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MICROBIAL METABOLISM AND MAMMALIAN PHYSIOLOGY: SUMMARY OF THE 29TH OLD HERBORN UNIVERSITY SEMINAR

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OVERVIEW AND KEY CONCEPTS

In this 29th edition of the Old Herborn University Seminar, microbial metabolism and mammalian physiology were discussed as one integrated biological system. The lectures, discussions, and contributions to this monograph emphasize the web of interactions between diet, the microbiome (placental, vaginal, oral, and intestinal), microbial metabolites, and mammalian cells (epithelial and immune). Central concepts included various holistic perspectives on microbial and mammalian (rodent, primate, and human) biology. Prenatal and postnatal nutrition may have a lasting impact on mammalian growth and development. In addition to the ingestion of active dietary components, microbes may generate bioactive metabolites by luminal conversion of dietary substrates or de novo biosynthesis in bacterial cells. These metabolites may impact mammalian physiology via the microbiome or directly on mammalian cell signalling pathways. Host-microbe metabolic axes must be considered in terms of metabolite flow across organ systems and across time in man's microbial ages (Figure 1). In this Seminar, leading scientists considered the contributions of microbial cells (species) and their metabolites on mammalian cell function, signalling pathways, and bidirectional communication networks. In this way, we are considering the microbial-mammalian interface of human biology, and how microbes can impact human health and susceptibility to disease.

MICROBES AND HUMAN DEVELOPMENT

New concepts to consider include the prenatal impact of the microbiome during foetal development and compositional relationships between microbiomes at different body sites. Microbial communities may impact human biology even during foetal development. During pregnancy the placenta grows and develops with its own microbiome forming a distinct collection of human-associated bacterial taxa. The placenta has long been considered sterile during gestation, and, similar to other previously "sterile" body sites, microbial communities have been characterized in the placenta (*Aagaard* et al., 2014).

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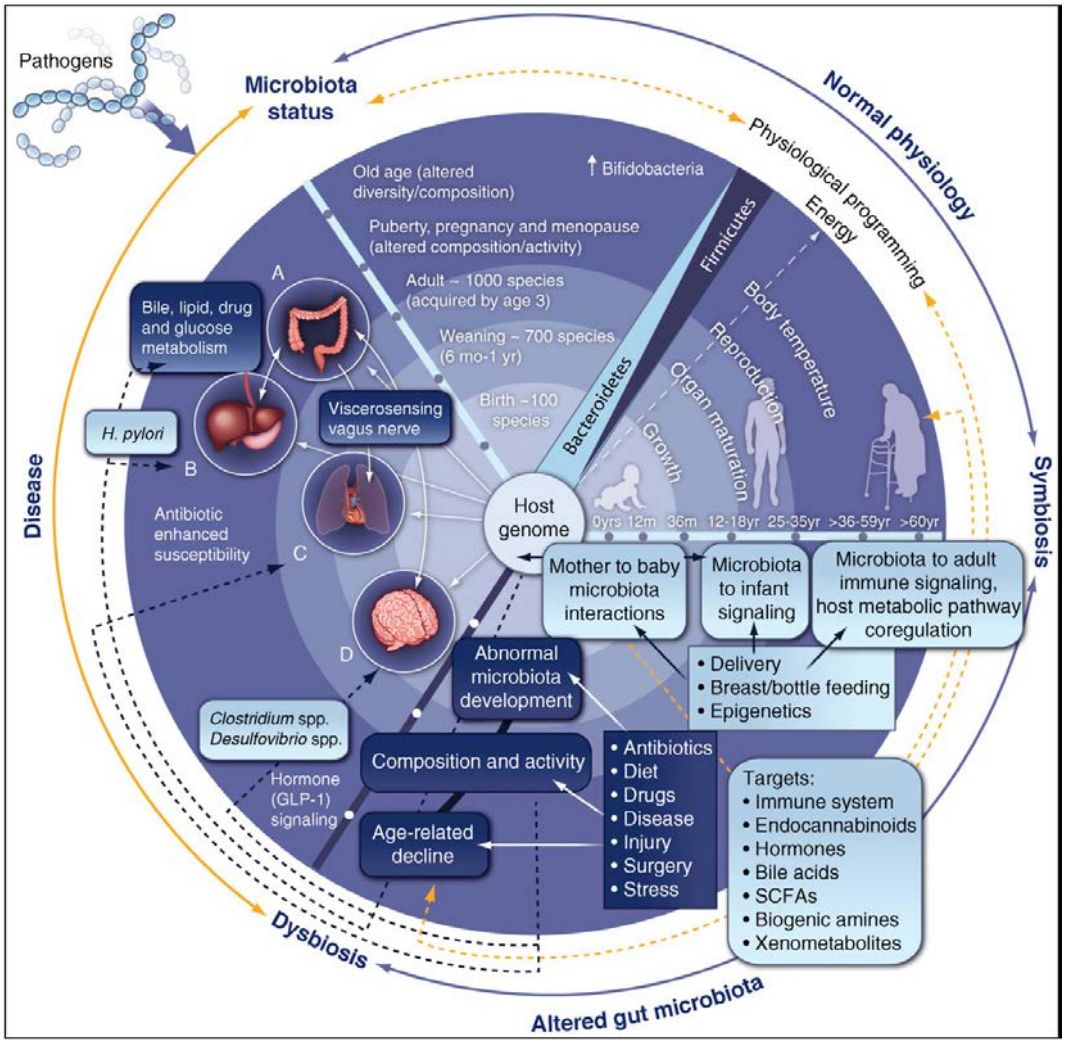

Figure 1: The human microbiome, microbial metabolism and mammalian physiology.
The gut microbiota affects human health from birth to elderly life stages and is important for maintaining human physiology and energy production. The composition of the gut microbiome in early life has an effect on the risk of developing disease in later life. (Reprinted with permission from *Nicholson* et al., 2012.)

The presence of pathogenic bacteria in placental cultures is considered diagnostic for intrauterine infection and a significant risk factor for preterm birth (*Hillier* et al., 1988). Findings in diagnostic microbiology must now be placed in the context of the placental microbiome and a broader array of commensal microbes in pregnancy. Term and preterm infants have discordant microbiomes, and future diagnostic tests may detect differences in placental microbial biomarkers that may be useful clinically. Taxa in the placental microbiome that may discriminate spontaneous preterm birth include members of the phyla Tenericutes and Fusobacteria. Could placental microbes or deficiencies in microbial features contribute to preterm birth along with maternal and environmental factors? In a practical sense, perhaps

microbial metabolites generated by placental bacterial taxa may be used to monitor women at risk of preterm birth.

Tantalizing evidence suggests that one microbiome may influence the composition and function of a distinct microbiome at a different body site. In other words, does the microbial community at a superficial body site potentially influence the microbiome established in a separate body compartment? In this case, the placental microbiome most closely resembles the oral microbiomes of the supragingival plaque and the dorsum of the tongue by bacterial composition (*Aagaard* et al., 2014). Seeding of the neonatal microbiome with bacteria from the placenta, which arises through hematogenous spread, may facilitate prenatal colonization of the foetus and *in utero* exposure to microbial metabolites. The oral-to-placental microbiome connection may help drive physiological and immunologic development of the foetus and help dictate susceptibility to preterm birth or neonatal disease (*King* et al., 2007; *Zeldovich* and *Bakardjiev*, 2012). We are beginning to make connections between microbial communities at different body sites and how microbial and human cells are linked together as one biological system.

This Seminar highlighted the importance of diet before and after birth with respect to microbial composition and function in the developing infant and child. Maternal diet during pregnancy may have a lasting impact on the composition of the gut microbiome postnatally. In foetal and postnatal life, offspring exposed to a maternal high fat diet yielded increased expression of inflammatory cytokines, Toll-like receptors, and their respective signalling pathways (*Yan* et al., 2011, *Xue* et al., 2014). Results of a study by *Aagaard* et al. (2014) on maternal diet modification of the primate foetal epigenome suggest that obesity caused by a high-caloric, high-fat maternal diet yielded changes in the juvenile intestinal microbiome of non-human primates. The quality of the diet during pregnancy may yield persistent effects on the gut microbiome during childhood several years after the initial dietary exposure. This phenomenon of diet's persistent effects on mammalian metabolism via changes in the human microbiome demands further attention.

After birth, neonatal nutrition may have a lasting impact on microbiome composition, function, and disease susceptibility. The intestinal microbiome of infants born vaginally and exclusively breastfed diminished the relative amounts of members of the phylum Firmicutes, while increasing the relative abundance of *Bacteroides* species (*Jost* et al., 2012). The maternal microbiome at birth and postpartum may be critical in the establishment and development of the neonatal microbiome via horizontal transmission. Human milk oligosaccharides (HMO) are the third most abundant component in human milk, and breastfeeding may serve as an important mechanism for dictating how the gut microbiome is "seeded" early in life. More than 150 different HMOs have been identified, and were discussed by Lars Bode in this Seminar (*Bode*, 2012). Only humans have such a high quantity and structural diversity of HMOs with inter- and intrapersonal differences in composition. These differences are determined partly by human genetics or maternal diet during lactation. HMOs are maternal compounds contributing to the development of the postnatal gut microbiome following childbirth. HMOs may function as prebiotics from human milk, and should be added to the list of plant-derived oligosaccharides considered as prebiotics (*Gibson* and *Roberfroid*, 1995; *Roberfroid*, 2007). HMOs also

have antimicrobial features partly due to anti-adhesive properties that may inhibit bacterial pathogens and alter the ability of commensal species to colonize the intestine. By altering colonization, HMOs may help shape the nature of bacterial communities. For example, HMOs reduced the ability of enteropathogenic *E. coli* (EPEC) to attach in a tissue culture adhesion assay and mouse EPEC infection (*Manthey* et al., 2014). HMOs may have indirect effects on the infant microbiome, namely as epithelial and immune cell modulators. Specific HMO structures such as disialyl-lacto-N-tetraose (DSLNT) improved survival and reduced pathology scores in a rat necrotizing enterocolitis (NEC) model (*Jantscher-Krenn* et al., 2012). The effects of DSLNT on intestinal pathology are highly structure-specific. Perhaps specific HMOs can prevent or suppress the pathology of severe NEC in infants, directly by acting on the intestinal mucosa, and raise the possibility that microbial metabolites may act directly as immunomodulators as well. The biology of HMOs serves as a reminder that key dietary components early in life may affect mammalian biology via changes in the composition and function of the microbiome, or directly on mammalian cells by modulating immune cell signalling via pattern recognition receptors (TLRs), production of antimicrobial peptides, mucin composition, and cytokine production in the intestinal mucosa.

THE BACTERIAL COMMUNITY: GUT MUCOSAL BIOLOGY INTERFACE

Dietary components such as carbohydrates and lipids clearly affect microbial composition in the intestine. Microbes that respond to elements of the diet, in turn, may influence whole body metabolism and affect the ability of mammals to absorb and process nutrients. This interplay of diet, the microbiome, and mammalian metabolism represents a central theme of this OHUS Monograph. In rodent models, diets differing in polysaccharide and fat content yielded major differences in gut microbial composition and correspondingly microbial metabolism. The caecal microbiota of genetically obese ob/ob mice, lean ob/+ and wild-type siblings, and their ob/+ mothers were compared after ingesting the same polysaccharide-rich diet (*Ley* et al., 2005). Regardless of kinship, ob/ob (obese) mice had approximately half the number of Bacteroidetes than lean mice and a proportional increase in Firmicutes taxa. Colonization of germ-free mice with obesity-associated microbiota resulted in increased total body fat, when compared to mice colonized by microbiota associated with leanness. The gut microbiota may produce metabolites, and directly or indirectly affect patterns of mammalian metabolism and weight gain (*Turnbaugh* et al., 2006).

Specific bacterial species have been identified that are associated with alterations in mammalian body metabolism and weight gain (*Le Chatelier* et al., 2013). Studies demonstrated that humans afflicted with diabetes and/or obesity harbour increased cell numbers of *Clostridium ramosum* (Erysipelotrichi) (*Qin* et al., 2012; *Karlsson* et al., 2013; *Le Chatelier* et al., 2013). The researchers investigated the role of *C. ramosum* in the pathophysiology of obesity in gnotobiotic mice. The results showed that Simplified Intestinal Human Microbiota (SIHUMI) mice with

C. ramosum had enhanced diet-induced obesity compared with SIHUMI mice without *C. ramosum*. This species of bacteria proliferated in SIHUMI mice fed a high-fat diet (HFD). Small intestinal glucose and fat transporters in these animals may be upregulated, leading to increase in body fat deposition. This upregulation in HFD-fed SIHUMI mice may be caused by *C. ramosum* increasing gene expression of the fatty acid transport proteins ileal FA-transport protein-4 (FATP4) and *CD36*. In addition, enterocytes of obese mice contained increased gene expression of perilipin, which is a fat storage protein (*Woting* et al., 2014). So, gut microbes may proliferate when exposed to dietary fat and may enhance the body's ability to absorb and metabolize simple sugars and triglycerides. Diet alters the microbiome, and the microbiome, in turn, changes the ability of mammals to absorb and metabolize nutrients.

The availability of nutrients and degree of flexibility of resident bacteria in finding nutrients determine the diversity and mutualistic cooperation among the component species in human gut microbiota (*Backhed* et al., 2005). Nutrient availability and microbial composition are complicated by the presence of different bacterial layers in the intestinal lumen (luminal) and adjacent to the mucosa (mucosal). These "layers" of microbes and luminal content are affected by hydrodynamics (fluid shear), oxygen content, and the presence of mucus (*Van den Abbeele* et al., 2011). The composition and function of the mucosal microbiome may directly affect the biology of the intestinal mucosa, but this area adjacent to the mucosa is difficult to access and dynamics of microbiome colonization are challenging to study. Butyrate-producing organisms of the groups *Clostridium* cluster IV and XIVa are enriched in the mucosal microbiome (discussed in this monograph by Tom van de Wiele) (*Nava* et al., 2011). Promotion of regulatory T cell accumulation in mice is caused by the spore-forming components of native intestinal microbiota, especially *Clostridium* clusters IV and XIVa (*Atarashi* et al., 2011). *In vitro* models, consistent with *in vivo* data, showed that strictly anaerobic bifidobacteria tend to reside in the upper part of the mucus layer, but the Firmicute, *F. prausnitzii*, mainly colonized the lower part of the mucus, at the anoxic/oxic interphase and adjacent to the intestinal epithelium. Intestinal bacteria and their metabolites are stratified in layers spanning the lumen and mucosa-associated spaces.

The metabolism of carbohydrates by specific microbes generates precursors of short chain fatty acids (SCFAs) such as butyrate. Generation of butyrate provides a fuel source for the intestinal epithelium, enhances tight junction formation and promotes intestinal "barrier" function. Deficiencies of butyrate-producing microbes such as *F. prausnitzii* (*Sokol* et al., 2008) have been demonstrated in disease states such as inflammatory bowel disease (e.g. Crohn's disease) (*Willing* et al., 2009). Models such as M-SHIME detected clear differences in healthy versus Crohn's disease-associated microbiomes, and data showed that human participants with active disease yielded lower butyrate production. In summary, deleterious effects on microbial composition of the mucosal microbiome caused by shifts in the intestinal environment may culminate in ineffective functioning of the gut mucosa and increased disease susceptibility. Conversely, abundance of microbial metabolites such as SCFAs may promote healthy immune and epithelial cell function, and disease resistance. The compositional and functional

stratification of the intestinal microbiome in "layers" can be studied with new model systems and provide insights into microbial-mucosal interactions.

MICROBIAL METABOLITES AND MAMMALIAN PHYSIOLOGY

Microbial metabolites represent the biochemical currency of communication between microbial and mammalian cells in the gut and the gut-brain axis. As we consider the links between diet and the microbiome, and the microbiome and mammalian physiology before and after birth, the roles of microbial metabolites in different body compartments must be considered. SCFAs are well-known examples of microbial metabolites affecting the intestinal epithelium. Three main SCFAs produced in the human colon are acetate, propionate, and butyrate. Propionate and acetate affect adipogenesis and leptin production. By modulating gut and adipocyte-derived hormones, SCFAs may affect the gut-brain axis and brain function, including appetite and satiety (*Xiong* et al., 2004; *Samuel* et al., 2008). Acetate is able to directly affect neuronal function in the brain, especially regions associated with appetite and satiety control (*Anastasovska* et al., 2012). Using carbon-11 labelled acetate as a potential PET marker, acetate is actively assimilated by many organs including the liver, skeletal muscle, spleen, heart, and adipose tissue. Acetate may reach the brain in small, yet significant amounts, regardless of the route of administration (*Frost* et al., 2014). Supplementation of fermentable carbohydrates has long been known to reduce appetite in animal studies, and the appetite suppressing effect of acetate was recently shown to occur via hypothalamic neuronal activation (*Frost* et al., 2014). Whether adminis
tered or produced in the colon, acetate has a direct effect on hypothalamic neurons by its incorporation into the glutamate-glutamine transcellular cycle, increasing GABAergic transmission and activating acetyl-CoA carboxylase (ACC). The increased ACC activity induces malonyl-CoA expression, which activates pro-opiomelanocortin neurons and therefore reduces food intake (see contribution by J. Bell in this monograph). The SCFA, acetate, provides a clear example of how one microbial metabolite mediates communication between different organs such as the gut and the brain.

In addition to appetite suppression and body metabolism, microbial metabolites may modulate the immune system by activating or suppressing immune responses in the gut mucosa. Fungi within the microbiome may promote resistance to pathogenic microbes and tolerance to commensal microbes. The mammalian tryptophan catabolic enzyme, indoleamine 2,3-dioxygenase 1 (IDO1), plays a key role in the conversion of dietary or microbial tryptophan into kynurenines and promotion of tolerance to commensal microbes. The capacity of IDO1-expressing dendritic cells, epithelial cells, and kynurenines to induce Tregs and inhibit Th17 has revealed their unexpected potential to control inflammation, allergy, and Th17-driven inflammation in fungal infections (*Grohmann* et al., 2007; *Romani* et al., 2008). Conversely, the exploitation of the IFN-γ/IDO1 axis for functional specialization of antifungal regulatory mechanisms may have enabled fungal microbiota to co-evolve with the mammalian immune system,

and to prevent dysregulated immunity (*Zelante* et al., 2009). In contrast, beneficial microbes may convert tryptophan into bioactive indole compounds. Indole derivatives act as endogenous ligands for the arylhydrocarbon receptor (AhR) (*Heath-Pagliuso* et al., 1998) and are generated through conversion from dietary tryptophan by commensal intestinal microbes (*Bjeldanes* et al., 1991). By binding AhR, the microbiome may stimulate mammalian immune cells to produce the cytokine lL-22 and thereby enhancing protective mucus production, suppressing IL-17 production, and stimulating production of antimicrobial peptides via STAT3 activation. The lesson in *Lactobacillus*-mediated indole-3-aldehyde (one example of indole derivative) production from tryptophan is that bacterial metabolites generated by the microbiome may have a profound impact on immunity. The metabiotic concept refers to metabolites produced by the microbiome or probiotic species that may confer specific benefits on mammalian physiology or immunity. Metabiotics such as indole derivatives may suppress inflammation and promote immunologic resistance to different pathogens.

Gamma-aminobutyric acid (GABA) is another important co-metabolite produced by glutamate decarboxylases (GADs) of mammals and bacteria. Amino acid decarboxylation systems, such as the GAD system, enable bacterial species to cope with acid stress, and GABA is enriched in the intestines of patients with recurrent *C. difficile*-associated disease (CDAD). Most intestinal GABA measured in stool is microbial in origin, and this metabolite (GABA) appears to potentiate the risk of *C. difficile* infection. Individuals consuming the $GABA_A$ receptor agonist, zolpidem (Ambien®), have increased risk of *C. difficile* infection, adding support to the pathogenic role of GABA in CDAD. Data from mouse models also support the ability of GABA receptor signalling to exacerbate intestinal inflammation. In contrast to elevated amounts of microbial GABA in recurrent CDAD, the bile acid metabolite lithocholate is associated with healthy controls and patients cured post-FMT. The scientific community is beginning to identify specific microbes and microbial metabolites that contribute to human health and alter disease susceptibility. New classes of microbial biomarkers are being characterized, and GABA is a good example. So we are beginning to identify specific microbial metabolites and how their receptors/signalling pathways may protect patients or enhance disease susceptibility.

SYSTEMS BIOLOGY AND THERAPEUTIC MICROBIOLOGY

Many published studies have explored associations between differences in microbial composition and human health and disease states, including but not limited to inflammatory bowel disease, necrotizing enterocolitis, and obesity. However, few microbiome studies could verify causation. Scientists should attempt to verify causation, try to identify microbial effector strains and metabiotics, and modify effector strains to improve mammalian health. To identify microbial effector strains, one must isolate individual microbes or rely on differences in metabolite profiles. *Faith* et al. (2014) approached the challenge of identifying microbial effector strains. One of several human faecal samples transmitted a metabolic/immunologic phenotype to

recipient germ-free mice and resulted in efforts to identify the key microbe(s) involved by metagenomic and microbial sequencing. Ninety-four bacterial consortia were taken from the culture collection and were introduced as humanized gut microbiota to germ-free mice. Feature selection algorithms and follow-up mono-colonization studies may identify effector bacterial strains. An unexpected range of bacterial strains was found to promote accumulation of colonic regulatory T cells (Tregs) and expand Nrp1 (lo/-) peripheral Tregs. Other strains modulated adiposity in mice and caecal metabolite concentrations (*Faith* et al., 2014). The greatest numbers of Tregs in the colonic lamina propria were associated with *B. intestinalis* colonization (*Faith* et al., 2014). Specific metabolites such as quinic acid, a cyclic polyol, were detected and correlated with effector bacterial strains. As effector strains are identified, their specific metabolic features may be useful in determining which effector strains (and metabolites) may be ideally suited for particular research and clinical applications. These studies point to the utility of combining intestinal microbiology with well-defined gnotobiotic mouse models so that specific functions of the microbiome can be linked to specific microbes.

Another key challenge in microbiome research is to understand the role of interpersonal microbiome variation in disease. Researchers investigated gut microbiota from twins with discordance for obesity that modulated metabolism in mice. They found that co-housing mice containing an obese twin's microbiota (Ob) with mice containing the lean co-twin's microbiota (Ln) led to suppression of body mass and adiposity increases in Ob cage mates. As mice are coprophagic, rescue is correlated with invasion of specific members of Bacteroidetes from the Ln microbiota into the Ob microbiota (*Ridaura* et al., 2013). Another study of interpersonal microbiome variation in disease showed that faecal microbiota transplantation induced 7 of 9 remissions in patients with active ulcerative colitis from one donor (*Moayyedi* et al., 2015). Thus, even if a donor does not have disease, this donor may not be an appropriate donor for someone that does have the disease. Thus, microbial composition is important in terms of determining whole body metabolism and careful selection of stool donors for faecal microbiota transplantation (FMT). Determining the causative drivers of diverse responses from diverse microbiota is a key challenge in microbiome research.

The relative diversity and abundance of each body habitat's microbes varie between individuals (*Human Microbiome Project Consortium*, 2012). On the other hand, metabolic pathways are relatively conserved between individuals in a healthy population (*Human Microbiome Project Consortium*, 2012). Diet has a large role in modulating microbiota, and it is a primary determinant of gut microbial composition. Although short-term dietary interventions might rapidly change community structures within the gut microbiome, long-term shifts in steady-state microbial communities require longer term and substantial dietary changes (*Voreades* et al., 2014). Antibiotics reduce the relative diversity within gut microbiomes. Researchers found substantial variations in microbial communities of subjects given a single antimicrobial agent (ciprofloxacin). These changes occurred between two courses of antibiotics administered to subjects. When the study ended, the composition of the gut microbiota in every subject had stabilized, although it was changed from its initial state. Thus, alternative stable states may shift depending on the

frequency, duration and nature of antimicrobial exposure (*Dethlefsen* and *Relman*, 2011).

Several options for microbial manipulation provide opportunities for changing microbial composition and function. Studies showed that probiotics were effective for infectious diarrhoea (*Francavilla* et al., 2012; *Dinleyici* and *Vandenplas*, 2014). A systemic review and meta-analysis showed significant beneficial effects of probiotics that reduced global symptoms or abdominal pain (*Ford* et al., 2014). A systematic review of probiotics, prebiotics, and synbiotics in inflammatory bowel disease demonstrated that some studies yielded improvement trends, but definitive treatments had been evasive (*Ghouri* et al., 2014). Diet of the host can have a large effect on responses to probiotics. Also, probiotics can alter microbial composition quite differently depending upon the diet (*Degirolamo* et al., 2014). The effects of probiotic treatment are strongly influenced by the existing intestinal microbial ecosystem. Confounding factors in human studies of probiotics include the individual's diet, established microbial communities in a particular individual, and the luminal environment.

Faecal microbiota transplantation (FMT) is a method to radically change microbial composition by introducing faecal microbiota from a healthy person to a person with an intestinal disorder. Researchers found that infusion of donor faeces was significantly superior to vancomycin in treatment of recurrent *C. difficile* infection (*van Nood* et al., 2013). FMT caused bacterial composition to normalize quickly from a state of dysbiosis. Although the microbiome in the subject reflected the donor implant material at 1 day post-FMT, the composition was highly varied at later time points (*Weingarden* et al., 2015). It is likely that mechanisms contributing to FMT's effectiveness as a treatment strategy in patients with recurrent *C. difficile* infection stem from the ability to interfere with pathogen spore germination and competition by *C. difficile* (*Britton* and *Young*, 2014). Importantly, FMT can be considered as the simultaneous administration of hundreds of bacterial taxa with different functional features. For example, functional classes of bacterial strains may include lactate producers, methanogens, mucin degraders, and short chain fatty acid producers. Restoration of many microbiome-related functional features after one or several procedures (FMT) may be necessary for restitution of a severely depleted microbiome. Disorders of microbial ecology (e.g. CDAD) secondary to antibiotic consumption in susceptible individuals emphasize the need for replacement strategies with consortia and pools of microbes harbouring diverse microbiologic functions.

Future perspectives on FMT include customization of bacterial combinations with defined and known specific biological properties to produce predictable responses. Additionally, development of standardized conditions for transplantation (inoculation) and maintenance of faecal-derived microbial communities, and analyses of donor-recipient microbiome compatibilities represent next steps in advancing FMT and other microbial consortia-based replacement strategies. In addition to global and multifunctional considerations, precision microbiome reconstitution has been shown to impact bile acid-mediated resistance to CDAD (*Buffie* et al., 2015). In this example, one species, *Clostridium scindens*, has 7-dehydroxylase activity resulting in production of bile acid metabolites that mediate resistance to *C. difficile* infection. One of these bile acid metabolites,

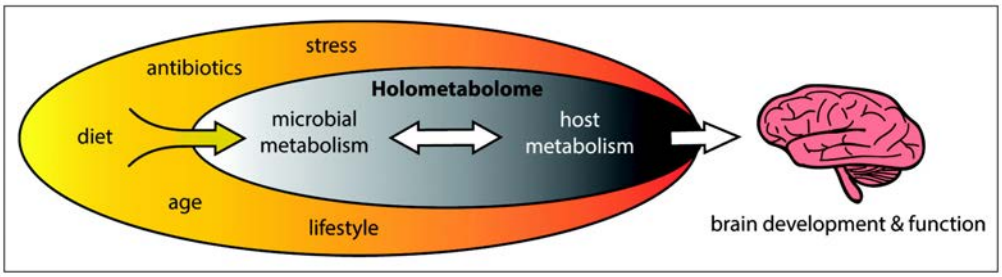

Figure 2: The human-microbiome holometabolome. Environmental factors including diet and medications influence microbial and human metabolism together as one physiological system. Changes in the holometabolome likely affect gut biology, the brain-gut axis and brain development and function. (Reprinted with permission from *Selkrig* et al., 2014.)

lithocholate, was found enriched in healthy controls and FMT-responsive patients by Tor Savidge and colleagues. So, we come full circle by considering microbiome replacement therapy (FMT), identifying individual effector bacterial strains (*C. scindens*), and detecting individual bile acid metabolites conferring disease resistance.

SUMMARY AND FUTURE DIRECTIONS

This 29th edition of the Old Herborn University Symposium (June 2015) placed the spotlight on the overlapping contact points between diet, the gut microbiome, microbial metabolism, and human metabolism/physiology. The "Holometabolome" concept as proposed in 2014 provides a conceptual framework by linking environmental factors with microbial and mammalian metabolism, and considering how metabolites from microbes and mammals impact the biology of the intestine and remote organ systems (e.g. brain) (Figure 2). The importance of the microbiome *in utero* and the impact of breast milk drive home the point that humans rely on microbes and microbial metabolites during the earliest weeks to months of human life. The exciting reality is that microbial composition and function can be manipulated by diet and that effects of the microbiome on early human development cannot be underestimated.

Beyond early human life, microbial taxa communicate effectively with mammalian cells at the mucosal interface, resulting in different metabolic and immunologic profiles later in life. Several important concepts in microbiome science have emerged. First, one microbiome (oral) may seed a different microbiome (placenta) and enable it to function with the foetus. Dietary impact of the microbiome may persist for many years. Compositional and functional stratification of the gut microbiome reminds us that the microbiome is a differentiated organ with different functional aspects. By describing various microbial metabolites and their potential roles, we must think in terms of microbiome-related contributions to the entire host. The gut microbiome will be tapped to understand the needs/identity/skills of individual microbes so that functions can be attached. The relative contribution of microbiomes to heredity is an interesting question to ponder. Finally, intentional manipulation of the gut

microbiome by introducing intact microbial communities (FMT), single or selected beneficial microbes (probiotics), or dietary shifts (including prebiotics) will hopefully yield opportunities to improve human health and prevent or cure diseases. Insights into microbial and human metabolism may help us identify new biomarkers, diagnostic tools, and therapeutic targets in the future.

LITERATURE

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., and Versalovic, J.: The placenta harbors a unique microbiome. Sci. Transl. Med. 6, 237ra265 (2014).

Anastasovska, J., Arora, T., Sanchez Canon, G.J., Parkinson, J.R., Touhy, K., Gibson, G.R., Nadkarni, N.A., So, P.W., Goldstone, A.P., Thomas, E.L., Hankir, M.K., Van Loo, J., Modi, N., Bell, J.D., and Frost, G.: Induction of colonic regulatory T cells by indigenous Clostridium species. Science 331, 337-341 (2011).

Backhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., and Gordon, J.I.: Host-bacterial mutualism in the human intestine. Science 307, 1915-1920 (2005).

Bjeldanes, L.F., Kim, J.Y., Grose, K.R., Bartholomew, J.C., and Bradfield, C.A.: Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Proc. Natl. Acad. Sci. USA 88, 9543-9547 (1991).

Bode, L.: Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology 22, 1147-1162 (2012).

Britton, R.A. and Young, V.B.: Role of the intestinal microbiota in resistance to colonization by Clostridium difficile. Gastroenterology 146, 1547-1553 (2014).

Buffie, C.G., Bucci, V., Stein, R.R., McKenney, P.T., Ling, L., Gobourne, A., No, D., Liu, H., Kinnebrew, M., Viale, A., Littmann, E., van den Brink, M.R., Jenq, R.R., Taur, Y., Sander, C., Cross, J.R., Toussaint, N.C., Xavier, J.B., and Pamer, E.G.: Precision microbiome reconstitution restores bile acid mediated resistance to Fermentable carbohydrate alters hypothalamic neuronal activity and protects against the obesogenic environment. Obesity (Silver Spring) 20, 1016-1023 (2012).

Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., Taniguchi, T., Takeda, K., Hori, S., Ivanov, II, Umesaki, Y., Itoh, K., and Honda, K.: Clostridium difficile. Nature 517, 205-208 (2015).

Degirolamo, C., Rainaldi, S., Bovenga, F., Murzilli, S., and Moschetta, A.: Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr-Fgf15 axis in mice. Cell Rep. 7, 12-18 (2014).

Dethlefsen, L. and Relman, D.A.: Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc. Natl. Acad. Sci. USA 108, Suppl. 1, 4554-4561 (2011).

Dinleyici, E.C. and Vandenplas, Y.: Lactobacillus reuteri DSM 17938 effectively reduces the duration of acute diarrhoea in hospitalised children. Acta Paediatr. 103, e300-305 (2014).

Faith, J.J., Ahern, P.P., Ridaura, V.K., Cheng, J., and Gordon, J.I.: Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. Sci. Transl. Med. 6, 220ra211 (2014).

Ford, A.C., Quigley, E.M., Lacy, B.E., Lembo, A.J., Saito, Y.A., Schiller, L.R., Soffer, E.E., Spiegel, B.M., and Moayyedi, P.: Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. Am. J.

Gastroenterol. 109, 1547-1561 (2014).
Francavilla, R., Lionetti, E., Castellaneta, S., Ciruzzi, F., Indrio, F., Masciale, A., Fontana, C., La Rosa, M.M., Cavallo, L., and Francavilla, A.: Randomised clinical trial: Lactobacillus reuteri DSM 17938 vs. placebo in children with acute diarrhoea--a double-blind study. Aliment. Pharmacol. Ther. 36, 363-369 (2012).
Frost, G., Sleeth, M.L., Sahuri-Arisoylu, M., Lizarbe, B., Cerdan, S., Brody, L., Anastasovska, J., Ghourab, S., Hankir, M., Zhang, S., Carling, D., Swann, J. R., Gibson, G., Viardot, A., Morrison, D., Louise Thomas, E., and Bell, J.D.: The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nat. Commun. 5, 3611 (2014).
Ghouri, Y.A., Richards, D.M., Rahimi, E.F., Krill, J.T., Jelinek, K.A., and DuPont, A.W.: Systematic review of randomized controlled trials of probiotics, prebiotics, and synbiotics in inflammatory bowel disease. Clin. Exp. Gastroenterol. 7, 473-487 (2014).
Gibson, G.R. and Roberfroid, M.B.: Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125, 1401-1412 (1995).
Grohmann, U., Volpi, C., Fallarino, F., Bozza, S., Bianchi, R., Vacca, C., Orabona, C., Belladonna, M.L., Ayroldi, E., Nocentini, G., Boon, L., Bistoni, F., Fioretti, M.C., Romani, L., Riccardi, C., and Puccetti, P.: Reverse signaling through GITR ligand enables dexamethasone to activate IDO in allergy. Nat. Med. 13, 579-586 (2007).
Heath-Pagliuso, S., Rogers, W.J., Tullis, K., Seidel, S.D., Cenijn, P.H., Brouwer, A., and Denison, M.S.: Activation of the Ah receptor by tryptophan and tryptophan metabolites. Biochemistry 37, 11508-11515 (1998).
Hillier, S.L., Martius, J., Krohn, M., Kiviat, N., Holmes, K.K., and Eschenbach, D.A.: A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N. Engl. J. Med. 319, 972-978 (1988).
Human Microbiome Project Consortium: Structure, function and diversity of the healthy human microbiome. Nature 486, 207-214 (2012).
Jantscher-Krenn, E., Zherebtsov, M., Nissan, C., Goth, K., Guner, Y.S., Naidu, N., Choudhury, B., Grishin, A.V., Ford, H.R., and Bode, L.: The human milk oligosaccharide disialyllacto-N-tetraose prevents necrotising enterocolitis in neonatal rats. Gut 61, 1417-1425 (2012).
Jost, T., Lacroix, C., Braegger, C.P., and Chassard, C.: New insights in gut microbiota establishment in healthy breast fed neonates. PLoS One 7, e44595 (2012).
Karlsson, F.H., Tremaroli, V., Nookaew, I., Bergstrom, G., Behre, C.J., Fagerberg, B., Nielsen, J., and Backhed, F.: Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 498, 99-103 (2013).
King, A.E., Paltoo, A., Kelly, R.W., Sallenave, J.M., Bocking, A.D., and Challis, J.R.: Expression of natural antimicrobials by human placenta and fetal membranes. Placenta 28, 161-169 (2007).
Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.M., Kennedy, S., Leonard, P., Li, J., Burgdorf, K., Grarup, N., Jorgensen, T., Brandslund, I., Nielsen, H.B., Juncker, A.S., Bertalan, M., Levenez, F., Pons, N., Rasmussen, S., Sunagawa, S., Tap, J., Tims, S., Zoetendal, E.G., Brunak, S., Clement, K., Doré, J., Kleerebezem, M., Kristiansen, K., Renault, P., Sicheritz-Ponten, T., de Vos, W.M., Zucker, J. D., Raes, J., Hansen, T., Bork, P., Wang, J., Ehrlich, S.D., and Pedersen, O.: Richness of human gut microbiome correlates with metabolic markers. Nature 500, 541-546 (2013).
Ley, R.E., Backhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., and Gordon, J.I.: Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. USA 102, 11070-11075 (2005).
Manthey, C.F., Autran, C.A., Eckmann, L., and Bode, L.: Human milk oligosaccharides

protect against enteropathogenic Escherichia coli attachment in vitro and EPEC colonization in suckling mice. J. Pediatr. Gastroenterol. Nutr. 58, 165-168 (2014).

Moayyedi, P., Surette, M.G., Kim, P.T., Libertucci, J., Wolfe, M., Onischi, C., Armstrong, D., Marshall, J.K., Kassam, Z., Reinisch, W., and Lee, C.H.: Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. Gastroenterology 149, 102-109 (2015).

Nava, G.M., Friedrichsen, H.J., and Stappenbeck, T.S.: Spatial organization of intestinal microbiota in the mouse ascending colon. ISME J. 5, 627-638 (2011).

Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., and Pettersson, S.: Host-gut microbiota metabolic interactions. Science 336, 1262-1267 (2012).

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., Liang, S., Zhang, W., Guan, Y., Shen, D., Peng, Y., Zhang, D., Jie, Z., Wu, W., Qin, Y., Xue, W., Li, J., Han, L., Lu, D., Wu, P., Dai, Y., Sun, X., Li, Z., Tang, A., Zhong, S., Li, X., Chen, W., Xu, R., Wang, M., Feng, Q., Gong, M., Yu, J., Zhang, Y., Zhang, M., Hansen, T., Sanchez, G., Raes, J., Falony, G., Okuda, S., Almeida, M., LeChatelier, E., Renault, P., Pons, N., Batto, J. M., Zhang, Z., Chen, H., Yang, R., Zheng, W., Yang, H., Wang, J., Ehrlich, S.D., Nielsen, R., Pedersen, O., and Kristiansen, K.: A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490, 55-60 (2012).

Ridaura, V.K., Faith, J.J., Rey, F.E., Cheng, J., Duncan, A.E., Kau, A.L., Griffin, N.W., Lombard, V., Henrissat, B., Bain, J.R., Muehlbauer, M.J., Ilkayeva, O., Semenkovich, C.F., Funai, K., Hayashi, D.K., Lyle, B.J., Martini, M.C., Ursell, L.K., Clemente, J.C., Van Treuren, W., Walters, W.A., Knight, R., Newgard, C.B., Heath, A.C., and Gordon, J.I.: Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341, 1241214 (2013).

Roberfroid, M.: Prebiotics: the concept revisited. J. Nutr. 137, 830S-837S (2007).

Romani, L., Zelante, T., De Luca, A., Fallarino, F., and Puccetti, P.: IL-17 and therapeutic kynurenines in pathogenic inflammation to fungi. J. Immunol. 180, 5157-5162 (2008).

Samuel, B.S., Shaito, A., Motoike, T., Rey, F.E., Backhed, F., Manchester, J.K., Hammer, R.E., Williams, S.C., Crowley, J., Yanagisawa, M., and Gordon, J.I.: Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proc. Natl. Acad. Sci. USA 105, 16767-16772 (2008).

Selkrig, J., Wong, P., Zhang, X., and Pettersson, S.: Metabolic tinkering by the gut microbiome: Implications for brain development and function. Gut Microbes 5, 369-380 (2014).

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermudez-Humaran, L.G., Gratadoux, J.J., Blugeon, S., Bridonneau, C., Furet, J.P., Corthier, G., Grangette, C., Vasquez, N., Pochart, P., Trugnan, G., Thomas, G., Blottiere, H. M., Doré, J., Marteau, P., Seksik, P., and Langella, P.: Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc. Natl. Acad. Sci. USA 105, 16731-16736 (2008).

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., and Gordon, J.I.: An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444, 1027-1031 (2006).

Van den Abbeele, P., Van de Wiele, T., Verstraete, W., and Possemiers, S.: The host selects mucosal and luminal associations of coevolved gut microorganisms: a novel concept. FEMS Microbiol. Rev. 35, 681-704 (2011).

van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E.G., de Vos, W.M., Visser, C.E., Kuijper, E.J., Bartelsman, J.F., Tijssen, J.G., Speelman, P., Dijkgraaf, M.G., and Keller, J.J.: Duodenal infusion of donor feces for recurrent Clostridium

difficile. N. Engl. J. Med. 368, 407-415 (2013).

Voreades, N., Kozil, A., and Weir, T.L.: Diet and the development of the human intestinal microbiome. Front. Microbiol. 5, 494 (2014).

Weingarden, A., Gonzalez, A., Vazquez-Baeza, Y., Weiss, S., Humphry, G., Berg-Lyons, D., Knights, D., Unno, T., Bobr, A., Kang, J., Khoruts, A., Knight, R., and Sadowsky, M.J.: Dynamic changes in short- and long-term bacterial composition following fecal microbiota transplantation for recurrent Clostridium difficile infection. Microbiome 3, 10 (2015).

Willing, B., Halfvarson, J., Dicksved, J., Rosenquist, M., Jarnerot, G., Engstrand, L., Tysk, C., and Jansson, J. K.: Twin studies reveal specific imbalances in the mucosa-associated microbiota of patients with ileal Crohn's disease. Inflamm. Bowel Dis. 15, 653-660 (2009).

Woting, A., Pfeiffer, N., Loh, G., Klaus, S., and Blaut, M.: Clostridium ramosum promotes high-fat diet-induced obesity in gnotobiotic mouse models. MBio 5, e01530-01514 (2014).

Xiong, Y., Miyamoto, N., Shibata, K., Valasek, M.A., Motoike, T., Kedzierski, R. M., and Yanagisawa, M.: Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. Proc. Natl. Acad. Sci. USA 101, 1045-1050 (2004).

Xue, Y., Wang, H., Du, M., and Zhu, M.J.: Maternal obesity induces gut inflammation and impairs gut epithelial barrier function in nonobese diabetic mice. J. Nutr. Biochem. 25, 758-764 (2014).

Yan, X., Huang, Y., Wang, H., Du, M., Hess, B.W., Ford, S.P., Nathanielsz, P. W., and Zhu, M.J.: Maternal obesity induces sustained inflammation in both fetal and offspring large intestine of sheep. Inflamm. Bowel Dis. 17, 1513-1522 (2011).

Zelante, T., Fallarino, F., Bistoni, F., Puccetti, P., and Romani, L.: Indoleamine 2,3-dioxygenase in infection: the paradox of an evasive strategy that benefits the host. Microbes Infect. 11, 133-141 (2009).

Zeldovich, V.B. and Bakardjiev, A.I.: Host defense and tolerance: unique challenges in the placenta. PLoS Pathog. 8, e1002804 (2012).