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Diet and gut microbiome: Impact of each factor and mutual interactions on prevention and treatment of type 1, type 2, and gestational diabetes mellitus

Davide Menafra [a,*,1], Mattia Proganò [a,1], Nicola Tecce [a], Rosario Pivonello [a,b], Annamaria Colao [a,b]

[a] Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Unità di Andrologia e Medicina della Riproduzione e della Sessualità Maschile e Femminile (FERTISEXCARES), Università Federico II di Napoli, Naples, Italy
[b] Unesco Chair for Health Education and Sustainable Development, Federico II University, Naples, Italy

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ABSTRACT

The gut microbiota (GM) plays a key role in health by influencing several physiological functions, including the digestive process, the immune system, vitamin production, and mental health. Dysbiosis in gut microbial composition and function has been linked to systemic inflammatory response and the development of metabolic diseases, including diabetes mellitus (DM). "Leaky gut" resulting from dysbiosis causes endotoxemia, leading to systemic inflammation and insulin resistance, which are pathogenetic agents of type 2 and gestational DM. Moreover, in children, gut dysbiosis has been associated with the immunitary dysregulation with increased risk of autoimmunity and development of type 1 DM. However, dietary changes in the GM and their role in DM are poorly understood. Plant-based diets that are low in fat and high in fiber have been associated with beneficial effects on the GM. Clinical trials of prebiotics and probiotics have shown promising, albeit mixed, results. This narrative review summarizes recent findings on the relationship between the GM, diet, and DM, focusing on the systems in which the microbiota is involved in the pathogenesis of this disease and its potential use as a therapy. In addition, we discuss immune dysfunction associated with gut dysbiosis and its role in type 1, type 2, and gestational DM. Further research is needed to evaluate the GM as a potential therapeutic target for the prevention and treatment of DM.

1. Introduction

The human gut microbiota (GM) is a complex and dynamic ecosystem in the human gut resulting from the mutualistic symbiosis of hundreds of different microbial species interacting with each other and with the human host [1]. With a weight of 1–2 kg and approximately 100 trillion resident microorganisms divided into bacteria, archaea and mycetes, the number of microbial cells that make up the gut is 10 times greater than the number of eukaryotic cells that make up the human body [2]. Given the oxygen-poor nature of the gastrointestinal tract, the microbial species best adapted to proliferate are anaerobic microorganisms, which are approximately 100 times more abundant than facultative anaerobes and aerobes; the dominant bacteria in adult humans belong to four predominant groups: Firmicutes, Bacteroides, Actinobacteria, and Proteobacteria [3]. Among them, Firmicutes bacteria account for 64 % and mainly include Enterococcus, Lactobacillus, and Clostridium species, whereas Proteobacteria include Escherichia, Salmonella, and Helicobacter [4]. *Bifidobacterium, Corynebacterium and Mycobacterium* are considered to be the main components of Actinomycetes [4]. The genus Bacteroides includes bacterial genera such as *Bacteroides, Prevotella, and Parabacteroides* [5]. The *Firmicutes/Bacteroidetes ratio* is a key index of the health and balance of the GM; a dysregulation in the relative abundance of some species of Firmicutes or Bacteroidetes could, in fact, contribute to obesity, intestinal inflammation and cancer [6–8]. Although the human genome consists of approximately 23,000 genes, the set of all microorganisms present in a state of dynamic equilibrium in the human gut encodes more than three million genes that produce thousands of metabolites that can influence

* Corresponding author.
E-mail address: menafradavide@gmail.com (D. Menafra).
[1] Co-first authors.

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various host functions, including energy metabolism, immunological and endocrinological responses; in particular, commensal bacteria regulate the expression of genes involved in several important intestinal and extraintestinal functions, including xenobiotic metabolism, postnatal gut maturation, nutrient absorption, and mucosal barrier strengthening [9,10]. Fermentation of undigestible food residues and endogenous mucus produced by the epithelium is the main source of energy in the colon [11]. The metabolic endpoint is the production of short-chain fatty acids (SCFA) such as acetate, propionate, and butyrate [12]. SCFAs have important functions in host physiology, including energy sources for the colonic epithelium, metabolization by the liver or peripheral tissues, particularly muscles, and may play a role as modulators of glucose and cholesterol metabolism [11,13]. Numerous research studies have shown that qualitative and quantitative alterations in gut microbial species and related metabolites play a crucial role in influencing the development of many diseases, including neurodegenerative diseases [14], cardiovascular diseases [15], gastrointestinal diseases [16], and metabolic diseases [17]. A change in the GM can affect the well-being of the organism through several mechanisms, such as maintaining vitality, choline, SCFAs, the gut-brain hub, and bile acids (BA) [18]. Metabolic diseases potentially associated with altered microbiota include diabetes mellitus (DM), which has been particularly associated with the reduction of butyrate-producing species, such as *Faecalibacterium prausnitzii* and *Roseburia intestinalis* [19]. DM is considered one of the most common metabolic disorders and is characterized by hyperglycemia resulting from defective insulin secretion by pancreatic β-cells, peripheral resistance to insulin action, or both [20]. The International Diabetes Federation estimates that 463 million people worldwide are living with the disease today and that this number could reach 700 million by 2045 [21]. Long-term complications resulting from elevated blood glucose concentrations include damage and impaired function of various organs in the body, resulting in conditions such as retinopathy, nephropathy, neuropathy, and increased cardiovascular risk [22]. DM is classified into 3 main sub-forms that account for almost all diagnosed cases: Gestational diabetes (GDM), type 1 diabetes mellitus (T1DM), and type 2 diabetes mellitus (T2DM). The central role of GM in the onset and development of metabolic dysregulation, such as insulin resistance and diabetes, is increasingly recognized in the scientific literature [23,24]. The mechanism through which an altered intestinal bacterial composition can trigger various pathological conditions, potentially including diabetes, is the disruption of its intestinal barrier function, which leads to metabolic endotoxemia [25]. Moreover, a compromised intestinal barrier allows the influx of inflammatory bacterial fragments into the bloodstream, causing chronic low-grade inflammation, a mechanism underlying increased host adiposity and insulin resistance [26]. Consistently, as suggested by numerous evidences, patients with different forms of DM have qualitative and quantitative differences in gut microbial composition compared with healthy people [27]. Therefore, with the aim of preventing diabetes and mitigating short- and long-term complications, as well as providing literature references on the contribution of the gut microbial population to the treatment of DM, this narrative review summarizes the most important state-of-the-art evidence and focuses on the systems in which the microbiota is involved in the pathogenesis of this disease, as well as its potential use as a therapy (see Fig. 1).

2. Methods

A narrative review was proposed to provide a condensed and filtered overview of the role of GM in the development and prognosis of different forms of diabetes mellitus and related complications. Multiple databases were used for the search, including Cochrane Library, PubMed, Medline (Web of Science), ProQuest, Scopus, and Medline Ovid. The keywords used (alone or in combination) in the databases were "GM", "diabetes", "microbiome", "prebiotics", "probiotics", "diabetes mellitus type 1″, "gestational diabetes", "diabetes mellitus type 2″, "obesity", "insulin resistance", "bacteria", "diet", "glucose homeostasis", and "gut health". For consistency, the search was limited to articles published in English. There were no restrictions on year of publication.

3. Diet, gut microbiome and diabetes

DM stands as one of the most widespread metabolic disorders, posing significant clinical and therapeutic challenges due to its potential for severe short- and long-term complications [28]. While the exact pathogenetic pathways remain incompletely understood, emerging evidence suggests that disturbances in intestinal microbiota, known as dysbiosis, may contribute to the onset or progression of DM [29]. Dysbiotic alterations in gut flora have been observed in individuals with T1DM [30], T2DM [27], and GDM [31], implicating various molecular and anatomical mechanisms. These include heightened intestinal permeability, which can compromise β-cell function, promote insulin resistance, and ultimately precipitate diabetes. Subsequent sections outline the changes in bacterial composition associated with each type of diabetes and explore new dietary strategies for diabetes management and prevention, emphasizing the potential of modulating the GM to alter the trajectory of the disease.

3.1. Pathogenesis of type 1 diabetes mellitus

T1DM is an autoimmune disease characterized by the destruction of pancreatic β-cells, leading to insulin deficiency. The disease results from a complex interplay between genetic predispositions and environmental factors, with epigenetic mechanisms, immune dysfunction and gut microbiome playing a crucial role in modulating this interaction.

3.2. Epigenetic modifications and their role in the development of type 1 diabetes mellitus

Epigenetic modifications such as DNA methylation, histone modifications, and non-coding RNAs (ncRNAs) are central to the regulation of gene expression without altering the DNA sequence itself [32]. DNA methylation involves the addition of a methyl group to the cytosine residues of CpG dinucleotides, which typically represses gene transcription. In T1DM, aberrant DNA methylation patterns have been observed in genes crucial for immune function and β-cell survival. For instance, hypermethylation in the promoter regions of the FOXP3 gene, essential for regulatory T cell (Treg) function, has been associated with reduced FOXP3 expression and impaired Treg function, contributing to the autoimmune attack on β-cells [32]. Additionally, studies on monozygotic twins discordant for T1DM have revealed differential methylation in genes such as HLA-DQB1 and GAD65, suggesting that these epigenetic changes might be early indicators of disease onset [32]. Histones, the protein components of chromatin, undergo various post-translational modifications, including acetylation, methylation, phosphorylation, and ubiquitination. These modifications can alter chromatin structure and gene expression. In T1DM, histone modifications have been implicated in the regulation of immune-related genes. For example, decreased histone acetylation and increased methylation at histone H3 lysine 9 (H3K9) in the promoter regions of immune regulatory genes have been associated with the pathogenesis of T1DM. The altered histone modification patterns can influence the expression of key cytokines and other mediators involved in the autoimmune response [32]. Non-coding RNAs, particularly microRNAs (miRNAs), play significant roles in the post-transcriptional regulation of gene expression. MiRNAs can bind to messenger RNAs (mRNAs) and either degrade them or inhibit their translation. In T1DM, several miRNAs have been found to be dysregulated. For instance, miR-375, which is highly expressed in pancreatic islets, is involved in the regulation of β-cell mass and insulin secretion. Dysregulation of miR-375 has been linked to β-cell apoptosis and the progression of T1DM. Other miRNAs, such as miR-146a and miR-21, have been shown to modulate immune responses, suggesting

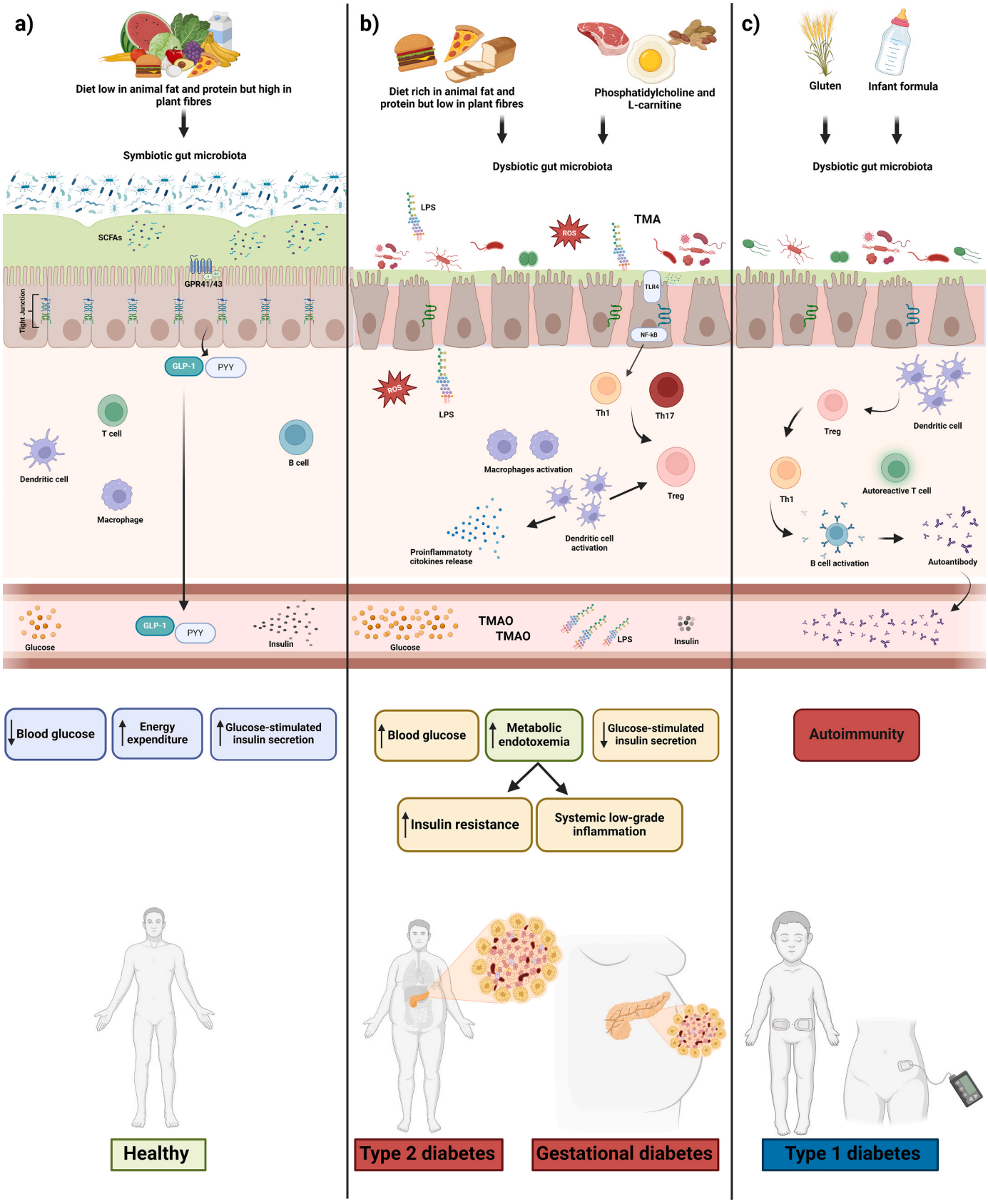

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Fig. 1. Overview of the pathophysiological mechanisms linking gut microbiota, diet and diabetes.
a) In individuals consuming a diet low in animal fat and protein and rich in plant fiber, carbohydrates accessible to the microbiota (MACs) reach the large intestine where they are fermented by intestinal bacteria. Fermentation of MACs by symbiotic bacteria produces SCFAs, mainly acetate, propionate, and butyrate. These SCFAs are taken up by colonocytes through passive diffusion and active transport mechanisms. SCFAs produced in the colon reach the systemic circulation and can exert beneficial effects in other organs and systems, such as regulating blood glucose by decreasing glucose uptake and increasing insulin secretion. They also assist in weight control through the regulation of appetite and energy homeostasis. **b)** Poor dietary habits, such as a diet high in animal fat and protein and low in plant fiber, or the intake of high levels of dietary methylamines, are important factors in the onset of metabolic diseases such as T2DM and GDM. An improper diet alters the balance of the intestinal microbiota, promoting the proliferation of Gram-negative bacteria and reducing the thickness of the mucosal layer. Gut dysbiosis causes increased intestinal permeability, allowing bacteria and bacterial fragments to pass into the bloodstream. Reduced levels of SCFAs in the intestine are associated with decreased secretion of the intestinal hormones GLP-1 and PYY causing altered insulin secretion and glucose metabolism. In addition, decreased SCFAs are linked to increased intestinal permeability and production of pro-inflammatory cytokines. The increase in LPS in the blood, termed metabolic endotoxemia, activates a chronic low-grade inflammatory response. Chronic low-grade inflammation, fueled by metabolic endotoxemia and high concentrations of TMAO produced by the liver, is a key factor in the development of obesity, adipose tissue inflammation, peripheral insulin resistance, and diabetes. **c)** The interaction between diet and intestinal microbiota significantly influences the onset of beta-cell autoimmunity, leading to the destruction of insulin-producing β-cells in the Langerhans islets. An imbalance or poor health of the intestinal microbiota may be a crucial factor in the development of autoreactive T-cells and the production of autoantibodies, which can result in the destruction of these insulin-producing cells, in individuals genetically predisposed to T1DM. Changes in the composition of the intestinal microbiota can lead to decreased mucus and butyrate production, increased gut permeability and bacterial translocation, subclinical intestinal inflammation, and an imbalance in T-cell homeostasis.

their potential as biomarkers for disease prediction and progression [32]. The understanding of epigenetic modifications in T1DM opens avenues for potential therapeutic interventions. Small molecule inhibitors of DNA methyltransferases and histone deacetylases (HDACs) are being explored for their ability to modulate epigenetic marks and restore normal gene expression patterns. For instance, the use of HDAC inhibitors has shown promise in protecting β-cells from cytokine-induced apoptosis and preserving their function. Additionally, miRNA-based therapies are being investigated to correct dysregulated miRNA profiles and mitigate the autoimmune response [32]. In conclusion, epigenetic mechanisms play a pivotal role in the pathogenesis of T1DM by regulating gene expression involved in immune responses and β-cell function. Understanding these mechanisms provides critical insights into disease development and opens up potential therapeutic strategies aimed at modifying epigenetic marks to prevent or treat T1DM [32].

3.3. *Immune dysfunction and its role in the development of type 1 diabetes mellitus*

The immune system plays a pivotal role in the pathogenesis of T1DM. This autoimmune disease is characterized by the targeted destruction of pancreatic β-cells, the cells responsible for insulin production. This process is initiated by a β-cell-specific autoimmune response, where β-cell autoantigens such as insulin, glutamic acid decarboxylase (GAD), tyrosine phosphatase (IA-2), and insulinoma-associated antigen are targeted by the immune system. The mechanisms underlying β-cell destruction are complex and not entirely understood, but they involve a coordinated attack by various immune cells including macrophages, dendritic cells, B lymphocytes, and T lymphocytes. Macrophages and dendritic cells act as antigen-presenting cells (APCs), capturing β-cell antigens and presenting them to T cells. This process activates T cells, particularly $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells. The $CD4^+$ T cells play a role in orchestrating the immune response by producing cytokines such as interferon-gamma (IFN-γ), which activates macrophages and further stimulates the immune response. The $CD8^+$ T cells directly attack and destroy β-cells through mechanisms that involve the release of cytotoxic granules containing perforin and granzyme, leading to β-cell apoptosis [33]. In addition to T cells, B cells also contribute to the autoimmune process in T1DM. B cells can present antigens to T cells and produce autoantibodies against β-cell antigens. These autoantibodies can form immune complexes that enhance the inflammatory response and contribute to β-cell destruction. The presence of autoantibodies against insulin, GAD65, IA-2, and zinc transporter 8 (ZnT8) is a hallmark of T1DM and can be detected years before the clinical onset of the disease [33]. The role of the innate immune system in T1DM is also significant. Natural killer (NK) cells, which are part of the innate immune system, have been found to be involved in the early stages of the disease. These cells can kill β-cells directly and produce cytokines that influence the adaptive immune response. Studies have shown that NK cells are present in the pancreatic islets of individuals with T1DM and may contribute to the initial inflammation that triggers the autoimmune attack [33]. Furthermore, genetic factors play a crucial role in the susceptibility to T1DM. The major histocompatibility complex (MHC) region, particularly the human leukocyte antigen (HLA) class II alleles DR3 and DR4, is strongly associated with an increased risk of developing T1DM. These HLA alleles are involved in the presentation of β-cell antigens to T cells and influence the immune response. Other genetic factors, including the insulin gene and various non-HLA genes, also contribute to disease susceptibility [33]. Understanding the interplay between the immune system and genetic factors in T1DM is essential for developing targeted interventions to prevent or treat the disease. Current research is focused on identifying the precise mechanisms of β-cell destruction and exploring new therapeutic approaches, such as immunomodulation and β-cell regeneration, to halt or reverse the progression of T1DM [33].

3.4. *Diet, gut microbiome and type 1 diabetes mellitus*

T1DM is a proinflammatory issue that results from the autoimmune assault on the insulin-producing β cells within the pancreas [34]. The increased mortality rates due to T1DM complications and the rising incidence rate among children, which has been reported to be ~3–5% per year worldwide since 1960, mostly in developing countries, underscore the urgent need for therapeutic interventions aimed at averting the onset of this enduring condition [35,36]. Whereas genetic predisposition plays a significant role and the interpretation of the interaction with environmental factors remains incomplete, recent studies suggest that the gut microbiome could be intricately involved in the mechanisms underlying this inflammatory phenomenon [37]. Several studies in both animal and human models have demonstrated differences in gut microbial composition between healthy individuals and those with T1DM or at risk of developing T1DM. In one study, the GM of Bio-Breeding diabetes-prone (BB-DP) rats, used as a model for T1DM, was profiled long before the clinical manifestation of diabetes using fluorescence in situ hybridization; specifically, BB-DP rats were given antibiotics and the resulting effect on the incidence of DM and the degree of insulitis was examined [38]. Mice destined to develop DM displayed a distinct gut bacterial composition compared to healthy mice, with lower levels of *Bacteroides* sp. Present well before disease onset; furthermore, antibiotic treatment, altering gut flora, reduced diabetes incidence and delayed its progression [38]. Likewise, another study reported a marked reduction in the abundance of *Lactobacillus*, *Bryantella*, *Bifidobacterium*, and *Turicibacter* in BB-DP rats, while there was an increase in the levels

of *Bacteroides*, *Eubacterium*, and *Ruminococcus* in BB-DP rats compared to Bio-Breeding Diabetes Resistant Rats (BB-DR) [39]. Consistently with results obtained in animal models, significant differences in the taxonomic composition of the gut have been found between individuals with T1DM and healthy individuals; in particular, an investigation conducted as part of the TEDDY study, which analyzed 10,913 metagenomes in stool samples from 783 mostly white, non-Hispanic children, showed a significant increase in the abundance of Bifidobacterium spp. And decreased abundance of Streptococcus thermophilus and Lactococcus lactis in children before seroconversion or onset of T1DM compared to their healthy counterparts [40]. Similarly, a case-control study involving 32 children, half with T1DM, found differences in fecal bacterial composition; compared to healthy children, those with diabetes showed a significant decrease in *Actinobacteria* and *Firmicutes* bacteria, as well as a lower *Firmicutes/Bacteroidetes* ratio and, conversely, the amount of *Bacteroidetes* bacteria was significantly higher in children with diabetes [17]. Moreover, previous research has shown that diet can rapidly change the gut microbial composition, affecting the development of T1DM in both humans and animals [41,42]. Some evidence in mouse model indicates that dietary glutamine could influence intestinal SIgA production through various mechanisms, including interaction with the GM, the process of antigen sampling and presentation, and the pathways involved in the induction of SIgA production by plasma cells, thus helping to modulate intestinal immunity [43]. A recent study showed that dietary lysine restriction played a significant role in the modulation of the GM and inflammatory response in piglets [44]. Through in-depth analysis of bacterial rRNA markers, it was observed that bacterial diversity increased in the lysine-restricted group; in particular, lysine restriction affected the abundance of several bacterial phyla in the gut, including Actinobacteria, Saccharibacteria, and *Synergistetes* [44]. At the household level, changes were found in the abundances of *Moraxellaceae*, *Halomonadaceae*, *Shewanellaceae*, *Corynebacteriaceae*, *Bacillaceae*, *Comamonadaceae*, *Microbacteriaceae*, *Caulobacteraceae*, and *Synergistaceae* in response to lysine restriction [44]. Gluten, a protein complex found in several major grains such as wheat, also has a high abundance of glutamine and proline [45] and intake of gluten early in life can lead to celiac disease (CD) and may assist in the onset of T1DM [46,47]. Interestingly, in a study the effect of gluten intake and changes in the GM on the incidence of T1DM was analyzed; the data reported that a gluten-free diet, compared with controls, can increase the number of regulatory T cells, delay the onset and decrease the number of new cases of T1DM in non-obese diabetic mice (NOD) [48]. This evidence suggests that the gluten-free diet could have a protective function of β-cell function through modulation of the GM [48]. Breastfeeding has a significant impact on the composition and diversity of the infant's gut bacteria, which also appear to be influenced by the amount and duration of breastfeeding [49]. Previous research has shown significant differences in the microbial composition and diversity of formula-fed versus breastfed infants [50]. In a comparative study, breastfeeding was found to promote the selective growth of the bacterium *Bifidobacterium longum* subsp. Infantis, which is involved in the development of the immune system and whose relative abundance was inversely correlated with the risk of developing T1DM [51]. Breastfed infants also have lower levels of potentially harmful bacteria such as E. coli than formula-fed infants [52]. Mechanisms by which breast milk would help maintain gut health in infants include specific milk components such as the genus Lactobacillus, oligosaccharides, and hormones such as insulin and leptin [30,53]. A notable finding from a case-control study involving 246 children revealed that each additional month of exclusive breastfeeding was associated with a 0.83-fold reduction (95 % CI 0.72, 0.96) in the risk of developing T1DM; conversely, introducing cereals into the diet at or before the sixth month was linked to a 2.58-fold increase (95 % CI 1.29, 5.16) in T1DM risk [54]. Overall, the results of the presented studies suggest that individuals with T1DM may have indications of gut dysbiosis. Indeed, diet-induced changes in the gut microbiome affect the balance of the immune system by altering the microbial composition and production of metabolites, such as the decreased presence of SCFA, which could promote an inflammatory environment when associated with a specific gut microbial pattern. Based on this evidence, it is reasonable to hypothesize that maintaining a healthy microbial composition early in life may help prevent the onset and progression of T1DM in genetically predisposed individuals. However, studies show mixed results on whether breastfeeding directly reduces the risk of T1DM, so more research is needed to fully understand the complex interactions between breastfeeding, gut bacteria, and the development of T1DM.

3.5. Diet, gut microbiome and diabetes: a role for treatment of type 1 diabetes mellitus?

Given the growing evidence suggesting that the gut microbiome is implicated in the pathophysiological mechanisms of DM, several studies have tested microbiota-targeted interventions like the use of prebiotics and probiotics as a new therapeutic strategy [55]. In this context, the term "targeted prebiotics" has been introduced to describe fibers with specific structures that predictably influence beneficial gut microbes; indeed, it has been highlighted the importance of selecting prebiotics based on their chemical structure and ability to interact with specific microbial groups, and, consistently, targeted prebiotics can induce predictable changes in microbial composition and short-chain fatty acid production, contributing to more effective nutritional strategies [56]. Moreover, mitigation mechanisms include modulation of gut microflora aimed at reducing intestinal permeability, systemic inflammation, and improving insulin sensitivity [57]. Prebiotics are non-digestible compounds, generally plant fibers that are easily fermented by intestinal bacteria; in particular, oligosaccharides such as inulin, xylose-oligosaccharide, galacto-oligosaccharide, and fructo-oligosaccharide are among the most commonly used prebiotics [58]. Probiotics are living microorganisms taken through supplements or fermented foods known for their beneficial effects on host health [59]. The most commonly studied probiotic microorganisms include *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium lactis*, and *Streptococcus* [60,61]. The effect of immune modulation operated by probiotic intake was evaluated in a previous work that observed a reduction in the number of splenic CD8+T cells and systemic inflammatory markers (such as interferon-gamma) in NOD mice treated with Lactobacillus Casei [62]. In a study conducted in 2016, these findings were confirmed by changes in the GM induced by oral treatment with a lactobacillaceae-enriched probiotic alone or in combination with retinoic acid (RA), which protected NOD mice from T1DM by affecting the inflammasome at the intestinal level [63]. Among the most promising results from clinical trials is that of the TEDDY cohort, which involved more than 7000 children and demonstrated a beneficial effect of early supplementation of mixtures of various *Lactobacillus* and *Bifidobacterium* species in the first 27 days of life in reducing the risk of developing T1DM in individuals with high-risk HLA-DR3/4 genotype [64]. The results obtained were confirmed in a randomized double-blind placebo-controlled trial conducted in children with newly diagnosed T1DM, who were orally administered one capsule per day containing 112.5 billion live, freeze-dried *lactic acid bacteria* and *bifidobacteria* for three months, which led to a significant reduction in HbA1c and insulin bolus doses in the intervention group compared with placebo, with no adverse reactions reported [65]. Similarly, other authors evaluated the impact of prebiotics on GM and barrier function in NOD mice; in particular, the results demonstrated that a diet supplemented with xylooligosaccharides was associated with delayed diabetes, decreased gut permeability, and fewer cellular infiltrations in pancreatic islets [57]. These findings were also evaluated in human models, specifically in a randomized controlled trial that investigated the potential benefits of prebiotics in the management of T1DM in 38 children [66]. After 3 months, children receiving prebiotics showed a significant increase in C-peptide levels and also experienced a modest improvement in

intestinal permeability compared to the placebo group [66]. The GM composition changed in both groups: the prebiotic group had a significant increase in beneficial *Bifidobacteria* at 3 months, which disappeared after stopping the washout period, while the placebo group experimented specific increase of *Streptococcus*, *Roseburia*, *Terrisporobacter*, and *Faecalitalea* compared with the prebiotic group at 3 months [66]. In conclusion, given the key role of GM in T1DM, recent studies explore the use of prebiotics and probiotics, which influence intestinal bacterial composition, as a potential therapy for T1DM [55]. Animal and human studies have shown that taking prebiotics and probiotics can improve gut health, reduce inflammation and enhance insulin sensitivity, showing potential benefits in the management of T1DM [57,63,65]. These encouraging results support continuous research into the use of prebiotics and probiotics as complementary therapeutic interventions for DM, with a focus on their long-term safety and efficacy.

3.6. Pathogenesis of type 2 diabetes mellitus

T2DM is a complex metabolic disorder characterized by chronic hyperglycemia resulting from a combination of insulin resistance and impaired insulin secretion. The pathogenesis involves genetic predispositions and environmental factors, such as diet and physical inactivity, which lead to alterations in glucose metabolism and insulin signaling pathways. Epigenetic modifications, immune dysfunction and dysregulation in the GM all play a critical role in mediating these effects, contributing to the development and progression of T2DM.

3.7. Epigenetic modifications and their role in the development of type 2 diabetes mellitus

Epigenetics plays a crucial role in the pathogenesis of T2DM by influencing the expression of genes involved in glucose metabolism and insulin signaling. The primary epigenetic mechanisms affecting T2DM include DNA methylation, histone modifications, and non-coding RNAs. These modifications regulate gene activity without altering the DNA sequence, and their disruption can contribute to metabolic diseases like T2DM. DNA methylation involves the addition of methyl groups to the cytosine residues of CpG dinucleotides, typically leading to gene repression. In T2DM, altered DNA methylation patterns have been observed in key metabolic tissues such as pancreatic islets, skeletal muscle, adipose tissue, and the liver. For instance, increased DNA methylation in the promoter regions of genes such as INS, PDX1, and PPARGC1A in pancreatic islets from T2DM patients is associated with decreased expression of these genes, contributing to impaired insulin secretion and β-cell dysfunction [32,67]. Additionally, DNA methylation changes in adipose tissue and skeletal muscle have been linked to insulin resistance, a hallmark of T2DM [67,68]. Histones are proteins around which DNA is wrapped, and their chemical modifications can significantly impact gene expression. Histone acetylation and methylation are two common modifications. Acetylation typically promotes gene expression by loosening the DNA-histone interaction, whereas methylation can either activate or repress gene expression depending on the specific amino acids modified. Genome-wide studies have identified differences in histone acetylation and methylation between diabetic and non-diabetic subjects, indicating that these modifications play a role in the regulation of genes involved in glucose metabolism and insulin signaling [67,69]. Non-coding RNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), regulate gene expression post-transcriptionally. In T2DM, dysregulation of specific miRNAs has been observed, affecting genes involved in insulin signaling and glucose metabolism. For example, miR-375 is known to regulate insulin secretion and β-cell function, and its dysregulation is linked to T2DM [67,70]. Environmental factors, diet, and lifestyle significantly interact with the epigenome, influencing the risk and progression of T2DM. For instance, diet can impact DNA methylation and histone modification patterns. High-fat diets and excessive calorie intake are associated with changes in the methylation of genes involved in metabolism and insulin signaling [67,68]. Physical activity, on the other hand, has been shown to induce beneficial epigenetic changes that improve insulin sensitivity and metabolic health [67,70]. Additionally, aging is associated with cumulative epigenetic changes that may predispose individuals to T2DM [67,69]. Understanding these epigenetic modifications offers insights into potential biomarkers for early detection and new therapeutic targets for managing T2DM. The reversible nature of epigenetic modifications holds promise for developing drugs that can modify these marks, potentially reversing or mitigating the progression of T2DM. For example, DNA methylation inhibitors and histone deacetylase inhibitors are being explored as potential therapies [67,68]. In conclusion, epigenetic mechanisms are integral to the development and progression of T2DM. They interact with genetic and environmental factors to regulate key metabolic processes. By understanding these mechanisms, researchers can develop targeted interventions to prevent or treat T2DM, ultimately improving patient outcomes [32,67].

3.8. Immune dysfunction and its role in the development of type 2 diabetes mellitus

Over the past three decades, research has increasingly underscored the significant influence of prenatal and early infant environmental exposures on the risk of adult health disorders, particularly T2DM, a concept known as developmental programming. One notable example of this is intrauterine growth restriction (IUGR), a condition that is associated with a considerable risk of developing T2DM, obesity, and cardiovascular disease in the offspring. IUGR frequently gives rise to a distinctive β-cell phenotype, typified by impaired function, which exerts a pivotal influence on the pathogenesis of T2DM. The observation of analogous β-cell dysfunctions in animal models of IUGR provides evidence of a shared pathway between humans and animals [71]. The fetal origin of disease hypothesis, as put forth by Professor David Barker, postulates that unfavorable intrauterine environments can give rise to long-term health concerns. For example, the Dutch famine of 1944–1945 provides a compelling illustration of this phenomenon. Offspring of mothers who experienced famine during early pregnancy exhibited a higher incidence of obesity, cardiovascular disease, and T2DM in adulthood. The risk of impaired glucose tolerance or T2DM was found to be sevenfold higher in men born with a low birth weight (2.5 kg or less) compared to those born with a birth weight of 3.5 kg [71]. The immune system also plays a pivotal role in the pathogenesis of IUGR-induced T2DM. The fetal immune system must maintain a balance between tolerance of maternal antigens and preparation for postnatal environmental challenges. IUGR can disrupt this balance, leading to alterations in immune pathways and cytokine levels, which in turn affect pancreatic islet development and β-cell function. In particular, cytokines such as IL-1β and IL-4 are altered in IUGR, contributing to the inflammatory milieu that predisposes individuals to T2DM [71]. Furthermore, research has indicated that immunomodulation may provide therapeutic advantages for the treatment of IUGR-induced T2DM. For example, the blockade of IL-4 in neonatal rat models has been demonstrated to prevent β-cell dysfunction and the subsequent development of T2DM. This evidence suggests that the targeting of immune pathways may represent a viable strategy for the mitigation of the effects of IUGR on long-term metabolic health. Furthermore, the administration of GLP1 agonists, which possess immunomodulatory properties, has demonstrated efficacy in enhancing β-cell functionality in IUGR models [71]. These findings highlight the necessity of elucidating the relationship between early developmental exposures and immune system alterations in the pathogenesis of T2DM. As research in this area continues, it has the potential to inform the development of targeted interventions to prevent or treat T2DM, particularly in individuals affected by adverse intrauterine conditions such as IUGR [71].

3.9. Diet, gut microbiome and type 2 diabetes mellitus

The development of T2DM is characterized by impaired insulin secretion by pancreatic β-cells and concomitant insulin resistance, which increases hepatic glucose production and hampers glucose uptake into tissues, leading to increased blood glucose [72]. Although the pathogenesis is multifactorial, the onset of T2DM is mainly associated with overweight and obesity [73,74]. Chronic low-grade inflammation, associated with excess weight and particularly increased visceral adiposity, is characterized by moderate production of cytokines, such as interleukin (IL-) 6, IL-1 and tumor necrosis factor alpha (TNFα), which negatively affect cellular insulin signals, promoting insulin resistance and T2DM [75,76]. Several studies have confirmed the association between GM dysbiosis, obesity, and T2DM [37,77]. Normally, GM plays a protective role in metabolic regulation and glucose and lipid metabolism [78]. However, in gut dysbiosis associated with obesity, these functions are impaired [79]. Studies in germ-free animals have shown that such animals are resistant to diet-induced obesity [80]. Conversely, exposure to bacteria such as obesity-associated *Enterobacter cloacae* or bacteria from obese donors induces increased energy storage, weight gain, and impaired glucose tolerance in germ-free mice [81,82]. These studies suggest a possible link between GM changes and obesity. In this sense, a comparative study showed that transplantation of GM isolated from obese donors into germ-free mice resulted in a significant increase in body fat content and insulin resistance in recipient mice [83]. A 2010 study involving 36 men, half with T2DM, revealed a link between GM and T2DM; they found positive correlations between blood sugar levels and specific bacterial groups: *Bacteroidetes* and *Firmicutes*, *Bacteroides-Prevotella*, and *C. Coccoides- E. Rectale* [27]. Additionally, the *Betaproteobacteria* class, more abundant in diabetic patients, also correlated with higher blood sugar [27]. Subsequently, several human studies have examined the effect of GM manipulation on obesity and T2DM. Transplantation of the GM from lean donors into subjects with metabolic syndrome was found to result in improved insulin sensitivity [84]. In addition, a recent study showed that obese participants who received fecal transplants from lean donors became more sensitive to insulin within 6 weeks, underscoring the causal role of a healthy GM in controlling insulin resistance [85]. Unlike the GM of healthy subjects, the GM of subjects with T2DM exhibits moderate gut dysbiosis with increased presence of opportunistic pathogens and decreased SFCA-producing bacteria, chief among them butyrate [19]. Similarly, recent evidences reported increased levels of *Akkermansia muciniphila* and the sulfate-reducing species Desulfovibrio, both of which are involved in intestinal barrier functions [86]. A recent study using quantitative real-time polymerase chain reaction (qPCR) showed an increase in *Enterococci* and a decrease in *Bacteroides*, *Bifidobacteria*, *Akkermansia muciniphila*, and *Lactobacilli* in patients with T2DM, which were associated with a worsening of anthropometric parameters, such as BMI, and cardiometabolic profile [87]. Based on this relationship, other works have advanced the hypothesis that reduced levels of butyrate-producing bacteria may be related to T2DM [19]. Indeed, bacteria such as *Faecalibacterium prausnitzii*, which plays a significant role in butyrate production, were found to be reduced in people with T2DM [86]. In addition, several studies have found a higher rate of intestinal microbes in the circulation of diabetic patients, suggesting that by affecting intestinal permeability, microscopic organisms may translocate from the digestive tract to the circulation, causing endotoxemia and inflammation [88]. Moreover, it has been observed that individuals with diabetes have higher fasting and postprandial lipopolysaccharide (LPS) concentrations than those without diabetes [89]. This subclinical pro-inflammatory state, resulting from the LPS-dependent production of inflammatory cytokines such as IL-1, IL-6, and TNF-α, could be associated with the development of insulin resistance and T2DM [89,90]. The conventional antidiabetic strategy recommends combining drugs with a healthier diet and less sedentary lifestyle, which promote GM remodeling and potentially enhance the therapeutic effects of drugs [91]. Some authors hypothesized the existence of three different enterotypes of microbiota, characterized by different species composition, including enrichment of *Bacteroides (enterotype I)*, *Prevotella (enterotype II)*, and *Ruminococcus (enterotype III)*, respectively [92]. Each enterotype was associated with a specific dietary regimen: the former appears to be related to the Western diet, which is characterized by a high content of calories and saturated fat, associated with a higher inflammatory profile and endotoxemia, as well as lower intestinal biodiversity, features also found in overweight and obese subjects [93]. In contrast, a Mediterranean diet, characterized by a low intake of saturated fatty acids and refined sugars but rich in fiber and unsaturated fatty acids, has been associated with enterotype II and shown to positively influence the microbiota, offering protection against diseases such as T2DM, cardiovascular disease, and cancer [94]. In this context, it is important to highlight that dietary fiber reduces intestinal permeability and consequently the proinflammatory state associated with endotoxemia [95]. Since fiber intake correlates with increased butyrate production [11], this mechanism could explain the preventive effect on the health of a fiber-rich Mediterranean diet [96]. Finally, the third enterotype is less frequent in the population and less constant in its composition and is not closely associated with a specific dietary profile [92]. The different response obtained from a given nutritional intervention in different enterotypes suggests that a personalized strategy, tailored to the composition of each individual's microbiota, should be offered. Finally, moderate-intensity physical activity can also influence GM composition with a positive impact on GM composition and endotoxemia through an increased relative abundance of *Akkermansia Muciniphila* and SCFA production [97]. In this regard, it is interesting to note that in a 2019 study of thirty patients with T2DM, a 6-month endurance, resistance, and flexibility physical activity intervention resulted in improved glycemic control, anthropometric variables, intestinal bacterial overgrowth, and systemic inflammation [98]. Based on these considerations, taking into account the gut biodiversity between healthy subjects and subjects with T2DM could lead to early identification of the risk of developing this disease and assist in the personalization of medical-nutritional therapy.

3.10. Diet, gut microbiome and diabetes: a role for treatment of type 2 diabetes mellitus?

Dietary and lifestyle habits change the qualitative and quantitative composition of the GM and metagenomic expression in a manner completely independent by the host genome [99,100]. Several studies have investigated the role of dietary approach on the composition of the GM related to the improvement of DM [101,102]. Studies focusing on the Mediterranean diet have provided evidence on the ability of this dietary regimen to change the microbial composition of the gut, and thus contribute to counteracting the progression of T2DM [103]. A low-fat, low-sugar diet causes a change in the *Bacteroidetes/Firmicutes* ratio in patients with overweight or obesity, with an increase in the former [104]. A randomized clinical trial also analyzed the effects of a high-fiber diet on gut dysbiosis, serum metabolism, and psychiatric comorbidities such as anxiety and depression in 26 patients with T2DM [105]. The high-fiber diet increased the abundance of beneficial gut bacteria such as *Lactobacillus*, *Bifidobacterium*, and *Akkermansia*, while reducing the levels of potentially harmful bacteria such as *Desulfovibrio* and *Klebsiella*, improving glycemic control, systemic inflammation, and mental health [105]. On the other hand, consumption of high-fat diets has been reported to alter the *Bacteroidetes/Firmicutes* ratio; these alterations have been associated with obesity and the subsequent development of chronic diseases such as DM and metabolic syndrome [106]. The use of probiotics and prebiotics appears to alter the GM and improve the digestion of certain macromolecules such as starch [107]. Bacteria such as *Bifidobacterium* and *Lactobacillus*, which can ferment sugars in the gut, have been prioritized in prebiotic-based therapeutic treatments of T2DM in populations of various ages [42]. Indeed, previous studies

have shown that the relative increase of *Bifidobacterium* spp. In the intestinal tract of obese mice reduces inflammation by increasing concentrations of glucagon-like peptide-1 (GLP-1), which is capable of modulating intestinal permeability [108]. It has also been shown in humans that the increase in *Bifidobacterium* induced by taking certain prebiotics is related to an increased incretin response by the intestine of GLP-1 and peptide YY [109]. These two molecules have a favorable prognostic impact on DM by reducing insulin resistance and improving β-cell function [109]. Recent research observed that when mice are subjected to a high-fat diet supplemented with prebiotics containing oligofructose, intestinal levels of *Bifidobacteria* are restored, with an improvement in endotoxemia and glucose tolerance [110]. Other research indicates a potential prebiotic effect of the polyphenols contained in red wine in humans, as a decrease in endotoxemia accompanied by an increase in species such as *Enterococcus*, *Prevotella*, *Bacteroides*, *Bifidobacterium*, *Bacteroides uniformis*, *Eggerthella lenta, Blautia coccoides*, and *Eubacterium* was observed [111]. The beneficial impact of prebiotics on dysmetabolic conditions was affirmed in a preliminary clinical study in which six obese volunteers with T2DM and hypertension were put on a vegetarian diet for one month [112]. Their metabolic parameters related to glucose and lipid metabolism, as well as anthropometric parameters, improved substantially; the vegetarian diet drove compositional changes in the GM, and improved *Firmicutes* ratio, which reduced intestinal irritation and increased SCFA levels [112]. In this regard, it has been indicated that supplementation with SCFAs, particularly butyrate, reduces inflammatory marker concentrations and reactive oxygen species production [113]. In an obese mouse model, butyrate supplementation was found to be protective against weight gain and insulin resistance while consuming a high-fat diet [114]. Another therapeutic approach using bacteria is the use of probiotic-based dietary supplements containing live bacterial strains of *Bifidobacterium* and *Lactobacillus* [115]. In mice, supplementation with this type of probiotic had an antidiabetic impact with a concomitant decrease in endotoxicosis [116]. Consistently, an anti-diabetic impact of probiotics was also found in mice fed a supplement of *Lactobacillus acidophilus* and *Lactobacillus cases* [117]. Another study that involved 32 patients demonstrated improved starch digestion following *Helicobacter pylori* destruction; in particular, HbA1c levels showed a strong correlation with GLP-1 levels after treatment at all time points measured following the oral glucose tolerance test (OGTT) [118]. It is also interesting to note that in a parallel-group, double-blind, randomized, placebo-controlled clinical trial that enrolled 120 prediabetic adults randomly assigned to receive probiotic supplements or placebo for 24 weeks, the group that received probiotics experienced a significant reduction in fasting plasma glucose levels, fasting insulin levels, HOMA-IR and HbA1C compared to the placebo group [119]. Other evidence suggested that daily ingestion of 300 g of yogurt containing *Lactobacillus acidophilus La5* and *Bifidobacterium lactis Bb12* for a period of 45 days reduced blood glucose and HbA1c levels in patients with T2DM [120]. Dietary interventions and probiotics have generally shown promise in alleviating T2DM. Additionally, the use of prebiotics derived from bacteria is an emerging trend in clinical exploration. However, further research is needed to determine the optimal formulation and dosage of prebiotics, as well as the specific efficacy of probiotics in managing T2DM.

3.11. Pathogenesis of gestational diabetes mellitus

Gestational Diabetes Mellitus (GDM) arises from a complex interplay of epigenetic factors, dysregulation in the GM, and immune dysregulation. Epigenetic modifications such as DNA methylation and histone modifications can alter the expression of genes involved in glucose metabolism and insulin signaling, predisposing women to GDM. Additionally, alterations in the GM, influenced by diet and other environmental factors, can affect metabolic health by modulating inflammation and insulin resistance. Immune dysregulation further exacerbates this condition, as immune system imbalances can lead to chronic inflammation and impaired insulin action. Together, these factors contribute to the development and progression of GDM [121].

3.12. Epigenetic modifications and their role in the development of gestational diabetes mellitus

Epigenetic mechanisms play a significant role in the pathogenesis of GDM, influencing both maternal and fetal health. GDM is characterized by glucose intolerance that arises during pregnancy and can lead to adverse outcomes for both the mother and the fetus. Epigenetic modifications, such as DNA methylation, histone modifications, and microRNAs, are critical in regulating gene expression without altering the DNA sequence, and these changes have been implicated in the development and progression of GDM. DNA methylation, the addition of methyl groups to cytosine residues in CpG dinucleotides, typically represses gene expression. In GDM, genome-wide DNA methylation studies have identified differential methylation in several genes. For instance, genes such as HOOK2, RDH12, and PIK3R5 exhibit altered methylation patterns in women with GDM compared to those with healthy pregnancies. HOOK2 is involved in organelle binding and endocytosis, RDH12 plays a role in short-chain aldehyde metabolism, and PIK3R5 is crucial for cell growth and survival [121]. These methylation changes can affect insulin resistance and β-cell function, key factors in the development of GDM. Histone proteins, around which DNA is wrapped, can undergo post-translational modifications such as acetylation, methylation, phosphorylation, and ubiquitination. These modifications can alter chromatin structure and gene expression. In the context of GDM, histone modifications have been shown to influence the expression of genes involved in glucose metabolism and inflammatory pathways. For example, altered histone acetylation and methylation patterns have been observed in key metabolic genes in placental tissues from GDM pregnancies, which can impact fetal development and increase the risk of metabolic disorders later in life [121]. Moreover, in GDM several miRNAs have been found to be dysregulated. For instance, miR-29a and miR-143 have been implicated in the regulation of insulin signaling pathways. Dysregulation of these miRNAs can contribute to insulin resistance and β-cell dysfunction, further exacerbating the metabolic disturbances seen in GDM [121]. Environmental factors and lifestyle choices also play a significant role in modulating epigenetic marks in GDM. Diet, physical activity, and exposure to environmental toxins can influence DNA methylation and histone modification patterns. For example, a high-fat diet during pregnancy has been associated with changes in the methylation of genes involved in lipid metabolism and inflammation, potentially predisposing offspring to obesity and T2DM [121]. Conversely, physical activity has been shown to induce beneficial epigenetic changes that improve insulin sensitivity and overall metabolic health [121]. One of the critical concepts in the context of GDM and epigenetics is metabolic memory, where even slight increases in maternal glycemia during pregnancy can cause lasting changes in gene expression through epigenetic modifications. These changes can predispose the offspring to metabolic disorders such as obesity and T2DM later in life. Studies have shown that placental DNA methylation patterns are adapted to maternal glycemic levels, affecting fetal development and increasing the long-term risk of metabolic diseases [121].

3.13. Immune dysfunction and its role in the development of gestational diabetes mellitus

GDM is a common pregnancy complication characterized by glucose intolerance and insulin resistance, often leading to significant health risks for both the mother and the fetus. One of the key factors in the pathogenesis of GDM is immune dysfunction, marked by low-grade systemic inflammation that exacerbates maternal immune responses. This inflammatory environment is characterized by alterations in

regulatory T cells (Tregs) and an imbalance in the Th17:Treg ratio, which fosters a pro-inflammatory state. These immune cell dysregulations significantly affect the maternal-fetal interface, potentially leading to adverse pregnancy outcomes such as fetal macrosomia, neonatal jaundice, and preeclampsia [122]. Research has highlighted the role of glycans and glycan-binding proteins, such as galectins, in modulating immune responses in GDM. Galectins, including galectin-1 (gal-1), galectin-2 (gal-2), galectin-3 (gal-3), and galectin-13 (gal-13), play crucial roles in maintaining immune tolerance during pregnancy. In GDM, there is a failure to upregulate gal-1, which disrupts immune cell function and contributes to inflammation. This dysregulation of galectins is associated with impaired placental function and increased risk of complications [122]. Furthermore, the etiology of GDM involves multifactorial pathways including inflammation, regulatory T cell dysregulation, proteinopathy, and altered autophagy. For instance, recent studies have shown that GDM is associated with changes in the levels and functions of various immune cells, such as an increase in pro-inflammatory macrophages (M1) and a decrease in anti-inflammatory macrophages (M2), contributing to the inflammatory milieu [122]. Additionally, the presence of protein aggregates in the serum of GDM patients indicates a link between metabolic stress and immune dysfunction, further complicating the disease pathology [122]. Recent findings also suggest that maternal immune activation during GDM can have long-lasting effects on the offspring, potentially increasing the risk of neurodevelopmental disorders such as autism and schizophrenia [122]. This underscores the importance of understanding the intricate interactions between immune and metabolic pathways in GDM to develop targeted therapeutic strategies that can manage the condition and improve pregnancy outcomes.

3.14. Diet, gut microbiome and gestational diabetes mellitus

Over the past two decades, a vast amount of scientific data has emphasized the role of GM in the development of GDM [123,124]. The diagnosis of GDM occurs when glucose intolerance is first detected during pregnancy [125]. This condition is one of the most common perinatal complications, often associated with the older age of pregnant women and the general increase in the number of overweight or obese women with metabolic disorders [126]. Hyperglycemia in pregnancy has serious and long-term clinical implications for the mother and fetus; these complications can include premature birth, high blood pressure during pregnancy (preeclampsia), cesarean delivery, admission to the neonatal intensive care unit, low blood sugar in the newborn (hypoglycemia), jaundice (hyperbilirubinemia), birth injuries, and even death of the baby (perinatal death) [127]. The gestational state, whether free or complicated by disease, induces changes in the composition and activity of the GM due to changes in body composition, hormonal fluctuations, and increased release of pro-inflammatory cytokines [128]. During pregnancy, there is a relative increase in bacteria belonging to the phyla *Proteobacteria* and *Actinobacteria*, accompanied by a decrease in bacteria such as *Roseburia intestinalis* and *Faecalibacterium prausnitzii*, which are known to correlate with good intestinal health [129]. In recent years, some studies have compared the GM of women with GDM and that of healthy pregnant women [123,130]. The main findings indicate reduced intestinal biodiversity, variations in the abundance of specific bacterial taxa, and consequent dysregulation of metabolic activity operated by the GM [131]. In a 2019 study, the authors found a higher abundance of *Ruminococcus* and *Prevotella* and lower numbers of *Bacteroides*, *Roseburia*, and *Akkermansia* in patients with GDM compared with healthy pregnant women [130]. A relative increase in the genus *Ruminococcaceae*, assisting in energy metabolism, insulin signaling, and inflammatory processes was correlated with increased fasting glucose concentrations and insulin resistance leading to an increased risk of developing GDM [132]. Another study including pregnant women also showed that the GM of patients with GDM shows a decrease in SCFA-producing bacterial species, such as the genus *Faecalibacterium* [133]. Bacteria belonging to the genus Faecalibacterium are important producers of butyrate, which promotes β-cell differentiation and proliferation, enhancing insulin resistance [123]. In a study involving 52 pregnant women at 24–28 weeks gestation, it was observed that the number of bacteria of the genus *Faecalibacterium* was negatively correlated with fasting blood glucose level, while the number of *Blautia*, generally increased in the microbiota of women with GDM, was positively correlated with fasting blood glucose level [134]. SCFAs deficiency is known to be linked to LPS-dependent metabolic endotoxemia, which leads to the production of proinflammatory cytokines, an additional factor predisposing women to the development of insulin resistance and GDM [134]. In addition, some gut microbes can metabolize methylamines contained in some foods (including red meat, fish, nuts, and eggs) such as choline, l-l-carnitine, and phosphatidylcholine, producing trimethylamine (TMA), which is subsequently converted to trimethylamine-N-oxide (TMAO) in the liver [135]. Elevated blood levels of TMAO are known to be associated with increased cardiovascular risk and diabetes [136]. A recent study showed a positive correlation between plasma concentrations of TMAO during pregnancy and an increased risk of developing GDM [137]. Studies in rodents fed a Western-style diet have shown that exposure to TMAO can impair glucose tolerance and promote inflammation in adipose tissue [138]. However, the exact role of TMAO plasma concentrations in glucose metabolism remains largely unknown, as does the taxonomic composition of TMA-producing bacteria in humans, which are found primarily in the phyla *Firmicutes*, *Actinobacteria* and *Proteobacteria*. Finally, it is important to note that scientific data show that the state of dysbiosis during GDM can be modulated through nutritional interventions [139,140]. In a prospective observational study was demonstraetd that a dietary intervention based on the recommendations of a healthy and balanced diet led to an increase in gut microbial α-diversity, an increase in *Firmicutes* and a reduction in the number of *Bacteroidetes* and *Actinobacteria* [141]. In summary, studies conducted in women with GDM have revealed obvious states of intestinal dysbiosis [142]. These imbalances involve several bacterial phyla such as *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* [129]. In contrast, a decrease in the relative abundance of butyrate-producing beneficial bacteria such as *Faecalibacterium* and *Bifidobacterium* has been suggested [123]. Furthermore, the dysbiotic status of the GM in GDM patients might promote the production of TMAO, which seems to be related to inflammation, increased adiposity and glucose intolerance [138]. Based on the provided evidence, further randomized controlled trials attesting the role of diet in GM manipulation to evaluate the possibility of preventing or controlling GDM are strongly recommended.

3.15. Diet, gut microbiome and diabetes: a role for treatment of gestational diabetes mellitus?

Despite the important role of dietary therapy in the treatment of GDM [143], there is still no consensus on the optimal dietary composition to recommend [144]. In the past, to limit postprandial glycaemic fluctuations and reduce fetal exposure to elevated glucose concentrations, the most common dietary strategy was carbohydrate restriction, particularly the reduction of simple sugars [145]. However, evidence regarding the benefits of a low-carbohydrate diet in the management of GDM is limited [145]. By keeping protein intake within recommended ranges, low-carbohydrate diets inevitably result in increased fat intake, with all the consequences associated with an excess of lipids, such as a potential increased risk of developing insulin resistance [146]. Furthermore, a study showed that a high-fat diet during pregnancy results in an unfavorable microbial pattern, with reduced biodiversity [147]. More recent studies have focused on the quality of carbohydrates rather than their quantity, exploring the impact of low glycaemic index (GI) and/or complex unrefined carbohydrate-rich diets on the composition of the GM, with promising results [148,149]. Different types of complex carbohydrates have variable effects on the composition of the

GM; for example, the administration of prebiotic fibers such as fructans, fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS) in young women with GDM have been associated with the selective growth of intestinal Bifidobacteria and Lactobacilli [150], while resistant starch promoted the growth of species such as *Ruminococcus*, *E. Rectale*, and *Roseburia* [151]. In animal studies, diets low in fiber and high in refined carbohydrates led to a thinning of the mucosal layer, increased intestinal permeability and increased susceptibility to pathogens [152]. The targeted use of specific probiotic fibers could have differential impacts on the composition of the host's GM. Therefore, in addition to the quality of carbohydrates consumed, the type of probiotic fibers and their impact on GM should be considered when defining the optimal diet for patients with GDM. Promising results have also emerged from the use of probiotics in the control of GDM [153]. Some studies suggest that probiotic supplementation during the second and third trimester of pregnancy in women with GDM would lead to a reduction in fasting blood glucose concentrations and improvements in insulin sensitivity parameters [154]. In a recent study, fifty-seven pregnant women were divided into two groups and randomly assigned to take probiotic supplements containing *Bifidobacteria* and *Lactobacilli* or a placebo [155]. The treated group showed significant benefits on glucose metabolism, including fasting glucose levels, fasting insulin and insulin resistance [155]. Furthermore, pro-inflammatory cytokine production, oxidative stress levels and lipid profile also appear to benefit from six weeks probiotic supplementation according to another study conducted on pregnant women [156]. However, another study including149 women with GDM found no significant effect on glycaemic control after intervention with probiotics [157]. In summary, probiotics could have a positive impact on glucose metabolism, lipid metabolism and inflammatory markers in GDM, despite contrasting evidence is available about this topic. In this context, it appears important to consider possible confounding factors including the duration of treatment with probiotics, the different dietary patterns employed and the physical activity performed. Further clinical studies are needed to more thoroughly evaluate the efficacy of probiotics in the treatment of GDM.

4. Conclusion

The importance of the GM in regulating host health has been increasingly recognized in recent years [10]. Because of the colonization that bacteria exert at numerous sites in the human body, the scientific literature suggests that there is an interchange between resident microbial populations and different organs [158]. Alterations in the GM may affect multiple metabolic pathways related to immunity, energy regulation, fat metabolism, and blood sugar control and have been related to several diseases, including DM [14–17]. The mechanism by which altered intestinal bacterial composition can trigger DM and various pathological conditions includes disruption of intestinal barrier function leading to metabolic endotoxemia, influx of inflammatory bacterial fragments into the bloodstream causing chronic low-grade inflammation, a mechanism underlying increased host adiposity, insulin resistance and autoimmune response [88–90]. States of gut dysbiosis were evidenced in all three types of DM compared with healthy people, suggesting that these imbalances may take part in the pathogenesis of the disease, and consequently may be taken into account during therapeutic intervention [27,129,159]. Diet plays a critically important role, as evidence from both animals and humans has outlined being able to influence not only the onset but also the course of the disease [57,105,154]. Promising results have come from studies using the Mediterranean diet, plant-based low-protein diets, or even the use of prebiotics and probiotics as potential therapeutic targets in the management of various types of DM [63,94,111,156]. In addition, large-scale human studies that control for important confounding factors such as age, gender, ethnicity, diet, and genetics are essential. These studies will allow us to fully exploit the potential of GM composition in the development of new diagnostic tools and personalized treatment approaches for DM and other metabolic diseases.

CRediT authorship contribution statement

Davide Menafra: Writing – review & editing, Writing – original draft. **Mattia Proganò:** Writing – review & editing, Writing – original draft. **Nicola Tecce:** Writing – review & editing, Visualization. **Rosario Pivonello:** Supervision. **Annamaria Colao:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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