The gut microbiota and inflammatory noncommunicable diseases: Associations and potentials for gut microbiota therapies

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The relationship between gut microbiota and inflammatory non-communicable diseases and the potential role for gut microbiota as therapy

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Keywords: fecal microbiota transplantation; gut microbiome; inflammation; non-communicable diseases; prebiotics; probiotics; short-chain fatty acids

List of abbreviations: CS-caesarean section; CRP-C-reactive protein; CVD-cardiovascular disease; DGGE -denaturing gradient gel electrophoresis; FISH- fluorescence in situ hybridization; FMT-fecal microbiota transplantation; HPA-hypothalamic-pituitary-adrenal; IBD- inflammatory bowel disease; NCD-non-communicable diseases; SCFA- short-chain fatty acid; VD-vaginally delivered; q-PCR- quantitative PCR; T-RFLP- terminal restriction fragment length polymorphism; TLR- Toll-like receptor; 16S- 16S rRNA gene

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ABSTRACT

Rapid environmental transition and modern lifestyles are likely to be driving changes in the biodiversity of human gut microbiota. With clear effects on physiological, immunological and metabolic processes in human health, aberrations in the gut microbiome and intestinal homeostasis have the capacity for multisystem effects. Changes in microbial composition are implicated in the rising propensity for a broad range of inflammatory diseases such as allergic disease, asthma, inflammatory bowel disease, obesity and associated non-communicable diseases (NCDs). There are also suggestive implications for neurodevelopment and mental health. These diverse multisystem influences have sparked interest in strategies that may favorably modulate the gut microbiota to reduce the risk of many NCDs. For example, specific prebiotics promote favorable intestinal colonization and their fermented products have anti-inflammatory properties. Specific probiotics also have immunomodulatory and metabolic effects. However, when evaluated in clinical trials the effects are variable, preliminary or limited in magnitude. Fecal microbiota transplantation (FMT) is another emerging therapy that regulates inflammation in experimental models. In humans this has been successfully used in Clostridium difficile infection and inflammatory bowel disease (IBD), although controlled trials are lacking for IBD. Here, we discuss relationships between gut colonization and inflammatory NCDs, and gut microbiota modulation strategies for their treatment and prevention.
Introduction

The health of our modern society is being threatened by a plethora of chronic inflammatory non-communicable diseases (NCDs) which share in common, an underlying low-grade inflammation. These include early onset NCDs such as allergy, asthma and some autoimmune diseases and later onset NCDs including cardiovascular disease (CVD), metabolic disease and neurodegenerative disorders – which also appear to share common environmental risk factors as well as common genetic risk variants. While inflammation and the pathways to disease are multifactorial, the altered gut colonization patterns associated with declining microbial diversity is a central theme, and increasingly implicated in the physiological, immunological and metabolic dysregulation seen in many NCDs. The adult gut harbors as many as 100 trillion resident microbes called the microbiota and the corresponding genome (microbiome) has been estimated to contain 150-fold more genes than the host genome. These complex communities have a symbiotic relationship with the host, and are involved in many aspects of host physiology. Generally, the two main phyla in adults are Bacteroidetes comprising Gram-negative bacteria and Firmicutes comprising Gram-positive bacteria. The cell wall of gram-negative bacteria contains lipopolysaccharides (LPS), which induce a strong host inflammatory response to protect from infection. Under normal conditions, finely tuned regulatory responses restrict excessive inflammation and maintain tissue equilibrium. The pattern of microbial exposure in early life appears to be important for the development of robust host immune regulation, and disruption in either the microbiota or the host response can lead to chronic inflammation. In this context, reduced exposure to commensals, helminths and other infectious agents in affluent countries is a likely contributor, at least in part, to the rising propensity for chronic low-grade inflammation and impaired immune-regulation. Our classical understanding of inflammation is of a normal physiological response to an infectious threat, or tissue injury. Inflammation leads to tissue repair,
resolution and restoration of the ‘homeostatic’ balance in the tissues. But the chronic inflammatory states of asthma, allergy, obesity, diabetes, atherosclerosis and other NCDs do not appear to fit this model. There is usually no acute or immediate threat; and there is no resolution. Instead there appears to be a chronic tissue malfunction and a shift of the normal homeostasis or ‘balance’ to adapt to new physiological or metabolic conditions. Disruption in the interrelationships between nutrition, and microbiome and host metabolism are likely to be key elements in the disruption of normal homeostasis. Dysbiosis – “an imbalance in microbiota structure and/or function that disrupts host-microorganism homeostasis” - is an emerging feature of many NCDs. This concept is driving strategies to favorably impact gut microbiota. However, a significant limiting factor is that the “normal, healthy gut microbiota” is yet to be clearly defined, including the range and variant profiles, and developmental windows that are likely to fall within this spectrum. To overcome this, large-scale investigations of the human microbiome e.g. the Human Microbiome Project and MetaHit are rapidly advancing our understanding by defining microbial compositions and their functions in large populations using coordinated strategies for project planning, analytic techniques and education programs. Clearly, experimental models have also provided invaluable insights, but our emphasis here is on the human dimension, with a focus on recent observational studies that have assessed intestinal colonization patterns and their associations to NCDs, and clinical trials that have explored gut microbiota modulation as a therapeutic strategy in both early and late onset NCDs.

**Methods for the investigation of the gut microbiota and microbiome**

Accelerating development of molecular biology methods to characterize the gut microbiome, has rapidly advanced knowledge in this field. Next-generation sequencing, computer technology, and advanced analytical strategies for the handling of ever-expanding biological
information (bioinformatics) can give an unprecedented detailed description of both the genome and metabolic activity of bacteria without requiring growth or isolation of bacterial species. Despite these advancements, differences in culture-independent DNA-based approaches and sequencing make direct comparison of studies difficult. This is an important consideration for clinicians when evaluating research in this field. Here, we provide a short description of modern molecular biology approaches used in observational studies and clinical trials (Table I), and more detailed reviews can be found elsewhere. Classical culture techniques can still provide a detailed depiction of a microbial community. However, as the majority of gut bacteria are anaerobic and challenging to cultivate, it is estimated that less than 20-30% of the gut microbiota have been cultured. This does not mean that all uncultured organisms are unculturable per se, rather that optimal growth conditions have not been designed or discovered.

Culture-independent techniques have the advantage that they can give a more representative view of the gut microbiota and their diversity. They are based on extraction of DNA and amplification of portions of the 16S rRNA gene (16S), which is highly conserved among bacterial species but variable in other internal regions of the gene and thus allows species identification. In clinical trials, methods such as quantitative PCR (q-PCR), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), fluorescence in situ hybridization (FISH), DNA microarrays, and next-generation sequencing of the 16S gene or its amplicons have been used. These methods not only provide qualitative and quantitative information on bacterial species, but also on microbial diversity and ordination, and are increasingly being used in clinical studies. Still, a level of imprecision in 16S-based microbial classification and the associated sequence data have currently hampered capacity to clearly define which specific microbes might be associated
with specific diseases, although this is being further developed\textsuperscript{15, 18}. It has also been argued that traditional measures of diversity in a microbial community are still relatively crude, and to overcome this new diversity profiles are being developed\textsuperscript{19}.

The 16S sequencing approach is now the most widely used. Although there are many benefits, there are also limitations; mainly that the precision is dependent on whether the proportions of the resulting 16S gene sequences accurately reflect the proportion of bacteria in the original sample - as this is affected by copy number variation, PCR primer and amplification bias\textsuperscript{15}. Cell lysis during DNA extraction can also lead to variation in taxa e.g. bifidobacteria. The magnitude of these biases varies substantially between studies\textsuperscript{15, 20}. Another limitation is that 16S sequencing does not give any information on the functional capacity, e.g. the metabolic capacity. One way of addressing this is through predictive bioinformatics methods. As the human intestine is a community with several available reference genomes, bioinformatics software packages can be used to predict an approximate metagenome\textsuperscript{15, 17, 21}. This is done by connecting functions of gene products encoded by the most closely related sequenced genomes with observed taxonomic profiles, thereby creating a functional profile\textsuperscript{4, 15, 21}.

Alternatively, metagenome or metatranscriptome sequencing is now being used to attain a functional profile and higher resolution of the composition of bacterial communities (identification to the species and also strain level). This is also known as shotgun sequencing, DNA-seq or RNA-seq and refer to sequencing the entire nucleotide pool isolated from a sample\textsuperscript{15}. In contrast to 16S studies, this technique also sequences a small amount of human DNA- and also archaea, fungi, and viruses found in a stool sample\textsuperscript{15, 16}. The latter can add valuable information since other microbes than bacteria may also have a role in forming mucosal immune responses\textsuperscript{5}, although less is known about these exposures in the context of
NCDs and will not be covered in this review. Compared with 16S studies, metagenome studies are more expensive and analysis of the massive amount of data generated requires additional computational expertise 16. Metaproteomic and metabolomic analyses are other emerging methods that further characterize the functional capacities of the microbiome 15,16.

The site and the nature of sample collection are also important. Most published studies have analyzed microbial communities in stool samples rather than using mucosal biopsies or luminal content analysis, which differ in composition. Although mucosal biopsy samples pose practical and ethical challenges, it is recognized that the mucosal microbiota might be more pertinent for human disease 17.

**Gut microbiota are essential for normal development and regulation; multisystem effects**

The critical role of the gut microbiota in immune development has been well documented in germ free animal models, demonstrating the failure of normal maturation and, in particular, failure of the systemic immune regulatory networks, that result in both allergic and autoimmune phenomena (as reviewed by) 5-8. Significantly, early experiments clearly demonstrated a critical early developmental "window" during which microbial colonization could induce appropriate maturation of the Th2 response and IgE regulation, and after which this was no longer possible 22. More recently, it has been shown that the diversity of colonization is crucial for the development of an immunoregulatory network that protects against IgE induction in the mucosa 23. It remains unclear if such a "window" exists also in humans, but this is nonetheless consistent with observations that gut microbial exposures in infancy may impact the maturation of mucosal and systemic immune responses in infants 8,24.
The proinflammatory and metabolic effects of altering the gut microbiota have also been observed in animal models of obesity. Notably, more subtle manipulation with low doses of antibiotics in early life (at levels likely to be encountered in human food) increased the risk of adiposity, with significant effects on short-chain fatty acid (SCFA) levels, and in the regulation of hepatic metabolism of lipids and cholesterol. Equally fascinating are results from experimental models reporting changes in behaviour and gene expression in the brain following changes in the gut microbiota. Possibly less well-known to immunologists, in another aspect of their work in germ-free animals, Sudo and colleagues also demonstrated reduced expression of the neuronal growth factor BDNF in the cortex and hippocampus, consistent with patterns seen in depression and anxiety. They also had heightened cortisol stress responses. This shows that without normal bacteria “germ free” animals will develop potentially maladaptive changes in brain function and in endocrine regulation of the stress response through the hypothalamic-pituitary-adrenal (HPA) axis. Critically, these effects can all be reversed by either giving probiotics, or by “fecal transplant” with normal mouse microbiota. Perhaps of even greater significance, timing was critical - reversal is only possible at an early stage of development. Once the response patterns become established with age, they cannot be readily changed. This is true of both the stress and immune responses, which remain abnormal once they are “committed”. It is also consistent with what we know about “critical early periods” for both immune and neural development and the declining plasticity of neural networks with age. Collectively, these findings formed a basis to further explore the role of gut microbiota in early programming of host responses also in humans.

**Establishment of the human gut microbiota**

The human gut microbiota evolves through ecological succession with compositional and
functional changes over the first years of life \(^28\), eventuating in a relatively stable microbial community \(^4\, 28\). This is influenced by interactions between the environment, diet, microbe-associated and host-related factors \(^29\, 30\). For the majority of humans, the first postnatal microbial exposures originate from maternal vaginal and perianal microbiota. Shortly after delivery, the gut microbiota of a vaginally delivered (VD) infant resembles that of their mother’s vagina \(^31\). In contrast, infants delivered by caesarean section (CS) initially acquire microbial communities typically found on maternal skin followed by the gradual acquisition of a more complex microbiota - typically more slowly than in VD infants. However there is high individual variability in the patterns of bacterial species, and the timing of acquisition \(^4\, 28\). Even so, across individuals and populations, infant microbiomes generally have a higher proportion of bifidobacteria and lower species richness than adults \(^28\) and share common functional characteristics – for example, infants typically show metagenomes enriched in genes for simple sugar breakdown and folate synthesis \(^4\, 28\). Microorganisms colonizing the gastrointestinal tract are involved in a number of vital processes \(^5\, 6\, 8\, 29\). With about 70% of the cellular component of the immune system present as gut-associated lymphoid tissue, the potential for crosstalk between microbiota and the immune system is substantial; and is likely to play a central role in enabling microbial imprinting and immune programming of the newborn microbiota \(^8\). Recent discoveries that bacterial DNA is present in the newborn’s first stool (meconium) \(^32\, 33\) and in the feto-placental unit \(^33\, 34\) with the placental microbiome profiles being most similar to the oral microbiome \(^34\), challenges the “sterile womb” paradigm. This suggests that the imprinting of gut microbiota may commence already in utero and is then further shaped by postnatal exposures. \(^8\, 30\, 33\)

**Environmental factors impact gut microbiota establishment in infants**

In addition to CS \(^31\, 35-37\), antibiotics to the mother or infant \(^29\), breastfeeding \(^35\, 37\) and
introduction of solid foods influence establishment of the gut microbiota. Infants born by CS have a higher incidence of respiratory distress and appear to be at higher risk for developing asthma and atopy, obesity and type 1 diabetes. It has been hypothesized that delayed and aberrant intestinal colonization might contribute to this risk conferred by CS. Recent studies that have applied DNA-based approaches have demonstrated perturbations in infant gut microbiomes with lower diversity of Bacteroidetes and abundance of the genus *Bacteroides* in CS-delivered infants suggesting that dysbiosis may, at least partly, contribute. Beyond its direct impact on microbial exposure, CS requires antibiotic treatment and can delay onset of breastfeeding, which can lead to further ecological perturbations. Retrospective studies have reported antibiotic treatment in early life to be associated with increased risk of asthma, although the results may still be influenced by reverse causation or confounding by indication.

The gut microbiota evolves over the preschool years with an adult-like profile emerging by 3-4 years of age in humans. Established microbiota of adult humans can be divided into different ”enterotypes” based on clusters of predominant bacterial genera in the gut microbiome. As yet, it is not clear how these enterotypes are formed, but this process is likely to be influenced by diet and other environmental factors. Recently, it has also been argued that there are gradients of key genera rather than distinct clusters.

**An aberrant gut microbiome in early and late onset NCDs**

Changes in the gut microbiome are the inevitable result of complex changes in both our nutritional patterns and our built environment. The consequences of early environmental change are evident in the dramatic rise in early onset inflammatory NCDs (such as allergic disease). Effects on the developing immune system are consistent with the ”hygiene hypothesis” which initially focused on declining exposures to ‘external’ infectious
microorganisms. A more recent focus on the "gut microbial deprivation hypothesis" has shifted the emphasis to the importance of gut microbial exposures for normal immune development and regulation. This concept is supported by culture-independent DNA-based studies demonstrating associations between reduced gut microbiota diversity and early onset NCDs including atopy, eczema, and asthma. However this has not been seen in all studies.

Also in intestinal inflammatory disease, several studies have now reported dysbiosis to be associated with IBD and celiac disease. Increased gut permeability and intestinal inflammation with impaired immune-regulatory mechanisms have been shown in children with allergic disease, and also in children with type 1 diabetes. Consistent with reports of dysbiosis in children with allergic manifestations, an aberrant gut microbiome has been associated with type 1 diabetes. 16S-sequencing revealed differences in the gut microbiome in children with signs of ß-cell autoimmunity compared to healthy children of the same age and sex, with similar feeding history and genetic risk. In particular, children with ß-cell autoimmunity had lower levels of bifidobacteria, which normally enhance intestinal epithelial barrier function and suppress inflammation. For comparison, using DGGE-PCR and q-PCR, dysbiosis was observed in diabetic children with the number of bifidobacteria, lactobacilli and the Firmicutes/Bacteroidetes ratio correlating negatively with plasma glucose levels whereas numbers of clostridia displayed a positive correlation.

It is also suggested that the early microbial environment drives more sustained predisposition to low-grade inflammation into adulthood and the propensity for later onset NCDs. In high-income countries baseline C-reactive protein (CRP), as a measure of inflammatory state, is higher than in traditional environments, and a risk factor for CVD, type 2 diabetes, and
have higher "all cause" mortality. A significant component of the risk of these disorders is programmed during early development, and many children already exhibit the inflammatory antecedents of cardio-metabolic risk. It has therefore been hypothesized that the early environment and early patterns of microbial exposure may influence the dynamics of inflammation in adulthood. While there has been a focus on infectious exposures, the commensal gut microbiota, which are also heavily influenced by early nutrition, are likely to play an even more important role in developing immune and metabolic homeostasis. Some support for this was recently reported in mice, where low-dose penicillin (LDP) in early life transiently impacted gut microbiota, affected ileal expression of genes involved in immunity and induced long-term metabolic perturbations. Notably, the combination of LDP and dietary excess further increased fat mass.

Although diet and a sedentary lifestyle are important contributors to the obesity epidemic, there is growing recognition that gut microbiota regulate metabolic function and energy balance. Studies in obese adults reported reduced diversity, an increase in Firmicutes and a corresponding decrease in Bacteroidetes, although this was not seen in all studies. Recently, a tendency towards decreased gut microbiota diversity in overweight/obese children assessed by q-PCR and T-RFLP was reported. Using FISH, reduced numbers of bifidobacteria in infancy have also been shown to precede development of obesity. Aberrations in the gut microbiota may also have implications for obesity-associated NCDs. In a recent report, adults with reduced gut microbial richness had higher overall adiposity, insulin resistance, dyslipidaemia and a more pronounced inflammatory phenotype than adults with higher gut microbial richness. Dysbiosis has even been associated with neurodevelopment and mental health in a series of experimental models. Whether microbial
variation is the cause or effect of these diseases is still the subject of conjecture, but this highlights the multisystem effects of the microbiome.

**Modulation of the gut microbiota for treatment and prevention of NCDs**

Collectively, this has led to intense interest in strategies to correct dysbiosis for treatment purposes and to favorably impact intestinal colonization to prevent NCDs (Figure I).

**Probiotics**

The most widely used approach has been to administer probiotics 69, (Table II). In most studies, single or several strains of lactobacilli and bifidobacteria have been used for treatment and prevention of allergic disease; the demonstrated effect of one probiotic strain cannot be extrapolated to another strain. Some probiotics exert immunomodulatory effects, mostly shown in experimental models but also in human intervention studies 8, 70. Mechanistic effects are thought to be mediated by Toll-like-receptors (TLR) to promote Th-1 cell differentiation, through enhanced production of IL-10, TGF-β and IgA, inhibition of antigen-induced T-cell activation and suppression of TNF 8, 70. The timing of probiotics for promoting immune tolerance appears critical 8, and a combination of pre and postnatal probiotic supplementation for allergy prevention (namely eczema) has shown the most consistent benefit, although their routine use cannot be recommended 8, 30, 70. To date, reports on later onset allergic manifestations e.g. respiratory allergic disease from initiated cohorts are limited and need to be prospectively collected and reported 8, 30, 70. If prenatal microbial exposure enhances the preventive effect, as also suggested by epidemiological 71, 72 and experimental studies 73, starting supplementation in the second trimester of pregnancy, when circulating fetal T cells have developed, may be more effective 8. This may include effects on asthma development, which so far probiotic interventions have failed to prevent 74-80. Using non-conventional probiotics such as butyrate and propionate producers, immunomodulatory strains of
bacteroides \(^8,^3^0\) or clostridia \(^8^1\) might also induce stronger effects. Another area to explore is that of probiotic treatment to CS-delivered infants, as data suggest that they might benefit the most \(^8^2\).

The preventive effects of probiotics on other NCDs, would be highly interesting to evaluate, but their low incidence and/or delayed onset compared with allergic diseases make such studies challenging to perform. Some probiotics have been demonstrated to exert beneficial metabolic effects in experimental models \(^8^3\) and in human intervention studies \(^8^4^-^8^7\). There is also growing appreciation that probiotics may have a role in gut-brain function and CNS-related conditions, (as reviewed by) \(^8^8\). However, more research is needed for the mechanistic understanding of probiotics and as of yet, probiotics do not have an established role in the treatment or prevention of any NCD. Many meta-analyses of clinical trials using probiotics have been carried out, however, there has been substantial heterogeneity in several aspects of these studies \(^1\). If we are to translate findings from conducted studies into recommendations in clinical practice, there is need for a more standardized approach using harmonized protocols and outcome measures in clinical trials \(^8,^7^0\).

**Dietary fiber and prebiotics**

Observational studies have reported an association between the high fat/low fiber Westernized diet and the prevalence of many NCDs, including allergic disease \(^8^9\). With these data in mind, modulation of the dietary substrate of gut microbiota (Table II) may be part of strategies for the prevention and treatment of NCDs. Dietary fiber pass through the upper intestine and are fermented by large bowel anaerobic microbiota to produce short-chain fatty acids (SCFAs) \(^9^0\). SCFAs promote gut epithelial integrity and exert immune effects including stimulation of G-protein-coupled receptors, promotion of innate (TLR2) immune responses, and induction of T regulatory cells in the colon \(^9^0\).
In experimental models, switching from a high to low fiber diet induces dramatic changes in gut microbiota composition; for example, a decrease in gut bacteria of the Bacteroidetes phylum \(^90\). Concordantly, infants in rural Africa, where mothers and infants have a traditional diet very high in fermentable carbohydrates, have substantially higher levels of Bacteroidetes and SCFAs in stool compared with infants from Europe, where mothers and infants have a Western diet \(^44\). In relation to early onset NCDs, there is preliminary evidence that feeding prebiotics (Table II), from early infancy may prevent eczema but these findings are yet to be confirmed \(^8,30,91\). In the most recent Cochrane review \(^91\), including 1428 infants at high risk of allergic disease, prebiotics added to infant feeds reduced the risk of eczema (risk ratio 0.68, 95% CI 0.48 to 0.97), with no effect on any other allergic outcomes.

There are also clinical trials that have assessed prebiotics for metabolic outcomes. In overweight adults, treatment with galactooligosaccharides induced “favorable” changes in gut microbial composition, increased secretory IgA (sIgA), reduced inflammation and measures of the metabolic syndrome \(^92\). Notably, the combination of a healthy dietary intervention (increased fiber/reduced fat) and probiotics in pregnancy decreased the risk of gestational diabetes compared with the healthy dietary intervention alone or no intervention \(^87\). Either alone, or in combination, dietary approaches may be a useful tool for gut microbiota modulation in both the treatment and prevention of NCDs. However, the effects of dietary modulation on gut microbiota and host response appear highly individual and difficult to foresee. Interestingly, in a proof of concept study combining microbiome data sets from different dietary intervention studies in obese adults, clostridial species in particular, indicated the amenability of the gut microbiota to dietary modulation, which in turn was associated with the host’s lipid metabolism \(^93\).
Fecal microbiota transplantation

There has been emerging interest in fecal microbiota transplantation (FMT) (Table II) in both the treatment and prevention of NCDs. It was first employed in the treatment of Clostridium difficile (C. difficile) diarrhea with a high rate of clinical resolution and no observed adverse events, although these initial trials were not controlled. The principle of FMT for this indication is predicated on the concept that antibiotic therapy (repeated courses or overuse of broad spectrum antibiotics) disrupts the normal ecology allowing colonization of C. difficile (Fig II). FMT offers both an investigational tool in rodent models to study the role of microbes in disease development and treatment response, as well as a new therapeutic intervention that has gained credibility in the clinical world following the publication of the first RCT demonstrating the effectiveness of FMT for treatment of antibiotic resistant C. difficile diarrhea. The authors also demonstrated a significant increase in Bacteroidetes species and Clostridium clusters IV and XIVa, and a decrease in Proteobacteria species in line with healthy donor profiles.

Despite the lack of clear evidence whether microbial variation is the cause or effect of IBD, there are now several trials of FMT for treatment of IBD with modest results, although no controlled trials have yet been published. FMT might be a future treatment option for many conditions, however, a truly controlled trial design might consider autologous versus allogeneic fecal transplant to ensure there is not a colonoscopy or placebo effect. There are also many recipient and donor factors to consider (Table III). Age is an important factor for both the donor and the recipient as there is significant increase in diversity and stability of gut microbiota over the first years of life. Compositional differences may affect susceptibility to disease, but also response to treatment. Factors that might influence the composition of the donor sample include diet, antibiotics/probiotics and a range of environmental exposures, as
well as underlying disease status. From a practical, clinical perspective the route of administration is important and at least for the treatment of *C. difficile*, colonoscopic and nasogastric administration appears similarly effective, although a higher stool volume was used for FMT in the colonoscopy group. Notably, standardized partially purified and frozen fecal microbiota might be just as effective as the current fresh harvest approach, which might facilitate its clinical use.

**Future directions**

While NCDs represent a wide range of diseases we can see common threads that trace back to increased tendency of inflammation, driven by virtually inseparable changes in nutrition and the gut microbiota. The end result of the inflammatory and metabolic changes might vary with genetics, epigenetics, the overall biodiversity and other risk factors, this provides an attractive shared explanation for why so many of these inflammatory conditions have risen during the same time period, under the influence of the same environmental changes. If we are to stand a better chance of solving the complex pathogenesis of NCDs and to understand the role of gut microbiota in this process, there is a call for interdisciplinary approaches, working collaboratively in networks. With standardized outcomes and methodologies we will be better apt to address current knowledge gaps (Table III):

1) Research should be focused on the ecological and functional properties of "a healthy gut microbiome"; results from the Human Microbiome Project and MetaHit are anticipated to provide more insight
2) Strategies to improve gut colonization patterns need to be developed since they may have life-long influences on health
3) Although dysbiosis and reduced diversity is centrally implicated in many NCDs, it still remains to be determined if microbial variation is the cause or effect
4) To overcome potential confounding, longitudinal studies
using the same protocols and methodological platforms are needed 5) Looking at the bacterial components of the microbiome might be overly simplistic and should ultimately include other aspects of the microbiome 6) A research priority should be the development of microbiota signatures in specific disease states 7) Ultimately, these can then be used in clinical practice for improved diagnostic, treatment and prevention strategies that may include optimized dietary interventions, prebiotics, non-conventional indigenous gut bacteria as “next-generation probiotics”, and FMT. Such multidisciplinary and integrative approaches may ultimately lead to improved strategies to overcome the disease epidemics of modern civilizations.

What do we know

- Experimental models demonstrate that gut microbiota exert physiological, metabolic and nutritional functions, and are vital for normal immune development and regulation
- Observational studies show variations of gut microbiota and reduced microbial diversity to be associated with various NCDs such as eczema, allergic rhinitis, asthma, autoimmune disease and obesity
- Meta-analyses of clinical trials using probiotics for allergy prevention have demonstrated a reduced incidence of eczema, but not any other allergic outcomes
- Meta-analyses of a limited number of clinical trials using prebiotics for allergy prevention suggest a reduction of eczema, but only in high-risk populations
- Fecal microbiota transplantation regulates inflammation in experimental models

What is still unknown?

- If the dysbiosis and reduced microbial diversity seen in many NCDs is causal
- How probiotics mediate their clinical effects in NCDs
• If prebiotics has a role in prevention of allergic disease in high-risk and low-risk populations

• What the long term effects of fecal microbiota transplantation are in humans- clinical benefit as well as safety aspects
**Table I.** Example of culture-independent methods used in studies assessing the role of gut microbiota in non-communicable diseases (NCDs)

<table>
<thead>
<tr>
<th>Method</th>
<th>Explanation</th>
<th>Benefits</th>
<th>Weaknesses</th>
<th>Insights from studies using the method in the context of NCDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>q-PCR</td>
<td>Amplification and quantification of 16S rRNA.</td>
<td>Confirms phylogenetic identity, quantitative, quick.</td>
<td>Cannot identify unknown species, PCR bias.</td>
<td>Mucosal biopsies from patients with inflammatory bowel disease revealed abnormal gastrointestinal microbiota composition depleted of commensal bacteria (Frank et al, 2007); Delivery mode and exposure to older siblings affected gut microbiota composition assessed in infant stool samples and impacted subsequent risk of allergic disease (van Nimwegen et al, 2011, Penders et al, 2013). Studies combining DGGE with q-PCR reported reduced microbial diversity in stool samples at 1 and 12 months of age in children developing sensitization, allergic rhinitis and blood eosinophilia but not eczema or asthma at school age (Bisgaard et al, 2011); and reported compositional changes in stool samples of diabetic compared with healthy children. The numbers of bifidobacteria and lactobacilli, and the Firmicutes/Bacteroidetes ratio correlated negatively with plasma</td>
</tr>
<tr>
<td>DGGE</td>
<td>Gel separation of 16S rRNA amplicons using denaturant.</td>
<td>Semi-quantitative, bands can be excised for additional analysis, quick.</td>
<td>Cannot confirm phylogenetic identification, PCR bias.</td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T-RFLP</strong></td>
<td>Amplification of fluorescently labeled primers, followed by digestion of the 16S rRNA amplicon by restriction enzymes. Separation of digested fragments by gel electrophoresis.</td>
<td>Semi-quantitative, quick, cheap.</td>
<td>Cannot confirm phylogenetic identification, PCR bias, weak resolution.</td>
</tr>
<tr>
<td><strong>FISH</strong></td>
<td>Hybridization of fluorescently labeled oligonucleotide probes with complementary target 16S rRNA sequences. Fluorescence enumerated by flow cytometry.</td>
<td>Confirms phylogenetic identity, semi-quantitative, no PCR bias.</td>
<td>Dependent on probe sequences and cannot identify unknown species.</td>
</tr>
<tr>
<td><strong>DNA microarrays</strong></td>
<td>Hybridization of fluorescently labeled oligonucleotide probes with complementary nucleotide sequences. Laser used to detect fluorescence.</td>
<td>Confirms phylogenetic identity, semi-quantitative, quick.</td>
<td>Cross hybridization, PCR bias, may miss species present in low levels.</td>
</tr>
<tr>
<td><strong>Sequencing of 16S rRNA ampli-</strong></td>
<td>Massive parallel sequencing of partial 16S rRNA amplicons.</td>
<td>Confirms phylogenetic identity but not at species level,</td>
<td>Labor-intensive, expensive, PCR and amplification bias. Need for</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reduced total microbial diversity and diversity of Bacteroidetes in stool samples at 1 month of age was associated with</td>
</tr>
<tr>
<td>Cons</td>
<td>Quantitative, quick, identifies unknown bacteria.</td>
<td>Computational expertise.</td>
<td>Increased risk of subsequent IgE-associated eczema (Abrahamsson et al., 2012); reduced total diversity was also associated with increased risk of asthma but not allergic rhinitis at school age (Abrahamsson et al., 2014). A low abundance of lactate- and butyrate-producing species in stool samples was associated with β-cell autoimmunity (de Goffau et al., 2013);</td>
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<tr>
<td>Meta-genome sequencing</td>
<td>Massive parallel sequencing of the whole genome.</td>
<td>Confirms phylogenetic identity, quantitative.</td>
<td>Most expensive method, generates large amounts of data that need computational expertise.</td>
</tr>
</tbody>
</table>

q-PCR- quantitative PCR, DGGE- denaturing gradient gel electrophoresis, T-RFLP- terminal restriction fragment length polymorphism, FISH- fluorescence in situ hybridization

Table II. Treatment strategies for gut microbiota modulation in non-communicable diseases (NCDs)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Definition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary fiber</td>
<td>Non-digestible carbohydrates of plant origin</td>
<td>Thorburn et al, 2014</td>
</tr>
</tbody>
</table>
Table III. Current knowledge gaps of the role of gut microbiota in non-communicable diseases (NCDs) and suggestions for further work

<table>
<thead>
<tr>
<th>Current situation</th>
<th>References</th>
<th>Future prospects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential confounding in observational studies because of lack of information on all exposures that may have affected the gut microbiota and/or short period of observation.</td>
<td>Lozupone et al, 2012 Gevers et al, 2012 Aagard et al, 2013</td>
<td>Longitudinal studies in multiple settings using the same protocols and methodological platforms, which may enable direct comparison of studies.</td>
</tr>
</tbody>
</table>

NCDs- non-communicable diseases, FMT- fecal microbiota transplantation
Figure legends

Fig I.
Dysbiosis- an “imbalance in the structure and/or function of the microbiota that leads to disruption of host-microorganism homeostasis” has been implicated in a broad range of inflammatory disease states. There is also suggestive evidence that changes in gut microbiota have implications for cognitive and mental health dysfunction, and stress responses. These diverse multisystem influences have sparked interest in strategies to favorably modulate the gut microbiota to attain homeostasis.

Fig II.
Overuse or repeated courses of broadspectrum antibiotics disrupts the normal ecology allowing colonization of Clostridium difficile (C.difficile) leading to dysbiosis. Fecal microbiota transplantation (FMT) with feces from a healthy donor administered via A) colonoscopic or B) nasogastric routes restores microbial ecology and resolves inflammation.
References

11. Wold AE. The hygiene hypothesis revised: is the rising frequency of allergy due to changes in the intestinal flora? Allergy 1998; 53:20-5.


