Reporting Requirements for Flavonoid Research: A critical component in enhancing our understanding

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Design/reporting of research has created challenges for development of recommendations for flavonoid intake.
Recommendations for designing, implementing, and reporting clinical studies for flavonoids

Klein MA et al., J Nutr 2010;140:1192S–1204S.

The Journal of Nutrition

Supplement: Guidance from an NIH Workshop on Designing, Implementing, and Reporting Clinical Studies of Soy Interventions

Guidance from an NIH Workshop on Designing, Implementing, and Reporting Clinical Studies of Soy Interventions

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CONSORT 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomized Trials

Kenneth F. Schulz, PhD, MBA; Douglas G. Altman, DSc; and David Moher, PhD for the CONSORT Group*

The CONSORT (Consolidated Standards of Reporting Trials) statement is used worldwide to improve the reporting of randomized, controlled trials. Schulz and colleagues describe the latest version, CONSORT 2010, which updates the reporting guideline based on new methodological evidence and accumulating experience.
Flavonoid Rich Foods/Diets & Health

Design & Reporting Consideration

Definition and assessment of Flavonoid Materials

Development of Evidence Based Dietary Guidance
Robbins et al.

Analysis of flavanols in foods: what methods are required to enable meaningful health recommendations?

Cardiovasc Pharmacol. 2006;47 Suppl 2:S110-8

“Underpinning the studies required to establish the relationship between the consumption of flavanol-rich foods and cardiovascular health is the need to have specific flavanol composition data of the foods employed in these studies.”
Gaps in definition, assessment and reporting of flavonoid test materials

1. Terminology/nomenclature for flavonoids and plant materials
2. Analytical methodologies
3. Clear definition of test materials used in clinical and preclinical studies
4. Description of potential food matrix effects including processing and stability
NIH NCCAM Guidance on Natural Product Integrity

- Guidance on description of complex botanical products and natural products for use in preclinical and clinical investigations
- Guidelines for establishment of product integrity and quality considerations for clinical trials and research
- FDA Guidance on investigative new drug

Impact of Guidance Document from FDA regarding review of Investigational New Drug (IND) status

• “…an edible product that might otherwise be a conventional food is intended for a use other than providing taste, aroma, or nutritive value... the product becomes a drug because the primary purpose of consuming it has changed”

• Investigation intended to evaluate other effects of a food on the structure or function of the body would require an IND
  – Example: “study of the effect of soy isoflavones on bone metabolism”

Key assessment and reporting considerations for flavonoid test materials used in clinical/preclinical studies

- **Botanical source**
  - Identification of plant origin and use of taxonomic nomenclature
  - Supplier source, batch, etc.

- **Flavonoid definition and quantitation**
  - Use of established and specific nomenclature for flavonoids
  - Use of advanced and specific methodologies for specific flavonoids

- **Food matrix form for intervention/trial**
  - Detailed compositional information of research materials, foods etc
  - Nutritional composition
  - Presence of other bioactive components

- **Stability of flavonoids to processing, storage and experimental conditions**
  - Establish and monitor archival materials
  - Assessment and reporting of stability of the test materials
Nomenclature for reporting key flavonoid subclasses

**Basic Flavonoid Subclasses (Aglycone)**
- Flavanol – (Catechin)
- Flavanone – (Hesperetin)
- Flavone – (Apigenin)
- Isoflavone – (Genistein)
- Flavonol – (Quercetin)
- Anthocyanidin – (Malvidin)

**Tannins**
- Condensed Tannins – (Procyanindin & Proanthocyanidins)
- Derived tannins – (Theaflavins, Thearubigins, Theasinensins)
Key reporting considerations for flavonoid test materials used in clinical/preclinical studies

• Use of “broad terms” should be avoided in reporting
  – e.g. Phenolics, Polyphenolics, Phytonutrients, Antioxidants etc.

• Use of standardized flavonoid nomenclature describing appropriate subclasses and specific form should be used
  – e.g. aglycone vs glycosides
  e.g. degree of polymerization (dp) in case of oligomers and polymers

• For quantitation in foods/diets, expression in “aglycone equivalents” can facilitate comparisons across studies
Specificity of common analytical methods applied in cocoa flavonoid research

Adapted from Robbins et al. (2006)
Specific method facilitate assessment and reporting of stability of archived flavonoid materials

**Archived Grape Seed Extract**

- BC/0712122001 - GSE crude

12 month Flavan-3-ol stability (mg/g, dry weight) of grape seed extract assayed using HPLC/UV (280 nm)

**Grape Seed Extract in drinking water**

Flavan-3-ol stability (% of initial) of Grape Seed Extract as a 1% solution in dd H₂O
Specific Methodology can differentiate changes in flavonoid profile that may impact functionality

Bioavailable

(+) Catechin

(-) Epicatechin

Roasting
Alkali Processing

(-) Catechin

Less Bioavailable

Hurst et al. Chemistry Central Journal 2011, 5:53
Donovan et al., Free Radic Res. 2006 Oct;40(10):1029-34
Development of databases (Intake Determination)
Flavonoid Rich Foods/Diets & Health

Design & Reporting Consideration

Design and outcomes of Human Studies

Development of Evidence Based Dietary Guidance
Specific Consideration for Dietary Intervention Trials

Test materials and Product

- Flavonoid materials
  - Single flavonoid, single class, mixture, food?

- Test Food or Product
  - Consider product formulation and structure
  - Use of commercially standardized product

- Nature of Control Product
  - Well-matched in sensory and quality attributes
  - Expected to be consumed in the context of a diet
  - Basal level of flavonoids or flavonoid free?
Specific Consideration for Dietary Intervention Trials

Participants

• Clearly state eligibility, exclusion criteria and rationale
  – High consumers of flavonoids, medication or habits that would influence absorption, metabolism, etc.
  – Healthy, “At Risk”, Disease state?

• Determine and report background flavonoid intake
  – Appropriate and focused use of Databases and Intake data

• Cite and/or assess and report bioavailability from similar foods
  – Influence of food formulation, processing as well as flavonoid source, content/composition must be considered
Complexity of bioavailability and metabolism of flavonoids must be considered in study design and reporting.
Specific Consideration for Dietary Intervention Trials

Study Design & Reporting

• Acute versus longer term intervention
  – Washout period or background flavonoid intake
  – Run in period
  – Targeted health status of participants

• Consumption frequency should be clearly reported
  – Short flavonoid half-life requiring repeated daily doses

• Absorption and compliance with intervention should be confirmed with plasma or urinary markers
  – Urinary Phenolics = total flavonoids
  – Theobromine = Cocoa
  – 4-O-methyl gallic acid = Tea
Flavonoid Rich Foods/Diets

Design & Reporting Consideration

Design and outcomes of Preclinical Studies (MOA)

Development of Evidence Based Dietary Guidance
Design and reporting of animal studies

Design

• Species differences in flavonoid “handling and processing”
• Duration and point of intervention in animal life cycle
• Doses relevance to humans?

Reporting: Provide the physiological context

• Test material administration mode (diet, water, gavage, etc)
• Dose reporting in unit per kg BW to facilitate comparisons between studies and models
• Human Equivalent Dose Calculation
**Considerations for in vitro and cell based studies**

**Relevance of material and in vitro/cellular conditions**
- Flavonoid forms in food ≠ Physiological forms
- Concentration in food/extract ≠ Physiological concentrations
  \[ \mu M \text{ to } mM \quad \text{pM to nM} \]

**Consideration of Flavonoid Metabolism**
- Commercial availability of metabolites
- When synthesized, structure and chemical nature of metabolites should be confirmed and documented
Metabolite characterization and commercial availability for quantification and MOA studies


Structure confirmed by NMR to be: 3’-OMe-(-)EC-5-β-glucuronide
Take Home Messages Related to Design and Reporting of Flavonoid Studies

• Apply consistency in reporting according to standardized flavonoid nomenclature and guidelines for material sourcing and QC

• Improved characterization of the specific identity, quantity and stability of constituents in test materials
  – (e.g. flavonoids, as well as other bioactive compounds)

• Development of appropriate controls for flavonoid materials and foods

• Need for development, characterization and application of metabolite standard materials
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Specific methodology would also detect subtle changes in qualitative flavonoid profile.

General Stability Flavanols in RTD beverages

Higher Stability Preserved RTD tea beverages

Hot Fill RTD tea beverages

Retort tea RTD beverages

Retort tea-milk RTD beverages

pH

3

7

Stability

Higher

Lower

~15-20% conversion may occur in some thermally processed beverages (Chen et al. 2001).