An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

14F828 - Dietary manipulation of microbiota diversity for controlling immune function (IMMUNOMET)

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

This project hypothesised that gut microbiota diversity could modulate inflammation and susceptibility to enteric infections; (2) that interventions with food ingredients could modulate inflammation, protect against infection and elicit positive effects on metabolism. We used in vitro and pre-clinical models, and human dietary challenge to test the ability of defined food ingredients to modulate microbiota diversity, to promote appropriate innate immune function, and to modulate inflammation-related disorders like type 2diabetes. We sought to identify food ingredients that promote microbiota diversity, and which can be incorporated by the Irish food industry into novel foods that promote health. We found that metabolic phenotype of IL-1RI-/- mice fed high fat diet (HFD) or low-fat diet (LFD) could be transferred to wild-type (WT) mice through gut microbiome exchange facilitated by co-housing. Transfer of short chain fatty acid (SCFA)-producing microbiota from IL-1RI-/- mice identified a new connection between diet, inflammatory signaling, and the gut microbiome, an association that is dependent on the nature of the dietary fat challenge, which is particularly relevant for human diet management. (2) Yeast β-glucans were potential modulators of the innate immune-metabolic response, by impacting glucose, lipid and cholesterol homeostasis. We examined whether yeast β-glucan interacted differentially with either an obese healthy or obese diabetic gut microbiome, to impact metabolic health through hepatic effects under high-fat dietary challenge. Obese diabetic (OBD) microbiome colonization adversely impacted metabolic health compared to obese healthy (OBH) microbiome engraftment. OBD mice were more insulin resistant and displayed hepatic lipotoxicity compared to weight matched OBH mice. Yeast β-glucan supplementation resolved this adverse metabolic phenotype, coincident with increasing the abundance of health-related bacterial taxa, and highlighting potential human therapeutic applications. In conclusion, the research has highlighted new mechanisms linking inflammation to obesity and to metabolic disease, and identified potentially therapeutic food ingredients to combat diabetes.

KEYWORDS

Diet, Microbiome, Diabetes

ACRONYM	IMMUNOMET
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Section 1 - Research Approach & Results

Start Date

01 January 2015

End Date

31 December 2020

Research Programme

Food Institutional Research Measure

TRL Scale

TRL 5: Technology validated in relevant environment

NRPE Priority area

Food for Health

Total DAFM Award

€1,246,995.10

Total Project Expenditure

€1,034,972.43

Rationale for undertaking the Research

The prevalence of diabetes continues to rise worldwide. Type 2 diabetes accounts for 90-95% of diagnosed diabetes cases which affected more than 500 million people worldwide in 2018, which is an underestimation since many cases are undiagnosed. Metformin is an effective therapeutic; however, there are opportunities for alternative diet-mediated therapeutic regimes which may interact with the gut microbiome. Effective dietary intervention is an increasingly important alternative to drug therapy and there is growing interest in how this may interact with the gut microbiome to improve metabolic health. Evidently there is a role for the gut microbiome and its SCFA products in the interaction between diet, obesity and metabolic-inflammation. The present investigation also tested the hypothesis that a co-housing model would enable interactions between IL-1RI, the gut microbiome and diet, with ensuing impact on metabolic tissue homeostasis. Finally, in work still to be published, we tested the ability of food ingredients to modulate levels of gut pathogens. The global unifying aim of this research was therefore to demonstrate the potential for food and food ingredients to display functional properties relevant to the major lifestyle diseases that challenge modern consumers, primarily metabolic disease, obesity and gut infections.

Methodology

1. Male WT (C57BL/J6) and IL-1RI-/- mice are fed HFD (45% kcal) or LFD (10% kcal) for 24 weeks and housed i) by genotype (single-housed) or ii) with members of the other genotype in a shared microbial environment (co-housed). The IL-1RI-/- gut microbiome is dominant to WT, meaning that co-housed WT mice adopted the IL-1RI-/- microbiota profile. This is concomitant with greater body weight, hepatic lipid accumulation, adipocyte hypertrophy, and hyperinsulinemia in co-housed WT mice, compared to single-housed counterparts. These effects are most evident following HFD. Primary features of microbiome differences are Lachnospiraceae and Ruminococcaceae (known producers of SCFA).

2. Male C57BL/6J mice were pre-inoculated with gut microbiota from obese healthy (OBH) or obese type 2diabetic (OBD) subjects, in conjunction with a high-fat diet (HFD) with/without yeast β-glucan. OBD microbiome colonization adversely impacted metabolic health compared to OBH microbiome engraftment. OBD mice were more insulin resistant and displayed hepatic lipotoxicity compared to weight matched OBH mice. Yeast β-glucan supplementation resolved this adverse metabolic phenotype, coincident with increasing the abundance of health-related bacterial taxa. Hepatic proteomics demonstrated that OBD microbiome transplantation increased HFD-induced hepatic mitochondrial dysfunction, disrupted oxidative phosphorylation, and reduced protein synthesis, which were partly reverted by yeast β-glucan supplementation.
3. The differences in the microbiome of Obese Healthy and Obese Diabetic human subjects were replicated in an intro system which allowed high-throughput screening of multiple food ingredients and multiple bacterial pathogens to be added. Glucose treatment led to an enrichment of both commensal and pathogenic proteobacteria. Fermentation of yeast β-glucan led to increased bacteroides and clostridial populations, which is largely desirable.

Project Results

Our results show that co-housing WT and IL-1RI−/− mice modified the gut microbiome of both genotypes, coincident with phenotypic transfer of hepatosteatosis and adipocyte hypertrophy from IL-1RI−/− to WT. This effect was most pronounced following a HFD, compared to low-fat diet (LFD). Moreover, microbiome transfer between genotypes was primarily driven by SCFA-producing bacterial taxa. These findings not only highlight a new connection between diet, inflammatory signalling and the gut microbiome, but also demonstrate that this association is dependent on the particular dietary challenge. OBD microbiome colonization adversely impacted metabolic health compared to OBH microbiome engraftment. OBD mice were more insulin resistant and displayed hepatic lipotoxicity compared to weight matched OBH mice. Yeast β-glucan supplementation resolved this adverse metabolic phenotype, coincident with increasing the abundance of health-related bacterial taxa. Hepatic proteomics demonstrated that OBD microbiome transplantation increased HFD-induced hepatic mitochondrial dysfunction, disrupted oxidative phosphorylation, and reduced protein synthesis, which were partly reverted by yeast β-glucan supplementation. Hepatic metabolism was adversely affected by OBD microbiome colonization with high-fat feeding, but partially resolved by yeast β-glucan. The yeast β-glucan was provided by Kerry Foods, through their (then recently acquired subsidiary) WellImmune. Novel Intellectual property was generated (patent application). A major conclusion of this exciting research was the necessity for performing a human trial, ideally in subjects with metabolic disease or Type 2 diabetes.

Section 2 - Research Outputs

Summary of Project Findings

High-fat diet-induced obesity drives a myriad of complex molecular perturbations in metabolic tissues, including dysregulated cytokine signalling (e.g., IL-1β) and a loss in tissue functionality. It is only now being appreciated that the relative impact of inflammatory signalling and obesity-associated complications maybe further impacted by the gut microbiota, which can in turn be modulated by diet. We demonstrated a novel connection between the gut microbiome and IL-1RI-mediated signalling, wherein SCFA-producing bacteria and metabolic phenotype are transmissible via co-housing with IL-1RI−/− serving as the “dominant” profile. Moreover, this effect was more significant with HFD compared to LFD-feeding. Studies examining diet-gut interactions in the context of metabolic inflammation should be encouraged to use a dietary challenge and to extend these findings in human interventions. Our findings will allow specific mechanisms to be investigated. The differential effect of the obese type 2 diabetes microbiome on insulin resistance, hepatic lipid and metabolite profiles, with associated hepatic

proteome profiles compared to an obese healthy microbiome, is novel. We show that obesity alone is not sufficient to impair metabolic health and indeed, within equivalent obese states the metabolic impact of diabetes on the microbiota can be substantial. Additionally, functional foods ingredients such as β-glucan supplementation can partly reverse the adverse induced metabolic phenotype. There is necessarily a lag time between a laboratory finding like this, and changes to clinical management of obesity and type 2 diabetes, but already we have provided a prima facie case for yeast beta glucan having a role in clinical management of metabolic disease. This is also applicable to pre-diabetes, which our initial human studies in Galway showed to be much more prevalent than expected.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students	1	0	1
Research Assistants/Technicians	0	4	4
Post Doctorates	2	0	2

Summary of Academic Outputs

Research Outputs	Total Number	Details
Publications in Peer Reviewed Scientific Journals	8	1. 1. Microbiome Transfer Partly Overrides Lack of IL-1RISignaling to Alter Hepatic but not Adipose Tissue Phenotype and Lipid Handling following a High-Fat Diet Challenge. Ralston JC, Mitchelson KAJ, Lynch GM, Tran TTT, Wang H, Strain CR, Lenighan YM, Kennedy EB, Stanton C, McGillicuddy FC, Su Q, O'Toole PW, Roche HM. Mol Nutr Food Res. 2021 Jan;65(1):e2000202. doi:10.1002/mnfr.202000202. Epub 2020 Jul 12 2. Kathleen A.J. Mitchelson, Tam T.T. Tran, Eugene T. Dillon, Klara Vlckova, Sabine M. Harrison, Alexandra Ntemiri, Katie Cunningham, Irene Gibson, Francis M. Finucane, Eibhlís M. O'Connor, Helen M. Roche and Paul W. O'Toole. Yeast β-glucan improves insulin sensitivity and hepatic lipid metabolism in mice humanized with obese type 2 diabetic gut microbiota. Mol. Nutr. & Food Research 2022, in press. 3. Strain, R., Stanton, C, & Ross, R.P. 2021. Effect of diet on pathogen performance in the microbiome. Microbiome Res Rep 2022;1:13. DOI: 10.20517/mrr.2021.10 4. 154. Tran TTT, Corsini S, Kellingray L, Hegarty C, Le Gall G, Narbad A, Müller M, Tejera N, O'Toole PW, Minihane AM, Vauzour D. APOE genotype influences the gut microbiome structure and function in humans and mice: relevance for Alzheimer's disease pathophysiology. FASEB J. 2019 Jul;33(7):8221-8231. 5. O'Toole, P.W. and M. Paoli. 2017. The Contribution of Microbial Biotechnology to Sustainable Development

		Goals: Microbiome Therapies. Microbial. Biotech. 10(5):1066-1069. 6. Pellanda P, Ghosh TS, O'Toole PW. 2020. Understanding the impact of age-related changes in the gut microbiome on chronic diseases and the prospect of elderly-specific dietary interventions. Curr. Opin. Biotechnol. 70: 48-55. 7. Salvetti E, and O'Toole PW. 2017. The genomic basis of lactobacilli as health-promoting organisms. Amer. Soc. Microbiol. Press, Washington DC. R.A. Britton, and P.D. Cani, ed.'s. Microbiol. Spectrum 5:BAD-0011-2016.doi:10.1128/microbiolspec.BAD-0011-2016. Under Review: 8. Strain, R., Stanton, C, & Ross, R.P. 2022. Pathogen performance in the presence of prebiotic fibres in an ex vivo model of the human gut. Microbiome Res Rep.
PhD Theses	1	1. PhD thesis, Ronan Strain, 2022, School of Microbiology, University College Cork. title: Novel insights into the influence of fibre and lipid substrates on the microbiome. PhD exam July 8th 2022.
Peer Reviewed Conference Papers	2	1. Nutrition Society Summer Conference: Getting Energy Balance Right in Leeds, England, July 2018 2. European Association for the Study of Diabetes Annual Meeting, Berlin, Germany, October 2018.

Intellectual Property

We submitted a European Patent Office Application on October 18th, 2021 as detailed below.
Title of the invention: Treatment or prevention of hepatic metabolic conditions associated with diabetes.
Field of the invention: The current invention relates to treatment or prevention of a metabolic condition comprising administering β-glucan, particularly β-(1→3)-glucan, to a subject. In particular, the invention relates to treatment or prevention of a metabolic condition. The metabolic condition is one associated with obesity, non-alcoholic fatty liver disease and/or diabetes.
Authors: Prof Helen Roche and Ms. Kathleen Mitchelson, UCD; Prof. Paul O'Toole, Dr Klara Vlckova and Dr Tam Tran UCC.
Prof. Roche also attracted an SFI grant for mechanistic investigations (basic science) associated with this project outcomes, while the issue of IP is being further developed with Kerry Ingredients.

Summary of other Project Outputs

Project Outputs	Details	Total Number
No Response	No Response	0

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
No Response	No Response
No Response	No Response

Dissemination Activities

Activity	Details
No Response	In order to protect IP, dissemination was restricted to formal publications whose timing was co-ordinated with patent application.

Knowledge Transfer Activities

Identify knowledge outputs generated during this project.	No Response
Identify any knowledge transfer activities executed within the project.	No Response
List any impacts resulting from the knowledge transferred during the project.	No Response

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource	Funding €	Summary
Exchequer National Funding	€994,587.00	Diet, Immune Training and Metabolism The SFI Frontiers Investigator Programme entitled ' Diet, immune Training & Metabolism ' is determining the interaction between nutrient stressors, the gut microbiome, inflammation and metabolism within the context of non-alcoholic fatty liver disease (NAFLD) a very common consequence of obesity, linked with type 2 diabetes mellitus(T2D). This project is in collaboration with Prof Fred Sheedy, School of Biochemistry & Immunology, Trinity College Dublin, Ireland (https://www.tcd.ie/Biochemistry/people/fsheedy/), Prof Paul O'Toole, UCC, Ireland(https://apc.ucc.ie/paul_otoole/) and Prof Suzanne Norris, St James' Hospital Dublin. The basic scientific concepts and approaches developed within our SFI Frontiers programme compliments other on-going basic and translational projects including, APPETITE, Precision Oncology Ireland (POI), ImmunoMet, FOODBALL, Nutrimal and FHI.

Future Strategies

Research is a linear incremental progress shaped by accumulating data. The relevant factors for determining Future strategies here are: (1) Prof. Roche's current project The SFI Frontiers Investigator Programme entitled 'Diet, immune Training & Metabolism' is determining the interaction between nutrient stressors, the gut microbiome, inflammation and metabolism within the context of non-alcoholic fatty liver disease (NAFLD); (2) the outcome of a dietary trial being performed in the FIRM 2017 project led by Dr. Eibhlis O'Connor called FIBREMET (this project is examining the role of dietary fibre in improving symptoms in Inflammatory Bowel Disease, and type 2 diabetes) (3) Ongoing commercial and IP evaluation by Kerry Foods. Subject to progress in all these areas, we anticipate application to Enterprise Ireland Commercialization Fund for potential commercial development of betaglucan-containing products to manage metabolic health.

Project Publications

1. Microbiome Transfer Partly Overrides Lack of IL-1RI Signalling to Alter Hepatic but not Adipose Tissue Phenotype and Lipid Handling following a High-Fat Diet Challenge. Ralston JC, Mitchelson KAJ, Lynch GM, Tran TTT, Wang H, Strain CR, Lenighan YM, Kennedy EB, Stanton C, McGillicuddy FC, Su Q, O'Toole PW,Roche HM. Mol Nutr Food Res. 2021 Jan;65(1):e2000202. doi: 10.1002/mnfr.202000202. Epub 2020 Jul 12
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8. Strain, R., Stanton, C, & Ross, R.P. 2022. Pathogen performance in the presence of prebiotic fibres in an ex vivo model of the human gut. Microbiome Res Rep, under review.