The role of intestinal microbiota in metabolic disease-a novel therapeutic target.
What is metabolic disease?

- Disease/disorder resulting from lifestyle choices
  - Diet / Exercise
- Obesity
- Cardiovascular Disease
- Certain Cancers
- Type 2 Diabetes
Metabolic syndrome

• The syndrome previously known as……
  – The Deadly Quartet
  – The Insulin Resistance Syndrome

• Factors used to evaluate risk of developing a metabolic disease

• Several Definitions
  – WHO
  – The European Group for study of Insulin Resistance (EGIR)
  – The National Cholesterol Education Program—Third Adult Treatment Panel (NCEP ATP III)
What are the factors?

The diagnosis of metabolic syndrome is given if 3 or more of these factors occur.

- Central obesity
- High blood pressure
- High triglycerides
- Low HDL-cholesterol
- Insulin resistance
Summary

• Metabolic syndrome - cluster of factors
  – Central obesity
  – LDL-cholesterol/ HDL-cholesterol
  – Blood pressure
  – TG
  – Insulin resistance

• used to describe the risk of developing metabolic disease

• Metabolic disease - conditions arising from poor lifestyle choices
What is the microbiota?

• We carry around with us approximately 1.25 Kg of microbes
• Evolved alongside microorganisms
• Present on our skin, hair, mouths, nose, and ears and within our gut.
  – intimate relationship
  – each one of us has a unique composition
  – known as our microbiota.
• Complex microbial world estimated
  – 1,500 different microbial phylotypes
  – 100 times more genes than the human genome
• Modern molecular techniques
Who is present and how many

- Dominated by strict anaerobes
  - *Bacteroides, Clostridium, Ruminococcus, Butyrovibrio, Fusobacterium, Eubacterium, Peptostreptococcus, Bifidobactium, Atopobium, Peptococci.*
- To a lesser extent, facultative anaerobes are present
  - *Lactobactillius, Enterococcus, Streptococcus,* and *Enterobacteriaceae*
  - Yeasts are also present but at even lower numbers, ranging from $10^2$-$10^4$ CFU/ml.
Effect on host

- Evidence that microbiota interacts with human body in a much more intermit manner
  - This relationship was often described as commensal
  - This is a mutualistic relationship with each partners best interest to enhance the other partners wellbeing

- In terms of health, Bifodobacteria are believed to be significant, 3% - 7% of the total population in the intestinal tract and up to 91% in newborns.
- Lactobacilli also considered health positive.
- Many other potentially beneficial bacteria,
Role of microbiota in metabolic disease

• Obesity:
  • Gut microbiota composition involved in regulation of energy homeostasis.
    – intestinal glucose absorption
    – energy extraction from non digestible
    – increase on blood glucose and insulin- Lipogenesis

• Related to obesity
  – reduced Bacteroidetes, increase Firmicutes in obese
  – Increase Bifidobacteria, reduced *S. aureus* - children

• Controls occurrence of metabolic disorders due to high fat diet
Metabolic Endotoxemia: low grade inflammation

- Metabolic disorders linked to increased inflammatory factors:
  - IL-1, TNF-α, MCP-1, IL-6
  - markers involved in insulin resistance
- Likely candidate involved- bacterial lipopolysaccharide (LPS):
  - LPS constituent of Gram negative bacteria
  - triggers secretion of proinflammatory cytokines
- *Bifidobacterium* spp./*E. rectale* decrease- high fat diet
- *Bifidobacterium* spp reduce intestinal endotoxin levels
Type 2 diabetes

- Study carried out using animal model
- Metabolic endotoxemia leads to insulin resistance
  - mice fed high fat diet
- Increased numbers of Bifidobacteria
  - positively correlated with improved glucose-tolerance, glucose-induced insulin-secretion and normalised low grade inflammation
  - metabolic endotoxemia correlated negatively with Bifidobacteria
Cardiovascular disease

- Metabolic endotoxemia linked to CVD through LPS
- Mice fed high fat diet
  - developed vascular inflammation
  - vascular insulin resistance
- Microbiota modulation proposed to reduce inflammation
- Changing gut microbiota improved atherogenic markers
  - LDL-cholesterol, IL-6
Colonic cancer

- May play a role in regulating colonic cancer development
- *Bacteroides* and *Clostridium* may produce genotoxic and carcinogenic substances
  - amine, indoles, *p*-cresol, ammonia and other N-nitroso compounds
  - fermentation of protein, peptide and amino acids
- Bifidobacteria possess range of activities against carcinogenesis
  - inhibit incidence colon adenocarcinomas
Short chain fatty acids

- By-product of gut carbohydrate fermentation
- Three major SCFA (90-95% total)
  - Acetate (*Bifidobacterium, Lactobacillus, Atopobium, Bacteroides, Ruminococcus*)
  - Propionate (*Bacteroides* and clostridial cluster IX)
  - Butyrate (*Faecalibacterium prausnitzii, Roseburia/Eubacterium rectale*)
- Acetate transported to liver, cholesterol synthesis
- Propionate, gluconeogenic, inhibits cholesterol synthesis
- Butyrate 70-90% used by colonocytes, improves gut permeability, reduced cancer
How to alter the balance

• Probiotics
• Live bacteria introduced to colon
• Cultures of ‘good’ bacteria
• *Lactobacillus* commonly used, *Bifidobacterium*
• Introduction to colon in large numbers hopefully to exert the same health benefits as microbiota
  – $10^6$-$10^7$ bacteria/ml supplement
• Detectable after 7 days consumption
• After consumption not detectable within a few weeks
• Shown to adhere *in vitro*
Prebiotics

• Alternative approach to use prebiotics
• Non-digestible carbohydrates fermented upon reaching the colon
• Fermentation is selective for beneficial modulation of microbiota
• Confirmed prebiotics
  – Fructo-oligosaccharide
  – Lactulose
  – Galacto-oligosaccharides
• Emerging prebiotics
  – Resistant Starch
  – Dietary Fiber
• Whole grain
Summary

• Gut microbiota has been associated to factors that are thought to lead to metabolic disease
  – Obesity
  – CVD
  – Cancer
  – Type 2 diabetes

• Can alter the microbiota composition through probiotics and prebiotic supplementation.

• Whole grains may have prebiotic potential
Whole grain cereals recommendations from United States Department of Agriculture (USDA)

- 1940’s: USDA Food Guide recommended – Eat grain products daily
- 1950’s-70’s: USDA Food Guide recommended – Eat 4 or more grain products daily
- 1984: USDA Food Guide Pyramid recommended – Eat 6-11 servings of grain products daily
- 1997: FDA allowed health claim
- 2000: USDA Food Guide Pyramid recommended – Eat 6-11 servings of grain products daily, including several servings of whole grain for their health benefits
- 2005: USDA Food Guide Pyramid recommended – Eat whole grain foods at most meals
Reasons for recommended increase intake and FDA health claim

• Diets rich in whole grains linked by Trowell to reduce hyperlipidaemia and heart disease.

• Epidemiological studies have linked increase whole grain/dietary fibre to reduced incidence of metabolic disease:
  – Obesity
  – CVD
  – Certain cancers
  – Type 2 Diabetes

• Rising incidence of metabolic disease
Whole grain consumption protects against metabolic disease

• The American Association of Cereal Chemists (AACC) has defined whole grain products
  – intact, ground, cracked, or flaked caryopsis,
  – main anatomical components (starchy endosperm, germ and bran) present in the same relative proportions

• Mechanisms of protection?
  – effect on the large bowel
  – effect on blood glucose and insulin levels
  – antioxidants
Why use whole grain instead of prebiotics?

• Common approach is to isolate individual components or one dietary ingredient and feed this to animal or human subjects.
  – supporting protective mechanisms for dietary fibre, trace minerals, vitamins or phytochemicals rather than the whole grain.

• Population studies focused on whole grain,
  – reason for attempting to isolate the magic component.
  – evidence whole foods are protective against metabolic disease
  – mechanism of protection is greater than any individual part.
Why a breakfast cereal?

- Diet patterns reported to have a link with risk of chronic disease in children, adolescents and adults
- Subjects who do not consume breakfast tend to gain weight as a result for overcompensating
- Obese adolescents skipped breakfast more often than non-obese
- Teenagers are more likely to consume ready-to-eat foods or if someone prepared the food for them.
  - influenced by what peers choose to eat and if family members would eat with them.
  - Baltimore Longitudinal Study of Aging (BLSA)- top contributor for whole grain intake were ready to eat breakfast cereals
Human faecal batch culture

- Short term fermentation studies
  - Fresh human faecal inoculum
  - Non-digestible components of whole grain
  - pH 6.8
  - temperature 37°C
  - anaerobic
Human feeding studies

• Two recent studies
  – Costabile *et al*
  – Carvalho-Wells *et al*

• Healthy subject
• Short feeding time
• No effect on markers of metabolic disease
• Bifidobacteria and Lactobacilli increase

• Present study
• Subjects at risk
  – elevated fasting glucose
  – elevated cholesterol
• Longer intervention
• Prebiotic effect
• Cholesterol decrease
Summary

- Metabolic disease incidence increasing
- Metabolic syndrome used to evaluate risk
- Microbiota has been associated with metabolic disease
- Prebiotics used to modulate microbiota
- Whole grain intake observed to protect against metabolic disease - mechanisms unknown
- Prebiotic potential may be one mechanism involved
- Evidence starting to accumulate that this is the case
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