M1: The upper airway microbiome in health and disease

Biswas, K.[1], Yin, T. [1], Broderick D.[1], Wagner Mackenzie, B. [1], Douglas, R. [1]

[1]Department of Surgery, University of Auckland, Auckland, NZ,

Factors which drive changes within the bacterial communities of the upper airway remain unclear. While some inconsistent associations have been reported between the microbiota and treatment, allergic rhinitis and asthma, most studies have not detected a relationship between the upper airway microbiota and clinical variables. This may relate to the limited numbers of samples typically collected from patients. To assess the stability of the sinonasal microbiota and how it interacts with medications and surgery, we performed a comprehensive longitudinal study in patients with and without sinus disease. Sinus swabs were collected from 20 chronic rhinosinusitis patients and 4 healthy controls over a year. Samples were subjected to DNA and RNA extraction and subsequent bacterial amplicon sequencing, host inflammatory profiling and quantification by droplet digital™ PCR (ddPCR).

Bacterial diversity significantly decreased during the time points immediately after surgery but returned to baseline levels after recovery (>3 months post-surgery). Relative and total abundances of *Staphylococcus aureus* were highest during recovery, suggesting not only a faster growth rate relative to other bacteria but also that it thrives in the immediate post-surgical environment. *Corynebacterium accolens* dominated the healthy sinonasal microbiota and was associated with milder symptom scores across all time points. Oral antibiotics had only a small effect on the microbiota, suggesting that empiric antibiotics may have limited effects on disease pathophysiology. This supports the idea that beneficial effects of empiric antibiotics are more likely related to their anti-inflammatory, rather than antimicrobial, properties. Cytokine analyses showed significantly higher inflammatory cytokine IL-6 and IL-8 levels during recovery.

This study demonstrated that sinonasal bacterial community composition in healthy participants is more stable over time compared to the highly variable sinus disease patients.

M2: Antibiotic exposure in early life and neurodevelopmental outcomes across childhood

Slykerman, R.F.[1], Neumann, D.[2], Underwood, L.[3], Hobbs, M.[3], Waldie, K.E.[2]

[1]Department of Psychological Medicine, University of Auckland, Auckland, NZ, [2]School of Psychology, University of Auckland, Auckland, NZ, [3]Centre for Longitudinal Research, University of Auckland, Auckland, NZ.

\Antibiotics reduce the diversity and composition of the human gut microbiota, common childhood infections are frequently treated with antibiotic medication which alters gut composition at a critical time of development. These antibiotic-induced changes have downstream effects for the central nervous system and could alter cognitive and behavioural outcomes. Using different birth cohorts we have demonstrated a significant association between early life antibiotic exposure and increased risk of behavioural problems and lower cognitive ability.

In the most recent study, we aimed to investigate the relationship between age at first exposure to antibiotics and cognitive and behavioural development at 4.5 years while controlling for multiple confounders, including otitis media. Study participants were 5589 children enrolled in the broadly generalisable Growing Up in New Zealand cohort study, with antibiotic exposure data, maternal antenatal information, and age 4.5-year behaviour and cognitive outcome data. Children were categorised as first exposed to antibiotics according to the following mutually exclusive ages: 0-2 months; 3-5-months; 6-8 months; 9-11 months; 12-54 months or not exposed by 54 months. Developmental outcome measures included the Strengths and Difficulties Questionnaire, Luria hand clap task, and the Peabody Picture Vocabulary Test-III.

In univariate analysis, there was an evident dose-response relationship where earlier exposure to antibiotics in the first year of life was associated with behavioural difficulties, lower executive function scores, and lower receptive language ability. After adjusting for confounders, pairwise comparisons showed that first antibiotic exposure between birth and three months or between six and nine months was associated with lower receptive vocabulary and increased behaviour difficulties.

In conclusion following adjustment for socioeconomic factors and otitis media, there is evidence that antibiotic exposure during potentially sensitive windows of development is associated with receptive language and behaviour later in childhood.

M3: Uncovering novel bacterial species in the microbiome of humans, mountain gorillas, and livestock in a region considered high-risk for emerging infectious diseases

Almeida, V.[1], Knox, M. A.[1], Ngabirano, A.[2], Rubanga, S.[3], Kalema-Zikusoka, G.[3], Midwinter, A. C.[1], Handley, K. M.[4], Biggs, P. J.[1,5], Hayman, D. T. S.[1]

[1]Molecular Epidemiology and Public Health Laboratory, School of Veterinary Science, Massey University, Palmerston North, NZ, [2]Bwindi Development Network, Buhoma, Kanungu, Uganda, [3]Conservation Through Public Health, Entebbe, Uganda, [4]School of Biological Sciences, The University of Auckland, Auckland, NZ, [5]School of Natural Sciences, Massey University, Palmerston North, NZ.

The emergence of zoonotic infectious diseases is a growing concern, particularly in areas where humans come into close contact with wildlife and domestic animals. Understanding why certain microbes switch hosts remains elusive, although factors such as contact rates between the different hosts are believed to play a role. To better comprehend the influence of spatiotemporal overlap on microbial sharing, we examined over 2,400 bacterial metagenome-assembled genomes obtained from the microbiomes of mountain gorillas, humans, and livestock in Uganda. Our analysis identified at least 500 novel bacteria, including novel bacterial species shared among different hosts, and others with potential implications as human pathogens. This study provides valuable insights into the diversity of microbiomes in a geographic interface with an elevated risk of emerging zoonotic diseases.

M4: Diet and disparities in shaping Indigenous microbiomes: the unexplored Māori gut microbiome

Silk, E.T.[1,2], Bayer, S.B.[1,2], Roy, N.C.[2,3,4], Taylor, M.W.[2,5], Foster, M.[2,6], Vatanen, T.[7], Gearry, R.B.[1,2]

[1]Department of Medicine, University of Otago, Christchurch, NZ, [2]High-Value Nutrition National Science Challenge, Auckland, New Zealand, [3]Department of Human Nutrition, University of Otago, Dunedin, NZ, [4]Riddet Institute, Palmerston North, New Zealand, [5]School of Biological Sciences, University of Auckland, Auckland, NZ. [6]Edible Research, Rangiora, NZ. [7] Helsinki Institute of Life Science, University of Helsinki, Helsinki, Finland.

The gut microbiome plays many vital roles in human health, including mediating metabolism, immunity, and the gut-brain axis. Gut microbiome research has been popularised in the last two decades due to decreasing costs and advances in sequencing technologies. Despite the increase in literature surrounding the gut microbiome, many ethnicities remain underrepresented. There are gut microbiome differences based on environmental factors that affect some ethnicities, such as Indigenous peoples. Current evidence suggests significant gut microbiome differences between many Indigenous and non-Indigenous peoples due to dietary, socioeconomic and health differences, particularly in the context of rural or remote Indigenous peoples compared to their urban, non-Indigenous, counterparts. Thus, these disparities, and in turn the gut microbiome, are influenced by urbanisation. Furthermore, distinct gut microbiome compositions have been found between Indigenous people of the same ethnicity based on urbanisation, introducing heterogeneity within Indigenous ethnicities. These factors harbour the capacity to impact gut microbiome diversity and composition.

While some literature on Indigenous gut microbiomes has been published over the last five years, literature on Māori microbiomes is absent despite the inequities Māori face in NZ. Māori suffer these disparities to a greater extent than other, non-Indigenous ethnicities of NZ, therefore differences likely exist between the gut microbiomes of Māori and non-Māori, potentially manifesting in worse health outcomes.

More research is needed to characterise the gut microbiomes of underrepresented populations in order to lessen health inequity gaps between Indigenous and non-Indigenous peoples. However, before studies can fully focus on Indigenous (i.e., Māori) microbiomes, it is crucial to improve study design for Indigenous peoples to protect their rights and interests, which will lead to increased study participation and adherence.

M5: Faecal microbiome transplants leave footprints in the plasma metabolome

Day, F. [1*]., O'Sullivan, J.M. [1,2,3,4,5*] Cutfield, W.S. [1*], & Pook, C. [1*]

Liggins Institute, Waipapa Taumata Rau—The University of Auckland, 85 Park Road, Private Bag 92019, Auckland 1142, New Zealand[1]; The Maurice Wilkins Centre, The University of Auckland, Auckland, New Zealand[2]; MRC Lifecourse Epidemiology Unit, University of Southampton, United Kingdom[3]; Australian Parkinson's Mission, Garvan Institute of Medical Research, Sydney, New South Wales, 384 Victoria Street, Darlinghurst NSW 2010, Australia[4]; A*STAR Singapore Institute for Clinical Sciences, Singapore, Singapore[5].

Microbes in the gut affect processes in their hosts far beyond the organ they inhabit. The mechanisms that allow them to do so are diverse and often poorly understood. Many microbes metabolise compounds fed to them through the host diet, excreted into the gastrointestinal tract by the host, or from the exogenous environment and form small (<1500 AMU) metabolites distinct from the host's endogenous metabolome. A great diversity of microbially derived metabolites [MDMs] are produced by different microbiota, each with specific functions. Many of the thousands of MDMs are found in circulation and have effects, positive or negative, upon host appetite, metabolism and adipogenesis. Faecal microbiota transfer [FMT] is a medical procedure where microbiota isolated from a healthy donor stool is transplanted into a recipient to partially replace and remediate health problems associated with a dysbiotic gut microbiome. The GutBugs Trial is a double blinded, randomised, controlled trial investigating the effects of a single FMT treatment on obese adolescents. At four years post-FMT the treatment was associated with weight loss, reduction in fat mass and improvement in metabolic syndrome (high waist circumference, blood pressure, plasma lipids and glucose). We have used liquid chromatography with mass spectrometry to apply an untargeted-metabolomic approach to the serum of trial participants to investigate changes in the abundance of MDM with phenotypic changes seen in participants. We identified 3,258 (ms^2 acquired) metabolites and were able to annotate 656. Sixty-one metabolites varied significantly with FMT-treatment over four years. These findings may provide mechanistic links between metagenomic and taxonomic changes in the microbiome and clinical outcomes of the GutBugs Trial.

M6: Novel application of a commercial agent to eradicate biofilms in sinusitis

Koefoed, A.M., Douglas, R., Biswas, K.

Department of Surgery, University of Auckland, Auckland, NZ

Chronic rhinosinusitis (CRS) is a sinonasal disease associated with a low quality-of-life and high economic burden. Antibiotics are prescribed widely for its treatment, however, the evidence supporting its efficacy is poor. This may be due to biofilms. Bacteria residing in biofilms are inherently more resistant to antibiotic activity than their planktonic counterparts. This is due to a low cell turnover rate (upon which antibiotics act) and the protective nature of the matrix. Furthermore, the subinhibitory antibiotic concentrations at the site of infection can lead to the development of antimicrobial resistant strains of bacteria. With approximately 50% of CRS patients having biofilms on their sinonasal mucosa, this necessitates the search for a novel antimicrobial agent (not an antibiotic) for biofilm eradication.

To investigate this, the following laboratory strains of clinically relevant bacteria were grown as biofilms in the Calgary Biofilm Device:

- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Moraxella catarrhalis*
- *Klebsiella pneumoniae*
- *Staphylococcus epidermidis*
- *Haemophilus influenzae*
- *Corynebacterium accolens*
- *Dolosigranulum pigrum*

The biofilms were exposed to the commercial agent and commonly prescribed antibiotics for CRS at concentrations found in the sinus tissue (0.27 µg/mL for doxycycline and 1.60 µg/mL for roxithromycin) at different time intervals. After treatment, colony forming units were enumerated.

Using the clinically relevant concentration of antibiotics, no significant change in biofilm presence relative to saline was recorded. In comparison, the commercial agent demonstrated rapid antibiofilm activity (>4 log reduction) within five minutes.

This study has demonstrated that commonly prescribed antibiotics for sinonasal disease are likely insufficient for eradicating biofilms. Therefore, treating patients with oral antibiotics could predispose them to adverse drug reactions and the emergence of antimicrobial resistant bacteria. Alternative approaches for antibiofilm treatment such as the tested commercial agent could be a potential way forward.

M7: Faecal Microbiota Transplant-mediated alteration of the phageome composition in a clinical trial for obesity

Zuppi, M.[1], Vatanen, T[1,2], Wilson, B.C.[1], Golovina, E.[1], Cutfield, W.S.[1], Vickers, M.H[1], O'Sullivan, J.M.[1]

[1]Liggins Institute, University of Auckland, Auckland, NZ

[2]Helsinki Institute of Life Science, University of Helsinki, Helsinki, FIN

Faecal microbiome transplant (FMT) is a medical procedure used to treat diseases associated with the gut microbiome. FMT aims at altering the gastrointestinal environment of its recipients through the introduction of donor faecal microbiome. Gastrointestinal phages are implicated with human health and successful engraftment of donor phages has been correlated with FMT treatment efficacy. Phages can impact the environment they inhabit by modulating bacterial communities, either through predation or by enhancing their fitness. These interactions can be described through population dynamics. In this study, we have investigated the effects of FMT on the phageome composition of participants within the Gut Bugs Trial (GBT), a placebo control clinical trial that investigated the efficacy of FMT in the treatment of adolescent obesity-related symptoms. Stool samples in the GBT were collected at baseline (prior to the transplant) and up to six months after the transplant (*i.e.,* 6 weeks, 12 weeks, and 26 weeks). Microbial DNA was sequenced using Illumina technology, and phage sequences were identified and characterised *in silico*. Donor phages engrafted stably in recipients following the FMT, and remained identifiable for the study time course. Phage engraftment was donor specific, and engraftment efficacy was positively correlated with donor phageome diversity. Engraftment of donor phages increased the abundance of temperate phages within FMT recipients and their phageome variability and diversity. An increase in variability and diversity was also observed in the bacteriome, suggesting that FMT altered microbial dynamics. Overall, FMT proved effective in modulating the gastrointestinal environment of the obese adolescents that participated in the Gut Bugs Trial, by altering their phageome composition in a donor-specific manner and by promoting shifts in microbial dynamics.

M8: Dietary interventions, digestive physiology, and microbiome modulation

Roy, N.C.[1,2,3], Bayer, S.[3,4], Maggo, J.[3,4], Ng H.M.[3,4], Wall C[3,4], Carco, C.[2,3,5], Young, W.[2,3,5], Mullaney, J.[2,3,5], Cabrera, D.[3,5], Fraser, K.[2,3,5], McNabb, W.C.[2,3], Cooney, J.[3,6], Guenther, C.[3,6], Trower, T[3,6], Foster, M.[3,7], Gearry, R.B.[3,4]

[1]Department of Human Nutrition, University of Otago, Christchurch, NZ, [2]Riddet Institute, Massey University, Palmerston North, NZ, [3]High-Value Nutrition National Science Challenge, [4]Department of Medicine, University of Otago, Christchurch School of Medicine, NZ, [5]AgResearch, Palmerston North, NZ, [6]Plant and Food Research, Hamilton, NZ, [7]Edible Research, Ohoka, NZ

Disorders of gut-brain interaction (DGBI) altered bowel habit and sometimes pain, significantly impacting a person's quality of life. The cause of DGBI remains unknown, but likely associated to alterations in the gut microbiome, host-microbial metabolism, immune function, sensitivity, and motility. Many individuals, therefore, resort to using food as a means of self-management, e.g., consuming green or gold kiwifruit to help with constipation.

Two randomised clinical studies were conducted to generate mechanistic insights on daily consumption of two green or gold kiwifruit over four weeks on gut symptoms and digestion in participants with constipation. The first study explored gold kiwifruit or psyllium (fibre-content matched) in participants with and without constipation, while the second study explored green kiwifruit or maltodextrin (calorie matched) in participants with constipation. In both studies, faecal microbiome and metabolome/metabolites were analysed. In the second study, gut physiome data (transit, gas profiles and colonic volume) were also collected.

Gold kiwifruit and psyllium had different effects on plasma and faecal metabolites in all participants, regardless of their bowel habits. Pathway mapping showed that in the kiwifruit group, plasma concentrations of neurotransmitters tryptophan and tyrosine, neurotransmitter precursors in the kiwifruit group were lower than downstream metabolites in their respective pathways. Conversely, the psyllium group accumulated tryptophan and tyrosine in plasma, while the concentrations of downstream metabolites were lower in their respective pathways. Both interventions had differential effects on the taxonomic composition (*Eggerthella* and *Bacteroides* genera) and predictive function (metabolism of carbohydrates, cofactors and vitamins, and transporters) of the faecal microbiome in all participants. Faecal microbiome, plasma metabolites and physiome data from the green kiwifruit intervention will also be presented.

Findings from both studies will provide insights into the molecules of the host-microbial interactions and physiological changes associated with the positive impacts of consuming kiwifruit for gut symptoms and digestion.

M9: The Aotearoa-New Zealand Hot Spring Microbiome

Stott, M.B.[1]

[1] Te Kura Pūtaiao Koiora | School of Biological Sciences, Te Whare Wānanga o Waitaha | University of Canterbury, Christchurch 8140, Aotearoa-New Zealand

Hot springs offer an ideal opportunity to understand biotic and abiotic interactions within often highly constrained and relatively non-complex ecosystems. In this presentation, I will discuss the observations and outcomes of a large multi-year hot spring sampling and analysis project in which we sampled over 1,000 hot springs from the Taupō Volcanic Zone[1]. We measured the microbial community composition and 56 physicochemical conditions. Our aim was to understand the primary drivers of hot spring microbial community composition and biogeographical processes, to define a core hot spring microbiome, and to identify hot springs of high conservation value. Surprisingly, as part of this research we discovered what appears to be an extremophilic bacterium endemic to Aotearoa-New Zealand. I'll cover these topics and also talk about related research projects where we observed hot spring microbiomes over time, to gain insights into ecosystem variability and drivers of change.

1. www.1000Springs.org.nz.

M10: Niche differentiation enables success of the Luna cluster across a river-to-sea system

West, A.G.[1,2], Astudillo-Garcia, C.[1,2], Tee H.S[1], Boey, J.S. [1,2], Hoggard M. [1,2], Lear G. [1], Handley, K.M. [1,2]
[1]Te Kura Mātauranga Koiora School of Biological Sciences, Waipapa Taumata Rau University of Auckland, Tāmaki Makaurau Auckland, Aotearoa NZ, [2]Genomics Aotearoa, Aotearoa New Zealand

Estuaries are a key interface between terrestrial and marine environments, funnelling freshwater and nutrients from land to sea. Microorganisms inhabiting these environments are strongly stratefied across freshwater and saline niches, necessitating genetic adaptations to tolerate salt stress. Estuaries represent an ideal system to study competition and niche differentiation within microbial lineages related to salt tolerance and resource utilisation. We investigated the microbial ecology of a river-to-sea transect via metagenomics and metatranscriptomics.

Nine filtered water samples were collected along the transect for sequencing and assembly. Metagenome-assembled genomes (MAGs) were identified using a multi-parameter binning approach with manual curation, and dereplicated at 99% average nucleotide identity. We obtained 265 unique MAGs >75% complete with 0-5% contamination, representing 17 phyla. RNA sequences were mapped to MAGs to determine transcriptional profiles across the estuary.

A single freshwater-to-marine bacterium, *Aquiluna* sp. Ww131 of the *Microbactericeae* Luna cluster dominated the saline aquatic environment, reaching relative abundances of >50%. Though *Aquiluna* Ww131 was present in freshwater, we observed a tradeoff with several closely-related *Rhodoluna* species whose abundance was greater in this environment. Members of the Luna cluster are known to utilise proteorhodopsin for photoheterotrophy, which may contribute to the success of this lineage in the estuarine system. Results suggest competition among freshwater members of the Luna cluster, expressing genes for a photoheterotrophic mode of life alongisde contributions to estuarine carbon degradation.

Transcriptomic evidence suggests *Aquiluna* Ww131 encodes a greater proportion of acidic amino acids in secreted proteins to tolerate salt stress in the saline reaches of the estuary. Extensive genetic adaptations of *Aquiluna* Ww131, which belongs to a genus originally described from freshwater environments, likely enables its colonisation of the saline reaches of the estuarine system. This study highlights the mechanisms through which Luna cluster members colonise an estuarine environment from river to sea.

M11: Development of an eDNA Metabarcoding Workflow to Estimate Time Since Deposition of Forensically Relevant Body Fluid Stains

Yugovich, $O.A.N.$[1,2], Bunce, M.[3], Cave, V.M.[4], Harbison, $SA.$[1,2]
[1] School of Chemical Sciences, University of Auckland, Auckland, NZ
[2] Institute of Environmental Science and Research, Auckland, NZ
[3] Department of Conservation, Wellington, NZ
[4] Department of Statistics, University of Auckland, Auckland NZ

Complex biological mixtures, such as environmental DNA (eDNA), are a rich source of information that can provide forensic science with new avenues of evidence to aid in the resolution of criminal cases. Recent studies have investigated the use of eDNA to solve forensic questions such as body fluid identification, predictive geolocation, and estimating postmortem interval. Identifying the presence of biological material at a crime scene and the body site of origin is an important aspect in forensic science. Although significant effort has been made to research techniques to identify body fluids, determining the time in which they were deposited remains uncertain. Providing an estimate of when a body fluid stain was deposited can aid in criminal investigations by revealing a time frame in which the crime may have been committed, or alternatively, can help to direct forensic scientists as to what stains are most relevant to a case. This study aims to investigate the use of methods commonly used in ecology, biodiversity and ancient DNA to determine the time since deposition of body fluid stains commonly encountered in forensic casework, such as saliva and blood. This was achieved by comparing suitable extraction methods to ensure representative DNA extraction from a broad range of taxonomic groups, appropriate quality control measures, marker selection, and sequencing platforms. This study also developed a suitable bioinformatics analysis workflow using freely available command-line tools. The results of this study highlight the influence of method choices on the generation of reliable and accurate results and demonstrates the need for standardised laboratory and analysis protocols to ensure these methods are fit for a forensic purpose.

M12: Spatial and temporal variation of soil microbiome across NZ dairy farms

Shengjing Shi[1], Bryony Dignam[1], Emily Gerard[1], Lee Aalders[2], Faith Mtandavari[2], Nigel Bell[2], Sean Marshall[1]

[1] Resilient Agriculture, Lincoln Science Centre, AgResearch Ltd., Christchurch, NZ, [2] Ethical Agriculture, AgResearch Ltd. Hamilton, NZ.

The soil microbiome plays critical roles for many soil functions. However, managing soil microbiome for agriculture gains is a serious challenge, partly due to the highly dynamic nature of soil microbes and unclear drivers of such variation. We aimed to understand soil microbial variations in dairy farms across New Zealand (NZ).

Soils from thirty farms over three regions across NZ were sampled. Two farms from each region were sampled seasonally. Detailed spatial variability was determined from 32 soil cores taken from a single field (100 × 50 m) in one region. DNA was extracted from soils and analysed using MiSeq 16S and ITS sequencing. High microbial diversity was detected within NZ pasture soils. Region had a strong influence on both soil bacterial and fungal communities, while seasons had a minor impact. Approximately 15% of bacterial OTUs and 19% of fungal OTUs were present in all 30 sites. The spatial variation of soil microbiome within a single field had similar levels of the seasonal variation in that region. Several soil properties (e.g., soil pH, moisture, Olsen P and C/N ratio) and nematode population significantly correlated to soil microbiomes. Our study revealed the different scales of soil microbial variations and potential drivers for soil microbiomes in NZ dairy farm soils. Common microbial taxa present across 30 sites were also identified. This knowledge is important in designing robust sampling strategies for soil microbiome studies and for moving towards managing soil microbiomes for productivity and environmental gains.

M13: The viral ecology and spatial niches of a river-to-sea salinity gradient

Hoggard, M. [1], Spoelstra E. [1], Palmer-Dale S. [1], Gios E. [1], Tee HS. [1], Handley K. [1]

[1]School of Biological Sciences, University of Auckland, Auckland, Aotearoa New Zealand.

Environmental viruses represent an important but understudied element in our understanding of microbial communities, as well as their active roles in ecosystem processes including nutrient cycling. The full diversity of the virosphere and viral contributions to the dynamics and biogeochemistry of ecosystems remain poorly understood. This study investigated the viral ecology of an estuary via metagenome and metatranscriptome sequencing, the reconstruction of viral genomes, and investigation of viral transcription, including genes that modify host metabolism.

Metagenomic and metatranscriptomic sequencing was conducted on filtered water and triplicate sediment samples from nine sites spanning the salinity gradient and large differences in nutrient availability. Viral sequences were identified by VIBRANT, VirSorter2, and deepVirFinder, and viral taxonomy and putative prokaryote hosts predicted. To further resolve virus-prokaryote host matching and dynamics across the estuary, a bacterial culture collection was generated, followed by long read sequencing, genome assembly, and identification of integrated viral genomes.

A total of 31,711 putative viral contigs were recovered, including representatives of *Caudoviricetes*, *Tectiliviricetes*, and *Malgrandaviricetes*. Few clustered closely with viral-RefSeq viruses, reflecting the vast degree of uncharacterised viral diversity. Salinity gradient, nutrient availability, and the benthic-pelagic divide were each strongly associated with patterns in viral community structure and diversity, mirroring observations for their putative microbial hosts based on 16S-rRNA gene sequencing. Additionally, anti-viral mechanisms were frequently identified in associated prokaryote metagenome-assembled genomes, suggesting interactions are common between viruses and a broad array of microbes in the estuarine system. Two thirds of the sequenced culture isolates contained integrated viral genomic regions. However, results indicated high rates of cryptic phage, highlighting a likely overestimation of active phage in metagenomic studies. Although unable to enter the lytic cycle, cryptic phage contribute to the genetic capability of their hosts.

This study highlights the interplay between diverse viruses and their prokaryote hosts within this dynamic ecosystem.

M14: Long-term therapeutic potential of fecal microbiome transfer in adolescent obesity: insights from a 4-year follow-up study

Wilson, B.[1], Derraik, J.[1,2], Tweedie-Cullen, R.[1], Albert, B.[1], Beck, K.[3], Creagh, C.[1], Depczynski, M.[1], Edwards, T.[1], Portlock, T.[1], Leong, K.[1], Jayasinghe, T.[1,4], Vatanen, T.[1,5], Cutfield, W.[1], & O'Sullivan, J.[1]

[1]Liggins Institute, University of Auckland, NZ, [2]Department of Paediatrics: Child & Youth Health, FMHS, University of Auckland, NZ, [3]School of Sport, Exercise and Nutrition, Massey University, Auckland, NZ, [4]Faculty of Medicine and Health, School of Dentistry and Charles Perkins Centre, The University of Sydney, Australia, [5]Helsinki Institute of Life Science, University of Helsinki, Finland.

The gut microbiome is dysbiotic in subjects with obesity and can induce weight gain and metabolic impairments when transplanted into germ-free mice. In a previous double-blind RCT, we assessed the effects of fecal microbiome transfer (FMT) from multiple healthy lean donors on BMI and metabolism among adolescents with obesity[1]. After 6 months, there were no effects on BMI or total body fat, but FMT lowered a marker of abdominal adiposity (A/G ratio) and transiently improved insulin sensitivity and glucose metabolism in a subset of participants with metabolic syndrome[1]. In parallel, we observed sustained shifts in the composition and functional potential of recipients' gut microbiomes[2], suggesting clinical improvements may manifest over longer time periods. To assess this, participants from the original trial were invited back for a 4 year follow-up assessment, of which 55/87 (63%) returned (27 FMT, 28 placebo). Compared to placebo, FMT recipients were 11 kg lighter, had lower BMI (-3.3 kg/m^2), less body fat (-4%), and lower insulin resistance (-25%), inflammatory markers (hsCRP), and metabolic syndrome scores. These effects appeared to be independent of diet which remained similar between groups. Intriguingly, we observed long-term persistence of donor-derived bacterial strains within the gut microbiomes of FMT recipients, while microbial diversity and gene richness declined within placebo microbiomes. Our findings indicate therapeutic potential for FMT to induce long-term changes in the context of metabolic disorders and obesity management. Future research will focus on identifying the key microorganisms and their associated metabolites driving these effects to assist in the development of novel therapies.

1. Leong et al. (2020). *Effects of fecal microbiome transfer in adolescents with obesity: the Gut Bugs randomized controlled trial*. JAMA Network Open, 3(12): e2030415.
2. Wilson et al. (2021). *Strain engraftment competition and functional augmentation in a multi-donor fecal microbiota transplantation trial for obesity*. Microbiome, 9(1): 107.

M15: Role of the gut microbiota and dietary intervention in the metabolically at-risk: insights from an Asian Chinese cohort

Saif Faraj[1,2*], Akarsh Mathrani[1,2*], Daniel Barnett[3], Michael Hoggard[1], Louise Lu[1,2,4], Ivana Sequeira-Bisson[1,2,4], Sally Poppitt[1,2,4], Mike Taylor[1,2]

[1]School of Biological Sciences, [2]High-Value Nutrition National Science Challenge, [3]Department of Statistics, and [4]Human Nutrition Unit, University of Auckland, New Zealand
[*]These authors contributed equally to this work

Metabolic disorders such as prediabetes and subsequent type 2 diabetes (T2D) are major global health issues. One concern is the increasing prevalence among many Asian peoples, often characterised by patterns of adipose deposition that may increase susceptibility to T2D and are termed the TOFI (Thin on the Outside-Fat on the Inside) profile. Disruptions in gut microbiota composition and functional capacity have been linked to T2D, yet the underlying mechanisms remain uncertain, especially in non-Caucasian populations. In a recent 3-month randomised controlled trial (Tū Ora study), we investigated the association between gut microbiota, dietary intervention, and metabolic health in Asian Chinese normoglycaemic and prediabetic cohorts. The Tū Ora intervention compared high-protein nut-based and isoenergetic high-carbohydrate cereal-based supplementation.

We performed 16S rRNA gene sequencing on 168 stool samples from 84 individuals, with additional shotgun metagenome sequencing on 64 stool samples representing a subset of 32 individuals. Our 16S rRNA gene data revealed no significant alterations in gut microbiota composition across either cohort. To a large extent our shotgun metagenome data supported these 16S-based findings, with no significant changes in bacterial community composition or functional capacity.

Notwithstanding the apparent lack of change in the composition or functional potential of the gut microbiota, these data contribute to the growing body of knowledge pertaining to the association between gut microbes and metabolic disorders.

M16: Assessing the dynamics of horizontal gene transfer after faecal microbiota transplantation in obese adolescents

Behling A[1], Wilson B[1], Ho D[1], Vatanen T[1,2,3], O'Sullivan J[1,4,5,6,7]

[1]Liggins Institute, University of Auckland, Auckland, New Zealand

[2]Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland

[3]Broad Institute of MIT and Harvard, Cambridge, MA, USA

[4]The Maurice Wilkins Centre, The University of Auckland, Auckland, Private Bag 92019, New Zealand

[5]Australian Parkinsons Mission, Garvan Institute of Medical Research, Sydney, New South Wales, 384 Victoria Street, Darlinghurst NSW 2010, Australia

[6]MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, SO16 6YD, United Kingdom

[7]Singapore Institute for Clinical Sciences, Agency for Science Technology and Research, Singapore

Horizontal gene transfer (HGT) describes the transmission of DNA outside of direct ancestral lineages. The process is best characterised within the bacterial kingdom and can enable the acquisition of genetic traits that support bacterial adaptation to novel niches. The adaptation of bacteria to novel niches has particular relevance for faecal microbiota transplantation (FMT), a therapeutic procedure which aims to resolve gut-related health conditions of individuals, through transplanted gut microbiota from healthy donors. This project aimed to assess the impact of HGT in the gut microbiomes of FMT recipients before and after treatment. HGT events across FMT and placebo recipient metagenomic samples from the Gut Bugs Trial[1] (Liggins Institute) were assessed using two complementary methodologies. First, all tentative HGT events, including historical HGT signatures, were quantified using the bioBakery tool WAAFLE (a Workflow to Annotate Assemblies and Find LGT Events). Second, metagenomic assembly and gene clustering were used to assess and quantify donor-specific genes transferred to recipients following the intervention. Both methodologies found no statistical difference between the level of tentative HGT events in the gut microbiomes of FMT and placebo recipients, post-intervention.

Quorum sensing molecules produced by bacteria are known to influence the efficiency of HGT. The pre-intervention bowel cleanse administered in the Gut Bugs Trial may have reduced the host bacterial population below a threshold, that subsequently hindered quorum sensing mediated HGT. Therefore, we anticipate that analysis of an additional FMT trial dataset with no participant bowel cleanse will show a disparity in the levels of HGT between FMT and placebo recipients, post-intervention.

1. Wilson, B.C., Vatanen, T., Jayasinghe, T.N. *et al.* (2021). *Strain engraftment competition and functional augmentation in a multi-donor fecal microbiota transplantation trial for obesity.* Microbiome **9**, 107.

M17: Carbohydrate degradation along an estuarine gradient

Boey, J.S.[1], Waite, D.[1], Astudillo-Garcia, C.[1], Tee, H.S.[1], Handley, K.M.[1]

[1]School of Biological Sciences, Faculty of Science, University of Auckland, Auckland, NZ

Estuaries are unique, mostly heterotrophic ecosystems characterised by highly active microbial biogeochemical cycling and primary production. Microbial carbohydrate metabolism catalysed by diverse groups of carbohydrate-active enzymes (CAZymes) substantially contributes to the global carbon budget. Hitherto, the structural diversity of carbohydrates hinders *in situ* substrate measurements. However, improved quality and quantity of gene annotation records in curated databases (e.g. CAZy) enables the use of sequence data to estimate the relative importance of carbohydrate substrates, their distribution, and putative microbial saccharolytic activity at an ecosystem level.

To characterise how carbohydrate degradation is distributed along an estuarine environment we paired metagenome-assembled genomes (MAGs), manually curated annotations, and metatranscriptomic analyses from water column and sediment samples. Samples were collected at 9 different salinities along the fresh-to-marine transect of the Waiwera estuary situated north of Auckland. Our study recovered 251 good quality (completeness > 70%, contamination < 5%), dereplicated MAGs spanning 16 phyla. Of these, 249 contain at least 1 putative GH or PL gene. Across all MAGs, we identified 6,537 putative degradative CAZyme genes from 114 unique GH and 27 unique PL families.

Transcripts for these CAZyme encoding genes were more abundant and variable in sediment (4.71 – 107.15 TPM) than in the water column (2.91 – 6.55 TPM). A comparison of the metagenomic and metatranscriptomic data also revealed that about half of the CAZyme-bearing community was unlikely to be active in carbohydrate degradation. This disconnect between capability and transcriptional activity for carbohydrate degradation was most prevalent in non-saline sediments. Finally, analyses of co-expression network centralities revealed distinct resource use strategies in saline and non-saline water column prokaryotic communities, and suggests differential substrate distributions across the estuarine salinity gradient. In sum, we show that salinity and habitat spatial heterogeneity patterns realised niches despite widespread genomic potential for carbohydrate degradation.

M18: Gut Bugs in Autism Trial: A double-blinded randomised placebo-controlled trial of faecal microbiome transfer for the treatment of gastrointestinal symptoms in autistic adolescents and young adults

Tweedie-Cullen, R.Y.[1], Leong, K.S.W.[1], Wilson, B.C.[1,2], Derraik, J.G.B.[1,i], Albert, B.B.[1], Monk, R.[3,4], Vatanen, T.[1,5], Creagh, C.[1], Depczynski, M.[1], Edwards, T.[1], Beck, K.[8], Thabrew, H.[6,7], O'Sullivan, J.M.[1,2*] Cutfield, W.S.[1*]

[1]Liggins Institute, University of Auckland, Auckland, NZ, [2]The Maurice Wilkins Centre, University of Auckland, Auckland, NZ, [3]Autism New Zealand, Wellington, NZ, [4]Department of Psychological Medicine, Faculty of Medicine & Health Sciences, University of Auckland, Auckland, NZ, [5]Research Program for Clinical and Molecular Metabolism, University of Helsinki, Finland, [6]Werry Centre for Infant, Child and Adolescent Mental Health, Department of Psychological Medicine, University of Auckland, Auckland, NZ, [7]Brain Research New Zealand, University of Auckland, Auckland, NZ, [8]School of Sport, Exercise and Nutrition, Massey University, Auckland, NZ, [9]Department of Paediatrics: Child and Youth Health, Faculty of Medical and Health Sciences, University of Auckland, Auckland, NZ

Autism (formally Autism Spectrum Disorder (ASD)) encompass a group of complex neurological conditions, characterised by differences in communication and social interactions. Co-occurring chronic gastrointestinal symptoms are common among autistic individuals and can adversely affect their quality of life.

We are currently undertaking a double-blinded, randomised, placebo-controlled trial to recruit 100 autistic adolescents and adults aged 16-45 years, who have moderate to severe gastrointestinal symptoms (Gastrointestinal Symptoms Rating Scale (GSRS) score ≥ 2.0). We have also recruited sixteen healthy donors (8 males and 8 females) aged 18-32 years, who have undergone extensive clinical screening. Recipients will be randomised 1:1 to receive FMT or placebo, stratified by biological sex. Capsules will be administered over two consecutive days following an overnight bowel cleanse with follow-up assessments at 6, 12, and 26 weeks post-treatment. The primary outcome is GSRS score at 6 weeks. Other assessments include anthropometry, body composition, hair cortisol concentration, gut microbiome profile, urine/plasma gut metabolites, plasma markers of gut inflammation/permeability, and questionnaires on general well-being, sleep quality, physical activity, food diversity, and treatment tolerability. Adverse events will be recorded and reviewed by an independent Data Monitoring Committee (DMC).

This study aims to evaluate the efficacy of oral encapsulated faecal microbiome transfer (FMT) in improving gastrointestinal symptoms and well-being among autistic adolescents and adults.

M19: 16S rRNA gene-based meta-analysis of the reptile gut microbiota reveals environmental effects, host influences and a limited core microbiota

Hoffbeck, C.[1], Middleton, D. MRL.[2], Nelson, N. J.[3], Taylor, M. W.[1]

[1]School of Biological Sciences, University of Auckland, Auckland, NZ, [2]Manaaki Whenua – Landcare Research, Lincoln, NZ, [3]School of Biological Sciences, Victoria University of Wellington, Wellington, NZ.

An animal's gut microbiota plays an important role in host health, reproduction, and digestion. However, many studies focus on only a few individuals or a single species, limiting our ability to recognise emergent patterns across a wider taxonomic grouping. Here we compiled and reanalysed published 16S rRNA gene sequence data for 746 gut microbiota samples from 91 reptile species using a uniform bioinformatics pipeline to draw broader conclusions about the taxonomy of the reptile gut microbiota and the forces shaping it. Our meta-analysis revealed significant differences in alpha- and beta-diversity across host order, environment, diet, habitat, and conservation status, with host environment and order contributing the most to these differences. We identified the principal bacterial phyla present in the reptile gut microbiota as *Bacteroidota*, *Proteobacteria* (mostly *Gamma* class), and *Firmicutes*, and detected the bacterial genus *Bacteroides* in most reptile individuals, thus representing a putative "core" microbiota. Our study provides novel insights into key drivers of the reptile gut microbiota, highlights existing knowledge gaps and lays the groundwork for future research on these fascinating hosts and their associated microbes.

M20: Looking at microbiomes through an evolutionary lens

Hugenholtz, P.

School of Chemistry and Molecular Biosciences & Australian Centre for Ecogenomics, University of Queensland, Brisbane, Australia

As evolutionary biologist Theodosius Dobzhansky famously wrote “Nothing in biology makes sense except in the light of evolution”, which also holds true for microbiomes. Many researchers understandably focus on a single host species, such as *Homo sapiens*, *Mus musculus* or *Arabidopsis thaliana*, and through their studies produce a wealth of data but also raise many open questions. Some of these questions can best be addressed by probing the evolutionary origins of a given microbiome. In this talk, I will give some examples where we have progressed our understanding, or at least provided new unexpected directions for our ongoing research, of rodent and marsupial gut microbiomes using an evolutionary lens, and conclude with an outlook on where this type of analysis may be headed.

M21: Deconstructing and reconstructing the captive kiwi gut microbiome

Dhami, M.K.[1], San Juan P.[1,2], Rowe S.[1,3], Brett B.[4], Bean E.[5], Stott M.[3]

[1]Biocontrol & Molecular Ecology, Manaaki Whenua Landcare Research; [2]Department of Biology, Stanford University; [3]School of Biological Sciences, University of Canterbury, [4]Willowbank Wildlife Reserve, Canterbury, [5]National Kiwi Hatchery, Rotorua.

Young kiwi face intense predatory pressure from stoats, cats and ferrets in the wild. Captive rearing programmes, where eggs are lifted in the wild and hatched and reared in facilities, have effectively removed this predation loss of kiwi chicks. As a result, the survival rate of chicks has increased to 96% percent in captive conditions. However, captive conditions are markedly different to those experienced by kiwi born in the wild.

We assessed whether near-sterile captive environments shaped the kiwi gut microbiome and whether reconstruction of a 'wild-like' microbiome was possible in captivity. We used metabarcoding approaches to characterise the gut microbiome of captive kiwi and compared it to their wild counterparts. We found that the captive microbiome was depauperate in species richness and diversity and more variable in composition than the wild gut microbiome. We provision natal soils, soils from the area of origin of the chicks, into the diet of chicks in captivity in order to reconstruct their gut microbiome. We found a small shift in the microbial community as a result. Importantly, some of the microbial partners with important metabolic roles were thus introduced into the captive kiwi gut microbiome.

Taken together, we conclude that kiwi microbiome is shaped by captive conditions. But it may be possible to reshape this through the provision of rich microbial communities via soil or other probiotics.

M22: Incorporating the microbiome dimension in host-parasite interactions

Priscila Salloum[1], Fátima Jorge[2], Robert Poulin[1]

[1]Department of Zoology, University of Otago, Dunedin, NZ, [2]Otago Micro and Nano Imaging, Electron Microscopy Unit, University of Otago, Dunedin, NZ.

We often consider the eco-evolutionary dynamics of host-parasite interactions as a two-racers contest: on the one hand, natural selection favours hosts resistant to parasitic infections; on the other hand, parasites that can bypass such resistance have a fitness advantage. However, like most living organisms, microbes associate with both hosts and parasites, potentially modulating host defence and parasite virulence and transmission[1-2]. Incorporating the microbiome dimension in this two-player evolutionary game is incredibly complex, but it starts by characterising the microbiome of both hosts and their parasites. In this short talk, I will present the tale of four parasitic trematodes that use the same mud snail species as an intermediate host. Differences in the microbiota composition and bacterial abundance among the four trematodes and between trematodes and their mud snail host support the hypothesis that parasites do not simply acquire microbes randomly from a pool available in their environment (or in their host). Ultimately, microbe-parasite associations that may favour microbial transmission (and evolution) are most likely tightly intertwined in the host-parasite evolutionary race.

1. Poulin, R., Jorge, F., and P. M. Salloum (2022). *Inter-individual variation in parasite manipulation of host phenotype: a role for parasite microbiomes?* Journal of Animal Ecology. 2(4): 807-812.
2. Salloum, P. M., Jorge, F., Dheilly, N. M., and R. Poulin (2023). *QGRS Eco-evolutionary implications of helminth microbiomes*. Journal of Helminthology. 97: E22-35.

M23: Gut microbiome of the ancient tuatara reveals variation with biogeography and host condition, but shows conserved bacteria across latitudinal gradient

Hoffbeck, C.[1], Middleton, D. MRL.[2], Nelson, N. J.[3], Taylor, M. W.[1]

[1]School of Biological Sciences, University of Auckland, Auckland, NZ, [2]Manaaki Whenua – Landcare Research, Lincoln, NZ, [3]School of Biological Sciences, Victoria University of Wellington, Wellington, NZ.

Tuatara are the sole extant species in the reptilian order Rhynchocephalia, and have been isolated geographically for ~84 million years and evolutionarily for ~250 million years. As an ancient and ecologically and evolutionarily unique reptile species, we sought to characterise the tuatara gut bacterial community for the first time. In this study, we took cloacal samples as a proxy for the gut microbiota of translocated tuatara at five sanctuaries spanning a latitudinal range of ~1000 km within New Zealand, as well as individuals from the source population on Takapourewa/Stephens Island. This represents a first look at the bacterial community of the order Rhynchocephalia, and provides the opportunity to address several key hypotheses, namely that the tuatara gut microbiota: (1) differs from those of other reptile orders; (2) varies among geographic locations but is more similar at sites containing similar habitats; and (3) is shaped by tuatara body condition, parasitism, and ambient temperature. We measured and weighed tuatara, estimated tick abundance, and identified translocated individuals at six sites around New Zealand, and took cloacal swabs to sample the gut microbiota. We found significant drivers of the microbiota in translocation site, tuatara body condition, parasitism, and ambient temperature, suggesting the importance of these factors when considering tuatara conservation. We also derived a “core” community of shared bacteria across tuatara at many sites, despite their geographic range and isolation. Finally, we identified 72% of ASVs obtained from sampling as unassigned genera, potentially indicating a largely undescribed gut bacterial community for this unique species.

M24: The role of hindgut microbiota in marine herbivorous fish

Clements, K.D.[1]

[1]School of Biological Sciences, University of Auckland|Waipapa Taumata Rau, Auckland, New Zealand

Herbivorous fishes are considered critical to ecosystem processes in shallow marine habitats including kelp forests and coral reefs. Most marine herbivorous fishes belong to a relatively small number of fish families, with well-known examples being surgeonfishes (Acanthuridae), parrotfishes and weed whitings (Labridae), rabbitfishes (Siganidae), damselfishes (Pomacentridae), mullets (Mugilidae) and chubs (Kyphosidae). The photoautotroph assemblage that constitutes potential foods for these fishes is phylogenetically diverse and biochemically disparate. Fish taxa differ in their capacity to harvest, digest and assimilate these resources, and specialise to varying degrees. These fishes can be viewed on a spectrum from rate maximisers to yield maximisers. Rate maximisers often target microscopic photoautotrophs, pass material through the gut rapidly, and assimilate mainly protein and soluble carbohydrate. Examples are parrotfishes and mullets. Yield maximisers typically feed on macroscopic algae and use lengthy gut retention times and hindgut microbiota to ferment refractory polysaccharides into short-chain fatty acids that are assimilated and metabolised by the host fish. Examples are some unicornfish and kyphosid chubs. This talk will discuss the importance of hindgut fermentation to digestion and how this relates to feeding behaviour and gut anatomy in various fish taxa, and describe the main bacterial taxa, algal substrates and carbohydrate-degrading enzymes involved in these processes with particular reference to the hindgut microbiome of kyphosid fishes.

1. Pardesi, B., A.M. Roberton, K.C. Lee, E.R. Angert, D.I. Rosendale, S. Boycheva, W.L. White and K.D. Clements (2022). *Distinct microbiota composition and fermentation products indicate functional compartmentalization in the hindgut of a marine herbivorous fish*. Molecular Ecology. 31: 2494-2509.

2. Stevenson, S.J., K.C. Lee, K.M. Handley, E.R. Angert, W.L. White and K.D. Clements (2022). *Substrate degradation pathways, conserved functions and community composition of the hindgut microbiota in the herbivorous marine fish* Kyphosus sydneyanus. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 272: 111283.

M25: New Zealand wide genetic and phenotypic characteristics of bovine *Staphylococcus aureus* isolates

Emma M. Voss[1,2], John Williamson[1], Scott McDougall[3], Ali Karkaba[3], Chad Harland[1], James E. Ussher[2], Scott A. Ferguson[2], Sergio E. Morales[2], Gregory M. Cook[2] and Christine Couldrey[1]
[1] Livestock Improvement Corporation (LIC) – Newstead, Waikato, NZ [2] Department of Microbiology and Immunology, University of Otago, Dunedin, NZ, [3] Cognosco, Animal Health Centre, Morrinsville, New Zealand

Staphylococcus aureus (S. aureus) is a common mastitis-causing bacteria in dairy cattle; studies in New Zealand and worldwide have focussed on its genetic diversity, virulence, clonal nature, and antimicrobial resistance (AMR) patterns. However, no studies have been conducted investigating the regional pattern of bovine *S. aureus* in New Zealand. We have used whole-genome sequencing to classify 865 bovine *S. aureus* isolates from 207 herds in the North Island and 66 herds in the South Island. The isolates were recovered from aseptic quarters and non-sterile bulk tank milk samples. All identified *S. aureus* isolates were sequenced via Illumina sequencing and analysed using a modified Nullarbor bioinformatic pipeline to classify isolates based on multilocus sequence typing (MLST) and provide details on the genes associated with virulence and AMR.

From 865 sequenced isolates, 65% were classified as sequence type 1 (ST1). ST1 was the predominant sequence type across all sampled regions. The dominance of *S. aureus* ST1/CC1 on a region and country wide scale is rare internationally where CC97 or CC151 dominates[1]. Bovine host-adaptation of ST1 has been documented but initial analysis indicates some ST1 isolates in this study do not carry bovine host adapted genes which warrants further investigation as ST1 is historically a human associated sequence type[1]. Further analysis will be presented comparing human and bovine *S. aureus* isolates to investigate if there is evidence of interhost transmission. Our approach highlights the importance of region and nationwide studies with a One-Health approach to investigate genetic and phenotypic characteristics.

1. Yebra, G., Harling-Lee, J. D., Lycett, S., . . . Fitzgerald, J. R. (2022). *Multiclonal human origin and global expansion of an endemic bacterial pathogen of livestock*. Proc Natl Acad Sci USA, 119(50).

M26: Dung beetle microbiome shifts with diet and parasiticide change

Biggs E.[1], Taylor M. W.[2], Donald, M.L.[1], Middleton, D. M. R. L.[1]

[1]Manaaki Whenua – Landcare Research, Lincoln, NZ, [2]School of Biological Sciences, University of Auckland, Auckland, NZ

Within the past decade New Zealand has introduced six species of dung beetle. This was to help with the degradation of mammalian dung, something to which the NZ ecosystem is not adapted. However, the establishment and survival of these beetles are threatened by parasiticide use in animal agriculture. It remains unknown whether dung beetles themselves are affected by the applied chemicals or rather as a secondary effect of disrupted microbiome due to parasiticide exposure.

In our pilot study, we tested the effect of three commercial parasiticides on the gut and exoskeleton microbiomes of *Onthophagus binodis*, a common introduced dung beetle species. Dung beetles were exposed to antiparasitic drugs mixed with fresh dung for 14 days, then sequencing of PCR-amplified 16S rRNA genes was used to detect changes in bacterial community composition and diversity.

Our findings indicate that, while parasiticides contributed to some microbial community changes, the most important determining factor was dung beetle diet. The dung beetles in our study came from one paddock and were subsequently fed with dung from a different paddock. Interestingly, dung beetles also harboured different microbiomes in their gut compared to their exoskeleton, despite dung being both its food and environmental niche.

These results indicate that dung beetles have a modifiable microbiome and that their sensitivity to parasiticides likely comes directly from the chemical exposure.

M27: Influence of management practice on the microbiota of a critically endangered species: a longitudinal study of kākāpō chick faeces and associated nest litter

West, A.G.[1], Digby, A.[2], Lear, G.[1], Kākāpō Recovery Team[2], Kākāpō Aspergillosis Research Consortium, Taylor, M.W.[1]
[1]Te Kura Mātauranga Koiora School of Biological Sciences, Waipapa Taumata Rau University of Auckland, Tāmaki Makaurau Auckland, Aotearoa NZ, [2]Kākāpō Recovery, Ta Papa Atawhai Department of Conservation, Waihōpai Invercargill, Aotearoa NZ.

The critically endangered kākāpō is a flightless, nocturnal parrot endemic to Aotearoa New Zealand. Recent efforts to describe the gastrointestinal microbial community of this threatened herbivore revealed a low-diversity microbiota that is often dominated by *Escherichia-Shigella* bacteria. Given the importance of associated microbial communities to animal health, and increasing appreciation of their potential relevance to threatened species conservation, we sought to better understand the development of this unusual gut microbiota profile.

To this end, we conducted a longitudinal analysis of faecal material collected from kākāpō chicks during the 2019 breeding season, in addition to associated nest litter material. Using an experimental approach rarely seen in studies of threatened species microbiota, we evaluated the impact of a regular conservation practice on the developing kākāpō microbiota, namely the removal of faecal material from nests. Artificially removing chick faeces from nests had negligible impact on bacterial community diversity for either chicks or nests ($p > 0.05$). However, the gut microbiota did change significantly over time as chick age increased ($p < 0.01$), with an increasing relative abundance of *Escherichia-Shigella coli* over the study period and similar observations for the associated nest litter microbiota ($p < 0.01$). Supplementary feeding substantially altered gut bacterial diversity of kākāpō chicks ($p < 0.01$), characterised by a significant increase in *Lactobacillus* bacteria.

Overall, chick age and hand rearing conditions had the most marked impact on faecal bacterial communities. Similarly, the surrounding nest litter microbiota changed significantly over time since a kākāpō chick was first placed in the nest, though we found no evidence that removal of faecal material influenced the bacterial communities of either litter or faecal samples. Taken together, these observations will inform ongoing conservation and management of this most enigmatic of bird species.

M28: Strain-level diversity of *Escherichia coli*, the dominant member of the kākāpō gut microbiome

Ayriss, N. J.[1], West, A.G.[1,2], Cookson, A.L.[3,4], Biggs P.J.[4,5], Digby, A.[6], Taylor, M.W.[1]

[1]School of Biological Sciences, University of Auckland, 3a Symonds Street, Auckland 1010.
[2]Genomics Aotearoa, New Zealand.
[3]Food Systems Integrity, Hopkirk Research Institute, AgResearch, Palmerston North 4410.
[4]School of Veterinary Science, Massey University, Tennent Drive, Palmerston North 4410.
[5]School of Natural Sciences, Massey University, Tennent Drive, Palmerston North 4410.
[6] Department of Conservation, Kākāpō Recovery Team, 33 Don Street, Invercargill, 9810.

Endemic to Aotearoa New Zealand, the critically endangered kākāpō (*Strigops habroptilus*) is a treasured taonga species. It is also highly unusual among parrots, being both the heaviest and only flightless parrot in the world, as well as nocturnal. The uniqueness of the kākāpō extends even to its gut microbiome, with our previous research indicating a low-diversity bacterial community that is atypical for a herbivore. 16S rRNA gene amplicon sequencing has revealed that the faecal microbiomes of many kākāpō individuals are dominated (up to ~90% of the obtained sequences) by *Escherichia coli.* Given the considerable phenotypic variation known to exist within this bacterial taxon, and the potential implications of harbouring different strains for kākāpō diseases such as exudative cloacitis, we sought to determine the strain-level diversity of *E. coli* within the kākāpō gut. This required analysis of a more discriminatory genetic marker than the 16S rRNA gene. To this end, we amplified then sequenced the 6-phosphogluconate dehydrogenase *(gnd)* gene from >150 kākāpō faecal samples using a recently developed methodology (Cookson et al., 2017). These data allowed us to document, for the first time, the influence of kākāpō age, sex, and geographic location on *E. coli* strains, with certain strains abundant in most kākāpō individuals while other strains were much more restricted in their distribution. This research should help inform future management and conservation of this iconic native species.

1. Cookson, A. L., Biggs, P. J., Marshall, J. C., Reynolds, A., Collis, R. M., French, N. P., & Brightwell, G. (2017). *Culture independent analysis using gnd as a target gene to assess Escherichia coli diversity and community structure*. Scientific Reports, *7*(1), 1-10.

M29: Buzz off: Discovery, Sequencing, and Cocktail Testing of Bacteriophages to Protect Honeybees in New Zealand.

Kok, D.N.[1,3] & Hendrickson, H.L.[2,3]

[1] School of Product Design, University of Canterbury, Christchurch, NZ. [2] School of Biological Sciences, University of Canterbury, Christchurch, NZ, [3] School of Natural Sciences, Massey University, Auckland, NZ.

American Foulbrood (AFB) is the most devastating disease of honeybees in New Zealand. The causative agent is *Paenibacillus larvae* a spore forming gram positive bacterium that can survive in soil in this dormant state for decades. Treating honeybee colonies with antibiotics is strictly forbidden and if AFB is detected the hive must be closed and burned within days. Bacteriophages (phages), the viruses of bacteria, have been demonstrated to be able to protect hives under threat of acquiring AFB and this protection can last at least four months. *P. larvae* phages that are a part of the microbiological milieu of honeybee colonies represent an approach to pathogen protection that would be acceptable to the honey industry and safe for the honeybee microbiome. In 2018, we set out to bring this prophylactic approach to New Zealand.

We have used a set of 30 isolates of *P. larvae* from across the country to enrich and isolate a set of 26 unique phages using a community science approach to sampling. All phages were isolated from healthy honeybee colonies. We developed a novel approach to adaptive laboratory evolution to improve the concentration of the phages by 10,000 times in only four days[1]. Complete genome sequencing confirms that these bacteriophages come from two phage clusters found overseas; Harrison and Vegas. In this talk I will describe the host range testing and the surprising results of some of our cocktail testing. This work forms the foundation required to approach rational formulation of phage cocktails that will be both effective in protecting honeybees and safe for use in the complex environment of these important pollinators.

1. Kok, D.N., Turnbull, J., Takeuchi, N., Tsourkas, P.K., & Hendrickson, H.L. (2023) *In Vitro Evolution to Increase the Titers of Difficult Bacteriophages: RAMP-UP Protocol.* PHAGE: Therapy, Applications, and Research. V4-2, DOI:10.1089/phage.2023.0005

M30: Helping navigate the climate crisis: The development of a tree-microbiome model system for conservation and sustainable forestry outcomes.

Wakelin, S.A.[1]

[1]Scion, Christchurch, NZ

From lab rats to *E. coli*, model systems have been instrumental in advancing biological sciences. Similarly, in the era of microbiome research, model systems based on Arabidopsis, rice, maize, and other crops are central in developing our understanding of the relationships between plants and their microbial associations. This work has shifted our fundamental perspective of plants existing as 'a host organism' colonised by microorganisms, towards one of a single co-evolved biological entity or 'metaorganism' with shared fitness and success outcomes.

Forests are a vital component of Earths biosphere. Indeed, the services these forests support are essential for sustaining the well-being of our planet. However, forests face significant threats from climate change and the rapid emergence of pests and diseases. These ecosystems are sessile and typically slow growing; their ability to adapt or shift ranges is slower than rates of change being imposed on them. As research on ornamental, horticultural and crop species is showing that the microbiome can expand and modify the functions, physiology and fitness of the plant, it is time to ask, 'what is the opportunity for the tree'?

There is an urgent need to establish tree microbiome model systems that be used to test and explore the complexities of tree-microbiome interactions under changing environmental conditions. In this context, *Pinus radiata* (Monterey pine) emerges as a promising candidate. It is already one of the most widely planted and well understood tree species globally. It is relatively fast growing and expresses phenotypically plasticity. By employing *Pinus radiata* as a model tree, we aim to gain crucial insights into microbial community assembly, functional contributions, and the impacts of environmental factors on the tree/forest microbiome. This will inform sustainable forest management practices and help the conservation of already at-risk tree species.

M31: Surveying wild *Daucus* seed for their bacteriota

Sandeep Kumar[1], Linda Johnson[1], Suliana Teasdale[1†], Yulia Morozova[1], Anouck de Bonth[1], Ruy Jauregui[1‡], Rina Hannaford[1] and Stuart Card[1]
[1]AgResearch Limited, Grasslands Research Centre, Palmerston North 4442, New Zealand
[†]Present address: Department of Conservation, Wellington, New Zealand
[‡]Present address: Ministry for Primary Industries, Upper Hutt, New Zealand

Daucus is a cosmopolitan genus of herbaceous plants, with *Daucus carota* subsp*. sativus,* the cultivated carrot, the most well-known member. New Zealand produces close to 50% of the world's carrot seed. As with many vascular plants, *Daucus* associates with endophytic microorganisms, which may confer advantageous traits to the host. Many authors hypothesize that crop domestication, coupled with the practice of intensive agriculture, has reduced the abundance and diversity of potentially beneficial endophytes within vegetative plants and their seed. The major motivation for investigating seed microbiomes is to understand how these microbiota impact plant function and ecology.

We surveyed the bacterial communities of 48 wild *Daucus* accessions, sourced mainly from New Zealand. Briefly, seeds from each accession were surface disinfected and germinated. DNA was extracted from healthy seedlings (i.e., those free of any symptoms) and used to prepare DNA libraries for the V3 to V4 region of the 16S rRNA gene using the 335F and 769R primer pair, then sequenced using the Illumina MiSeq platform.

Actinobacteria, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were the most abundant and prevalent bacterial phyla across all samples, together comprising >97% of the total reads. These phyla are often the most abundant in soil ecosystems and therefore have ample opportunity to associate with seed. Despite this, bacterial seed endophytes may be highly conserved in some plant species. *Bacillus*, *Massilia*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Sphingomonas*, and *Xanthomonas* were the most abundant and prevalent genera within our wild *Daucus* samples, comprising >67% of the total reads. Several of these genera are known to be vertically transmitted. Although several genera identified in our study contain pathogenic species, the seed microbiome can contain nonpathogenic relatives of these bacteria. For example, nonpathogenic strains of *Xanthomonas* with bioprotection activities have been isolated from ryegrass seed. This study has provided the first insight into the *Daucus* microbiome.

M32: The effect of *Epichloë* strain in established New Zealand perennial ryegrass (*Lolium perenne*) pastures.

Prout, B.M.[1], Bell, N.L[1], Shi, S.[3], Maclean, P.[2], Mtandavari, Y. F.[1], Hume, D.E.[2], Jauregui, R.[2],

[1]AgResearch, Ruakura, NZ, [2]AgResearch, Palmerston North, NZ, [3]AgResearch, Lincoln, NZ.

Perennial ryegrass (*Lolium perenne*) is a critical agricultural plant supporting New Zealand's intensive pasture-based dairy industry. A significant breakthrough in perennial ryegrass research was the discovery of *Epichloë*, a genus of ascomycete fungi that form an endophytic symbiosis with grasses and reduce invertebrate pest damage. Plant breeders have incorporated several strains of *Epichloë* in perennial ryegrass, including AR1 and AR37, which exhibit variations in their alkaloid production to target different pasture pests[1]. The impact of *Epichloë* on ryegrass production and invertebrate pests has been extensively studied; however, little attention has focussed on its effects on other associated microorganisms, particularly in established pastures.

This study utilised 16S and ITS rRNA amplicon sequencing to determine the influence of two *Epichloë* strains, AR1, AR37, and a 'without endophyte' treatment, on the bacterial and fungal communities in three-year-old perennial ryegrass swards from three locations in New Zealand[1].

The results revealed that farming location and ryegrass niche were significant factors driving microbiome variation. Evidence of *Epichloë* effects on microbiota communities was observed in the shoot endosphere, root endosphere, and rhizosphere niches. Differences in the relative abundance of individual fungal and bacterial genera could also be seen between *Epichloë* strains.

This study provides evidence that *Epichloë* has minimal effects on the overall structure and composition of the perennial ryegrass microbiome, with only minor variations observed between different endophyte-infected plants. These findings strengthen our understanding of the influence of *Epichloë* on the other microbiota of perennial ryegrass. Moreover, the study unveils the diverse microbial interactions within established ryegrass pastures. This information can assist future research on microbial solutions and plant breeding targets for the mitigation of soil diseases and poor ryegrass persistence across different farming regions in New Zealand.

1. Prout, B. (2023). *The microbiome of Perennial Ryegrass Lolium perenne* (Masterate dissertation, The University of Waikato).

M33: Effect of *Epichloë* on ryegrass plant and soil microbiome

Shengjing Shi[1], Sandra Young[1], Emily Gerard[1], Russell Croy[1], David Hume[2]

[1] Resilient Agriculture, Lincoln Science Centre, AgResearch Ltd., Christchurch, NZ, [2] Resilient Agriculture, Grasslands, AgResearch Ltd. Palmerston North, NZ.

Perennial ryegrass (*Lolium perenne*) is the most common livestock forage species for pastures in New Zealand and other temperate countries. It can form mutualistic symbiosis with a shoot endophytic *Epichloë* fungus which produces a diverse range of alkaloid metabolites protecting plants from invertebrate pest damage. There is limited understanding of the effect of *Epichloë* on plant-associated and soil microbiomes. We aimed to address this knowledge gap and examine whether such effects are *Epichloë* strain dependent.

A field trial was established with six ryegrass cultivar/ *Epichloë* strain combinations, enabling comparison of *Epichloë* strain specific effects as well as cultivar/strain interactions on plant and soil microbiomes. Samples were collected multiple times across 1.5 year, both before and after grazing. Microbiomes associated with bulk soil, rhizosphere soil, endophytic roots and shoots were analysed using MiSeq 16S and ITS sequencing. Additionally, the microbiomes associated with seeds used for the trial were examined.

Strong *Epichloë* effects were detected for seed microbiome. However, in samples obtained from the field trial, sampling niches and time played more substantial roles in shaping the microbiome compared to cultivar and *Epichloë*. Different strains of *Epichloë* showed various impacts on specific microbial phylum and classes in individual niches, including shoots, root, rhizosphere and soil microbiomes. Our study demonstrated that the aboveground shoot endophyte *Epichloë* only had minor impact on shoot microbiome as well as belowground microbiomes (root, rhizosphere and soil). Some of the impacts were strain specific. Further studies or metagenomic analyses are required to examine biological impact of *Epichloë* on plant and soil microbiome.

M34: Exploring the wood microbiome of *Pinus radiata*

Wigley, K.[1], Daley, K.[1], Eslami, Y.[1], Armstrong, C.[2], Wakelin, S.A.[2]

[1]Scion, Rotorua, NZ, [2]Scion, Christchurch, NZ

Wood microbiomes are one of the most understudied habitats in microbiome research, although tree stems hold over half the total biomass carbon on Earth. Trees are a sizable potential habitat for microbes, and understanding the microbial community within wood may provide valuable insights into the wood formation and quality, nutrient cycling, tree growth and health. *Pinus radiata* is a model conifer which makes up over 90% of planted forests here in New Zealand. The microbiome associated with *P. radiata* wood has not previously been explored. This research aimed to investigate the microbiome of the different *P. radiata* tree tissues from the outer bark through to the pith. The influence of sampling height on the composition of the microbiome was also investigated.

Initially, wood cores were taken from a single tree from near-ground level to 6 m. Wood cores were divided into different tissue types: outer bark, inner bark, cambium, Year 8, 6 and 2 growth rings, and the pith. High throughput sequencing revealed the bacterial and fungal community in each tissue type. Sampling height was found not to influence community composition. Sampling was repeated across 11 trees. Wood cores were taken at a height of 140 cm and divided by tissue type. Bark tissues, cambium, growth rings and pith all had differing microbial communities ($p<0.001$). Inner and outer bark tissues had high richness and the most distinct communities. Microbiome richness was lowest in Year 2 through Year 8 wood, and the communities in these samples had a similar composition. Microbiomes of cambial and pith tissues were distinct to those niches. This study provides evidence of the *P. radiata* wood microbiome and provides novel insights into how it is structured.

Kai mō Aotearoa Food Science Joint Session with AWCBR and Microbiomes

The effect of fruits and vegetables on children's mental and cognitive health: A systematic review of intervention studies and perspective for future research

Gillies, N.A[1], Lovell, A.L[1], Waldie, K.E[2], Wall, C.R[1]

[1]Department of Nutrition, University of Auckland, Auckland, New Zealand, [2]School of Psychology, Faculty of Science, University of Auckland, Auckland, New Zealand.

Background: Childhood is a critical period to promote and protect mental well-being. Observational evidence suggests that higher fruit and vegetable (FV) intake could benefit children's mental and cognitive health, but comparatively little is known from intervention studies about the causative effects. We therefore conducted a systematic review of FV intervention studies that investigated mental and/or cognitive health outcomes in children aged 10 years or younger, both to understand the current evidence base and inform future intervention trial design.

Systematic Review Methods and Results: A systematic search of the Cochrane, Embase, PubMed, and CINAHL databases was conducted for articles published before August 2022. A total of 4686 articles were identified, with only 7 of the 17 full texts screened included in the final review. Included studies used either a cross-over (n=4) or parallel (n=3) design, with sample sizes ranging from *n* of 14 to 54. Six of the 7 studies were conducted in the UK by the same research group using a drink made from fresh or freeze-dried blueberries as the intervention, the other study was conducted in Thailand and used a drink made from mulberry powder as the intervention. The majority of studies were acute interventions with outcomes measured in a 2–3-hour postprandial window (n=6, 85.7%). Positive Affect increased 2 hours after blueberry consumption (sample size=52), though longer-term effects were not found with daily supplementation for four weeks (sample size=15). Measures of Executive Function were sensitive to the effects of mulberry and blueberry interventions, with improvements observed in both acute and longer-term interventions and particularly with more cognitive demanding tasks.

Future Research perspectives. Despite a strong theoretical basis for mental and cognitive health benefits of increased FV intake, the closest available intervention evidence was for studies providing supplemental doses of vitamins and polyphenols through beverages made of fresh or freeze-dried blueberries and mulberries. The benefits of increasing FV in the diet are undisputed and therefore highly suitable to improve childhood mental health at a population level, yet intervention studies are needed to confirm causation and efficacy. Acknowledging the challenges of conducting rigorous trials of this nature in children, we will discuss future research opportunities to advance this critical field of research.

Parkinson’s disease and nutrition: what is the link?

Lithander F.E. [1, 2]

Liggins Institue[1] and Department of Nutrition and Dietetics [2], University of Auckland, New Zealand

Parkinson’s disease is the second most common neurodegenerative disease after Alzheimer's disease and affects 1% of people over the age of 60 years in industrialised countries (1). In New Zealand, the number has increased from ~ 7,000 in 2006 to ~11,000 in 2020. Whilst the lowest rate is in Māori, given that the life expectancy of Māori is increasing at a greater rate than that of non-Māori, the prevalence of Parkinson’s among Māori will increase faster than others in the New Zealand population (2).

Parkinson’s is heterogeneous in nature and results in a spectrum of motor and non-motor symptoms which include issues related to adverse nutritional status. Diet is often overlooked despite being an important factor in the management of the disease, and people with Parkinson’s (PwP) face many issues which can negatively impact their nutritional status (3). Poor nutritional status is multifactorial and different symptoms can occur over the course of Parkinson’s which vary in the degree to which they impact adversely on quality of life. These symptoms include anosmia, dysgeusia, dysphagia, orofacial issues, gastrointestinal dysfunction, cognitive effects, poor bone health, and an increased risk of sarcopenia. Each of these symptoms can individually or together impair dietary intake, negatively impact nutritional status and importantly, impair quality of life in PwP.

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Linking the Gut Microbiome to Neurocognitive Development in Bangladesh Malnourished Infants

Portlock, T.P.[1], Ho, D.[1], Pook, C.[1], Wilson, B.C.[1], Shama T.[2], Kakon S.H.[2], Shennon I.[1], R. Haque[2,3], T. Forrester[4], C.A. Nelson[3], O'Sullivan J.M.[1,5,6,7], on behalf of the M4EFaD consortium

[1]The Liggins Institute, The University of Auckland, NZ., [2]Infectious Diseases Division, International Centre for Diarrheal Disease Research, Bangladesh, [3]Intellectual and Developmental Disabilities Research Center, Boston Children's Hospital, Boston, USA, [4]Faculty of Medical Sciences, University of the West Indies (UWI), Jamaica, [5]The Maurice Wilkins Centre, The University of Auckland, Auckland 1010, New Zealand, [6]MRC Lifecourse Epidemiology Unit, University of Southampton, University Road, Southampton, UK, [7]Singapore Institute for Clinical Sciences, Agency for Science Technology and Research, Singapore

Malnutrition is a significant global health issue that affects millions of infants every year. Malnutrition has serious implications on cognitive and neurological development, leading to long-term deficits in learning, memory, and behaviour[1]. While much is known about these implications, there remains a crucial need to understand the mechanism by which they arise.

To this end, we trained AI Random Forest classification models with a combination of gut microbiome species and functional profiles, serum metabolites, and neurological electroencephalogram (EEG) metrics from 1-year-old infants with Moderate Acute Malnutrition (n = 70) and healthy infants (n = 70) to predict brain development; specifically to classify high and low quantiles of Expressive Communication (EC) score. Data were obtained as part of the M4EFaD trial, run in Dhaka, Bangladesh (NCT05629624).

The models predict EC effectively (AUCROC = 0.71). By interpreting these models, serum metabolites had the highest proportion of highly predictive features. Interestingly, presence of *Bifidobacterium* species in the gut and their fermentation pathways were strong predictors of high EC (SHAP score = 0.20). Through co-abundant network analysis, *Bifidobacterium* species were correlated with serum fatty acids and EEG measurements forming a distinct cluster involved in the breakdown of cholesterol esters and sugars for sphingomyelin biosynthesis - an essential precursor for cognitive maturation[2]. Depletion of this cluster was predictive of malnutrition.

The intricate and non-overlapping connections among gut microbiome composition, nutritional status, and behaviour highlight the significance of targeted interventions in addressing both the short and long-term impacts of malnutrition.

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The effect of milk fat globule membrane supplementation on cognitive and psychological outcomes in adults

Rebecca Slykerman1, Naomi Davies[1], Christopher Frampton[2], Maher Fuad[3]

[1]Department of Psychological Medicine, The University of Auckland, Auckland, New Zealand; [2]Department of Medicine, University of Otago, Christchurch, New Zealand; [3]Fonterra Research and Development Centre, Fonterra Cooperative Group Limited, Palmerston North, New Zealand

r.slykerman@auckland.ac.nz

Polar lipids are present in abundance in the nervous system and are essential for the maintenance of membrane integrity, neuronal membrane health, and signalling capacity. Milk fat globule membranes (MFGM) are a rich source of complex lipids and membrane proteins. Pre-clinical studies demonstrate that phospholipid supplementation can mitigate age-related cognitive decline and alter the hypothalamic pituitary adrenal axis function. Polar lipids for improvement of cognitive and psychological health in humans has not been widely investigated. We aimed to test the effect of two doses of MFGM supplementation on stress, cortisol response and cognitive performance. One-hundred-and-twenty-two healthy adults were enrolled in a randomized, double blind placebo controlled trial. Participants received MFGM 600mg, MFGM 1200mg, or placebo daily for 12 weeks. At baseline, 6, and 12 weeks, salivary cortisol, cognitive test performance, and psychological outcomes were assessed. Participants supplemented with MFGM had significantly lower stress ($p=0.002$) and anxiety ($p=0.06$) scores than the placebo group after 12 weeks. No group differences in cortisol or cognitive test performance were detected. Evidence suggests MFGM supplementation can improve psychological health.

Effects of intermittent access to high-fat, high-sugar diets on behaviour and gut microbiota

Kendig, M.D.[1,2], Leigh, S.J.[2,3], Hasebe, K.[2], Kaakoush, N.O.[2], Westbrook, F.R.[4], Morris, M.J.[2]

[1]School of Life Sciences, University of Technology Sydney, Sydney, Australia; [2]School of Biomedical Sciences, The University of New South Wales, Sydney, Australia; [3]APC Microbiome, University Cork College, Cork , Ireland; [4]School of Psychology, The University of New South Wales, Sydney, Australia

michael.kendig@uts.edu.au

Studies in humans and animal models show that consuming foods high in fat and sugar (HFHS) can impair cognition and alter composition of the gut microbiota. However, HFHS foods are rarely eaten exclusively, and more commonly form part of diverse diets that vary in composition over the short- and long-term. The cognitive effects of HFHS foods eaten under these conditions are less well understood. Here we tested whether HFHS diet-induced cognitive impairments were sensitive to (a) diet withdrawal; (b) intermittent or (c) time-restricted access; and (d) individual differences in consumption of HFHS foods. Adult male Sprague-Dawley rats were fed a 'cafeteria-style' diet comprised of palatable sweet and savoury HFHS foods and 10% sucrose solution, provided in addition to standard chow and water. Short-term memory was assessed via hippocampal-dependent place recognition and perirhinal cortex-dependent object recognition tests; faecal microbiota diversity was analysed via 16S rRNA gene amplicon sequencing. The HFHS diet-induced place memory impairment was ameliorated by withdrawing the HFHS diet for 11 but not 4 days. Place memory and microbiota diversity were progressively altered by intermittent access to HFHS diets, and were still evident when HFHS diet access was time-restricted (8h/day). Changes in cognition were associated with microbiota beta diversity but were not reliably predicted by the proportion of energy derived from fat or sugar, nor by total caloric intake. Results demonstrate that even intermittent or limited intake of HFHS foods may lead to changes in cognitive function.