Naturally Functional: overview of new science

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Functional Ingredients

- Traditional/functional - with added health benefit
  - Ascorbic acid and tocopherol (preservative)
  - Colour additives (including berry extracts)
  - Fibre (gelling, thickeners)
- Functional – provide health benefit exclusively
  - Polyunsaturated fatty acids
  - Pro- and prebiotics
  - Dark chocolate
  - Berry extracts
Naturally Functional
Flavonoids in Foods

- Isoflavones
  - Daidzein
  - Genistein
  - Glycitein
  - <5 mg/d

- Flavan-3-ols
  - Catechin
  - Procyanidins
  - Epicatechin
  - 157 mg/d

- Flavanones
  - Hesperetin
  - Naringenin
  - 14 mg/d

- Flavonols
  - Quercetin
  - Kaempferol
  - 13 mg/d

- Flavones
  - Apigenin
  - Luteolin
  - <5 mg/d

- Anthocyanins
  - Cyanidin
  - Pelargonidin
  - Malvidin
  - 3-215 mg/d

Total intake of polyphenols approximately 3 g/day
Average serving berries – 400-500 mg
Flavonoids

**Roles in Plants**
- Provides colour
- Bitter taste
- Attracts symbiotic bacteria
- Antimicrobial action

**Effects in Humans**
- Improved blood lipid profile
- Decrease in BP
- Decrease in CRP
- Decrease in CVD Mortality
Scientific Evidence

- **Population Studies**: generally epidemiological studies support the notion that foods rich in flavonoids provide cardiovascular benefit

- **Randomised Controlled Trials**: intervention studies show activities of flavonoids on circulating biomarkers of CVD risk

Cassidy et al., 2010-2011; Kay et al., 2012
Review of Red Grapes and Red Wine

• Red wine & purple grape juice ↓ inflammation

• Chronic or acute consumption of purple-grape-juice or red grape extract causes improvement in FMD (in CAD patients)

(Cassidy et al., 2011; Hooper et al., 2008)
Review Of Olive Oil

- High polyphenol oil (acute, 4h) ↑ NO and ischemic reactive hyperemia

(Cassidy et al., 2011; Hooper et al., 2008; Kay et al., 2006)
Review of Tea

- ↓ CVD risk biomarkers

- Acute & chronic consumption tea 2 to 5 cups/d ↑ fmd response

(Cassidy et al., 2011; Hooper et al., 2008; Kay et al., 2006)
Review Of Chocolate/Cocoa

- Reduced biomarkers of CVD risk
  - ↓ platelet aggregation
  - ↑ FMD

(Cassidy et al., 2011; Hooper et al., 2008; Kay et al., 2006)
Field of Research

• Reviewed favourable results of a few individual dietary interventions

• What happens when we summarise the entire field of research?
Summary of Intervention Studies/ Meta-Analysis

- Structured search:
  - MEDLINE, EMBASE, Cochrane
- Data extraction/quality assessment:
  - 2 independent reviewers
  - Inclusion criteria eg suitable control arm, randomised
- Examine relative importance of different subclasses on biomarkers of risk

6393 abstracts
↓
582 papers
↓
133 included interventions (242 papers)

Hooper et al., 2008 – Kay et al., 2012
Flavonoid Subclasses & FMD

RCTs: 15 chronic, 14 acute

- Red wine, grape, black tea - modest benefit on CVD risk
- Chocolate & cocoa - also significant chronic intake effect, when sub-grouping by epicatechin dose

Hooper et al 2008
Flavonoids & Diastolic BP

- Chocolate ↓ diastolic BP (3mmHg) & systolic BP (6mmHg)
- Differential effects with different soy sources
Overall Finding

• Studies are difficult to compare
  • Design, population, control/placebo, dose, duration…

• Global comparisons difficult
Updated Analysis of Harvard Cohorts

• 87,242 women NHS
• 46,672 men HPFS
• Unpublished data

Adjusted for:
age & smoking, BMI, physical activity, alcohol, family history of hypertension, aspirin, multivitamins, sodium, magnesium, potassium, fibre, wholegrains, folate and caffeine
Habitual Intake Of Flavonoids & Incident Hypertension

A. NHS II

Total anthocyanin intake

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<tr>
<th>Flavone</th>
<th>Apigenin</th>
<th>Pooled RR</th>
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<td>Flavan-3-ol</td>
<td>Catechin</td>
<td>0.93 (0.88-0.97)</td>
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<td>Epicatechin</td>
<td>0.95 (0.91-1.00)</td>
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<td>Anthocyanin</td>
<td>Cyanidin</td>
<td>0.88 (0.82-0.94)</td>
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<td>Malvidin</td>
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## Habitual Intake Of Flavonoids & Parkinson’s Disease Risk In Men

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<th>Case = 347</th>
<th>RR</th>
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<tr>
<td>Total Flavonoids</td>
<td>0.55 (0.38 - 0.80)</td>
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<td>Flavonols</td>
<td>0.64 (0.44 - 0.94)</td>
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<td>Flavones</td>
<td>0.91 (0.65 - 1.29)</td>
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<td>Flavanones</td>
<td>0.86 (0.61 - 1.21)</td>
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<td>Flavan-3-ols</td>
<td>0.59 (0.40 - 0.86)</td>
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<tr>
<td>Anthocyanins</td>
<td>0.61 (0.43 - 0.86)</td>
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<tr>
<td>Polymers</td>
<td>0.49 (0.33 - 0.71)</td>
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</table>

- Higher Anthocyanin intake associated with slower rates of cognitive decline
- (preliminary analyses)

Adjusted for age, smoking, BMI, alcohol, aspirin, energy, caffeine, lactose

Gao, Rimm et al – unpublished data
Clearly Activity: but…

- must consider a few complexities
  - Bioavailability
Relative Bioavailability

- Isoflavones
- Flavan-3-ols (Catechins)
- Flavanones
- Flavonols
- Anthocyanins
- Procyanidins
- Flavones

- Bioavailability
  - Isoflavones: ~30%
  - Flavan-3-ols (Catechins): ~9.7-18.5%
  - Flavanones: ~8.7%
  - Flavonols: ~2.5%
  - Anthocyanins: ~0.4%

- Important to understand bioavailability & metabolism

Manach et al 2005; D'Archivio et al., 2007;43:348-361
What Mechanisms of Action?

- What types of mechanisms could be affected at these low plasma concentrations?
- If we are going to design functional products we need to know target mechanisms of action
Antioxidant Capacity

Oxygen Radical Absorbance Capacity (ORAC)

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<tr>
<th>Fruit</th>
<th>Trolox Equivalents per 100 g of Fresh Fruit</th>
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<td>Pears</td>
<td>134</td>
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Oxidative Stress Theory

\[ \text{Oxidant} = \text{Antioxidant} \]

Equilibrium

Inflammation

Oxidative Stress

Chronic Disease

Antioxidant

Oxidant
Are Flavonoids Likely To Affect Global Redox Status Directly?

- LIKELY NOT…WHY?

- Concentration relative to endogenous antioxidants
Contribution of plasma antioxidants to total antioxidant capacity, expressed as a percentage of total plasma FRAP

Hollman PCH (2014) Unravelling of the health effects of polyphenols is a complex puzzle complicated by metabolism; Archives of Biochemistry and Biophysics; Volume 559, 1 October 2014, Pages 100–105. Polyphenols and Health.
What We Need is More Advanced Models

13C5-C3G (500 mg)

Bioactivity Screen
HUVEC, SMC, HAEC: NOX, eNOS, endothelin, HO-1, ET-1, VCAM, IL6, TNF-α, Nrf2, NfkB

RCT
Cardiovascular bioactivity (vascular reactivity)

Metabolite Pharmacokinetics

- Phase II conjugates
- Degradants
- Hippuric acid
- C3G
- Phenylacetic/
- Phenylpropenoic

13C-Tracer Study: ADME

Blood & breath
Urine & faeces

Relative bioavailability: IRMS
Metabolite Identification
HPLC-MS/MS Q-Trap

Metabolite Synthesis

Synthesis
Overview of study design. 8 healthy men 18-45y. 7 days of diet restrictions (no anthocyanin and low $^{13}$C containing foods). 500mg of $^{13}$C$_5$-labeled cyanidin-3-glucoside bolos followed by 48 hours of urine, blood, faeces and breath collection.
Metabolism
Serum Metabolite Pharmacokinetics

**Study Design**

- Metabolites
- Time points
- Study Design

**Concentration (nM)**

- 2500.00
- 2000.00
- 1500.00
- 1000.00
- 500.00
- 0.00

**Metabolites**
- Hippuric acid
- Ferulic acid
- Ferulic acid (+3)
- PCA
- PCA-sulfate
- M34dhbz
- M34dhbz (+3)
- 4-HydroxyBAL (+2)
- PGA (+3)
- C3G
- BA-4-GlcA
- VA-4-GlcA
- IsoVA-3-GlcA

**Time points**

- 0
- 6
- 12
- 18
- 24
- 30
- 36
- 42
- 48

**LC-MS/MS**

De Ferrars et al, *Br J Pharmacol* 2014
Conclusions from $^{13}$C approach

- Anthocyanins are extensively metabolised
- Anthocyanin metabolites are found in much higher concentration than their parent structures and often have much longer $t_{1/2}$
Implications for Product Development

- Characterising the bioactivity of ‘altered/metabolised’ components provides commercial/economic benefit by:
  - Informing study design
  - Provide evidence for future health claims
  - Aiding in establishing ‘new’ functional-products
Are They Bioactive?

• Are The Metabolites Of Anthocyanin Degradation Products Bioactive?
Structural Activity

3-hydroxybenzoic acid

Vanillic acid (VA)

Hippuric acid

Salicylic acid

Apocynin

Tryptophan
Flavonoid metabolism: The synthesis of phenolic glucuronides and sulfates as candidate metabolites for bioactivity studies of dietary flavonoids


1a \( R^1 = \text{CO}_2\text{H}, R^2 = \text{H}, R^3 = \text{H} \)
1b \( R^1 = \text{CO}_2\text{H}, R^2 = \text{H}, R^3 = \text{OCH}_3 \)
1c \( R^1 = \text{H}, R^2 = \text{CO}_2\text{H}, R^3 = \text{OCH}_3 \)
1d \( R^1 = \text{H}, R^2 = \text{CO}_2\text{H}, R^3 = \text{OH} \)
1e \( R^1 = \text{CO}_2\text{H}, R^2 = \text{H}, R^3 = \text{OH} \)

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2e \( R^1 = \text{CO}_2\text{H}, R^2 = \text{H}, R^3 = \text{OH} \)
Screening Models

THP-1 cells – monocyte model

Western Blot
ELISA
PCR

Inflammatory Stimulus
What Is Novel About This Work?

1. Use of physiologically relevant levels
2. Use of physiologically relevant metabolites
3. Studying the effects of combinations
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<th>Combination treatment</th>
<th>Hesperetin</th>
<th>Peonidin-3-glucoside</th>
<th>Epicatechin</th>
<th>Cyanidin-3-glucoside</th>
<th>4-hydroxybenzoic acid</th>
<th>Benzoic acid-4-gluconuride</th>
<th>PCA-3-glucuronide</th>
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<th>PCA-3-sulfate</th>
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<th>Isovanillic acid</th>
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Vascular Function

Vasodilation

Vasoconstriction

Increased Transport of oxygen & nutrients
Removal of waste products
Vascular Inflammatory Damage

Negative Stimulus

Single molecule release

Vessel lumen

Blood flow

Neutrophil

Rolling

Activation and adhesion

Migration

Endothelial cells

Tissue
Parent Flavonoids

Peonidin-3-glucoside
Combinations

Protocatechuic acid & 4-Hydroxybenzoic acid

n ≥ 3. *p≤ 0.05, **p≤0.01 ANOVA, Post-Hoc Tukey
Combinations

Protocatechuic acid, 4-Hydroxybenzoic acid & Vanillic acid

**TNF-α Production (pg/mL) Normalised to LPS Control**

- VC
- 0.1 μM
- 1 μM
- 10 μM
- 100 μM

*n ≥ 3. *p≤ 0.05 ANOVA, Post-Hoc Tukey
Dose-response effect of PCA on the secretion of sVCAM-1 in TNF-α stimulated HUVEC

Columns represent the mean of three biological replicates normalised to a TNF-α stimulated control (dashed line) ± standard deviation. † p≤ 0.15, * p≤ 0.05, **p≤0.01 (ANOVA with Post-Hoc Tukey).
Summary

• Important to investigate the effects of combinations as well as single compounds in isolation.
  
  • Possibility of additive and/or synergistic effects must not be overlooked.

• Important to consider physiological relevance of compounds and concentrations assessed.
Nutritional Relevance

• Dietary relevance:

  ✓ The more physiologically relevant metabolites are active at much lower concentrations

  ✓ The fact that combination treatments were active indicates the use of flavonoid-rich foods to promote health over the need for powdered-extracts or pure/pharmaceutical preparations

  ✓ Prevention model
Industrial Relevance

• This suggests that bioactivity *in vivo* may result from a cumulative low-exposure to a variety of structurally similar metabolites:

  ✓ Health effects may be achievable following simple changes in dietary patterns or supplementation with flavonoid-rich products
Present and Future Research Focus

- CVD
- Cancer
- Vascular function/reactivity
- Induction of antioxidant response element
- Inhibition of cytokine and adhesion molecule secretion
- Colonic speciation & health
- Age-related cognitive decline
  ✓ Neuronal: inflammation, morphology, vascular effects

Rodriguez-Mateos et al., 2014; Archives of Toxicology. 88;10:1803-1853
Future Research into Health Claims

• Ultimately, evidence will:
  ✓ inform future dietary interventions exploring optimal dietary intakes of functional foods/products containing flavonoids
  ✓ *paradigm shift: target foods as precursors for establishing optimal blood metabolite profiles*
  ✓ provide data required to establish health claims for flavonoid-rich foods and beverages
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DRINC - DIET AND HEALTH RESEARCH INDUSTRY CLUB
THANK YOU
Replacing synthetic ingredients with natural ingredients which also have added health benefits such as omega-3, fibre, proteins and antioxidants, enables manufacturers to tap into other super trends and offset some of the higher costs associated with sourcing and formulating natural ingredients. Through case studies, market analysts and manufacturers will discuss how to overcome technical and financial obstacles to realise the value of ingredients with 2 benefits in 1.

Chair: Lu Ann Williams, Director of Innovation, Innova Market Insights

12.30 - 12.50 Commercial benefits and opportunities in functional natural ingredients

Lu Ann Williams, Director of Innovation, Innova Market Insights

12.50 - 13.10 Delivering health and energy through functional beverages

Tricia McNeilly, Founder/Directory, CocoMojo

13.10 - 13.30 High Lipid Algal Flour for healthier nutrition with no compromise

Valerie Barfoot, Business Development Manager, Roquette UK

13.30 - 14.00 Naturally functional – overview of new science

Dr Colin Kay, Senior Lecturer, Department of Nutrition and Director of LTQ for Post Graduate Research, Norwich Medical School, University of East Anglia