TEXAS A&M UNIVERSITY
NUTRITION & FOOD SCIENCE

"PREVENTION OF OBESITY RELATED DISEASES THROUGH CHERRY CONSUMPTION: *WHAT WE CAN SAY SO FAR?*"

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Outline

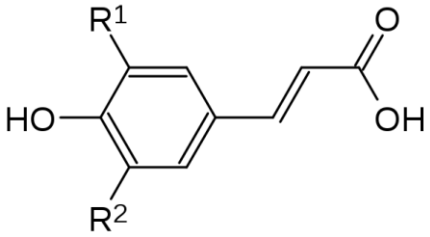
Cherries for prevention and treatment of diseases associated with obesity

1. Intestinal health
2. Prevention and treatment of ***diabetes and non-alcoholic liver disease***
3. Prevention and treatment of ***breast cancer***

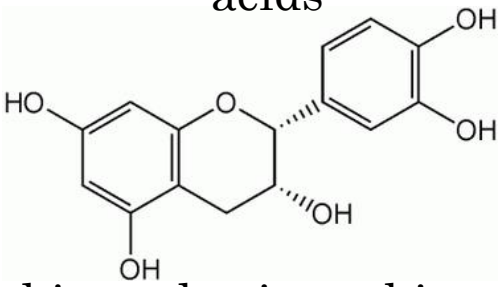
1. CHERRIES for INTESTINAL HEALTH

HYPOTHESIS

Bioactive compounds in cherries



Hydroxycinnamic acids



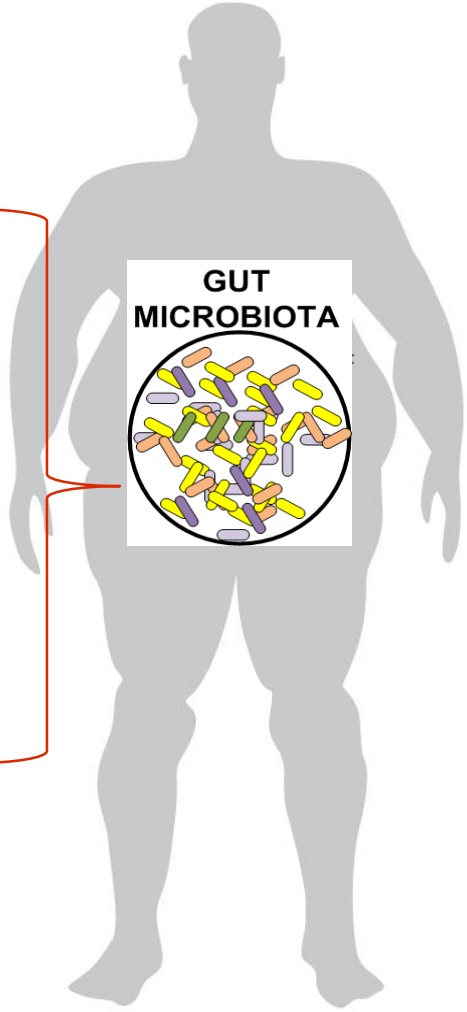
Catechin and epicatechin: building blocks of *proanthocyanins*

Obese

Polyphenolic compounds with low availability:

Dietary Fiber

- Soluble fiber
- Insoluble fiber



EXPERIMENTAL APPROACH

Obese diabetic (db/db)/mice

Lean mice

Diet supplemented with cherry powder (10%)

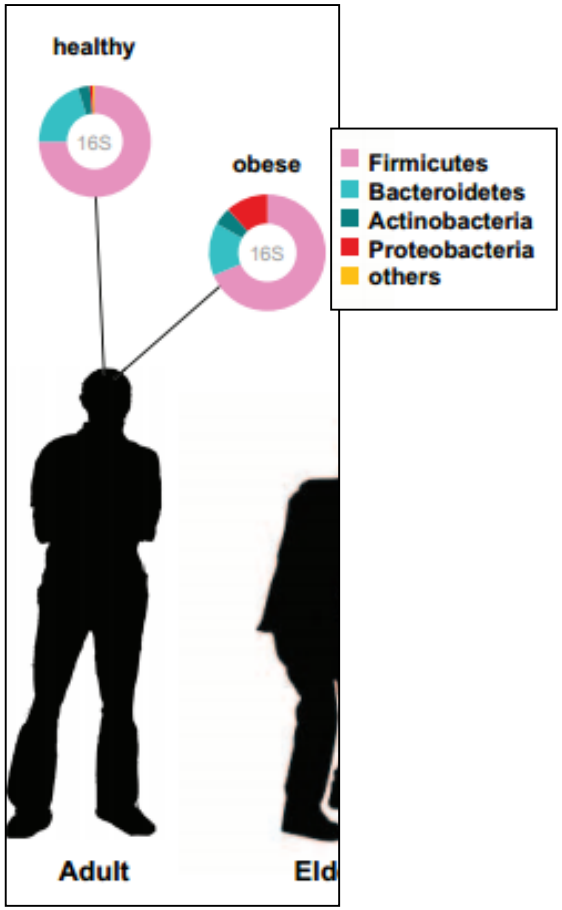
Control isocaloric diet

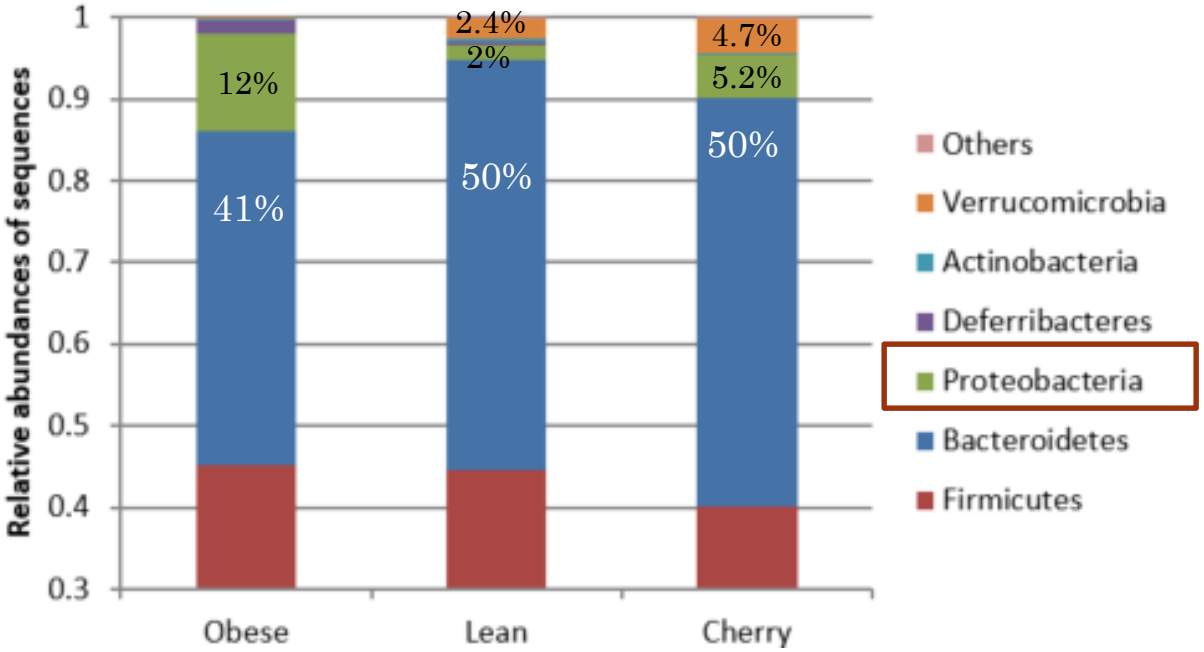
Colon microbiota

Microbiota metabolites

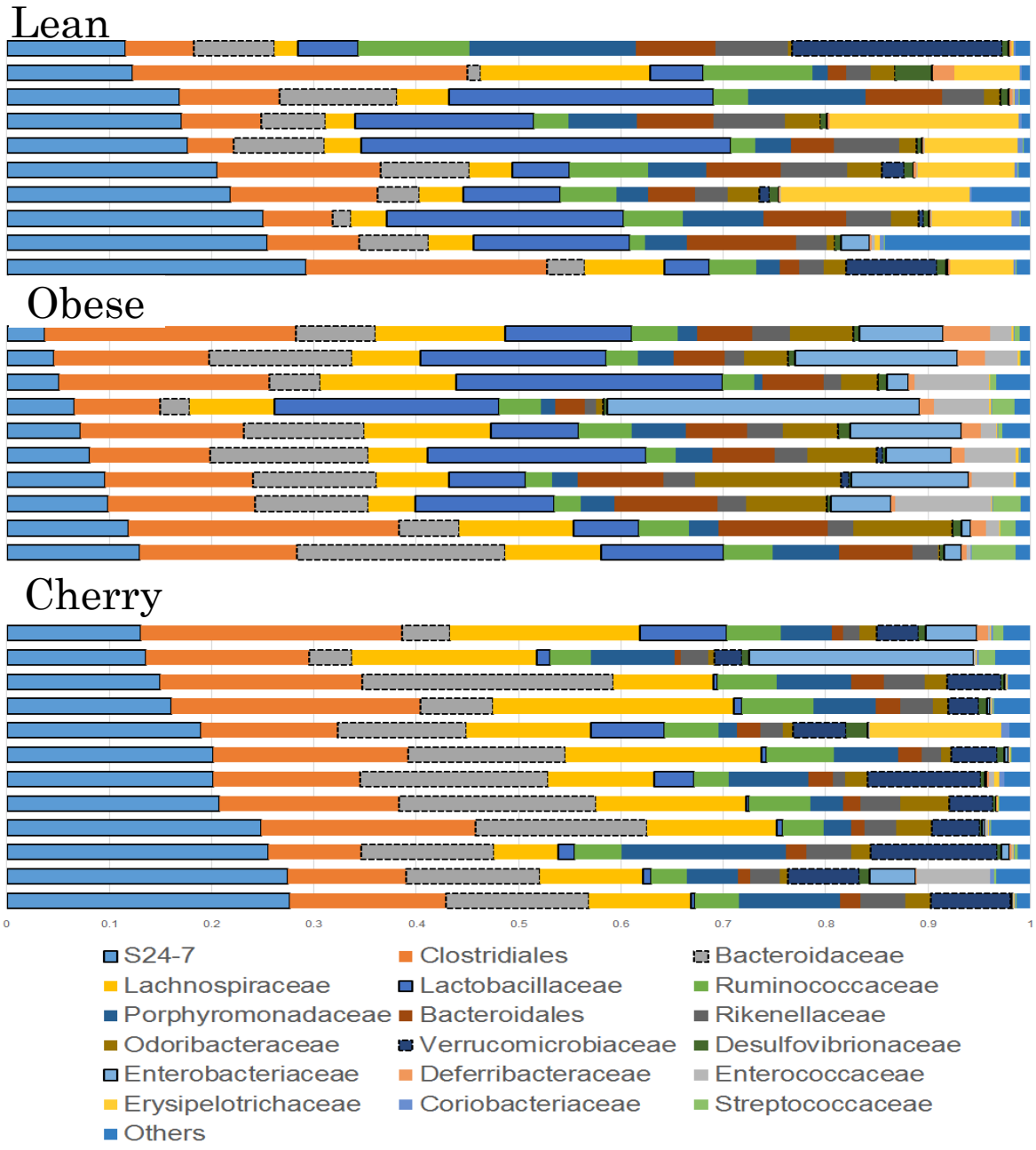
Biomarkers of intestinal health

RESULTS: CHERRY CONSUMPTION MODIFIED FECAL MICROBIOTA RELATIVE ABUNDANCES



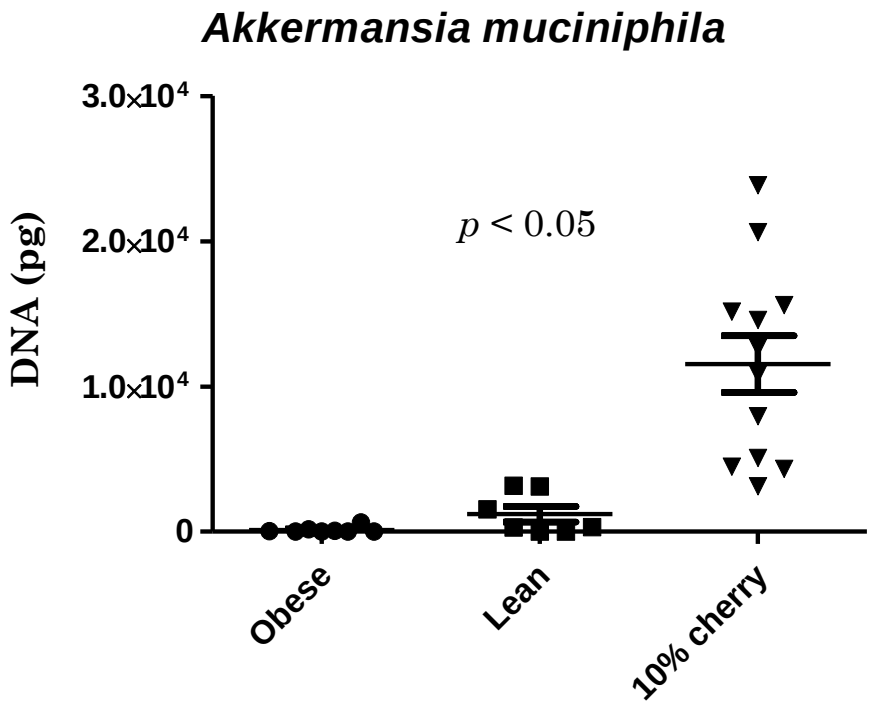


Results: *Cherry supplementation changed colonic microbiota abundances at the family level*



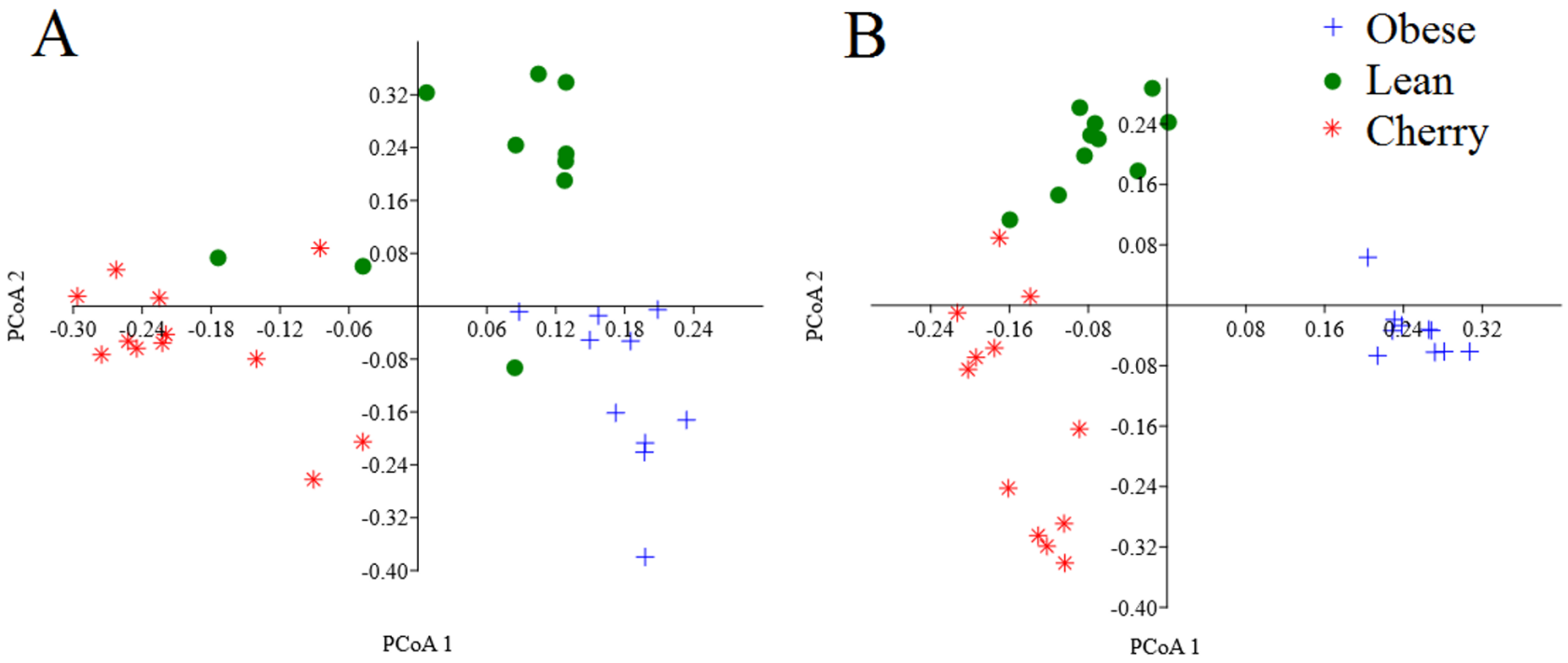
Bar plots showing relative abundance (percentages, x axis) of the most abundant bacterial taxa at the family level.

VERRUCOMICROBIA



- Believed to have anti-inflammatory effects in humans
- Inverse relationships between colonization and inflammatory conditions
- <u>May be used to combat obesity and type 2 diabetes</u>

Microbial communities clustered according to treatment



PCoA plots of weighted (A) and unweighted (B) UniFrac distance matrices.

RELEVANCE

GUT MICROBIOTA

Bacteria metabolites

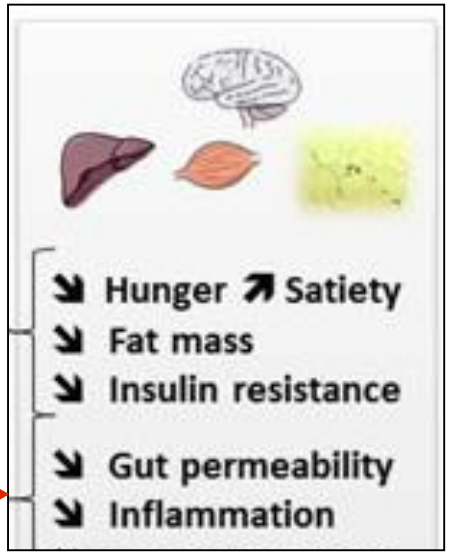
Nutrients for colonic epithelial cells

Improved barrier function

Decreased endotoxins in the blood

↑ *Endocrine function*

↓ *Systemic Inflammation*



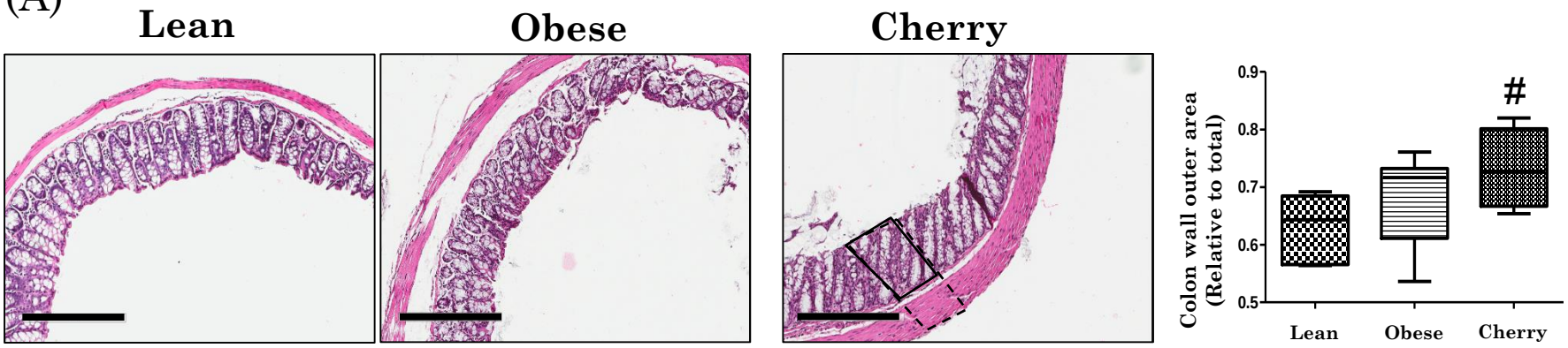
Cherry supplementation increased production of SHORT CHAIN FATTY ACIDS (SCFAs)

	Obese controls	Cherry	Lean controls	*P* value
Caproate	1.2(0.4–3.9)[a] ($n=9$)	285(217–437)[b] ($n=12$)	1.0(0.4–652)[a] ($n=10$)	0.0033
Methyl butyrate	–	116(17–405) ($n=12$)	62(43–92) ($n=3$)	NA
Butyrate	6.2(5.3–20)[a] ($n=9$)	–	11.9(6.1–16.2)[a] ($n=7$)	0.3511
Propionate	–	384(258–649) ($n=12$)	356(281–438)($n=4$)	NA
Acetate	1.9(1.4–1.9) ($n=3$)	269.4(128–672) ($n=12$)	273.2(40–351) ($n=3$)	NA
Valerate	–	15.4(4–48) ($n=10$)	–	NA

How these modifications were translated into intestinal health?

Colon barrier function

(A)



	Obese	Cherry	Lean	p value
Relative thickness of outer colon wall	0.64 (0.6-0.7)	0.73 (0.7-0.8)	0.72 (0.5-0.8)	0.08#

Values are median (min, max). #, $p < 0.1$

…And biomarkers involved in inflammation and cellular stress in colonic mucosal cells

Parameter/biomarker	Obese	Cherry	Lean	p value
ATF4*	4.10 (1.1; 8.2)	3.33 (1.7; 6.2)	5.85 (2.7; 10.2)	< 0.05
VCAM-1 *	6.42 (1.0; 32.0)	3.98 (2.6; 7.0)	8.51 (3.8; 12.0)	< 0.05

*mRNA levels of ATF4 (activating transcription factor 4) and VCAM-1 (vascular cell adhesion molecule-1). Values are median (min, max).

Conclusions

- Cherry supplementation for 12 weeks can modify the colon microbiota and the concentrations of SCFAs
- In general, these changes did not influence biomarkers of inflammation, cellular stress, and gut barrier function in colonic mucosal cells and colon tissues.
- This study has provided insights for future studies investigating cherry intake within the context of acute and chronic intestinal inflammation.

1. Cherries for intestinal health

- *Completed and published January 3rd, 2018*

PeerJ

Effect of dark sweet cherry powder consumption on the gut microbiota, short-chain fatty acids, and biomarkers of gut health in obese db/db mice

Jose F. Garcia-Mazcorro[1,2], Nara N. Lage[3,4], Susanne Mertens-Talcott[4], Stephen Talcott[4], Boon Chew[4], Scot E. Dowd[5], Jorge R. Kawas[6] and Giuliana D. Noratto[4]

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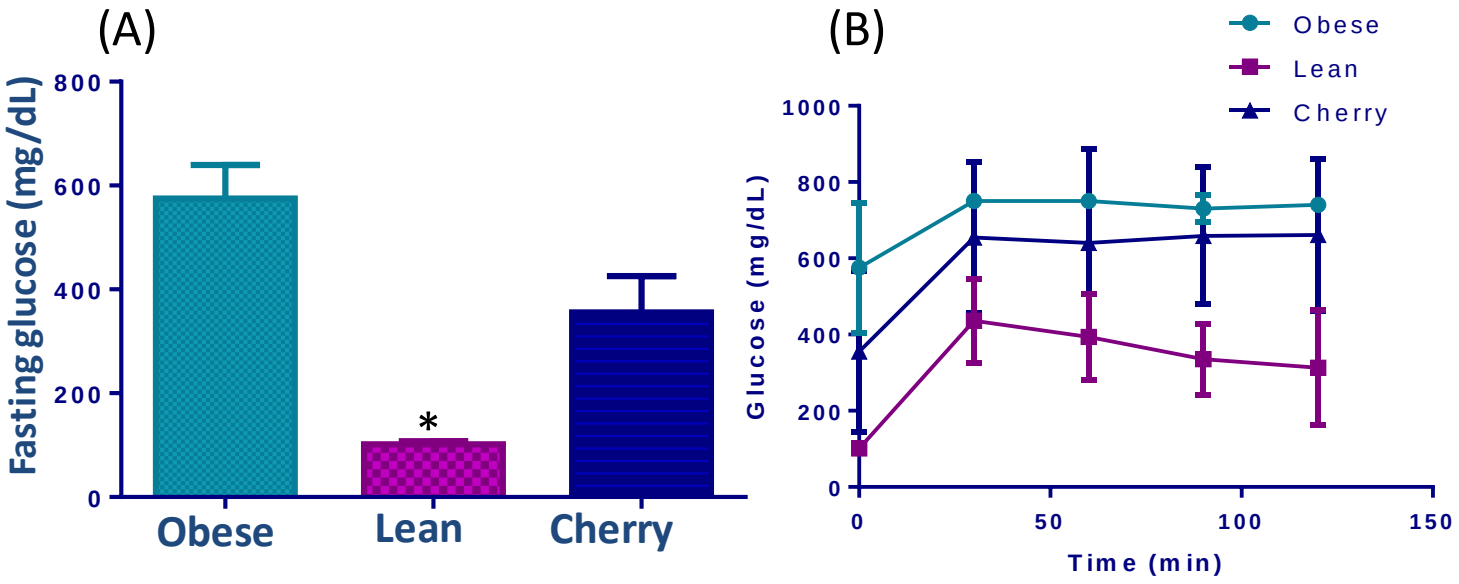
Project funded by the NORTHWEST CHERRY GROWERS/ WASHINGTON STATE FRUIT COMMISSION and the *WASHINGTON STATE DEPARTMENT OF AGRICULTURE*.

2. Cherries for Diabetes and non-alcoholic fatty liver disease (NAFLD)

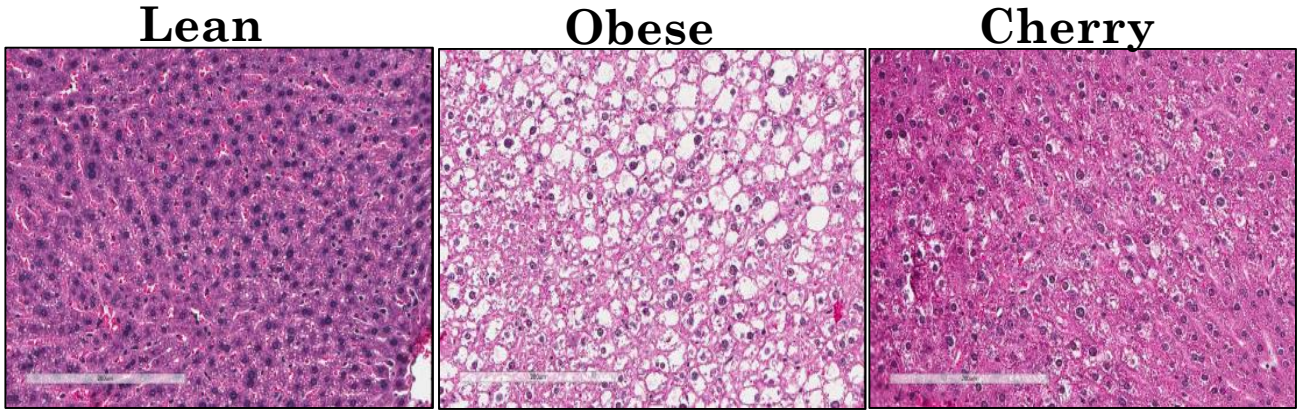
What are the specific benefits of anthocyanin-depleted cherry powder?

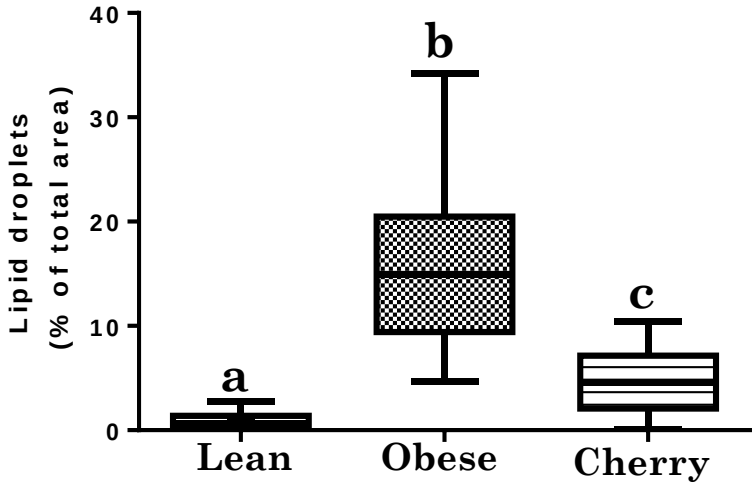
Improved some blood biomarkers of inflammation and diabetes

Parameters	Lean	Obese	Cherry	*p value*
IL-6 (ρg/mL)	*12.84[a] (7.26; 17.79)*	*38.6[b] (20.64; 185.60)*	*21.35[a] (3.15; 32.84)*	*<0.0001*



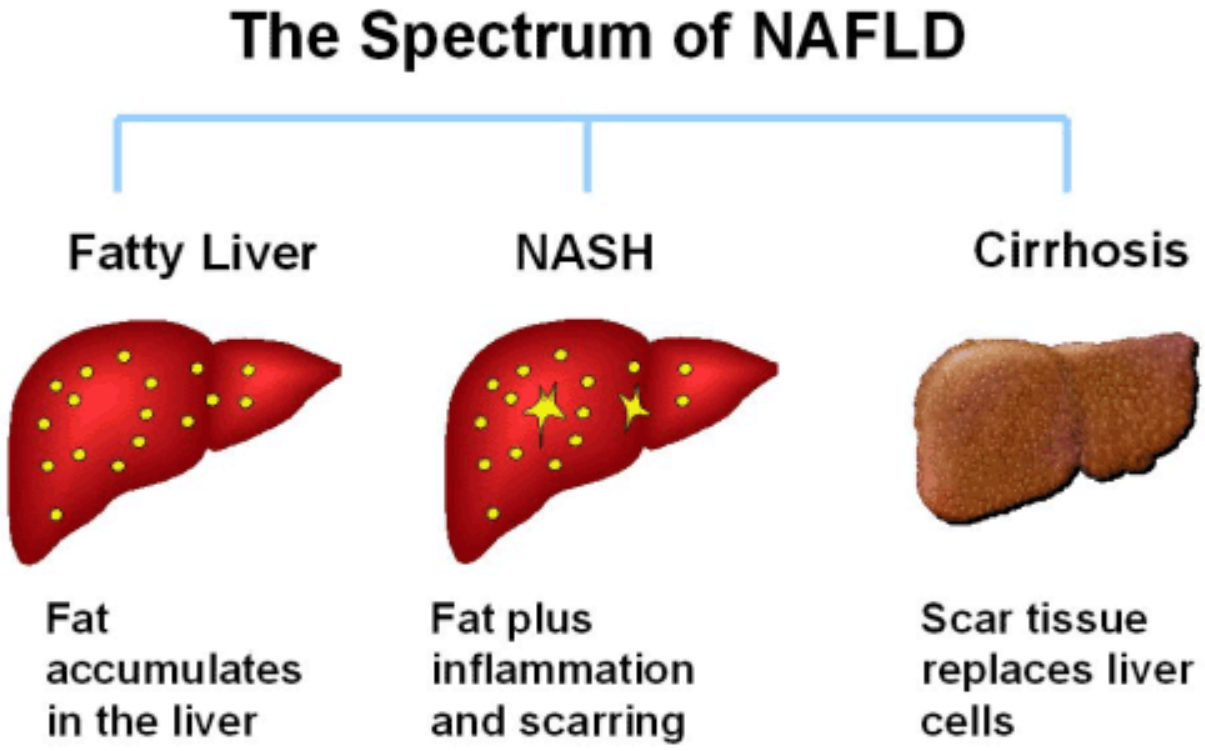
Protected liver from hepatic steatosis (NAFLD)



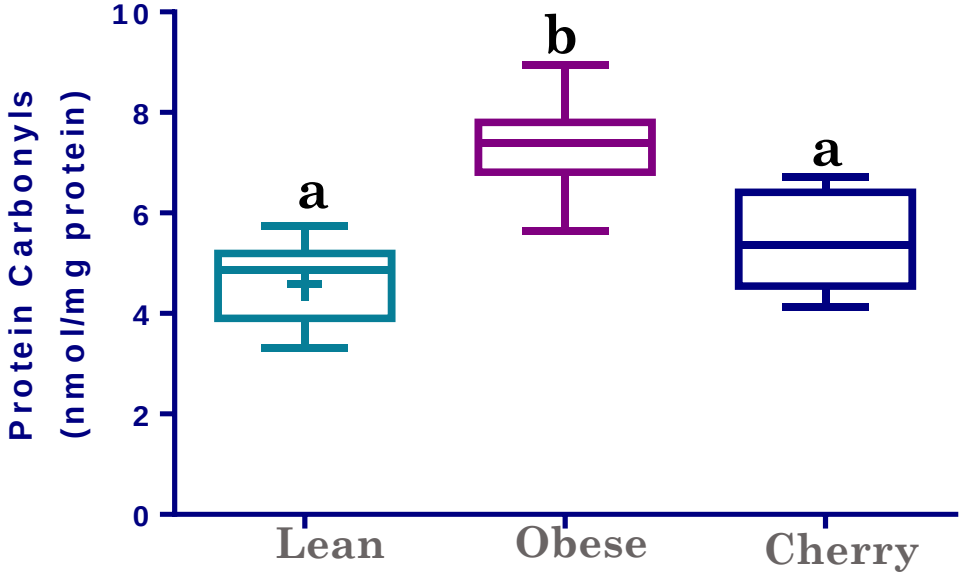


Cherry dietary supplementation decreased liver lipids in ~ 69% compared to obese control ($p < 0.0001$)

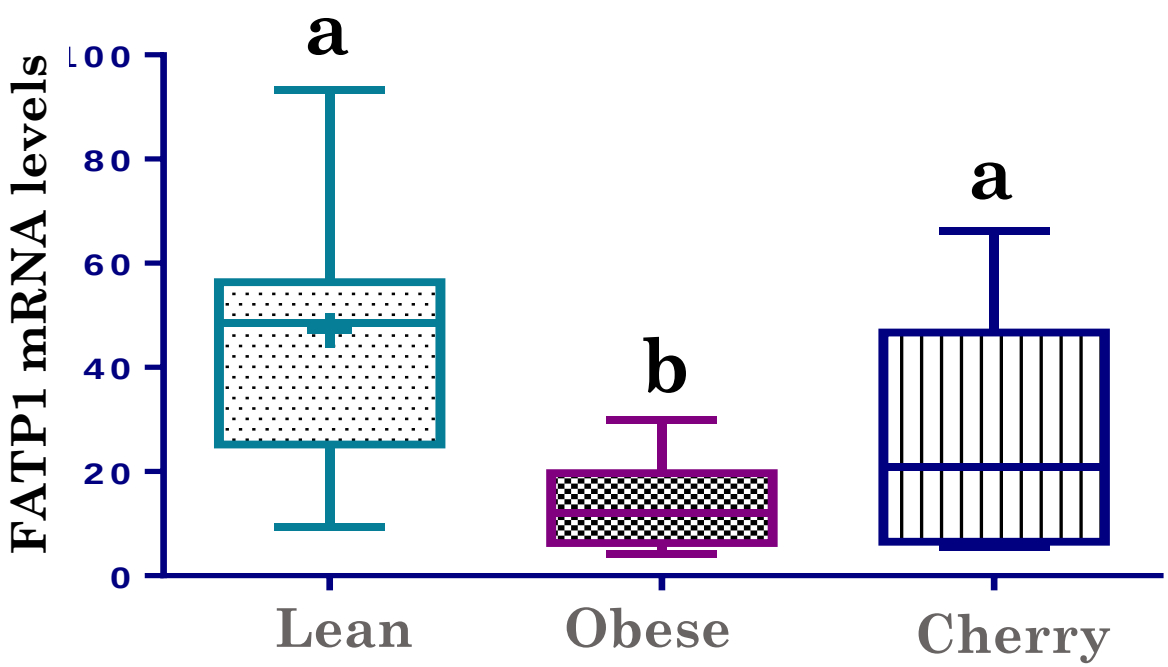
NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)



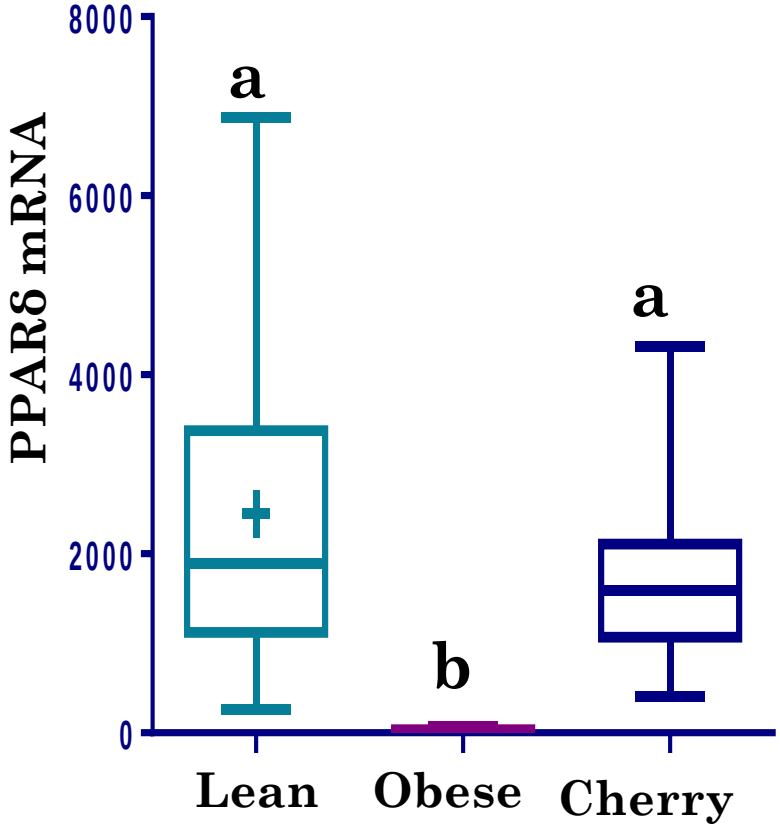
Decreased Protein carbonyls in liver

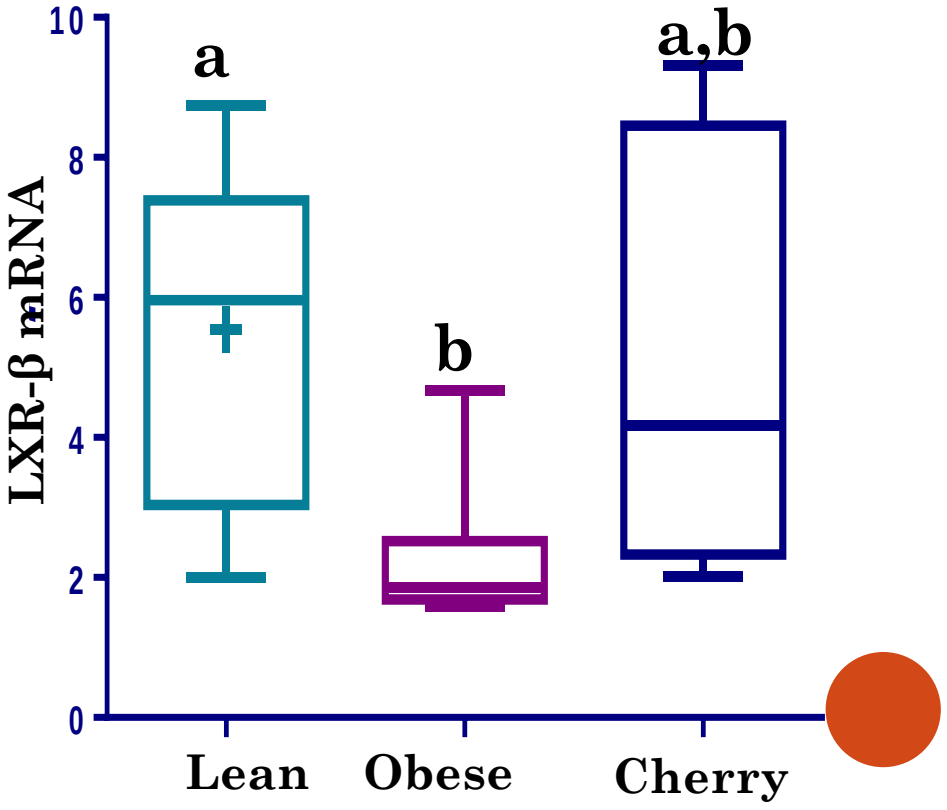


EXPRESSION OF AN ENZYME ASSOCIATED WITH LIPID METABOLISM IN LIVER



EXPRESSION OF TRANSCRIPTION FACTORS INVERSELY ASSOCIATED WITH HEPATOSTEATOSIS, INSULIN SENSITIVITY AND INFLAMMATION





Conclusions

- We elucidated specific benefits of non-anthocyanin polyphenolics in cherries linked to hepatosteatosis and inflammation reduction (PPARδ and LXRβ).
- We identified some of the benefits that overlap those reported for cherry anthocyanins, implying complementary activities (plasma IL-6 inflammatory cytokine reduction).

Manuscript currently under revision

3. Cherries for Breast cancer

Most common malignant disease in women

266,120 new cases of invasive breast cancer are expected to be diagnosed in USA in 2018

Obesity increases risk postmenopausal breast cancer

U.S. Breast Cancer Statistics,
Huang, Hankinson et al. 1997; Breastcancer.org 2017

Dark sweet cherry
(*Prunus avium*)

Polyphenolic Compounds

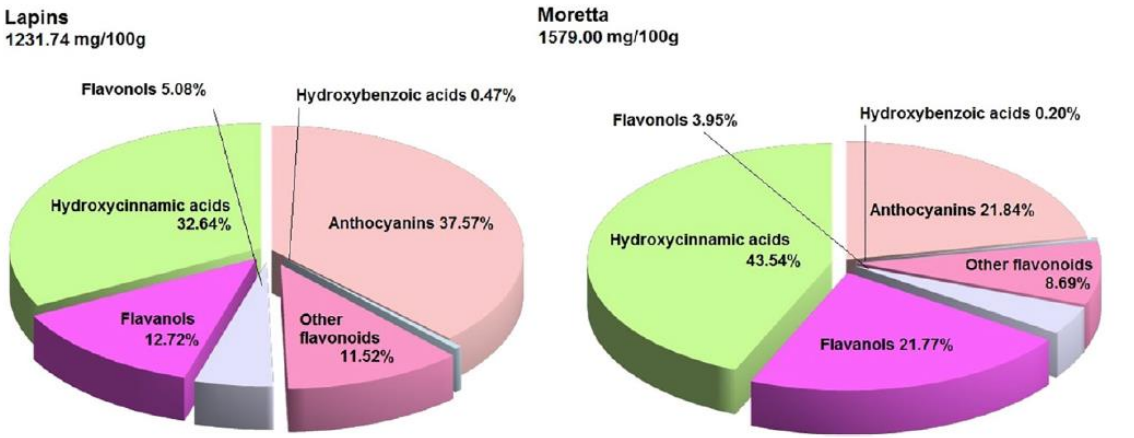


Fig. 1. Global percentage of hydroxycinnamic acids, flavan-3-ols, flavonols, hydroxybenzoic acids, anthocyanins and other flavonoids in the six sweet cherry cultivars.

Health Benefits

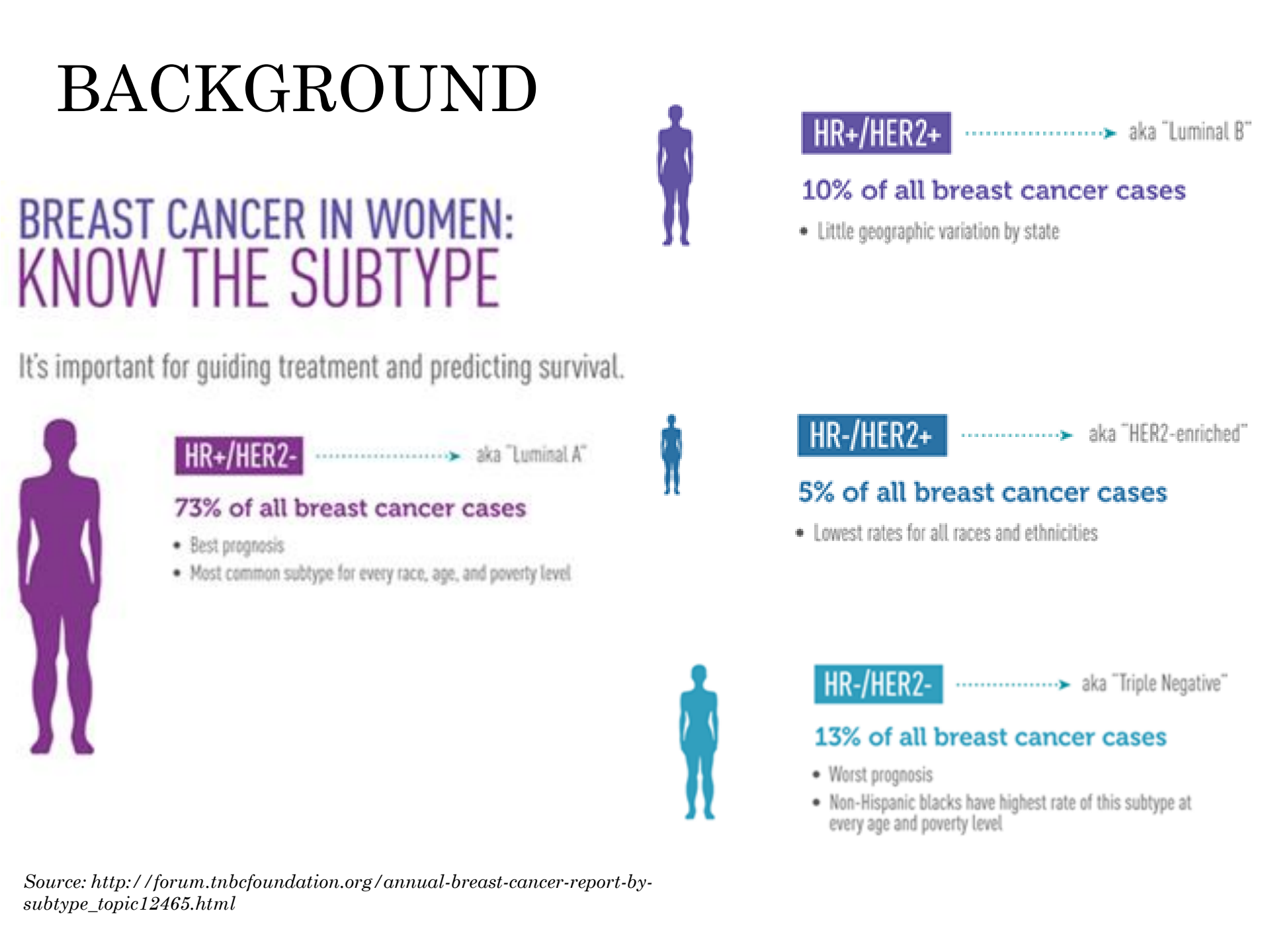
Breast Cancer?

Compounds	Anti-cancer studies	Source
Tart cherry anthocyanins (TCA)	**Tart cherry anthocyanins inhibit tumor development in Apc(Min) mice and reduce proliferation of human colon cancer cells**	Kang et al, 2003[1]
	Tart cherry juice induces differential dose-dependent effects on apoptosis, but not cellular proliferation, in MCF-7 human breast cancer cells	Martin KR and Wooden A, 2012[2]

[1] *Kang SY, Seeram NP, Nair MG, Bourquin LD.* ***Cancer*** *Lett. 2003 May 8;194(1):13-9.*

[2] *Martin KR and Wooden A, 2012. J Med Food. 2012. Nov;15(11):945-54*

BACKGROUND



Source: http://forum.tnbcfoundation.org/annual-breast-cancer-report-by-subtype_topic12465.html

Exploration of cherry polyphenolics as chemopreventive dietary agents to fight breast cancer

- Hypothesis
  - *Polyphenolics from red cherries have chemopreventive activity for human breast cancer*

(A)

EXPERIMENTAL APPROACH

Quantification of total polyphenolics and identification of individual phenolic compounds by HPLC

Aim 1: Breast cancer anti-proliferative activity *in vitro*

- Luminal B (BT474)
- HER2+ (MDA-MB-453)
- Triple negative (MDA-MB-231)
- Non-cancer breast cells (MCF-10A)

MDA-MB-453 (isolated from 48 years old female derived from metastatic site)

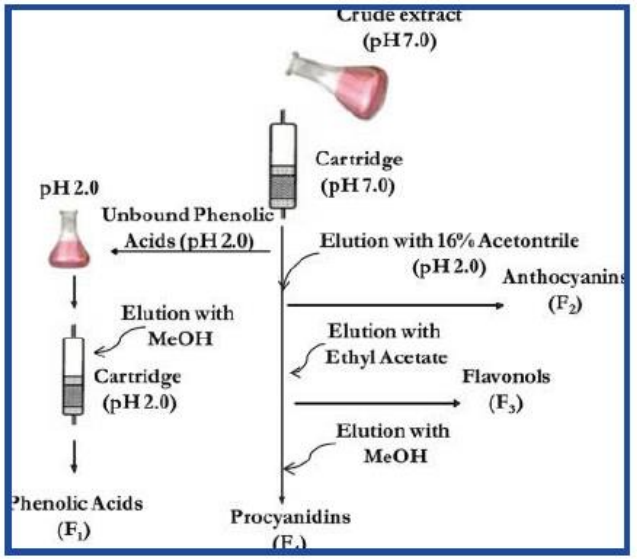
BT-474 (isolated from 60 years old adult female, derived from breast/duct carcinoma)

MCF-10A CELLS (isolated from 51 years old adult female, derived from metastatic site)

(B)

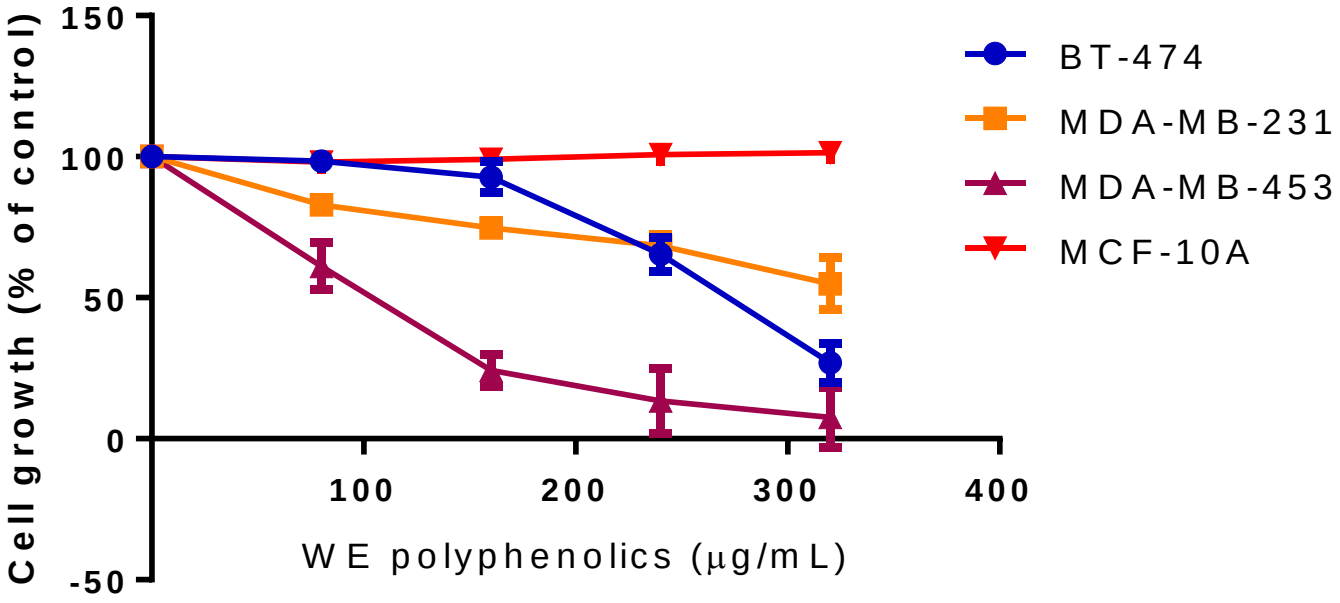
DARK SWEET CHERRY JUICE POLYPHENOLICS

Noratto et al.



(A) RESULTS

CELL GROWTH INHIBITION EXERTED BY CHERRY JUICE POLYPHENOLICS (WE)



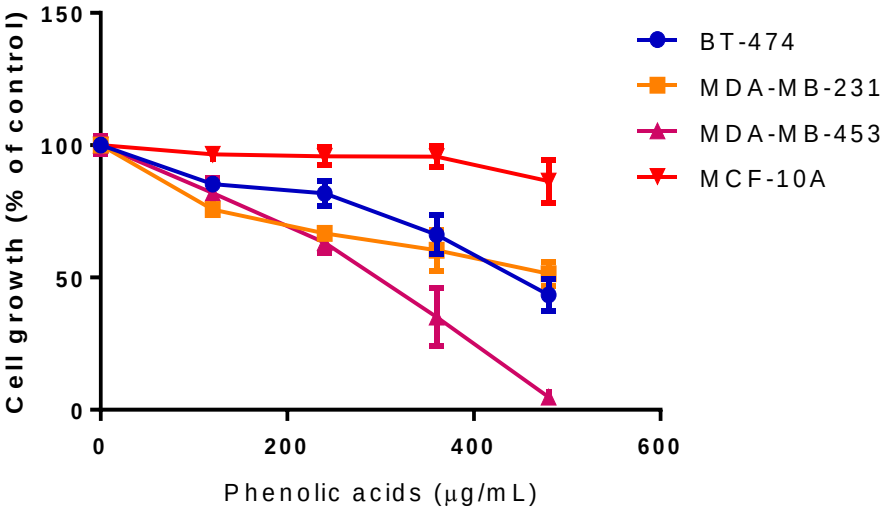
Summary of cell growth inhibitory potencies of WE cherry polyphenolics (IC_{50}) among breast cell lines

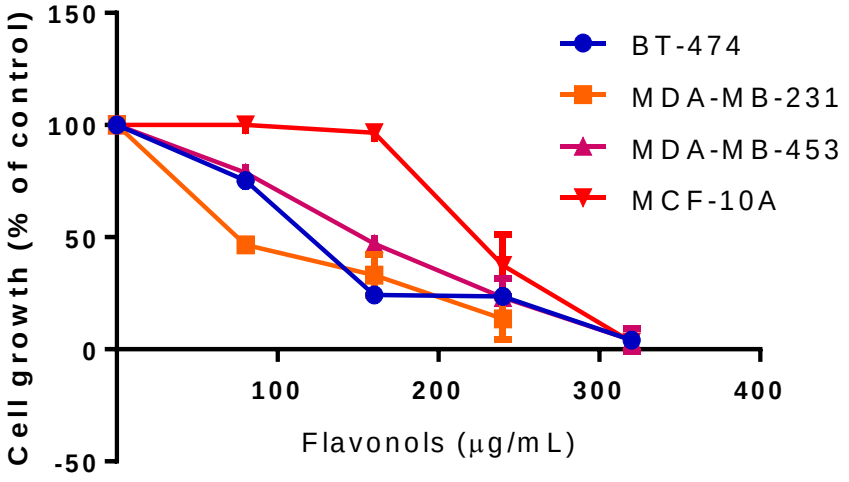
IC_{50} values for inhibition of cell growth	
Cell Line	WE (µg GAE/mL)
MDA-MB-231	[a]281 ± 56
BT-474	[a]289 ± 19
MDA-MB-453	[b]83 ± 33
MCF-10A	ND

Data are average of three or more independent determinations ± SD. Data was analyzed with ANOVA followed by Holm-Sidak's multiple comparisons test. Different superscript letters indicate significant difference between cell lines ($p<0.05$).

(B) RESULTS

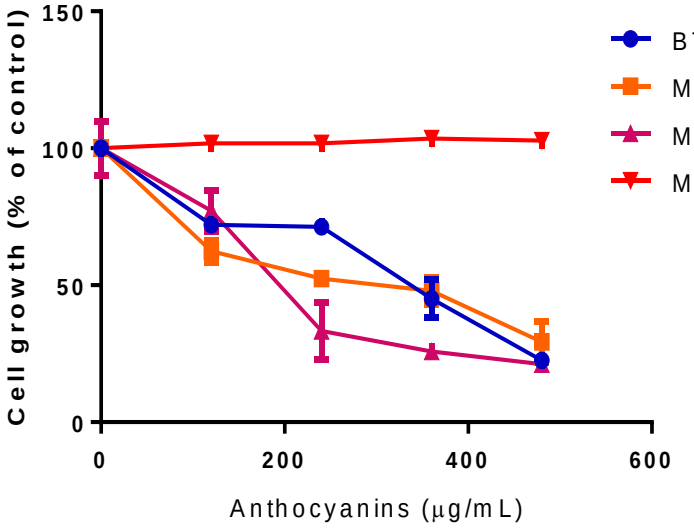
Cell growth inhibition exerted by phenolic acids and flavonols enriched fractions

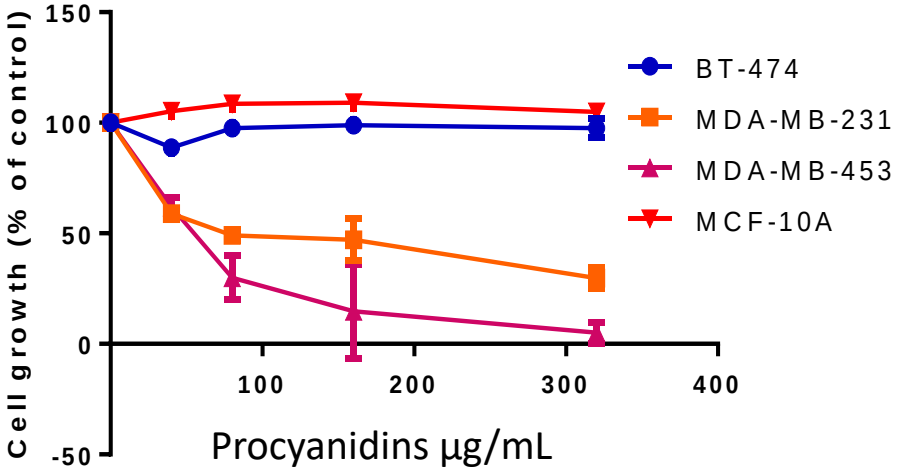




(B) RESULTS

Cell growth inhibition exerted by anthocyanins and procyanidins enriched fractions





Summary of IC_{50} among cherry polyphenolic fractions and cell lines

Cells Line	IC_{50} (µg GAE/mL)				
	Phenolic Acids	Anthocyanins	Flavonols	Procyanidins	WE
BT-474	$^{a}458^{A} \pm 25$	$250^{A,B,C} \pm 127$	$^{a}133^{B} \pm 23$	ND	$^{a}289^{C} \pm 19$
MDA-MB-453	$^{b}85^{A} \pm 35$	$70^{A} \pm 14$	$^{b}47^{A} \pm 5$	$45^{A} \pm 7$	$^{b}83^{A} \pm 33$
MDA-MB-231	$^{c}280^{A} \pm 57$	$205^{A,B} \pm 21$	$^{b}69^{B} \pm 1$	$149^{A,B,C} \pm 41$	$^{a}281^{A,C} \pm 56$
MCF-10A	ND	ND	$^{a}187 \pm 73$	ND	ND

Data are average of two or more independent determinations ± SD.
Different superscript lower case letters within each column indicate significant difference between cell lines ($p < 0.05$).
Different capital letters within each raw indicate significant difference among polyphenolic fractions ($p < 0.05$). ND: no determined within dose range tested

CONCLUSSIONS

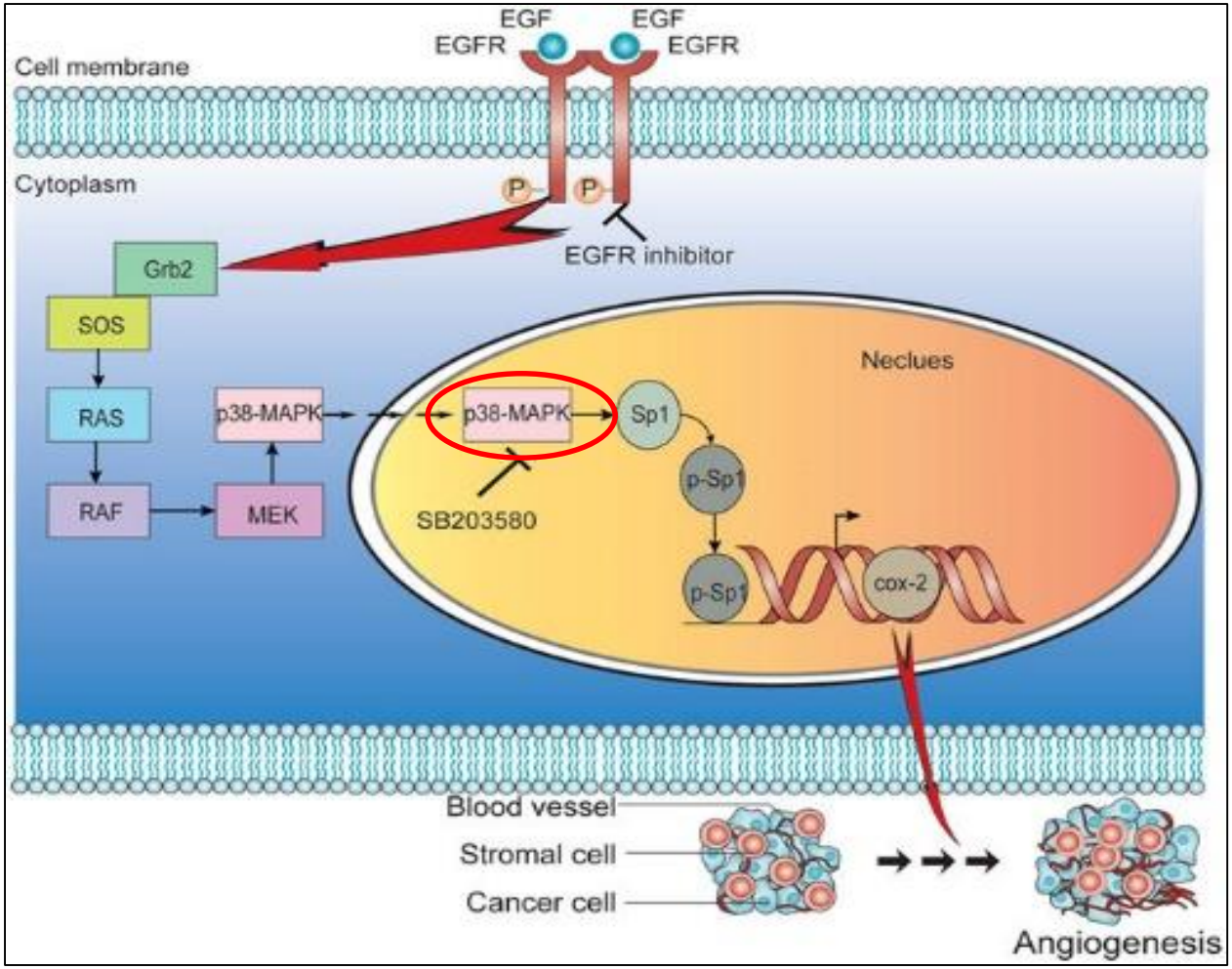
1. Dark cherry polyphenolics (WE) inhibit the growth of breast cancer cells with potency:

   MDA-MB-453 > MDA-MB-231 ~ BT-474 with no toxicity to non-cancer cells.

2. Anthocyanins and procyanidins enriched fractions inhibit the growth of ***MDA-MB-453 and MDA-MB-231 with similar potency without toxicity to non-cancer cells***

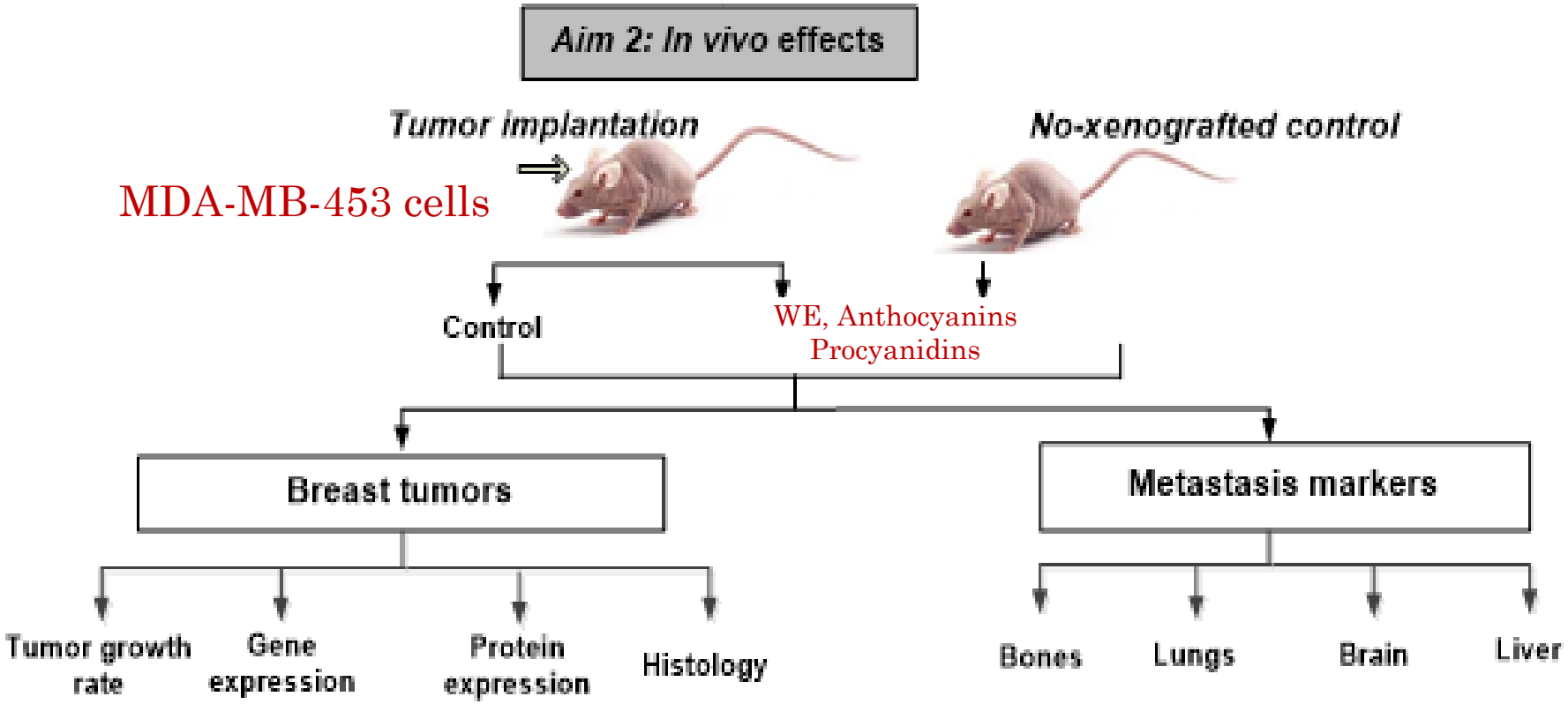
Ongoing studies:

Elucidating the molecular mechanisms targeted by cherry polyphenolics



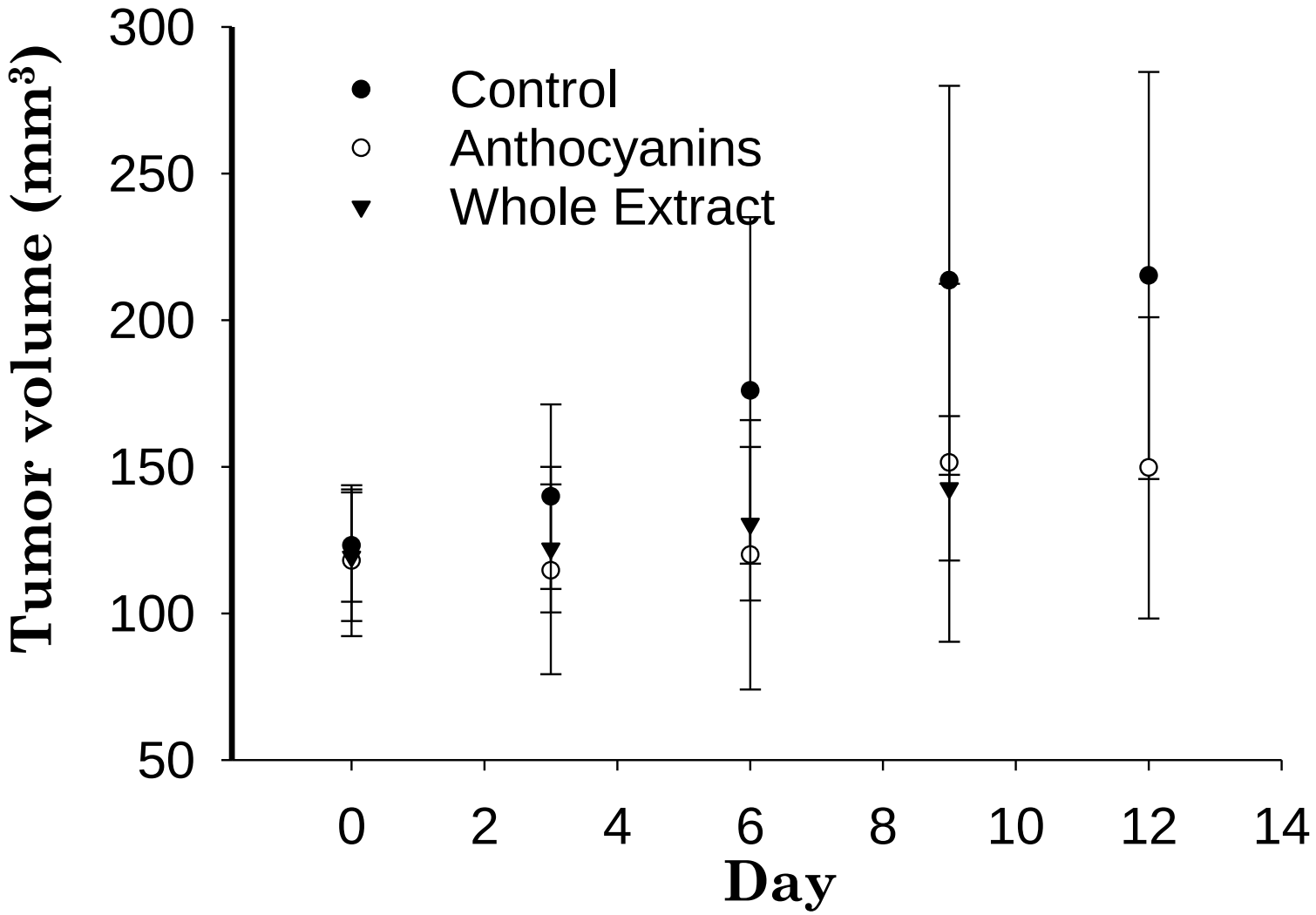
(source: Hu et al, 2017: doi:10.1038/s1598-017-00288-4)

Ongoing studies: Tumor growth inhibition *in vivo*



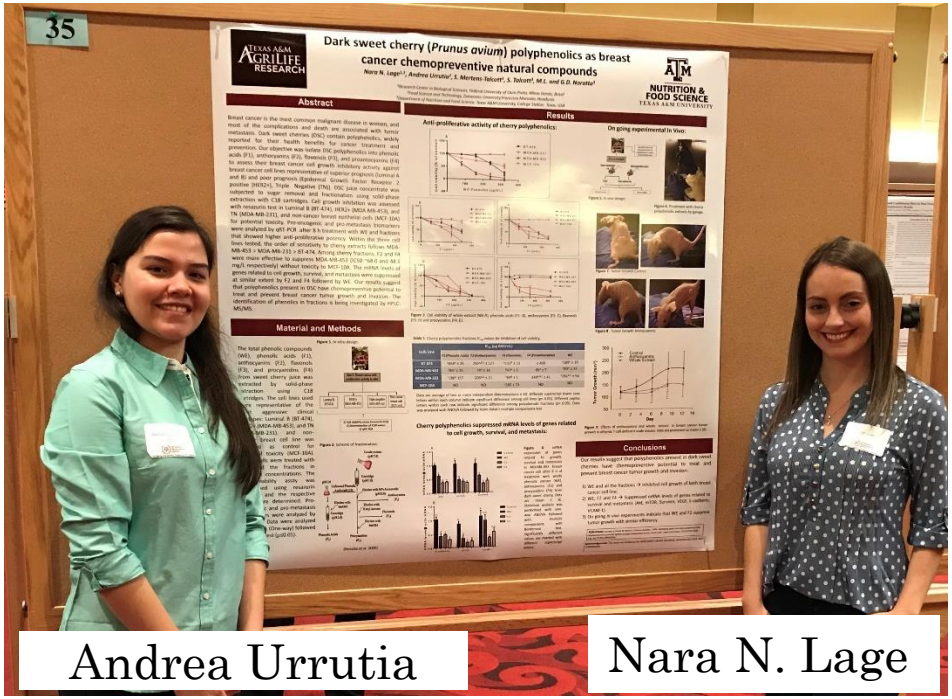
Preliminary in vivo results support the in vitro data

Preliminary results



ACKNOWLEDGEMENTS

- Students:



Mentors and Collaborators:

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Stephen Talcott, Ph.D.

Jose Garcia-Mazcorro, Ph. D.

FOR YOUR VALUABLE SUPPORT

- *NORTHWEST CHERRY GROWERS/ WASHINGTON STATE FRUIT COMMISSION*
- *WASHINGTON STATE DEPARTMENT OF AGRICULTURE*


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&
A