Anthocyanin supplementation improves serum LDL- and HDL- cholesterol concentrations associated with the inhibitions of cholesteryl ester transfer protein in dyslipidemic subjects

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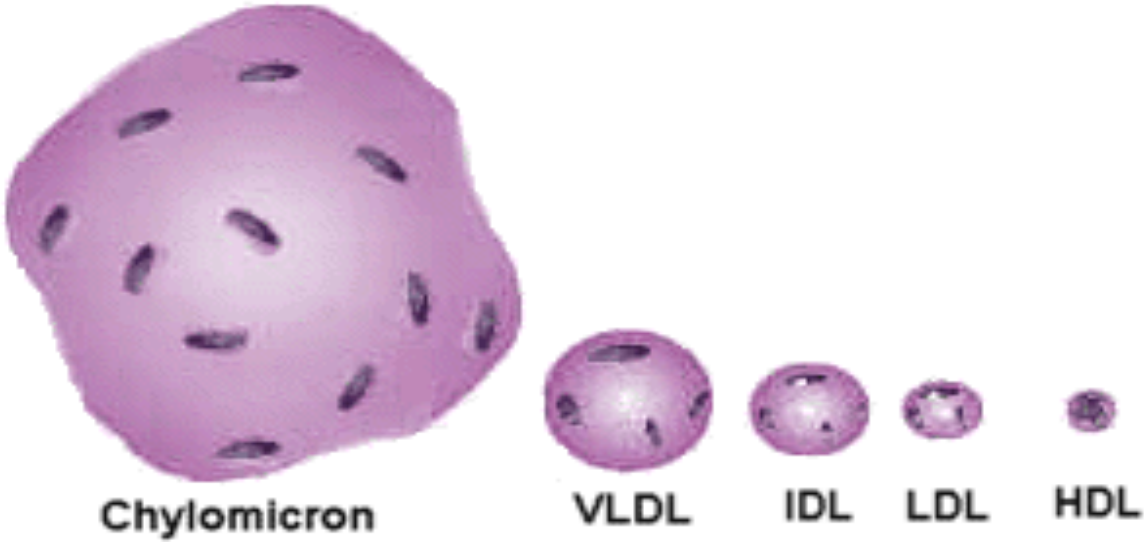
Itinerary

- Background
- Objectives
- Materials and Methods
- Results
- Conclusions
- Application to practice

Background

- Many intervention studies of CVD have focused on lowering LDL and triglyceride concentrations
- Studies show that raising HDL cholesterol is inversely related to the development of CVD
  - independent of serum LDL and triglyceride concentrations

Background



https://www.google.com/webhp?sourceid=chrome-instant&ion=1&espv=2&ie=UTF-8#q=cetp%20cholesterol

Background

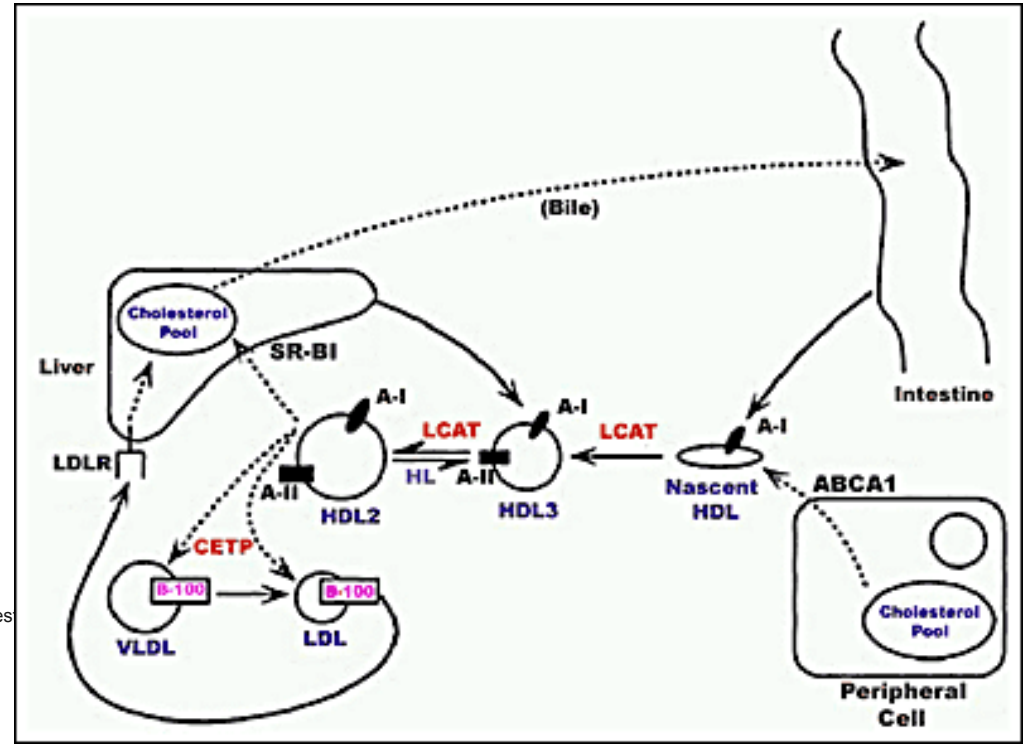
Functions of HDL:

- Promotes and facilitates reverse cholesterol transport (RCT)
  - cholesterol is effluxed from macrophages, foam cells, and atherosclerotic plaques and is delivered back to the liver and eliminated as bile salts or bleary cholesterol

Efficiency dependent on:	-Macrophage cholesterol efflux -Lecithin cholesterol transferase (LCAT) -Cholesteryl ester transfer protein (CETP) -Selective Uptake of cholesteryl esters from HDL to liver

Background

CETP



http://drugline.org/img/term/choles

Background

Functions of HDL:

https://www.youtube.com/watch?v=q0YiPqmsXRg

Background

- Dietary composition is considered the first approach in treating and controlling CVD
  - studies have shown phytochemicals help prevent CVD
- Anthocyanin-rich extracts from foods have effective antioxidant properties in vitro and in animal models in vivo

Background

In previous studies (Xia M et al. Ling WH et al.), supplementation of anthocyanin has resulted

In animals:

Increased	Serum HDL cholesterol, apolipoprotein A-1 (apo A-1)
Decreased	Serum triglyceride, Total Cholesterol, non-HDL cholesterol

In vitro: promotion of cholesterol efflux from macrophages

Objectives of the Study

1) To attempt to evaluate the effects of pure anthocyanins derived from berries on the serum lipid profiles in dyslipidemic patients
2) To use the human HepG2 cell line to investigate the mechanism by which cyanidin 3-*O*-B-glucosides (Cy-3-G) improve lipid profile

Materials and Methods-Subjects

- 120 dyslipidemic patients
  - 40-65 years old
  - From Guangzhou, China
  - Excluded if: History of CVD/chronic disease/medications to alter lipid metabolism
- Met at least two of the below:

Fasting total cholesterol concentration	Fasting triglyceride concentration	Fasting LDL cholesterol concentration	Fasting HDL cholesterol concentration
>200 mg/dL	>150 mg/dL	>100 mg/dL	<40 mg/dL

Materials and Methods-Design

- Randomized, double-blind, placebo-controlled trial
  - randomly assigned to anthocyanin group (n=60) or control group (n=60)
  - 12 weeks long
- Anthocyanin group- two 80 mg capsules 2x daily
- Control group- 2 placebo 2x daily
  - every 4 weeks compliance was assessed by recalling the remaining capsules and obtaining related information

Materials and Methods-Design

At baseline and every 4 weeks:

- Body weight, blood pressure, and waist and hip circumferences were measured
- Completed a 3-day food intake log
  - analyzed using CDGSS3.0 software
- A questionnaire regarding dietary habits
- Only at baseline and wk 12- blood sample collection (fasting the night before)

Materials and Methods

- The anthocyanin capsules contained 17 different natural purified anthocyanins from bilberry and black currant
- Total content 80 mg/capsule
  - anthocyanin capsules: pullulan, maltodextrin and citric acid
  - placebo: pullulan and maltodextrin

Materials and Methods-

biochemical assays

RBCs/WBCs/HgB	Blood Cell Analyzer
serum total protein conc	biuret method
serum albumin	dye binding method
aspartate/alanine transaminase	kinetic method
urea nitrogen/creatine	trinitrophenol method
total cholesterol and TAG conc	peroxidase-antiperoxidase method
HDL-C and LDL-C conc	clearance method
Apo A-1 and apo B conc glucose conc	immunoturbidimetry glucose oxidase method

Materials and Methods-

biochemical assays

- Cholesterol efflux from macrophages
  - after 24 hrs of incubation, activity calculated in the medium as a percentage
- Human HepG2 cells
  - seeded in 6 well plates, 24 hr incubation
  - re-incubated with different levels of Cy-3-G
  - analyzed for autofluorescence CETP levels

Methods-Statistical Analysis

Kolmogorov-Smirnov Test	Tested normal distributions in variables Expressed as mean±SD and 95% CI $P<0.05$
Student's *t* Test for independent samples	Differences in variables between both groups at baseline
One factor analysis of covariance	Compared difference in effects of the anthocyanins and placebo on blood markers with 12-week and baseline values
One factor analysis of covariance	Differences in mass and activity of CETP in HepG2 cell-conditioned media between doses of Cy-3-G
Tukey test	Differences in mass and activity of CETP in HepG2 cells conditioned media between both groups
Spearman's Rank Correlation Coefficients	Calculated changes in LDL cholesterol, HDL cholesterol, mass and activity of CETP, and cholesterol efflux to serum

Results-Anthropometric characteristics and dietary intake of the dyslipidemic patients

- No differences were observed in age or anthropometric markers (refer to table 1)
- Uniform distribution of age, sex and blood lipid profile
- 1 patient in each of two groups had T2DM
- 17 subjects in placebo and 18 in anthocyanin group had high blood pressure
- No significant differences in daily mean energy and nutrient intake or dietary intake between 2 groups(table 2)
- No adverse events from consuming anthocyanin or placebo capsules

TABLE 1
Anthropometric characteristics of the dyslipidemic patients at baseline and during the 12-wk intervention by group[1]

	Placebo (n = 60)				Anthocyanin (n = 60)				
	Baseline	4 wk	8 wk	12 wk	Baseline	4 wk	8 wk	12 wk	P^2
Weight (kg)	66.1 ± 10.1	66.5 ± 10.1	66.0 ± 10.0	66.4 ± 10.3	63.7 ± 9.7	65.0 ± 9.7	64.1 ± 9.7	63.8 ± 9.5	0.327
BMI (kg/m^2)	26.7 ± 4.0	27.4 ± 4.2	27.5 ± 4.1	26.8 ± 3.9	25.5 ± 3.1	26.2 ± 3.1	25.8 ± 3.0	25.5 ± 2.9	0.063
Waist circumference (cm)	89.7 ± 9.6	89.9 ± 10.2	89.8 ± 10.6	89.5 ± 10.8	87.1 ± 7.1	87.1 ± 7.1	86.8 ± 6.4	86.4 ± 7.2	0.159
Hip circumference (cm)	98.9 ± 6.4	99.4 ± 6.6	98.8 ± 6.8	98.5 ± 6.2	97.5 ± 6.7	97.8 ± 6.0	97.1 ± 6.0	97.5 ± 6.0	0.306
Waist-to-hip ratio	0.91 ± 0.06	0.88 ± 0.06	0.91 ± 0.06	0.91 ± 0.08	0.90 ± 0.06	0.89 ± 0.06	0.89 ± 0.05	0.88 ± 0.06	0.598
Systolic blood pressure (mm Hg)	129.1 ± 19.0	124.2 ± 17.0	123.7 ± 16.5	124.8 ± 17.0	126.5 ± 17.8	124.4 ± 17.3	121.7 ± 17.0	125.3 ± 20.0	0.888
Diastolic blood pressure (mm Hg)	82.4 ± 10.6	80.5 ± 9.4	77.7 ± 10.6	81.6 ± 10.6	82.7 ± 10.0	79.0 ± 9.9	79.8 ± 11.7	82.7 ± 11.1	0.343

TABLE 2
Mean daily intake of nutrients by the subjects at baseline and at 12 wk by group[1]

	Placebo (n = 60)		Anthocyanin (n = 60)		
	Baseline	12 wk	Baseline	12 wk	P^2
Energy (kcal/d)	2168 ± 98	2199 ± 133	2145 ± 95	2189 ± 111	0.726
Protein					
(g/d)	88.8 ± 5.1	87.0 ± 4.3	89.8 ± 6.0	86.9 ± 4.5	0.648
(% of energy)	17.5 ± 2.2	17.2 ± 3.2	17.5 ± 2.0	17.3 ± 3.0	0.846
Total carbohydrate					
(g/d)	269 ± 18	270 ± 22	272 ± 19	267 ± 21	0.098
(% of energy)	51.8 ± 1.4	51.4 ± 2.8	51.8 ± 1.4	51.6 ± 2.7	0.809
Total fat					
(g/d)	85.0 ± 2.9	83.0 ± 4.5	85.3 ± 3.2	82.6 ± 3.9	0.363
(% of energy)	31.9 ± 1.5	31.1 ± 1.6	31.7 ± 1.4	31.2 ± 1.6	0.516
Cholesterol (mg/d)	325 ± 17	326 ± 19	327 ± 21	325 ± 21	0.295
Fiber (g/d)	22.0 ± 22.3	22.3 ± 2.4	22.0 ± 2.1	22.1 ± 2.1	0.669

Results-Compliance

- All subjects completed the study
- Rates of capsule intake:
  - 99.2% in placebo group
  - 99.4% in anthocyanin group

Results-Effects of the consumption of anthocyanin on hematologic measures and liver enzyme markers

- Anthocyanin consumption had no significant effects on (Table 3):
  - red and white blood cell counts
  - hemoglobin
  - total protein
  - albumin
  - urea nitrogen
  - creatine concentrations
  - aspartate and alanine transaminases

TABLE 3

Blood chemistry results, hematologic measures, and liver enzyme values for the dyslipidemic patients at baseline and at 12 wk by group

	Placebo (n = 60)		Anthocyanin (n = 60)		
	Baseline	12 wk	Baseline	12 wk	P^1
Red blood cell ($\times 10^{12}$/L)	4.39 ± 0.42[2]	4.49 ± 0.37	4.27 ± 0.40	4.39 ± 0.31	0.657
White blood cell ($\times 10^{9}$/L)	5.59 ± 0.96	5.75 ± 1.25	5.31 ± 0.89	5.48 ± 0.94	0.860
Hemoglobin (g/L)	130.7 (123.0, 137.0)[3]	137.2 (130.3, 143.0)	129.1 (122.0, 137.8)	137.1 (129.3, 145.0)	0.299
Total protein (g/L)	75.7 ± 2.5	74.9 ± 3.8	74.9 ± 3.3	74.1 ± 3.1	0.115
Albumin (g/L)	43.7 ± 2.1	43.7 ± 2.0	44.0 ± 2.0	43.9 ± 2.0	0.819
Urea nitrogen (mmol/L)	5.18 ± 1.03	4.90 ± 0.85	5.41 ± 0.86	5.15 ± 0.84	0.347
Creatinine (μmol/L)	74.9 (68.8, 79.6)	72.8 (67.0, 80.6)	74.8 (66.3, 84.7)	74.6 (66.8, 83.1)	0.095
Alanine transaminase (U/L)	19.4 (15.0, 26.0)	18.8 (15.0, 26.0)	17.5 (13.0, 23.0)	17.1 (13.0, 22.0)	0.724
Aspartate transaminase (U/L)	20.4 (16.0, 25.0)	19.8 (16.0, 25.0)	20.3 (16.3, 25.0)	18.7 (16.0, 21.8)	0.167

Results-Effects of anthocyanin consumption on fasting serum lipid profile and glucose concentrations

- Serum HDL concentration levels after intervention
  - anthocyanin group: 13.7% increase
  - placebo group: 2.8% increase (P<.001)
- Serum LDL concentration levels after intervention
  - anthocyanin group: 13.6% decrease
  - placebo group:0.6% increase
  - significantly different change (P<0.001) (Table 4)

Results-Effects of anthocyanin on plasma CETP and LCAT

Table 4

- CETP mass
  - anthocyanin group: decreased by 10.4%
  - placebo group: increased by 3.5%
- change in mass significantly different ($p<.001$)
- CETP activity:
  - anthocyanin group: decreased by 6.3% ($p<.001$)
  - placebo group: decreased by 1.1%
- no significant difference in mass and activity of LCAT

TABLE 4
Blood lipid profile and lipid metabolic enzymes at baseline and after consumption of anthocyanins or placebo for 12 wk[1]

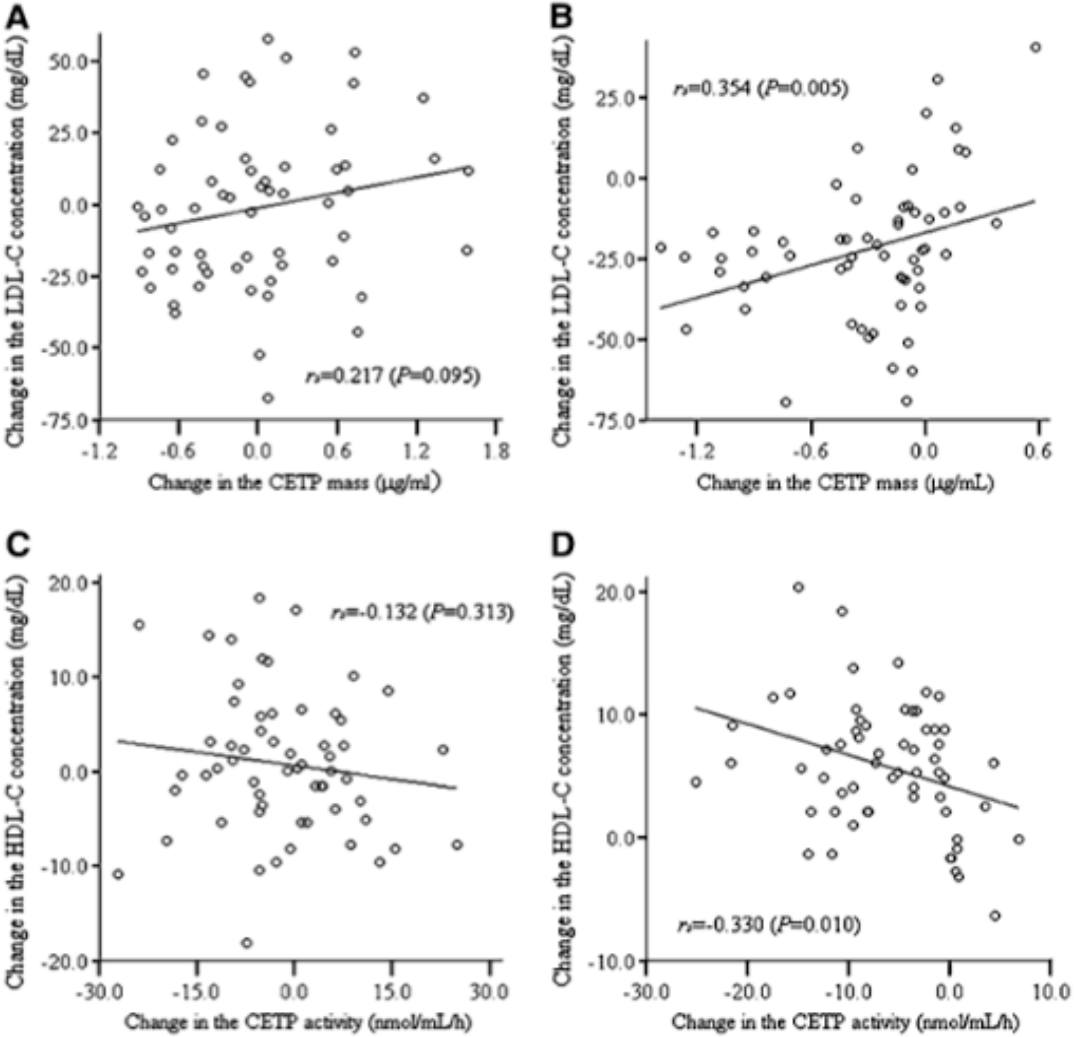
	Placebo (n = 60)			Anthocyanins (n = 60)			
	Baseline	12 wk	Change[2]	Baseline	12 wk	Change	P[3]
			%			%	
Total cholesterol (mg/dL)	224.3 ± 36.4[4]	222.4 ± 39.8	−0.4 (−3.6, 2.7)[5]	226.2 ± 35.5	220.5 ± 34.0	−2.1 (−4.5, 0.3)	0.435
Triacylglycerol (mg/dL)	205.8 ± 83.0	200.4 ± 91.2	2.0 (−7.0, 11.1)	197.9 ± 87.0	189.5 ± 85.6	−0.4 (−8.2, 7.4)	0.576
LDL cholesterol (mg/dL)	158.5 ± 37.8	157.3 ± 36.6	0.6 (−4.1, 5.2)	159.2 ± 34.4	139.9 ± 35.5	−13.6 (−17.1, −10.1)	<0.001
HDL cholesterol (mg/dL)	46.1 ± 9.6	46.9 ± 10.1	2.8 (−1.6, 7.2)	45.9 ± 8.5	51.2 ± 8.7	13.7 (10.4, 16.9)	<0.001
Apolipoprotein A-I (mg/dL)	124.9 ± 19.5	126.3 ± 18.7	1.7 (−0.7, 4.0)	125.7 ± 17.4	126.5 ± 15.6	1.4 (−1.5, 4.3)	0.842
Apolipoprotein B (mg/dL)	111.9 ± 24.0	114.1 ± 23.8	3.5 (−1.0, 8.0)	110.8 ± 21.8	112.5 ± 19.5	2.8 (−0.6, 6.3)	0.773
Glucose (mmol/L)	5.68 ± 1.68	5.79 ± 2.45	1.8 (−3.2, 6.8)	5.64 ± 1.42	5.59 ± 1.58	−0.8 (−3.3, 1.6)	0.458
LCAT mass (μg/mL)	8.31 ± 2.42	8.65 ± 2.36	9.4 (−0.1, 18.9)	8.66 ± 2.29	8.94 ± 2.29	8.8 (−2.4, 20.0)	0.773
LCAT activity (nmol · L^{-1} · h^{-1})	79.5 ± 7.3	79.4 ± 6.6	0.2 (−1.8, 2.1)	80.5 ± 9.1	80.6 ± 7.6	0.6 (−1.2, 2.5)	0.440
CETP mass (μg/mL)	2.52 ± 0.60	2.52 ± 0.58	3.5 (−3.6, 10.5)	2.55 ± 0.67	2.23 ± 0.50	−10.4 (−14.1, −6.7)	<0.001
CETP activity (nmol · L^{-1} · h^{-1})	96.9 ± 9.4	95.3 ± 10.0	−1.1 (−4.0, 1.6)	96.7 ± 9.9	90.3 ± 9.4	−6.3 (−8.0, −4.6)	0.001

Results-correlation between changes in serum HDL- or LDL-cholesterol concentrations and the mass and activity of plasma CETP

Figure 1

- change in HDL-C concentration was negatively correlated with change in CETP activity (rs=-.330, P=.010)
- change in LDL-C concentration was positively correlated with change in CETP mass (rs=.354, P=.005)
- no correlation between these changes in placebo group

Figure 1


A
Change in the LDL-C concentration (mg/dL)
50.0
25.0
0.0
-25.0
-50.0
-75.0
-1.2
-0.6
0.0
0.6
1.2
1.8
Change in the CETP mass (μg/ml)
r_s=0.217 (P=0.095)
B
Change in the LDL-C concentration (mg/dL)
25.0
0.0
-25.0
-50.0
-75.0
-1.2
-0.6
0.0
0.6
Change in the CETP mass (μg/mL)
r_s=0.354 (P=0.005)
C
Change in the HDL-C concentration (mg/dL)
20.0
10.0
0.0
-10.0
-20.0
-30.0
-15.0
0.0
15.0
30.0
Change in the CETP activity (nmol/mL/h)
r_s=-0.132 (P=0.313)
D
Change in the HDL-C concentration (mg/dL)
20.0
10.0
0.0
-10.0
-30.0
-20.0
-10.0
0.0
10.0
Change in the CETP activity (nmol/mL/h)
r_s=-0.330 (P=0.010)

Results-Effects of anthocyanin consumption on cellular cholesterol efflux into serum

- No differences in baseline cholesterol efflux capacity between each group
- Post intervention cholesterol efflux capacity
  - anthocyanin group: 20.0% increase
  - placebo group: 0.2% increase

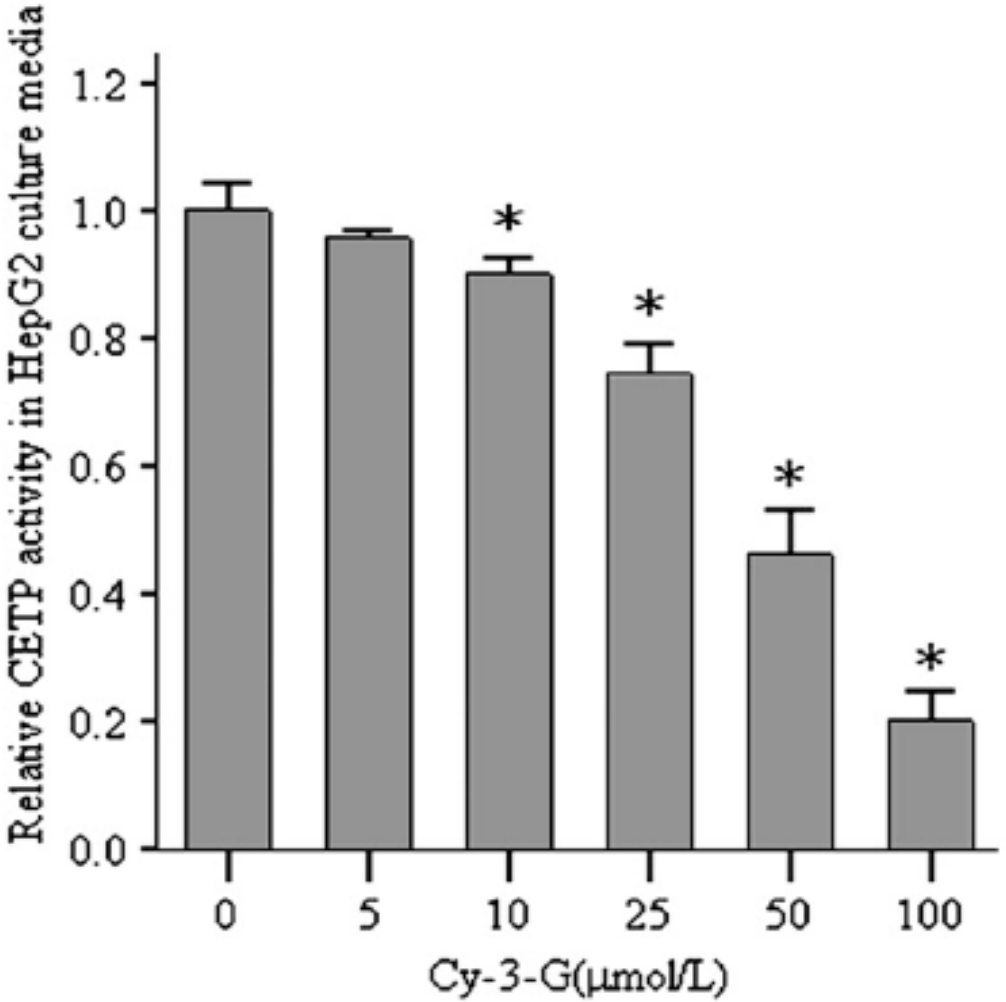
Results-Correlation between changes in cellular cholesterol efflux and serum HDL cholesterol

- Anthocyanin group:
  - positive correlation between change in cholesterol efflux capacity from baseline to 12 wks and change in serum HDL-C concentration (rs=.485, P=.008)
- Placebo group:
  - no correlation between previous two measures
- no correlation between change in cholesterol efflux capacity and change in other lipids in each study

Results- Effects of Cy-3-G on the mass and activity of CETP in HepG2 cells

- Cy-3-G inhibited the activity of CETP in an in-vitro dose-dependent action in HepG2 cells
- No significant CETP mass change after Cy-3-G treatment (Figure 2)

Figure 2


Relative CETP activity in HepG2 culture media
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
*
*
0
5
10
25
50
100
Cy-3-G(μmol/L)

Conclusions

- Anthocyanins are beneficial in both lowering LDL and raising HDL
- Anthocyanins may be considered CETP inhibitors
- Cy-3-G decreased CETP activity in human HepG2 culture media

Conclusions

- In a similar study, Chen et al. found that apple polyphenols lowered CETP and non-HDL and raised HDL in hamsters

Lam CK, Zhang Z, Yu H, Tsang SY, Huang Y, Chen ZY. Apple Polyphenols Inhibit Plasma CETP Activity and Reduce the Ratio of Non-HDL to HDL Cholesterol. *Mol Nutr Food Res* 2008;52:950-8.

Table 3. Changes in serum TC, total TG, HDL-C, non-HDL-C, and non-HDL-C/HDL-C in hamsters fed the control diet, and two experimental diets supplemented with 0.3AP and 0.6AP

	Control	0.3AP	0.6AP
Week 0			
TC	212.4 ± 28.8	219.9 ± 42.0	213.7 ± 36.5
HDL-C	105.7 ± 22.8	101.8 ± 12.2	101.1 ± 11.2
Non-HDL-C	101.1 ± 20.8	112.8 ± 29.1	112.6 ± 35.1
Non-HDL-C/HDL-C	1.01 ± 0.37	1.10 ± 0.21	1.12 ± 0.35
Triglycerides	108.3 ± 40.4	105.7 ± 58.4	102.6 ± 45.6
Week 6			
TC	199 ± 28	197 ± 26	184 ± 12
HDL-C	109 ± 18[a]	125 ± 15[b]	127 ± 13[b]
Non-HDL-C	90 ± 13[a]	72 ± 15[b]	57 ± 11[c]
Non-HDL-C/HDL-C	0.84 ± 0.12[a]	0.58 ± 0.10[b]	0.46 ± 0.11[c]
Triglycerides	144 ± 31[a]	142 ± 45[a]	98 ± 28[b]

Values are expressed as mean ± SD, $n = 13$.
a–c) Means at the same row with different superscripts differ significantly at $p < 0.05$.

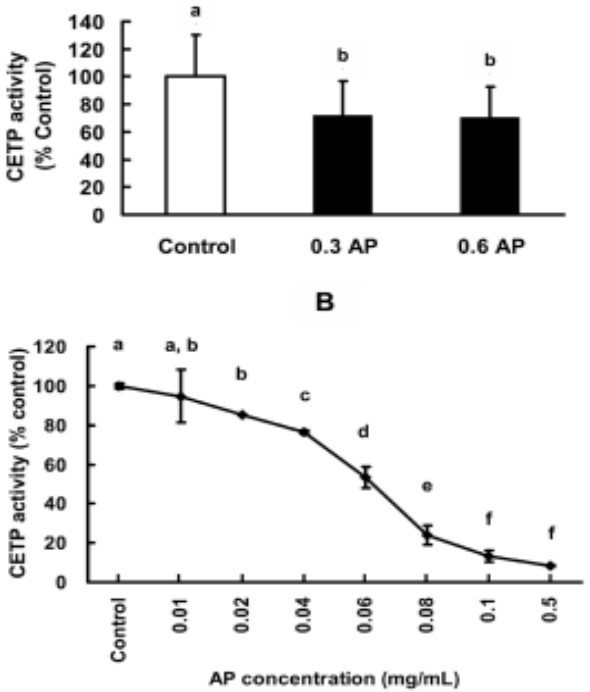


Figure 3. (A) Plasma CETP activity in the hamsters fed the control and AP-supplemented diets. Data were normalized so that CETP activity of the control group was regarded as 100%. Values were expressed as means ± SD ($n = 13$). Means with different superscript letters (a, b) differed significantly at $p < 0.05$. (B) Concentration–response curves of AP on *in vitro* CETP activity. Values were expressed as means ± SD ($n = 3$). Values with different letters (a–f) were significantly different from that of the control at $p < 0.05$.

Lam CK, Zhang Z, Yu H, Tsang SY, Huang Y, Chen ZY. Apple polyphenols inhibit plasma CETP activity and reduce the ration of non-HDL to HDL cholesterol. Mol Nutr Food Res 2008;52:950-8.

Limitations

- no detection of anthocyanins or their metabolites in blood or urine samples
- Cy-3-G treatment on CETP activity conducted in-vitro, not in humans

Application to Practice

- According to Brown BG et al. CVD events are reduced by about 1% for each 1% reduction in LDL and by ≥ 1% for each 1% increase in HDL
- 13.6% decrease in LDL and 13.7% increase in HDL in this study would result in 27.3% reduction in CVD

Discussion Questions

- According to this study what would you recommend a patient with dyslipidemia add to his or her diet?

References

Lam CK, Zhang Z, Yu H, Tsang SY, Huang Y, Chen ZY. Apple Polyphenols Inhibit Plasma CETP Activity and Reduce the Ratio of Non-HDL to HDL Cholesterol. *Mol Nutr Food Res* 2008;52:950-8.

Qin Y, Xia M, Ma J, Hao Y, Liu J, Mou J, Cao L, Ling W. Anthocyanin Supplementation Improves Serum LDL- and HDL-cholesterol Concentrations Associated with the Inhibition of Cholesterol Ester Transfer Protein in Dyslipidemic Subjects. *Am J Clin Nutr.* 2009;90:485-92.

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Ling WH, Cheng QX, Ma J, Want T. Red and black rice decrease atehroslcertoic plaque formation and increase antioxidant status in rabbits. *Journal of Nutrition*. 2001;131:1421-6.

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