The role of diet-induced gut dysbiosis in the development of Type 2 diabetes

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Abstract

Metabolic disorders are a group of diseases resulting from changes in normal metabolic processes. Since the completion of the Human Microbiome Project, further research has identified the important contribution of the gut microbiota to the host's overall metabolism. Subsequent study has revealed relationships between different compositions of gut microbiota and metabolic diseases, such as Type 2 diabetes (T2D). T2D is characterised by high blood glucose levels, because of impaired glucose metabolism. This review aims to highlight key studies that link changes in diet to differences in gut microbiota and the role of certain genera in T2D. It will then cover the current understanding of the molecular detail in which their metabolites act, which is understood to be important in the development of T2D, specifically their effect on insulin sensitivity, and therapeutic strategies.

Introduction

Type 2 diabetes (T2D) is a highly preventable metabolic disease characterised by high blood glucose levels and low-grade inflammation. The number of people living with diabetes has doubled to 4.6 million over the last 20 years in the UK[1]. Almost 90% of people diagnosed with diabetes suffer from type 2 with a further 12.3 million people at increased risk of developing it[1]. T2D causes complications around the body, particularly in the cardiovascular system[2]. The characteristic high blood glucose is a result of the body developing insulin resistance and not producing enough insulin[2]. The most common cause is thought to be a combination of excessive body weight and insufficient exercise[2]; however, the exact cause is unknown. Given breakthroughs in sequencing technologies, there is an expanding body of literature linking gut dysbiosis, a state of microbial imbalance, to metabolic diseases[3]. This literature suggests metabolites of the gut microbiota have roles in the pathogenesis of metabolic diseases, like T2D, which coordinate different functions throughout the body[4]. The fields of genomics, transcriptomics, proteomics and metabolomics are advancing rapidly allowing for the gut microbiome to be better understood. This review highlights how different diets result in different compositions of the microbiota, how dysbiosis is implicated in T2D and the potential mechanisms of microbial metabolites' action on insulin sensitivity. The metabolites are also thought to be important in low-grade inflammation and increased gut permeability, which are both associated with T2D and other metabolic disorders, such as alcoholic fatty liver disease

and irritable bowel syndrome[4]. Finally, this review also explores how the gut microbiome is being targeted for therapeutic intervention.

Gut microbiota

The gut microbiota refers to a hugely dynamic community of diverse bacteria, archaea and eukarya that can be found lining the digestive tract of humans. These organisms make up an estimated 90% of all the cells in a human body[5]. At birth, the entire digestive tract is sterile before it is quickly colonised by different sources of bacteria from the mother and the environment[3]. At around 2.5 years old the composition of the gut resembles that of an adult with 90% of bacteria belonging to the *Bacteroidetes* (gram-negative) or *Firmicutes* (gram-positive) phyla and is believed to remain stable throughout life[3]. The specific compositions are unique to an individual due to their genotype and phenotype, colonisation history and environmental factors, food, drugs and pollutants[6].

A mutualistic relationship is seen between the gut microbiota and the host as it plays many beneficial roles. These include protection from pathogens, immunomodulation, maintenance of the intestinal epithelium, vitamin production, xenobiotic and drug metabolism and the breakdown of compounds that would otherwise be excreted[7]. The metabolites derived from the microbial breakdown of substrates in the gut can function in a similar way to hormones, shown in figure 1. The gut microbiota is hence beginning to be considered as an endocrine organ[5]. If so, it would be the most biochemically heterogeneous endocrine organ in the body due to its potential to produce hundreds of different metabolites, dependent on the available substrate[5]. The highest number of microorganisms is seen in the large intestine with 10^{12} per gram of content compared to 10^{7} in the small intestine[8]. The microorganisms in the proximal colon ferment indigestible carbohydrates via saccharolytic fermentation mostly into short-chain fatty acids (SCFAS)[4]. As the indigestible carbohydrates are used up, microbes in the distal colon gain energy from residual protein and peptides instead, utilising proteolytic fermentation producing a wide range of metabolites including branched-chain amino acids (BCAA)[4]. The specific genera that contribute towards each pathway are not very well understood but some species have been identified and are shown in figure 2.

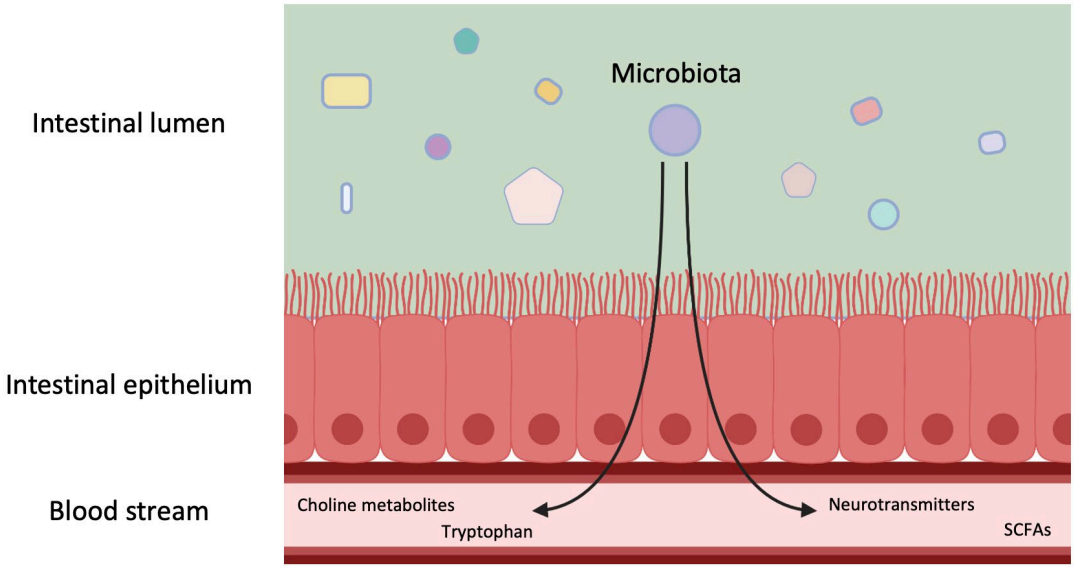

Figure 1: *The passage of gut microbial metabolites across the epithelium into the blood where they behave like hormones, coordinating various responses around the body. Created with BioRender.com.*

Dysbiosis

The microbiota composition has been shown to remain stable yet diverse throughout life. The microbiome of healthy adults is dominated by *Bacteroidetes* and *Firmicutes*[3]. However, the use of antibiotics, alcohol abuse and poor diet can result in a state of dysbiosis, an unbalanced microbiota displaying an increased presence of detrimental microbes, lower species diversity and potentially pathobionts[3].

There are significant differences between the combined genetic material of gut microorganisms, the microbiomes, in adults from the US and adults from Malawi[9]. These can be explained by differences in diet. US adult microbiomes are overrepresented by enzymes that break down amino acids and simple sugars because of a diet rich in protein and simple sugars, but deficient in fiber[9]. The enzymes of the microbiome of Malawian adults are proportionally represented by glutamate (an SCFA) synthase enzymes, but overrepresented by enzymes involved in the breakdown of starch as a result of a diet high in complex carbohydrates and relatively lower in protein[9]. These differences are reflected in the microbiome of both carnivorous and herbivorous mammals, showing that diet is a major influencer of the microbiota[9]. The microbiota of a US adult has become dominated by species that utilize the increased supply of protein and simple sugars.

Dysbiosis influences the progression of metabolic disorders because low species diversity is associated with insulin resistance and low-grade inflammation, the markers in T2D[10]. Two large metagenome-wide association studies carried out in Europe and China found a moderate dysbiosis in the microbiota of subjects with T2D, with variation between the two

compositions also a result of other factors[11,12]. A consistent observation was the reduced amount of butyrate (an SCFA) producing bacteria, *Roseburia intestinalis* and *Faecalibacterium prausnitzii*, and an increased amount of pathogenic strains and certain *Lactobacillus* species in the microbiota of T2D subjects of both studies. In a separate study, the oral administration of vancomycin to obese males decreased the amount of *Faecalibacterium prausnitzii* and other butyrate-producing bacteria and also insulin sensitivity, suggesting a protective role for butyrate[13]. This protective role of butyrate was also shown in a study where the microbiota of lean donors was infused into the microbiota of obese males[14]. After six weeks, the obese males showed a significant increase in microbiota across 16 different genera with a 2.5-fold increase in the amount of butyrate-producing *Roseburia intestinalis* and an increase in insulin sensitivity.

A Danish study found increased levels of BCAAs in the serum of patients with decreased insulin sensitivity[15]. This can be explained by increased numbers of certain microorganism species that utilise protein as their primary substrate such as *Prevotellacopri* and *Bacteroides vulgatus* and also their reduced transport into cells. This paper links a low diversity microbiome enriched in BCAA biosynthesis, like the aforementioned microbiome of a US adult, to T2D whilst also suggesting a causative role for BCAAs. However, the role of *Bacteroides vulgatus* has been shown to be beneficial in another study by upregulating the genes involved in tight junctions and decreasing gut permeability[16]. Increased gut permeability is another hallmark of T2D and can cause endotoxemia[3]. Contrasting studies often suggest opposite roles for the same genera, creating a highly contradictory body of literature. The ratio of Firmicutes to Bacteroidetes is believed to be a good biomarker for T2D. However, the two previously mentioned metagenome-wide association studies[11, 12] both report a lower ratio in T2D, but more recent studies have found the ratio to be higher in patients with T2D[17, 18].

The literature shows a consistent link between dysbiosis and T2D. However, specific microbiota species have been linked to both protective and causative roles in different studies. Even *Bifidobacterium,* the most commonly reported genus in playing a protective role has been contradicted by M. Sasaki *et al*[19]. The differences seen across the literature in identifying individual species are a result of the huge number of different species of bacteria that can inhabit our digestive tracts as well as the influences of other factors that are impossible to control in large scale studies. These discrepancies highlight the caution required when defining a species role in T2D and the need for further study. A study that tracks the change in the composition of microbiota with a change in diet as T2D progresses would provide useful data for identifying key species and understanding the pathology of T2D.

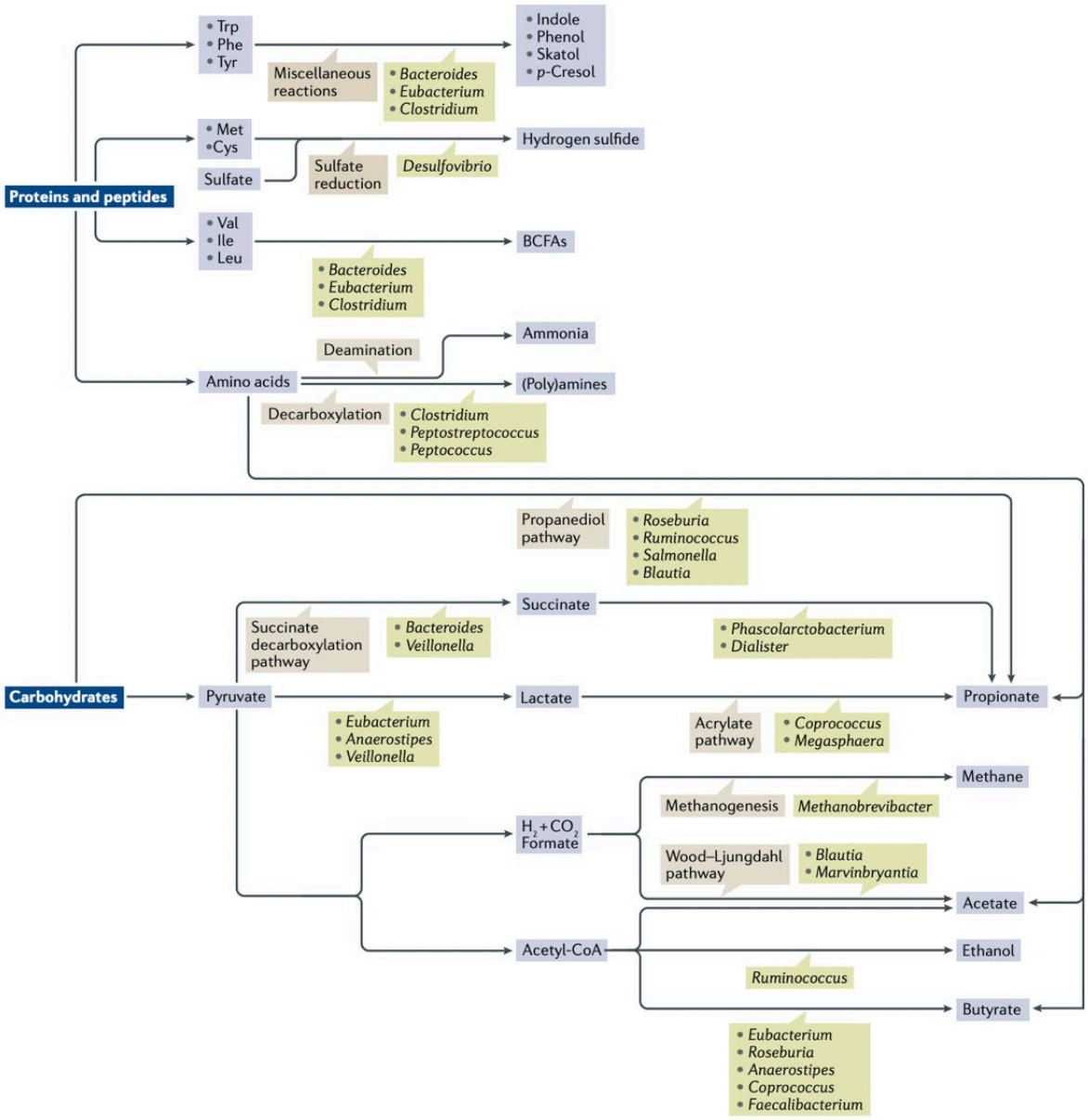

Figure 2: *A few of the metabolic pathways for the gut microbial fermentation of proteins and carbohydrates. Certain genera are also shown alongside some examples of the range of products of the two main fermentation pathways (Figure 1 from[4]).*

Metabolites

In the previous section, an altered microbiota composition has been linked to T2D, due to the action of their metabolites, shown in figure 3, with certain genera suggested as protecting against insulin resistance. This is potentially due to their ability to produce SCFAs.

These SCFA (saccharolytic fermentation products) have been shown to improve insulin sensitivity in obese animals[20] and play important roles in insulin sensitivity and inflammatory

status in humans[21]. Insulin is a peptide hormone, secreted from beta-cells found in the islets of Langerhans[22]. Insulin acts on skeletal muscle and fat cells causing them to take up glucose, reducing blood glucose concentration[22]. SCFA can act both indirectly and directly to improve insulin sensitivity. They bind primarily to two receptors GPCR41 and GPCR43 which are expressed on the membrane of enteroendocrine cells and in the islets of Langerhans[23, 24]. Insulin secretion depends on the correct function and mass of beta-cells. Sodium butyrate has been shown to trigger the expression of early pancreatic development genes in embryonic stem cells, suggesting a protective role by maintaining b-cell mass[25]. In line with this finding, administrating butyrate to diabetic rats was followed by an increase in b-cell differentiation and a reduction in apoptosis and also lower blood glucose levels[26]. This finding suggests that butyrate might function in a similar way to HDAC inhibitors as they promote the development and proliferation of beta-cells.

However, insulin secretion is affected indirectly by SCFA as they first trigger an increase in glucagon-like peptide-1 (GLP-1) to enhance insulin secretion[27]. GLP-1 is an incretin hormone secreted from enteroendocrine cells, found in the epithelium of the intestines[27]. In *vitro*, acetate and propionate stimulate GPCR43 and not GPCR41, in mice colon cultures, to secrete GLP-1[28]. In *vivo*, GPCR43 knockout mice show a significant decrease in GLP-1, highlighting the importance of GPCR43[28]. Further study into the long-term effect of this knockout would be beneficial in elucidating the relative contribution of SCFA dependent GLP-1 secretion in total insulin secretion. GPCR41 stimulation has been shown to result in the secretion of peptide YY in animals, which induces satiety, an important factor in T2D and obesity[29]. The three most common SCFAs, propionate, butyrate and acetate, have been shown in numerous studies to display protective roles against the development of T2D. SCFA can display anti-inflammatory effects by reducing the secretion of proinflammatory cytokines which also improves insulin sensitivity[4], but is not discussed in this review.

The microbiota can change and the increased capacity for proteolytic fermentation has been linked to T2D[15], again through metabolites functioning like hormones. Proteolytic fermentation produces a wider range of metabolites that are considered to be detrimental to metabolic health[4]. Histidine, a common amino acid, is converted to Imidazole propionate, a BCAA, by gut bacteria which can impair insulin sensitivity through mTORC1[30]. P-cresyl sulfate is a product of tyrosine metabolism by the gut microbiota and its direct administration to mice resulted in peripheral insulin and ectopic fat storage in the liver and glucose[31]. These two studies start to offer potential molecular mechanisms of detrimental metabolites as a result of a change in microbiota composition. Hydrogen sulphide, another product of proteolytic fermentation, has also been shown to have a detrimental effect by causing apoptosis in

cultured rat beta-cells[32]. However, further study in mice has suggested a contrary protective role, by preventing beta-cell apoptosis[33]. This result has been reflected in human studies, with T2D patients showing lower blood concentrations of hydrogen sulphide than the controls[34]. These contradictory results show the importance of the further study of the proteolytic metabolites contribution to T2D, as a lot less molecular detail is known relative to saccharolytic metabolites.

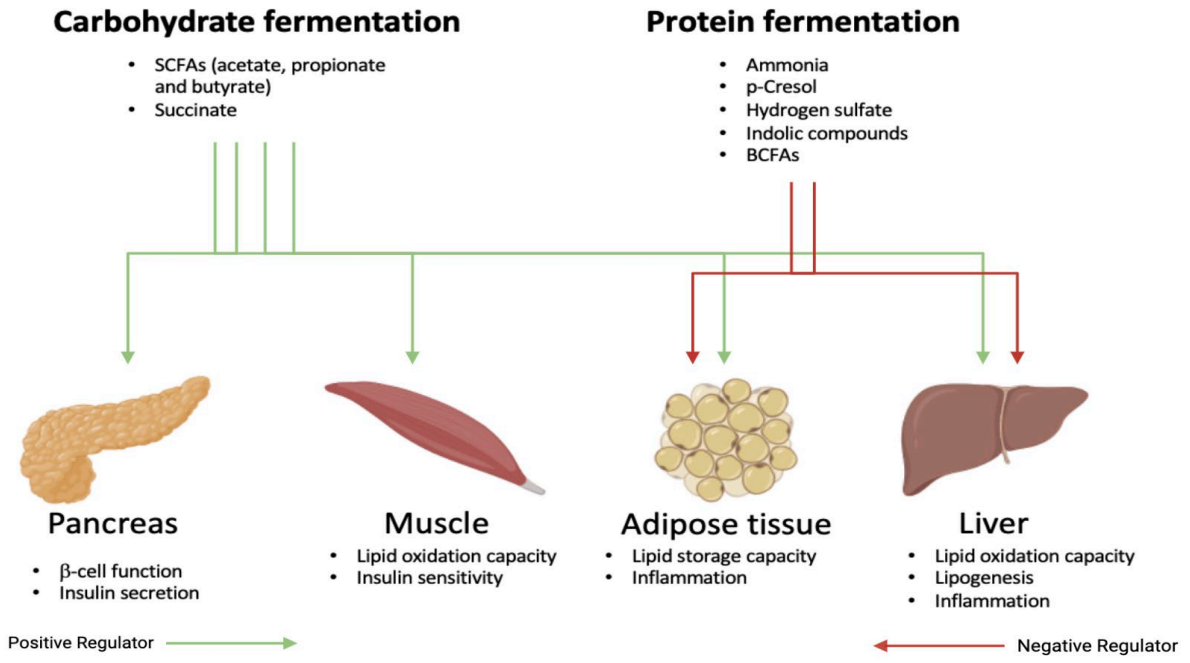

Figure 3: *The different tissues affected by microbial metabolites that result in changes in insulin sensitivity and the other hallmarks of T2D. Created with BioRender.com.*

Therapeutics

The simplest and most common treatment for T2D is weight loss[2]. This involves changing to a diet low in sugar and simple carbohydrates (glucose) which aims to change the gut microbiota. This change attempts to create a microbiota composition associated with the previously described 'protective state' by increasing the number of beneficial microorganisms. Epidemiological studies consistently show that diets low in fibre are positively associated with T2D hence the maintenance or reintroduction of fibre into the diet is key in the prevention or treatment of T2D[35]. Treatments like faecal matter transplantation (FMT) and probiotics involving the direct administration of considered beneficial bacteria have increased in popularity, but their benefits are backed only by limited human studies. There has only been one FMT study in humans, which caused a positive change in composition and an increase in

insulin sensitivity[14]. New treatments are being developed due to the increased understanding of the relationship between gut microbiota and T2D. Antibiotics have been shown to greatly modify the gut microbiota in diabetic mice but also improve insulin sensitivity[36]. A future strategy may involve the administration of a highly specific antibiotic to reduce the number of detrimental species. It is known that gut microbiota breaks down drugs which affect their pharmacokinetics and pharmacodynamics[37]. Interestingly the major drug for the treatment of T2D metformin, which has multiple mechanisms of reducing blood glucose levels and has been used in humans since 1957, has recently been shown to alter the composition of gut microbiota[38, 39]. One study reported that the combination of anti-diabetic drugs metformin and sitagliptin with prebiotic polysaccharide reduced hyperglycaemia in Zucker diabetic rats more than just the drugs alone[40]. Another similar study also reported similar success in using a combination of metformin and prebiotic mannan-oligosaccharide to improve insulin sensitivity in diabetic mice over just the drug alone[41]. Further studies are required to see how different anti-diabetic drugs affect the microbiota and to identify the species. A genetically engineered *Lactococcus lactis* strain, containing the GLP-1 gene, was delivered to mice on high-fat diets which resulted in an increase in insulin sensitivity and secretion compared to the controls[42]. Further study is needed to enable its use for humans, but this experiment is key to showing the potential for another type of treatment. There is huge potential for highly personalised treatment for T2D but only when the changes in composition and the molecular mechanisms are fully understood.

Discussion

There is a vast amount of evidence supporting the role of diet-induced gut dysbiosis in T2D pathology. However, the finer details of the mechanisms and magnitude behind the changes in composition and metabolite action are yet to be solved. The presence of other factors, other than diet, that influence the composition of microbiota increase the difficulty of the challenge of solving the finer details. Hence, they contribute to the inconsistencies seen in the literature. Most of the studies into microbiomes analyse the genomes from faecal samples in which the gut microbiome is not accurately represented. Also, the current sequencing and analysis techniques do not identify microorganisms at a species level. Substantial animal data is suggesting a protective role for the metabolites produced from saccharolytic fermentation. However, the metabolites of proteolytic fermentation have been shown to be detrimental in a limited number of studies. Similarly, the effects of metabolites on the metabolism also show inconsistencies, mostly proteolytic fermentation metabolites. The studies mostly involve the effect of a single metabolite and do not consider the accumulative effect that would be seen in *vivo*. It is not known whether it is the loss of the SCFA's protective role, the gained effect of

potentially detrimental proteolytic metabolites or the balance of both being the reason for metabolites for causing T2D. Crucially, the development of a non-invasive technique for collecting samples from the digestive tracts of patients would allow for the whole gut microbiome to be characterised. The simultaneous development in sequencing technologies would allow for better resolution in identifying microbial species. These advancements would result in a better understanding of different compositions and the effects of different compositions at distinct locations within the gut itself. The use of labelled substrates would enable us to fully characterise the metabolome, see their targets in the body and decipher the molecular mechanisms behind T2D pathogenesis.

This emerging field is in its early stages but is nonetheless very exciting. The inconsistencies highlight the need for further study, with the development of new techniques important to understand the details of T2D progression and potentially pave the way for personalised treatment.

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