

What are the risks/benefits of prebiotic carbohydrates

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Outline

- Prebiotic - definition, concepts & scientific rationale, selection criteria
- Status - accepted and emerging prebiotics
- Weight of evidence for health benefits of confirmed prebiotics
- Candidate prebiotics – resistant starches
- Possible adverse effects
- Opportunities: alternative prebiotics & synergies
- Directions for future R&D

Prebiosis – Historical Background

Current definition:

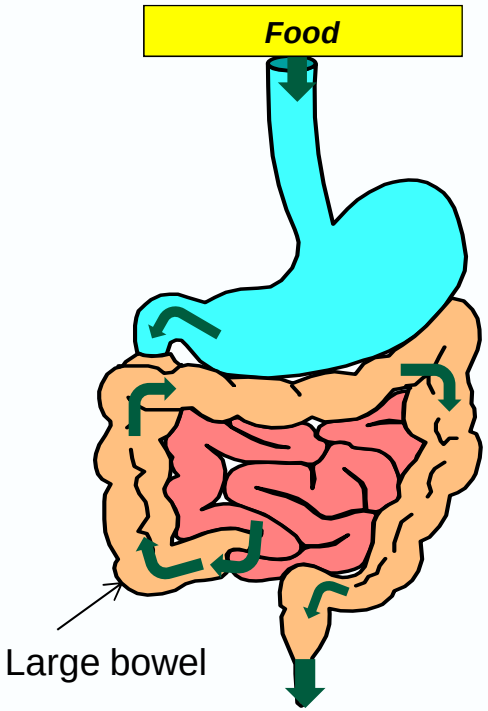
*Prebiotic : a **selectively** fermented ingredient that results in **specific** changes in the **composition** and/or **activity** of the gut microbiota, thus conferring benefit(s) upon host **health***

New area of nutrition research (the term 'prebiotic' coined in mid 1990s)

Concept dates back to 1950s

"bifidus factor" in human milk promoted the growth of bifidobacteria in bottle-fed infants

Gut Microbiota & Host Health



Colonic microbiota plays an important role in human health

- Complex & dynamic microbial ecosystem
- Taxonomically & metabolically diverse (10^{13} bacteria, ~500 g, >1000 spp)
  - Barrier function (colonisation resistance – outcompetes pathogens)
  - Maturation and maintenance of intestinal and systemic immune responses
  - Salvages energy & provides nutritional support (vitamins, SCFA)

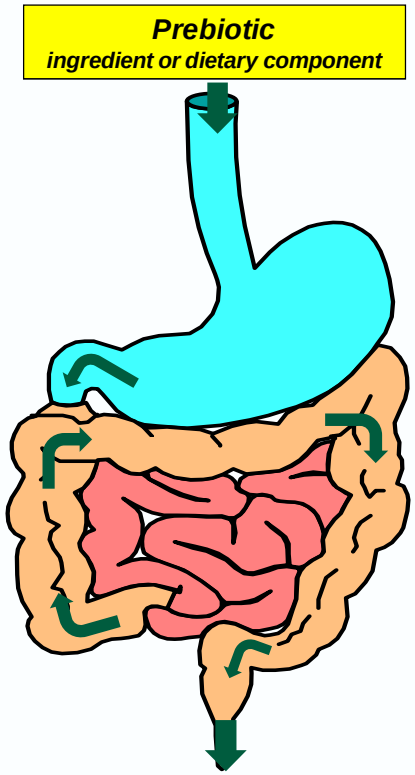
Microbiota composition & metabolism impacts host health

- Microbial metabolites regulate host physiology, biochemical pathways, gene expression
- Microbiota can also have adverse effects (infections, toxins)
  - Compositional changes (dysbiosis) linked to disease (obesity, IBD, IBS)
  - "Westernized" microbiota – reduced taxonomic diversity and richness
- Delicate balance between homeostasis and dysbiosis

Diet determines microbial populations & metabolic function

- Diet-based strategies to achieve "eubiosis" and promote host health and reduce disease risk

Prebiotic Carbohydrates, Microbiota & Health



Faecal samples
Surrogate for luminal bacterial community

Prebiosis: manipulation of the composition of the indigenous colonic microbiota to promote health

Colonic microbiota utilises (ferments) food escaping assimilation in upper gut

Carbohydrates are the major food source (glycophiles/saccharolytics)

- Dietary fibre – nonstarch polysaccharides, resistant starches, fructans

Proteins, lipids, phytochemicals - diet determine their relative importance as substrates

Endogenous substrates (epithelial cells, digestive enzymes, mucins)

Saccharolytic fermentation conducive to host health

Favourable bacterial spp, metabolites (SCFA) & luminal conditions (low pH)

Type & amount of dietary carbohydrate shapes microbiota composition & function

Intense competition for carbohydrates

Heterogeneous environment – spatial colonisation & metabolite gradients

Prebiotic - substrate for saccharolytic (beneficial) bacteria

Prebiotics: Qualifying Criteria

Digestion resistant carbohydrates

"Prebiotics are dietary fibres but not all dietary fibres are prebiotics"

Selectivity

Composition

- Prebiotics support the growth of only beneficial (probiotic) bacteria
- Bacterial targets have been bifidobacteria (and lactobacilli)

Metabolic activity of the microbiota?

Prebiotic claim - supported by evidence from more than one human study

- Mechanistic studies in animal models important for building the evidence base
- Quantitative molecular methods are essential

Established and Candidate Prebiotics

Nondigestible oligosaccharides (eg inulin-type fructans, FOS, GOS)

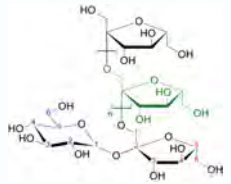
Commercial ingredients - production of foods, beverages and nutraceuticals

Established Prebiotics	Candidate	Do not qualify?
Fructans Inulin, Oligofructose, FOS	Resistant starch	Nonstarch polysaccharides
Galactans Galactooligosaccharides (GOS) Transgalactooligosaccharides	Arabinoxylan oligosaccharides	
Lactulose	Soy-oligosaccharide	
	Isomaltooligosaccharides	
	Xylosaccharides	
	Mannooligosaccharides	
	Lactosucrose Nigeroligosaccharides Gentiooligosaccharides	

Inulin-type fructans

Linear, fructose oligomers and polymers; ± terminal glucose; glycosidic bonds are β-(2→1) configuration

- Inulin– number of sugar moieties varies from 2-60, mean dp 12
- Oligofructose - dp<9; produced from enzymatic hydrolysis of inulin
- Fructooligosaccharide (FOS) dp 3-5; synthetic (from sucrose)



Commercial sources: primarily chicory root

Dietary sources: wheat, onions, garlic, banana, artichokes, leeks, asparagus

- Intake: 3-11 g/d Europe; 1-4 g/d USA; wheat accounts for 70% of total fructan intake

Inulin-type fructans are well established and widely used prebiotics

- Concentrates/Isolates - crude and purified fractions
- Consistently increase intestinal bifidobacterial populations
- Dosage: 4-15 g/d (but as little as 1 g in some people is effective; response highly individualised)
- Augment other potentially beneficial bacterial populations (eg Lactobacilli, Eubacteria, Roseburia) but effect is less consistent
- Bifidobacterial populations return to baseline in 1-2 wk after cessation of prebiotic intake

Inulin-type fructans

Health Benefits (claimed)

- **Gastrointestinal**
  - Bowel function – normalise colonic transit time, increase stool frequency, faecal bulk
  - Colonic health – IBD, IBS, diarrhoea (antibiotic-associated; traveller's), colorectal cancer
- **Metabolic**
  - blood lipids: TG, LDL cholesterol
  - attenuate blood glucose & insulin levels
- **Bone**
  - Mineral bioavailability – increased calcium absorption & colonic uptake of Ca, Mg in target cohorts
  - Bone mineral density
- **Immune System**
  - Modulation of gut immune system
  - Immune function enhanced in adults and children; may help combat infections and inflammatory conditions
- **Weight management, satiety**

Inulin-type fructans

Health Benefits - Evidence

- **Gastrointestinal**
  - **Bowel function** – normalise colonic transit time, increase stool frequency, faecal bulk✓
  - Colonic health – IBD, IBS, diarrhoea (antibiotic-associated; traveller's), colorectal cancer,
- **Metabolic**
  - blood lipids: TG, LDL cholesterol
  - attenuated blood glucose & insulin levels
- **Bone ✓**
  - **Mineral bioavailability** – increased calcium absorption & colonic uptake of Ca, Mg in target cohorts
  - **Bone mineral density**
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Are other types of fibre prebiotics?

Oligosaccharides✓

e.g. Jerusalem artichoke, chicory, agave

Non Starch Polysaccharides?

NSP are the major fibre component of the Australian diet

β-glucans, pectin, gums, cellulose, arabinoxylan, hemicelluloses.....

Soluble & insoluble fibres

Limited evidence of prebiosis - only a few human studies, mixed results, methodological deficiencies

Recent evidence that certain wholegrain foods are prebiotic

Resistant starches

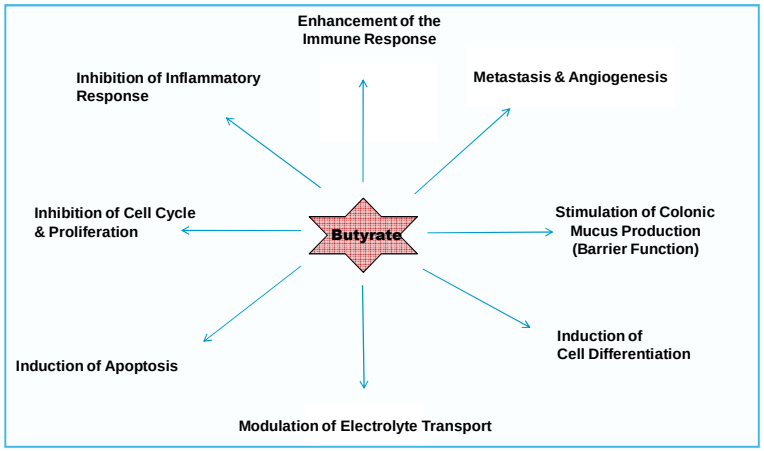
The fraction of dietary starch that escapes from the small intestine into the large bowel

Resistant Starches

Unlike nonstarch polysaccharides, resistant starch acts principally through the products of its fermentation by the commensal microflora

- short chain fatty acids – acetate, propionate and butyrate

Resistant starch is butyrogenic
- fermentation yields more butyrate than other fibres



Types of Resistant Starch

Type	Source
RS_1 – physically inaccessible	Whole or partly milled seeds or grains
RS_2 – resistant granules	Raw starches (eg banana), high amylose starches
RS_3 – retrograded	Cooked and cooled potato, cornflakes
RS_4 – chemically modified	Food ingredients
RS_5 –V form	Inclusion complexes formed by amylose and polar lipids

Resistant Starch, Prebiosis & Gut Microbiota

Molecular methods have shown that diets containing resistant starch produce significant changes in the faecal microbial population structure

In vitro studies demonstrate that RS types 1 to 4 are bifidogenic

In vivo studies – mainly using resistant maize starches

- Rats: RS2 (HAMS), RS4 (butyrylated maize starch), RS1 & 2 (high amylose wheat) *Conlon et al., 2011 J Nutr*
- Pigs: RS2 (HAMS) *Bird et al., 2007 Brit J Nutr*
- Humans: RS 2 (HAMS), RS4 (phosphorylated, cross-linked wheat starch) *Martinez et al., 2010 PLoS ONE*
  - Bifidogenic efficacy & time course dependent on starch type; bacterial attachment to granules important
  - Large interindividual responses (host factors: physiology & microbiota composition; indigenous spp absent? diet?)

Resistant starches had positive effects on microbial metabolic endpoints and host health biomarkers – but only is some studies was a prebiotic (bifidogenic) effect observed

Resistant Starch, Prebiosis & Gut Microbiota

Resistant starches increase various genera of saccharolytic bacteria (other than bifidobacteria) known to facilitate starch fermentation and SCFA production:

- Butyrate-producing Clostridia cluster – enrichment of phylotypes related to:
  - *Faecalibacterium prausnitzii*
  - Fusobacterium/Roseburia cluster
  - *Ruminococcus bromii*

- Q-PCR confirmed a significant increase in *R. bromii* in response to RS (not NSP alone) (*Abell et al. 2008)*

- *Eubacterium rectale and R. bromii (Walker et al., 2010)*

Resistant starch also reduces numbers of pathogenic bacteria

- Coliform and E coli populations depleted in the proximal colon of young pigs fed cooked white & brown rice high in resistant starch (RS1 & 3) reduced

Resistant Starch & Gut Microbiota

Resistant starch alters the luminal environment of the large bowel

Changes are consistent with improved bowel health

- Increases SCFA levels, especially butyrate
- Lower luminal pH & levels of toxic metabolites
- Benefits extend beyond the proximal colon

Resistant starch has profound effects on the population structure of the microbiota:

Phylum to species level changes

Increase Bacteroidetes/Decreased Firmicutes

Bifidobacteria, Ruminococcus & Eubacteria increased

Also *Ruminococcus bromii* and *Faecalibacterium prausnitzii*

Is resistant starch a prebiotic?

Responses dependent on the type of RS, varied markedly between individuals

Microbial population shifts occurred together with favourable changes in indices of bowel health

(eg increased butyrate levels with resistant starch diets)

Possible Adverse Effects of Prebiotics

Same as for high intakes of (other) dietary fibres:

- abdominal discomfort, bloating, flatulence, soft stools

Low molecular weight prebiotics (inulin type fructans):

- Side effects at doses >15 g/d, but individual specific (usual intakes 2-4 g/serving)
- Osmotic effects: watery stool, diarrhoea, nausea (depend on MW)
- Toxicological studies – no to mild adverse effects at high doses
- Long term use as food ingredients in many countries (eg Japan, Europe)
- Bifidobacteria (and lactobacilli) do not produce gas
    - Cross-feeding reactions, substrates fermented by other spp

Resistant starch is well tolerated (>30 g/d)

High intakes of dietary fibre (>40 g/d) from mixed sources:

- Large intra- and interindividual variation in response to dietary fibre consumption
- Incidence & severity of reported gut discomfort no different to low fibre controls

Opportunities: Other Fructans, Dietary Fibres & Prebiotic Combinations

Fructans widespread in nature, occur in 15% of flowering plants:

Traditional prebiotics are linear inulin-type β(2-1) fructans, but there are other types:

- Levan with β(2-6) linkages found in bacteria & fungi; high MW, also β(2-1) linkages
- Graminans, highly branched, mixed fructans containing both of the above linkages eg wheat
- Inulin neo-series eg onion, asparagus
- Levan neo-series

Molecular characteristics influence prebiotic efficacy – molecular size and branching

- Different chain lengths stimulate different bacterial (sub)groups

Synergies - combinations of different types of prebiotics (RS + FOS), dietary fibres + prebiotics

Evidence to Support a Prebiotic Claim

Strong evidence from in vitro and animal studies but data for humans is limited – fewer intervention trials investigating prebiosis and clinical endpoints; inconsistent results

No evidence that simply increasing any group(s) of microorganisms (including bifidobacteria & lactobacilli) is in and of itself a beneficial physiological effect

- Bifidogenic response is strain specific (not all spp can use fructans)

More research is needed on prebiotic-induced alterations in gut microbial populations and health outcomes:

- Human RCTs of suitable quality: adequate power, diet controlled, longer duration, dose-response
- Address methodological limitations – fecal sampling protocols, mucosal samples?
- Comprehensive (molecular) mapping of taxa (groups -strains) and function (microbiome)
- Supported by evidence from mechanistic studies in animals
- Many determinants of prebiotic response (esp baseline microbiota)
- Target cohorts - healthy individuals or those with a disrupted intestinal microbiota

Summary & Conclusions

- Manipulation of the microbiota in favour of health is an appealing strategy
- Commercial oligosaccharides (inulin, OF, FOS, GOS) are established prebiotics:
  - Greater abundance of one or more groups of commensal bacteria is not evidence of a health benefit
  - Soundly designed human studies required to confirm/extend the range of health benefits
- Resistant starches are promising prebiotics – but more studies in humans are required
- Prebiotic (and fibre) combinations more effective
- Personalised prebiosis is an imperative
- Traditional prebiotic approach too simplistic - what constitutes a healthy microbiota?
  - Broader focus: consider health promoting bacteria other than bifidobacteria (& lactobacilli)
  - Deleterious components of the microbiota – pathogens & toxigenic bacteria?
  - Metabolic activity (SCFA fermentation patterns, putrefactive metabolites?) –focus on microbial functional biomarkers (bacterial genes, biochemical endpoints)?
  - Prebiotic index?

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