Fermentable Carbohydrate Alters Hypothalamic Neuronal Activity and Protects Against the Obesogenic Environment

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The role of Inulin and Oligofructose

- **Inulin**: heterogeneous blend of fructose polymers found widely distributed in nature as plant storage carbohydrates.
  
  **Oligofructose**: subgroup of inulin, consisting of polymers with a degree of polymerization (DP) ≤10

- Inulin and oligofructose are not digested in the upper gastrointestinal tract; therefore, they have a reduced caloric value. They are also known to act as prebiotics on different parts of the large intestine.
Where can I get my Inulin?

- Agave
- Jicama
- Wild Yam
- Garlic
- Onions
- Bananas
- Leeks
- Chicory Root
- Wheat
- Jerusalem Artichoke
- Rye
- Barley
- Bananas
- Super Green Algae
Eat Your Vegetables

Oligofructose-Enriched Inulin IN – gives the beneficial bacteria in your body the nutrition it needs to flourish (act as a prebiotic).

The Importance of a high fiber diet.

- hyper-caloric food enriched with fermentable fibre caused reduction in weight gain and an improvement in body composition and metabolic function in mice.
- linked to decreases in visceral adipose tissue and hepatic lipid storage, production of small insulin sensitive adipocytes and an overall increase in insulin sensitivity.

Fermentable carbohydrates such as inulin (In) and oligofructose are fermented by the colonic bacteria to short chain fatty acids (SCFA). → Travel across BBB

- acetate = 60%
- propionate = 20% → Oligofructose and In increase acetate and propionate circulation in the periphery
- butyrate = 20%

amples were subjected to fluorescent in situ hybridization with Cy-3 labeled genus specific probes, Lab158, Bif 164, Erec 482, and Mib 663 (17,18) to enumerate Lactobacillus-Enterococcus, Bifidobacteria, Eubacterium rectale-Clostridium coccoides, and mouse intestinal bacteria, respectively, as previously described.
Current Study

Hypothesis: Male C57Bl/6 mice with a high fat (HF) diet supplemented with fermentable fibre will experience a reduction in body weight and improvement in cognitive function, compared to obesogenic mice on a HF diet.

3 conditions: HF+In, HF+CS, and control (Fed regular rat chow).

<table>
<thead>
<tr>
<th></th>
<th>HF + In</th>
<th>HF + Cs</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ/g)</td>
<td>19.0</td>
<td>19.2</td>
<td>11.4</td>
</tr>
<tr>
<td>Protein (% by wt.)</td>
<td>17.3</td>
<td>17.3</td>
<td>20.2</td>
</tr>
<tr>
<td>Carbohydrates (% by wt.)</td>
<td>42.3</td>
<td>49.0</td>
<td>33.9</td>
</tr>
<tr>
<td>Fat (% by wt.)</td>
<td>21.2</td>
<td>21.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Fibre (% by wt.)</td>
<td>12.5</td>
<td>5.0</td>
<td>15.4</td>
</tr>
</tbody>
</table>
Experimental One

Aim: To study the effects of HF diet supplementation with oligofructose-enriched In and Cs on body weight, energy intake, plasma GLP-1, and microbiota.

- Significant change in body weight in 15 days: HF+Cs = 28.9g & HF+In = 27.3g
  - No sig change between the In enriched and control.
  - Sig daily changes in body weight between Cs and In HF diets.
- Energy levels for both HF groups remained sig higher than the control. The HF+Cs group also experienced an increase in energy at day 19, which lasted until the end of the experiment.
- HF+In had a sig increase in *Bifidobacteria* and *Lactobacillus-Enterococcus* over the control and HF+Cs.
- GLP-1 increased but the results were not significant.
- Significantly higher SCFA in the HF+In group
  - Acetate and propionate were significantly higher - butyrate was not.
<table>
<thead>
<tr>
<th></th>
<th>HF + In (a)</th>
<th>HF + Cs (b)</th>
<th>Control (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt. Gain after 15 days</td>
<td>27.3g</td>
<td>28.9g</td>
<td>~(a)</td>
</tr>
<tr>
<td>Daily Wt. Gain</td>
<td>0.19g</td>
<td>0.26g</td>
<td>0.09g</td>
</tr>
<tr>
<td>cecal bifidobacteria (8 wks)</td>
<td>8.5%</td>
<td>7.1%</td>
<td>?</td>
</tr>
<tr>
<td>fecal bifidobacteria (diff.)</td>
<td>+2.3%</td>
<td>+0.7%</td>
<td>?</td>
</tr>
<tr>
<td>GLP-1 conc.</td>
<td>57.8 pmol/l</td>
<td>c&lt;(b)&lt;(a)</td>
<td>35.2 pmol/l</td>
</tr>
<tr>
<td>SCFA (acetate+propionate)</td>
<td>34.4mmol/l</td>
<td>25.6mmol/l</td>
<td>?</td>
</tr>
</tbody>
</table>
Experiment two:

Aim: To study the effects of HF diet supplementation with oligofructose-enriched In on whole body adiposity and intrahepatocellular lipid.

- Whole body adiposity: HF+In= 24.9% & HF+Cs= 30.7%
  - Subcutaneous fat: HF+In= 24.9% & HF+Cs= 30.7%
  - Internal fat: HF+In= 24.9% & HF+Cs= 30.7%
  - Intrahepatocellular lipid was sig lower in the HF+In condition
    - HF+In= 11.7% & HF+Cs= 23.8%
    - There was not a significant difference in IHCL between the control and the HF+In groups.
<table>
<thead>
<tr>
<th></th>
<th>HF + In (a)</th>
<th>HF + Cs (b)</th>
<th>Control (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body Adiposity</td>
<td>24.9%</td>
<td>30.7%</td>
<td>?</td>
</tr>
<tr>
<td>Subcutaneous fat</td>
<td>4.5g</td>
<td>6.0g</td>
<td>?</td>
</tr>
<tr>
<td>Internal fat</td>
<td>3.4g</td>
<td>4.9g</td>
<td>?</td>
</tr>
<tr>
<td>IHCL (Liver fat)</td>
<td>11.7%</td>
<td>23.8%</td>
<td>9.0%</td>
</tr>
</tbody>
</table>
Experiment three:

Aim: To study the effects of HF diet supplementation with oligofructose-enriched In on hypothalamic activity as measured by MEMRI

- significant increase in SI in the ARC of HF+In animals compared to the HF+Cs group (ARC SI: HF+Cs vs. HF+In, $P < 0.05$)

- No additional, significant differences were observed between the SI profiles of HF+In and HF+Cs groups in the ventromedial hypothalamus ($P = 0.86$), paraventricular nucleus ($P = 0.66$), or the brainstem ($P = 0.20$)

But what is causing this difference in ARC signal intensity?
Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats

Jill A. Parnell¹ and Raylene A. Reimer²*

Given that prebiotics alter the composition of the gut microbiota, there is significant interest in their ability to do so in a manner that promotes weight loss. Here, we report increases in Lactobacillus spp. with prebiotic supplementation. These results are promising as perinatal probiotic treatment with Lactobacillus rhamnosus has been shown to attenuate excessive weight gain in the first years of life (50) and Lactobacillus gasseri reduced abdominal adiposity and body weight in adults with obese tendencies (51). Our 1:1 mixture of inulin and oligofructose also increased Bacteroides, suggesting that prebiotics may be able to normalise the reduced Bacteroidetes numbers reported in obesity. While others have shown that prebiotic fibres increase gut Bifidobacterium spp. numbers (26), the present study is the first to show that this response is dose-dependent in lean and obese JCR:LA-cp rats. The gut microbiota has been linked to body fat through a variety of mechanisms including increased energy harvesting, hepatic de novo lipogenesis, adipocyte fatty acid storage, presumably through lipoprotein lipases, and suppression of AMP-activated protein kinase-dependent fatty acid oxidation (20). Cani et al. (52)
The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism


Acetate reduces hypothalamic AMPK catalytic activity
Mammalian neuronal tissue is thought to rapidly convert acetate to acetyl-CoA, which then enters the Krebs cycle\(^{13}\). Accumulating evidence suggests that hypothalamic AMP-activated protein kinase (AMPK) has an important role in energy and nutrient sensing\(^{18}\). Phosphorylation of threonine 172 (T172) within the alpha subunit of AMPK directly activates the kinase, which in turn phosphorylates and inactivates acetyl-CoA carboxylase (ACC), leading to decreased levels of malonyl-CoA. Studies have shown that increased hypothalamic malonyl-CoA concentrations are associated with an increase in the expression of POMC\(^{19}\) and suppression of Neuropeptide Y (NPY) and AgRP with a subsequent reduction in rodent food intake. Thus, we hypothesized that acetate administration may mediate the changes in appetite we had observed and also more specifically changes in POMC and AgRP expression. We measured the phosphorylation of key residues of AMPK (T172) and ACC (S79) in mouse hypothalamic lysates following i.p. acetate injection as a surrogate of their activities. We found a significant reduction in levels of pT172 of AMPK and pS79 of ACC (Fig. 3g–i), suggesting that acute acetate administration inactivated AMPK, thereby leading to increased ACC activity. Increased ACC activity has been shown to elevate malonyl-CoA that can stimulate expression of POMC and cocaine- and amphetamine-regulated transcript and decrease NPY and AgRP leading to a reduction in food intake\(^{19}\). This therefore suggests that the anorectic effects of acetate administration are mediated through a change in hypothalamic ACC and AMPK activities and downstream changes in neuropeptide expression. A schematic illustration of this relationship is shown in Fig. 3j.
Glucagon-like peptide 1 (GLP-1) is secreted from the intestinal tract in response to nutrients and is also produced in the nucleus of the solitary tract (NTS) of the brainstem with projections throughout the CNS.

It has captivated interest due to its incretin (aiding the action of insulin) and anorectic effects.

Findings suggest that activation of central GLP-1Rs strikingly suppresses food reward/motivation by interacting with the mesolimbic system.

This indicates an entirely novel mechanism by which the GLP-1R stimulation affects feeding-oriented behavior.
A long-acting GLP-1 analog, EX4, was peripherally and also centrally administered to rats in two conditions.

- **Condition 1:** rats need to push lever to get rat chow.
- **Condition 2:** rats are given conditional preference between two visually distinctive levers - one lever yields normal rat chow and the other 1g chocolate pellets.

**Results:** EX4 suppresses food intake & weight gain in the first condition. In the second condition, EX4 also suppresses the ability of chocolate pellets to condition a place preference and also has a suppressive effect on how hard a rat is willing to work for a sweet reward.
- Effects are also exerted centrally as direct injection of EX4 into the brain ventricles.

- In addition to food reward, selective GLP-1R stimulation in the VTA and the NAc reduced free-feeding over 24 hours.

- At 24 h after injection, the VTA GLP-1R activation potently (50%) reduced chow intake and body weight gain at both doses tested,

- NAc shell injection produced a small effect only on chow intake at the highest dose tested, potentially indicating differential sensitivity of those two sites to anorexic effects of GLP-1 stimulation
References (DISCLAIMER: Only useful if read)

Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats
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The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism
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The Glucagon-Like Peptide 1 (GLP-1) Analogue, Exendin-4, Decreases the Rewarding Value of Food: A New Role for Mesolimbic GLP-1 Receptors
http://www.jneurosci.org/content/32/14/4812.full.pdf

Neurobiology of food intake in health and disease
http://www.nature.com/nrn/journal/v15/n6/abs/nrn3745.html