Cardiovascular health benefits of apples and apple polyphenols

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Background

Cardiovascular disease (CVD):

Leading cause of death in Australia
Impacts quality of life
Impacts Australia’s economy
Background

5% ↓ in risk of CVD mortality for each additional serving of fruit per day

Relationship of fruit intake with atherosclerosis in humans is not clear

Atherosclerosis contributes to CVD events in post-menopausal women

Abdominal Aortic Calcification (AAC) is a subclinical measure of atherosclerosis

AAC predicts CVD mortality.
Observational Study

Aim
Examine the cross-sectional associations of total and individual fruit (apple, pear, orange and other citrus, and banana) intake with AAC in a cohort of women over 70 years of age.
Participants

Western Australian women from the general population aged over 70 years

Calcium Intake Fracture Outcome Study (CAIFOS).

Food frequency questionnaire and AAC measured at baseline (1998)

n=1052
Abdominal Aortic Calcification (AAC)

Measured using dual energy X-ray absorptiometry

Scored from 0-24
Then categorized:
- not severe (AAC 0-5);
- severe (AAC >5)
### AAC and Fruit intake

<table>
<thead>
<tr>
<th>AAC Score</th>
<th>Total fruit</th>
<th>Apples</th>
<th>Pears</th>
<th>Oranges</th>
<th>Bananas</th>
</tr>
</thead>
<tbody>
<tr>
<td>spearman’s rho (ρ)</td>
<td>−0.061</td>
<td>−0.089</td>
<td>−0.016</td>
<td>−0.012</td>
<td>−0.035</td>
</tr>
<tr>
<td>p-value</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>0.60</td>
<td>0.70</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Severe Abdominal Aortic Calcification

The odds ratio for each 50 g/day increase in fruit intake.

24% decrease in odds
Summary

AAC was negatively associated with apple intake, but not with the intake of pears, oranges and other citrus or bananas.

A 50 g/day increase in apple intake was associated with 24% lower odds of having severe AAC.
Flavonoid concentration (mg/100g) in whole apple

Western Australian Apple Varieties

- Anthocyanin
- Chlorogenic Acid
- Phloridzin
- Epicatechin
- Total Quercetin
Mechanism

Flavonoids

Nitric oxide
Aim

To determine if acute and long–term (4 weeks) ingestion of apples will improve blood vessel function and BP in volunteers with at least one risk factor for cardiovascular disease.
Participants

30 non-smoking men and women (20-70 years of age) from the general population with one or more of the following:

- Slightly elevated blood pressure (120 < systolic BP < 160)
- High blood sugar (5.6 < glucose < 6.5)
- High cholesterol (5 < total cholesterol < 8)
- Central obesity (men > 94cm; women > 80cm)
Study design

Phase 1: screening & learning visits

Phase 2: 2 weeks

Phase 3: 4 weeks

- apples
- control

Visit 4

Acute study

Acute study
Study design

- Screening & learning visits
- Phase 1: Acute study
- Phase 2: 4 weeks
- Phase 3: Control
- Visit 4: apples

Diagram:
- 2 weeks
- 4 weeks
Study design

Phase 1:
- 4 weeks
- apples
- wash-out
- control
- Visit 1
- Acute study

Phase 2:
- 2 weeks
- wash-out
- control
- Visit 2

Phase 3:
- 4 weeks
- apples
- control
- Visit 3
- Acute study
- Visit 4
## Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.0 ± 10.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.7 ± 13.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5 ± 3.9</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>124.6 ± 14.1</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>72.0 ± 7.2</td>
</tr>
<tr>
<td>Total cholesterol (mM)</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td>Triglyceride (mM)</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>LDL- density lipoprotein cholesterol (mM)</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol (mM)</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Fasting plasma glucose (mM)</td>
<td>5.2 ± 0.3</td>
</tr>
</tbody>
</table>

n=30; males=10, females=20
Concentration of key flavonoids in apples (µg/g)

- **Total Quercetin glucosides**
  - Apple Flesh
  - Apple Flesh + Skin

- **Epicatechin**
  - Apple Flesh
  - Apple Flesh + Skin

- **Phloridzin**
  - Apple Flesh
  - Apple Flesh + Skin

- **Chlorogenic acid**
  - Apple Flesh
  - Apple Flesh + Skin
Concentration in plasma (nmol/L)

**Acute**

- Quercetin: 40 nmol/L
- Isorhamnetin: 10 nmol/L

**Chronic**

- Quercetin: 25 nmol/L
- Isorhamnetin: 20 nmol/L

Significance levels:
- ***: p < 0.001
- **: p < 0.01
- *: p < 0.05
Flow-mediated dilatation (FMD)
Pulse Wave Velocity
# Pulse Wave Velocity

<table>
<thead>
<tr>
<th></th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flesh</td>
<td>Flesh + Skin</td>
</tr>
<tr>
<td>Office Systolic Blood Pressure</td>
<td>128.5 ± 2.1</td>
<td>127.4 ± 2.1</td>
</tr>
<tr>
<td>Augmentation Index</td>
<td>8.1 ± 1.0</td>
<td>9.3 ± 1.0</td>
</tr>
<tr>
<td>Pulse Wave Velocity</td>
<td>7.6 ± 0.1</td>
<td>7.7 ± 0.1</td>
</tr>
</tbody>
</table>
24 hr Ambulatory Blood Pressure
24 hr Ambulatory Blood Pressure

No difference in 24 hour blood pressure between the two groups
# Blood Test Results

<table>
<thead>
<tr>
<th></th>
<th>Flesh</th>
<th>Flesh + Skin</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.29 ± 0.04</td>
<td>5.33 ± 0.04</td>
<td>0.46</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>6.03 ± 0.10</td>
<td>5.84 ± 0.10</td>
<td>0.13</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.27 ± 0.05</td>
<td>1.25 ± 0.05</td>
<td>0.84</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>4.00 ± 0.08</td>
<td>3.84 ± 0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.45 ± 0.02</td>
<td>1.42 ± 0.02</td>
<td>0.36</td>
</tr>
<tr>
<td>F₂-Isoprostanes</td>
<td>728.34 ± 18.56</td>
<td>690.46 ± 18.56</td>
<td>0.14</td>
</tr>
</tbody>
</table>
We saw significant improvements in FMD, both acutely and chronically
Not accompanied by a change in BP
No change in arterial stiffness
No change in plasma cholesterol, glucose or markers of oxidative stress (chronic)
Concentration of key flavonoids in apples (µg/g)

- Total Quercetin glucosides
- Epicatechin
- Phloridzin
- Chlorogenic acid

Apple Flesh

Apple Flesh + Skin
Quercetin

Found in skin and leaves

Richest sources are onions, curly kale, leeks, broccoli, apples and tea

Biosynthesis is stimulated by light

Usually found in its glycosylated form

Persons with higher quercetin intake had lower mortality from ischemic heart disease.

Reduces BP in stage 1 hypertensives
Human Intervention Study 2

Aim

Determine if there is a dose-related effect of dietary derived quercetin-3-O-glucoside on blood vessel function and blood pressure in healthy human volunteers.
Study Design

5 visits; one week apart
Volunteers receive each treatment in a random order:

- 0 mg quercetin + 2 g maltodextrin (control)
- 50 mg quercetin + 2 g maltodextrin
- 100 mg quercetin + 2 g maltodextrin
- 200 mg quercetin + 2 g maltodextrin
- 400 mg quercetin + 2 g maltodextrin

Endothelial function (FMD), blood pressure and quercetin levels were measured before and one hour after treatment
Participants

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>15 (men=6; women=9)</td>
</tr>
<tr>
<td>Age</td>
<td>60.8 ± 9.3</td>
</tr>
<tr>
<td>BMI</td>
<td>24.1 ± 3.1</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>27.7 ± 7</td>
</tr>
<tr>
<td>SBP</td>
<td>117.4 ± 9.5</td>
</tr>
<tr>
<td>DBP</td>
<td>66.9 ± 10.2</td>
</tr>
</tbody>
</table>
Concentration in plasma (µM)

- Free Quercetin
- Isorhamnetin

Quercetin dose (mg)
# Results

Increases in plasma quercetin metabolites

No changes in:
- FMD
- BP

Maybe it’s not quercetin?

Pure compound rather than whole food?
Whole food vs pure compound

Benefit of higher apple intake apparent in observational studies

Whole apple can improve vascular function compared to apple flesh only

Clear apple juice is associated with adverse effects

Synergistic relationship between the fibre and flavonoids

This is likely mediated by the gut microbiota
Mouse study

Blood glucose (mmol/L)

- Healthy diet
- High-fat diet
- High fat diet + flavonoid
- High fat diet + fibre
- High fat diet + flavonoid + fibre
Mouse study

Insulin (ng/mL)

- Healthy diet
- High-fat diet
- High fat diet + flavonoid
- High fat diet + fibre
- High fat diet + flavonoid + fibre
Interpretation

Participants who consumed more apples had a lower abdominal aortic calcification score

This was not seen with pears, oranges or bananas

Apples skin is rich in flavonoids

Apples plus skin improve endothelial function compared to apple flesh only

These results were not replicated using pure quercetin

Possibly flavonoid + fibre interaction driving beneficial effects of apples
If indeed it can be shown that
“an apple a day keeps the doctor away”
this could prove to be a simple and economic way of
reducing cardiovascular disease incidence worldwide.