Resistant Starch: physiological effects and *in vitro* fermentability

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Topics

- Introduction

- Physiological effects of RS in the GI tract:
  - Effects on blood parameters
  - Effects on colonic function

- *In vitro* fermentability of resistant starch:
  - *In vitro* fermentation method
  - Comparison of RS preparations
  - RS and butyrate production
  - Degradability of RS throughout life

- Summary
Definition of resistant starch

- Resistant starch (RS) is the sum of starch and starch degradation products not absorbed in the small intestine of healthy individuals

  - **RS 1: physically inaccessible starch**
    - partly milled grains, seeds
  - **RS 2: resistant starch granules**
    - raw potato, banana
  - **RS 3: retrograded starch**
    - processed foods
  - **RS 4: chemically modified starch**
    - gelling and thickening agents
Definition of dietary fibre
(AACC and AOAC approved)

- Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine.

- Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances.

- Dietary fibre promotes beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.
Resistant starch and lipid metabolism: Summary of studies

<table>
<thead>
<tr>
<th></th>
<th>Positive effect*</th>
<th>No effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animal</td>
<td>Human</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>LDL fraction</td>
<td>3</td>
<td>--</td>
</tr>
<tr>
<td>HDL fraction</td>
<td>3</td>
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</tbody>
</table>

*: increase in HDL; but decrease in all others

➢ On balance RS does not appear to influence these markers of lipid metabolism in humans

RS and glucose & insulin metabolism: Effects in humans

- Most studies have focused on postprandial glycaemic and/or insulinaemic responses
- Lack of consensus regarding precise effects:
  - 15 studies: improvement in responses following RS-rich test meals
  - 10 studies: no or physiologically irrelevant effect
- Possible reasons for inconsistency:
  - differences in source, type and dose of RS
  - varying diet composition (digestible starch, dietary fibre, fat .... ; physico-chemical properties of foods)
- No reports of RS worsening glucose and insulin responses

RS and glucose & insulin metabolism: Suggestions for further research

- More studies are needed on:
  - effects of RS-rich diets on long-term glucose response and insulin sensitivity
  - effects of RS on glucose and insulin in individuals with impaired glucose tolerance
  - effects of chemically modified RS (i.e. RS 4)
  - studies to objectively measure satiety

- Standardised test meals matched for macronutrients but containing different levels of clearly defined RS

Substrates entering the colon [g/day]

- **Dietary substrates**
  - non-starch polysaccharides 8-18
  - resistant starch 8-40
  - oligosaccharides 2-8
  - unabsorbed sugars 2-10
  - proteins and peptides 3-9

- **Endogenous sources**
  - pancreatic secretions 4-6
  - small bowel mucins 2-3
  - bacterial secretions, epithelial cells & colon mucins ??

Sites of fermentation in the human colon

- Slower fermentation rate
- Reduced substrate availability
- End product concentration reduced

**Proximal Colon**
- Active site of carbohydrate fermentation
- Rapid transit
- Low pH (c.a. 5.5/6.0)

**Distal Colon**
- Little carbohydrate fermentation
- pH more neutral
- Higher proteolysis

Short chain fatty acids (SCFA)

- Formation is regulated by host, environmental, dietary and microbiological factors
- Mainly derived from bacterial breakdown of undigested carbohydrates in the proximal colon
- Different carbohydrates lead to distinct SCFA patterns:
  - Pectins favour acetate production
  - Galactomannans as well as arabinogalactans favour propionate production
  - Resistant starches favour butyrate production
- Branched chain fatty acids mainly from protein and peptide breakdown in the distal colon
Resistant starch and colonic function

Improved colonic function is associated with:

- Reduced transit time
- Increased faecal weight and output
- Lower pH due to:
  - Increased short chain fatty acid (SCFA) production
  - Decreased production of ammonia and phenols
- Decreased secondary bile acid production
- Reduced toxicity of faecal water
- Altered activity of bacterial enzymes and bacteria
Resistant starch and colonic function: Effects in humans

- Most human studies have shown positive effects on:
  - transit time, faecal weight and output
  - faecal pH
  - faecal ammonia and phenols

- Inconsistent results have been reported as far as SCFA are concerned:
  - 7 reports suggested beneficial effects
  - 3 studies did not show significant changes

- No clear effects on markers of colon cancer risks

- More research to elucidate exact effects

Definition of prebiotics

- A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon, and thus improves host health.

- Among the generally recognised beneficial species are bifidobacteria and lactobacilli.

- Non-digestible carbohydrates are classified as “colonic food”, but not all are prebiotics.
Resistant starch as a prebiotic

- Potential prebiotic effect based on:
  - many bifidobacteria can utilise resistant starch *in vitro*
  - consumption of high RS diets causes shift in faecal (human) and large bowel (pig) SCFA profiles
  - granular RS may provide microenvironments protecting probiotic bacteria (adhesion; micro-encapsulation)

  RS is a prebiotic and synbiotic

- RS seems to function differently than well known prebiotics

- Prebiotic concept update (2004):
  ... evidence pointing towards any prebiotic effect is too sparse to justify .... classification as prebiotic at present

- More research in humans is needed
**In vitro fermentation (1)**

**Model experiments in a static system**

- 4 substrates per experiment (digestion residues)
- Lactulose and blank as controls
- Sampling usually after 2, 4, 6, 8 and 24 h

Incubation with fresh human faecal samples under strictly anaerobic conditions
**In vitro fermentation (2)**

- **Quantification of fermentation products:**
  - total gas production (overpressure)
  - hydrogen accumulation by GLC
  - changes in pH
  - short chain fatty acids (SCFA) by GLC

- **Substrate disappearance:**
  - neutral sugars by HPAEC-PAD or specific enzymatic determinations

- **Optional:**
  - structural characterisation of substrates
  - enumeration of colonic bacteria

In vitro fermentability of indigestible oligo- and polysaccharides (example)

![Graphs showing in vitro fermentability of various substrates](image)

Fructo-Oligosaccharide
Xylo-Oligosaccharide
Gluco-Oligosaccharide
Resistant Starch
Polydextrose

57. Starch Convention
Detmold, 27 April 2006

In vitro fermentability of RS preparations

Substrate disappearance [mg/100mg]

Unpublished results
In vitro fermentability of RS preparations: Discussion

- In vitro fermentability turned out to differ according to RS type and preparation:
  - RS 3 preparations are more easily susceptible to colonic microbiota than RS 2 preparations
  - Within RS 3: starch source as well as degree of polymerisation influence degradability
  - Within RS 2: modified HAM is more resistant to bacterial attack than untreated HAM

- Pyrodextrinated maltodextrins showed a different behaviour:
  - More easily fermentable at the beginning, but rather resistant in a later phase of fermentation
  - Elevated propionate production
Resistant starch and butyrate production

![Graph showing Actistar (tapioca) and Lactulose production](image)

- **Actistar (tapioca)**
  - Donor 1: Increase
  - Donor 2: Increase
  - Donor 3: Increase
  - Donor 4: Increase
  - Donor 5: Increase

- **Lactulose**
  - Donor 3: Increase

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Resistant starch and butyrate production: Discussion (1)

- Despite good reproducibility of overall fermentability, high variability in butyrate production from RS 3 was found:
  \[158 \pm 95 \mu\text{mol/100mg substrate (n=5)}\]

- A donor-related effect is assumed to be responsible for this effect

- Other *in vitro* experiments on RS 3 led also to large donor-specific variations*:
  - Incubation with faecal samples from two individuals
  - well-fermenting flora: \(~ 0.88 \text{ mmol total SCFA/g, acetate:propionate:butyrate ratio: 63:16:21}~\)
  - medium-fermenting flora: \(~ 0.65 \text{ mmol total SCFA/g, acetate:propionate:butyrate ratio: 1:24:75}~\)

*Rössler et al. (2002). Ernährung/Nutrition 26, 297-305.*
Resistant starch and butyrate production:
Discussion (2)

- 16S rRNA sequencing techniques have revealed a remarkable diversity of butyrate producing bacteria
- In studies, in which butyrate production is of particular interest, it is recommended:
  - To screen faecal slurries for butyrate producing bacteria
  - To measure bacterial changes during fermentation experiments
  - To include a well characterised RS as an additional standard
- Under *in vivo* condition, butyrate production may vary similarly between individuals
Changes in faecal microbiota during age

Fermentability changes during life: Short chain fatty acid production [µmol/100mg]

- Breast-fed infants
- Formula-fed infants
- Infants at weaning
- Adults
- Elderly

Scheiwiller et al. (submitted).
Fermentability changes during life: Substrate degradation [mg/100mg]

Breast-fed infants

Formula-fed infants

Infants at weaning

Adults

Elderly

Actistar (tapioca)

Lactulose

Scheiwiler et al. (submitted).

57. Starch Convention
Detmold, 27 April 2006
Fermentability changes during life:
Hydrogen production [ml/100mg]

Breast-fed infants

Formula-fed infants

Infants at weaning

Adults

Elderly

Actistar (tapioca)
Lactulose
Blank

Scheiwiller et al. (submitted).
Fermentability changes during life: Discussion

- Microbiota of all age groups were found to be able to degrade lactulose, but clear qualitative differences were observed:
  - $\text{H}_2$ was accumulated as an end product on incubation with breast-fed and formula-fed infants’ microbiota
  - Acetate was produced nearly exclusively from breast-fed infants’ inocula

- The ability to ferment RS 3 is only established during weaning:
  - Actistar resisted degradation by microbiota obtained from breast-fed and formula-fed infants
  - At weaning and with the elderly the ability to degrade RS 3 seemed to be somewhat delayed
Summary

- Resistant starch may play an important role to increase dietary fibre (DF) consumption.
- As part of the DF fraction RS shows:
  - A slight influence on blood parameters
  - Positive effects on colonic health
- RS is fermentable in the colon:
  - It is classified as a butyrogenic substrate
  - It may act as a prebiotic and/or synbiotic
- RS preparations vary in their properties, depending on type and structural features.
- More research is needed to elucidate their physiological characteristics in detail.
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