SPEAKER ABSTRACTS

Session I: Metabolic Function

Moderator: **Samuel Klein, MD**, William H. Danforth Professor of Medicine and Nutritional Science; Director, Center for Human Nutrition; Director, Center for Applied Research Sciences; Chief, Division of Geriatrics and Nutritional Science; Medical Director, Weight Management Program, Washington University School of Medicine, St. Louis, MO

PREGNANCY: "Factors that Influence the Perinatal Microbiome"

Kjersti Aagaard, MD, PhD
Associate Professor, Department of Obstetrics & Gynecology, Baylor College of Medicine, Houston, TX

Hominids and *hominins* serve as remarkable hosts to microbes, and we have co-evolved over the past 4.5 million years as highly plethoric communities. Precisely when and how these microbes take up residence during development and over the span of an individual's lifetime remains unclear. Moreover, the role of the microbiome in parturition is relatively unexplored.

Human microbial communities are characterized by their metagenomic and metabolic diversity, which varies by distinct body sites and influences human physiology. We are only beginning to characterize the complex set of interactions which alters both community membership and function in early development. With respect to the potential source of microbiota at birth, it has generally assumed that the majority of seeding microbes originate in the lower genital tract, with microbiota ascending into the otherwise sterile intrauterine. However, we and others have recently demonstrated that (1) the vaginal and gut microbiome communities are distinctly structured in pregnancy, and (2) the placenta is in fact not sterile, but rather harbors a low-abundance microbiome, and (3) the maternal diet, and notably a high fat diet, has a particularly strong impact on the developing and early in life microbial community structure. Through ongoing longitudinal metagenomic studies characterizing the human and primate antenatal and perinatal microbiome, we are piecing together new understandings as to **when, how and where** it varies in the course of human gestation.

We have taken two approaches to answering these questions in our studies. First, we use longitudinal cohorts of maternal-infant dyads collected across gestation and through 6 weeks post-delivery. Second, we utilize our well established primate models of maternal high fat dietary exposure, both in the absence and presence of maternal obesity. Collectively, these recent experimental vignettes are examples of where understanding phenotype-genotype associations render insight to evolution and human reproductive traits.

With mindful design of clinical translational studies accompanied by robust molecular and clinical data, we and others will continue to assemble the capacity to significantly discern causal inference of the role of the maternal and placental microbiome in the timing of parturition and establishment of the early microbiome. Key remaining questions include understanding what the true role of Cesarean delivery is in shaping the infant microbial community, or alternately understanding what maternal or pregnancy factors resulting in Cesarean delivery are actually the drivers of microbial variation. Finally, future efforts need to focus on ongoing studies relating perinatal exposures to both metabolic and behavioral health in the offspring.

INFANT: "Manipulating the Infant Microbiome to Improve Metabolic Health"

Joseph Neu, MD
Professor of Pediatrics, Division of Neonatology, University of Florida, Gainesville, FL

Interaction between microbes, metabolites, developing immune system and host that can have life long consequences. Multi-omic technologies and techniques of evaluating these results in patient care settings will be critical in future studies of Developmental Origins of Health and Disease (DOHaD). One major roadblock to advancing this field is obtaining adequate samples for evaluation in pregnant women, fetuses and infants. Preterm infants remain in the hospital for prolonged periods and provide windows to the fetal microbiome (using gastric aspirate and meconium samples) and effects of various environmental factors postnatally. Such factors include antibiotics, diet (donor versus mother's own milk versus formula), total parenteral nutrition, mother baby interaction including early stress, skin to skin care, and the use of various medications that may alter the intestinal microbiota. Some recent data from human pregnant mothers and preterm as well as term infants will be presented that discuss these environmental factors on the "multi-ome". Studies in animals will also be discussed that suggest early microbial influences on later outcomes. This lecture will summarize some of these interactions, then we will attempt to extrapolate these to perturbations of intestinal microbiota in early life that may lead to obesity, diseases associate with loss of intestinal mucosal integrity (such as Type 1 diabetes) with a highly permeable barrier and neurobehavioural difficulties. Understanding these early interactions can lead to efforts for more rational use of medications such as antibiotics, interactions between infant and mother, diets and stress during pregancy. This may also lead to therapeutic interventions that mitigate these effects.

ADULTHOOD: "Influence of the Microbiome on Body Weight Regulation"

Rob Knight, PhD
Professor Departments of Pediatrics and Computer Science & Engineering, University of California, San Diego, La Jolla, CA

We change our microbiomes every day through the foods we eat, the environments we experience, even the people we live and work with. The implications of these changes in the microbiome for our health are just beginning to be understood. Through the American Gut Project, the largest crowdsourced and crowdfunded citizen-science project yet conducted, we now know about the microbiomes of many types of people, from the healthiest (student-athletes, centenarians) to the sickest (cancer patients, ICU patients, those with C. diff). Amazingly, diet has an especially profound effect on our microbiomes, often outweighing the effects of disease or medications. This raises the prospect of a system for real-time analysis of our microbiomes that helps guide our daily decisions in a way that optimizes our microbiomes for life-long wellness.

ADULTHOOD: "Dissecting the Role of the Gut Microbiome in Obesity and Type 2 Diabetes"

C. Ronald Kahn, MD
Mary K. Iacocca Professor of Medicine, Harvard Medical School; Chief Academic Officer, Joslin Diabetes Center, Boston, MA

Diet, genetics and the gut microbiome are crucial factors in determining individual metabolic status. This occurs, in part, through the production of various metabolites by gut microbiota. We have previously shown that different strains of mice and mice from different vendors exhibit different rates of obesity following high fat diet (HFD) challenge. C57BL/6J (B6J) mice from Jax Labs and 129 mice from Taconic (129T) are obesity prone, while 129 mice from Jax (129J) are obesity resistant. This difference between strains of 129 mice correlates with differences in the gut microbiome. More recently, we have treated these 3 strains with either vancomycin, which kills gram positive bacteria, or metronidazole, which kills anaerobic bacteria, and challenged them with HFD to further explore the role of the microbiome in obesity. 16S rRNA sequencing analysis showed both antibiotics changed microbiome composition in all strains, but with differing end results. In B6J mice, which tend to become obese and insulin resistant, antibiotics improved glucose metabolism, decreased serum TNFa levels, decreased inflammatory markers in liver, adipose tissue and colon and improved insulin signaling. This improvement of glucose metabolism could be reproduced by transferring gut bacteria isolated from metronidazole-treated donors to HFD-fed B6J mice and was associated with improved insulin signaling at a molecule level. On the other hand, in 129J mice, which tend to be lean and insulin sensitive, antibiotics increased inflammatory markers and impaired insulin sensitivity. These metabolic changes were also associated with behavioral changes. Metabolomic analysis showed HFD and antibiotic treatments changed multiple serum metabolites including prominent change in bile acids. Indeed, bile acid receptor, TGR5 level was decreased in liver of HFD-fed mice and restored by metronidazole treatment. Treatment with TGR5 agonist lowered inflammatory gene expression of peritoneal macrophages in response to LPS. Thus, antibiotic treatment modifies the gut microbiome, and impacts on various metabolites and inflammation induced by HFD leading to improved insulin signaling and glucose metabolism. These effects are strain dependent, indicating an important interaction of the gut microbiome with host genetics in determining metabolic state.

ADULTHOOD: "Gut Microbiota and Chronic Inflammatory Diseases"

Andrew Gewirtz, PhD
University Center Professor, Center for Inflammation, Immunity & Infection, Institute of Biomedical Sciences, Georgia State University, Atlanta, GA

The intestinal tract is inhabited by a large diverse community of bacteria collectively referred to as the gut microbiota. Alterations in gut microbiota composition are associated with a variety of disease states including obesity, diabetes, and inflammatory bowel disease (IBD). Transplant of microbiota from diseased persons (or mice) to germfree mice transfers some aspects of disease phenotype, indicating that altered microbiota plays a role in disease manifestation. There are myriad potential mechanisms by which alterations in gut microbiota might promote disease including increasing energy harvest, production of toxic metabolites, and molecular mimicry of host proteins. However, our research indicates that an overarching mechanism by which an aberrant microbiota negatively impacts health is by driving chronic inflammation. More specifically, we hypothesize that the histopathologically-evident gut inflammation that defines IBD is a severe but relatively rare outcome of an altered host-microbiota relationship while a much more common consequence of such disturbances is "low-grade" inflammation, characterized by elevated proinflammatory gene expression, that associates with, and may promote, metabolic syndrome. In this context, a variety of chronic inflammatory diseases may stem from inability of the mucosal immune system to properly manage a stable healthy relationship with the gut microbiota. While one's ability to manage their gut microbiota is dictated in part by genetics, it can be markedly influenced by the composition of the microbiota one inherits from their early environment. Moreover, the host-microbiota relationship can be perturbed by dietary components, such as emulsifiers that may prove to play a role in promoting chronic inflammatory disease states.

ADULTHOOD: "Host Microbiome Interactions in Health and Disease"

Eran Elinav, MD, PhD
Senior Scientist, Immunology Department, Incumbent of the Rina Gudinski Development Chair, Weizmann Institute of Science, Rehovot, Israel

The mammalian intestine contains trillions of microbes, a community that is dominated by members of the domain Bacteria but also includes members of Archaea, Eukarya, and viruses. The vast repertoire of this microbiome functions in ways that benefit the host. The mucosal immune system co-evolves with the microbiota beginning at birth, acquiring the capacity to tolerate components of the community while maintaining the capacity to respond to invading pathogens. The gut microbiota is shaped and regulated by multiple factors including our genomic composition, the local intestinal niche and multiple environmental factors including our nutritional repertoire and bio-geographical location. Moreover, it has been recently highlighted that dysregulation of these genetic or environmental factors leads to aberrant host-microbiome interactions, ultimately predisposing to pathologies ranging from chronic inflammation, obesity, the metabolic syndrome and even cancer. We have identified various possible mechanisms participating in the reciprocal regulation between the host and the intestinal microbial ecosystem, and demonstrate that disruption of these factors, in mice and humans, lead to dysbiosis and susceptibility to common multi-factorial disease. Understanding the molecular basis of host-microbiome interactions may lead to development of new microbiome-targeting treatments.

ADULTHOOD: "Effect of Diet on the Microbiome and Cardiometabolic Health"

Karine Clément, MD, PhD
Professor of Nutrition, ICAN: Institute of Cardiometabolism and Nutrition/INSERM/Universté Pierre et Marie Curie, Paris, France

The human body—composed of about 10 trillion cells—is host to 100 trillion bacteria, the majority of which constitute an extremely rich and diverse gut microbiota. Environmental factors such as diet and physical activity have a clear demonstrated effect on cardiometabolic health. In this context, gut microbiota can thus be considered a key organ playing a major role linking dietary changes and cardiometabolic disorders. These effects could be driven by a variety of mechanisms, such as the gut microbiota's ability to metabolize nutrients, which may influence bioavailability and intestinal absorption. Gut microbiota may also synthesize or derive metabolites, or may change in composition and/or function as a result of various environmental stimuli. Thus understanding the relative impact of gut microbiota and environmental factors on health is of particular importance in the management of obesity-associated cardiometabolic diseases. Recently, we contributed to work demonstrating gut microbiota imbalance (or dysbiosis) in patients with cardiometabolic risk factors or disorders. As such obese/overweight people with a loss of bacterial diversity and/or specific decrease in some bacterial groups (such as *Akkermansia muciniphila*) had more cardiometabolic risk factors such as dyslipidemia (including changes in lipidomic species) or low-grade inflammation. Recent unpublished data from our team also showed that gut microbiota richness worsens in the more severe forms of obesity and associates with an aggravation of comorbidities. Both dietary interventions and bariatric surgery are procedures known to ameliorate cardiometabolic health. We observed that these interventions can actually correct some abnormalities (richness and composition) but in different manners depending on intervention type and duration. Some bacterial species clusters are associated with improved cardiometabolic traits. Such integrative approaches also shed light on the relative contribution of microbiota and environmental factors to cardiometabolic health, paving the way for future personalized care.

Open question: Several current approaches in humans, including dietary intervention, have shown that changes in gut microbiota richness and composition are associated with cardiometabolic traits. However the question remains whether there are gut-derived modifiable factors that may be used in personalized care to better stratify cardiometabolic disorders and improve response to weight loss interventions.

ADULTHOOD: "Manipulating the Adult Gut Microbiome for Metabolic Health"

Patrice D. Cani, PhD
Professor, Research Associate FRS-FNRS; Louvain Drug Research Institute (LDRI), Metabolism and Nutrition, Walloon Excellence in Life sciences and BIOtechnology (WELBIO), Universite catholique de Louvain (UCL), Brussels, Belgium

Obesity is associated with cardiometabolic disorders and inflammation. Over the last 15 years, our work has been devoted to identify the mechanisms by which the bacteria present in the gut interact with nutrients and host biology to control energy, glucose and lipid homeostasis in the context of metabolic disorders associated with obesity. By using prebiotics, we found that changing the microbiota in rodents and in humans induces specific beneficial effects on metabolism (e.g., food intake, fat mass, hepatic steatosis, gut peptides).

In 2007, we have described the concept of metabolic endotoxemia (increase in plasma LPS levels) as one of the putative factor involved in the onset of low-grade inflammation and insulin resistance (Cani et al Diabetes 2007). Following this discovery, we have proposed that the development of metabolic endotoxemia during diabetes is mainly due to a complex alteration of the gut barrier function.

By using metagenomic approaches, we have identified novel bacterial candidates. Among them, we discovered the key role of prebiotics on the abundance of *Akkermansia muciniphila* (Everard et al Diabetes 2011) and more importantly we described the beneficial effects of *A. muciniphila* (Everard et al. PNAS 2013). Novel mechanisms explaining the beneficial effects of *A. muciniphila* and related products will be discussed.

In conclusion, evidence suggests that the identification of specific microbes such as *A. muciniphila*, but also the use of specific nutrients such as prebiotics might be helpful to discover novel mechanisms involved in the crosstalk between gut microbiota and host.

The future promise of the topic is directly related to my motto: "In Gut we Trust"©

Pharmacognosy was and is still the science using plants to isolate active compounds, but today I would like to introduce the "microbiotagnosy" has the future medicine, that is a strategy based on the use of microbes and/or products isolated from microorganisms to treat diseases.

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OLDER ADULT: "The Microbiota of Older Adults: Clinical Perspectives on Health and Disease"

Vincent J. Quagliarello, MD
Professor of Medicine, Clinical Chief, Infectious Disease, Yale University School of Medicine, New Haven, CT

Studies of the human microbiota, particularly of the gastrointestinal tract, has revolutionized thought about health, disease, and what it means to be human. The Human Microbiome Project spawned multiple investigations that demonstrated the diversity, complexity, and stability of the microbiota and its associated metabolic pathways. The older adult (i.e., > age 65 years) represents a unique human host due to specific age-associated disease, increased exposure to group living, multiple medication use and alteration in swallowing that can affect nutrition. These predisposing and precipitating factors can all affect the microbiome and the development of a variety of chronic clinical issues (e.g., frailty) and acute illness (e.g., *Clostridium difficile* colitis). Prospective cohort studies including the ELDERMET consortium and the NU-AGE project have provided important information linking diet, the microbiota, systemic inflammation, and functional impairments that can lead to frailty. Changes in gut microbiota (e.g., with antibiotic exposure) have been linked to changes in intestinal colonization resistance, and the risk for antibiotic-resistant infection (e.g., vancomycin-resistant *Enterococcus*) as well as *C. difficile* colitis – an important cause of morbidity and mortality among older adults. Fecal microbiota transplantation, as a form of probiotic adoptive bacteriotherapy, has biological plausibility and clinical trial evidence for effectiveness in the therapy of recurrent *C. difficile* infection. More targeted probiotic approaches, based on animal models, are a realistic future for *C. difficile* infection, and they serve as a proof of principle that similar probiotic approaches may prevent the major chronic public health problems affecting our aging population.

SPEAKER ABSTRACTS

SESSION 2: BRAIN FUNCTION

Moderator: **Emeran A. Mayer, MD**, Professor, Department of Medicine, Physiology and Psychiatry, David Geffen School of Medicine, Executive Director, Oppenheimer Family Center for Neurobiology of Stress, Co-Director, CURE: Digestive Diseases Research Center University of California, Los Angeles, Los Angeles, CA

PREGNANCY: "Transmission of maternal stress signals in neurodevelopment via the microbiome"

Tracy Bale, MD
Professor of Neuroscience, School of Veterinary Medicine, Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Prenatal stress is associated with an increased risk for neurodevelopmental disorders, such as schizophrenia and autism spectrum disorders. In our established mouse model of early prenatal stress (EPS), long-term programming effects on offspring development have been demonstrated, including reprogramming of the hypothalamic-pituitary-adrenal axis, stress responsivity, cognition, and post-pubertal growth in male, but not female, offspring. Mounting evidence points to a likely influence of maternal stress experience on reprogramming of the gut-brain axis, especially that of the hypothalamus. Innoculation of the intestinal microbiota occurs at birth during passage through the birth canal. As such, maternal disruption to this vaginal microbiome is poised to influence this transmission, and is associated with lasting effects on offspring immunity, metabolism, neurodevelopment, and behavior. Using our mouse model of EPS, we find that maternal stress experience shifts offspring gut microbiome composition towards a higher representation of pro-inflammatory taxa, a hallmark of gastrointestinal dysfunction in children with many neurodevelopmental disorders. Using c-section delivery, we have demonstrated a causal role of the maternal vaginal microbiota in promoting offspring stress dysregulation by a partial recapitulation of the EPS phenotype. As the mucosal immune system shapes postnatal host-microbe interactions, we tested the hypothesis that EPS disrupts the transcriptional and immune profiles of the fetal gut. Early prenatal stress exposure reprogrammed fetal intestinal transcriptional profiles in a sex-specific manner, including disruption of genes encoding innate immunity pathways in EPS male offspring. Specifically, EPS exposure altered sex-specific frequency of myeloid cells in the fetal gut and brain. Taken together, these studies support a link between maternal stress experience during pregnancy and sex-specific programming of the immune system in shaping the developing gut-brain axis, and subsequent disease risk.

INFANT: "Does Obstetric Mode of Delivery Alter Brain Function?"

Ted Dinan, PhD
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While the physical health of individuals born by caesarean section (c section) has been the subject of considerable recent scrutiny very little attention has been paid to the mental health consequences of such delivery. Given the global increase in c section rates this may be of major significance.

We have conducted three studies which address the issue. We initially conducted a rodent study in which animals were delivered by c section or *per vaginum*. We examined a variety of behavioural, endocrine and immune parameters. Overall, the animals born by c section displayed significantly increased anxiety levels. We then recruited healthy male volunteers half of whom were born by c section and half *per vaginum*. On a variety of rating measures the former group had far higher anxiety scores. They underwent the Trier Social Stress Test and in this paradigm showed exaggerated release of cortisol. The stress responses in the human subjects were similar to those obtained in the rodent.

Does the increased stress responsivity observed following c section delivery translate into higher levels of depression and anxiety? To determine this we used the Swedish National Registry data to identify all singleton, live births which occurred from January 1st 1982 and December 31st 2001. We found a slight increased risk of depression and anxiety in those who had been delivered by c section.

Our data support the view that c section delivery impacts on mental health outcome.

ADULTHOOD: "Indigenous Bacterial Species from the Gut Microbiota Regulate Host Serotonin Biosynthesis "

Elaine Hsiao, PhD
Assistant Professor, Department of Integrative Biology and Physiology, Department of Medicine, University of California, Los Angeles, Los Angeles, CA

There is growing evidence that the microbiota fundamentally regulates the development and function of the nervous system, but the mechanisms underlying indigenous microbe-nervous system interactions are largely unknown. We explore fundamental interactions between the indigenous microbiota and mammalian host that regulate the bioavailability of neuroactive molecules, including neurotransmitters and neuropeptides. In particular, we reveal that a striking ~60% of peripheral serotonin (5-hydroxytryptamine, 5-HT) is regulated by the microbiota. We have identified a limited microbial consortium that sufficiently and reversibly modulates host serotonin biosynthesis in specific cell subtypes of the gastrointestinal tract, and that corrects enteric and hemostatic abnormalities related to serotonin deficiency in germ-free and genetically-altered mice. We further identify particular microbial metabolites that confer this serotonergic effect of gut microbes, representing the first account of the molecular mechanisms by which a limited bacterial consortium from the mouse or healthy human microbiota modulates host serotonin levels and serotonin-related disease phenotypes in mice.

ADULTHOOD: "Gut Microbiota Brain Interactions in IBS"

Kirsten Tillisch, MD
Associate Professor, University of California, Los Angeles, Los Angeles, CA

Increasing clinical and preclinical evidence suggests that the bidirectional brain-gut axis can be extended to include the gut microbiota. Via this axis the gut microbiota may influence brain structure and function with a subsequent influence on behavior. This is of particular interest in irritable bowel syndrome, which is considered to be a disorder of brain-gut communication and which has recently been associated with alterations in gut microbiota profiles. During this presentation we will review the evidence for brain-gut-microbiota interactions in health and disease, the existing knowledge on microbiota changes in irritable bowel syndrome, and the early evidence for brain-gut-microbiota associations in IBS.

There is great promise for the future use of microbiota manipulation and probiotics in IBS. We know that patients who have gastrointestinal symptoms can improve with probiotics. As we develop a greater understanding of which probiotic species will help individual patients, based on their specific symptoms or fecal microbiota profiles, we expect the treatment outcomes to improve. Further, it appears that gut microbes have the potential to influence both ends of the brain-gut axis, therefore the co-morbid psychological symptoms often observed in IBS may also be helped in the future by modulation of the gastrointestinal microbiota.

OLDER ADULT: "Gut Microbiota Related to Parkinson's Disease"

Filip Scheperjans, MD, PhD
Neurologist, Helsinki University Hospital, Helsinki, Finland

In Parkinson's Disease (PD), the neuropathology extends beyond the brain involving essentially the whole autonomic nervous system and olfactory structures. Accordingly, PD patients suffer from a broad range of non-motor symptoms, in particular gastrointestinal dysfunction and hyposmia, frequently years before emergence of motor symptoms. PD patients show alpha-synuclein deposits and neurodegeneration in the enteric nervous system as well as breakdown of the mucosal barrier, bacterial invasion, and mucosal inflammation in the colon. Pathological forms of alpha-synuclein may show prion-like spreading and neuron-to-neuron transmission and can be transported from the gut to the brain via axonal transport through the vagal nerve. It has been proposed that local inflammation in the gut mucosa and / or olfactory structures in PD could be a precipitating event leading to alpha-synuclein toxicity. Gut bacteria do not only affect gut physiology, but there is also an intense bidirectional interaction with the brain influencing neuronal activity, behavior, as well as levels of neurotransmitter receptors, neurotrophic factors, and inflammation. According to one hypothesis, gut microbiota could be implicated in the initiation of inflammation and protein misfolding in PD. Recently, gut microbiome alterations in PD subjects and a connection between gut microbiota and akinetic-rigid symptoms have been described. This talk will give an overview of recent findings regarding the gut-microbiota-brain axis in PD and how this may reshape our understanding of disease etiology and pathogenesis and could lead to new therapeutic approaches.