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Anti-inflammatory effect of purified dietary anthocyanin in adults with hypercholesterolemia: A randomized controlled trial

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Abstract *Background and aim:* Atherosclerosis is a chronic inflammatory disease and previous studies have demonstrated that anthocyanin inhibits atherosclerosis. In the present study, we explored the effects of anthocyanins on inflammatory cytokines in hypercholesterolemic adults and cell lines.

Methods and results: A total of 150 subjects with hypercholesterolemia consumed a purified anthocyanin mixture (320 mg/d) or a placebo twice a day for 24 weeks in a randomized, double-blind trial. Anthocyanin consumption significantly decreased the levels of serum high sensitivity C-reactive protein (hsCRP) (−21.6% vs. −2.5%, $P = 0.001$), soluble vascular cell adhesion molecule-1 (sVCAM-1) (−12.3% vs. 0.4%, $P = 0.005$) and plasma IL-1 β (−12.8% vs. −1.3%, $P = 0.019$) compared to the placebo. We also found a significant difference in the LDL-cholesterol (−10.4% vs. 0.3%, $P = 0.030$) and HDL-cholesterol level changes (14.0% vs. −0.9%, $P = 0.036$) between the two groups. In cell culture assays in vitro, purified anthocyanin mixture, delphinidin-3-O- β -glucoside (Dp-3g) and cyanidin-3-O- β -glucoside (Cy-3g) inhibited IL-6 and IL-1 β -induced CRP production ($P < 0.05$) in HepG2 cell line and LPS-induced VCAM-1 secretion ($P < 0.05$) in porcine iliac artery endothelial cell line respectively in a dose-dependent manner.

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In addition, the reduction of inflammatory cytokines associated with anthocyanin mixture was stronger when compared with the effects of Dp-3g and Cy-3g separately ($P < 0.05$).

Conclusions: Anthocyanin mixture reduced the inflammatory response in hypercholesterolemic subjects. In addition, different anthocyanin compounds were found to have additive or synergistic effects in mediating anti-inflammatory responses in vitro cell culture assays.

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Introduction

Atherosclerosis is a major cause of the mortality worldwide and a substantial number of studies have now demonstrated that inflammatory reactions are involved in all stages of atherosclerotic development [1]. Inappropriate inflammatory reactions are thought to be mediated by a series of risk factors, including hypercholesterolemia, which is typically an early biomarker of atherosclerosis [2]. A number of studies have also examined the role of various proinflammatory cytokines in the progression of atherosclerosis, including C-reactive protein (CRP) [3], vascular cell adhesion molecule-1 (VCAM-1) [4], TNF- α [5] and IL-1 β [6]. Among these inflammatory biomarkers, C-reactive protein is currently the best validated inflammatory biomarker [7,8]; VCAM-1 and TNF- α may provide additional information for cardiovascular risk stratification and prediction [7–9].

Anthocyanins are water-soluble flavonoid pigments, abundant in various fruits, vegetables and beverages, and they are a commonly consumed phenolic compound in the human diet [10,11]. Previous human studies have demonstrated that anthocyanin-rich foods or beverages inhibit atherosclerosis due to their anti-oxidant and anti-inflammatory properties [12,13]. However, it has been reported that the anti-inflammatory properties of anthocyanin-rich beverages or anthocyanin extracts do not exhibit the same benefits in populations with different health conditions [14–16]. The levels of inflammatory molecules, the purity of the anthocyanin and the period of the intervention trial may be the important factor resulting in the controversial conclusion. Therefore, the influence of anthocyanins on inflammation in subjects with cardiovascular risk factors needs further investigation. In addition, evidence is emerging that specific combinations of phytochemicals may be far more effective in protecting against risk factors for cardiovascular diseases than single phenolic compound [17,18]. Whether purified anthocyanin mixtures could cause more significant inhibitory effects on inflammatory responses compared with a single anthocyanin compound has not been previously investigated.

We thus investigated whether long-term supplementation with purified anthocyanins derived from bilberries and blackcurrants inhibits the inflammatory response in subjects with hypercholesterolemia. Then, we further examined the synergism efficacy of an anthocyanin mixture compared with single anthocyanin compounds (cyanidin-3-O- β -glucosides, Cy-3g and delphinidin-3-O- β -glucosides, Dp-3g) in affecting the secretion of inflammatory cytokines in cultured HepG2 cell and endothelial cell.

Methods

Materials

The anthocyanin and placebo capsules and the single anthocyanin (Dp-3g and Cy-3g) were provided by Polyphenols AS (Sandnes, Norway). All chemicals, unless otherwise specified, were purchased from local suppliers (see Supplementary materials and methods).

Subjects

One hundred and fifty hypercholesterolemic subjects aged 40–65 y were recruited into this clinical trial between October 2008 and December 2010 from three hospitals and by advertisement leaflets in several communities in Guangzhou, Guangdong, China. Inclusion criteria were a fasting total cholesterol level of the participants of >5.17 mmol/L and <8.01 mmol/L (about 200 mg/dL to 310 mg/dL). Exclusion criteria included a history of cardiovascular disease, diabetes mellitus, hypertension, thyroid disorders, smoking or the use of any drugs that could influence lipid parameters and inflammatory markers. This study was approved by the Ethics Committee of Sun Yat-Sen University, and signed informed consent was obtained from all participants. All procedures were performed according to institutional guidelines and the Helsinki Declaration.

Study design

The study was a randomized, double-blind, placebo-controlled 24-week trial. After evaluation of exclusion/inclusion criteria, the eligible participants entered a 7-day run-in phase, which excluded anthocyanin-rich foods and beverage including berry juice and red wine; a list of these foods and beverages was given to each participant. At the end of the run-in period, all the participants were randomly assigned to either the anthocyanin group ($n = 75$; aged 56.2 ± 6.7 y) or the placebo group ($n = 75$; aged 55.8 ± 6.0 y). The randomization code was computer-generated by the study biostatistician. All researchers and participants were unaware of the randomization list or treatment assignment except for the project manager. During the trial, the subjects were asked to maintain their habitual diet and lifestyle except for consuming anthocyanin-rich foods and beverage. The anthocyanin group was instructed to orally consume two 80 mg anthocyanin capsules twice daily (30 min after breakfast and supper) for a total intake of 320 mg anthocyanins/d. The

placebo group took 2 placebo capsules twice daily. Each of the participants came back every 4 weeks, the adherence of the subjects to the protocol was assessed by recalling the empty packages and obtaining related information and reinforcing the requirements if necessary. The capsules were dispensed at these visits, and body weight, blood pressure, and waist and hip circumferences were measured. At baseline and weeks 12 and 24 of the trial, all subjects were required to fast overnight to enable blood sample collection the next morning.

A three-day 24-h dietary recall and a validated questionnaire were undertaken to assess nutrient intake and dietary habits in all subjects at baseline and at weeks 12 and 24 (see [Supplementary materials and methods](#)).

Cell culture and treatments

The effect of anthocyanins on the secretion of inflammatory cytokines (CRP and VCAM) was also carried out in cultured HepG2 cell and endothelial cell (see [Supplementary materials and methods](#)).

Laboratory measurements

Assessments of the levels of hsCRP, sVCAM, TNF- α , IL-1 β , lipids and glucose in human blood samples and the levels of CRP and VCAM in cell culture medium were described in [Supplementary materials and methods](#).

Statistical methods

The sample size, randomization and blinding for the intervention study was described in [Supplementary materials and methods](#).

Normal distributions were tested using the Kolmogorov–Smirnov test. Variables that deviated from the normal pattern were logarithmically transformed for statistical analyses. Variables that followed a normal distribution were expressed as the means \pm SD, means \pm SE or means with 95% CIs. Transformed data, including the levels of triacylglycerol, hsCRP and insulin were presented as geometric means and upper and lower quartiles. The percentage changes of lipid profile, glucose, insulin and inflammatory cytokine levels were calculated as follows: (the value at week 24 – the value at baseline)/value at baseline \times 100. The average level of the percentage change was displayed as the mean and 95% CI.

Differences of the variables at the baseline and of the percentage changes after 24-week intervention in the two groups were evaluated using the unpaired Student's *t* test. Multivariate analysis of variance (MANOVA) on repeated measures was carried out using General Linear Model (GLM) for comparison of group anthropometric characteristics and dietary intake. Multivariate analysis of covariance (MANCOVA) on repeated measures with the BMI value as covariate was used to compare the difference of the effects of the treatment on lipid profile, glucose and insulin. Differences in the inflammatory molecule levels between the two treatment groups were analyzed using the repeated-measures MANCOVA with the BMI and lipid profile values as covariates in human study. Pearson correlation

coefficients (*r*) were performed to analyze for correlations in changes in the variables tested. Differences in the production of CRP and VCAM in HepG2 and PIEC cell-conditioned media between different doses of the same treatment such as anthocyanin mixture, Dp-3g or Cy-3g and between different treatments of the same dose were evaluated using one-factor ANOVA followed by post-hoc (Bonferroni) statistical tests. Statistical significance was set at *P*-values < 0.05 . All statistical analyses were performed using SPSS for Windows software (version 16.0; SPSS Inc, Chicago, IL).

Results

Anthropometric characteristics and dietary intake

One hundred and forty six subjects out of a total of 150 individuals originally enrolled in the trial completed the study (*n* = 73 in the anthocyanin group, 31 male and 42 female; *n* = 73 in the placebo group, 30 male and 43 female). Four participants (3 females, 1 male) withdrew from the trial due to a clinical requirement to take lipid-lowering drugs or because moving far away ([Supplemental Fig. 1](#)). There were no differences in age, anthropometric characteristics or the mean daily intake of nutrients ([Supplemental Table 1](#)) between the two groups. The distribution of these variables was uniform between the two groups at baseline. No adverse effects were reported by any of the participants consuming anthocyanins or placebo throughout the intervention period.

Effects of anthocyanin supplementation on serum lipids in human subjects

As shown in [Table 1](#), after the 24-week intervention, no significant differences in the levels of total cholesterol, HDL-cholesterol, LDL-cholesterol and triacylglycerol between the two groups at baseline were observed (all *P* > 0.05). There were significant increases in the HDL-cholesterol level (baseline vs week 24, *P* = 0.018), and significant decreases in the LDL-cholesterol level (*P* = 0.038) after the 24-week anthocyanin supplementation. There were no significant changes in the level of HDL- and LDL-cholesterol in the placebo group. When changes in the two variables were compared between the anthocyanin and the placebo groups, we observed significant differences in the changes of HDL-cholesterol level (*P* = 0.036), and LDL-cholesterol level (*P* = 0.030). No significant differences in the levels of triacylglycerol, total cholesterol, glucose and insulin were observed between the two groups after the 24-week intervention.

Effects of anthocyanin supplementation on inflammatory biomarkers in human subjects

We examined serum inflammatory molecule levels (hsCRP, sVCAM-1 and TNF- α) in the study participants at baseline, as well as at 12 and 24 wk after the intervention ([Table 2](#)). There were no significant differences at baseline of hsCRP, sVCAM-1 and TNF- α levels between the anthocyanin and the

Table 1 Changes in the lipids profile of the participants at baseline and at week 24 of the trial.^a

	Placebo (<i>n</i> = 73)			Anthocyanin (<i>n</i> = 73)			<i>P</i> -value ^c
	Baseline	24 wk	Mean change, % (95%CI) ^b	Baseline	24 wk	Mean change, % (95%CI)	
Total cholesterol (mmol/L)	6.48 ± 0.84	6.25 ± 0.83	−3.6 (−7.8–0.6)	6.45 ± 1.02	6.18 ± 0.82	−2.9 (−6.3–0.5)	0.556
HDL-cholesterol (mmol/L)	1.24 ± 0.21	1.23 ± 0.20	−0.9 (−5.2–3.4)	1.22 ± 0.23	1.37 ± 0.22 ^d	14.0 (7.9–20.2) ^e	0.036
LDL-cholesterol (mmol/L)	3.29 ± 0.47	3.30 ± 0.52	0.3 (−2.9–3.5)	3.36 ± 0.58	3.01 ± 0.41 ^d	−10.4 (−14.8 to −6.0) ^e	0.030
Triacylglycerol (mmol/L) ^f	2.41 (1.47–2.70)	2.34 (1.35–2.62)	−3.2 (−7.6–1.2)	2.45 (1.53–2.74)	2.35 (1.37–2.61)	−4.8 (−9.8–0.2)	0.462

^a The data, unless otherwise specified, were expressed as mean ± SD. No significant differences were found for any variable between the two groups at baseline via the unpaired Student's *t* test.

^b Calculated as (value at 24 wk – value at baseline)/value at baseline × 100.

^c The effects of the intervention on these variables were tested by repeated-measures MANCOVA with the BMI value as covariate.

^d *P* < 0.05 vs baseline, assessed by paired Student's *t* tests.

^e *P* < 0.05 vs percentage changes in the placebo group, assessed by unpaired Student's *t* tests.

^f Geometric mean; upper and lower quartiles in parentheses (all such values).

placebo groups (*P* > 0.05). Anthocyanin treatments for 24 weeks led to appreciable decreases in serum hsCRP level [baseline vs week 12 and week 24, *P* = 0.007 and *P* < 0.001, respectively], in serum sVCAM-1 level (*P* = 0.026 and *P* = 0.014, respectively) and in plasma IL-1β level (*P* = 0.016 and *P* = 0.017, respectively). However, no changes in the levels of hsCRP, sVCAM-1 and IL-1β were observed in the placebo group after intervention. When changes in the two variables were compared between the anthocyanin and the placebo groups, we observed significant differences in the changes of hsCRP [−21.6% (*P* = 0.001), sVCAM-1 level (*P* = 0.005) and IL-1β level (*P* = 0.019). Nevertheless, no significant changes of TNF-α level were found between the two groups at the end of the study (Table 2).

The decrease in hsCRP level after 24-week anthocyanin intervention was found to positively correlated with the change in LDL-cholesterol level (*r* = 0.286, *P* = 0.024). No such correlations were found in the placebo group. The intervention, however, did not cause significant correlations between the changes of HDL-cholesterol levels and the changes of hsCRP, sVCAM-1 and IL-1β levels.

Effects of anthocyanins on inflammatory factors in vitro

To examine the effects of anthocyanins on cytokine-induced CRP expression in vitro, HepG2 cells were stimulated with IL-6 (20 ng/mL) plus IL-1β (10 ng/mL) either alone, or in the presence of Dp-3g, Cy-3g or the anthocyanin mixture from 0.1 to 50 μg/mL for 24 h. Compared with control cells (DMSO pretreated cells, 1.46 ± 0.22 ng/mL), the basal CRP levels were significantly increased (9.24 ± 0.45 ng/mL) by stimulation with IL-6 and IL-1β (*P* < 0.001). As shown in Fig. 1, the addition of either the anthocyanin mixture, Dp-3g or Cy-3g, significantly decreased the production of CRP in HepG2 cells in a dose-dependent manner (all *P* < 0.001). Also, treatment with the anthocyanin mixture (50 μg/mL) from berries resulted

in a much stronger reduction of CRP than either Dp-3g or Cy-3g alone (50 μg/mL) (*P* < 0.05). The observed reductions in CRP were also far greater in cells treated with the anthocyanin mixture than with either Dp-3g or Cy-3g at 1 and 10 μg/mL (*P* < 0.05). Whereas, the placebo capsule had no inhibitory effects on cytokine-induced CRP secretion (data not shown).

To determine whether anthocyanins could inhibit VCAM-1 secretion in endothelial cells, we investigated their effects on PIECs. The basal VCAM-1 levels were significantly increased (7.61 ± 0.40 ng/mL) by stimulation with LPS (10 μg/mL), compared with control cells (DMSO pretreated cells, 1.62 ± 0.26 ng/mL). The treatment of anthocyanin mixture, Dp-3g or Cy-3g lowered LPS-induced VCAM-1 expression levels in PIECs in a dose-dependent manner (all *P* < 0.001) (Fig. 2). Meanwhile, treatment with the anthocyanin mixture (50 μg/mL) from berries resulted in a much stronger reduction of VCAM-1 than either Dp-3g or Cy-3g alone (50 μg/mL) (*P* < 0.05). It was noteworthy that the observed reductions in VCAM-1 were also far greater in cells treated with the anthocyanin mixture than with either Dp-3g or Cy-3g alone at 0.1, 1 and 10 μg/mL (*P* < 0.05). However, no such changes were observed in cells pretreated with the placebo capsule (data not shown).

Discussion

In our current study, supplementation with purified anthocyanins for 24 weeks reduced serum levels of hsCRP, sVCAM-1, IL-1β and LDL-cholesterol and increased the HDL-cholesterol levels in subjects with moderate hypercholesterolemia. Furthermore, the changes in the LDL-cholesterol level positively correlated with hsCRP change in the anthocyanin group. This is the first report that long-term supplementation with purified anthocyanins can inhibit the inflammatory response in hypercholesterolemic subjects. The addition of single anthocyanins (Dp-3g and Cy-3g) also decreased IL-6 plus IL-1β-induced CRP production in HepG2 cells and LPS-induced VCAM-1 secretion in endothelial

Table 2 Changes in the inflammatory cytokines of the participants at baseline and at weeks 12 and 24 of the trial.^a

	Placebo (n = 73)			Anthocyanin (n = 73)			P-value ^c
	Baseline	12 wk	24 wk	Mean change, % (95%CI) ^b	Baseline	12 wk	24 wk

hsCRP (mg/L)^d 2.26 (0.97–3.72) 2.23 (1.08–3.76) 2.19 (0.93–3.82) –2.5 (–7.0–2.1) 2.25 (1.06–4.25) 1.95 (0.92–2.84)^e 1.74 (0.86–2.60)^e –21.6 (–37.5 to –5.7)^f 0.001

sVCAM-1 (ng/mL) 544.2 ± 107.8 546.3 ± 106.9 547.6 ± 109.5 0.4 (–4.6–5.4) 542.9 ± 103.6 481.0 ± 91.8^e 478.7 ± 97.8^e –12.3 (–21.5 to –3.1)^f 0.005

TNF-α (pg/mL) 18.0 ± 6.0 19.1 ± 6.7 18.5 ± 5.4 2.8 (–3.4–9.1) 18.7 ± 6.4 17.9 ± 5.1 18.4 ± 5.6 –1.6 (–5.6–3.4) 0.673

IL-1β (pg/mL) 4.77 ± 1.71 4.23 ± 0.91 4.71 ± 1.60 –1.3 (–5.3–2.7) 5.18 ± 2.11 4.62 ± 1.20^e 4.51 ± 1.60^e –12.8 (–24.4 to –1.2)^f 0.019

^a hsCRP: high-sensitive C-reactive protein. sVCAM-1: soluble vascular adhesion molecule-1. TNF-α: tumor necrosis factor-α. The data, unless otherwise specified, were expressed as mean ± SD. No significant differences were found for any variable between the two groups at baseline via the unpaired Student's *t* test.

^b Calculated as (value at 24 wk – value at baseline)/value at baseline × 100.

^c The effects of the intervention on these variables were tested by repeated-measures MANCOVA with the BMI and lipid profile (including HDL- and LDL-cholesterol, triacylglycerol and total cholesterol) values as covariates.

^d Geometric mean; upper and lower quartiles in parentheses (all such values).

^e *P* < 0.05 vs baseline, assessed by paired Student's *t* tests.

^f *P* < 0.05 vs percentage changes in the placebo group, assessed by unpaired Student's *t* tests.

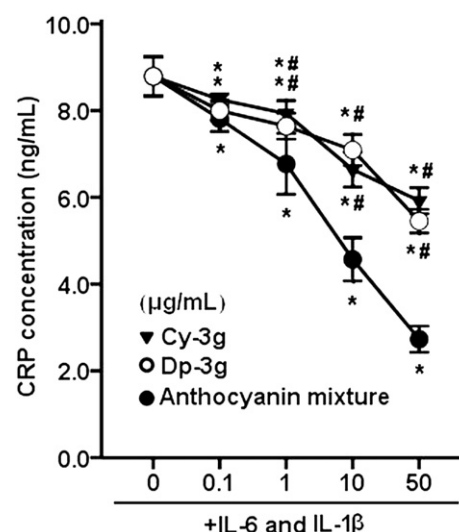


Figure 1 Effects of the anthocyanin mixture, Dp-3g and Cy-3g on CRP expression. HepG2 cells were pretreated for 2 h with varying levels of anthocyanin mixture, Dp-3g or Cy-3g and then stimulated for 24 h with IL-6 (20 ng/mL) and IL-1β (10 ng/mL). At the end of this treatment period, the conditioned media were collected and the CRP levels were measured by ELISA. Data are representative of three independent experiments and expressed as the means ± SE, **P* < 0.05 compared with stimulus treatment alone (0 μg/mL anthocyanins), #*P* < 0.01 compared with the anthocyanin mixture-treated group at the same level.

cells, respectively. In addition, a purified anthocyanin mixture from berries caused a greater reduction of inflammatory cytokines in vitro than single anthocyanin.

Our previous studies have demonstrated that anthocyanin supplementation for 8 weeks and 12 weeks improved lipid profile like increasing HDL-cholesterol level and decreasing LDL-cholesterol level [19,20]. The present study conducted for 24 weeks further confirmed the beneficial effect of anthocyanin on the lipid profile in subjects with dyslipidemia. Additionally, the change of LDL-cholesterol positively correlated with the hsCRP change after 24-week anthocyanin intervention. A recent meta-analysis of LDL-C-lowering intervention trials also showed a strong correlation between the change in LDL-cholesterol and change in CRP [21]. It has been reported that CRP can bind to native LDL [22] or modified forms of LDL such as oxidized LDL [23], which promote the uptake of these molecules by macrophages and may contribute to the development of the atherosclerotic lesion. We, therefore, conclude that the effect of anthocyanin on the improvement of lipid profile may in part contribute to the decrease of inflammation in humans with hypercholesterolemia.

It has been reported that the elevated CRP levels is one of the most actively studied inflammatory biomarkers of cardiovascular diseases [7,24,25]. Additionally, the levels of soluble adhesion molecules (e.g. VCAM-1), TNF-α and IL-1β have been regarded as useful biomarkers in assessing cardiovascular events in populations with various disease settings [6–9]. Our present study has revealed that the administration of anthocyanins in subjects with hypercholesterolemia significantly reduces their CRP, VCAM-1 and IL-

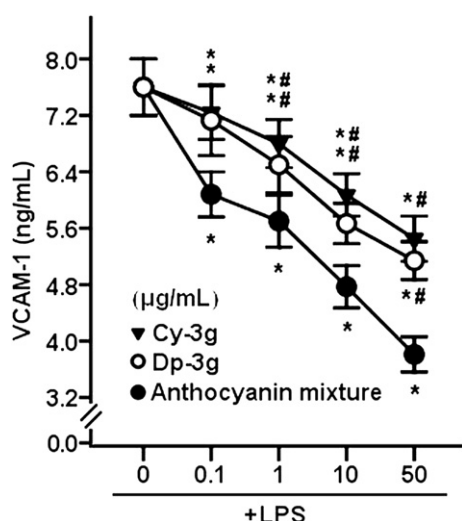


Figure 2 Effects of the anthocyanin mixture, Dp-3g and Cy-3g on VCAM-1 expression. PIEC cells were pretreated for 2 h with varying levels of anthocyanin mixture, Dp-3g or Cy-3g and then stimulated for 24 h with LPS (10 µg/mL). At the end of this treatment period, the conditioned media were collected and VCAM-1 levels were measured by ELISA. Data are representative of three independent experiments and expressed as the means \pm SE, * P < 0.05 compared with stimulus treatment alone (0 µg/mL anthocyanins), # P < 0.01 compared with the anthocyanin mixture-treated group at the same level.

1 β levels at 12 and 24 weeks after intervention. Consistent with our findings, a previous epidemiological study showed that dietary anthocyanin intake was associated with reduced risk of coronary heart disease (CHD) or cardiovascular disease (CVD) in a prospective postmenopausal women study [26]. Karlsen *et al.* reported that supplementation with anthocyanin-rich bilberry juice for 4 weeks resulted in significant decreases in plasma levels of CRP, IL-6, IL-15, and monokine induced by interferon-gamma in subjects with elevated levels of at least one risk factor for CVD [16].

However, our findings are inconsistent with two previous studies on healthy volunteers. Curtis *et al.* reported that there was no improvement of inflammatory biomarkers, including hsCRP, TNF- α and 'regulated upon activation, normal T cell expressed and secreted' (RANTES), following 12-wk consumption of anthocyanin extract (500 mg/d) from elderberry in healthy postmenopausal women [15]. Another study conducted by Karlsen *et al.* showed that 3-week intake of purified anthocyanins (300 mg/d), derived from bilberries and blackcurrants, decreased plasma IL-8 and regulated RANTES expressed and secreted by T cells upon activation, but did not result in significant alterations in CRP, TNF- α and IL-1 β levels in healthy adults [14]. It might be possible that the efficacy of anthocyanin on inflammatory responses depends on, to some extent, the studied populations and the duration of the intervention. It has been determined that elevated serum cholesterol is associated with a higher level of proinflammatory cytokines [27,28], thus making it much easier to detect the changes in inflammation caused by anthocyanin intervention. In the subjects with dyslipidemia in the present study, supplementation with anthocyanin

indeed reduced the inflammatory response, which may differ from studies with healthy individuals.

A growing number of evidence that consumption of anthocyanin-rich fruits and beverages, as well as anthocyanin extracts, is strongly associated with reduced risk of CVD and other chronic diseases. This has led to the hypothesis that specific anthocyanins may be responsible for the observed beneficial effects [15,16,19]. As a result, various pure anthocyanin compounds have been isolated and identified, and their potential health-promoting effects have been evaluated extensively, both in vitro and in vivo [17,29,30]. However, whether single anthocyanins, anthocyanin-rich foods or multiple purified anthocyanin extracts produced different effects remains incompletely understood. To elucidate this point, we employed HepG2 and endothelial cell cultures to investigate the individual, additive or synergetic effects of anthocyanin on CRP and VCAM-1 in vitro. In comparison with Dp-3g or Cy-3g alone at the same level, purified anthocyanin mixture (containing 17 anthocyanin compounds) from berries produced a stronger inhibitory effect on CRP production in HepG2 cells and VCAM-1 secretion in endothelial cells. This suggests that the various anthocyanins in the mixture may synergistically operate to inhibit the inflammatory response. Hence, incorporating plant-based foods rich in different anthocyanin compounds in the diet is likely to be more beneficial than consuming a single anthocyanin supplement. Further, these in vitro findings support our observations in human subjects that anthocyanins can remarkably regulate the levels of inflammatory cytokines in hypercholesterolemic subjects.

In conclusion, the supplementation of an anthocyanin mixture to hypercholesterolemic subjects for 24 weeks reduced serum levels of CRP, VCAM-1 and IL-1 β , which involved the improvement of the lipid profile. In addition, different anthocyanin compounds were found to have additive or synergistic effects in mediating anti-inflammatory responses in vitro cell culture assays.

Conflict of interest statement

Authors have no conflict of interest to be disclosed.

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Appendix A. Supplementary material

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.numecd.2012.06.005>.

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