Targeted delivery of dietary flavanols for optimal human cell function: Effects on cardiovascular health

SFRBM Annual Meeting, Pre-meeting Workshop II
Flavanols in Health and Disease

Flavanol and Procyanidin Intake and Cardiovascular Health: Outcomes from Clinical Intervention Studies

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Flavanol research in humans

• Do flavanols enhance vascular function?

• Are flavanol effects age-dependent?

• Can flavanols be protective in CV disease?
Causality criteria

1. Vascular effects not attributable to confounders?
2. Flavanols absorbed and transported to vasculature?
3. Dose-responsiveness? Temporality?
4. Pure flavanol mimicking vascular effects?
5. Inhibition of mediator pathway attenuates vascular effects?
6. Withholding of flavanols reverses vascular effects?
1. Clinical trial standards control confounders

Randomized controlled trial (RCT) according to Good Clinical Practice (GCP)

– Subjects randomized to treatment
– Tested against placebo control
– Subjects and investigators blinded to allocation
– Relevant groups
– Sufficiently powered
– CONsolidated Standards Of Reporting Trials*

*http://www.consort-statement.org/
1. Clinical trial standards control confounders

Use accredited (surrogate) endpoints#

— Blood pressure

— Endothelial function (FMD)

— Pulse wave velocity

#EFSA Journal 2011
2. Monomers, but not oligomers are absorbed and circulate primarily as metabolites

![Graph showing concentration of flavanol metabolites in plasma over time]

- DP1-10
- DP2-10
- DP1

**Time after consumption (h)**

**Concentration of flavanol metabolites in plasma (nmol/L)**

- Monomers + Oligomers
- Monomers
- Oligomers

**Variables**

- Weight (kg): 76
- Age (y): 31
- BMI (kg/m²): 25
- Diastolic blood pressure (mm Hg): 73

**Table 3: Characteristics of the study population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study population</td>
<td></td>
</tr>
<tr>
<td>Subject characteristics</td>
<td></td>
</tr>
</tbody>
</table>

**Statistical analysis**

- *Significantly different from the rest of the drinks ingested (P < 0.05; 2-factor repeated-measures ANOVA, Oligomers vs. Monomers).
- Data are presented as means ± SEMs.
- The primary test for an effect was a test of the interaction in a 2-factor repeated-measures ANOVA (in which the 2 factors were the test drink compared against the urine collection period (0–7 h and from 7 to 24 h after consumption) (2-factor repeated-measures ANOVA, DP1-10 vs. other drinks).
3. Dose-dependent improvement of endothelial function following flavanol-rich intervention

![Graph A: Time course of flow-mediated dilation (FMD) after consumption of cocoa drinks with different amounts of total flavanols (36, 330, and 918 mg).](image)

![Graph B: Dose-response curve of peak flow-mediated dilation (FMD) after ingestion of flavanol-rich cocoa containing 28 to 918 mg total flavanols.](image)

* Indicates significance compared with baseline at 0 time.
# Indicates significance compared with respective time point after consumption of low-flavanol control drink.

4. Pure (-)-epicatechin acutely improves endothelial function
5. Inhibition of NO Synthase by L-NMMA inhibits vascular effects of flavanols

Heiss JACC 2005, Schroeter PNAS 2006
6. Withholding reverses vascular effects

Acute application (975 mg)

Time (h)

Flow-mediated dilation (%)

0 2 4 6 8 10 12

0 2 4 6 8

1 2 3 4 5 6

Repetitive Application (3x375 mg/d)

Time (days)

Flow-mediated dilation (%)

1 3 5 7 15

Heiss JCVP 2007
Flavanol research in humans

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Age-related vascular changes

**FMD**

![Flow-mediated dilation (Δ%)](image)

- Young subjects
- Old subjects

**Nitrite increase**

![Nitrite increase (nM)](image)

- Young subjects
- Old subjects

**Intima media thickness**

![Intima Media Thickness (mm)](image)

- Young subjects
- Old subjects

**Pulse wave velocity**

![Pulse wave velocity (m/s)](image)

- Young subjects
- Old subjects

**Study design:** 4-armed randomized controlled double blind parallel group intervention study

**Inclusion**
- Healthy, male
- 18-30 (YOUNG)
- 50-80 (ELDERLY)
- BMI 23-30 kg/m²

**Exclusion**
- Cardiovascular disease (signs, symptoms, meds)
- Diabetes mellitus
- Smoking
- Acute inflammation
- Cardiac arrhythmia
- Malignancies
- Heart failure
- Renal failure

**FLAVANOL (2x410 mg)**
- YOUNG
- ELDERLY

**CONTROL (2x0 mg Flavanols)**
- YOUNG
- ELDERLY

Day
- 0
- 14

Hours after single drink
- 0
- 1
## Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>YOUNG</th>
<th>ELDERLY</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>26 ± 3</td>
<td>60 ± 7</td>
<td>&lt;0.001</td>
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<tr>
<td>BMI (kg/m2)</td>
<td>24.9 ± 3.0</td>
<td>26.5 ± 3.0</td>
<td>0.013</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.83 ± 0.06</td>
<td>1.81 ± 0.04</td>
<td>0.453</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81 ± 10</td>
<td>88 ± 11</td>
<td>0.079</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.991</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>184 ± 33</td>
<td>225 ± 32</td>
<td>&lt;0.001</td>
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<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>129 ± 26</td>
<td>157 ± 27</td>
<td>0.005</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>53 ± 16</td>
<td>54 ± 12</td>
<td>0.900</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>97 ± 44</td>
<td>118 ± 39</td>
<td>0.104</td>
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<td>Fasting plasma glucose (mg/dl)</td>
<td>89 ± 8</td>
<td>95 ± 8</td>
<td>0.027</td>
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<tr>
<td>HbA1c (%)</td>
<td>4.8 ± 1.1</td>
<td>4.6 ± 2.0</td>
<td>0.554</td>
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<tr>
<td>SBP (mmHg)</td>
<td>120 ± 9</td>
<td>131 ± 11</td>
<td>0.006</td>
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<td>DBP (mmHg)</td>
<td>77 ± 7</td>
<td>82 ± 9</td>
<td>0.011</td>
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<td>HR (bpm)</td>
<td>56 ± 7</td>
<td>56 ± 7</td>
<td>0.908</td>
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<tr>
<td>CRP (mg/dl)</td>
<td>0.1 ± 0.2</td>
<td>0.1 ± 0.2</td>
<td>0.692</td>
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<td>Hb (mg/dl)</td>
<td>15.3 ± 1.0</td>
<td>15.4 ± 1.1</td>
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<td>Leucocytes (1000/ul)</td>
<td>5.5 ± 1.3</td>
<td>5.8 ± 1.4</td>
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<tr>
<td>Smoking history</td>
<td>0</td>
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<tr>
<td>Medication</td>
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</tbody>
</table>

MV ± SD
Flavanols improve age-related impairment in endothelial function

YOUNG (18-30)
- Flavanols
- Control

ELDERLY (50-80)

* p<0.05 vs. 0 h, day 1 baseline, # p<0.05 vs. Control drink, † p<0.05 vs. Young

FLAVIOLA session at the annual SFRBM meeting – 14 November 2012
www.flaviola.org
FLAVIOLA Health Study

- **Aims**: General applicability, larger population at increased cardiovascular risk, in the context of cardiovascular health, and identify influencing factors.

- **Study Design**: 2-armed, randomized controlled parallel-group study

- **Sample Size**: n=100

- **Study Population**: middle-aged, male, healthy subjects

- **End points**
  1° Endothelial function
  2° Blood pressure, cholesterol, glucose
  3° Exploratory end points

- **1 month Intervention**
  » Flavanol intervention 2x410 mg
  » Flavanol free control
Flavanol research in humans

• Do flavanols enhance vascular function? ✔

• Are flavanol effects age-dependent? (✔)

• Can flavanols be protective in CV disease?
Endothelial homeostasis

Injury and Regeneration

Endothelial injury & dysfunction

Endothelial regeneration

NO

NO
Study design

- Randomized controlled crossover double blinded trial
- N=16 Patients with **coronary artery disease on optimal medical therapy**
- **30 days** high flavanol intervention (2 x 375 mg/d) & low flavanol control (2 x 9 mg/d)
Flavanol intervention reverses endothelial dysfunction in CAD patients

Flavanol intervention (2x375 mg 30d)
C: low flavanol control (2x9 mg 30d)

* p<0.05 vs respective pre, # p<0.05 vs C
Flavanol intervention mobilizes functional CACs

Heiss JACC 2010
Flavanol research in humans

- Do flavanols enhance vascular function? ✔

- Are flavanol effects age-dependent? (✔)

- Can flavanols be protective in CV disease? ✔
Clinical intervention studies

Conclusion
Flavanols and their metabolites are vasoactive in young, elderly, healthy and diseased humans
Clinical intervention studies

Conclusion
Flavanols and their metabolites are vasoactive in young, elderly, healthy and diseased subjects

Perspectives
Flavanols and their metabolites harbor vasculoprotective potential for primary and secondary CVD prevention
Take-home Message: *Flavanols Session*

- *Bioactives* ‘ rather than (only) ’Antioxidants‘

- *Active Compounds*: Flavanol *Metabolites from Monomer; (-)-Epicatechin; Oligomers (Procyanidins) do NOT contribute*

- *Epidemiology*: blood pressure lowering in human intervention studies at >50 mg flavanol (monomer)/day
• **Mechanism of Action**: prooxidant enzymes, reactive oxygen species (*in vitro*, cell culture expts); inflammatory markers; master switches: *Nrf2, NFκB*  

• **Cardiovascular Health**:  
  Flavanols and metabolites vasoactive are vasoactive in young, elderly, healthy and diseased subjects  

• Flavanols and their metabolites harbor vasoprotective potential for primary and secondary CVD prevention
FLAVIOLA international workshop on Flavanols in Cardiovascular Health

Brussels, 24 January 2012

www.flaviola.org/project/events/workshop/index.php