Gut Microbiome and T2DM

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Type 2 diabetes mellitus (T2DM) is a disease that affects over 9% of the United States population and is closely linked to obesity. While obesity was once thought to stem from a sedentary lifestyle and diets high in fat, recent evidence supports the idea that there is more complexity pertinent to the issue. The human gut microbiome has recently been the focus in terms of influencing disease onset. Evidence has shown that the microbiome may be more closely related to T2DM than what was originally thought. High fat diets typically result in poor microbiome heath, which then shifts the gut into a state of dysbiosis. Dysbiosis can then lead to metabolic deregulation, including increased insulin resistance and inflammation, two key factors in the development of T2DM.

gut health inflammation insulin resistance microbiome microbiota type 2 diabetes mellitus

1. Introduction

An estimated 39.8% of the US population is considered obese, as defined by the body mass index (BMI), hence a BMI > 30.0 [1]. This includes 35% of people under 40 and over 40% of middle-aged adults, and these numbers are only on the rise [2]. Obesity is a global epidemic interestingly seen both in developed and developing countries [2]. Unhealthy, often hypercaloric diets and sedentary lifestyles, typically coinciding from a behavioral standpoint, have been deemed major contributors to the development of the obesity epidemic in America and the world. Obesity can, over time, lead to various health complications, such as heart disease, high blood pressure, and type 2 diabetes mellitus (T2DM). While obesity has historically been thought of as the result of external factors, there is evidence to suggest that a significant component of obesity may be within a person's own gut. Recent research on the human microbiome supports the notion that an individual's microbiome profile could favor obesity, inflammation, and insulin resistance, eventually inducing T2DM.

The human microbiome is comprised of two primary phyla, namely Bacteroidetes and Firmicutes, typically in a ratio favoring Bacteroidetes over Firmicutes (B/F > 1). However, several studies have shown that in obese individuals, this ratio is altered, resulting in a higher prevalence of Firmicutes to that of Bacteroidetes [3][4]. Research has also demonstrated that transplanting microbiota from obese mice to germ-free (GF) mice resulted in significant weight gain in the latter compared to controls [5], suggesting the B/F ratio difference could contribute significantly to obese phenotype development. It is proposed that the specific demography of the gut microbiome in obese individuals causes increased energy harvest by the host organism, with any surplus leading to an overall significant increase of adiposity [6]. This function of the gut bacteria is likely attributed to the increased presence of Firmicutes, which

have the ability to metabolize insoluble carbohydrates resulting in a higher energy harvest. The specifics of such a link and the exact mechanism remain largely elusive [5][7]. Nonetheless, the evidence suggesting a favorable link between the microbiome as per its demography and obesity is substantial, making the investigation of the microbiome and by extension its role in T2DM an interesting field of inquiry with potential therapeutic applications.

2. Major Metabolic Contributors to Microbiome Profile Identity (SCFA, BCAA, LPS)

2.1. LPS

LPS translocation is considered one of the first steps in the pro-inflammatory cascade response. To further emphasize the importance of dysbiosis, LPS, and inflammation, Whelan et al., fed mice either a high fat diet, a diet supplemented with LPS (a low dose), or a control diet. The mice fed LPS developed obesity in a similar way as those that were fed a high fat diet. However, when mice missing CD14, an immunoprotein responsible for inflammatory reactions, were fed LPS, no weight gain was observed [8]. In both the HFD and the diet supplemented with LPS, the binding to TLR-4 was able to occur. However, in the absence of CD14, the inflammatory response was never initiated. Similar results have been obtained in mice not expressing TLR-4 [9].

2.2. Short Chain Fatty Acids (SCFAs)

As previously mentioned, SCFAs are highly important in the regulation of the inflammatory response, and a decrease in Bacteroidetes results in a decrease of SCFAs. Butyrate, a type of SCFA, is a major metabolite and important for gut health. While its role is not completely understood, its known importance is highlighted by a series of experiments. In a study by Gao et al., mice were given sodium butyrate as a dietary supplement. Their insulin sensitivity and energy metabolism were both monitored over the course of 16 weeks. It was observed that mice which consumed an HFD but were given butyrate supplements did not develop insulin resistance or obesity [10].

Interestingly, a study explored the gut microbiota of urban Italians versus a community of hunter-gatherers called Hadza, located in Tanzania, analyzing how the former’s gut microbiota compares to that of a foraging lifestyle, one that all human ancestors took part in. Fecal samples from 27 Hadza and 16 Italians were analyzed, and while much of this study focused on microbiota demographics, SCFA profiles were also analyzed. Conclusively, it was found that urban Italians generate significantly more butyrate, whereas Hadza generate more propionate [11]. This is particularly interesting, as butyrate is typically associated with Firmicutes, and propionate with Bacteroidetes [11], but excess Firmicutes are associated with weight gain. Based on this association, it could be argued that butyrate supplementation in the discussed study would not be beneficial. However, by the same token it can be argued that this further emphasizes the importance of SCFAs. Even in adverse conditions, the phenotype is still improved, showing that SCFA functions in a corrective way in the gut. It is also important to note that some Bacteroidetes produce butyrate as well, meaning that in an ideal B/F ratio, the butyrate producing Bacteroidetes do produce ample butyrate to compensate for the lack of Firmicutes, thus restoring, at least partially, a metabolite balance in the gut environment [12].

Based on current knowledge, the microbiome seemingly plays an important role in inflammatory responses, both in its own right as well as in an interplay with the diet [13]. Mackay and colleagues studied colitis in GF- and CONV-raised mice. The mice were treated with DSS, to chemically induce colitis. The GF mice faired significantly worse than the CONV mice, displaying much worse colonic inflammation. Additionally, when GF mice were then colonized with CONV gut microbiota, their inflammation was reduced. To identify the cause of this reduction in inflammation, GF mice that were not colonized were treated with acetate, a SCFA, and known to be produced by Bacteroidetes. This also caused a decrease in colitis symptoms, further emphasizing the importance of SCFA in the inflammatory response [14]. This again underscores the importance of SCFA, especially those produced by Bacteroidetes, and argues in favor of the proposition that SCFA production is a plausible mechanism for salvaging desirable phenotypes as per the gut health and related metabolism. Furthermore, numerous studies have indicated that the gut environment is highly responsive to a variety of bioactive compounds found in food items (typically fruits and vegetables) in ways that reduce risk for several chronic diseases, including T2DM, CVD, and cancers [15].

2.3. Branched Chain Amino Acids (BCAAs)

While increased levels of SCFAs may be beneficial in the prevention of T2DM, this is not necessarily the case with BCAAs. Three of the nine essential amino acids are BCAAs (leucine, isoleucine, and valine) [16], and they must be obtained through diet in humans. Elevated levels of BCAAs have been observed in obese individuals and those with T2DM [17], while obese individuals demonstrate increased BCAA catabolism [18].

The effects of BCAA production and insulin resistance are fairly complex. BCAAs have been shown to interfere with insulin signaling by stimulating mTOR, a kinase complex that plays an important role in protein synthesis [19], S6K1, a kinase important for cell growth [20], and phosphorylation of insulin receptor substrate 1 (IRS1) [18][21].

3. Dysbiosis and the Development of T2DM

T2DM develops when, systematically, the pancreas is forced to produce gradually increasing amounts of insulin to achieve postprandial glucose clearance reaching a point of such low insulin responsiveness from peripheral tissues (insulin resistance) normoglycemia cannot be achieved [22]. The exact mechanism of this malfunction is unknown, however many factors, such as obesity, a sedentary lifestyle, genetics, diet, and other environmental factors, and now, the microbiome, seem to influence the onset and development of this disease [23]. Insulin postprandially stimulates cells to uptake glucose by binding to insulin receptor on cellar membrane initiating a signaling cascade that normally leads to the translocation of glucose transporter type 4 (GLUT4) to the cellular membrane, thus initiating glucose clearance, as GLUT4 transports glucose into the cell down a concentration gradient [24]. How precisely glucose undergoes this transportation is not entirely understood, while it is important in the attempt to control onset of T2DM. Once inside the cell, glucose is either used for energy production or stored as glycogen within specific cells (hepatocytes and myocytes). Notably, if insulin is not present, there is no effective alternative mechanism for glucose clearance, resulting in hyperglycemia [25].

All responses described above appear to be linked to the microbiome as well, while more specifically dysbiosis in the gut appears to be a risk factor for T2DM development. In a metagenome-wide study of 345 Chinese individuals with T2DM, 60,000 T2DM-associated markers were validated, and all correlated with gut dysbiosis, decrease in butyrate producing bacteria, and an increase in oxidative stress [26]. This pioneering study provided solid evidence to suggest that the microbiome plays an important role in the development of T2DM, and dysbiosis is a contributor to the disease.

3.1. Inflammation

There is no clear, direct, known pathway by which inflammation relates to T2DM, but there is increasing evidence supporting a definite relationship between induced inflammation and increased risk for insulin resistance, which in turn leads to T2DM. Individuals in a pre-diabetic state compensate for insulin resistance by β-cells insulin hypersecretion [27], but as the disease progresses, β-cells progressively grow less able to supply the needed amount of insulin, gradually become exhausted, and eventually die. In this context, β-cells dedifferentiation is being investigated as a means of β-cells failure in T2DM [28], but this pathway is not confirmed, while anti-inflammatory diets are considered to help reduce risk of diabetes [29]. Several inflammatory cytokines, such as IL-1, IL-6, NF-κB, and TNF-alpha, also have been linked to obesity. Specifically, IL-6 biosynthesis functions as an initial state of inflammation. Upon generation, it moves to the liver triggering the rapid protein synthesis of C-reactive protein (CRP), which will be discussed further later. IL-1 inhibits β-cell function by inducing the destruction of β-cells hence reducing β-cell mass over-time, which is primarily seen in T2DM development at the late stages of the disease. Higher levels of IL-1 have also been commonly observed in obese individuals. TNF-alpha IL-6, IL-1, are all adipokines, a subset of cytokines. They are secreted by adipose tissue and can function as pro-inflammatory signaling agents. As a result, dysregulation has been linked to obesity and T2DM onset, especially considering inflammation. In obese individuals, it has been consistently observed that expression of pro-inflammatory cytokines is subsequently commonly followed by insulin resistance as well [30], hence making cytokines an important area of investigation when considering T2DM risk, onset, and disease management. Based on this approach, the microbiome and inflammation have been a focus of study when looking for causes and treatments regarding obesity and T2DM.

The effect of RYGB on mice microbiomes was previously illustrated as an example [31], but such an effect on human microbiomes and the body as a whole is an important area of investigation for fully understanding how the microbiome and T2DM development are dynamically interrelated. In a study by Bornstein et al., five individuals with T2DM and one obese individual who had all undergone RYGB were studied for changes in microbiota as an effect of RYGB and how observed changes influenced disease management [32]. Researchers showed that the RYGB procedure resulted in a decrease in both Firmicutes and Bacteroidetes, and a concurrent increase in Proteobacteria. It is important to note that while Bacteroidetes decreased, the phylum was still present in higher amounts than in Firmicutes, and the ideal B/F ratio was actually more closely achieved towards desirable post-operation, suggesting a favorable effect of the surgical operation (RYGB) [32]. The RYGB and subsequent microbiota change in the body was also observed when association with the inflammatory state was tested. Out of all the detected species, 9 of the 22 species were significantly correlated to C-reactive protein, a biomarker for

systemic inflammation commonly tested in the blood to assess inflammatory status [33]. Since nine bacterial species demonstrated a significant correlation with CRP levels in the blood, it was suggested that gut microbiome closely relates to the inflammatory state. Furthermore, a significant correlation of inflammatory state as assessed by CRP levels was seen with BMI, suggesting that a lower BMI correlates with a less inflamed state [28]. This is consistent with the proposition that obesity due to increased cytokine excretion induces a chronic mild pro-inflammatory state.

3.2. Insulin Resistance

While insulin resistance is a metabolic condition that typically eventually leads to T2DM, the microbiome appears to extend significant influence over the course of events and ultimately risk towards T2DM outcome. Work by Nieuwdorp et al., investigated the effects of microbial infusion in men with metabolic syndrome, defined as “a cluster of conditions that occur together… including increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels” [34]. Intestinal microbiota from lean donors were transferred to male recipients suffering from metabolic syndrome, and recipient microbiota and glucose metabolism were monitored post-transfer. Six weeks after trans-inoculation, insulin sensitivity of the recipients almost doubled, suggesting significant improvement in metabolic syndrome, while desirable butyrate producing microbiota also increased significantly [35].

Moreover, in a recent study, 291 non-diabetic Danish individuals underwent microbiome analysis, with their results being compared to those of 75 individuals with T2DM [36]. After analysis, insulin resistance levels and metabolic syndrome metabolites were investigated and compared between the two groups of focus [37]. The microbiome composition of both groups was then clustered based on metabolite production, where it was found that 19 of the 74 clusters were significantly associated with insulin resistance and metabolic syndrome. The correlated clusters were consistent across all 291 individuals and were also confirmed in the T2DM patients [36]. This suggests that certain metabolites produced by microbial clusters are strongly associated with higher insulin resistance, reinforcing the idea that certain microbiome configurations contribute to the development of insulin resistance.

Metformin is commonly prescribed medication to help manage T2DM, where it functions to suppress glucose production and increase insulin sensitivity. In a study evaluating the effects of metformin on metabolic improvement and the microbiome, mice on HFDs were evaluated. Mice were fed: (i) an HFD, (ii) an HFD and then switched to an ND, or (iii) an HFD supplemented with metformin. These dietary regimes were provided to induce obesity; hence obesity was the goal point, and not the development of T2DM. Results showed that upon administration of metformin to the HFD mice, the number of Bacteroidetes increased significantly, from 43% in the HFD group to 77% in the HFD–met group. Additionally, 18 metabolic pathways were also upregulated as a result of metformin administration [37]. While metformin is used primarily because of its positive effect on insulin sensitivity, interestingly it is shown to also alter the microbiome significantly in a desirable fashion. It cannot be ruled out that one of the potential mechanisms via which insulin sensitivity is improved upon metformin administration is mediated by metformin-induced changes in the microbiome.

While the direct connection between the microbiome and insulin resistance is not clear, it is evident that the microbiome plays an important role in regulating insulin resistance. These discussed findings provide a foundation for understanding this pathway, although more work needs to be done in the field to elucidate potential mechanistic pathways and series of events establishing how metformin may be influencing the microbiome leading to improved insulin sensitivity.

3.3. Oxidative Stress

The human body naturally produces free radicals when exposed to outside agents, such as food, alcohol, and air pollutants [38]. Reactive oxygen species (ROS) form as a result of metabolism, and transfer unpaired electrons causing oxidation of cellular machinery [27]. In a healthy individual, antioxidants, to a large extent, counter this process, neutralizing ROS and hence defending body homeostasis [39]. Imbalance, due to ineffective antioxidant defense, results in oxidative stress, which is closely related to glycation phenomena and diabetes onset [40]. A sedentary lifestyle and Western-type diets have been associated with overabundance of glucose and fatty acids, resulting in excess ROS. Glucose also reacts with plasma proteins to form glycation end-products, again producing ROS [40]. Oxidative stress induces inflammation, which in turn increases the risk for T2DM among other pathologies.

A recent study aimed to further understand the association between the microbiome and oxidative stress examining mice on HFDs [41]. Mice were either fed an HFD or HFD supplemented with lipoic acid, an antioxidant known to decrease oxidative stress [42]. ROS and total antioxidant capacity were assessed, as well as the microbiome of all mice in the study. Interestingly, in the mice supplemented with lipoic acid, lactobacilli were present in much lower numbers than in the mice on the HFD with no lipoic acid supplementation group. This constituted an important finding, as lactobacilli are members of the Firmicutes phylum. Thus, low numbers of lactobacilli observed also corresponded with decreased oxidative stress and better ROS levels, suggesting that antioxidants can ameliorate the microbiome profile and subsequently oxidative stress, and hence lower the risk for associated chronic disease such as T2DM [42].

While the correlation between the microbiome and onset of T2DM appears strong, there are several aspects of the microbiome that influence the development of disease. More specifically, microbiomes of patients with T2DM have begun to be evaluated in an attempt for a new search treatment. It has been revealed that the microbial composition of T2DM patients is quite different compared to non-T2DM individuals. The importance of diet in combination with disease state is critical in the establishment of a microbiome's demography. As such, lifestyle and dietary intake factors need to be considered when evaluating the microbiome, in addition to disease state and medication. In a 2010 study, a group of 36 men, half of which had T2DM, with a wide range of BMI, underwent gut microbiota analysis [41]. Bacterial composition was analyzed using 16S rRNA sequencing, and it was found that the diabetic patients had significantly less Firmicutes present than their non-diabetic counterparts, specifically of the class Clostridia. Additionally, T2DM patients also displayed a higher B/F ratio (higher Bacteroidetes to Firmicutes) [41]. These results taken together may appear surprising at first, as one would believe the opposite to be true after considering the literature as a whole. However, lifestyle and diet were not considered in this study. Commonly,

T2DM patients must follow a strict diet, low in simple carbohydrates and refined sugars, rich in complex carbohydrates, and low glycemic index foods/meals [43], whereas non-diabetic individuals are typically not on as strict of a dietary regime. The improved B/F ratio and overall lower numbers of Firmicutes observed, may be attributed to differences in the dietary regime plausibly followed by T2DM patients, as well as medication effects.

Overall, the thus far available evidence underlines a clear relationship between the type and state of the microbiome and the onset of chronic diseases, including T2DM. Further investigation considering the microbiome as a target for treatment towards chronic disease, particularly T2DM and ensuing CVD, is important and potentially highly valuable. The food industry and healthcare industry need to be involved in the development of potential foods [43] or systems [44] to provide potential therapeutic solutions enhancing and/or positively modifying the microbiome's profile into an optimal, desirable state that would minimize risk of disease.

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