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PANEL:
**Natural Colors: Overcoming
 Technical Challenges &
 Consumer Perceptions**

UNDERWRITTEN BY

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Anthocyanins – More than Nature's Colors!

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DISCLOSURES

- Think Healthy Group
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- Journal of the American College of Nutrition – Deputy Editor

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PRESENTATION OUTLINE

- What are anthocyanins?
- Utility as an alternative to synthetic colors
- Estimated population intakes
- Tissue distribution using animal models
- Bioavailability issues
- Effects on cardiovascular disease
- Future research



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What are Anthocyanins?

- The orange-red to blue-violet pigments in many plants (anthos = flower; kyanos = blue).



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What are Anthocyanins?

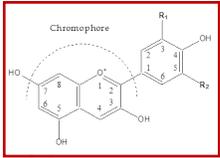
- Secondary plant metabolites produced via the phenylpropanoid pathway as a response to the environment.
 - UV light
 - Frost
 - Soil acidity
 - Pollination attractants
 - Deter insects (e.g. purple potatoes)



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What are Anthocyanins?

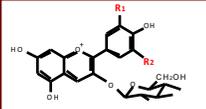
- Members of the flavonoid family
 - (C6-C3-C6) skeleton
- Their **positive charge** allows them to absorb light in the visible region.



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What are Anthocyanins?

- > 700 anthocyanins identified
 - 96% are glycosylated
 - Approximately 50% are acylated
- 27 anthocyanidins (aglycons)
- R_1 and R_2 conjugations shift the λ_{max} .
- Acylation also shifts the λ_{max} but results in a more stable compound.

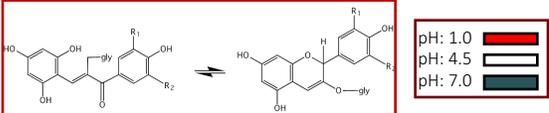


Aglycon	R ₁	R ₂	λ_{max}
Pelargonidin	H	H	494 nm
Cyanidin	OH	H	506 nm
Peonidin	O-CH ₃	H	506 nm
Delphinidin	OH	OH	508 nm
Petunidin	O-CH ₃	OH	508 nm
Malvidin	O-CH ₃	O-CH ₃	510 nm

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Alternative to Synthetic Colors

- Anthocyanins are stable at **low pH environments**. They are very unstable at neutral to higher pH ranges. Thus their use in food products has been thought to be somewhat limited.



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Alternative to Synthetic Colors

- Interaction with proteins/fat in yogurt may help them achieve higher stability in foods.

J Food Sci. 2010; 73(4):C238.

Estimated Population Intakes

- Average intake of anthocyanins by U.S. adults has been estimated to be **11.6 mg/d**. Approximately **31%** of adults have zero intake.
- Top sources in the U.S. diet include:
 - Berries
 - Wine
 - Grapes
 - Red/purple vegetables
 - 100% juice, non-citrus
 - Yogurt

POPULATION	MEET FRUIT RECOMMENDATIONS		MEET VEGETABLE RECOMMENDATIONS		MEET FRUIT AND VEGETABLE RECOMMENDATIONS	
	N	PERCENT	N	PERCENT	N	PERCENT
M 19-44Y	309	15	293	18	63	3
F 19-44Y	266	15	255	17	59	3
M 45-64Y	209	20	297	26	64	6
F 45-64Y	346	27	326	28	122	9
M 65+Y	241	23	222	23	67	7
F 65+Y	369	36	278	27	126	12
ALL ADULTS	1740	21	1631	22	501	6

J Nutr. 2015; 145:1239

Estimated Population Intakes

- Adults who meet fruit and vegetable recommendations set by the 2010 Dietary Guidelines consume about **34.4 to 36.0 mg/d** of anthocyanins.

AGE GROUP (YEARS)	Men (%)	Women (%)
19-44	85%	90%
45-64	85%	90%
65+	85%	90%

J Acad Nutr Diet. 2012; 112(2):222.

Tissue Distribution

- Stomach is a major absorption site.
- Anthocyanins tightly bind to bilirubin and other proteins.
- 7.5% entered intestinal tissue.
- Anthocyanins, their metabolites and/or breakdown products cross the **blood-brain barrier** and have been also identified in various animal tissues *in vivo*.

J Agric Food Chem. 2009; 57:3141.

Bioavailability Issues

- Evidence from *in vitro* gastric and microbial fermentation studies suggest that after ingestion anthocyanins are broken down either **spontaneously** or **enzymatically** into phenolic degradation products, which may be then further metabolized.
- Anthocyanins do appear in circulation intact or as glucuronated, sulfoconjugated or methylated derivatives.

Nutr Cancer. 2006; 54(1):3.

Bioavailability Issues

- It has been previously thought that only about **>0.1%** of anthocyanins cross the basolateral membrane and enter circulation.
- A new ¹³C-tracer study indicates that cy-3-glu has a minimal relative bioavailability of 12.38%.

Serum Metabolites	C _{max} (µmol/L)	T _{max} (h)
Cy-3-glu	0.14 ± 0.05	1.81 ± 0.16
PCA + PGA*	0.72 ± 0.23	6.06 ± 0.75
Phase II Conjugates of PCA	2.35 ± 0.15	13.44 ± 2.46
Ferulic acid	0.94 ± 0.37	11.29 ± 4.23
Hippuric acid	1.96 ± 1.39	15.69 ± 4.07

*PCA, Protocatechuic acid; PGA, Phloroglucinolaldehyde.

AJCN. 2013; 97:995.

CVD – Observational Data

- Framingham Heart Study Offspring Cohort** (AJCN. 2015; 102(1):172.)
 - Anthocyanin intake (Q1 vs. Q5) ↓ inflammation score, markers of inflammation, and insulin levels.
- Nurses Health Study and Health Professionals Follow Up Study** (AJCN. 2011; 93(2):336.)
 - Highest quintile of anthocyanin intake showed **8% reduced risk of hypertension.**
- Iowa Women's Study** (AJCN 2007;85:895.)
 - Intake of 0.2 mg/d and ↓ CVD mortality.

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CVD - Systematic Review of RCTs

nutrients MDPI

Systematic Review of Anthocyanins and Markers of Cardiovascular Disease

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Abstract: Anthocyanins are dietary flavonoids commonly consumed in the diet, which have been suggested to have a preventative effect on cardiovascular disease (CVD) development among cross-sectional studies. We systematically reviewed randomized controlled trials (RCTs) testing the effects of purified anthocyanins and anthocyanin-rich extracts on markers of CVD (lipoproteins, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and blood pressure) in both healthy and diseased populations. Eligible studies included RCTs of adults published in English. We searched PubMed, Web of Science Core Collection, and BIOSIS Previews for relevant articles from inception until 1 July 2015. Twelve RCTs representing 10 studies were included in this review. Supplementation with anthocyanins significantly improved LDL cholesterol among diseased individuals or those with elevated biomarkers. Supplementation did not significantly affect other markers of CVD in either healthy individuals or those with elevated markers. No adverse effects of anthocyanins were reported across studies at levels up to 160 mg/day. Limitations of trials in this systematic analysis include short trial duration and large variability in the dose administered within the trials. Longer duration trials assessing these responses are needed to adequately determine whether an effect of supplementation exists.

Keywords: anthocyanins; cardiovascular disease; LDL cholesterol

Nutrients. 2016; 8:32.

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CVD - Systematic Review of RCTs

- 10 included RCTs** (12 manuscripts) testing the effects of purified anthocyanins and/or anthocyanin-rich extracts on markers of cardiovascular disease.
- Consistent **decrease in LDL** among unhealthy individuals or those with elevated markers.
- No significant effects lipid profiles, triglycerides and blood pressure in healthy individuals; however, **trends of improvement among subjects in the treatment group** were noted.

Nutrients. 2016; 8:32.

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3 High Quality RCTs

- Inhibition of cholesterol ester transfer protein in dyslipidemic subjects.**

TABLE 4
Blood lipid profile and lipid metabolic enzymes at baseline and after consumption of anthocyanins or placebo for 12 wk¹

	Placebo (n = 60)			Anthocyanins (n = 60)			p ²
	Baseline	12 wk	Change ³	Baseline	12 wk	Change ³	
Total cholesterol (mg/dL)	224.3 ± 36.4 ^a	222.4 ± 39.8	-0.4 (-3.6, 27) ⁴	226.2 ± 35.5	220.5 ± 34.0	-2.1 (-4.5, 0.3)	0.435
Triglyceride (mg/dL)	205.8 ± 83.0	200.4 ± 91.2	-2.0 (-7.0, 11.1)	197.0 ± 87.0	189.5 ± 85.6	-4.6 (-10.4, 1.2)	0.570
LDL cholesterol (mg/dL)	158.5 ± 37.8	157.3 ± 36.6	0.6 (-4.1, 5.2)	159.2 ± 34.4	139.9 ± 33.3	-15.6 (-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 ± 8.1	15.7 (10.4, 16.9)	<0.001
Apolipoprotein A1 (mg/dL)	124.9 ± 19.5	126.3 ± 18.7	1.7 (-0.3, 4.0)	125.7 ± 17.4	125.5 ± 15.6	-0.2 (-1.9, 1.5)	0.942
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 (mg/mL)	8.31 ± 2.42	8.65 ± 2.26	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 ± 0.65	-10.4 (-14.1, -6.7)	<0.001
CETP activity (nmol · L ⁻¹ · h ⁻¹)	96.8 ± 9.4	95.3 ± 10.0	-1.1 (-4.0, 1.6)	96.7 ± 9.9	90.3 ± 9.1	-6.3 (-8.8, -4.6)	<0.001

AJCN. 2009; 90:485.

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3 High Quality RCTs

- Whortleberry anthocyanins (45 mg/d) significantly **decreased total cholesterol, LDL, and triglycerides** over 4-wk among hyperlipidemic individuals.
 - No significant effects on HDL
 - Baseline status was in higher range (~46 mg/dL)



Evid Based Complement Alternat Med. 2014. Article 217451.

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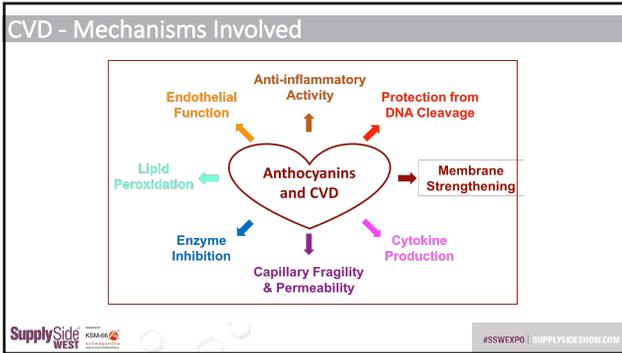
3 High Quality RCTs

- Elderberry extract anthocyanins (500 mg/d) **did not affect plasma levels of inflammatory biomarkers, vascular reactivity, plasma lipid profiles, or glucose concentrations** in an RCT of 57 individuals over a 12-week period.
 - Healthy population (markers in normal range)
 - Threshold effect?



J Nutr. 2009; 139:2266.

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Conclusion

- Although research is emerging, the current literature collectively supports that consumption of anthocyanin-rich diets may help to maintain a healthy vascular system (non-US populations).
- We need better population exposure data such and more data on the actual amounts of anthocyanins in branded products.
- As research continues to expand we need to consider how to appropriately translate findings into dietary guidance and public policy.

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Final Thoughts

- **Inflammation** is a part of the immune response network that is essential, but can be damaging when sustained!
- Can anthocyanins, their metabolites and/or breakdown products **accumulate** in certain tissues over time?
- What **amount** of anthocyanins are needed to achieve optimal vascular health?
 - Dose response human intervention studies.

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Future Research

- Continue to use the USDA Flavonoid Database and NHANES dietary recall information to better understand population intakes of anthocyanins.
- Small cross-over RCT of purified anthocyanins.
 - Could be accomplished alongside a GRAS safety study.



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Thank You!

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