Examining human health effects of berries

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MRC Human Nutrition Research labs, Cambridge, 14th May 2012
About JHI – formed last year as a merger between SCRI and Macaulay Institutes

Long-established breeding program for berries
Berry research at JHI

We breed market-leading varieties

- **Blackcurrants** – the “Ben” series
- **Raspberries** – the “Glen” series
- **Strawberry** – “Symphony, Rhapsody....”
- **Blueberries for UK conditions**

- Research into Health Benefits of Berries
- Feedback and direct breeding of new varieties
Scheme of talk

Background

Biological activities of berry components

- **MODEL SYSTEMS**
- Cardiovascular Health
- Neuroprotection
- Cancer
- Control of nutrient availability
- Diabetes & Obesity

Analytical studies

- Correlate bioactivity with polyphenol composition
- Examine bioavailability of components

The 3 main causes of premature death in Scotland led to the “5 a day” programme - Government-led Mass Intervention to alter our diet.

How do FAV affect health?

Minerals (Zinc)? Vitamins (C and E)? Fibre? Displacement? Lower Fat? Antioxidants? Phytochemicals?
Berries contain a diverse and species specific mixture of antioxidants – the two main types are **Polyphenols** and **Vitamin C**.
Availability of nitric oxide (NO) in ex vivo rat carotid arteries

Raspberry extract effective at 50-fold less than Vitamin C or Quercetin derivatives – known effectors of CV performance

Protection of NO bio-availability maintains blood vessel flexibility

Prof. Carlene Hamilton, BHF, University of Glasgow
Cardiovascular disease (CVD)

- Protection of vasodilatory responses of rat aorta against inflicted oxidative damage
- Protective effect of polyphenols
  - not predicted by *in vitro* antioxidant activity
- Anthocyanins particularly effective
- Modelled breakdown products also effective

*Prof. Ian Megson, Univ. Highland and Islands*
Cardiovascular function and intake of soft fruit: Effects of qualitative and quantitative variation in berry antioxidant status

**Intervention trial** – assess effects of six week ingestion of

- blackcurrant berries with low vitamin C content
- blackcurrant berries with high vitamin C content
- blueberries (No vitamin C)
- coloured flavoured water (control)

**Effects on cardiovascular function**

Positive effects on intima media thickness and *in vivo* markers for endothelial cell function and oxidative stress
Oxidative stress, Alzheimer’s and the Brain

Brain = 2 % adult body mass but uses **20 %** oxygen inhaled

Poorer antioxidant mechanisms

High levels of PUFAs, minerals and neurotransmitters – good targets for free radicals

Brain cells don’t renew by cell division - accumulate FR-induced damage with age & FR damage implicated in AD

*EU project*

**BrainHealthFood**

*Bioactive compounds from blackcurrant processing waste for brain health*

MTT Agrifood

JHI

TTZ

Univ. Kuopio

& SME partners
Protective effect of anthocyanins in Alzheimer’s model system

Further studies with berry extracts suggest positive results in behavioural studies in mice

Vepsäläinen et al. in press. Univ. Kuopio
Effects on cancer cells

All berry extracts tested at 50 μg/ml
Inhibition not related to *in vitro* antioxidant capacity

McDougall et al. (2008) JAFC 56; 3016-3023
Anti-cancer effects

Raspberry inhibits cancer cell growth at low levels (μg/ml)

Cell number vs. Days graph showing different concentrations of raspberry extract: 0, 7.5, 15, and 30 μg/ml.
Most effective components are tannins
Joint project on berry polyphenols & colon cancer

Emma Brown and Dr Chris Gill, Biomedical Sciences, University of Ulster, Coleraine
Colon cancer and polyphenols

Matrigel invasion by HT115 colon cancer cells was inhibited by raspberry polyphenols in the μg range.

Invasion related to ability to spread from initial site.

Colon cancer and polyphenols

Berry extracts inhibit genotoxic effects of H$_2$O$_2$ in HT29 cells in a dose-dependent fashion;

SB = BC > RB
Colon cancer and polyphenols

Fermentation with faecal bacteria produces berry-specific polyphenol products

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control</th>
<th>Raspberry</th>
<th>Strawberry</th>
<th>Blackcurrant</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzoic acid</td>
<td>2.44 ± 0.50</td>
<td>2.32 ± 0.41</td>
<td>2.32 ± 0.08</td>
<td>1.71 ± 0.10</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>n.d.</td>
<td>0.26 ± 0.01*</td>
<td>0.52 ± 0.07*</td>
<td>0.25 ± 0.01*</td>
</tr>
<tr>
<td>3, 4-dihydroxybenzoic acid</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.49 ± 0.08*</td>
</tr>
<tr>
<td>tyrosol</td>
<td>0.15 ± 0.03</td>
<td>0.32 ± 0.02*</td>
<td>0.32 ± 0.07*</td>
<td>0.17 ± 0.20</td>
</tr>
<tr>
<td>phenylacetic acid</td>
<td>16.34 ± 4.20</td>
<td>34.05 ± 3.5*</td>
<td>20.57 ± 1.4</td>
<td>7.35 ± 0.11</td>
</tr>
<tr>
<td>4’-hydroxyphenylacetic acid</td>
<td>1.52 ± 0.20</td>
<td>3.65 ± 0.5*</td>
<td>3.04 ± 0.3*</td>
<td>5.33 ± 0.30*</td>
</tr>
<tr>
<td>3-(phenyl)propionic acid</td>
<td>0.99 ± 0.30</td>
<td>2.10 ± 0.30</td>
<td>3.60 ± 0.21*</td>
<td>20.73 ± 0.51*</td>
</tr>
<tr>
<td>3-(4’-hydroxyphenyl)propionic acid</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>1.66 ± 0.21*</td>
</tr>
</tbody>
</table>
Colon cancer and polyphenols

After fermentation with faecal bacteria, the berry-derived components were still genoprotective even though the original polyphenols were completely degraded.
Faecal metabolism of berry polyphenols

- Profiling of faecal water metabolites in 10 free-living students after intake of raspberry puree (200 g/d for 14 d) by gas-chromatography mass spectrometry (GC-MS)

- Substantial ingestion of polyphenols (anthocyanins, ellagitannins etc)

- Focus on major phenolic metabolites
Faecal metabolism of berry polyphenols

- Phenylacetic acid increased in 7/10 subjects
- 4-hydroxy phenylacetic acid increased in 6/10 subjects
- 3-hydroxy phenylacetic acid increased in 5/10 subjects
- 3-Phenylpropionic acid increased in 6/10 subjects
- 3(4-hydroxy) phenylpropionic acid increased in 5/10 subjects
- 3, 4-dihydroxy benzoic acid increased in 7/10 subjects
- 4-hydroxy benzoic acid increased in 2/10 subjects

But not the same subjects

Fits evidence from model studies with faecal inoculates but shows large inter-individual variation - Due to differences in diet or microflora?

Gill et al, JAFC (2010) 58, 10389–10395
DGGE analysis of faceal microbiota revealed that this supplementation did not alter the composition.

Samples before and after supplementation are more alike than between individuals.
Control of nutrient availability

- Polyphenols can inhibit digestive processes and slow or modulate nutrient release from food
- Inhibition of starch digestion – blood glucose control and type 2 diabetes
- Inhibition of lipid digestion – control of hyperlipidemia, CVD and obesity
Lipid digestion and lipase

Inhibitory at 50 μg/ml
Inhibition by cloudberry extracts is saturable

Due to ellagitannins (ETs) in cloudberry, arctic bramble and raspberry and

procyanidins and ETs in strawberry

Mainly procyanidins in lingonberry

ASTRIGENT EFFECT?

Ties in with animal studies on obesity

McDougall et al. (2009) Food Chemistry 115, 93–199
Inhibition of starch digestion

Amylase chops into fragments
Glucosidase nibbles off glucose
α-amylase inhibition

Strawberry and raspberry most effective

McDougall et al (2005) JAFC 53, 2760-2766; Grussu et al., 2011
Co-incubation with acarbose

Co-incubations at ratios of IC\textsubscript{50} – rowanberry PACs first

% Control amylase activity

- Rep 1
- Rep 2
- Rep 3

Each at IC\textsubscript{50}

At half IC\textsubscript{50}

100/100
50/50
75/25
25/75

IC\textsubscript{50} ratios
α-glucosidase inhibition by berries

Not all berries equal?
Akin to pharmaceutical inhibition with acarbose?

α-glucosidase inhibition by berries

Inhibition by black currant

IC$_{50}$ = 20 μg/ml

Co-incubation with acarbose

% Activity

Black currant/acarbose (μg/ml)
Inhibition by rowanberry

IC\textsubscript{50} = 30 \, \mu g/ml
Co-incubation with acarbose

Additive/synergistic effect between rowan polyphenols and acarbose

Rowanberry/Acarbose (µg/ml)
Mixing of berry extracts

Lack of additive effect suggests components are operating at same site on enzyme?
Summary – α-glucosidase inhibition

- Berry polyphenols inhibit glucosidase activity *in vitro* at low levels
- Inhibition depends on polyphenol composition
- Tannins are not important and astringency is probably not the main mechanism
- Anthocyanin-rich and chlorogenic acid-rich black currant and rowanberry are similarly effective
- The active components potentiate effect of acarbose but different berries do not act additively – sites of action?
Human trial – modified glycemic response

Volunteers given sucrose-loaded black currant (BC) juice or sucrose-loaded BC juice supplemented with crowberry juice

The supplemented juice (●) caused a reduction in peak height of plasma glucose and extended the area under the curve

Törrönen et al. submitted
The insulin responses showed a similar pattern to the glucose response.

Possible role for inhibition of glucosidase/glucose transport?
Urinary polyphenol metabolites after intake of fortified juice quantified by LCMS

Increases noted within 2hrs
### Human trial – urinary metabolites

<table>
<thead>
<tr>
<th></th>
<th>[M-H] m/z</th>
<th>MS²</th>
<th>Putative Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>244</td>
<td>164, 162, 80</td>
<td>Phenolic acid sulphate</td>
</tr>
<tr>
<td>B</td>
<td>233, 153, 109</td>
<td>189, 153, 97</td>
<td>Dihydroxybenzoic acid sulphate</td>
</tr>
<tr>
<td>C</td>
<td>247, 167</td>
<td>203, 167, 123</td>
<td>Dihydroxyphenylacetic acid sulphate</td>
</tr>
<tr>
<td>D</td>
<td>189, 109</td>
<td>109</td>
<td>Catechol sulphate</td>
</tr>
<tr>
<td>E</td>
<td>259, 273</td>
<td>179, 193 resp.</td>
<td>Mix of ferulic &amp; caffeic acid sulphates</td>
</tr>
<tr>
<td>F</td>
<td>178</td>
<td>134</td>
<td>Hippuric acid</td>
</tr>
<tr>
<td>G</td>
<td>287</td>
<td>207, 163</td>
<td>Dimethoxy cinnamic acid sulphate</td>
</tr>
<tr>
<td>H</td>
<td>Multiple</td>
<td>-</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

**Identities were confirmed by exact mass determination at 4 decimal places**
Breakdown products of anthocyanins?

Suggestive of ring fission as reported in other studies.
Nutrient Digestion

- Berry polyphenols inhibit enzymes involved in starch and lipid digestion \textit{in vitro}
- The inhibition occurs at concentrations easily reached in the GIT
- The active components are unknown but differ between enzymes and in potential mechanisms (↑ synergy?)
- Berry components can potentiate inhibition by acarbose at low levels
- Initial human studies show promise
Various polyphenols stimulate the phosphorylation and activation of FOXO1A, a transcription factor involved in regulating insulin responses and controlling glucose mobilization.

Two different berry extracts (1 & 2) stimulate phosphorylation of FOXO1A but the active ingredients fractionate differently on SPE.
Summary

- Berry polyphenols have bioactivities that may influence human health.
- Their mechanisms of action are not well defined but efficacy not always related to antioxidant capacity.
- Structure-activity relationships are beginning to be gleaned.
- Their stability and bioavailability *in vivo* is not fully understood but components can be identified in blood, urine and faeces that are characteristic of their uptake and metabolism.
Acknowledgements

All staff in CPU, JHI

B.Sc and M.Sc students
Questions?

Visit http://www.hutton.ac.uk

JHI is located in Invergowrie on the north bank of the River Tay
Other areas

Effect of tea and coffee polyphenols on neurodegeneration and obesity models resp.

Analysis of carotenoids in sea buckthorn & carrot

Anti-parasitic effects of berry and vegetable extracts

Natural products as anti-inflammatory agents
Developing high-through-put methods for assessing inheritance of polyphenols

- Link to genetic maps and markers to speed up selection
- Improve on traditional means of assessing polyphenol levels slow
- Develop and validate new methods
- Use power of mass spectroscopic and metabolic profiling methods

How can polyphenols affect human health?

Antioxidant theory? Low serum bioavailability!

Majority of polyphenols remain in gut
Are these components inactive?

Possible roles
Modulating colonic microbiota?
In-gut antioxidants?
Benefit gut epithelia function / colon cancer

Modulate digestive processes
Targeted analysis
Yield, flavour, aroma taste, texture, disease resistance, bioactivities, antioxidant capacity, polyphenol content, ascorbate, anthocyanins

Untargeted analysis
Put metabolic profiling

Hierarchical cluster analysis: Measure of (phytochemical) biodiversity - link to genetic map

Correlation Network: Interrelate metabolite changes.

Principal component analysis of MS data

Two environments, 5 seasons

MS spectra

Direct Infusion MS
No chromatography

Chromatogram
(10^2-10^3 compounds)
The inhibitory components in raspberry are Ellagitannins

Tannins bind to amylase and prevent starch digestion

? Full story?
Effect of different cooking regimes on rhubarb polyphenols

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ABSTRACT

Polyphenolic components, such as anthraquinones and stilbenes, from species of the genus Rheum have been shown to have a range of bioactivities relevant to human health. This paper outlines the polyphenolic composition of edible peels of garden rhubarb (Rheum rhabontigen) and describes the effects of common cooking methods on total polyphenolic content, anthocyanin content and total antioxidant capacity.

Most cooking regimes (fast stewing, slow stewing and baking) except blanching increased total polyphenol content and overall antioxidant capacity, compared to the raw material. The patterns of anthocyanin content and total polyphenol content between the different cooking regimes suggested a balance between two processes: cooking facilitated the release of polyphenol compounds from the rhubarb but also caused breakdown of the released compounds.

Baking and slow stewing offered the best maintenance of colour through preservation of anthocyanin and the highest antioxidant capacity. Baking for 20 min provided well-cooked rhubarb with the highest antioxidant capacity and the highest anthocyanin content, which is important for the aesthetic quality of the dish.

Liquid chromatography–mass spectrometric (LC–MS) analysis putatively identified over 40 polyphenol components in raw rhubarb, including anthraquinone, stilbene, anthocyanin and flavonol derivatives. Baking caused selective effects on the stability of the different polyphenol components. Initially, the yield of all components increased but there was a drastic decline in the relative stability of anthraquinone aglycones with increasing cooking time and initial evidence for the turnover of other anthraquinone derivatives was obtained.

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Stability and Bioavailability

Raspberry ellagitannins inhibit cancer cell growth
Ellagitannins bind to proteins in media
Not taken up by cells!
Breakdown to release ellagic acid
What is the active anti-cancer component?
In vitro digestion

Stability not related to aglycone or sugar moiety

For Pelargonidin
Glc > GRut > Rut > Soph

For Cyanidin
GRut > Soph > Rut > Glc

Stability dependent on components in mixture

*Total Recovery wrt gastric figures - McDougall et al. (2005) JAFC 53 5896-5904
In vitro digestion

Which components stable and bioavailable?

Simulation of human digestive system

1. Gastric digestion – 2 hrs at 37°C at pH 1.7 with pepsin

2. Pancreatic digestion – 2 hrs at 37°C with digestive enzymes and bile salts

Analyse recovery of components
Ellagitannins – most effective anti-cancer polyphenols in raspberry

Are these components bioavailable?

Do they get from berries – gut – site of action?

Ellagic acid
