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# Faecal Transplantation As a Treatment For Gut Microbiome Dysbiosis in Diabetes Type 2

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Bachelor thesis

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## Summary

The incidence of diabetes is rising quickly and becoming a larger burden on healthcare. Out of three types, diabetes type 2 (T2D) is the most common and characterized by insulin resistance and an impairment in secretion of insulin. Insulin is a peptide hormone that regulates blood glucose levels through upregulation of glucose transporter GLUT4 in target cells with insulin receptors. An impairment in secretion and resistance to this hormone are attributed to dysbiosis of the gut. Diets high in fats, sugars and products with a high glycaemic load and low intake of fibres have shown to cause this gut dysbiosis through reduced microbiota diversity, less short-chain fatty acid (SCFA) production and subsequently reduced production of the glucagon-like peptide-1 (GLP-1) hormone that is involved in sensitization of insulin vesicles. A reduction in GLP-1 therefore results in decreased insulin sensitivity. Furthermore, reduced SCFA levels are associated with increased inflammation, since this increases production of pro-inflammatory agents KC, IL-6, IL-8 and TNFα and reduced production of anti-inflammatory Treg cells, which also attributes to insulin resistance. Both previous and current interventions, including oral drugs that target the gut, have not been able to treat T2D but only to alleviate symptoms and risk for further complications. A new proposed treatment is faecal microbiota transplantation (FMT), which has shown great results in treating gut dysbiosis in Clostridium difficile infection. Therefore, this thesis investigates the exact role of gut dysbiosis in T2D and how FMT can aid in treatment of said dysbiosis and thus insulin resistance in T2D. Results have shown that FMT indeed has a lot of potential. Studies showed that there was an increase of microbiota diversity, especially fibre metabolizing species, an increase in SCFA production and a decrease in gut inflammation after FMT. However, without a lifestyle intervention targeted at diet and level of exercise, the microbiota composition is likely to change back as it was in the T2D state before FMT. Though, lifestyle interventions are not always feasible interventions. Therefore, it is proposed that more research needs to be focussed on how to keep the microbiota composition rather stable after FMT, possibly through supplementation of pre- and/or pro-biotics.

**Keywords: faecal transplantation, gut microbiome, microbiota, insulin resistance, inflammation, diabetes, T2D, SCFA, GLP-1, dietary fibre, high fat diet**

## Introduction

Healthcare systems all over the globe have been struggling with the rapidly increasing amount of patients diagnosed with diabetes mellitus for the past few decades. Especially the economic burden on low- and middle-income countries has been demonstrated to be enormous. A large portion of the annual income of citizens in these countries is spent on treatment costs, significantly affecting patients' wellbeing (Seuring et al., 2015). Additionally, diabetes is often the foundation of more serious issues, including micro- and macrovascular complications. In over 90% of patients suffering from such complications, diabetes was determined to be the onset. This has proven to produce a great stress for patients on both physiological and psychological level, which exhibits the vast need for new possible interventions to help combat the current diabetes epidemic (Chatterjee et al., 2017).

Diabetes mellitus is a collective name for three chronic metabolic disorders; type 1 diabetes, type 2 diabetes and gestational diabetes. Type 2 diabetes, or T2D, is by far the most common type found in diagnosed patients. This disease is characterized by impairment of insulin secretion by the pancreas and resistance to insulin (DeFronzo et al., 2015). Gut microbiota dysbiosis is commonly found in T2D patients and seems to play a central role in development of insulin resistance (Aw & Fukuda, 2018). This appears to be the result of an imbalance in microbiota and bad dietary habits such as low dietary fibre intake and a diet high in saturated- and trans-fats and sugars that shift microbiota composition from fibre metabolizing to mainly carbohydrate metabolizing species, which in turn affects insulin sensitivity and secretion (Hu et al., 2001; Ojo et al., 2020). This would indicate that the gut could be an important target for therapy.

Currently, the main drug that is used in treatment is metformin, which inhibits gluconeogenesis, reduces fasting blood glucose by 20% and preserves the barrier of the intestines. However, disadvantages of long term use are reduced B12 uptake in the gut, which might lead to a deficiency and gastrointestinal issues (Marín-Peñalver et al., 2016). Other oral agents are sulfonylureas and meglitinides or glinides, which all have stimulative effects on secretion of insulin by the β-cells. Nonetheless, efficacy can greatly reduce after long term use and other related side effects include hypoglycaemia, increased risk of cardiovascular disease and weight gain. Though, none of these drugs or other currently used interventions were able to treat T2D. They were only able to reduce symptoms and risk of worse complications that could arise when left untreated, such as cardiovascular disease (Marín-Peñalver et al., 2016).

Research regarding possible new interventions has recently been focussed on faecal microbiota transplantation, seeking to solve gut issues that bring about insulin resistance in T2D. This treatment is not invasive, cheap and was previously successful in treating gut dysbiosis in Clostridium difficile infection. It works through establishing a more diverse microbiota composition, which indicates a huge potential for T2D treatment (Borody et al., 2013). Faecal transplantation seems to be a promising intervention that could increase insulin sensitivity and decrease gut inflammation as a result of this compositional shift. It is clear that diet, gut microbiota, gut function and insulin resistance are all interconnected and involved in the development of T2D. Therefore, the intention of this thesis is to determine the exact role of gut dysbiosis in T2D and how faecal microbiota transplantations can aid in treatment of said dysbiosis and thus insulin resistance in T2D.

## Diabetes Type 2

The incidence of individuals with diabetes mellitus has been on the rise for the past few decades, from 108 million in the 1980s to a soaring 422 million in 2014. Not only has the number of diabetics increased massively, there was also an increase of 5% in premature mortality from this disease between 2000 and 2016 (WHO, 2021). Type 2 is the most common form of diabetes and is found in over 90% of all patients (DeFronzo et al., 2015). All types are chronic metabolic diseases with numerous serious consequences for both health of diagnosed patients and the healthcare system in general. In 2015, the global economic pressure of diabetes was estimated at US$825 billion, which demonstrates the need for new interventions (Chatterjee et al., 2017).

Some of the health consequences for patients include dysregulation of metabolism, especially that of proteins, carbohydrates and lipids. Furthermore, these patients suffer from both insulin resistance and an impairment in secretion of insulin (DeFronzo, 2015). Impairment of secretion is usually a result of dysfunction of the β-cells in the pancreas and insulin resistance is found in target organs like skeletal muscle, liver and adipose tissue (Chatterjee et al., 2017; DeFronzo, 2015). Moreover, these issues can result in even worse complications if left untreated; which involve kidney disease, organ damage, damage to the cardiovascular system, coronary heart disease, myocardial infarction and ischaemic stroke (Emerging Risk Factors Collaboration, 2010; Saltiel, 2001; WHO, 2021). Additionally, another important pathology that is less well-known compared to previously mentioned morbidities is gut diseases and/or dysbiosis. Gut disease and T2D often coincide and therefore the gut can be an important target for diabetes treatment. For instance, diabetes is the single most associated co-morbidity of patients suffering from ulcerative colitis, a chronic disease characterized by inflammation of the mucosa in the colon (Maconi et al., 2014). Research by Kappelman et al. (2011) in children with ulcerative colitis indicated that this illness is related to a higher incidence of diabetes compared to the controls. This suggests that gut dysbiosis likely plays an important role in the development of diabetes.

Health consequences as stated above are the effect of several factors. Although genes related to insulin secretion and sensitivity are partially responsible for the insulin resistance and dysfunction of β-cells, they cannot explain the enormous rise in patients in such a short period of time (Kahn et al., 2014). Therefore, environmental changes should be central in finding a new treatment. Important environmental factors that can facilitate the development of diabetes are increased intake of calories, decreased energy expenditure as a result of a sedentary lifestyle, nutrient composition of the patients' diet, stress and old age (Kahn et al., 2014; Ozougwu et al., 2013). Especially an unhealthy diet containing large amounts of fats and sugars and rather low amounts of fibres is a massive risk factor for developing insulin resistance along with affected glucose metabolism (Ojo et al., 2020). Such a diet can negatively affect insulin sensitivity through the gut microbiota, which will be dissected further in the next chapters. A faecal transplantation targeting the microbiome could be a new potential treatment, increasing microbiota diversity in the gut of diabetes patients and therefore targeting the gut dysbiosis that causes insulin resistance (Aron-Wisnewsky et al., 2019).

It is clear that diabetes is a serious global issue that needs to be targeted. The increasing number of diabetic patients indicates that a potential new treatment should be considered. Since gut dysbiosis likely caused by dietary habits and/or gut disease like ulcerative colitis is often found in diabetes patients, the gut could be an important target for this new strategy, possibly in the form of faecal microbiota transplantation.

## Insulin resistance in diabetes

To understand how insulin resistance develops from dietary habits, the functioning of insulin and its role in healthy individuals needs to be assessed first. Insulin is a peptide hormone produced in the β-cells of the islets of Langerhans, which are found in the pancreas, and stored there until a signal for release reaches the cells. This signal is glucose entry into the cells, after which it facilitates glucose uptake into cells and stimulates different metabolisms (Wilcox, 2005). It is clear that insulin plays a role in homeostasis of blood glucose levels. Therefore, insulin resistance in diabetes can result in hyperglycaemia, or high blood glucose levels. If left untreated, this can be the reason of even more serious health issues, such as damage to the cardiovascular system and damage to organs as mentioned in the previous chapter (Saltiel, 2001).

Insulin was first discovered in 1889, by two scientists who hypothesized that certain metabolic aspects must be regulated by a substance secreted by the pancreas (Wilcox, 2005). Several decades later, insulin was revealed to be a dipeptide hormone made up of an A and B peptide chain linked by disulphide bonds with in total 51 amino acids (Dodson & Steiner, 1998). Biosynthesis of insulin occurs in a few steps; production starts when mRNA is transcribed into pre-proinsulin. Pre-proinsulin is already made up of an A and B chain, but also a connecting C-peptide and a

signal peptide. The latter is removed, creating a new molecule called proinsulin. Zinc molecules are then integrated into the proinsulin, creating soluble hexamers. In the final step, proinsulin is converted into insulin through cleavage of the connecting C-peptide, now being stored in mature secretory granules (Wilcox, 2005). The release signal for excretion of the insulin is glucose entering the β-cells. In the cell it is phosphorylated by glucokinase, creating glucose-6-phosphate and ATP in this process. This increase in ATP subsequently results in closing of the $K^+$ channels, after which the membrane is depolarized and $Ca^{++}$ channels are opened. Finally, a $Ca^{++}$ influx stimulates exocytosis of the insulin-containing granules (de Lonlay & Saudubray, 2012). Once insulin is released into the bloodstream, it circles through the body until it binds to tyrosine kinase receptors present on the surface of target cells (Fig. 1) (Lizcano & Alessi, 2002; Pessin & Saltiel, 2000). In total, the receptor contains 4 subunits; two β-units on the inside of the cell and two α-units on the outside, to which insulin can bind. When binding occurs, the receptor phosphorylates several tyrosine kinase domains of its intracellular β-units, thereby being activated (Pessin & Saltiel, 2000). Following this, a cascade of phosphorylation reactions by kinases and other activating and recruiting reactions takes place, which eventually activates translocation of the glucose transporter GLUT4 (Fig. 1) (Lizcano & Alessi, 2002). GLUT4 is the main glucose transporter involved in blood glucose homeostasis, removing the excess exogenous glucose and transporting it into skeletal and cardiac muscle and adipose tissue. In the normal state, most of the GLUT4 is stored inside the cell, rather than at the surface of the cell membrane. However, when insulin binds to the receptor and activates a signalling cascade, GLUT4 is ultimately translocated to the cell membrane. Here it transports glucose into the cell, where it is metabolized and/or stored (Richter & Hargraeves, 2013).

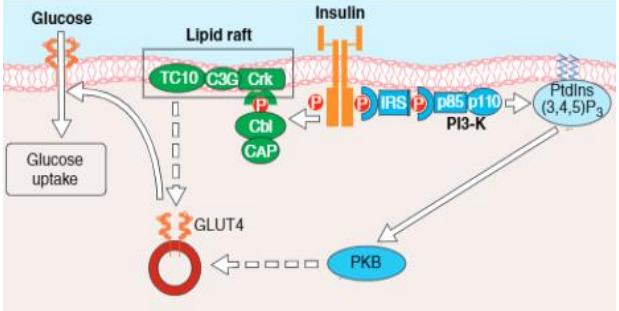


*Figure 1: An overview of the signalling pathway after insulin binding to its receptor. Eventually, glucose transporter GLUT4 translocation to the membrane is promoted, which transports glucose into the cell for storage and metabolization. Dashed arrows represent pathways that are speculated but have yet to be fully clarified. Figure adapted from Lizcano & Alessi, 2002).*

However, sometimes normal circulating levels of insulin do not provoke a reaction strong enough to sufficiently lower blood glucose. This phenomenon is referred to as insulin resistance and the strength of the reaction to insulin is referred to as insulin sensitivity. Both resistance to insulin in target cells and a deficiency in insulin secretion by the β-cells is needed to develop T2D (DeFronzo et al., 2015). In the case of gut dysbiosis as discussed in this thesis, diet plays an important role in insulin resistance. A high dietary glycaemic load from sugar-sweetened drinks for example, has been linked to insulin resistance in different studies. A 3-year study in women suggested that the dietary glycaemic load was 18% higher in those diagnosed with insulin resistance compared to the controls [$P < 0.001$]. Additionally, a regression model based on the same data indicated that a rise of 15 glycaemic load units heightened the risk of insulin resistance by 109% [$P < 0.001$] (O'sullivan et al., 2010). Salmerón et al. (1997) even found an increased risk of 150% when high glycaemic load and low cereal fibre intake were combined, which is the case in most diabetics (Fig. 2).

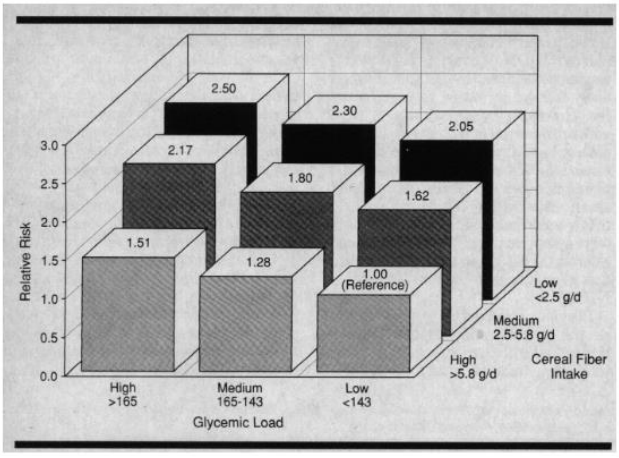


*Figure 2: The relative risk (RR) of diabetes mellitus depending on different levels of cereal fibre intake and glycaemic load. RR is highest (RR = 2.50) for the combination of high (> 165) glycaemic load and low (< 2.5 g/d) fibre intake. Figure taken from Salmerón et al., 1997.*

Furthermore, high saturated-fat diets showed an increased glucose infusion rate compared to chow-fed controls in a study by Storlien et al. (1991). Firstly, saturated-fats showed to drastically reduce glucose infusion rate (GIR), indicating how fast carbohydrates are received by an individual, from 16.1 ± 1.0 mg · $kg^{-1}$ · $min^{-}1$ in the control group to 6.2 ± 0.9 mg · $kg^{-1}$ · $min^{-1}$. Consequently, this group was found to be considerably more insulin resistant than all other research groups but one. Insulin resistance was indicated by both peripheral glucose disposal and insulin suppression of the hepatic glucose output, which were both impaired in the saturated-fat diet group. This is possibly due to elevated triglyceride levels in skeletal muscle in this group, since the glucose-fatty acid cycle plays a role in insulin sensitivity in muscle (Storlien et al., 1991). This has been suggested by Randle et al. (1965), who indicated that diabetic rats showed quickened oxidisation of fatty acids, leading to an impairment of glycolysis and glucose oxidation by insulin action.

Altogether, it is clear that insulin secretion is a complex process, modulated by a large amount of molecules. It is also evident that different dietary habits, such as low fibre intake and high fat and sugar intake can have a large impact on insulin sensitivity. In the next chapters it will be discussed in more depth how exactly diet can have this impact on insulin sensitivity and secretion.

## Dietary habits of diabetes patients

As touched upon in the previous chapter, dietary habits play a central role in development of insulin resistance and diabetes. Poor nutritional diets, like the Western diet, are major risk factors, as they induce insulin resistance through gut microbiome dysbiosis and inflammation. The Western diet is associated with microbial dysbiosis and one of the explanations is low fibre intake (Ojo et al., 2020). To illustrate, the recommended daily intake of fibres for children up to 8 years is around 19-25 grams, that of children and adolescents between 9 and 18 years old is around 26-38 grams and that of adults older than 18 is 21-38 grams. A ten-year trend survey using data from 14.973 children (4 - 18y) and 24.809 adults (> 18y) in the U.S. revealed that although there was a significant increase of fibre intake between 2001 and 2010 in children aged 4-18y with approximately 1.1 g/day and adults of 51y or older with 0.8 g/day, overall daily intake was still well below the recommended intake (Table 1). The mean daily intake for the children was 13.2 (±0.1) grams of fibre and for adults of both 19-50y and > 50y 16.1 (±0.2) grams (Table 1) (McGill et al., 2015). This shows that all age groups had a rather low fibre intake compared to the dietary recommendations, which can lead to disrupted gut function. Dietary fibres contain accessible carbohydrates for microbiota and thus play a role in the composition of the microflora in the gut. Soluble fibres are fermented by microbiota, during which short-chain fatty acids (SCFAs) are produced. When less of these microbiota-accessible carbohydrates, or MACs, are consumed in diet, the microbiome can reduce in diversity and even loss of certain species could occur (Makki et al., 2018).

*Table 1: Fibre intake by age over a ten year period. Table has been adapted from McGill et al. (2015)*

| Dietary Fiber (g/day) | | | | | |
|---|---|---|---|---|---|
| **Age (y)** | ***n*** | **2001–2010** | **2001–2002** | **2009–2010** | ***p*-value for trend** |
| 4–18 | 14,973 | 13.2 ± 0.1 | 12.8 ± 0.2 | 13.9 ±0.3 | **0.016** |
| 19–50 | 13,268 | 16.1 ± 0.2 | 16.2 ± 0.4 | 17.0 ± 0.4 | 0.16 |
| 51+ | 11,541 | 16.1 ± 0.2 | 16.2 ± 0.5 | 17.0 ± 0.2 | **0.03** |

Besides a lower fibre intake, Western diets are often rich in fats. Although, not all fats present in diet have been linked to T2D development, the lipid environment in which fats are present plays a key role in risk factor. The normal GIR in the control group had a value of 16.1 ± 1.0 mg · $kg^{-1}$ · $min^{-1}$ in the study of Storlien et al. (1991). When comparing this to several different dietary fats, GIR was greatly reduced. Mainly diets high in saturated- [GIR 6.2 ± 0.9 mg · $kg^{-1}$ · $min^{-1}$], monounsaturated ω-9 [GIR 8.9 ± 0.9 mg · $kg^{-1}$ · $min^{-1}$] and polyunsaturated ω-6 [GIR 9.7 ± 0.4 mg · $kg^{-1}$ · $min^{-1}$] fats showed a significantly reduced GIR compared to the controls, suggesting a profound resistance to insulin (Fig. 3) (Storlien et al., 1991). These findings indicate how certain dietary habits can have a large impact on health, especially regarding insulin sensitivity. Surely, Western diets seem to contain large quantities of especially saturated-fats and trans-fats, explaining the large number of patients with insulin resistance in Western countries (Hu et al., 2001).

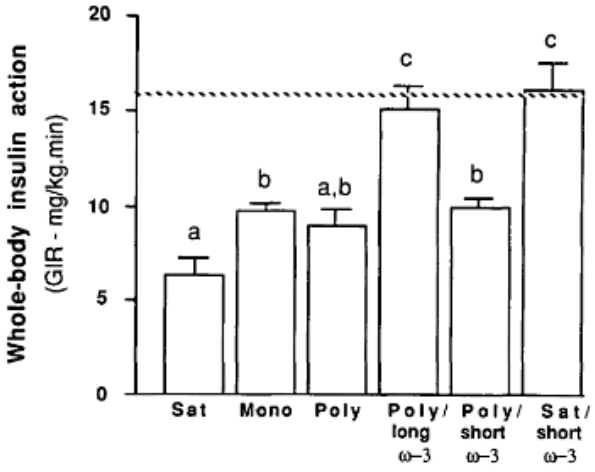


*Figure 3: Whole body insulin action expressed in glucose infusion rate (GIR). Values are means of n= 5 or 6 per group. Saturated fatty acids shown as sat, monounsaturated fatty acids as Mono and polyunsaturated fatty acids as Poly. Dashed line represents GIR of the control group. GIR was significantly reduced in Sat, Mono and Poly groups, being the lowest in the Sat group. Figure taken from Storlien et al. (1991).*

Furthermore, a nested case-control study of women with T2D recognized a dietary pattern that was associated with markers of inflammation found in blood samples. Inflammatory markers have been linked as predictors of T2D development in the past. Likewise, the dietary pattern associated with the markers was strongly correlated to a high risk of T2D development. This diet included high amounts of sugar-sweetened drinks, processed meat, refined grains and very little cruciferous and yellow vegetables. The study also indicated that such diets were linked to endothelial dysfunction, which in turn is likely caused by the inflammation indicated by these markers. Furthermore, diets containing relatively high amounts of refined grains have been indicated as a risk factor for T2D development in the past. This is likely due to the low fibre content and high glycaemic index of the grain, which are both associated with inflammatory markers as well (Schulze et al., 2005). Additionally, sugar-sweetened drinks have also been linked to T2D development in another cohort study performed in 91.249 women, in which T2D incidence was investigated over a 4-year period of following certain dietary patterns. This study suggested that the risk of T2D was increased with 83% in women that drank 1 or more sugar-sweetened drinks on a daily basis. Sugar-sweetened drinks contain vast amounts of high-fructose corn syrup, which acts very similar to sucrose in increasing blood glucose levels. The increased risk is most likely the result of a substantial increase in glycaemic index and glycaemic load, which are measures of how fast glucose is absorbed and the actual impact on blood sugar levels respectively (Schulze et al., 2004a). A high glycaemic load and high glycaemic index in consumed foods have been associated with increased insulin resistance (Villegas et al., 2007). Villegas et al. (2007) also exhibited in 64.227 Chinese women with no previous chronic disease diagnosis that intake of high glycaemic load and glycaemic index both increased T2D risk by 21% and 34% in the highest quintile respectively.

Thus, it can be assumed that common dietary habits of diabetes patients, consisting of a rather low intake of fibres, high intake of saturated-fats and an increased glycaemic load and index compared to healthy individuals have a profound role in T2D development. These dietary habits have indicated to largely increase risk of developing insulin resistance. Additionally, these dietary habits have shown to increase gut inflammation. Therefore, diet plays an important role in risk of diabetes development. Again, it is unmistakable that the gut plays an important role in the body's response to insulin and should be considered when providing treatment for T2D.

## The role of the gut microbiome

Since this thesis focusses on insulin resistance as a result of certain dietary habits that negatively affect the gut microbiome, it is also important to deepen the understanding of how these processes in the gut work. Our gut is home to $10^{14}$ bacteria of several hundred species, alongside viruses, fungi, phages and yeast (Adlerberth & Wold, 2009; Lee et al., 2020). They help ferment and metabolize the food that humans consume, therefore living in symbiosis with us. In healthy individuals, the most abundant bacterial phyla are Bacteroidetes, Firmicutes and Proteobacteria. The establishment of the gut microbiome starts with facultative bacteria at birth, when the new-born is exposed to different bacteria depending on a.o. environment and type of delivery (Lee et al., 2020). Oxygen levels in the gut of the infant are still quite

high, making a great environment for *E. coli* to be one of the first to colonize it, after which other facultative anaerobe bacteria follow (Fig. 4A). Such bacteria can perform both anaerobic and aerobic metabolisms. During the first weeks after birth, new bacteria are able to enter the gut through breast milk of the mother, human contact and antibiotics (Lee et al., 2020). The composition of the microflora continues to change and facultative anaerobic bacteria, such as the *Bifidobacterium, Bacteroides* and *Clostridium* start colonizing the gut as well (Fig. 4B). The facultative bacteria are eventually greatly reduced in quantities, since competition by the anaerobic bacteria is too potent (Adlerberth & Wold, 2009). At around 2 years old, an individual's unique microbiome is developed and remains stable for the most part. In healthy individuals, the microbiome can slightly shift as a result of diet or antibiotics, but will return to the original established microbiome when these factors return to normal (Lee et al., 2020).

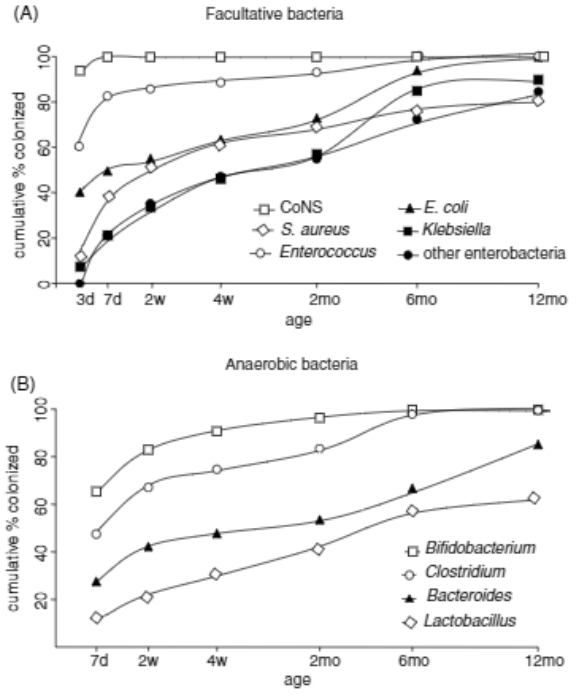


*Figure 4: Frequency of colonizing facultative bacteria (A) and anaerobic bacteria (B) in the first 12 months after birth. Figure taken from (Adlerberth & Wold, 2009).*

However, the previous chapter indicated that bad dietary habits can have a profound impact on the gut microbiome when continued for too long. For instance, the average Western diet nowadays contains only half the amount of fibres that should be consumed daily and thus a greatly reduced amount of microbiota-accessible carbohydrates (MACs). Normally, these MACs are fermented by microbiota in the gut, serving as an energy and carbon source for the microbiota that consume them. Sonnenburg et al. (2016) exhibited in humanized mice that were fed a low-MAC diet for 7 weeks, a decrease in occurrence of 60% of the microflora species. Additionally, the microbiota composition diverted even more with new generations that were fed the same diet. Although they did show that this was mostly reversible in the first generation when switching to a high-MAC diet, the microflora remained changed compared to controls when doing so in later generations. The taxa that had mostly disappeared from the gut were Bacteroidales and Clostridiales, profound fibre consuming species (Sonnenburg et al., 2016). Furthermore, Turnbaugh et al. (2008 & 2009) indicated an increase of Firmicutes and a decrease of Bacteroidetes [$P < 0.01$] in humanized mice when fed a HF/HS Western diet (Fig. 5A). Overall diversity was also reduced in the Western diet group compared to the controls (Fig. 5B). This suggests a shift to mainly metabolizing simple sugars as glucose, fructose and sucrose that are frequently consumed through the Western diet (Turnbaugh et al., 2008). The fibre consuming species that disappeared from the gut normally use enzymes to ferment fibres, generally using the glycolytic pathway, during which short-chain fatty acids (SCFAs) are produced as metabolites (Tan et al., 2014). SCFAs are saturated fatty acids mainly made up of acetate, propionate and butyrate and are in turn also used as energy sources in colonocytes (Makki et al., 2018; Tan et al., 2014). These colonocytes mainly consume butyrate, while hepatocytes in the liver utilize propionate. Acetate is either released into the venous system or remains at the site of the liver (Tan et al., 2014). Propionate can be used directly for glucose synthesis and therefore serves as an energy source for the body. Butyrate also has a part in metabolism and insulin resistance, stimulating the epithelium of the colon and whole body energy expenditure when added to high-fat diets. Through these mechanisms, butyrate is able to prevent or even reverse resistance to insulin in the obese mice model (Kootte et al., 2012). Therefore, consuming less fibre will result in gut dysbiosis through reduced SCFA production. Furthermore,

patients also have much less SCFA producing bacteria compared to non-diabetes patients, further contributing to dysbiosis (Aw & Fukuda, 2018).

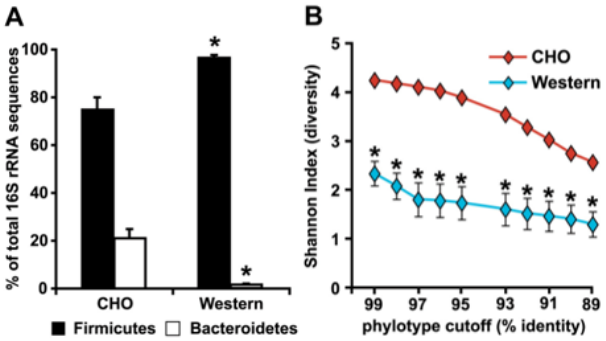


*Figure 5: The abundance of Firmicutes and Bacteroides in the gut microbiota of wild-type mice fed either a standard chow diet (CHO; n=5) or a Western HF/HS diet (n=5). Mice in the Western diet group show an increase in Firmicutes and a decrease in Bacteroides (A). The diversity of the microbiota measured by the Shannon index of diversity. Diversity is significantly lower in the Western diet group (B). Figure taken from Turnbaugh et al. (2008).*

Additionally, SCFAs in turn work as anti-inflammatory agents and can activate receptors in the gut mucosa, liver and adipose tissue, which trigger secretion of gut hormones involved in glucose homeostasis. (Cani et al., 2013). One of these hormones is glucagon-like peptide-1 (GLP-1) (Ojo et al., 2020). The GLP-1 polypeptide is a glucose-dependent peptide that inhibits glucagon secretion and liver gluconeogenesis and increases sensitivity to insulin (Aw & Fukuda, 2018; Ojo et al., 2020). GLP-1 is one of the main incretin hormones, which altogether provide 60% of the response of insulin secretion to oral glucose administration and is therefore able to influence insulin sensitivity. It is secreted when SCFAs bind to G-protein coupled receptors (GPCRs) like GPR43 and GPR41, of which microbiota themselves seem to regulate expression levels. These receptors are present on enteroendocrine cells that release GLP-1. GPR41 receptors are found in the mucosa, particularly the murine L cell line (Cani et al., 2013). When released by the L cells, GLP-1 is capable of promoting insulin secretion, hence lowering blood glucose levels. The mechanism by which this operates is through an increase of cAMP levels (Fig. 6), a second messenger that activates protein kinase A (PKA) and Epac 1 and 2 (Ahrén, 2011). These two molecules then increase secretion of insulin; Epac2 through forming a complex with another molecule, Rim2, which then binds to the insulin secretory vesicles and results in exocytosis of the vesicles. On the other hand, the $Ca^{+}$ sensitivity of said vesicles is increased by PKA. Consequently, glucose entry into the cell will lead to improved insulin secretion because of the higher vesicle sensitivity (Doyle & Egan, 2007). Lower GLP-1 levels can therefore greatly reduce insulin secretion, relating back to an impaired response to increased blood glucose levels. Therefore, the gut microbiome, especially regarding SCFA and GLP-1 production, can be a very important target to treat insulin resistance in diabetes.

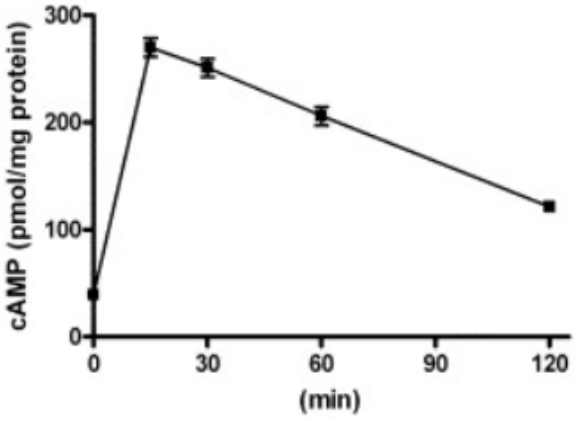


*Figure 6: Increase in intracellular cAMP levels in response to 10nM GLP-1 administration. Figure taken from Doyle & Egan (2007).*

Although the role of GPR41 in glucose metabolism is not well known, knockout mice revealed that GLP-1 secretion from L cells as a result of butyrate binding was reduced, suggesting that this receptor mediates GLP-1 secretion stimulated by butyrate SCFAs (Fig. 7) (Lin et al., 2012). However, both oral glucose and insulin tolerance remained unchanged in the GPR41 knockout mice, which indicates that glucose metabolism is not dependent on this receptor (Lin et al., 2012). Another knockout study found increased acetate and propionate levels in the distal gut, along with increased total faecal SCFA levels. This indicates that reduced amounts of SCFAs are absorbed in the intestines, which could explain the reduced GLP-1 levels (Samuel et al., 2008). Furthermore, knockout mice of the GPR43 receptor exhibited a reduced GLP-1 response of 70% to propionate [$P < 0.001$] and

no response to acetate [$P < 0.001$]. Consequently, colonic GLP-1 levels were significantly reduced compared to the wild-type mice [$P < 0.05$]. Likewise, in vivo blood GLP-1 levels in response to oral glucose were decreased by 40% in the knockout model [$P < 0.01$]. These mice also seemed to have reduced glucose tolerance and plasma insulin levels, which is likely related to the reduced GLP-1 response (Tolhurst et al., 2012). Not only does a low fibre diet affect this through reduced MACs, a combined high fat, high sugar (HF/HS) diet has also indicated to reduce expression levels of the GPR43 receptor in mice by 1.5-fold [$P < 0.0001$], along with reduced SCFA levels. Additionally, a HF/HS diet has shown to induce inflammation in the gut, along with dysbiosis of the mucosa and reduced SCFA concentrations (Agus et al., 2016). Furthermore, the link between high dietary GI and T2D as discussed in the previous chapter is most likely related to the GLP-1 hormone in the gut as well. Sugar-sweetened beverages with a high glycaemic index [GI = 90] have been shown to reduce GLP-1 levels in healthy men compared to sugar-sweetened beverages with a low glycaemic index [GI = 32] (Keller et al., 2016). Since GLP-1 is involved in insulin sensitivity and secretory responses, reduced levels will result in an increased insulin resistance (Aw & Fukuda, 2018; Salvatore et al., 2019).

Agus et al. (2016) also investigated the potential protective role of the GPCRs in relation to inflammation in the gut and found that indications of colitis and pro-inflammatory KC and IL-6 cytokines were vastly reduced when mice were treated with a GPR43 agonist. This suggest that SCFA activation of GPR43 does indeed confer protective properties against inflammation of the gut mucosa (Agus et al., 2016). Butyrate also affects the epithelial barrier through stimulation of mucus production and influencing expression of tight-junctions in the cells. Besides that, butyrate activation of G-protein coupled receptors can regulate pathways involved in inflammation. When activating GPR43, it can stimulate production of anti-inflammatory Treg cells that have demonstrated to reduce insulin resistance through a decrease in macrophage infiltration of white adipose tissue (Saad et al., 2016). SCFAs are also able to influence inflammation through inhibition of histone deacetylases or HDACs, changing gene expression. Butyrate is the most prominent HDAC inhibitor out of all SCFAs, likely acting as a competitive inhibitor to prevent HDAC binding to substrates and possibly also through activation of GPR41. Additionally, acetate inhibition of HDAC was associated with reduced levels of inflammatory cytokines IL-6, IL-8 and TNFα. This is likely due to decreased activity of NF-κB activity, which activates the main pathway through which these cytokines are released (Tan et al., 2014).

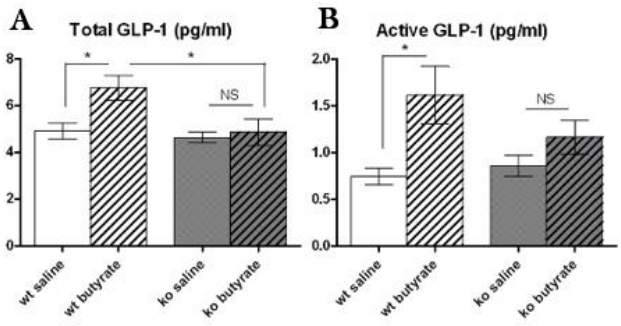


*Figure 2: Total GLP-1 (A) and active GLP-1 (B) response to saline and sodium butyrate (400mg/kg) in both wild-type and GPR41 knockout mice (n=8). Response to butyrate was vastly reduced in the knockout mice. Figure taken from Lin et al. (2012).*

These findings indicate that a shift in microbiome composition and reduced SCFA levels resulting from a Western diet can result in increased inflammatory cytokine levels, reduced levels anti-inflammatory cells, such as Treg cells, and reduced GLP-1 levels. These effects consequently induce inflammation of the gut, and increased insulin resistance, thereby increasing risk of diabetes development. Hence, this could be a potential target for treating T2D.

## Faecal microbiota transplantation treatment

It has become clear that diet-induced dysbiosis of the gut microbiome is an important factor in development of diabetes. Since a microbiome shift and reduced SCFA production are at the base of this dysbiosis, it would be useful to look at possible interventions targeting this issue. The treatment proposed is faecal microbiota transplantation, also known as FMT. So far, this treatment has been very effective in treating 90% of cases with gut dysbiosis in Clostridium difficile (CD) infection through increasing the diversity of the microbiota and could

possibly do the same for T2D treatment. FMT is a cheap and non-invasive treatment, mainly executed by an enema, colonoscopy, naso-jejunal or naso-duodenal tube or multiple of these (Aron-Wisnewsky et al, 2019; Borody et al., 2013). Nasal tubes that end in the upper gut are not the most practical though, as most patients do not find it pleasant to receive it this way and might even vomit. The transplant material consists of microbiota from the lower gut and administrating this into the upper gut may dysregulate the microbiome in that area. The advantage of colonoscopy is that the state of the mucosa can be assessed precisely and it allows for bowel preparation, during which the host microbiota can be partly removed, enabling easier settling of the transplant. Enema is the most effective, with a success rate of 95% compared to 89% for colonoscopy and 76% for administration through nasal tube. Healthy donors without risk factors for disease or antibiotic use give transplant material in the form of fresh stool, which is first diluted with a saline solution, then smoothened with a blender and lastly filtered to remove big particles. It is then administered through either of the aforementioned methods (Borody et al., 2018). Although screening for donors is necessary, since stool of some donors has no effect and that of others has a very significant effect (super-faecal donor) (Udayappan et al., 2014). FMT is a rather safe treatment as well; no adverse events were identified in patients receiving an enema, only very few patients have been reported with internal bleeding and peritonitis when using a nasal tube and less than 1% of 1000 patients ended up being hospitalized after colonoscopy (Aron-Wisnewsky et al., 2019).

The mechanism through which FMT could aid in treating T2D is through changing microbiota composition, which could in turn increase SCFA production and alleviate dysbiosis causing insulin resistance. This has indeed been shown in several studies; FMT via a duodenal tube in males suffering from metabolic syndrome was able to increase insulin sensitivity after 6 weeks (Fig. 8), increasing the glucose disappearance rate from 26.2 to 45.3 μmol/kg/min [$P < 0.05$]. The diversity of microbiota had increased from 178 ± 62 to 234 ± 40 species [$P < 0.05$], in particular butyrate producing bacteria, such as *Roseburia intestinalis* by 2.5-fold (Vrieze et al., 2012).

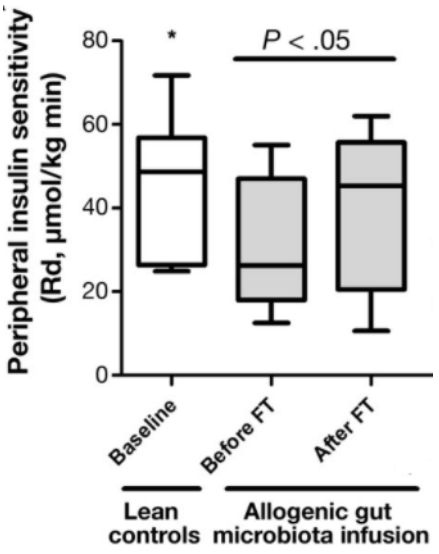


*Figure 3: Box plots at baseline and at 6 weeks showing peripheral insulin sensitivity. Insulin sensitivity is greatly increased after faecal transplantation (FT), $P < 0.05$. Figure taken from Vrieze et al. (2012).*

A recent study in T2D patients also indicated that there was a significant increase in abundance of over 20 butyrate-producing species after FMT [$P < 0.05$] (Ng et al., 2021). This shift can in turn increase SCFA levels, as shown in irritable bowel syndrome patients. Faecal butyric acid levels increased after both 30g and 60g of FMT, while in the 60g group the total SCFA levels also increased [$P \leq 0.001$] (El-Salhy et al., 2021). This would improve insulin sensitivity through the mechanisms mentioned in the previous chapter; mainly GLP-1 action and reduced inflammation resulting from increased SCFA production. In a T2D mice model receiving FMTs for 8 weeks, insulin resistance was reduced and sensitivity was improved. This model also indicated that islets were damaged in the control T2D group but when given FMT both the number and size of said islets was significantly increased [$P < 0.05$] (Fig. 9). Furthermore, pro-inflammatory cytokines IL-6 and TNFα were greatly reduced after FMT, while anti-inflammatory cytokine levels were increased. This indicates a vast reduction in inflammation of the gut and could explain the improvement of the islets (Wang et al., 2020). These findings indicate that FMT can improve both insulin resistance and inflammation pathologies in T2D. It can consequently be concluded that there

is a large potential in FMT treatment mediated through an increase in microbiota diversity, particularly SCFA-producing species. When metabolism is shifted towards SCFA production again, thereby increasing its levels, it could reduce gut inflammation through pro- and anti-inflammatory agents. Lastly, it could improve or possibly even reverse insulin resistance through GLP-1 action as explained before, thus effectively treating T2D.

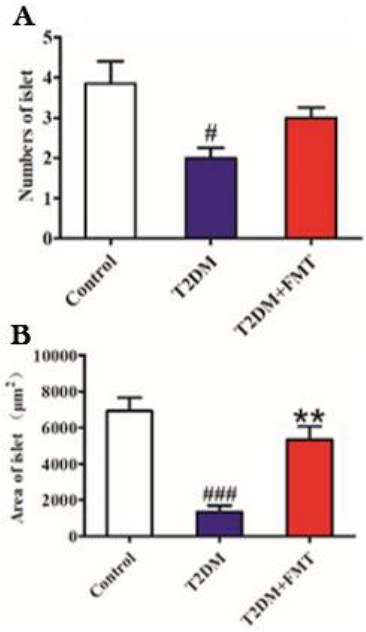


*Figure 9: Number of islets (A) and area of islets in (B). Both number and area of islets is decreased in T2D and increased again after FMT, P < 0.05. Figure taken from Wang et al. (2020).*

## Discussion and conclusion

Although the findings presented in this thesis can be applied to the human setting, most of the research has only been performed in animal models. Although the human gut system was mimicked, results may still differ or mechanisms may be more complex in an actual clinical setting. Besides that, not all mechanisms or actions of metabolites involved are completely known yet, calling for more research on microbiological level. Furthermore, diabetes is a very complex disease that involves not only gut dysbiosis, but multiple different factors possibly causing or contributing to insulin resistance. The disease is so complex that all factors cannot be evaluated in a single thesis, which logically limits the extent of research proposed in this paper. Thus, the treatment proposed here might not yield the most effective result, since other factors are involved as well.

Furthermore, although there seems to be a general consensus on most of the findings discussed, there is some controversy in the literature regarding the effect of a high glycaemic index and load on risk of insulin resistance and T2D. The studies mentioned in this thesis all found a positive correlation, discovering an increased risk of insulin resistance by 109% up to 150% when high GI was combined when low fibre intake (O'Sullvian et al., 2010; Salmerón et al., 1997). However, some studies only found a significant increase in risk for only one of the two factors, like Schulze et al. (2004b) who only found an increase of T2D for glycaemic index [P = 0.001], but not for glycaemic load [P = 0.21]. Furthermore, both Stevens et al. (2002) and Meyer et al., (2000) found no increased risk for either factor, with a relative risk of around 1.0 for both factors. The sample size of these studies was quite large; 12.251 in that of Stevens et al. (2002) and 35.988 in that of Meyer et al. (2000), indicating that it was not an error on this level. The majority of the studies seemed to find a positive correlation though, especially for glycaemic load, so it can be assumed that there is indeed a connection between glycaemic load or index and insulin resistance.

Additionally, FMT has mainly been used to treat CD infection in the clinic so far and further studies are needed to point out if it can actually function as T2D treatment as well. One significant difference between CD infection and T2D is that an infection is not a continuous issue unlike dietary habits in T2D. Because of this, the microbiota composition is likely to return to the same state as before FMT if diet is not changed. A FMT would be a great head start to assist the gut microbiome in becoming more like the healthy microbiome again, but a larger lifestyle intervention is needed to keep it there on the long term. Without a change in diet and preferably also increased exercise, FMT transplantations would be needed regularly to maintain gut symbiosis, which is not very convenient considering the massive amount of patients. This was investigated in a study as well, which showed that ≥20% of donor-associated microbiota was present in 100% of T2D patients receiving FMT each 4 weeks up to week 12 along with

lifestyle intervention, compared to 88.2% in the FMT only group at week 24 [$P < 0.001$] (Ng et al., 2021). Though, a change in lifestyle is not always feasible either. Some patients are bedridden or might have allergies that make lifestyle intervention more difficult. Besides, a shift in diet can be very hard to establish for some individuals due to low income or inability to break certain habits. Hence, it would also be useful to provide further research on ways to keep the microbiota composition rather stable for a longer period of time after FMT, which could possibly be through supplementation of pre- and/or pro-biotics.

After all, it can be concluded that bad dietary habits are often the onset of insulin resistance and development of diabetes through gut dysbiosis, which can be improved by faecal transplantation. Low intake of fibres, along with a high intake of fats and sugars have indicated a shift in microbiota composition from fibre metabolizing to mainly carbohydrate metabolizing as a result of low MAC availability and presence of vast amounts of carbohydrates. This suggests reduced fermentation of fibres by the microbiota, during which SCFAs are normally produced. Since SCFA binding to GPCRs activates GLP-1 secretion by L cells, levels of this peptide are also reduced. GLP-1 has shown to increase insulin sensitivity by improving insulin secretion in response to increased blood glucose levels, which indicates decreased sensitivity or even resistance if levels are diminished. Furthermore, SCFAs themselves influence the epithelial of the gut through stimulating production of anti-inflammatory cells and inhibiting assembly of pro-inflammatory cytokines. Low SCFA levels can therefore increase inflammation in the gut. This dysbiosis can be treated by faecal transplantations, which shifts the microbiota composition towards a more favourable composition again, improving SCFA and GLP-1 production. However, the microbiota can change back when diet and lifestyle are not intervened. Therefore, the final conclusion is that faecal transplantation can be a great start in treatment of T2D by targeting the gut microbiome, but patients themselves have to work on their habits as well in order for it to work as a treatment on the long term.

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