EDITORIAL

Diabetes Management

Microbe-based approaches for the treatment of diabetes

"Until now most of the studies conducted have been association studies without proof of causality."

Mikael Knip*[1,2,3,4] & Heli Siljander[1,2]

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The adult human intestinal microbiota comprises 10^{13}–10^{14} microorganisms, and the gut microbiome, in other words, the aggregate genome of all microorganisms, is around 100-times larger than the human genome. Intestinal dysbiosis is defined as a state of imbalance of the gut microbial ecosystem, including the overgrowth of some organisms and the loss of others. Dysbiosis has been implicated in a series of major chronic diseases such as Type 1 diabetes and obesity and Type 2 diabetes [1,2]. These observations raise the issue of whether measures aimed at restoring the balance of the microbiota and correcting the dysbiosis could be applied for the prevention and/or treatment of these metabolic disorders.

In the context of Type 1 diabetes it has been reported, based on prospective studies, that autoantibody-positive children en route to clinical disease are characterized by a decreased bacterial and functional diversity and a reduced community stability when compared with autoantibody-negative controls matched for gender, date of birth and HLA genotype [3,4]. Bacteroidetes seems to be the dominant phylum among autoantibody-positive children and at the genus level an increased abundance of *Bacteroides* and *Clostridium* was observed [3,5], although not consistently so [6]. In addition, the autoantibody-positive children, who later progressed to clinical Type 1 diabetes or carried a high risk of progression, were characterized by a decreased abundance of butyrate-producing bacteria [5,7]. A recent prospective study with monthly stool samples starting from birth showed that the difference in microbial diversity between seroconverters and controls emerged after seroconversion to autoantibody positivity [4] indicating that the altered microbiota may be involved in the progression from β-cell autoimmunity to overt diabetes rather than in the initiation of β-cell autoimmunity. A very recent study comparing the intestinal microbiota in children with HLA-conferred diabetes susceptibility derived from six centers taking part in the international TEDDY study showed clear significant differences in the bacterial diversity and in the abundance of several bacterial

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[1]Children's Hospital, University of Helsinki, Helsinki University Hospital, Helsinki, Finland
[2]Research Programs Unit, Diabetes & Obesity, University of Helsinki, Helsinki, Finland
[3]Folkhälsan Research Center, Helsinki, Finland
[4]Tampere Centre for Child Health Research, Tampere University Hospital, Tampere, Finland
*Author for correspondence: Tel.: +358 947 172 701; Fax: +358 947 175 299; mikael.knip@helsinki.fi

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genera such as *Bifidobacterium*, *Veillonella* and *Faecalibacterium*, although *Bacteroides* was the predominant genus in all six centers without any significant difference in its abundance [8]. This observation underscores that geographical and ethnic background should be taken into consideration when analyzing microbiome data.

Obesity is a strong risk factor for Type 2 diabetes, and it is associated with alterations in the composition of the gut microbiome [9]. Experimental data indicate that the obese microbiome is more efficient in harvesting energy from the diet [10]. Initial studies [11] reported an increased abundance of *Firmicutes* and a decreased abundance of *Bacteroidetes* in obese subjects. More recent studies have, however, been unable to confirm that observation [12] or found an opposite association [13]. This controversy may be due to differences in study design and/or in the methodology applied. Both obesity and Type 2 diabetes are associated with a decreased microbial diversity and a reduced abundance of butyrate-producing bacteria in the gut [10]. Patients with Type 2 diabetes have been observed to have an increased abundance of *Firmicutes* and a decreased abundance of *Bacteroidetes* in their intestinal microbiota. They are also characterized by a reduced abundance of butyrate-producing bacteria such as *Roseburia*, *Eubacterium halii* and *Faecalibacterium prausnitzii* as reported by both Qin *et al.* [13] in a Chinese study population and by Karlsson *et al.* in a European population [14]. There were, however, also some differences in the outcomes of these two studies. The Chinese study reported an enrichment of *Proteobacteria* in patients with Type 2 diabetes and a reduced number of genes involved in cofactor and vitamin pathways, while the European study observed an increased abundance of *Lactobacillus gasseri* and *Streptococcus mutans* and higher numbers of genes playing a role in the oxidative stress response in subjects affected by Type 2 diabetes. Taken together there is a clear evidence that the gut microbiota influences the host through its effect on bodyweight, bile acid metabolism, proinflammatory activity and insulin resistance as well as modulation of gut hormones [10].

"Diet has been shown to have a strong effect on the composition of the gut microbiome."

The expanding knowledge of the intestinal microbiome and its role in Type 1 and Type 2 diabetes paves the way for exploring therapeutic modalities to fight these diseases characterized by an increasing incidence and prevalence globally. The type of potential therapeutic approaches ranges from interventions based on dietary changes or the use of single bioactive produced by microbes to ecosystem-level interventions that completely reset an individual's microbiota such as fetal microbiota transplantation (FMT) [15]. Diet has been shown to have a strong effect on the composition of the gut microbiome [16]. Diet-induced weight loss was associated with an increased microbial gene richness and a reduced systemic inflammation when compared with a weight stabilization intervention in overweight and obese subjects [17]. Probiotics are microorganisms that are ingested either in combination or as a single organism in an effort to normalize gut microbiota and potentially improve intestinal barrier function. Early administration of a probiotic compound comprising nine bacteria reduced the incidence of autoimmune diabetes substantially in NOD mice [18]. Observational data from the TEDDY study suggest that early probiotic supplementation may reduce the risk of β-cell autoimmunity in children at increased genetic risk for Type 1 diabetes [19]. On the other hand, probiotic supplementation was most common in Finland, the country with the highest incidence of Type 1 diabetes in the world. There are no intervention studies assessing the effect of probiotic administration on β-cell autoimmunity and progression to clinical Type 1 diabetes. Antidiabetic effects have been observed in mice after administration of probiotics containing certain *Lactobacillus* strains [20]. This was associated with reduced endotoxemia, in other words, endotoxic compounds from gram-negative intestinal bacteria entering the peripheral circulation, resulting from a reduced abundance of *Bifidobacteria* in the gut [21]. Endotoxemia is linked to decreased insulin sensitivity and impaired glucose tolerance. Prebiotics are nondigestable substances acting as a nutritive substrate to stimulate the growth and metabolism of protective endogenous enteric bacteria. This group of substances includes, for example, galacto-oligosaccharides, fructo-oligosaccharides and inulin. A high-fat diet is associated with insulin resistance induced by endotoxemia. When oligofructose, a prebiotic, was fed to mice on a high-fat diet, the intestinal abundance of *Bifidobacteria* was restored leading to decreased endotoxemia and improved glucose tolerance [22].

One step up from the use of probiotic preparations is the application of consortia of defined, cultured bacterial strains [23]. Such an approach

has been used successfully in experimental studies to prevent autoimmune diseases and to treat relapsing *Clostridium difficile* infections (CDI) [24,25]. A proof-of-principle human study was performed recently in two patients with antibiotic-resistant CDIs resulting in a rapid and persisting cure in both patients [26]. This therapeutic alternative has not so far been tested in diabetes. Although this approach provides advantages when compared with FMT in terms of safety, stability, controllability and palatability, there are still a series of open questions to be answered such as what happens to the displaced microbiota and what are the long-term effects of microbial ecosystem therapeutics of this type before this strategy may be integrated into mainstream medicine.

The first clinical use of FMT, which is the most dramatic manipulation of the intestinal microbiome composition, was the successful treatment of unremitting CDI. Subsequently, FMT has been observed to be effective in other chronic gastrointestinal infections and inflammatory bowel diseases [27,28]. The therapeutic potential of FMT has been attributed to its ability to restore the intestinal microbial balance by replacing pathogens with beneficial bacterial strains. A recent randomized clinical trial in insulin-resistant male with the metabolic syndrome, who were given either autologous or allogenic feces infusion from a lean donor via a gastroduodenal tube, demonstrated improved insulin sensitivity in those receiving 'lean' feces [29]. That treatment also resulted in a significantly increased gut microbial diversity and in an increased abundance of butyrate-producing bacteria, such as *Roseburia* in stool and *E. halii* in the small intestine. The trial also showed that not all lean donors exerted the same beneficial effect in the obese host raising the issue whether there are 'super' donors. Although the first results are promising we need larger, well-designed trials to confirm efficacy and lack of severe adverse events before moving FMT into the clinical tool box for treating obesity and Type 2 diabetes.

Our knowledge of the intestinal microbiota and microbiome and their potential role in the development of diabetes and other chronic diseases has advanced rapidly over the last decade. Still there is a lot more to learn. Standardized operating procedures should be applied for sampling, storage of samples and extraction of DNA and RNA. Better working tools, such as improved DNA sequencing technology and advances in data analysis tools, will further accelerate the generation of new knowledge. Optimization of study design is another important consideration. Until now most of the studies conducted have been association studies without proof of causality. Some of the identified associations may turn out to have no role in driving disease processes. Nevertheless new therapeutic strategies have been identified. Various interventions are conceivable, such as the administration of prebiotics, probiotics or cultured gut microbial components or the application of FMT.

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