“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

Polyphenolic compounds with low availability:

Dietary Fiber

- Soluble fiber
- Insoluble fiber
EXPERIMENTAL APPROACH

Obese diabetic (db/db)/mice
Diet supplemented with cherry powder (10%)
Control isocaloric diet

Lean mice

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.
• Believed to have anti-inflammatory effects in humans
• Inverse relationships between colonization and inflammatory conditions
• May be used to combat obesity and type 2 diabetes
MICROBIAL COMMUNITIES CLUSTERED ACCORDING TO TREATMENT

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

- Decreased endotoxins in the blood
- Improved barrier function
- Nutrients for colonic epithelial cells
- Endocrine function
- Systemic inflammation
- Gut permeability
- Inflammation

Gut microbiota

- Hunger ↔ Satiety
- Fat mass
- Insulin resistance
- Gut permeability
- Inflammation
Cherry supplementation increased production of SHORT CHAIN FATTY ACIDS (SCFAs)

<table>
<thead>
<tr>
<th></th>
<th>Obese controls</th>
<th>Cherry</th>
<th>Lean controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caproate</td>
<td>1.2(0.4–3.9)&lt;sup&gt;a&lt;/sup&gt; (n = 9)</td>
<td>285(217–437)&lt;sup&gt;b&lt;/sup&gt; (n = 12)</td>
<td>1.0(0.4–652)&lt;sup&gt;a&lt;/sup&gt; (n = 10)</td>
<td>0.0033</td>
</tr>
<tr>
<td>Methyl butyrate</td>
<td>–</td>
<td>116(17–405)  (n = 12)</td>
<td>62(43–92)     (n = 3)</td>
<td>NA</td>
</tr>
<tr>
<td>Butyrate</td>
<td>6.2(5.3–20)&lt;sup&gt;a&lt;/sup&gt; (n = 9)</td>
<td>–</td>
<td>11.9(6.1–16.2)&lt;sup&gt;a&lt;/sup&gt; (n = 7)</td>
<td>0.3511</td>
</tr>
<tr>
<td>Propionate</td>
<td>–</td>
<td>384(258–649) (n = 12)</td>
<td>356(281–438)(n = 4)</td>
<td>NA</td>
</tr>
<tr>
<td>Acetate</td>
<td>1.9(1.4–1.9) (n = 3)</td>
<td>269.4(128–672) (n = 12)</td>
<td>273.2(40–351) (n = 3)</td>
<td>NA</td>
</tr>
<tr>
<td>Valerate</td>
<td>–</td>
<td>15.4(4–48)  (n = 10)</td>
<td>–</td>
<td>NA</td>
</tr>
</tbody>
</table>
**How these modifications were translated into intestinal health?**

Colon barrier function

(A)

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Cherry</th>
<th>Lean</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative thickness of outer colon wall</td>
<td>0.64 (0.6-0.7)</td>
<td>0.73 (0.7-0.8)</td>
<td>0.72 (0.5-0.8)</td>
<td>0.08#</td>
</tr>
</tbody>
</table>

Values are median (min, max). #, p < 0.1
AND BIOMARKERS INVOLVED IN INFLAMMATION AND CELLULAR STRESS IN COLONIC MUCOSAL CELLS

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

*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

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

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3 Research Center in Biological Sciences, Federal University of Ouro Preto, Minas Gerais, Brazil
4 Department of Nutrition and Food Science, Texas A&M University, College Station, TX, United States of America
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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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Lean</th>
<th>Obese</th>
<th>Cherry</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/mL)</td>
<td>12.84&lt;sup&gt;a&lt;/sup&gt; (7.26; 17.79)</td>
<td>38.6&lt;sup&gt;b&lt;/sup&gt; (20.64; 185.60)</td>
<td>21.35&lt;sup&gt;a&lt;/sup&gt; (3.15; 32.84)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Cherry dietary supplementation decreased liver lipids in ~69% compared to obese control (p<0.0001)
NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

The Spectrum of NAFLD

- **Fatty Liver**
  - Fat accumulates in the liver

- **NASH**
  - Fat plus inflammation and scarring

- **Cirrhosis**
  - Scar tissue replaces liver cells
DECREASED PROTEIN CARBONYLS IN LIVER

![Box plot showing decreased protein carbonyls in liver across different conditions.](image-url)
EXPRESSION OF AN ENZYME ASSOCIATED WITH LIPID METABOLISM IN LIVER

FATP1 mRNA levels

Lean  Obese  Cherry

0  20  40  60  80  100

Legend:
a
b
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**

**Lapins**
1231.74 mg/100g

- Hydroxycinnamic acids 32.64%
- Anthocyanins 37.57%
- Flavonols 12.72%
- Other flavonoids 11.52%
- Flavonoids 5.08%
- Hydroxybenzoic acids 0.47%

**Moretta**
1579.00 mg/100g

- Hydroxycinnamic acids 43.54%
- Anthocyanins 21.84%
- Flavonols 21.77%
- Other flavonoids 0.69%
- Flavonoids 3.95%
- Hydroxybenzoic acids 0.20%

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


BACKGROUND

BREAST CANCER IN WOMEN: KNOW THE SUBTYPE

It’s important for guiding treatment and predicting survival.

HR+/HER2- —— aka “Luminal A”
73% of all breast cancer cases
• Best prognosis
• Most common subtype for every race, age, and poverty level

HR+/HER2+ —— aka “Luminal B”
10% of all breast cancer cases
• Little geographic variation by state

HR-/HER2+ —— aka “HER2-enriched”
5% of all breast cancer cases
• Lowest rates for all races and ethnicities

HR-/HER2- —— aka “Triple Negative”
13% of all breast cancer cases
• Worst prognosis
• Non-Hispanic blacks have highest rate of this subtype at every age and poverty level

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

Polyphenolics from red cherries have chemopreventive activity for human breast cancer
**EXPERIMENTAL APPROACH**

**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)

**MATERIALS**

- DARK SWEET CHERRY JUICE POLYPHENOLICS

**DARK SWEET CHERRY JUICE POLYPHENOLICS**

- Noratto et al.

**METHODS**

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

**RESULTS**

- **MDA-MB-453** (isolated from 40 years old female, derived from metastatic site)
- **BT-474** (isolated from 60 years old adult female, derived from breast/duct carcinoma)
- **MDA-MB-231 CELLS** (isolated from 51 years old adult female, derived from metastatic site)
(A) RESULTS

Cell growth inhibition exerted by cherry juice polyphenolics (WE)
### Summary of Cell Growth Inhibitory Potencies of WE Cherry Polyphenolics (IC<sub>50</sub>) Among Breast Cell Lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>WE (μg GAE/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-MB-231</td>
<td>a281 ± 56</td>
</tr>
<tr>
<td>BT-474</td>
<td>a289 ± 19</td>
</tr>
<tr>
<td>MDA-MB-453</td>
<td>b83 ± 33</td>
</tr>
<tr>
<td>MCF-10A</td>
<td>ND</td>
</tr>
</tbody>
</table>

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

![Graph showing cell growth inhibition](image)
Summary of IC$_{50}$ among cherry polyphenolic fractions and cell lines

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

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.
CONCLUSIONS

1. Dark cherry polyphenolics (WE) inhibit the growth of breast cancer cells with potency: 
   \[ MDA-MB-453 > MDA-MB-231 \sim BT-474 \]
   with no toxicity to non-cancer cells.

2. Anthocyanins and procyanidins enriched fractions inhibit the growth of \[ MDA-MB-453 \text{ and } MDA-MB-231 \]
   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

![Graph showing tumor volume (mm$^3$) vs. day, with data points for Control, Anthocyanins, and Whole Extract]
ACKNOWLEDGEMENTS

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Stephen Talcott, Ph.D.
Jose Garcia-Mazcorro, Ph. D.
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- WASHINGTON STATE DEPARTMENT OF AGRICULTURE