower in the HF+C3G group than in all other groups.[3] The different findings on hepatic and adipose GPAT1 expression in mice fed high fat diet with C3G in this study and the other study[3] might be due to also again low dose of C3G and decreased activity of C3G during processing of pellets, i.e., at >100°C for 2½ hours.

The findings of increased hepatic and adipose tissues TAG concentrations and decreased hepatic and adipose GPAT1 expressions in high fat diet groups were inconsistent in this study. It is possible that the increased TAG content in liver and adipose tissues in high fat diet mice was probably due to increased activity or amount of other isoforms of glycerol 3 phosphate acyltransferase. However, it could be proved if expressions of other isoforms were determined in this study.

One study has shown that GPAT1 activity and protein expression were discordant, particularly in liver and heart, suggesting a mechanism of acute regulation.[12] One possible mechanism of acute regulation is phosphorylation, particularly by AMP-activated kinase or sudden changes in substrate availability. GPAT1 expression might be modulated according to specific needs for triacylglycerol storage. It would be elucidated if GPAT1 activity and GPAT1 protein expression were determined in this study.

In conclusion, twelve-week dietary supplementation of cyanidin-3-glucoside rich purple corn color (0.25%) could not significantly decrease concentrations of hepatic

and adipose triacylglycerol and tissue glycerol phosphate acyltransferase 1mRNA levels in ddy strain mice fed high fat diet and control diet. Significantly increased hepatic and adipose triacylglycerol levels were found although there were decreased expressions of glycerol 3 phosphate acyltransferase 1 enzyme in mice with high fat dietary group because triacylglycerol can be synthesized by glycerol 3 phosphate acyltransferase 1 as well as other isofroms. Therefore, it could be inferred that dietary supplementation of purple corn color rich with cyanidin-3-glucoside had no preventive effect on triacylglycerol accumulation by suppression of glycerol 3 phosphate acyltransferase 1 only.

*Recommendations*

Expression of other isoforms of glycerol 3 phosphate acyltransferase enzymes should be studied. Not only the study on enzymes expression, but also the activity of enzymes should be determined. The lowest but highest effective dose of cyanidin-3-glucoside for decreased tissues contents of triacylglycerol should be identified.

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