inVIVO PLANETARY HEALTH

6th Annual Workshop
New York, USA
May 3-5th 2017

2017 Report

Building Resiliency

Inflammation, Ecosystems and the Transformation of Global Health

THE UNIVERSITY OF WESTERN AUSTRALIA

WORLDWIDE UNIVERSITIES NETWORK

DANONE NUTRICIA RESEARCH

Our Meeting in New York!

In 2017 our growing global network converged on New York City for our 6th Annual Workshop – for yet another enormously popular and stimulating multidisciplinary gathering.

The meeting was focused on many of our established popular themes, as well as developing our recent 'Global Environments' initiative which examines the links between human health and environmental health. This aimed to provide an integrative systems framework for understanding the eco-biological impact of living environments (including biodiversity) on microbial diversity and life-course human health from the perspective of omics and nature relatedness. As previously, we focused on aspects of the early environment which mediate immune, metabolic and neurodevelopment– as these pertain to many aspects of health and well-being.

Continuing the themes of our previous workshops, this meeting had a core focus on key early exposures - namely nutrition, the microbiome, early microbial diversity, nature relatedness, pollutants and the built environment - and how these interact to modify early immune development, to impact many aspects of development. Our multisystem focus included a range of early outcomes including allergy and asthma, obesity and metabolism, mental health and behaviour.

And, as always, this meeting was as fun as it was productive, leading to many more friendships and collaborative projects.

Susan Prescott
Director, inVIVO

Dianne Campbell
Co-Director, inVIVO

Anita Kozyrskyj
Chair, LactoActive

Alan C. Logan
Local Organiser
(New York)

Ganesa Wegienka
Local Organiser
(Detroit)

Our expanding horizons

In recent years our initiative has expanded to recognize the vital importance of approaching complex environmental issues from a more holistic and integrated perspective – extending and combining the typical focus on the biological and psychological level, with the wider sociological and environ-mental determinants of human health, and understanding how these are inter-related to societal health.

We recognize that human health challenges are the result of a dual burden: not only are we exposed to many 'new' things that are 'bad' for us (e.g. fast food, toxins and stress) we are also losing what was 'good' in our ancestral environments. This is having consequences for our mental and physical health - at individual and societal levels - with unsustainable consequences (social, economic and environmental). The facets of 'loss' are often overlooked in this agenda and extend from the physical (loss of biodiversity, species, local foods and produce) to the loss of community (loss of language, tradition, and stories) and the far less tangible aspects of loss (such as loss of value systems, loss of privacy and solitude, loss of purpose, peace, respect, spirituality, compassion, awe and wonder). In essence, we are increasingly disconnected from natural environments and traditional cultures - and in the processes losing appreciation for them. Our goal is contributing to meaningful societal change using the tools we have to best collaborative advantage. These philosophies will underpin our formal and informal workshop discussions.

Meeting Sponsorship

This meeting was supported by a Research Collaborative Award through the University of Western Australia, and in-kind support from the University of Sydney and the Henry Ford Health System.

Secretariat support

Special thanks to **Jenny Boden** and **Ben Thompson** for assisting in the meeting organization. We also thank the volunteer ECR from Detroit's Henry Ford Health System, **Andrew Bossick** and **Kyra Jones**, who are assisting in the conference registration desk. We are also grateful to **Ganesa Wegienka** for her help with the program and logistic support.

Our program and meeting abstracts

The details of our full 2017 program and speakers are outlined below (pages 10-18) and selected meeting abstracts are included on pages 19-36)

Our Publications

Our 2017 publications are listed below. A full list of publications (since 2012) can be found at http://www.in-flame.org/publications.html

Our Key Contributers

More information about the Key Contributors to the network can be founds at http://www.in-flame.org/people.html

About the Network

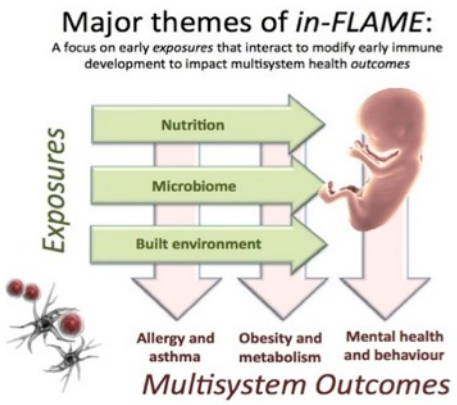
Launched in 2012 our Network addresses the risk factors, pathways and strategies to overcome the rising propensity for chronic inflammatory disorders, with a focus on early effects on the developing immune system. Led from UWA by Professor Susan Prescott, it involves 9 WUN universities and WUN+ partners from >50 institutions, and 20 countries around the world. Together our >350 current members are working on an integrated program of population studies, biological studies and intervention studies aimed at preventing inflammation and the burden of subsequent disease.

'…There has been an unprecedented rise in non-communicable diseases (NCDs) such as allergies, asthma, cancer, diabetes, mental ill health and obesity. Inflammation and immune dysregulation are common features, often associated with similar environmental and lifestyle risk factors such as dietary patterns, environmental pollutants, microbial patterns and stress. Given the central role of the immune system in health and development, inflammation must be examined as both a common element and target for the prevention of NCDs…'

This initiative recognises that the health of humanity (in every sense) is interdependent on the health of the environment. We see the need to have a more expansive (broad and long ranging) vision in addressing human, environmental and planetary health. It draws on the overarching premise that 'it is time to shape a better future' – emphasizing the imperative for change: on all levels. Paradoxically, in a culture where 'there is a never enough' we are 'losing' everything that once gave us deeper value, and our sense of purpose, place and identity. It may be argued that our shifting values and loss of deeper purpose are a root cause of social and economic instability and underlie the more superficial drivers of environmental and societal degradation. This erosion is gradual and associated with a shift in the 'normative' position to one of greed and self-interest. To some extend this has 'radicalized' empathy, kindness and compassion. At the same time, we are carrying the increasing burden of technology and a culture of unhealthy 'excess':

ultra-processed food, sedentary indoor behaviour, air and water contamination, excessive noise and light pollution, stress, electromagnetic radiation, screen-time, sleep disruption and many other adverse exposures that were not present in traditional environments. Of great concern, the burden and consequences of these adverse exposures is greatest in the socially disadvantaged - amplifying the disparities in health and opportunity, and further widening social inequity. Together with the 'missing' elements (e.g. absence of green space) the many adversities associated with urbanicity are eroding health in built environments with the higher burden of NCDs shouldered by disadvantaged populations. Viewed through the lens of 'connectedness' (i.e that we are all interconnected), this inequity can (and should be) viewed as a fundamental imbalance in our 'social ecosystem' which systemic consequences for all of us and beyond to our 'environmental ecosystems'.

inVIVO PLANETARY HEALTH



2017 publications

1. Jeffrey M Craig, Susan L. Prescott, Planning ahead: the mental health value of natural environments. The Lancet Planetary Health. 2017 1(4) e128-129

2. Susan L. Prescott and Alan C. Logan. Down to Earth: Planetary Health and Biophilosophy in the Symbiocene Epoch. Challenges. 2017, 8 (2): 19

3. Alan C. Logan, Susan L. Prescott. Astrofood, Priorities and Pandemics: Reflections of an Ultra-Processed Breakfast Program and Contemporary Dysbiotic Drift. Challenges, 2017. 8(2), 24

4. Susan L. Prescott, Alan C. Logan Each Meal Matters in the Exposome: Putting the Biological and Community into the Fast-Food-Socioeconomic Associations. 2017. Nov; 27 (Pt B):328-335; PMID: 29107462

5. Alan C. Logan, Martin Katzmann. Quo Vadis, Probiotics? Human Research Supports Further Study of Beneficial Microbes in Mental Health. EBioMedicine. in press

6. Susan L. Prescott. History of Medicine: Origin of the Term Microbiome and why it Matters. Human Microbiome Journal, 2017; 4:24-25.

7. *Daniel Munblit, Marina Treneva, Ilya Korsunskiy, Alan Asmanov, Alexander Pampura, John O Warner, A national survey of Russian physicians' knowledge of diagnosis and management of food-induced anaphylaxis. BMJ Open 2017;7:e015901.

8. *Kirsty Le Doare, Katie Bellis, Amadou Faal, Jessica Birt, Daniel Munblit, Holly Humphries, Stephen Taylor, Fiona Warburton, Paul T. Heath, Beate Kampmann and Andrew Gorringe. SIgA, TGF-**β**1, IL-10, and TNF**α** in Colostrum Are Associated with Infant Group B Streptococcus Colonization. Front. Immunol., 20 October 2017, ePub

9. Catrina L. McStay, Susan L. Prescott, Carol Bower, and Debra J. Palmer Maternal folic acid supplementation during pregnancy and childhood allergic disease outcomes: a question of timing? Nutrients 2017, 9, 123 (1-13)

10. Harald Renz, Patrick G. Holt, Michael Inouye, Alan C. Logan, Susan L. Prescott, Peter D. Sly. An exposome perspective: early life events and immune development in a changing world. J Allergy Clin Immunol. 2017 Jul;140(1):24-40

11. *Logan CA, Brandt S, Wabitsch M, Brenner H, Wiens F, Stahl B, Marosvölgyi T, Decsi T, Rothenbacher D, Genuneit J. New approach shows no association between maternal milk fatty acid composition and childhood wheeze or asthma. Allergy. 2017 Mar 17. doi: 10.1111/all.13161. [Epub ahead of print]. PMID: 28306160.



2017 publications……cont.

12. *L Drago, M Toscano, R De Grandi, E Grossi, EM Padovani, DG Peroni· Microbiota network and mathematic microbe mutualism in colostrum and mature milk collected in two different geographic areas: Italy versus Burundi. The ISME Journal (2016), in press

13. * H Harb, J Irvine, M Amarasekera, C Hii, DA Kesper, Y Ma, N D'Vaz, H Renz, A Ferrante, Daniel P. Potaczek, SL Prescott. Epigenetic regulation of PKCζ (by fish oil) and its role in cord blood T-cell maturation towards Th1 cytokine profile. Bioscience Reports Mar 27;37(2). pii: BSR2016048.

14. *Munblit D, Treneva M, Peroni DG, Colicino S, Chow LY, Dissanayeke S, Pampura A, Boner AL, Geddes DT, Boyle RJ, Warner JO. Immune Components in Human Milk Are Associated with Early Infant Immunological Health Outcomes: A Prospective Three-Country Analysis. Nutrients. 2017; 9(6):53

15. *CA. Logan, R Bornemann, W Koenig, F Reister, V Walter, G Fantuzzi, M Weyermann, H Brenner, J Genuneit D Rothenbacher. Gestational Weight Gain and Fetal-Maternal Adiponectin, Leptin, and CRP: results of two birth cohorts studies. Scientific Reports 2017;7:41847.

16. Debra J. Palmer and Susan L. Prescott, and Michael R Perkin. Early introduction of food reduces food allergy – Pro and Con. Pediatr Allergy Immunol. 2016 Dec 31. 2017 May;28(3):214-221.

17. Daniel Munblit, Alba Boix-Amorós, Robert J. Boyle, Maria Carmen Collado, Johan Garssen, Melvin C.L. Gay, Donna T. Geddes, Peter S. Hsu, Ralph Nanan, Carolyn Slupsky, Belinda Van't Land, John O. Warner, Ganesa Wegienka, Diego G. Peroni, Anita L Kozyrskyj. Human milk and allergic diseases: an unsolved puzzle. Nutrients 2017, 9, 894.

18. Christina E. West, Majda Dzidic, Susan L. Prescott, Maria C. Jenmalm. Bugging allergy; role of pre-, pro- and synbiotics in allergy prevention. Allergology International, 2017, in press

19. Susan Prescott, Danica Larcombe, Alan C Logan, Christina West, Wesley Burks, Luis Caraballo, Michael Levin, Eddie Van Etten, Pierre Horwitz, Anita Kozyrskyj, Dianne Campbell, on behalf of the members of the WAO Committee on Barrier Disease Issues and the Microbiome. The skin microbiome: impact of modern environments on skin ecology, barrier integrity, and systemic immune programming. World Allergy Org J. 2017 10:29 PMID: 28855974



2017 publications……cont.

20. H. Harb, M. González-de-la-Vara, L. Thalheimer, U. Klein, H. Renz, M. Rose, J. Kruse, D.P. Potaczek, E.M.J. Peters. Assessment of Brain Derived Neurotrophic Factor in hair to study stress responses: A pilot investigation. Psychoneuroendocrinology 86 (2017) 134–143

21. Richard Mitchell, Julia Africa, and Alan Logan. Vulnerable populations, health inequalities, and nature.Oxford Textbook of Nature and Public Health -The role of nature in improving the health of a population Edited by Matilda van den Bosch, William Bird. Oxford University Press. Oxford. 2017.

22. SL Prescott, AC Logan "The Secret Life of Your Microbiome: Why Nature and Biodiversity are Essential to Health and Happiness" First published in 2017 by New Society, Canada, Copyright © Susan L. Prescott and Alan C Logan (ISBN 978-0-86571-851-7)

Travel grants

Early Career Researchers (ECR) are a fundamentally important part of our network and key contributors to our program. We thank Danone Nutricia Research for once again supporting our ECR travel grants. These were be awarded based on abstract rankings and we are proud to congratulate the successful candidates, who all presented their work at the meeting.

- Janne Marie Laursen (Denmark)
- Hein Min Tun (Canada)
- Nina Iszatt (Norway)
- Majda Dzidic (Spain)
- Jonathan Thorsen (Denmark)
- Joacy Magdalene Gerard-Mathias (USA)
- Ling Xiao (Netherlands)
- Neils Soto-Ramirez (USA)

Program at a glance:

Tuesday May 2 Arrival and Check in	Day 1 Wednesday May 3	Day 2 Thursday May 4	Day 3 Friday May 5
FREE TIME **Note: Regular ferry links to to Manhattan leave from the front of the Hotel (7 minute journey)**	Welcome and introduction	**Session 4:** The First Food We Eat (breast milk and early life nutrition) (including presentations and progress from the LACTOACTIVE interest group)	**Session 5:** Food Systems and Diversity (connections between our food, environment and microbiome)
	Session 1: Establishing healthy microbial habitats in early life (developmental human ecology)		**In a nut shell (v)** (Bus Stop Abstract presentations)
	Session 2: Improving the Ecology of Built Environments (microbial diversity in postmodern societies)		**Session 6:** Systems biology (providing granularity to the big picture)
	In a nut shell (i) (Bus Stop abstract presentations)	**In a nut shell (iii)** (Bus Stop Abstract presentations)	**In a nut shell (vi)** (Bus Stop Abstract presentations)
	Lunch	Break	Reporting Back And Next Steps
	Session 3: Changing the Exposome – Resiliency (stress and microbes and behavior)	**LUNCH CRUISE AROUND MANHATTAN** (Hudson River, Statue of Liberty, Brooklyn Bridge) Will return around 4pm	Lunch
Registration opens 4pm	**In a nut shell (ii)** (Bus Stop Abstract presentations)	**Travel Award abstracts** In a nut shell (iv)	Goodbyes and Departures
6pm "Meet and Mingle" Informal light meal (included in registration)	**FREE TIME**	Keynote Lecture Working groups (light meal)	
	DINNER	**FREE TIME** **Suggest visits to Manhattan or Hoboken**	

07.30 -08.30 **BREAKFAST AND REGISTRATION**
(Hotel Breakfast Room is opposite the Meeting Room)

Welcome and Introduction:

08.30	Welcome and Setting the Scene for our Workshop	Susan Prescott (Australia)

Session 1: Establishing healthy microbial habitats in early life

	Chairs Dianne Campbell (Australia) Maria Jenmalm (Sweden)	
08.45	KEYNOTE: Setting the scene for health - The impact of the early microbiome on development	Maria Gloria Dominguez Bello (USA)
09.15	Tracking the microbiome in the first 7 years: new data from Swedish cohorts	Karsten Kristiansen (Denmark)
09.30	Breaking News from Barwon: Maternal Prevotella carriage during pregnancy and offspring allergic disease	Peter Vuillermin (Australia)
09.40	Transgenerational effects or parasites and helminths. Don't forget the future fathers	Cecilie Svanes (Norway)
09.50	Human microbiota mouse transplant models to study the effects of the early microbiome on development of inflammation, autoimmunity and allergy	Linda Mansfield (USA)
10.00	Questions and Discussion	

10.30 -11.00 **MORNING TEA**

Session 2: Improving the Ecology of Built Environments

	Chairs: Ganesa Wegienka (USA) Felice Jacka (Australia)	
11.00	Cities of Tomorrow: Improving microbial ecology for healthier cities and healthier humans	Jeff Craig (Australia)
11.15	How human habitats alter human microbial ecology – urban strategies to improve ecology and human health	Jenni Lehtimäki (Finland)
11.30	Healthier Homes – understanding the impact of the indoor microbial environment on health	Julian Crane (New Zealand)
11.45	The coalition of man and microbes: understanding and modulating the microbial-host interplay in the context of chronic inflammatory disease	Julia DURACK (USA)
12.00	Questions and Discussion	

In a nut shell – rapid fire oral poster session (i)		
Goal: Short and snappy 3 minute 'bus stop' presentations *Your world in 3 minutes! Will be strictly timed! FIVE slides only*		Chairs: Alan Landay (USA) Peter Hsu (Australia)
12.15	Longer outside, better inside: outdoor exposure and its impact on the gut microbiota and prevention of allergy	Cristina Gamez (Australia)
12.19	The Baby & Microbiota of the Intestine Cohort Studies: Baby & Mi and Baby & PreMi	Katherine Morrison (Canada)
12.23	The effect of environmental biodiversity on human skin microbial health	Danica-Lea Larcombe (Australia)
12.27	Transcriptional rewiring in human DC by gut microbial metabolite butyrate is associated with propagation of a tissue-sustaining Type 2-like immune response	Janne Marie Laursen (Denmark)
12.31	Dog Introduction Alters the Home Dust Microbiome	Alexandra Sitarik (USA)
12.35	Postnatal development of the murine gut microbiota	Niels van Best (Netherlands
12.39	Effects of fermented food diet on immune response of human faecal flora-associated mice	Noriko M. Tsuji (Japan)
12.43	From Infancy to Childhood: The Intersection of Gastrointestinal Microbial Communities, Diet and Health	Eileen Hutton (Canada)
12.47	PANEL DISCUSSION (Q AND A for all presentations)	

13.00 LUNCH

Session 3: Changing the Exposome – Resiliency

		Chairs Harald Renz (Germany) Marjorie Aelion (USA)
14.00	Positive Affect, Inflammation and the meta-exposome	Alan C Logan (USA)
14.15	Psychosocial determinants of allergic disease - pioneer in maternal stress and childhood asthma	Rosalind Wright (USA)
14.30	Infant gut microbiota characteristics relates to child developmental delay	Merete Eggesboe (Norway)
14.45	Maternal depression and infant gut immunity (fecal IgA)	Anita Kozyrskyj (Canada)
15.00	Probiotics in Pregnancy - a window for maternal and infant health	Kristen Wickens (New Zealand)
15.10	Questions and Discussion	

15.30 -15.45 AFTERNOON TEA

In a nut shell – rapid fire oral poster session (ii)		
Short and snappy 3 minute 'bus stop' presentations *Your world in 3 minutes!* *Will be strictly timed! FIVE slides only*		Chairs: Johan Garsson (Netherlands) David Fleischer (USA)
15.45	Repeated lipopolysaccharide administration develops depressive and anxious behaviors in obese mice	Shun-Fen Tzeng (Taiwan)
15.49	The role of TLR4 in Gut-Brain cross talk in a murine model for Parkinson's disease	Paula Perez Pardo (Netherlands)
15.53	The effects of short chain fatty acids on lipopolysaccharide or TNFα-induced endothelial activation and dysfunction	Meng Li (Netherlands)
15.57	Placental histone acetylation at promoters of several immune regulatory genes is a potential predictor of allergy development	Daniel P. Potaczek (Germany)
16.01	The role of early-life vitamin D in childhood allergy or allergen sensitization is controversial	Naoki Shimojo (Japan)
16.05	The role of cutaneous Staphylococcus aureus in the development of atopic dermatitis during infancy	Shuichi Suzuki (Japan)
16.13	PANEL DISCUSSION (Q AND A for all presentations)	

16.30- 18.30 FREE TIME

18.30-21.00 DINNER – SHERATON HOTEL

07.00 -08.00 BREAKFAST

Session 4: The First Food We Eat (LactoActive Session)

Chair: Anita Kozyrskyj (Canada)

08.00	Introduction to the LactoActive Group	Anita Kozyrskyj (Canada)
08.10	Intimate link between milk and the infant microbiome. What goes in, what comes out, and what does it mean?	Carolyn Slupsky (USA)
08.35	The problem is the mixture of infant feeding, not the duration of breastfeeding	Wilfried Karmaus (USA)
08.55	The impact of breastmilk composition on regulation of appetite and infant body composition	Donna Geddes (Australia)
09.05	Composition of fats in human milk and influential factors	Merete Eggesbo (Norway)
09.15	LactoActive breast milk metabolomics study update	Anita Kozyrskyj (Canada)
09.25	Breastfeeding and DNA methylation in the offspring: a mechanism for long term effects of breast feeding?	John Holloway (UK)
09.35	Even more to breast milk than meets the eye	Naoki Shimojo (Japan)
09.45	Discussion	

10.00 – 10.15 MORNING TEA

In a nut shell – rapid fire oral poster session (iii)

Chairs: Anita Kozyrskyj (Canada) and John Penders (Netherlands)

10.15	Impact of breastmilk and solid food on human gut microbial colonization: the lucki birth cohort	Niels van Best (Netherlands)
10.19	Breastfeeding duration modifies effect of smoking during pregnancy on eczema in offspring	Nandini Mukherjee (USA)
10.23	Longitudinal study of persistent organic pollutants in human milk in relation to allergy	Donna Geddes (Australia)
10.27	Relationship of human milk lysozyme with body composition of term breastfed infants	Zoya Gridneva (Australia)
10.31	Maternal body composition and appetite hormones influence macronutrients in human milk	Zoya Gridneva (Australia)
10.35	Breast Milk IgA specificity in older order mennonites (OOM): lower allergy prevalence	Kirsi Jarvinen-Seppo (USA)
10.39	Immune-modulatory properties of HMO on human monocyte and mouse bone marrow derived DC	Ling Xiao (Netherlands)
10.43	PANEL DISCUSSION (Q AND A for all presentations)	

Working Group Discussions

Chairs:

11.00 12.00	Suggest topics for small Working Groups to develop collaborative projects. Initial discussions.	Michael Levin (South Africa) Jacob Stockholm (Denmark)

LUNCH CRUISE – boarding from jetty by 12.15 for 12.30 Departure

16.00 -16.15 RETURN TO HOTEL

An afternoon at the Bus Stop (Travel Award Presentations)

In a nut shell – rapid fire oral poster session (iv)

The following presenters were awarded travel awards (for the best early career abstracts) – Sponsored by Danone Nutricia		Chairs: Hasan Arshad Susan Prescott
16.15	Prediction of endotoxin variants in the human gut microbiome and their relation to metabolic disease	Janne Marie Laursen (Denmark)
16.19	How pets make our babies healthier: their influence on the gut microbiome of infants following various birth scenarios	Hein Min Tun (Canada)
16.23	Brominated flame retardants in breast milk are associated with decreased infant gut microbiota diversity at 1 month of age in a Norwegian birth cohort	Nina Iszatt (Norway)
16.27	The oral microbiota during childhood and its relationship to allergy development	Majda Dzidic (Spain)
16.31	Airway microbiome-immune interactions in the origins of childhood asthma	Jonathan Thorsen (Denmark)
16.35	Mixed Infant Feeding and allergy Risk – effects of Direct Breastfeeding, Pumping and Feeding, and Formula Food	Joacy Magdalene Gerard-Mathias (USA)
16.39	Human milk oligosaccharides early in life modulate and program intestinal microbiota and immunity in an autoimmune mice model	Ling Xiao (Netherlands)
16.43	Modes of infant feeding and occurrence of eczema at 6 years of age	Neils Soto-Ramirez (USA)
16.47	PANEL DISCUSSION (Q AND A for all presentations)	ALL

Keynote Lecture

17.10-17.40	KEYNOTE LECTURE – BUILDING RESILIENCY: Addressing Inflammation through Ecosystems in the Transformation of Global Health	Alan C. Logan (USA) (Chair: Dianne Campbell)

Light Dinner and Working Groups

18.00 19.30	Opportunity for further project discussions by Working Groups (at discretion of each Working Group)	ALL

07.00 -08.00 BREAKFAST

Session 5: Food Systems and Diversity		
	Chairs: Laufey Hrolfdottir (Iceland) Eileen Hutton (Canada)	
08.00	Diet diversity and healthy eating: One and the same thing?	Carina Venter (USA/UK)
08.15	Fish oils and other specific nutrients in pregnancy: update and new data	Jakob Stokholm (Denmark)
08.25	Associations with food sensitization and food allergy in urban and rural African children.	Mike Levin (South Africa)
08.35	Diet quality during pregnancy and child emotional dysregulation at 2 yrs: Results from Barwon Infant Study	Felice Jacka (Australia)
08.45	Developments in food allergy	Ania Nowak-Wegrzyn (USA)
08.55	Questions and Discussion	

In a nut shell – rapid fire oral poster session (v)		
Short and snappy 'bus stop' presentations		Chairs: Yu Jiang (US) John Holloway (UK)
09.00	Perinatal determinants of naïve Treg during the first year of life in healthy and food allergic infants	Fiona Collier (Australia)
09.04	Interleukin 2 enhances the gut homing potential of human naïve regulatory T cells early in life	Peter Hsu (Australia)
09.08	Fish oil in pregnancy is positive associated with gestational age, birth weight and intra uterine growth	Rebecca Vinding (Denmark)
09.12	The relationship between microbiota complexity, SCFA and epigenetic changes in the gut and other tissues	Jeff Craig (Australia)
09.16	Characterisation of ovalbumin-specific regulatory T & B cells in egg-allergic children	Catherine Lai (Australia)
09.20	Maternal diet, obstetric complications and inflammatory markers during pregnancy	Laufey Hrolfsdottir (Iceland)
09.24	Early biomarkers of allergic phenotype: TSLP and other epithelium-derived cytokines (IL-25 and IL-33)	Cristina Gamez (Australia)
09.28	PANEL DISCUSSION (Q AND A for all presentations)	

09.45 -10.15 MORNING TEA

Session 6: Metabolomics and Systems Biology

	Chairs: Marion Groetch (USA) Alexandra Sitarik (USA)	
10.15	Allergy and asthma: Towards precision medicine and requirement for biomarker development	Harald Renz (Germany)
10.25	Precision Medicine meets Birth Cohorts: Next Generation Studies	Christine Cole Johnson (USA)
10.35	Gut microbial metabolites limit autoimmune T cells and protect against type 1 diabetes	Charles Mackay (Australia)
10.45	Update on the metagenomic and metabolomic data-analyses in KOALA	John Penders Liene Bervoets (Netherlands)
10.55	Host-microbe extracellular cellular talk: more going on than we think	Annika Scheynius (Sweden)
11.05	Microbial metabolites and host interactions	Susanne Brix (Denmark)
11.15	Questions and Discussion	ALL

In a nut shell – rapid fire oral poster session (vi)

	Short and snappy 'bus stop' presentations Chairs: Lawrence Gray (Australia) Peter Vuillermin (Australia)	
11.25	The combinatorial activity of a defined microRNA network shapes Th2 phenotype in airway inflammation.	Ayse Kilic (Germany)
11.29	Metabolic profiling of type 1 diabetes mellitus in children by proton NMR-based metabolomics	Liene Bervoets (Netherlands)
11.33	Maternal poly(I:C) mouse model to study effects of prenatal maternal infection on the offspring	Johan Garssen (Netherlands)
11.37	Use of Infant African Green Monkeys To Model Early Life Influences on Chronic Disease	Alan Landay (USA)
11.41	Raw cow's milk prevents airway inflammation in a murine house dust mite-induced asthma model	Betty van Esch (Netherlands)
11.45	The interaction between infant eczema, dietary diversity and allergic outcomes in the FAIR birth cohort	Carina Venter (USA/UK)
11.49	PANEL DISCUSSION (Q AND A for all presentations)	

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PLANETARY HEALTH

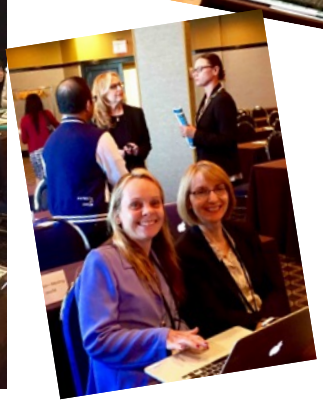
Session 7: Reporting Back

Chairs: Susan Prescott and Dianne Campbell

12.10-13.00	Working Group Presentations: new plans Summary outcomes Planning next steps 2018 and Beyond THANKS AND FAREWELLS

13.00 **LUNCH and FINAL WORKING GROUP DISCUSSIONS**

14.00 **MEETING CLOSE**
Departures and Sight Seeing



2018 meeting abstracts
(Not available for all speakers and presentations)

From Infancy to Childhood: The Intersection of Gastrointestinal Microbial Communities, Diet and Health

Eileen Hutton,[1] Katherine Morrison,[2] Alison Holloway,[1] Jennifer Stearns,[3,4] Michael Surette,[3,4,5] Helen McDonald,[6] Elyanne Ratcliffe,[2,4] Jonathan Schertzer,[2,5] Lehana Thabane,[7,8] Monique Mommers,[9] Liene Bervoets,[10] Niels van Best,[10] Susanna Lau,[11] Eckard Hamelmann,[12] John Penders[10]

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[2] Department of Pediatrics, McMaster University, Hamilton, Canada
[3] Department of Medicine, McMaster University, Hamilton, Canada
[4] Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, Canada
[5] Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Canada
[6] Midwifery Education Program, McMaster University, Hamilton, Canada
[7] Department of Clinical Epidemiology and Biostatistics, McMaster University, Hamilton, Canada
[8] Centre for Evaluation of Medicines, St. Joseph's Healthcare Hamilton, Hamilton, Canada
[9] Department of Epidemiology, Maastricht University, Maastricht, The Netherlands
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Background: The composition and function of the human gut microbiome are increasingly recognized as important contributors to immunological and metabolic health and to disease susceptibility across the lifespan. Importantly, widespread non-communicable diseases such as allergy and obesity have been linked to the early life establishment of the gut microbiome. The transition to a solid food based diet in infants occurs during a critical window for the development of the gut microbiome and thus may influence obesity and allergy outcomes later in life.

Objectives: Our primary objectives are to describe the impact of the timing and nature of the introduction of solid foods and cessation of human milk on colonization patterns of the gut microbiome in infancy and to examine linkages with subsequent fat accretion, metabolism, inflammation and allergy.

Method: Our international consortium of researchers in the area of infant and child health and the gastrointestinal microbial community brings together 4 cohorts that are different in terms of biology, culture, and geographical location. The first cohort is recruiting 240 Canadian infants born at term to low-risk pregnant women who have experienced minimal intervention during birth and have a high rate of breastfeeding. The second Canadian cohort will recruit 60 infants who are born preterm. Both Canadian cohorts plan to follow participants until 3 years of age. The third cohort is composed of 606 German born infants, with at least one atopic parent, half of whom were randomly allocated to receive oral bacterial lysates in infancy. Follow up to 3 years of age was completed for 80% of participants and over half of the children provided another stool sample at 6-12 years. The fourth cohort is embedded in the Dutch Child and Youth Health Care System and will include100 healthy term infants followed until the age of 14 months, with the possibility of linkage to medical registries for continued data collection in childhood and beyond. All 4 cohorts have collected or plan to collect repeated faecal samples at frequent intervals and comparable detailed information about perinatal exposures, dietary intake, medications and supplement use. These prospectively collected data will enable longitudinal characterization of microbial communities using 16S amplicon sequencing of all faecal samples. We will also do intensive sampling in a small subgroup in the two week period around weaning from breastmilk and the two week period when solid foods are first introduced. These samples will be analysed using metagenomic and metabolomics methods. We will study the influence of early life dietary exposures on the gut microbiome community structures and the subsequent association with allergy and obesity.

Implications: We anticipate that this work will contribute to understanding how differences in genetic predisposition and early life events influence the trajectories of the gut microbiome, and the persistence and influence of these trajectories with subsequent health outcomes. We will also develop novel analytic approaches to assist our understanding of the development of the gut microbiome. Our findings will inform infant nutrition guidelines and may lead to future research on correctional therapies for gut microbiome dysbiosis.

Baby & Mi and Baby & PreMi

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Background: Following birth, the newborn gut is rapidly colonized in the first hours and days by a wide variety of bacteria, typically after exposure to maternal vaginal and rectal microbiota. Different exposures in early life may affect how the infant gut becomes colonized with microorganisms. The organisms that establish in the gut early in life are thought to persist for the long term, making early exposure to the appropriate colonising organisms potentially critical to long term health.

Approximately 50% of low risk, full-term infants born in Canada are exposed to intrapartum antibiotic prophylaxis (IAP). In adulthood, short-term antibiotic therapy usually causes temporary changes to the microbiota. However, little is known of the influence of IAP on early gut colonization, longer term microbiota differences or health outcomes later in life.

Preterm infants are at increased risk for disruption in the normal development of the gut microbiome due to differential nutritional and medical exposures in the first days of life (including antibiotics), a higher prevalence of health conditions that may lead to dysbiosis and, in infants born with extreme prematurity, many days spent in an altered microbial environment.

Objectives: The primary objective of the Baby & Mi cohort is to determine what factors influence the type and quantity of organisms found in the intestinal microbiome of full-term infants during the first three years, with a particular interest in the impact of IAP. Secondary objectives are to link gut microbiota profiles to health outcomes. The Baby & PreMi study is a pilot study to assess the feasibility of enrollment, follow up and data collection in preterm infants over a 3 year period. We will also determine what sample size is required to compare the microbiota and health outcomes of preterm infants to full-terms from the Baby & Mi cohort.

Method: The Baby & Mi cohort is currently recruiting pregnant women from midwifery practices who are planning a vaginal birth. Women who have a known multiple pregnancy or have a preterm birth are excluded. The Baby & PreMi cohort will begin recruiting preterm infants in January 2017. The cohort will exclude triplets, higher order multiples, infants born with structural bowel abnormalities, and those diagnosed with a bowel disease that will require surgical intervention.

Data is collected from the birth record and questionnaires completed by the parents. Eight stool samples are collected between 3 days and 3 years of age. In addition, stool samples will be collected every other day from preterm infants from birth to hospital discharge and in-hospital progress (nutrition, medications and intercurrent illness) will be recorded weekly. Growth is monitored and body composition is measured using PEA POD and dual energy x-ray absorptiometry.

Implications: Our studies will examine the trajectory of change in the gut microbiota over the first 3 years of life and the influence of gestational age, early life exposures, diet, probiotic and antibiotic use on this trajectory. We will begin to explore potential clinical ramifications of differences in gut microbiota on growth, adiposity and the child's systemic inflammatory status.

Longer outside, better inside: outdoor exposure and its impact on the gut microbiota and prevention of allergy.

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Background: The incidence of childhood allergic diseases has dramatically increased. The early environment is thought to have a significant impact on the development of the immune system and consequently on allergy risk. Spending time outdoors has the potential dual benefit of exposing children to both a greater diversity of microbes and to UV light.
We propose that increased time spent outdoors during early life will be associated with an optimal intestinal microbial ecology and beneficial metabolites, helping with the development of the immune system during this critical period and decreasing the risk of allergic diseases (eczema, food allergy, allergic rhinitis, asthma).

Aims: To determine whether increased time outdoors and measures of UV light exposure in the first 6 months of life influences the gut microbiota, gut metabolites, as short chain fatty acids (SCFA), inflammatory markers and the development of allergic diseases.

Material & Methods: We will analyze stool samples collected from infants at 6 months of age in relation to the duration of the time spent outdoors, according to parent-reported questionnaires. UV exposure, obtained by UV dosimeters will also be analyzed to determine whether UV exposure may independently associate with faecal microbial, metabolic and immune parameters. With ongoing follow-up of the children we will examine associations between the gut microbiota, time spent outdoors, and the development of allergic diseases.

Conclusions: This study will provide essential pilot data for future investigations of early life environmental exposures, the developing microbiome, and childhood allergic disease.

Modes of infant feeding and the occurrence of eczema at 6 years of age

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Abstract
Background: Modes of infant feeding such as direct, indirect, formula feeding, and their combinations may play a role in child health. The aim was to investigate whether such patterns in the first six months of infancy pose different risks for eczema/skin allergy in children up to 6 years compared to direct breastfeeding (at the breast) for at least 3 months.

Methods: The Infant Feeding Practices Study II (1,387 infants) in the United States and its follow-up provided data on feeding modes in the first six months of infancy and doctor's diagnosed eczema/skin allergy in the first 6 years of life (6 years follow-up). Different feeding patterns were identified. Log-linear models were used to estimate relative risks (RRs) of feeding patterns for doctor's diagnosed eczema/skin allergy in the first 6 years of life, adjusting for confounders.

Results: Compared to 'direct breastfeeding for at least 3 months' (DBF3m), the combination of direct feeding at the breast (DBF), pumping and feeding breast milk (BM), and formula (FF) in the first months' (DBF/BM/FF) showed a statistically significant higher risk for eczema/skin allergy in the first 6 years of life (RR= 1.46; 95%CI: 1.01, 2.11), adjusting for confounders. Among the confounders, paternal eczema and race/ethnicity (Hispanic vs White) were associated with a higher risk of eczema/skin allergy.

Conclusions: Mixed infant feeding may carry a higher risk of eczema/skin allergy compared to direct feeding at the breast. Further investigations are necessary to determine why mixed feeding practices may pose a risk for the occurrence of eczema.

Abstracts

Human milk oligosaccharides early in life modulate and program intestinal microbiota and immunity in an autoimmune mice model

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Objective: Early life nutrition such as breast milk is known to be an independent protective factor against development of type 1 diabetes. Human Milk Oligosaccharides (HMOS) are important bioactive components of human milk. The effect of early supplementation with HMOS on the autoimmune diabetes incidence in Non-obese diabetic (NOD/ShiLtJ) mice was evaluated and correlated to the protective effect with regulation of immune responses and modulation of gut microbiota.
Methods: NOD mice were fed diet containing human milk derived oligosaccharides from week 4 to 10 or normal diet. Diabetes incidence was determined by urine glucose tests. Pancreatic insulitis was characterized histologically. Naturally occurring regulatory T cell and T helper cell frequencies in the spleen were analyzed by flow cytometry. Intestinal microbiome composition was analyzed by 16S rRNA amplicons derived from fecal samples collected from different time points. Short chain fatty acids (SCFAs) were measured in cecum content and fecal samples during intervention and diabetes development.
Results: Early supplementation with HMOS significantly reduced the incidence of diabetes up to the age of 30 weeks ($p<0.05$). Suppressive effects were corroborated by lower pancreatic insulitis, lower Th1 and Th17 induction and decreased T-cell activation markers (CD25 and CD69) expression in the spleen. Spleen regulatory T cells (CD4+CD25+FOXP3+) were reduced in the HMOS receiving mice. The composition of gut microbiota was found significantly different between the groups: an increased ratio of Firmicutes/Bacteriodetes and increased richness of bacterial genera was observed in HMOS receiving mice; more importantly, more SCFAs producing bacteria in the HMOS group was flourished. Corresponding to the microbiota community, SCFAs, known as immunomodulatory, in the cecum content and fecal were elevated by HMOS diet, and significantly different during intervention.
Conclusion: Temporary dietary exposure of NOD mice to HMOS in early life reduced the incidence of autoimmune diabetes beyond the intervention period. These results implicate that human milk derived oligosaccharides modulate the immune development and microbiome composition leading to suppression of spontaneous autoimmune development later in life.

RELATIONSHIP OF HUMAN MILK LYSOZYME WITH BODY COMPOSITION OF TERM BREASTFED INFANTS IN FIRST YEAR OF LIFE

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Human milk (HM) components, such as immune, growth and anti-inflammatory factors may influence infant feeding behavior, regulation of appetite and body composition (BC). Lysozyme in HM is well known for its immune effects. It is also implicated in gut health, inflammatory status and homeostasis of bone and cartilage growth. Oral administration of lysozyme in preterm infants increases weight gain, thus HM lysozyme may influence the BC of breastfed infants. This study investigated associations between breastfed term infant BC and the concentration and calculated daily intake (CDI) of HM lysozyme in first 12 months of life.

BC of term infants that were fully breastfed at 2 and 5 months and continued to breastfeed at 9 and 12 months (n = 20) was measured with ultrasound 2- (US2SF) and 4-skinfolds (US4SF) (biceps, triceps, subscapular, suprailliac). Lysozyme concentration in HM was determined using modified turbidimetric assay. 24-h milk intake between 2 and 5, at 9 and/or 12 months was measured and CDI of lysozyme was calculated. Linear regression/linear mixed effect models were used to determine associations between infant BC and HM lysozyme concentration/CDI.

Lysozyme concentration (g/L) increased throughout the 12 months of lactation ($p<0.001$) [2mo: 0.11±0.13 (n=14); 5mo: 0.12±0.06 (n=18); 9mo: 0.14±0.02 (n=19); 12mo: 0.14±0.01 (n=14)], while CDI (g) did not differ (p=0.65) [2mo-5mo: 0.09±0.08 (n=15); 9mo: 0.06±0.01 (n=6); 12mo: 0.07±0.02 (n=6)]. Higher lysozyme CDI (g) was associated with larger infant triceps (mm) (122.3±51.3 g; p=0.027), higher percent fat mass (US2SF: 71.7±33.7, p=0.046; US4SF: 23.1±10.9, p=0.046), higher fat mass (kg) (US4SF: 3.0±1.2, p=0.023), and lower fat-free mass (kg) (US2SF -10.2±3.0, p=0.014).

HM lysozyme is differentially associated with infant fat and lean mass. Via increase in concentration infant CDI of lysozyme remained unchanged throughout the first 12 months of lactation offering continuous protection. These findings further support the importance of HM components in the programming of infant BC.

LONGITUDINAL STUDY OF PERSISTENT ORGANIC POLLUTANTS IN HUMAN MILK in relation to allergy in mothers from Western Australia: An egg intervention study

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Egg is one of the most common food allergens in infants and young children. Besides allergenic proteins, the presence of persistent organic pollutants (POPs), such as pesticides, found in eggs can also be found in human milk (HM). Many of these pesticides, such as DDT and DDE, have been identified as endocrine disruptors and induce cytokine production and increase inflammatory mediators (e.g. IgE) in humans. Thus, potential associations between pesticides and maternal allergic status may exist. This longitudinal study aimed to investigate the relationship between maternal egg consumption, HM POP and maternal allergy.

HM samples were collected from 60 Western Australian (WA) mothers at 2, 4, 6 and 16 weeks postpartum. A scale-down version of the QuEChERs extraction optimized for HM (1mL) was used, followed by quantitative GC-MS/MS analysis for 88 pesticides. Mothers were randomized into 3 egg groups: High egg group (consumption of 4-6 eggs per week), Low egg group (1-3 eggs per week) and egg-free group. Maternal allergies (hay fever, eczema, asthma and food allergy) were identified via questionnaire. Linear mixed models was used to investigate association between HM pesticides and egg groups, type of allergies and lactation periods.

The organochlorine pesticide, p,p'-DDE, was detected in 92.6% of the milk samples with an average concentration of 73.9 ± 69.6 ng/g fat (range: 7.6 – 551.0 ng/g fat). Overall, a 10.5% increase in HM p,p'-DDE was observed from mothers in the high egg group at week 16 compared to week 2. Whereas a 3.2% increase in HM p,p'-DDE was observed in the low egg group and a decrease of 5.8% in the egg-free group. However these differences are not significant ($P > 0.78$). No significant association was observed between individual average p,p'-DDE and presence of maternal hay fever ($P = 0.49$), eczema ($P = 0.17$), asthma ($P = 0.14$) and food allergy ($P = 0.51$).

Overall, HM p,p'-DDE in WA is approximately 4-fold below the regulatory limit. Increased egg consumption was not associated with increased HM POP. Maternal pesticides burden (measured by HM) was not related to the type of maternal allergy.

Mixed Infant Feeding - Direct Breastfeeding, Pumping and Feeding, and Formula Food Pose a Risk for Food Allergy in Early Childhood

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Rationale: Food Allergy is a growing epidemic in developed countries which has acquired the attention of the research community for a while. The role played by various infant feeding patterns (IFP) in food allergy is still in contention. The effect of breastfeeding on food allergy has been analyzed by few studies only; but these have not differentiated between direct feeding at the breast (DBF) and pumping and feeding of breast milk (BM) when compared to formula feeding (FF). In addition, parallel feeding from these different sources have not been taken into account, but oversimplifications such as duration of exclusive breastfeeding or month of weaning have been used to estimate risks. We want to determine if mixed modes of IFP (DBFBMFF) in the first six months, bear a higher risk of food allergy in early childhood.

Methods: The Infant Feeding Practice Study 2, a longitudinal study conducted by CDC and FDA, enrolled pregnant women from 38 states in the US in their third trimester. Infant feeding information was ascertained using nine monthly questionnaires during infancy. Participants were re-contacted after 6 years. Food allergy related information was collected at 4, 9, 12, and 72 months. A total of 1,387 participants had complete IFP data and information on food allergy symptoms (FAS) and doctors' diagnosed food allergy (DDFA). Generalized estimating equation models were used to analyze repeated measurements of FAS and DDFA. We controlled for confounders such as baby's sex, maternal smoking status during pregnancy and post-pregnancy, mode of delivery, race/ethnicity, maternal education, and maternal and paternal history of food allergy.

Results: Prevalence of FAS and DDFA (in %) were 9, 8, 8, and 8 and 1, 1, 2, and 1, respectively, at the four time points. For FAS, children who were exposed to DBFBMFF have a higher risk (RR=1.57, 95%CI:1.09-2.25) of food allergy compared to the DBF group. No significant risk of IFP was found for DDFA. However, only 18-37% of children with food allergy symptoms were taken to the doctor for diagnosis. Paternal and maternal food allergy also pose a higher risk in the incidence of food allergy among children ((RR=1.58, 95%CI:1.19-2.09; RR=1.21, 95%CI:0.88-1.67), respectively).

Conclusion: Considering the recommended six months of breast-feeding by WHO, most studies do not investigate actual modes of parallel infant feeding of food from different sources (direct at the breast, stored breast milk, and formula). In this prospective birth cohort, it appears that multiple sources of allergens due to mixed feeding may pose a higher risk for food allergy.

Breastfeeding duration modifies the effect of smoking during pregnancy on eczema from childhood till adolescence

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Introduction: Maternal cigarette smoke during pregnancy and breast feeding are two important environmental factors for the occurrence of eczema. Interestingly, certain components similar to coal tar, a traditional remedy of eczema, are also found in cigarette smoke. Additionally, these components are also present in breastmilk if the mother smoked during pregnancy. However, studies linking prenatal exposure to gestational smoking, and breastfeeding duration, respectively, to eczema, have produced contradictory findings. This motivated us to investigate the interplay of these factors for the development of eczema from childhood to adolescence.

Methods: Information on eczema symptoms was obtained at 1 and 2 (n=974), 4 (n=897), 10 (n=960), and 18 (n=924) years of age from the Isle of Wight (IOW) birth cohort, UK. Eczema was defined as chronic or chronically relapsing, itchy dermatitis lasting more than 6 weeks with characteristic morphology and distribution. Gestational smoking and breastfeeding data were collected at birth and the 1- and 2-year follow-up. DNA was genotyped using GoldenGate Genotyping Assays (Illumina, Inc, SanDiego, CA) for FLG variants. Repeated measurements of eczema were analyzed using logistic regression with the generalized estimating equation (GEE) approach, adjusting for maternal and paternal history of eczema, gender, FLG loss of function variants, weeks of gestation, and socioeconomic status of the offspring.

Results: The interaction of duration of breastfeeding and gestational smoking was significantly associated with the eczema indicating that the risk of maternal smoking varies with duration of breastfeeding. Maternal smoking was not associated with offspring eczema if the mothers did not breastfeed (RR: 0.97 (95%CI: 0.70, 1.36)). However, it was significantly associated with a lower risk of eczema if the mother breastfeeds for a longer duration. The risk ratios and 95% CI for 3, 9, 15, 21 weeks are 0.88 (95%CI:0.66, 1.20), 0.74 (95%CI: 0.54, 1.01), 0.62 (95%CI:0.41, 0.93), and 0.51 (95%CI: 0.3, 0.88), respectively. Additionally, a stratified analysis by gestational smoking showed that breastfeeding is protective of eczema only when the mother smoked.

Conclusions: Our study highlights the importance of breastfeeding by showing that longer duration of breastfeeding is protective of eczema if the mother smoked during pregnancy. Future studies should investigate the underlying mechanism by assessing common molecular pathways related to eczema that are influenced by both breastfeeding and cigarette smoking in order to develop novel interventions to prevent eczema in childhood and adolescence.

Fish oil supplementation in pregnancy is positive associated with gestational age, birth weight and intra uterine growth

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Background and Objective Randomized trials have reported that supplementation with n-3 long chain polyunsaturated fatty acids (LCPUFA) in pregnancy can prolong pregnancy and thereby increase birthweight.
We aim to examine the relationship between fish oil supplementation in pregnancy and duration of pregnancy and birthweight, and furthermore investigate effects on intrauterine growth, measured as size for gestational age.

Methods The Copenhagen Prospective Studies on Asthma in Childhood 2010 is an ongoing Danish cohort study of 738 unselected pregnant women and their children. The women were recruited at 24 weeks of pregnancy and were randomized to take 2.4 g/day of n-3 LCPUFA (fish oil) or control (olive oil) supplementation until 1 week postpartum. ClinicalTrials.gov number NCT00798226.
Exclusion criteria were intrauterine death, disabling disease and twin pregnancy.
Ultrasound determined due date was obtained at the 24 weeks pregnancy visit in the clinic. Gestational age and birthweight were obtained at the child's 1-week visit in the clinic and verified against national registry data. Intra uterine growth were measured as birth weight for gestational age

Results 700 woman/infant pairs were included. Fish oil supplementation in pregnancy was associated with increased intrauterine growth compared to control supplementation measured as birth weight for gestational age (g/day mean (SD); 12.8 (1.7) vs. 12.6 (1.7); p=0.04), a 2 day longer pregnancy duration (days median [IQR]; 282 days [275-288] vs. 280 [273-286], p=0.02) and a 97 g higher birthweight (g; mean (SD); 3601 (534) vs. 3504 (528), p=0.02). We observed no effects on preterm birth.

Conclusion Fish oil supplementation in the third trimester of pregnancy was associated with increased intra uterine growth and prolonged gestation, leading to a higher birthweight.
Future studies should focus on fish oil supplementation's potential for improving fetal and infant health worldwide.

Characterisation of ovalbumin-specific regulatory T & B cells in egg-allergic children

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Background - Egg allergy affects around 9% of Australian children. The underlying cause of allergies is thought to be associated with the breakdown of immune tolerance development in early life. In murine models, both regulatory T (Tregs) and B (Bregs) cells are implicated. However, few studies have examined the role of these cells in humans, especially in terms of allergen-specific regulatory and allergic responses. We aim to characterise ovalbumin-specific Tregs and Bregs in at risk infants randomized to receive egg or placebo from 4-6 months of life.

Methods - Peripheral blood mononuclear cells were collected from infants participating in two randomised controlled trials (BEAT and STEP) examining the effect of early regular egg introduction on egg allergy development. The cells were analysed by flow cytometry for ex vivo FoxP3$^+$ Tregs phenotype. In vitro assays were conducted to observe cytokine production, ovalbumin-specific Breg frequency and ovalbumin-specific FoxP3$^+$ Treg responses. Cells were acquired using the BD LSRII flow cytometer and analysed by Flowjo.

Results – In healthy and egg allergic individuals, ovalbumin-reactive Tregs were identified as CD4$^+$CD137$^+$CD40L$^-$FoxP3$^+$ cells, whereas ovalbumin reactive effector T cells were identified as CD4$^+$CD137$^-$CD40L$^+$Foxp3$^-$. Ovalbumin-specific Bregs were identified as CD19$^+$fluorescence-conjugated ovalbumin$^+$IL-10$^+$ B cells.

Conclusion - Analysis of these cohorts is on-going and will assist in understanding the mechanisms of natural tolerance acquisition to foods during infancy.

How pets make our babies healthier: their influence on the gut microbiome of infants following various birth scenarios

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Introduction
Early-life exposure to household pets has the capacity to alter risk for overweight and allergic diseases in children, both of which link to gut microbiota changes. However, the mechanisms are unclear. We, and others, have found that household pets can alter infant gut microbial diversity and composition. In this study, we aimed to investigate the impact of differential pet exposures on infant gut microbiota following cesarean section and vaginal delivery with and without maternal antibiotic propylaxis.

Materials and Methods
The study population comprised 746 infants enrolled at the Edmonton, Vancouver and Winnipeg sites of the Canadian Healthy Infant Longitudinal Development (CHILD) population-based birth cohort. Gut microbial diversity and composition at 3 months and 1 year after birth was assessed using high-throughput 16S rRNA sequencing. Delivery method recorded in the birth chart was defined as vaginal (with or without intrapartum antibiotic prophylaxis, IAP), elective cesarean and emergency cesarean. Pet exposure (prenatal and postnatal at 3 months of infant age), ethnicity and other covariates were retrieved from the standardized questionnaires completed by mothers. Statistical analyses were performed in SAS V9.4. Infant gut microbial profiles according to pet exposure and delivery mode, and adjusted for ethnicity, were examined by linear discriminant analysis effect size (LEfSE) with an LDA log score cut-off of 2.

Results
Among 746 infants in this study, 53.8% of infants were exposed to at least 1 furry pet either prenatally and/or postnatally. Eight percent were exposed during pregnancy alone and 46. 8% exposed during both the pre- and postnatal time periods. As a common association in all birth scenarios, pet exposure enriched the abundance of Oscillospira and Ruminococcus ($P<0.05$) with more than a two-fold increased risk. In vaginally born infants with maternal IAP exposure, Streptococcaceae were substantially and significantly reduced by prenatal pet exposure ($P<0.001$, FDRp=0.03), reflecting a 80% decreased likelihood of high abundance (OR 0.20, 95% CI, 0.06-0.70) for infants who were exposed pets prenatally only and a 69% reduced likelihood (OR 0.31, 95%CI, 0.16-0.58) for infants exposed to pets at both time periods.

Conclusions
This study provides new evidence that exposure to pets can modify gut microbial composition of infants following all birth modes, which may confer future health benefits for infants. Reduced abundance of streptococci after vaginal delivery and intrapartum antibiotic prophylaxis may provide more immediate protection.

Maternal diet, obstetric complications and inflammatory markers during pregnancy

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Background/Objectives: Weight gain and poor dietary habits have been associated with low- grade inflammation in non-pregnant subjects. Whether the same holds for pregnant women has not been properly examined, but exploring this may be of importance as elevated maternal inflammatory response has been associated with pregnancy complications. Our objective was to examine the associations of maternal diet and obstetric complications with low-grade inflammation.

Design: A cross-sectional analysis of 671 pregnant women in normal weight. Information about gestational weight gain (GWG) and obstetric complications was retrieved from the Danish Medical Birth Registry and hospital records. Diet was assessed in gestational week 30 with food frequency questionnaires and interviews. Markers of inflammation, high sensitivity C-reactive protein (hsCRP), Serum amyloid A (SAA), Interleukin-6, (IL-6), IL-8, IL-1**β** and Tumor necrosis factor **α** (TNF-**α**) were quantified with multi-arrays in serum from week 30.

Results: Median concentrations of markers of inflammation tended to be higher in women exposed to different obstetric complications compared to women with uncomplicated pregnancy. For example, in gestational diabetes (hsCRP: 4.4 vs. 3.0 µg/mL), hypertension (hsCRP: 4.9 vs. 3.0 µg/mL) and preeclampsia (Il-6:1.5 vs. 1.1 pg/ml and TNF-**α**: 7.9 vs. 5.6 pg/ml) these differences were statistically significant. Moreover in adjusted models, each 1-kg increase in GWG was associated with 3% (95%CI: 1, 5) higher hsCRP and 3% (95%CI: 1, 4) higher SAA concentrations, which corresponded to ~18-25% increase in these biomarkers among those with excessive GWG. With respect to diet, women in the highest compared to lowest quintile of protein intake had 26% (95%CI: 3, 54) higher hsCRP concentrations. This increase appeared to be driven by intake of animal protein. A similar pattern was observed for SAA.

Conclusions: In a cohort of lean pregnant women, obstetric complications, excessive GWG as well as high intake of animal protein during pregnancy was associated with higher concentrations of markers of inflammation.

Title: The interaction between infant eczema, dietary diversity and allergic outcomes in the FAIR birth cohort

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Background: Dietary diversity (DD) during infancy may prevent allergic diseases by exposing the gastrointestinal microbiota to a diversity of foods and their bacterial load. Eczema is known to increase the risk of food allergy (FA), but it is not known if the risk may be modified by DD.

Objective: To investigate the association between DD during infancy and risk of subsequent allergic outcomes in childhood, and to assess whether atopic eczema during the first year of life modified this association.

Methods: A birth cohort was established on the Isle of Wight, UK in 2001/2002 (n = 969). Variable intake of 21 different foods was prospectively recorded at 3, 6 and 9 months of age. Intake of the major 8 common allergenic foods only was recorded at 12 months. Skin prick testing, clinical history and oral food challenges (where indicated) were performed at 1, 2, 3 and 10 years. The presence of eczema and other allergic diseases were determined using standardised questions. The association between DD in infancy and risk of allergic outcomes at 3 and 10 years was assessed using logistic regression models.

Results: The presence of eczema at 6, 9 and 12 months was associated with lower DD. A higher DD at 12 months was associated with a lower likelihood of eczema at 3 years taking into account confounding variables ($p = 0.048$). There was no association between DD and asthma or rhinitis at 3 or 10 years. In children with eczema present at 9 months, a higher DD reduced the likelihood of developing FA at 3 years.

Conclusion Increased DD in infancy may decrease the likelihood of FA and the effect may be modified by eczema status.

Early biomarkers of allergic phenotype: TSLP and other epithelium-derivated cytokines (IL-25 and IL-33) levels.

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Background: The previous paradigm of Th1/Th2 imbalance driving allergic disease has been expanded by the identification of novel T helper families, like Th17 and type 2 innate lymphocyte cells (ILC2); with their signatures cytokines. The newly discovered epithelium-associated cytokines thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 are thought to play a role in the early progression towards Th2 immunity. These cytokines have been shown to be up-regulated in allergic conditions such as asthma or atopic dermatitis, but their involvement in food allergy remains to be investigated. Also, there is a lack of knowledge regarding any correlations of these cytokines in the development of the immune system, in utero or early life, to promote an allergic or tolerant phenotype.

Aims: To investigate whether the epithelium-associated cytokines in utero or early in life are associated with the development of egg allergy later in infancy.

Material & Methods: TSLP, IL-25, IL-17 and IL-33 levels from cord blood serum and plasma samples from infants with IgE-mediated egg allergy at 12 months (positive pasteurized raw egg challenge and egg sensitization) are being measured and compared with control infants (negative pasteurized raw egg challenge and negative egg sensitization). A total of 216 samples at different time points (birth, 6 weeks, 5 months and 12 months of age) are being tested for both groups of infants. An enzyme-linked immunoabsorbent assay (ELISA) and Luminex Technologies are being used to measure TSLP levels, and IL-25, IL-17 and IL-33 cytokines, respectively.

Results: To be submitted at the second round of abstracts.

Conclusions: This study will add to our understanding of other pathways that can initiate Th2 immunity in utero or early in life that will lead to food allergen sensitization and development of food allergy during infancy. With this study we hope to identify new biomarkers as early life predictors for the development of egg allergy.

The effects of short chain fatty acids on lipopolysaccharide or TNFα-induced endothelial activation and dysfunction

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Aim: To investigate the effects of short chain fatty acids (SCFA) on lipopolysaccharide (LPS) or TNF**α**-induced endothelial activation and dysfunction which is involved in several diseases including sepsis and atherosclerosis.

Methods: The effects of SCFA on primary human umbilical vein endothelial cells (HUVEC) were tested. The HUVEC were pre-incubated with acetate (1mM, 10mM and 100mM), propionate (0.3mM, 1mM and 5mM, 10mM) and butyrate (0.1mM, 0.3mM and 1mM, 5mM) for 1 hour, 16 hours or 24 hours and then stimulated with lipopolysaccharide (LPS, 1ug/ml or 10ug/ml) or TNF**α** (100pg/ml or 1ng/ml) for 6 hours, 12 hours or 24 hours. Inflammatory cytokines secreted by cells in the supernatant were measured by ELISA. Besides, the adhesion molecules expressed on HUVEC, ICAM-1 and VCAM-1, were detected by flow cytometry with one more time point, 24h pre-incubation with 8h stimulation. Meanwhile, the effects of phenylacetamide (PA, specific G-protein coupled receptor 43 agonist) and trichostatin A (TSA, class I and II histone deacetylase inhibitor, HDAC) were studied.

Results: Under this experimental set-up, the highest concentrations of SCFA used showed no cytotoxic effects on HUVEC after 48 hours of stimulation. Interleukin-6 (IL-6) and IL-8 secretion were significantly increased by stimulation with LPS or TNF**α**, but were decreased or increased by different concentrations of SCFA treatment with different pre-incubation and stimulation time points. ICAM-1 and VCAM-1 expression were upregulated after LPS or TNF**α** stimulation, but VCAM-1 expression was unaltered after 24 hours stimulation. Acetate had no effects on LPS- or TNF**α**-induced ICAM-1 and VCAM-1 expression. In contrast, high concentrations of butyrate (5mM) and propionate (10mM) decreased VCAM-1 expression after long pre-incubation (16 or 24 hours) and short stimulation times (6 , 8 and 12 hours) but showed no effects on ICAM-1 expression. Some results of inflammatory cytokines and adhesion molecules obtained in PA and TSA treated cells are similar to SCFA, which suggest that the effects of SCFA are mediated by activation of GPCR43 or inhibition of HDAC.

Conclusions: These data demonstrate the effects of SCFA on LPS- and TNF**α**-induced endothelial activation and dysfunction are dependent on the concentrations of SCFA as well as pre-incubation and stimulation time. Thus, we demonstrate that SCFA improve endothelial activation and dysfunction after LPS and TNF**α** stimulation through modulation of the inflammatory process, which may be associated with activation of GPCR43 or inhibition of HDAC.

Airway microbiome-immune interactions in the origins of childhood asthma

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Background - Asthma is believed to originate in early life through aberrant immune development in response to environmental exposures. The human microbiome is thought to be an essential exposure in this regard, but the complex interactions between the early airway ecology and the host airway immune response are still unknown.

Objective - To examine the airway microbiota and its interplay with the topical immune system in early life in relation to asthma in childhood.

Methods - The population-based Copenhagen Prospective Studies on Asthma in Childhood 2010 ($COPSAC_{2010}$) cohort consists of 700 children prospectively monitored for the development of asthma. Airway aspirates from the children taken during the first three months of life were characterized using 16S rRNA gene amplicon sequencing. At one month of age, unstimulated nasal mucosal immune profiles were determined based on 20 immune mediators.

Results - We found that children who developed asthma within the first five years of life had an increased diversity in the airway microbiota at age one month, compared to children with no asthma (Median Shannon index, 1.66 vs 1.56, p = 0.02). Relative abundances of the taxa Veillonella and Prevotella at age one month were associated with development of asthma (Veillonella, Hazard Ratio per z-score (HR_z) 1.40 [1.16-1.67], p=0.0003; Prevotella, HR_z 1.35 [1.15-1.59], p=0.0003), which was confirmed in a multivariate cross-validated sparse Partial Least Squares model (HR_z 1.32 [1.11-1.57], p=0.0017). Presence of these airway bacteria coincided with significantly reduced mucosal secretion of immunological mediators involved in potentiating mucus production and bacterial clearance mechanisms, including IL-10, IL-13 and IL-17.

Conclusion - The airway microbiota composition in healthy neonates may predispose to later development of asthma in childhood through perturbation of the developing airway immune system.

The role of TLR4 in Gut-Brain cross talk in a murine model for Parkinson's disease

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Parkinson's disease (PD) clinical picture is usually dominated by motor impairments. However, non-motor symptoms, such as gastrointestinal dysfunctions, often precede the motor symptoms by many years and their occurrence in otherwise healthy people is associated with an increased risk of developing PD. TLRs are expressed by innate immune cells in the gut and its activation results in proinflammatory and chemotactic cytokines. TLR4 is involved in intestinal permeability regulation. It has been previously shown that abnormal intestinal permeability correlates with **α**-synuclein accumulations in the enteric nervous system (ENS). The gut might be an early site for PD in response to an environmental toxin or pathogen. In our study we aimed to investigate whether intestinal TLR4 dysregulation plays a role in PD pathology. Rotenone exposure in rodents is a frequently used model for studying PD since it is able to reproduce many pathological features found in PD patients. Moreover, rotenone exposure is known to be associated with an increased risk of developing PD in humans. TLR4 deficient and wild type mice received 10mg/kg of rotenone orally once a day for 28 days. Control animals received vehicle. Readout parameters included motor function using the rotarod test, intestinal transit time and histological examination of the brain and the gut to assess PD-like pathology. Oral administration of rotenone induces motor deficits accompanied with a reduction of dopaminergic neurons in the substantia nigra, delayed intestinal transit time and **α**-synuclein accumulation in the ENS in mice. Moreover, we observed inflammation in the gut in rotenone-treated mice characterized by a reduction in colon length and an increase in the number of T-cells. Zonula Occludens 1 (ZO-1) tight junction protein was evaluated in order to assess the intestinal barrier integrity in the gut. Rotenone treatment resulted into completely diminished levels of ZO-1 expression at the epithelial junctions of the colon suggesting dysfunctional barrier integrity. TLR4 deficient mice were partly protected against rotenone-induced motor deficits, delayed intestinal transit time, gut inflammation and ZO-1 expression reduction. TLR4 is partly involved in the motor dysfunction and intestinal phenotype found after rotenone administration. Our results support the hypothesis that gut/brain cross talk possibly via TLR4 plays a central role in **α**-synuclein-induced PD pathology.

Prediction of endotoxin variants in the human gut microbiome and their relation to metabolic disease

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Exposure to lipopolysaccharide (LPS) has been associated with the low-grade systemic inflammation seen in insulin resistance and other metabolic diseases. Low levels of LPS produced by bacteria in the gut microbiome are thought to enter systemic circulation and activate TLR4 to induce inflammatory processes that can lead to type 1 polarization of key tissues such as liver and adipose tissue. However, gram-negative bacteria from different taxonomical families produce lipopolysaccharides which differ in their immunogenicity due to variations in their LPS structure. LPS produced by certain families in the Gammaproteobacteria (hexa-acylated LPS) is highly endotoxic while LPS produced by species e.g. in the Bacteroidetes phylum (penta-acylated LPS) has no TLR4-agonistic activity and can inhibit hexa-acylated LPS-induced TLR4 activation in a dose-dependent manner. In the vast majority of the current literature, only the effects of hexa-acylated LPS have been assessed without taking the presence of penta-acylated LPS into account. In the current work, we have developed a computational pipeline for identification of the enzymes responsible for production of the penta- and hexa-acylated LPS variants. We used this pipeline to identify bacteria producing either LPS variant within shotgun sequenced fecal samples from Danish and Chinese individuals. In non-diabetic and type 2 diabetic Danes and Chinese, we find that penta-acylated LPS species outnumber hexa-acylated LPS species on average by over 1,000-fold. In congruence with previous reports, the total abundance of LPS-producing bacteria was associated with LPS-binding protein and C-reactive protein in plasma, and systemic insulin resistance. Intriguingly, the two latter correlations were however driven by species producing the non-endotoxic penta-acylated LPS and not by the expected inflammation-promoting hexa-acylated LPS-producing species. Bioinformatics pathway analysis revealed that penta-acylated LPS production potential was confounded with production of other metabolites known to influence metabolic disease, such as branched chain amino acids. In conclusion, our results suggest that LPS production by the gut bacteria may be less pro-inflammatory than previously thought. Moreover, the association between LPS and metabolic disease was found to be driven by intestinal penta-acylated LPS-producing species, hence pointing to functions secondary to the inflammatory potential of LPS as contributors to the link between gut microbiota changes and metabolic disease.

Transcriptional rewiring in human dendritic cells by the gut microbial metabolite butyrate is associated with propagation of a tissue-sustaining type 2-like immune response

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Depletion of short-chain fatty acid (SCFA)-producing bacterial species in the gut microbiota is associated with poor health and higher disease prevalence in multiple non-communicable diseases, such as obesity, type 2 diabetes, and asthma. Butyrate is one of the major SCFAs in the human gut and has anti-tumoral and anti-inflammatory effects, but little is known about the molecular mechanism governing the anti-inflammatory effects of butyrate on primary immune cells.

Using human monocyte-derived dendritic cells (moDCs) activated with LPS and IFN-**γ** (strong inducers of a type 1 immune response), we performed an investigation of butyrate's effects on 1) early intracellular signaling using global shotgun phospho-proteomics, 2) transcriptional regulation using RNA-sequencing, and 3) production of cytokines, chemokines and co-stimulatory molecules by activated moDCs using flow cytometry and high-sensitivity immunoassays.

In moDCs, butyrate redirected the development of a pro-inflammatory type 1 immune response in moDCs to a tissue-sustaining type 2-like phenotype. Integrative data analysis showed that butyrate had profound effects on the early transcriptional responses, which were in concordance with the phenotype that subsequently developed in butyrate-treated moDCs. These moDCs were characterized by IL-18 production, inhibition of expression of pro-inflammatory cytokines such as IL-12p70 and IL-23, and upregulation of expression of prostaglandin E_2 synthase and efferocytotic genes, which together contribute to a tissue-sustaining and non-inflammatory type 2-like phenotype. Together with data on butyrate-induced changes in genome-wide chromatin states, the proposed mechanism for butyrate's effective diversion of the LPS/IFN-**γ**-induced type 1 response in moDCs will be presented. In conclusion, the potent anti-inflammatory effects of butyrate to sustain tissue integrity in the gut may contribute to the association of butyrate with homeostatic gut conditions.

Actual infant feeding patterns constitute a risk for asthma, but not their simplifications

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Background: Traditionally, infant feeding is defined as duration of exclusive or total breastfeeding, as age of weaning, or as the age when solid food was introduced. However, these definitions are simplifications of actually more diverse patterns including combinations of direct feeding at the breast (DBF), pumping and feeding of breast milk (BM), formula feeding (FF). The aim was to investigate whether various infant feeding patterns in the first six months of infancy are associated with a different risk of asthma at 6 years of age.

Methods: The Infant Feeding Practices Study II (n=1,387) in the United States provided data on feeding modes in the first six months of infancy and doctor's diagnosed asthma at the 6-years follow-up. Log-linear models were used to estimate relative risks (RRs) of feeding patterns for asthma adjusting for confounders including maternal smoking during pregnancy, race/ethnicity, sex of the child, and maternal and paternal history of asthma.

Results: Compared to 'direct breastfeeding for at least 3 months' (DBF3m), the combined occurrence of direct feeding at the breast (DBF), pumping and feeding breast milk (BM), and formula (FF) in the first months' (DBF/BM/FF) showed a statistically significant higher risk for asthma in the first 6 years of life (RR= 3.55, 95%CI: 1.54, 8.2). In addition, short duration of DBF and also FF in the first 2-3 months increased the risk of asthma (RR= 3.21, 95%CI: 1.25, 8.23, RR= 2,48; 95%CI: 1.04, 5.87; respectively). Among the confounders, paternal and maternal history of asthma and being a boy were associated with asthma. Against that, simplifications such as duration of (exclusive) breastfeeding did not gain statistical significance.

Conclusions: Patterns of mixed infant feeding, short duration of direct breastfeeding, and initial formula feeding carry a higher risk of asthma compared to direct feeding at the breast for at least three months. These actual feeding patterns provide a better explanation of the occurrence of asthma compared to simplification based on duration of one mode of feeding.

Postnatal Development Of The Murine Gut Microbiota

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The enteric microbiota represents a dense and highly dynamic microbial community consisting mainly of bacteria but also viruses, phages and archaea. It exerts a major influence on many aspects of the host's organism including structural and functional aspects of the immune system, tissue maturation and remodeling as well as metabolism. Emerging epidemiological and experimental evidence suggests that alterations of the enteric microbiota are linked to highly prevalent human diseases such as the susceptibility to infection, as well as several non-communicable inflammatory diseases. In the adult intestine, the microbiota displays a dense bacterial community with relatively stable composition. In contrast, neonates are born essentially sterile with the establishment of the microbiota starting immediately after birth. Since the most dramatic changes in the density and composition of the microbiota are observed during the postnatal period and early childhood, this developmental period might critically influence the ultimate composition of the enteric microbiota and the life-long maintenance of host-microbial homeostasis. Therefore, we conducted a systematic analysis of the time kinetic of murine bacterial colonization during the immediate postnatal period (day 1, 3, 7, 14, 21 and 28 after birth). Particular attention was paid to the longitudinal course of the colonization of both the small and large intestine in mice. Our analysis included 16S rDNA V4 sequencing, the use of bacterial group specific PCR primers at various time points and anatomical sites after birth.

We observed a rapid colonization of the neonate intestine, decrease in richness (choa1) early after birth, and increase in richness combined with a major shift in composition during weaning. The post-weaning microbiota was closely related to the maternal adult microbiota. The microbiota composition was found to be highly individual directly after birth, but shifted towards a more homogenous pattern within one week. Small intestine and colon harbored a comparable microbiota composition during the pre-weaning period. Our results are consistent with the existence of selective host mechanisms that shape the initial, largely environment-dependent colonization pattern and ensure the development of a beneficial mature microbiota composition.

The effect of environmental biodiversity on human skin microbial health

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Background:
Diversity of the ancient human microbiota was vast in comparison to humans of today living in urban environments. Many studies have used the biodiversity hypothesis to attempt to explain how chronic inflammatory diseases result from a lack of human microbiota diversity. The links between high density living, environmental biodiversity and microbial health and human immunology have not been established. The skin microbiome, as one expression of this relationship, has only just begun to be explored.
Objective:
This study investigates the relationships between high rise apartments, environmental biodiversity and the biodiversity of the human skin microbiota. Participants have received real, artificial or no plants, and are being tested for stress, lifestyle factors and communities of skin bacteria over a 12 month period.
Discussion:
Many studies used the biodiversity hypothesis to attempt to explain how chronic inflammatory diseases result from a lack of human microbiota diversity. The nature of evidence is varied and further human studies of the natural environment, the commensal microbiota and their interaction with the immune system as an interdisciplinary approach need to be explored.
The relationship between stress, inflammation and chronic diseases is well known, however the relationship of biodiversity to immunology is less clear. The human skin microbiota is linked with innate and adaptive immunity, and recent findings have found that humans give off a microbial cloud.
Conclusion:
This study presents the opportunity to focus on exploring direct links between the environment and skin microbiota. A central pathway between nature and health may be enhanced immune function which has a positive effect on preventing chronic health diseases. Effective remedial strategies towards optimal immune function may be found, and new environmental policies can be encouraged to benefit high-rise apartment residents.

Metabolic profiling of type 1 diabetes mellitus in children by proton NMR-based metabolomics

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Objectives
Type 1 diabetes mellitus (T1DM) is one of the most common pediatric diseases and its incidence is rising in many countries. Recently, it has been shown that metabolites other than glucose play an important role in insulin deficiency and the development of diabetes. The aim of our study was to look for discriminating variation in the concentrations of small-molecule metabolites in the plasma of T1DM children compared with non-diabetic matched controls using proton nuclear magnetic resonance (^{1}H-NMR)-based metabolomics.

Methods
A cross-sectional study was set-up to examine the metabolic profile in fasting plasma samples from 7 T1DM children and 7 non-diabetic controls aged 8 to 18 years, and matched for gender, age and BMI. The obtained plasma ^{1}H-NMR spectra were rationally divided into 110 integration regions, representing the metabolic phenotype. These integration regions reflect the relative metabolite concentrations and were used as statistical variables to construct (train) a classification model in discriminating between T1DM patients and controls.

Results
The total amount of variation explained by the model between the groups is 81.0% [R2Y(cum)] and within the groups is 75.8% [R2X(cum)]. The predictive ability of the model (Q2(cum)) obtained by cross-validation is 50.7%, indicating that the discrimination between the groups on the basis of the metabolic phenotype is valid. Besides the expected higher concentration of glucose, the relative concentrations of lipids (triglycerides, phospholipids and cholinated phospholipids) are clearly lower in the plasma of T1DM patients compared to controls. Also the amino acids serine, tryptophan and cysteine concentrations are slightly decreased.

Conclusions
The present study demonstrates that metabolic profiling of plasma by ^{1}H-NMR spectroscopy allows to clearly discriminate between T1DM patients and controls. The metabolites that significantly differ between both groups might point toward disturbed biochemical pathways including i) choline deficiency, ii) increased gluconeogenesis, and iii) glomerular hyperfiltration. Early preventive and therapeutic strategies aimed at restoring or improving the plasma metabolic profile, e.g. by re-establishing lipid and amino acid availability or by modulating gut microbial composition, might prevent **β**-cell destruction and delay T1DM progression in children and adolescents.

In vitro evidence for immune-modulatory properties of authentic human milk oligosaccharides: direct effects on human monocyte and mouse bone marrow derived dendritic cells

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Background: Breastfed infants have lower risk of infection and inflammatory diseases than formula-fed infants. Human milk oligosaccharides (HMOs), the third most abundant bioactive component in human milk, have been detected in the systemic circulation and can produce short chain fatty acids (SCFAs) after fermentation. Both synthetic oligosaccharides and SCFAs have been shown to directly modulate DCs, leading us to investigate the direct immunomodulatory effects of authentic HMOs. To mimic the real situation in the gut, combination of HMOs with SCFAs were also tested.
Methods: HMOs were isolated from pooled matured human milk and reduced lactose. Immature DCs (iDC) generated from human monocytes or C57BL/6 mouse bone marrow cells were collected after culturing and stimulated with preparations of HMOs, and HMOs+SCFAs in the presence or absence of LPS for 24h. DCs were analyzed for their surface marker expression and cytokines production after different stimulations. The different DC types were tested for the capacity to prime naïve T cells in an allogeneic co-culture system. Cytokines secreted by the co-cultured cells were measured. Ultimately, the suppressing capacity of DC-induced regulatory T cells was tested.
Results: In both human and mouse iDCs, HMOs- and HMOs+Acetate induced elevated levels of CD80, CD86, CD40, PD-L1 and PD-L2 on their membrane, the phenotype was maintained in the presence of LPS stimulation. Modulated DCs are equipped to migrate to inflamed tissues as shown by higher expression of CCR7 and CXCR3. Furthermore, cytokines measurements revealed significantly lower levels of IL-12p70 and IL-6, but higher levels of IL-10. Consistently, HMOs and HMOs+Acetate exposed DCs attenuated LPS primed Th1- and Th17-cells induction, but induced remarkably higher percentage of IL-10 secreting regulatory T cells equipped with suppressive functionality.
Conclusion: Overall, this study shows that HMOs are capable of inducing regulatory DCs, and subsequently modulating the adaptive immune cells; combination of HMOs and Acetate might represent a useful dietary ingredient for the maintenance of intestinal homeostasis. These data support the critical role that HMOs play in the health benefits of human milk for the breastfed infants in early life.

The Impact Of Breastmilk And Solid Food On Gut Microbial Colonization In Humans: The Lucki Birth Cohort

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Compared to adults, the infant microbiome is quite volatile, has greater intra-individual variation, has a lower number of species and a higher proportion of Bifidobacterium spp. Bacterial communities in the young child begin to resemble the adult by 3-years of age, and it is hypothesized that the primary shift away from infant towards adult-like microbiota occurs with weaning and introduction of solid foods. Although exploratory research has linked infant nutrition to alterations in microbiota characteristics on one hand, and on the other to health outcomes like body mass index and allergenic responses in children, none of these studies directly link the timing of cessation of breastfeeding or solid food introduction to the establishment and composition of gut microbiome and subsequent development of obesity or allergy. There is little foundation for guidelines regarding the introduction of solid foods and duration of breastfeeding for term infants. Therefore, we aim to describe the colonization patterns of the human gut microbiome in the first year of life, with specific focus on after the period surrounding the introduction of solid food and weaning from breastmilk within the Dutch LucKi Birth Cohort Study. This study is part of an international collaboration in which data collected from three prospective observational birth cohorts in Canada (Baby & Mi, Baby & Pre-Mi), Germany (PAPS) and The Netherlands (LucKi) will be combined. In The Netherlands, 100 newborns (gestational age >37 weeks) will be included before birth and fecal samples of the infant will be collected at ages 1-2, 4, 8 weeks, 4, 5, 6, 9, 11 and 14 months. In addition, a maternal fecal sample will be collected. The microbial composition of fecal samples will be analyzed by next-generation sequencing and linked to perinatal determinants, diet, medication use and life style factors. Integration of the LucKi study within the routine of the Baby Well Clinics enables detailed follow-up on infant development (e.g. anthropometrics). Additional information on determinants and health outcomes (such as atopic diseases) will be collected by repeated parental questionnaires. A subgroup of 10-15 participants will be asked to collect additional infant fecal samples for a 10 day sampling period around the time when solids are first introduced and again around the time of weaning from breastmilk. Each day during the 10-day sampling periods, the women will complete a diary outlining the diet, medication use, and microbial exposure details. To identify the short-term changes to the microbial community function and host metabolism during weaning and introduction of solid food we will use molecular profiling, a targeted strategy for bacterial culturing, (functional) metagenomics and metabolomics. The primary outcome will be dynamics over time of the microbial community structure, in terms of bacterial membership, abundance and phylogeny, bacterial gene profiles and microbial metabolism.

The oral microbiota during childhood and its relationship to allergy development

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Allergic diseases have become a major public health problem in affluent societies. Microbial colonization occurring early in life seems to be critical for instructing regulation on the maturation of the immune system and allergy development in children. While gut microbial dysbiosis during infancy has earlier been associated with the aberrant development of immune responses, the influence of oral bacteria on allergy development have yet not been revealed. The oral cavity is the first site of encounter between a majority of foreign antigens and the immune system. In this study we aimed to determine the significance of oral microbiota composition during the first 7 years of life in relation to asthma and allergy development. Deep Illumina sequencing of the 16S rDNA gene was used to characterize the bacterial composition and diversity in saliva samples collected at 3, 6, 12, 24 months and 7 years of age from children staying healthy (n=33) and children developing allergic symptoms and sensitization (n=47) up to seven years of age. The preliminary results indicate that the bacterial richness and diversity, at the OTU level, are increasing with time in both healthy children and children developing allergies. Additionally, children staying healthy tended to have higher oral bacterial diversity, particularly seen at 7 years of age (p=0.03, Mann-Whitney U test). Canonical correspondence analysis, here used to detect the relationship between microbial community composition, health status and age, showed that the oral microbiota evolved through time and that the differences in microbial composition between healthy and allergic children were predominantly noticeable at the later ages. However, further analyses (including the influence of confounding factors and bacterial density) are still in progress. Taken together, the impact of oral microbiota composition and diversity in modulating allergic immunity seem to increase with age, in contrast to previous findings on the gut microbiota.

Perinatal determinants of naïve regulatory T cells (Treg) during the first year of life in healthy and food allergic infants

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Background: Alterations in regulatory T cell (Treg) number and function have been associated with a range of early life immune-related outcomes including allergy and autoimmune disease. We recently showed that duration of labour was inversely associated with the proportion of cord blood naïve Treg (nTreg), and this relationship was more pronounced in infants that developed food allergies by one year of age. To investigate this further we assessed the relationship between key perinatal variables and nTreg proportions over the first year and related these data to the development of food allergy.

Methods: Blood samples were collected from infants in a population-derived birth cohort and proportions of nTreg (% of CD4 T-cells) were measured by flow cytometry at birth (n=463), 6 (n=600) and 12 (n=675) months of age. Relationship between perinatal factors and nTreg at each time point were examined by linear regression.

Results: Males had a slightly higher proportion of nTreg at each time point. Birthweight, adjusted for sex and gestational age, was positively associated with an increase in nTreg proportion at birth and 6 months but not at 12 months. Increasing gestational age and exposure to labour were associated with a reduced proportion of nTreg at birth but not at later time points. Among these variables, exposure to labour resulted in a strong association between a lower proportion of nTreg at birth and subsequent food allergy (-0.87% (95% CI, -1.14, -0.61), p<0.001). There was no evidence that nTreg at 6 or 12 months associated with food allergy.

Conclusion: These data indicated that sex and birthweight have long-lasting influence on infant nTreg levels, while gestational age and exposure to labour influenced nTreg levels at birth but not at 6 and 12 months of age. A relationship between low nTreg levels and food allergy was only evident at birth, thereby suggesting in utero priming of nTreg levels as a factor contributing to risk of food allergy at one year of age.

Brominated flame retardants in breast milk are associated with decreased infant gut microbiota diversity at 1 month of age in a Norwegian birth cohort

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Background- Environmental toxicant exposure during gestation and through breastfeeding is associated with adverse health outcomes in childhood and later life. In animal studies, toxicant exposure negatively effects gut microbiome composition possibly contributing to poor health. This is the first investigation of whether breastfeeding exposure to individual or a mixture of environmental toxicants negatively impacts the human infant gut microbiome at one month.

Methods - We used data from the Norwegian Microbiota Cohort (NoMIC, n=552, recruitment 2002-2005). Mothers collected breast milk samples and their own and their infants' fecal samples and completed questionnaires on maternal and infant characteristics. This analysis included 296 mother-child pairs of singleton births with information at one month on both toxicant concentrations in breast milk and gut microbiota composition from fecal samples, and no antibiotic use between 2-4 weeks of age. Our exposures were 28 individual chemicals from five classes of compounds (dioxin-like mono-ortho and non-dioxin-like polychlorinated biphenyls (PCBs), organochlorine pesticides, polybrominated diphenyl ethers (PBDEs), and per- and polyfluoroalkyl substances (PFAS)). We investigated three measures of alpha diversity: Shannon Diversity, Phylogenetic Diversity and number of observed operational taxonomical units (OTUs). To assess the association between toxicant exposure and infant alpha diversity, we used three methods: principal component analysis (PCA) to identify mixtures, elastic net regression modelling to select individual toxicants and generalized linear models to get unbiased estimates. We adjusted for confounding factors: maternal Shannon diversity at 4 days after birth, proportion of breastfeeding, and gestational age.

Results -The principle component dominated by PBDEs was significantly associated with a reduction in all three diversity measures. The elastic net modelling selected PBDE-100 as the toxicant associated with reduced infant gut diversity. In the generalized linear regression models a 1 ng/g increase in PBDE-100 was significantly associated with a -0.13 (-0.20, -0.07) change in Shannon diversity, equivalent to a 13 % decrease in the interquartile range (IQR) of infant gut diversity. We also detected similar changes in phylogenetic diversity and observed OTUs associated with PBDE-100.

Conclusion - In a multipollutant study, we detected a modest decrease in infant alpha gut diversity associated with low breast milk concentrations of PBDEs at 1 month.

Maternal poly(I:C) mouse model to study effects of prenatal maternal infection on the offspring

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The prenatal environment plays an important role in neurocognitive development. Epidemiological studies show that disturbance of this environment by infections increases the risk of neuropsychiatric disorders in the offspring. Activation of the maternal immune system, rather than a direct infection of the fetus, appears to cause these changes. However, the underlying mechanisms are largely unknown and potential protective interventions are not much studied. Here, we assessed the effects of polyriboinosinic-polyribocytidilic acid (poly(I:C)) injected during early gestation on the cognitive and behavioral development of the offspring, as well as on their central immune system.

Pregnant C3H/HeOuJ mice were treated with poly(I:C) or control on GD9. Their offspring were tested using cognitive and behavioral tests (ultrasonic vocalizations, prepulse inhibition and social recognition test) at 1,5,12, and 15 weeks, after which they were sacrificed and microglia density and morphology were assessed.

Our preliminary data demonstrate that poly(I:C) causes a dose-dependent acute proinflammatory response with long-lasting effects in the offspring. Although the model is still being optimized, our current data shows that it is a promising model to study maternal immune activation, as well as potential protective interventions. Ongoing studies aim to investigate whether maternal dietary supplementation, aimed at restoring the inflammatory balance, can protect against the effects of maternal poly(I:C) injection.

Raw cow's milk prevents the development of airway inflammation in a murine house dust mite-induced asthma model

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Background: Numerous epidemiological studies show an inverse relation between raw cow's milk consumption and the development of asthma. This protective effect seems to be abolished by milk processing. Evidence for a causal relationship is however still lacking and also direct comparisons between raw and heat treated milk are hardly studied. In the present study we therefore investigated the preventive capacity of raw milk and heated raw milk on the development of house dust mite (HDM)-induced allergic asthma in mice.

Method: Six- to seven-week-old, male BALB/c mice were intranasally (i.n.) sensitized with 1 µg HDM or PBS on day 0, followed by an i.n. challenge with 10 µg HDM or PBS on days 7 to 11. In addition, mice were orally treated with 0,5 mL raw cow's milk, heated raw cow's milk (10 minutes, 80°C) or PBS three times a week throughout the study. At the end of the study (day 14), airway hyperresponsiveness (AHR) in response to increasing doses of methacholine was measured in order to assess lung function and bronchoalveolar lavage fluid (BALF) was examined to study the extent of airway inflammation. T helper (Th) cell subpopulations were quantified in lung cell suspensions using flow cytometry and chemokine and cytokine concentrations were determined in lung homogenates and supernatants of ex vivo HDM re-stimulated lung cells.

Results: Sensitization and challenge with HDM resulted in AHR and pulmonary eosinophilic inflammation. Raw milk prevented both typical features of allergic asthma, whereas heated raw milk did not. Epithelial- and DC-derived mediators, IL-33, CCL20, CCL17 and CCL22, were significantly increased in the lungs of HDM-mice. Both milk types reduced the concentration of CCL17. Pulmonary concentrations of Th2 cytokines, IL-5 and IL-13 were also increased in HDM-mice, but only raw milk prevented this increase. Upon re-stimulation of lung cells with HDM, both raw and heated raw milk were able to significantly reduce the production of IL-4 and IL-13. The percentage of Th2 cells in lung cell suspensions was also significantly reduced by both milk types.

Conclusion: Raw cow's milk prevents the development of asthma in a murine HDM-induced allergic asthma model. Heat treated raw milk did not show this protective effect. Besides an abundant amount of epidemiological evidence, this study now also suggests a causal relationship between raw cow's milk consumption and the prevention of allergic asthma.

Effect of microbiota on immunoregulation in reproductive tissues

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Pregnancy requires a well balanced local immune system. Immune cells need to tolerate the semi-allogeneic fetus and still maintain robust immune reactivity against pathogens. Recently, it has become apparent that the uterine environment contains a microbiome which could play a critical role in this required local immune homeostasis. Disturbance of this homeostasis could potentially lead to reproductive complications. Our aim is to investigate whether different microbiota (commensal or non-commensal) can immunologically influence the uterine microenvironment. To this end endometrial lymphocytes isolated from menstrual blood (MMC) and peripheral blood mononuclear cells (PBMC) were stimulated with different micro-organisms (Escherichia coli, Candida albicans and Fusobacterium nucleatum). Cytokine production was measured by Luminex. Results show that MMC produce less pro-inflammatory cytokines (IL-6 and IL-1**β**) compared to PBMC and more IL-10 in response to E.coli and F.nucleatum. While both PBMC and MMC produce pro-inflammatory cytokines (TNF-**α**, IFN-g, IL-17F, IL-22 and IL-13) in response to C.albicans stimulation. This suggests that F.nucleatum and E.coli (present in the placental microbiome) induce primarily a tolerogenic cytokine profile in MMC, while C.albicans induces a more pro-inflammatory response. This experimental setup now offers unique opportunities to study the influence of other micro-organisms on the local uterine immune response.

Use of Infant African Green Monkeys To Model Early Life Influences on Chronic Disease

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Studying early life exposures and chronic disease risk in the clinical setting is difficult due to variability in exposures, inability to collect invasive samples and successfully track long term consequences. Old world nonhuman primates provide a relevant animal model which share genetic, reproductive, immunological, and disease phenotypes with people. The Vervet Research Colony (VRC) at Wake Forest University is NIH-supported national resource for investigators and has unique attributes that lend itself to the study of early life exposures. The VRC is a multi-generational, pedigreed and genomically sequenced colony of Caribbean-origin vervets/AGMs (Chlorocebus aethiops sabaeus), originally founded in 1975. The current population contains approximately 310 US-born animals (0-28 years old), descended from 57 founders. The majority of the population is housed in matrilineal social groups, with separate cohorts available for use in studies requiring intervention or other experimental and environmental controls. In addition to a reference animal, the genomes of every adult animal in the colony have been sequenced and are available in public databases along with the individual pedigree to assess heritability. Additionally, blood and tissues from every animal are collected three times per year along with an annual physical and biochemical health examination. All animals are subjected to daily visual health checks. The VRC is one of only two monkey nursery facilities in the United States which facilitates interventional studies of neonates such as vaccine exposures, formula feeding, and intensive observation and sampling. Pregnancies are monitored and amniocentesis and other maternal sampling enables in utero health tracking and manipulations. Infant collections (examples include microbiomes, blood, biopsies of lymph nodes, fat, muscle, bronchoalveolar lavage, body composition estimates) are able collectable in the neonate, juvenile, and adult stages to achieve a life-course approach to disease risk.

We have previously documented fecal microbiome shifts with consumption of typical Western diets. The microbiome is now known to underpin many pathologies, thus beginning in 2017 a large proportion of the VRC will switch to a Western diet for 12 months to capture an entire birth season (LabDiet 5L3K). The quantitation of dietary influences on neonatal outcomes, and the trajectory of their subsequent development, will be comparable between Western diet exposed and unexposed pregnancies, neonates, mothers, and juveniles. We encourage collaborations and aim to support investigators interested in early life development. We have on-site research facilitation and resources which include pathology, imaging, surgical and technical support. All requests and questions should be directed to primates@wakehealth.edu.