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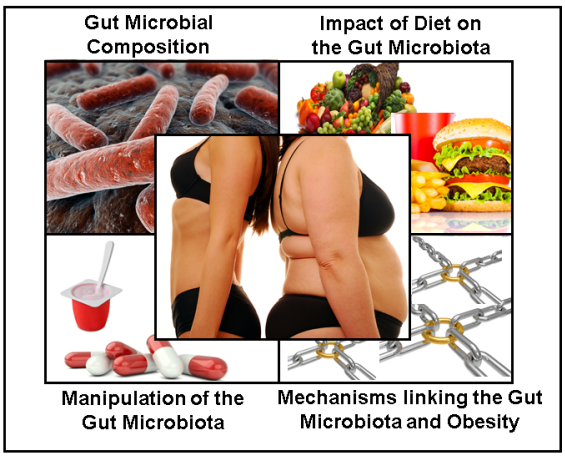
Controlling obesity-associated gut microbes



Key external stakeholders:
Irish food industry, Irish consumers

Practical implications for stakeholders:
The outcome/technology or information/recommendation is……

- The microbes in our gut (gut microbes) can contribute to weight gain. There is an opportunity to develop new weight management strategies by altering these microbial populations in a beneficial way. Through the course of this research we have provided evidence that antimicrobial-producing probiotics, whey protein and exercise have the potential to bring about such beneficial changes.

Main results:
Through a variety of animal and human studies, we have investigated the obesity associated gut microbiota and have employed a variety of approaches with a view to changing this population in a beneficial way.

- We have established that a bacteriocin (antimicrobial) producing probiotic (*Lactobacillus salivarius* UCC118) more considerably alters the gut microbiota than a non-bacteriocin producing equivalent and that the changes induced bring about a short-term reduction in weight gain.

- We have revealed a desirable, high microbial diversity in the gut of elite athletes and have revealed a correlation between this high diversity with exercise and protein consumption, respectively.

Opportunity / Benefit:
As a consequence of our studies, the potential benefits of employing bacteriocin-producing probiotics, protein and exercise to modulate the gut microbiota in a beneficial way have been highlighted. Further research will focus on optimizing the use of these intervention strategies (individually and in combination) to control obesity associated gut microbes.

Collaborating Institutions:
University College Cork
Alimentary Pharmabiotic Centre

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External collaborators: Prof. Paul O'Toole, University College Cork, Alimentary Pharmabiotic Centre
Prof. Fergus Shanahan, UCC, APC
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1. Project background:

There are now several emerging lines of evidence to suggest that the gut microbiota has a role in energy and metabolic homeostasis. Recent evidence from animal and human studies indicates that the composition of the gut microbiota may be involved in the development of obesity. Other studies highlight the role of the gut microbiota in the regulation of energy homeostasis, insulin resistance, non-alcoholic fatty liver disease and energy, lipid and amino acid metabolism. These findings highlight the opportunity for new research to examine the ability of selected bioactives and interventions to modulate the composition of the gut microbiota in a manner that may contribute to the prevention of obesity and obesity-related conditions.

2. Questions addressed by the project:

To what extent can (bacteriocin producing) probiotics, diet and exercise alter the obesity-associated gut microbiota in a beneficial way?

3. The experimental studies:

Study 1: Increased efficiency of energy harvest, due to alterations in the gut microbiota (increased Firmicutes and decreased Bacteroidetes), has been implicated in obesity in mice and humans. However, a causal relationship is unproven and contributory variables include diet, genetics and age. Therefore, we explored both diet-induced obesity (DIO) and genetically-determined obesity (ob/ob) for changes in microbiota and energy harvesting capacity over time. Methods: Seven-week old male ob/ob mice were fed a low-fat diet and wild-type mice were fed either a low-fat diet or a high-fat diet (DIO) for 8 weeks (n=8/ group). They were assessed at 7, 11 and 15 weeks of age for: fat and lean body mass (NMR); fecal and cecal short chain fatty acids (SCFA, gas chromatography); fecal energy content (bomb calorimetry) and microbial composition (metagenomic pyrosequencing).

Study 2: The gut microbiota is an environmental regulator of fat storage and adiposity. Whether the microbiota represents a realistic therapeutic target for improving metabolic health is unclear. This study explored two antimicrobial strategies for their impact on metabolic abnormalities in murine diet-induced obesity: oral vancomycin and a bacteriocin-producing probiotic (*Lactobacillus salivarius* UCC118 Bac(+)). Male (7-week-old) C57BL/J6 mice (9-10/group) were fed a low-fat (lean) or a high-fat diet for 20 weeks with/without vancomycin by gavage at 2 mg/day, or with *L. salivarius* UCC118Bac(+) or the bacteriocin-negative derivative *L. salivarius* UCC118Bac(-) (each at a dose of 1×10(9) cfu/day by gavage). Compositional analysis of the microbiota was by 16S rDNA amplicon pyrosequencing.

Study 3: Since extremes of exercise often accompany extremes of diet, we assessed the impact of diet and exercise on the gut microbiota by studying professional athletes from an international rugby union squad. Two groups were included to control for physical size, age and gender. Compositional analysis of the microbiota was explored by 16S rRNA amplicon pyrosequencing. Each participant completed a detailed food frequency questionnaire.

4. Main results:

Study 1: A progressive increase in Firmicutes was confirmed in both DIO and ob/ob mice reaching statistical significance in the former, but this phylum was unchanged over time in the lean controls. Reductions in Bacteroidetes were also found in ob/ob mice. However, changes in the microbiota were dissociated from markers of energy harvest. Thus, although the fecal energy in the ob/ob was significantly decreased at 7 weeks, and cecal SCFA increased, these did not persist and fecal acetate diminished over time in both ob/ob and DIO mice, but not in lean controls. Furthermore, the proportion of Firmicutes and Bacteroidetes did not correlate with energy harvest markers. Conclusion: The relationship between the microbial composition and energy harvesting capacity is more complex than previously considered. While compositional changes in the fecal microbiota were confirmed, this was primarily a feature of diet-induced rather than genetically-induced obesity. In addition, changes in the proportions of Firmicutes and Bacteroidetes were unrelated to markers of energy harvest which changed over time. The possibility of microbial adaptation to diet and time should be considered in future studies.

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Study 2: Analysis of the gut microbiota showed that vancomycin treatment led to significant reductions in the proportions of Firmicutes and Bacteroidetes and a dramatic increase in Proteobacteria, with no change in Actinobacteria. Vancomycin-treated high-fat-fed mice gained less weight over the intervention period despite similar caloric intake, and had lower fasting blood glucose, plasma TNFα and triglyceride levels compared with diet-induced obese controls. The bacteriocin-producing probiotic had no significant impact on the proportions of Firmicutes but resulted in a relative increase in Bacteroidetes and Proteobacteria and a decrease in Actinobacteria compared with the non-bacteriocin-producing control. No improvement in metabolic profiles was observed in probiotic-fed diet-induced obese mice but a short-term reduction in weight gain was apparent.

Study 3: As expected, athletes and controls differed significantly with respect to plasma creatine kinase (CK, a marker of extreme exercise), inflammatory and metabolic markers. More importantly, athletes had a higher diversity of gut microorganisms, representing 22 distinct phyla, which in turn positively correlated with protein consumption and CK. The results provide evidence for a beneficial impact of exercise on the gut microbiota diversity but also indicate that the relationship is complex and related to accompanying dietary extremes.

5. Opportunity/Benefit:

The research team has developed expertise with respect to undertaking animal and clinical studies to assess the impact of a variety of gut microbiota targeting strategies to control weight gain. Other interventions, foods, supplements etc could be investigated in a similar way and the team is available for collaboration in this area.
The specific benefits of employing probiotics, exercise and protein will be the focus of further attention and, again, the team is open to collaboration in these areas.

6. Dissemination:

The results of this project have been transferred through presentations by the associated researchers to companies and the general public. In addition, a number of peer-reviewed and popular press publications have resulted with the study involving the Irish rugby team, attracting a significant amount of attention in the national and international press.

Main publications:

Clarke S.F., Murphy E.F., O'Sullivan O., Lucey A.J., Humphreys M, Hogan A., Hayes P., O'Reilly M, Jeffery I.B., Wood-Martin R., Kerins D.M., Quigley E., Ross R.P., O'Toole P.W., Molloy M.G., Falvey E., Shanahan F and Cotter, P.D. (2014) 'Exercise and associated dietary extremes impact on gut microbial diversity' Gut 2014 Published online first 09/06/2014 doi: 10.1136/gutjnl-2013-306541.

Clarke S.F., Murphy E.F., O'Sullivan O., Ross R.P., O'Toole P.W., Shanahan F. and Cotter P.D. (2013) 'Targeting the microbiota to address diet-induced obesity: a time dependent challenge' PLoS One. 8(6):e65790.

Murphy E.F., Clarke S.F., Marques T.M., Hill C., Stanton C., Ross R.P., O'Doherty R.M., Shanahan F. and Cotter P.D.(2013) 'Antimicrobials: Strategies for targeting obesity and metabolic health?' Gut Microbes 4:48-53.

Murphy E.F., Cotter P.D., Hogan A., O'Sullivan O., Joyce A., Fouhy F., Clarke S.F., Marques T.M., O'Toole P.W., Stanton C., Quigley E.M., Daly C., Ross P.R., O'Doherty R.M. and Shanahan F. (2013). 'Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity.' Gut 62:220-6.

Clarke S.F., Murphy E.F., Nilaweera K., Ross P.R., Shanahan F., O'Toole P.W. and Cotter P.D. (2012) 'The gut microbiota and its relationship to diet and obesity: new insights' Gut Microbes 3:186-202.

Murphy EF, Cotter PD, Healy S, Marques TM, O'Sullivan O, Fouhy F, Clarke SF, O'Toole PW, Quigley EM, Stanton C, Ross PR, O'Doherty RM, Shanahan F. (2010) 'Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models' Gut 59:1635-42.

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Popular publications:
http://issuu.com/spin35/docs/spin_61_all/24

Cotter P.D. (2013) 'Gut microbes and obesity' TResearch Autumn 2013 p. 28.

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