Colonic Propionate, Appetite, and Weight Loss (ProAp)

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1. INTRODUCTION

DIET AND OBESITY:

Obesity has reached epidemic proportions worldwide. In the United Kingdom, 58% of adult women and 65% of adult men are either overweight or obese (Health and Social Care Information Centre 2014), with the expectation that rates will increase (Ng et al. 2014). Obesity, mainly caused by a chronic positive energy balance, is a known risk factor that contributes to the development of type 2 diabetes and cardiovascular diseases, and the subsequent morbidities and mortalities (Kopelman 2007). A positive energy balance is inevitable with the composition of current western diet that contain refined (fibre-depleted) carbohydrates that are consumed quickly, and have minimal effects on long-term satiety (Cleave 1974; Heaton 1973).

NONTDIGESTIBLE CARBOHYDRATES:

The quality and quantity of diet has changed dramatically over the last fifty years. Of which, carbohydrates have the most significant changes; from unprocessed fruits and vegetables based carbohydrates to more processed cereal based ones (Cordain et al. 2005). Epidemiological studies have shown that, as a result, ancestral diets had greater amounts of Non-Digestible Carbohydrates (NDC) than modern diets (>100g/day compared to <15g/day) with the inclusion of more indigestible intact cell wall plant material, such as tubers, grasses and sedges (Eaton 2006). A strong association has been established between increased weight gain with decreased NDC intake (Liu et al. 2003). Furthermore, increasing intake of NDC have shown to induce satiety and improve body composition in animals (So et al. 2007) and humans (Bouché et al. 2002).

LOW CALORIC HIGH NDC DIET IN LOSING WEIGHT

Applying energy-restricted diets is one of the first steps to treat obesity. However, weight regain in weight maintenance period could happen due to resting energy expenditure reduction and lean mass loss. The inclusion of specific foods to the nutritional intervention is being investigated in order to produce persistent weight losses (Abete et al. 2008). It was suggested earlier that the addition of high fibre NDC foods to a hypocaloric diet would help reducing the incidence of metabolic syndrome and increasing the dietary compliance (Hermama et al, 2011). The high fibre content in combination with a low glycemic index diet, can both result in higher satiety effects with an increased mitochondrial oxidation, all favour weight loss.
PROPIONATE AND THE FFAR2 RECEPTOR:

Production of short chain fatty acids (SCFA) is the main by-product of NDC colonic fermentation (Pawlak et al. 2004). Of them, colonic propionate was found to play a critical role directly in the colon and systematically after its absorption into the circulation (Wong et al. 2006). Free Fatty Acid Receptor 2 (previously known as orphan G-coupled Protein Receptor 43) has been discovered to have high affinity to propionate (Brown et al. 2003). It is expressed on the distal ileum and colon L-enteroendocrine cells (Karaki et al. 2008), adipocytes and certain monocytes lineage (Hong et al. 2005).

THE ROLE OF PROPIONATE IN GUT HORMONE RELEASE AND APPETITE REGULATION:

The L-enteroendocrine colonic cells are responsible for secreting anorexic gut hormones (PYY and GLP-1) physiologically in response to food. These hormones are capable of inducing satiety through signalling the brain appetite centre (Murphy & Bloom 2006). Propionate-induced FFAR2 activation have found to cause the release of these hormones in vitro. Moreover, NDC ingestion was found to stimulate the release of these hormones in animals, resulting in decreasing food ingestion (Delzenne et al. 2005). Propionate was shown to replicate the same effects in further studies, suggesting the owning of the beneficial effects of NDC on appetite and gut hormones to propionate production.

SHORT CHAIN FATTY ACID ESTER:

Administering NDCs that promote the production of propionate is an attractive way to increase the level of propionate over an extended period of time. However, due to variability of the gut microbiota activity, administering high proportions (≥ 35g/d) of dietary fibres in human diets to increase colonic propionate does not reliably or predictably provide the same SCFA levels in colon or systemically (Cummings 1981). Oral propionate supplementation is another method, but unfortunately its short plasma half-life, poor palatability, and the fact that its main absorption happens in the small intestine limit its use as a food supplement (Frost et al. 2003).

A novel system has been developed by Dr Douglas Morrison, (Scottish Universities Environmental Research Centre (SUERC)). This molecule of inulin (β(2-1) linked polymer of fructose) carrier with an ester linked to propionate (propionate ester) was demonstrated to reliably and reproducibly increase colonic propionate while prevent the side effects of NDC (Chambers et al. 2015). Thus, propionate is only released when the carrier molecule inulin is fermented by colon microflora, which was estimated to be 180 minutes post-ingestion. (Chambers et al. 2014). Chambers et al. (2015) were able to show that 10g of Inulin-Propionate Esters (IPE) has the ability to deliver 2.4g of propionate to the colon, that is an increment of 2.5-fold of propionate production in the colon, a level that is achieved only with 60g/d via traditional dietary fibre supplementation.
The same group has also demonstrated that while the acute administration of this supplement caused increments in Plasma GLP-1 and PYY and reduced food ingestion, the long term (24 weeks) supplementation of propionate ester significantly reduced body weight gain and the development of abdominal adipose tissue (Chambers et al. 2014). Interestingly, none of the long term participants reported any side effects from the intake of propionate esters. Increasing colonic propionate is, therefore, one of the attractive strategies to manage weight and diabetes risk factors.

The current protocol aims to investigate the impact of the propionate ester in conjugation with restricted diet on appetite and weight loss.
2. Study: Effect of increased colonic propionate on weight loss during a hypo-caloric diet.

**Study methodology:** A randomised, control, paralleled study.

**Participants:** 70 overweight and obese healthy male and female volunteers aged between 18 and 65 years with body mass index (BMI) of 25-35 kg/m².

**Support of number of volunteers:** Based on previous work we anticipate that a hypo-caloric diet alone will cause a weight loss of 3.0 ± 2.5 kg, whilst the addition of propionate-ester will cause a weight loss of 5.0 ± 2.5 kg. A power calculation shows that 26 volunteers will be need per group to detect a significant difference between groups (p=0.05, 0.80 power). 70 volunteers will be recruited to allow a 25% drop-out rate.

**Visit 1: Health Screening**

Participants will attend the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital where their eligibility will be assessed. They will have a blood test (FBC, ALT, HbA1C, renal function, and lipids) and height and weight measurements will also be taken. They will also have an electrocardiogram (ECG) and blood pressure will be recorded. All women of childbearing age will have a pregnancy test.

Participants will be told not to start any other new diets or intensive exercise regimes during the study period as this may give us conflicting results.

**Visit 2: Acclimatization visit (Week 0)**

The main objective of this visit is to fully acclimatize the participants to the study before the baseline visit.

The day prior to the study visit, participants will be requested to refrain from strenuous exercise and alcohol. Participants will then be requested to fast overnight (they are allowed to drink water) and will come to the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital the following morning. Fasting saline flushes will be done through an intravenous peripheral cannula at -10 and 0 min. Resting energy expenditure will be measured with a standard indirect calorimeter. The calorimeter captures expired air from a large, transparent canopy, worn over the head and thorax, and is typically very well tolerated. Each participant will be asked to lie in a semi-recumbent position under the canopy. Once the carbon dioxide content of the air entering the chamber has stabilised, measurements will be taken for 20 min in order to assess the participants’ resting metabolic rate (RMR) and respiratory quotient (RQ). The calories needed per day for each subject will be estimated and a -500 kcal/day diet will be provided to the participants to follow for the rest study period. Participants will be asked to complete a 7days-food diary in order to form a suitable individualised diet. A weight management booklet will be designed to each participant, where their resting energy requirement will be estimated and a healthy diet plan will be designed. This will also include a supplementary material of the portions required and list of different substitutes to be taken per food group. The same booklet content was previously designed and used in a trial that was ethically approved with a reference number of 12/LO/0139 version 2: 31/07/2012.

Participants will then receive a standardised breakfast (0 min). Postprandial saline flushes will be given at 15, 30, 60, 90, 120, 180, and 240 min.
Appetite and satiety will be assessed by visual analogue scale every 60 min and breath hydrogen concentration will be measured at the same time, as a marker of colonic fermentation. Food intake will be assessed at 240 min by offering a free buffet meal served in excess.

Visit 3: Study visit 1 (Week 1)

The same measurements collected during the acclimatization visit will be repeated. The only difference would be in collecting 10 ml of postprandial blood, instead of flushing saline, at each time point of 15, 30, 60, 90, 120, 180, and 240 min to measure hormones and metabolites. These will include glucose, insulin, gastrointestinal and appetite hormones (GLP-1, pro-glucagon-derived peptide, PYY, PP, Ghrelin, Cholecystokinin), and short chain fatty acids level.

Participants will be randomised to take 10 g of Propionate ester or Inulin (control) dietary supplement once a day for 84 days (12 weeks). Supplements will be given to participants in ready-to-use sachets and instructed to mix the contents into their -500 kcal/day individual diet. An independent researcher (i.e. not linked to the study) will be given the task of randomisation, which will be by sealed envelopes.

Urine will be collected throughout the study visit to measure propionate and urea concentrations. Subjects will empty their bladder before the test breakfast and collect all urine thereafter for a period of 240 min. Subjects will be provided with an appropriate measurement container to collect urine and will be asked to note the total volume following each collection.

Subjects will be asked to collect a stool sample to assess propionate levels. Subjects will collect a sample of the first stool they pass after 10:00 pm on the study day. Participants will be provided with detailed instructions of how to do this and will be given appropriate containers.

Visits 4 and 5: Study visits 2, 3 (Week 4 and week 8):

Will involve short visits to monitor weight and dietary compliance to the diet, through collecting 7-days food intake diary and taking anthropometry measurements.

Visit 6: Study visit 4 (Week 12):

At the end of 84 day supplementation period, participants will attend the NIHR/Wellcome Trust Imperial Clinical Research Facility at Hammersmith Hospital to and all measurements collected during Study Visit 1 will be repeated.
PARTICIPANT ENTRY

INCLUSION CRITERIA

- Healthy overweight and obese volunteers (body mass index (BMI) of 25-35 kg/m²)
- Age between 18-65 years (inclusive)
- Having blood results for the following tests in the ranges of:
  - HbA1C 20-41 mmol/mol haemoglobin
  - ALT 0-40 IU/L
  - FBC:

<table>
<thead>
<tr>
<th>TEST</th>
<th>MALE</th>
<th>FEMALE</th>
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<tbody>
<tr>
<td>WBC (x10⁹/L)</td>
<td>4.2-10.6</td>
<td>4.2-11.2</td>
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<tr>
<td>RBC (x10¹²/L)</td>
<td>4.23-5.46</td>
<td>3.73-4.96</td>
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<tr>
<td>Hb(g/L)</td>
<td>130-168</td>
<td>114-150</td>
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<tr>
<td>PCV (Ratio)</td>
<td>0.390-0.500</td>
<td>0.350-0.450</td>
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<tr>
<td>MCV (fl)</td>
<td>83.5-99.5</td>
<td>83.5-99.5</td>
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<tr>
<td>MCH (pg)</td>
<td>27.5-33.1</td>
<td>27.5-33.1</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>315-350</td>
<td>315-350</td>
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<tr>
<td>RDW</td>
<td>10.0-16.0</td>
<td>10.0-15.9</td>
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<tr>
<td>PLATELETS x(10⁹/L)</td>
<td>130-370</td>
<td>135-400</td>
</tr>
<tr>
<td>NEUTS (x10⁹/L)</td>
<td>2.0-7.1</td>
<td>2.0-7.1</td>
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<tr>
<td>LYMPHs (x10⁹/L)</td>
<td>1.1-3.6</td>
<td>1.1-3.6</td>
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<td>MONOs (x10⁹/L)</td>
<td>0.3-0.9</td>
<td>0.3-0.9</td>
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<td>EOSINs (x10⁹/L)</td>
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<tr>
<td>RETIC#</td>
<td>20.0-92.0</td>
<td>12.0-96.0</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>1-10</td>
<td>1-20</td>
</tr>
</tbody>
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- Renal function:
  - Creatinine (umol/L)  60-125     55-110
  - eGFR >59 mL/min/1.73m²

EXCLUSION CRITERIA

- Weight change of ≥ 3kg in the preceding 2 months
- Undergone any weight loss surgery or gastric procedures to promote weight loss
- Current smokers
- Substance abuse
- Excess alcohol intake
- Pregnancy
- Diabetes
- Cardiovascular disease
- Cancer
- Gastrointestinal disease e.g. inflammatory bowel disease or irritable bowel syndrome
- Kidney disease
- Liver disease
- Pancreatitis

- Use of medications likely to interfere with energy metabolism, appetite regulation and hormonal balance, including: anti-inflammatory drugs or steroids, antibiotics, androgens, phenytoin, erythromycin or thyroid hormones.

Any participants with the above conditions would already have an altered pattern of hormones and inflammatory molecules because of their disease process and would therefore give us confounding or misleading results.
WITHDRAWAL CRITERIA
The safety of the study participants takes priority. Any significant adverse event (as assessed by the researchers) will halt the study and the ethics committee and sponsor will be informed as per standard protocol. All adverse events will be recorded and investigators will review each adverse event as it arises. In addition, participants will be free to withdraw at any time and are not required to give a reason.

STATISTICAL PLAN
Statistical analysis perform with IBM SPSS version 23. Graphs prepare with GraphPad Prism version 7.04. Data will be checked for normality using a Shapiro-Wilk test (defined as P≥0.01). Percentage of weight loss will be calculated using the weight at the end of the intervention divided by the baseline weight multiplied by 100. The results express as mean difference with ± SEM. Mean of data will be compared within and between groups using a paired t-test or Wilcoxon signed-rank test where applicable. Analysis of variance will assess the impact of two different interventions (IPE and Inulin) on participant blood parameters across the 12-week supplementation period (baseline and week 12), with the supplementation group used to determine between-subjects differences. A p-value ≤0.05 will consider significant.
References:


Ng, M. et al., 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global Burden of Disease Study