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Modulation of gut microbiota by dietary macronutrients in type 2 diabetes: A review

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Abstract: Dietary interventions have been a first-line strategy in the long-term management and prevention of type 2 diabetes mellitus (T2DM). Recently, low carbohydrate diets and ketogenic diets emerged as common dietary approaches in managing T2DM. Literature suggests that dietary macronutrients result in significant impacts on the gut microbiome related to glucose tolerance, insulin resistance and inflammation. Although there is insufficient literature on gut microbiota modulation via macronutrient management, this area of study has been gradually gaining interest amongst researchers. This review describes the current evidence and summarizes specific macronutrients effects in sculpting the diverse gut microbiome. Potential crucial research concepts could help develop macronutrient-specific diets to modulate gut microbiota beneficial to T2DM management and pathogenesis.

Keywords: type 2 diabetes mellitus; gut microbiota; macronutrients; modulation

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is defined as a metabolic disorder identified by the presence of hyperglycemia and other hemostatic control systems such as insulin secretion defects, disturbance in insulin action, and increased hepatic glucose production[1]. As a result, the metabolism of macronutrients becomes disrupted within the body. The World Health Organization has defined the diabetes diagnostic criteria by four main measurements, 2-hour (2-h) post-load plasma glucose after a 75 g oral glucose tolerance test (OGTT); fasting plasma glucose; random blood glucose in the presence of diabetes signs and symptoms and Hemoglobin A1c (HbA1c), with respective cut-off values of ≥11.1 mmol/L (200 mg/ dl); ≥ 7.0 mmol/L (126 mg/dl); ≥ 11.1 mmol/L (200 mg/dl); ≥ 6.5% (48 mmol/mol)[1]. T2DM is estimated to be the most common type of diabetes, accounting for 90%–95% of its patients,

with an expected significant quadruple increase since 1980 from 422 million to 693 million by 2045[2].

The etiology of T2DM can largely be credited to one's alterable characteristics, especially nutrition, body weight, and various metabolic profiles[3]. Henceforth, dietary strategies focus on energy restriction and dietary quality to improve glycemic control by helping individuals with T2DM adopt healthy eating habits[3]. Recent studies have attributed the effect of dietary factors, specifically macronutrient intake, on diabetes parameters and their ability to manage diabetes.

Gut microbiota in healthy individuals conveys multiple beneficial functions within the body. For instance, it aids in host nutrient metabolism, immunomodulation and crucially maintains gut mucosal barrier structure and integrity. Therefore, a slight disrupt in the microbiome's ecosystem may lead to the pathogenesis of major diseases including irritable bowel syndrome, osteoporosis, myasthenia gravis, colon cancer, spleen deficiency syndrome, and hives[4–9].

As such, the gut microbiota's potential role in one's health and the development of diabetes will be further explored in this review. A summary of the narrative review can be found in Figure 1.

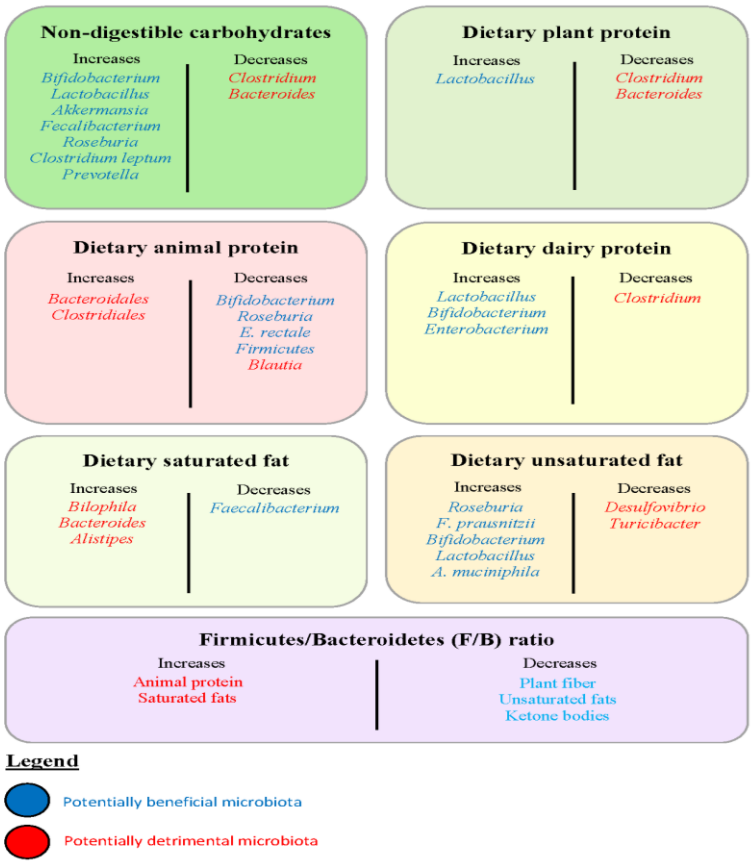

Figure 1. Effects on dietary macronutrients on gut microbiota that have potential impact on diabetes.

2. Role of Macronutrients in Diabetes

Carbohydrate intake has been sparking interest amongst researchers as a critical macronutrient in diabetes management. Diets enriched with high carbohydrate composition have often been associated with heightened glycemic index and T2DM risks[10]. Increased insulin secretion required to reduce dietary carbohydrate-induced hyperglycemia may ultimately lead to glucose intolerance and T2DM[11]. However, certain non-digestible carbohydrates like dietary fibers in the diet have been associated with protective effects against T2DM[12]. Thus, while many studies have discussed the beneficial effects of low carbohydrate diets (LCDs) in T2DM, more studies are now focused on specific carbohydrate types, especially non-digestible carbohydrates.

Dietary fat is another macronutrient heavily focused on due to the rising popularity of the ketogenic diet (KD). Like carbohydrates, the diverse sources of dietary fats instead of the amount of fats consumed can significantly impact diabetes parameters. Many papers have discussed that saturated fats derived from animal sources could negatively affect people with T2DM and should be minimized in diets[13]. In contrast, recent studies have pointed out the beneficial effects of consuming unsaturated vegetable fats by improving T2DM patients' lipid profiles and glycemic control[14].

Recent studies have also observed a possible association between protein intake and the success of T2DM management. While the effects of total protein intake on diabetes are inconclusive, differing protein sources have displayed different outcomes in T2DM patients. For example, animal proteins have been indicated to result in inconclusive effects on diabetes parameters, while plant-based protein sources, specifically from legumes, may positively affect the parameters[15–17]. However, the limited number of studies on dietary protein's effects on T2DM indicates that detailed studies should further establish the potential associations.

3. Role of Gut Microbiota in Diabetes

Recently, studies on T2DM have begun to speculate the diverse effects that specific gut microbiota populations may have on improving or worsening diabetes parameters. Studies have shown that individuals with T2DM present with lower gut microbial diversity than healthy individuals[18]. Although substantial research has yet to be conducted, gut microbiota modulation could become a potential avenue for diabetes management.

Specific gut microbiota species have been found to have a negative correlation with T2DM development. *Bifidobacterium* spp., *Roseburia* spp., *Akkermansia* spp., *Faecalibacterium* spp. and distinct strains of *Bacteroides* spp. and *Lactobacillus* spp. are negatively associated with T2DM with varying degrees of significance[19-23]. For example, *Bifidobacterium* spp., specifically *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, and *Bifidobacterium pseudocatenulatum* may have the most protective potential towards diabetes[20–22]. Strains of *Lactobacillus* spp. such as *L. amylovorus*, *L. plantarum*, *L. reuteri*, *L. casei*, *L. curvatus*, *L. gasseri*, *L. paracasei*, *L. rhamnosus* and *L. sakei* indicated negative correlations with T2DM[19]. A majority (21 out of

23) of operational taxonomic units *Bacteroides* spp. were also indicated to correlate with diabetes[23] negatively. Studies have also suggested that *Bifidobacterium* spp. and *Lactobacillus* could potentially complement each other to improve diabetes parameters[24,25].

On the other hand, several gut microbiota species like *Ruminococcus* spp., *Fusobacterium* spp., *Blautia* spp. and certain *Lactobacillus* strains have shown positive correlations with T2DM development and worsening T2DM symptoms[19]. While results remained inconsistent due to varied T2DM treatments, *Ruminococcus* spp. and *Blautia* spp. were observed to be elevated in T2DM patients.

Surprisingly, the association between *Firmicutes*/*Bacteroidetes* (F/B) ratio, a standard marker used to identify the metabolic disease, and T2DM was inconsistent, with 6 out of 14 studies indicating no association between both factors[19]. Despite this lack of consistency, this ratio may still be used in many studies as one of the means of suggesting T2DM development.

4. Macronutrients and Gut Microbiota

Specific macronutrient-based diets help reduce blood glucose levels, prevent any long-term diabetes-related complications, and contribute to shaping and restoring healthy human gut microbiota.

Multiple human and experimental-animal studies claim that carbohydrates enriched diets are proven to have a positive effect on health-beneficial gut microbes[26]. For instance, high-fiber diets increase the abundance of *Bifidobacterium* population, reduce the F/B ratio, and improve gut microbiota diversity[27–31]. Besides, increased *Lactobacillus* spp., *Akkermansia* spp., *Faecalibacterium* spp., *Roseburia* spp., *Bacteroides* spp., and *Prevotella* post-high carbohydrate diets (HCD) illustrates their potential prebiotic effect. Similarly, arabinoxylan, resistant starch, and inulin-type fructans, other types of dietary fibers modulate health-beneficial bacteria such as *Bifidobacterium*, *Faecalibacterium*, and *Lactobacillus* by increasing their pool size[32]. Further studies on other dietary fibers, such as oligofructose and polydextrose, were also found to modulate many health beneficial bacteria such as *Ruminococcus intestinalis*, *Clostridium leptum*, and *Roseburia*[26]. Therefore, such carbohydrates discussed in multiple studies have provided rigorous evidence on their potential ability to be used as a therapeutic intervention for metabolic diseases such as T2DM according to their multiple beneficial outcomes.

Clinical and preclinical studies on protein-enriched diets suggest that the quality and quantity of proteins have a varying effect on gut microbiota. For example, plant protein consumption such as mung beans in a high-fat diet (HFD)-fed mice reduced the HFD-induced F/B ratio[26]. It was also evident that animal-based protein could increase detrimental gut microbiota compared to plant-based protein, increasing intestinal inflammation sensitivity[33].

Similar to protein, types, and quantities of dietary fats may have substantial effects on either beneficial or detrimental gut microbiota in individuals with diabetes. Unlike

carbohydrates, the results of fifteen studies examining the effect of HFD on gut "microbiota" diversity and richness suggest that both total and saturated fats have negatively affected microbiota[26]. For instance, studies show that saturated fats cause a consistent decrease of health-beneficial microbes such as *Faecalibacterium* and *Bifidobacterium* and increase F/B ratio. Multiple studies supported these findings where the diet consisted of 44% to 72% of fat[34]. On the contrary, 20%–40% of dietary fat consumption was evident to reduce F/B ratio[35]. Consumption of unsaturated fats also decreased the F/B ratio, alongside detrimental bacteria such as *Escherichia* spp. and *Streptococcus* spp.[26]. In summary, HFD and saturated fat-enriched diet are mainly detrimental to gut health, as they reduce the population of beneficial microbes. However, this can be reversed if T2DM patients consume an unsaturated fat diet or low-fat diet (LFD).

The subsequent parts of this narrative review assess scientific studies that evaluated the effect of dietary macronutrients on the gut microbiome composition using *in vitro* and *in vivo* models, human and animal clinical trials. The F/B ratio, the shift in potential detrimental and beneficial gut microbiota species, and gut microbial diversity are the significant findings discussed in this review.

5. Dietary Carbohydrates

5.1. Low Carbohydrate Diet in Type 2 Diabetes

A recommended dietary pattern for people with diabetes includes carbohydrates from various sources for good health, and monitoring carbohydrate intake is a critical strategy in achieving glycemic control[36].

Low carbohydrate diets (LCDs) are common among people with diabetes. For example, Ma and colleagues reported the adoption of low-carbohydrate, low-fiber and high-fat diet in patients with T2DM enrolled in a dietary intervention[37]. However, the authors warned that though patients may find reducing weight and controlling blood glucose appealing, such nutritional patterns may have severe cardiovascular implications. Several studies have shown the effectiveness of LCDs in controlling blood glucose. One such trial by Haimoto *et al*. reported a remarkable reduction in HbA1c levels after a 30% carbohydrate diet over six months[38]. The researchers suggested the LCDs effectiveness to be comparable to insulin therapy. This finding is supported by similar studies[39–41]. Most of the reported trials were, however, short-term in nature.

Dyson *et al*. and colleagues reviewed six studies investigating the effects of hypocaloric reduced carbohydrate diets in patients with T2DM and found all studies reported reductions in body weight and HbA1c[42]. Although studies were few and small in sample sizes, LCDs are safe and effective over the short term for patients with T2DM. Meta-regression of 13 studies showed that HbA1c and fasting blood glucose (FBG), improved with LCDs in patients with T2DM[43]. A more recent systematic review of 9 randomized-controlled trials (RCTs) by Meng *et al*. (2017) showed LCDs were beneficial in controlling blood glucose levels of patients with T2DM, assessed by significant reduction on HbA1c level (weighted mean difference (WMD): -0.44; 95% CI: -0.61, -0.26)[44]. A similar finding

was reported by Sainsbury et al. (2018)'s meta-analysis of 25 RCTs, where a significant reduction in HbA1c level was noted at three months (WMD: -0.47%, 95% CI: -0.71, -0.23) and six months (WMD: -0.36%, 95% CI: -0.62, -0.09)[45]. However, LCDs were not associated with a significant effect on long-term weight loss in T2DM[43,45].

A meta-analysis has shown an improvement in patients' lipid profile with T2DM after restricting their carbohydrate intake. Triglyceride levels (TG) of these patients have been shown to decrease after LCD trials (WMD: -0.33; 95% CI: -0.45, -0.21)[44]. The analysis also showed that LCDs with calorie limitation effectively increased high-density lipoprotein (HDL)-C levels (WMD: 0.07; 95% CI: 0.03, 0.11).

Patients with T2DM are regarded as an ideal target group for LCDs. However, during the diet, insulin requirement needs to be reviewed closely because the LCDs can be very effective at lowering blood glucose. Patients on diabetes medication who use this diet should be under close medical supervision or capable of adjusting their medication[46].

5.2. Gut Microbiota and Dietary Carbohydrate

Dietary carbohydrates can be differentiated into digestible and non-digestible carbohydrates. This review focuses on the effects of non-digestible carbohydrates, referred to as dietary fibers, on gut microbiota. Non-digestible carbohydrates (plant fiber and resistant starch) are the main form of energy to the large intestinal microbiota mainly because enzymes found in the upper gastrointestinal tract cannot digest resistant starch and are fermented by the colonic microbiome[47].

Under macronutrients, carbohydrates form the major modulator for health-beneficial microbes. Dietary fiber, arabinoxylan, galactooligosaccharides (GOS), inulin-type fructan, resistant starch, and polydextran have significant bifidogenic effects (increase the growth of *Bifidobacteria*) and positively modulate health-beneficial microbes in the gut[12]. Significant health-beneficial microbes modulated by these major carbohydrates are *Bifidobacterium* spp., *Lactobacillus* spp., *Akkermansia* spp., *Fecalibacterium* spp., *Roseburia* spp., *Bacteroides* spp. and *Prevotella*, *Roseburia*, *Clostridium leptum* and *Ruminococcus intestinalis*[12,26].

Diets involving non-digestible carbohydrates like whole grains, traditional Chinese medicine food plants, and foods rich in dietary fibres increased *B.pseudocatenulatum* C1 levels, *B. pseudocatenulatum* C62, and mostly *B.pseudocatenulatum* C15 (accounted for more than 50% of *Bifidobacterium* population), while *B.pseudocatenulatum* C55 and *B.pseudocatenulatum* C95 were unresponsive. *B. pseudocatenulatum* C15 correlates with improved inflammatory markers, where leptin levels are decreased, and adiponectin levels are increased[48].

Human clinical trials showed that increased resistant starch consumption positively correlated with increasing bacterial species like *Bifidobacterium* spp., *Fecalibacterium* spp., *Eubacterium* spp., *Ruminococcus* spp. and *Parabacteroides distasonis*, which improves T2DM. When combined with arabinoxylan, resistant starch could alter gut microbiota by

increasing *Bifidobacterium* spp. concentration and reducing dysbiotic bacterial species while concurrently increasing short-chain fatty acids availability, thus improving metabolic syndromes and colonic health[12,26]. Oligosaccharides, which include fructans, raffinose-oligosaccharides, and GOS, has shown positive impacts on enhancing gut microbiota, mainly due to its bifidogenic effects[26]. Fructans alter gut microdiversity by increasing *Bifidobacterium* spp. and *Faecalibacterium* spp, while simultaneously decreasing detrimental microbes like *Bacteroides* and *Clostridium*[12]. Clinical trials *in vitro* fermentation of galactooligosaccharides, a potential prebiotic, have shown an increase in *Bifidobacterium* spp. and specific *Lactobacillus* strains[26]. These bacteria promote and positively modulate a healthy gut microbiome, indicating GOS's bifidogenic potential. Moreover, GOS simultaneously reduces gut inflammation by increasing gut mucosa immunoglobulin A and plasma C-reactive proteins[12].

Butyrate, a complex carbohydrate digestion product, is responsible for modulating inflammation, immune cell function, and migration. Butyrate can also be produced by specific gut microbiomes (*Clostridiales* spp. SS3/4, *F. prausnitzii*, *Eubacterium rectale*, *Roseburia intestinalis* and certain *Lactobacillus* species) within healthy individuals[46]. In contrast, T2DM patients have a reduced population of butyrate-producing bacteria[46]. Therefore, a high non-digestible carbohydrate diet aids in modulating butyrate-producing bacteria, *Roseburia* spp. and *Fecalibacterium prausnitzii*, associated with improving insulin sensitivity[12]. Consumption of foods high in polyphenols such as flavonoids, stilbenes, and lignans from vegetables, fruits, coffee, wine, tea, and cereals has indicated a rise in mucopolysaccharide-degrading *A.muciniphila* populations, which plays a potentially protective role in T2DM development[46]. *A. muciniphila* maintains gut mucosa integrity, reduces inflammation, improves glucose tolerance, and reduces insulin resistance[19]. Furthermore, polyphenols can modulate microbial diversity and gut microbes by inhibiting potential pathogenic organisms.

Overall, most non-digestible dietary carbohydrates are positively related to the beneficial gut microbe species in T2DM patients.

6. Dietary Protein

6.1. Dietary Protein in Type 2 Diabetes

Observation of T2DM patients' blood amino acid content suggests increased circulation of high branched-chain amino acids and aromatic amino acids [49]. According to the American Diabetes Association, an optimum amount of protein consumption should be personalized on a case-by-case basis due to the lack of compelling evidence-based conclusions derived from the latest research[50].

Currently, available literature on the effects of animal and plant protein on diabetes parameters are inconsistent. A systematic review and meta-analysis of 13 RCTs investigating the impact of substituting animal protein with plant-based proteins, a majority of its soy, indicated a drastic reduction in HbA1c levels (MD: -0.15%; 95% CI: -0.26, -0.05%), FBG (MD: -0.53 mmol/L; 95%-CI: -0.92, -0.13 mmol/L), and fasting insulin (MD: -10.09 pmol/L;

95%-CI: -17.31, -2.86 pmol/L) compared to control arms[51]. This evidence suggests plant proteins to be more beneficial than animal proteins in T2DM.

Plant proteins' potentially protective effects on T2DM have been recorded in several studies, specifically pulses. For example, tree nuts consumption indicated improvements in FBG (MD: -0.15 mmol/L, 95% CI: -0.27, -0.02 mmol/L) and HbA1c levels (MD: -0.07%, 95% CI: -0.10, -0.03%)[52]. However, the beneficial effect of soy protein on glycemic endpoints is not entirely supported. While some human and animal studies on soy protein's effects on T2DM suggest that soy protein consumption can improve insulin resistance, reduce serum very-low-density-lipoprotein and low-density-lipoprotein cholesterol, and triacylglycerol studies are indicating that soy protein's effects on HbA1c, FBG, and insulin are insignificant[17,53,54].

Similarly, dairy protein is another type of protein reported to improve insulin resistance and reduce inflammation in overweight patients with T2DM[55]. More specifically, insulin sensitivity enhancement accompanied by a 55% increase in adiponectin, as well as reductions in oxidative stress and several inflammatory markers were discovered to be associated with the dairy-based protein consumption. Whey protein, a milk product, has also been shown to lower blood glucose concentration by stimulating insulin secretion postprandial. It is hypothesized that glucose-regulation is possible due to gut hormones and incretins[55].

On the contrary, animal protein, including red meat, poultry, and fish, have been widely reported to increase the risk of T2DM development upon inclusion into the diet[17]. Yet, some studies report that its overall effects on glycemic and metabolic control in T2DM patients remain relatively insignificant, despite having a statistically negligible negative association with HbA1c levels[15,56].

For instance, trials conducted on animal and plant protein found improved glycemic and metabolic control of T2DM patients. In this trial, the HbA1c levels of both patient groups subjected to high plant and animal protein diets after six weeks were reduced by ~0.5%, although the plant protein diet group had a more significant association with reduced Hb1Ac levels. Also, there was an improvement in the whole-body insulin sensitivity after following animal and protein diets, without substantial differences[16]. The findings were supported by another trial that reported high animal and plant protein diets to decrease HbA1c and FBG, while animal protein diets improved whole-body insulin sensitivity[57].

Hence, it seems that while animal protein may worsen T2DM management and plant proteins may have protective effects against T2DM, studies specifically detailing the results of each protein type and quantity could entail a much more valid conclusion. In summary, the different qualities rather than quantities of proteins play different roles in T2DM management and development.

6.2. Gut Microbiota and Dietary Protein

As mentioned earlier, plant-based proteins have illustrated a positive association in T2DM management. This is because specific plant-based proteins have produced promising results in altering gut microbiota to benefit T2DM patients.

Different types of protein (animal, plant bases, and dairy) and the quantities have varied effects on gut microbes. During amino acid catabolism, enterocytes play a role in modulating intestinal barrier function, such as the type of bacteria present[58]. For instance, an animal-based protein diet displayed an increase in Bacteroidales and Clostridiales population within the gut[26]. Similarly, an increased trimethylamine N-oxide level, a proatherogenic microbial metabolite, was associated with high red meat intake, which was proven to be a precursor for T2DM risk[59]. Furthermore, a 70-day supplementary study of blend whey isolate and beef hydrolysate was conducted to examine gut microbiota's effect. The results suggested a decreased health-beneficial microbiota level of *B. longum*, *Blautia* and *Roseburia*, *E. rectale*, as well as Firmicutes[26]. Further human studies on dietary protein intake indicate a positive relationship between animal protein consumption and reduced Bacteroidetes, increasing F/B ratio, which is positively related to T2DM[60-61].

Conversely, consumption of vegetable proteins in individuals increased the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. while decreasing *Bacteroides* and *Clostridium* spp.[61]. Mung bean proteins were evident to increase the Ruminococcacea family abundance in HFD mice models. This aid in mediating bile acid metabolism is hypothesized to provide some health benefits[26]. Overall, plant-based protein can potentially shift the gut microbiome to benefit T2DM patients.

In contrast to plant based-diet, dairy protein consumption such as casein in piglets was found to increase the fecal Enterobacteriaceae and decrease beneficial fecal *Lactobacillus* spp. Some clinical studies suggested that caution must be taken while exposed to a casein protein diet [26]. This is because it appears to affect overweight "individuals' rectal mucosa by disturbing the regular gene expression of bacteria. On the other hand, cheese whey protein, another form of dairy protein, potentiates the fecal counts of beneficial *Lactobacillus* and *Bifidobacterium* spp. while reducing the *Clostridium* spp. population[26]. Therefore, both beneficial and detrimental metabolites were produced by specific microbes, stimulating different gut microbiome effects in T2DM patients.

Overall, most studies have evidenced that plant proteins display beneficial effects on gut microbiota modulation, while studies on animal protein's impact on diabetes have conflicting results.

7. Dietary Fats

7.1. High Fat Ketogenic Diet and Type 2 Diabetes

While low-carbohydrate ketogenic diets (KD) have risen in prominence among T2DM patients, the debatably beneficial effects of reducing carbohydrates intake in these patients are yet to be firmly proven. A KD is defined as a very low carbohydrate (VLC) diet

consisting of less than 50 g of daily carbohydrates from non-starchy vegetables, resulting in ketosis and reduced insulin levels[62]. Typically, KD consists of 5% carbohydrates, 80% fats, and 15% protein[63].

Multiple studies have illustrated a positive weight loss outcome due to KD. Sahama st al. study suggests that KD likely to result in weight loss three times more than LFD[64]. This is due to the excessive fatty acid metabolism resulting in high ketone bodies. Moreover, Hba1c levels were also proven to be significantly reduced in multiple studies due to the possibility of ketone bodies' ability to decrease glucose metabolism[64–65]. A prospective cohort study suggested a statistically significant association between consumption of a low-carbohydrate ketogenic diet with a decrease in LDL, TG, and cholesterol levels and an increase in HDL level[65].

A trial comparing high carbohydrate LFD or very low carbohydrate, high-unsaturated fat or low-saturated-fat diet among obese T2DM patients showed a significant decrease in body weight and HbA1c levels in both groups[14]. On the other hand, the lipid profile in a VLC diet improved at a higher level than HCD due to the possible fat quality. A similar study also noted that both diets were clinically significant in reducing HbA1c (-0.7%; 95% CI: -1.0, -0.5%)[66]. A more significant improvement was also marked by diabetes medication reduction, which could reach up to half the amount (anti glycemic medication effect score (MeS): -0.5; 95% CI: -0.6, -0.3), HC: -0.2; 95% CI: -0.4, -0.02units), as well as blood glucose variability[66].

While there are concerns on long-term KDs increasing the risk of diabetic ketoacidosis, a trial with follow up after two years indicated its beneficial weight loss effects void of potentially adverse renal effects[14].

7.2. Gut Microbiota and Dietary Fat

Dietary fat plays a significant role in modulating the gut microbiome in T2DM patients. Specifically, dietary fats (saturated and unsaturated) and the quantity consumed may have distinctly different gut microbiota effects.

A human study suggested that a diet rich in polyunsaturated fat protects against T2DM development by increasing bacterial populations of *Roseburia* and *F.prausnitzii*[26]. Similarly, a mice study indicated that saturated fatty acids like lard could promote harmful bacteria like *Bilophila* and increase the F/B ratio. In contrast, polyunsaturated fatty acids increased the beneficial bacteria genus of *Bifidobacterium*, *Lactobacillus* and *A. muciniphila*[26]. A systematic review of fifteen studies reported HFD or saturated fat diet negatively associated with gut microbiota's diversity[26,67].

According to Qi *et al.*, HFDs increase the F/B ratio indicating an opposite effect on KD[63]. This could be attributed to ketone bodies' presence in KD compared to HFD. Elevated ketone bodies like βHB can increase the *Bacteroides* population, reducing the F/B ratio[63]. A trial also indicated that a 40% fat diet caused a rise in the detrimental *Bacteroides* and *Alistipes* genus while simultaneously reducing the beneficial *Faecalibacterium* genus[26].

Moreover, the stimulation of ketone body production in KDs inhibits *Bifidobacterium* spp's growth, resulting in decreasing the levels of pro-inflammatory Th17 cells and adipose tissues. The reduced levels of Th17 cells in both adipose tissue and gut could improve metabolic syndrome aspects such as glycemic control[63]. Similarly, mice studies have indicated that KD reduces *Desulfovibrio* and *Turicibacter*, potential pro-inflammatory bacteria genus, and increases the abundance of *A. muciniphila* and *Lactobacillus* spp. *A. muciniphila* results in low blood glucose concentration caused by the increase in insulin sensitivity[68].

Interestingly, KD's potential benefits to T2DM patients may be overshadowed by a few possible adverse effects. KD stimulates ketogenesis, reverting the body's primary energy source from glucose to ketone bodies. As a result, the serum concentration of ketone bodies like beta-hydroxybutyrate (βHB) becomes elevated. According to a human study, βHB displays bacteriostatic effects and a negative correlation with *Bifidobacterium* spp. through a pH-dependent mechanism[63]. *Bifidobacterium* spp. has a role in the metabolism of non-digestible carbohydrates; hence a reduction in carbohydrate intake explains the reduced *Bifidobacterium* population[48]. Since *Bifidobacterium* spp. are beneficial to T2DM patients, KD's overall benefits are potentially reduced.

Also, several human studies concluded that a reduction in dietary fat to 35% of one's diet aids in normalizing the gut microbiome. This suggests that the gut microbiome's adverse changes may result from diets with a dietary fat composition of more than 35%[26].

8. Conclusions

This current research review summarized multiple studies on the different macronutrients and their role in modulating T2DM gut microbiota. However, further research is required to provide evidence and supporting data to the questions in doubt. For instance, it is unclear why macronutrients affect individuals differently and their mechanism of action within the gut to alter the microbiome. Some studies have suggested Gnotobiotic or humanized mouse models, as they could be used to overcome these issues despite their several limitations. This is because they enable careful and close control of dietary nutrients and evaluate gut microbiome changes[70]. Additionally, a diverse range of individuals can undergo a well-defined, closely monitored dietary intervention to understand better how their microbiome responds to specific macronutrients according to their intra- and inter-variability. Moreover, a long-term dietary analysis must be integrated into different macronutrient dietary interventions with intensive longitudinal data collection to improve research results.

Although extensive research has already been conducted, dietary macronutrients' role in gut microbiota modulation as an effective management mechanism for T2DM remains uncertain. However, gut microbiota modulation by dietary macronutrients should still be thoroughly evaluated as a means of improving T2DM complications. In this review, we have identified and analyzed each dietary macronutrient's effects on specific strains of gut microbes and the management of T2DM. Thus far, our discoveries indicate that the integration of non-digestible carbohydrates and polyunsaturated fats in KDs best modulate

the gut microbiome in managing the disease via the regulation of HbA1c levels, body weight, insulin resistance, and secretion.

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