MSI Journal of
Medicine and Medical Research (MSIJMMR)

Mechanistic Insights, Interventions, Challenges, and Innovations in Gut Microbiota and Type 2 Diabetes Mellitus (T2DM)

Umar. A. I.[5], Jidda, M.L[1*], Bunza J.M[1]. Dallatu M.K.[1], Ngaski A.A[1]. Aliyu K. B.[2]. Rufai, M. A.[3], Maryam Kasimu.[1], Kwaifa, I. K[4] Ogunwale K.A.T.[5], Yusuf H.D.[6], Giwa J.I.[7], Oladele B.S.[8]

[1] Department of Chemical Pathology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

[2] Department of Chemical Pathology, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

[3] Kebbi State Ministry of Health.

[4] Department of Hematology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

[5] Department of Medical Microbiology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

[5] Department of Chemical Pathology & Immunology, University of Ilorin Teaching Hospital, Ilorin,

[6,8] Nigeria, Department of Medical Microbiology & Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria.

[7] Nigeria Army Reference Hospital, Yaba, Lagos, Nigeria.

[8] Department of Biochemical Science, University of Salford Manchester,United Kingdom.

*** Correspondence:** Jidda, M.L

The authors declare that no funding was received for this work.

OPEN ACCESS

Received: 10-November-2025
Accepted: 12-December-2025
Published: 17-December-2025

This article is published in the **MSI Journal of Medicine and Medical Research (MSIJMMR)**
ISSN 3049-1401 (Online)

The journal is managed and published by MSI Publishers.

Volume: 2, Issue: 12 (December-2025)

ABSTRACT: The pathophysiology of type 2 diabetes mellitus (T2DM) is increasingly linked to the dysregulation of the gut microbiome, a crucial component of the host's metabolic health. Evidence regarding the compositional and functional changes of the gut microbiome in T2DM is compiled in this

semi-systematic review. These changes include decreased microbial diversity, an increased Firmicutes/Bacteroidetes ratio, and a decrease in the number of beneficial short-chain fatty acid (SCFA)-producing bacteria. We elucidate the mechanistic pathways linking dysbiosis to T2DM, including raised intestinal permeability and ensuing systemic inflammation via lipopolysaccharide (LPS) translocation, impaired SCFA signalling affecting glucagon-like peptide-1 (GLP-1) secretion and insulin sensitivity, and dysregulated bile acid metabolism. Furthermore, we explore how these microbial disturbances activate immune pathways (e.g., TLR4 signaling) and disrupt hormonal regulation, supporting resistance to insulin and dysfunction of β-cell. The review also evaluates the therapeutic potential of microbiota-targeted interventions such as probiotics, prebiotics, and synbiotics to restore eubiosis and improve glycemic control. Despite encouraging results, there are still many obstacles to overcome before this research can be applied in clinical settings. These obstacles include the individualized nature of the host-microbiome interaction, methodological variability in microbiome studies, and challenges in establishing causality. Overcoming these hurdles through standardized methods, advanced multi-omics integration, and innovative therapeutic delivery systems is crucial for harnessing the gut microbiome as a novel diagnostic and therapeutic target for T2DM management.

Keywords: *gut microbiome, dysbiosis, type 2 diabetes, insulin resistance, short-chain fatty acids (SCFAs), probiotics, prebiotics, metabolic inflammation*

Introduction

Through a variety of physiological functions, including nutrient metabolism, immune regulation, and pathogen defense, the human gut microbiota is essential to preserving host health (Sender et al. 2016). There is growing evidence that the dysregulation of gut microbiota, or "dysbiosis," plays a role in the onset and progression of a number of diseases, including type 2 diabetes (T2DM), a complex metabolic disorder that affects over 463 million adults worldwide and has substantial morbidity and mortality burdens (IDF 2021).

Studies have shown that the gut microbial profiles of people with type 2 diabetes differ from those of healthy controls. Reduced microbial diversity and changes in the

ratios of bacterial phyla (e.g. *Firmicutes/Bacteroidetes*), as well as modifications in particular genera and species associated with inflammation and compromised glucose homeostasis (Cani et al. 2007). However, due to variations in study populations, methodologies, and confounding variables like medication use and diet, there are inconsistencies between studies. The purpose of this semi-systematic review is to identify gaps in the literature and compile current information on changes in gut microbiota in T2DM patients.

Mechanistic Insights: The Role of Gut Microbiota in Health and Disease

The Gut Microbiota Ecosystem

Trillions of microorganisms, including bacteria, viruses, fungi, and archaea, make up the gut microbiota, a dynamic ecosystem (Le Chatelier et al. (2013). This community influences many physiological processes, including metabolism, immunity, and homeostasis, and is essential to host-microbe interactions. It communicates via neural, endocrine, humoral, immunological, and metabolic pathways (Afzaal *et al*., 2022, Maciel-Fiuza *et al*., 2023). Short-chain fatty acids (SCFAs) are produced, bile acid metabolism is modulated, and systemic inflammation is regulated as a result of the gut microbiome's numerous interactions with the host (Le Chatelier *et al*., 2013). In T2DM, dysbiosis may disrupt these interactions, contributing to insulin resistance, chronic low-grade inflammation, and metabolic dysfunction (Karlsson *et al*., 2013).

The gut microbiome supports host physiology and immunity by aiding in nutrient metabolism, pathogen defense, and immune cell signaling (Afzaal et al. 2022). A healthy person needs a varied gut microbiome that includes important bacterial populations from the phyla *Firmicutes, Bacteroides, Actinobacteria, Proteobacteria, and Verrucomicrobia*. Genuine immune function and the prevention of autoimmune disorders depend on the early development of the gut microbiome (Afzaal *et al*., 2022).

Dysbiosis and Disease

Alterations in the gut microbiome's composition (see Fig. 6) are linked to a number of illnesses, such as cancer, inflammatory bowel disease, obesity, diabetes, anxiety,

depression, and cardiovascular conditions (Afzaal et al. 2022). Dysbiosis can result in inflammatory and immunological diseases that impact organs other than the intestine, including the skin, brain, and lungs (Maciel-Fiuza et al. 2023). Hepatocellular carcinoma, chronic kidney diseases, inflammatory bowel diseases, and non-alcoholic fatty liver diseases are all associated with the gut microbiota and their metabolites (Afzaal *et al*., 2022).

The gut microbiota has an impact on the immune system, mental health, autoimmune diseases, endocrine disorders, gastrointestinal disorders, and even sleep patterns. According to Maciel-Fiuza et al. (2023), the gut microbiota can fight pathogens, help with digestion, control epithelial cells, and change insulin resistance. Research on the function of gut microbiota in health and disease is ongoing and may revolutionize disease pathogenesis and therapeutic approaches (see Fig. 1) (Afzaal *et al*., 2022).

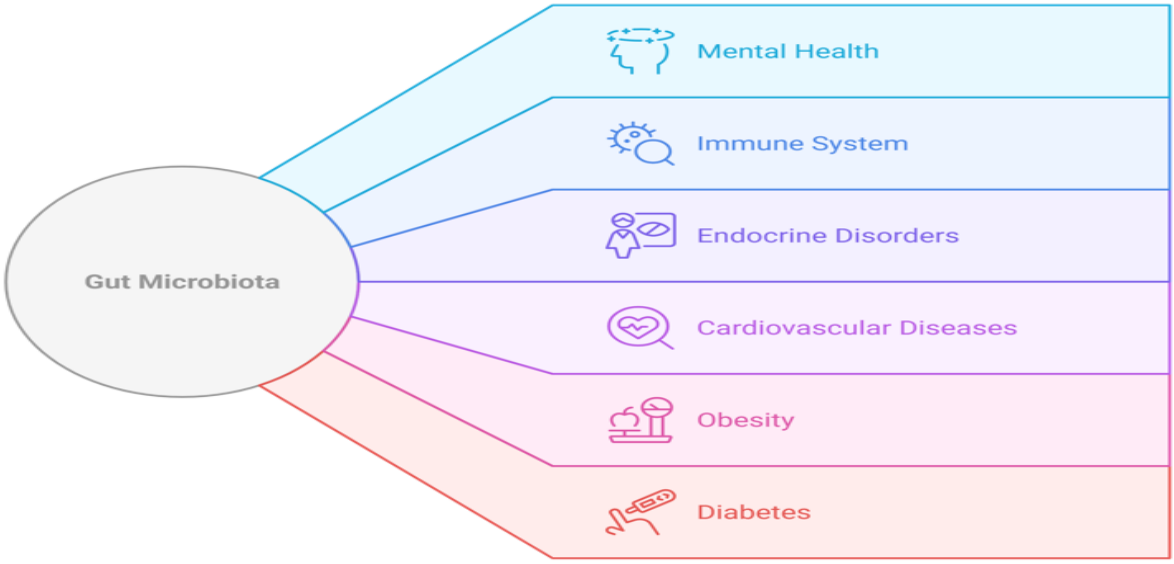

Figure 1: Multifaceted impact of gut Microbiome

Despite growing interest in the gut microbiota-T2DM axis, there remains a lack of consensus regarding the specific microbial signatures associated with T2DM.

Mechanistic Links between Gut Microbiome Dysbiosis and T2DM Pathogenesis

The mechanistic links between gut microbiome dysbiosis and T2DM pathogenesis involve a complex interplay of altered microbial composition, increased intestinal

permeability, metabolite production, immune system interactions, and hormonal regulation (see Fig. 2). Understanding these mechanisms furnishes further insights into the pathophysiology of T2DM, and opens avenues for potential therapeutic interventions aimed at restoring gut microbiota balance to improve metabolic health (Cunningham *et al*., 2021). The mechanisms through which gut microbiome imbalances contributes to T2DM are multifaceted, involving alterations in microbial composition, increased intestinal permeability, and changes in metabolic processes (Wu et al., 2021).

The relationship between gut microbiome dysbiosis and the pathogenesis of T2DM is complex and multifaceted (Bandopadhyay and Ganguly 2022). Beneficial gut bacteria plays a pivotal role in the breaking down of indigestible carbohydrates, by producing enzymes, thus ensuring the body absorbs nutrients effectively. They also assist in breaking down dietary fibers and metabolizing bile (Rowland *et al*., 2018). Bacteria produce SCFAs, which are important nutrients that feed the cells in the gut lining and help maintain a healthy gut environment. According to Rowland et al. (2018), they also provide enzymes to synthesize certain vitamins, including B1, B9, B12, and K. The immune system is trained to distinguish between helpful and harmful microorganisms by beneficial microbes. Up to 80% of the body's immune cells reside in the gut, which relies on these microbes to clear out pathogens (Rowland et al., 2018).

The SCFAs produced by bacteria, are important nutrients that feed the cells in the gut lining and help maintain a healthy gut environment. They also provide enzymes to synthesize certain vitamins, including B1, B9, B12, and K (Rowland *et al*., 2018). Beneficial microbes help train the immune system to distinguish between helpful and harmful microorganisms. The gut, containing up to 80% of the body’s immune cells, relies on these microbes to clear out pathogens (Rowland *et al*., 2018). By competing with harmful types of gut microbes for resources, these beneficial microbes prevent harmful bacteria from taking over the gut (Rowland *et al*., 2018).

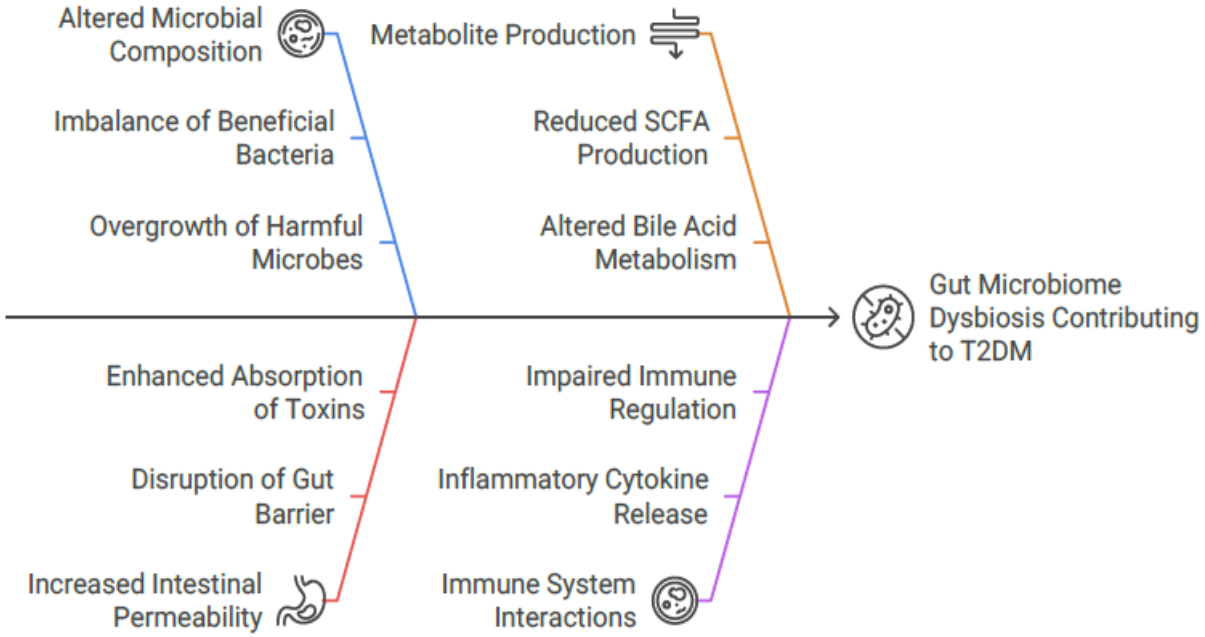

Figure 2: Gut microbiome dysbiosis in T2DM

Altered Microbial Composition

The gut microbiome, plays a critical role in maintaining host health through digestion, immune regulation, and pathogen defense (Collado et al., 2024). Dysbiosis, characterized by an overabundance of harmful bacteria (e.g., *Proteobacteria, Firmicutes*) and depletion of beneficial taxa (e.g., *Bifidobacterium, Bacteroides*) is strongly associated to T2DM. Individuals with T2DM exhibit distinct microbial profiles, marked by reduced diversity, elevated Firmicutes/Bacteroidetes ratios, and diminished levels of butyrate-producing species such as *Akkermansia muciniphila* and *Roseburia*, which are critical for anti-inflammatory and insulin-sensitizing effects (Zhou et al., 2022; Jeyaraman et al., 2024). Specific pathogens, such as Fusobacterium spp., promote pro-inflammatory cytokines, while *Prevotella copri* elevates branched-chain amino acids, increasing obesity and T2DM risk (Cunningham et al., 2021). Conversely, beneficial strains like Lactobacillus and Bifidobacterium enhance insulin sensitivity via β-cell expansion (Zhou et al., 2022) and SCFA production (Jeyaraman et al., 2024). Dysbiosis also disrupts metabolic pathways, including AMPK inhibition via GPR43 activation, further impairing glucose homeostasis (Zhou et al., 2022). These findings underscore the gut microbiota's dual role in T2DM pathogenesis and therapeutic potential.

Increased Intestinal Permeability

Preventing chronic inflammation and controlling immune responses depend on gut barrier function. In order to prevent excessive absorption of dangerous substances into the bloodstream, a healthy gut microbiome supports the integrity of the intestinal barrier (Zhou et al., 2023). This barrier is compromised by dysbiosis, which increases intestinal permeability. Bacterial endotoxins like lipopolysaccharides (LPS) are absorbed into the bloodstream as a result of increased intestinal permeability, which increases the production of pro-inflammatory cytokines and causes systemic inflammation (Crudele et al. (2023). This systemic inflammation leads to the destruction of pancreatic beta cells and the onset of insulin resistance, contributing to T2DM pathogenesis (Utzschneider *et al*., 2016, Jeyaraman *et al*., 2024). By boosting the immune system, LPS causes systemic inflammation, which exacerbates the metabolic dysregulation linked to type 2 diabetes by raising levels of pro-inflammatory cytokines like TNF-α and IL-6. Insulin resistance and pancreatic β-cell dysfunction are closely associated with this inflammation (Utzschneider *et al*., 2016).

Metabolite Production

The host's metabolism and immune response are significantly influenced by the different metabolites that the gut microbiota produces. Metabolites derived from short-chain fatty acids, like butyrate and propionate, which are produced when beneficial bacteria ferment dietary fibers, are essential for controlling glucose homeostasis and enhancing insulin sensitivity. Additionally, these metabolites have anti-inflammatory qualities and support the differentiation of regulatory T cells (Tregs), which is crucial for preserving immunological homeostasis (Lv et al. 2022). SCFA production may be reduced as a result of gut dysbiosis, which would lessen their advantageous impact on metabolism. These SCFAs increase insulin sensitivity and anti-inflammatory responses by promoting fatty acid oxidation and reducing inflammation; however, reduced SCFA production aggravates metabolic disorders (Lv et al. Jeyaraman et al., 2022. 2024). Additionally, the liver can convert high levels of metabolites like trimethylamine (TMA), which is produced by some gut bacteria, to trimethylamine N-oxide (TMAO), which exacerbates insulin resistance

and cardiovascular problems linked to diabetes (Ye et al., 2022, Jeyaraman *et al*., 2024).

Immune System Interaction

The pathophysiology of T2DM is significantly influenced by the interaction between the gut microbiome and the immune system, which affects immune function through a variety of mechanisms (Al Bataineh et al., 2023). An imbalance between pro-inflammatory and anti-inflammatory signals within the immune system may result from dysbiosis. This imbalance may intensify inflammatory responses that are detrimental to insulin signalling pathways, further contributing to insulin resistance and β-cell damage (Ye *et al*., 2022, Al Bataineh *et al*., 2023). This significantly influences immune responses, contributing to metabolic dysfunction. Moreover, interactions between gut microbiota and CD4+ T cells have been shown to affect systemic inflammation levels, closely related to T2DM pathogenesis, highlighting the significance of immune-microbiota crosstalk in metabolic regulation (Jeyaraman *et al*., 2024).

a. Inflammatory Response Activation

Pro-inflammatory cytokines are elevated as a result of dysbiosis. Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) on immune cells are bound by substances produced by some gut bacteria, such as LPS. The hallmark of type 2 diabetes, chronic low-grade inflammation, is fostered by this binding, which sets off a series of inflammatory reactions. For example, exposure to LPS can increase the expression of inflammatory mediators such as TNF-α and IL-6, which are linked to pancreatic β-cell dysfunction and insulin resistance (Huda et al., 2021, Zhou *et al*., 2023).

b. Immune Cell Modulation

The maturation and functions of various immune cells are mostly shaped by gut microbiota. Specific bacterial species can trigger the production of anti-inflammatory cytokines, such as IL-10 and IL-22, which helps in counteracting pro-inflammatory signals. For example, beneficial bacteria like *Akkermansia* and *Roseburia* have been

shown to promote anti-inflammatory responses that can mitigate insulin resistance. Conversely, pathogenic bacteria like *Fusobacterium nucleatum* may enhance inflammatory responses, further exacerbating metabolic dysregulation (Huda *et al.,* 2021, Zhou *et al*., 2022).

c. Host-Microbiota Interactions

The interplay between host genetics, diet, lifestyle, and gut microbiota composition further complicates immune modulation in T2DM. Specific dietary patterns can influence microbial diversity and functionality, thus affecting immune responses. For instance, a high-fiber diet promotes beneficial bacteria that enhance SCFA production and reduce inflammation, while a high-fat diet may favor pathogenic bacteria associated with increased inflammatory markers (Conlon and Bird 2014).

Gut Microbiota and Hormonal Regulation in T2DM

Through its effects on insulin sensitivity and glucose homeostasis, the gut microbiota has a major impact on hormonal regulation in type 2 diabetes. Key hormonal pathways, such as those involving glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2), which control insulin secretion, appetite, and gut barrier integrity, are disrupted by dysbiosis, a microbial imbalance (Lv et al., 2022; Zhou et al., 2022). Gut bacteria produce short-chain fatty acids (SCFAs), which increase insulin secretion, suppress glucagon, and promote satiety by stimulating intestinal L-cells to release GLP-1 (Wu et al. 2023). Dysbiosis impairs GLP-1 secretion and exacerbates hyperglycemia by lowering SCFA production (see Fig. 3) (Lv et al., 2022).

Additionally, gut microbiome modulate the metabolism of bile acid, which activates receptors like Farnesoid X Receptor (FXR) and Takeda G Protein-Coupled Receptor 5 (TGR5). These receptors regulate glucose/lipid metabolism and amplify the effects of GLP-1 (Wu et al., 2023). Dysbiosis alters bile acid profiles, disrupting FXR/TGR5 signaling and promoting insulin resistance (Jeyaraman et al., 2024). Furthermore, dysbiosis may dysregulate ghrelin (an appetite-stimulating hormone), increasing food intake and obesity risk (Massey & Brown, 2021). Together, these mechanisms

underscores the gut microbiota's significant role in pathogenesis of T2DM through hormonal crosstalk.

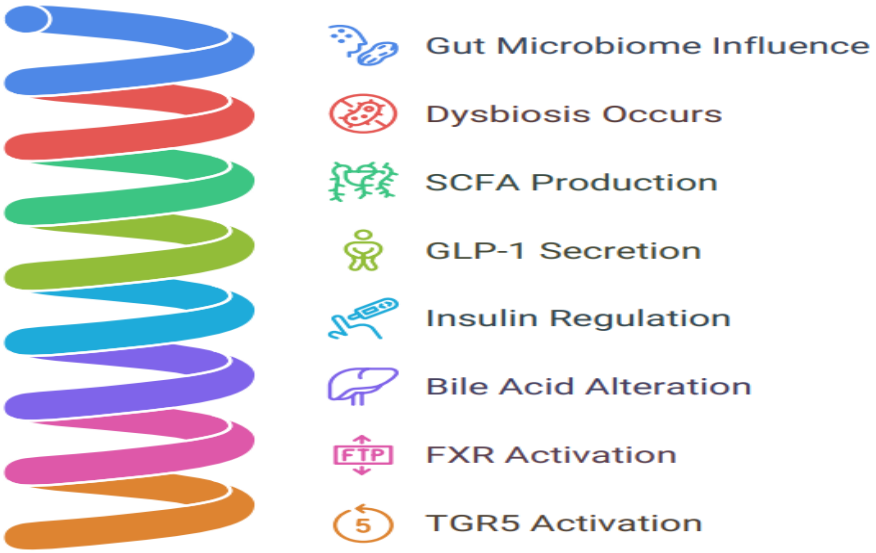

Figure 3: Influence of Gut microbiota on hormonal regulation.

Inflammatory Cytokine Production

An imbalance in the composition of the gut microbiota causes an increase in the production of pro-inflammatory cytokines, which disrupt hormonal signaling. Systemic inflammation is triggered by LPS translocation into the bloodstream as a result of increased intestinal permeability. Insulin resistance is encouraged and insulin signaling pathways are weakened by persistent inflammation (Massey and Brown 2021). In people with type 2 diabetes, pro-inflammatory cytokines like TNF-α and IL-6 can interfere with insulin's action on target tissues, making metabolic regulation even more difficult (Zhou *et al*., 2022).

Interaction with Host Receptors

Gut microbiota-derived metabolites engage with host receptors that modulate hormonal responses. These metabolites acts on host receptors in an endocrine-like manner, influencing hormone secretion and metabolic processes. This interaction represents a novel area for therapeutic intervention in T2DM management (Massey and Brown 2021). Furthermore, components of microbial cell wall are sensed by Pattern Recognition Receptors (PRRs) on host cells, leading to immune responses

that can either promote or defends against inflammation associated with T2DM complications (Massey and Brown 2021).

Molecular Pathways

Several molecular pathways connect dysbiosis, to insulin resistance. Some of these pathways include AMPK activation, TLR4 signaling, and resistin (Hrncir 2022).

AMPK ACTIVATION

Activation of AMPK can enhance metabolic health and insulin sensitivity (Coughlan et al. [2014]. Tumor-suppressor liver kinase B1 (LKB1), one of AMPK's three upstream kinases, must phosphorylate Thr172 on the α-subunit's "activation loop" in addition to raising the intracellular AMP:ATP ratio. Humans and animals with metabolic syndrome or type 2 diabetes (T2D) exhibit dysregulation of AMPK; however, in insulin-sensitive cells, AMPK activation can increase insulin sensitivity. AMPK has an impact on metabolic syndrome and insulin resistance (Coughlan *et al*., 2014). Reduced and elevated AMPK activity are associated with insulin resistance and sensitivity, respectively, in model systems (Ruderman et al. (2013)). Additionally, AMPK increases the uptake of glucose by skeletal muscle over time by upregulating GLUT4 expression and translocating GLUT4 to the plasma membrane in the short term (see Fig. 4) (Hardie 2013). Reducing the amount of glucose produced by the liver while increasing fatty acid oxidation in adipose tissues (Coughlan et al. (2014). The activation of AMPK can counteract increased triglyceride storage and deficiencies in mitochondrial function, both of which are associated with insulin resistance, by promoting fat oxidation and suppressing fat synthesis (Hardie 2013). As a metabolic sensor, AMPK interacts with molecular pathways like PGC-1α, PI3K/Akt, NOX4, and NF-κB to modulate glucose and lipid metabolism. In humans or rodents with type 2 diabetes or insulin resistance, pharmacological activation of AMPK increases skeletal muscle glucose uptake via an insulin-independent mechanism (Zachariah *et al.,* 2014).

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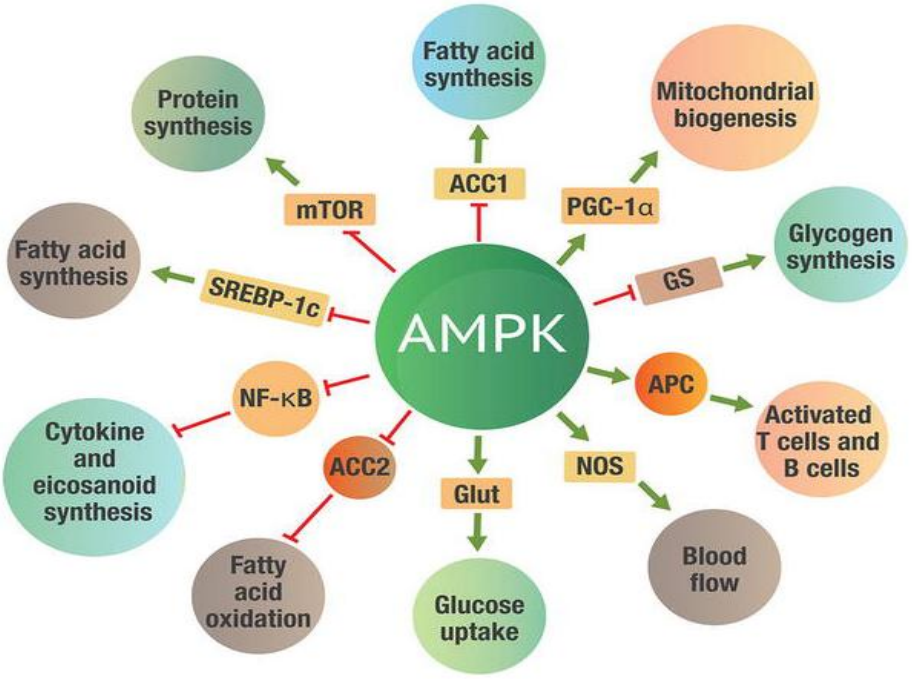

Figure 4: Metabolic effects of AMPK activation. Green arrows indicating activation, red lines indicating inhibition (Sears, and Saha, 2022).

TLR4 Signaling

Resistin-TLR4 Pathway in Hypothalamic Inflammation

Resistin, an adipokine elevated in obesity, activates Toll-like receptor 4 (TLR4), a pattern recognition receptor integral to innate immunity. This interaction triggers hypothalamic inflammation, impairing central insulin signaling (Benomar & Taouis, 2019). Insulin action is desensitized when resistin binds to TLR4, inhibiting tyrosine phosphorylation of the insulin receptor (IR) and insulin receptor substrate (IRS) and encouraging serine phosphorylation of IRS1/2 via MAP kinases. Crucially, TLR4 knockdown in the arcuate nucleus highlights its role in metabolic dysfunction by protecting against diet-induced weight gain and glucose intolerance (Benomar & Taouis, 2019). Resistin further exacerbates insulin resistance by suppressing adiponectin receptors (AdipoRs) and autophagy, disrupting energy balance and inflammatory responses.

TLR4 Signaling in Peripheral Tissues

In peripheral tissues, TLR4 activation by dietary fatty acids or lipopolysaccharides (LPS) recruits pro-inflammatory kinases (JNK, IKK, p38), which phosphorylate IRS on inhibitory serine residues, blocking insulin signal transduction (Kim & Sears, 2010). Mice lacking TLR4 are shielded from lipid-induced insulin resistance in

muscle and liver, emphasizing TLR4's role in metabolic inflammation (Shi et al., 2006). TLR4 activation also amplifies pro-inflammatory cytokines and reactive oxygen species, further impairing glucose uptake and lipid metabolism.

Protein Tyrosine Phosphatases (PTPases) and Insulin Resistance

PTPases, such as PTP1B and LAR, dephosphorylate the insulin receptor, reducing its kinase activity. Elevated PTP1B levels in insulin-resistant individuals correlate with impaired glucose homeostasis. Notably, PTP1B knockout mice exhibit enhanced insulin sensitivity, positioning PTPase inhibition as a promising therapeutic strategy (Pessin & Saltiel, 2000).

Gut Dysbiosis and Intramyocellular Lipid Metabolites

Gut dysbiosis in obesity increases intramyocellular lipid metabolites (e.g., fatty acyl CoAs, diacylglycerol), which activate serine/threonine kinases. These kinases phosphorylate IRS-1, disrupting insulin signaling and promoting muscle insulin resistance (Morino et al., 2006). This mechanism links gut microbiota imbalance to systemic metabolic dysfunction.

The interplay between obesity-induced inflammation (via resistin/TLR4), phosphatase dysregulation, and gut microbiome imbalance converges on IRS dysfunction, driving insulin resistance. Therapeutic strategies targeting TLR4, PTPases, or microbial balance may restore metabolic homeostasis.

Insulin Signaling and GLUT4 Trafficking in Health and Disease

By initiating a phosphorylation cascade involving insulin receptor substrate (IRS), phosphoinositide 3-kinase (PI3K), and Akt, insulin increases the uptake of glucose (see Fig. 5) which encourages the glucose transporter GLUT4 to move to the plasma membrane. Aberrant serine phosphorylation of IRS proteins impairs GLUT4 translocation in insulin-resistant states by interfering with downstream signaling (van Gerwen et al., 2023). A combination of flawed insulin signaling (e.g.) causes this dysfunction. (g). IRS inhibition) as well as new pathways that involve kinases like AMPK and GSK3 and end at effectors like Rho GTPases. Insulin resistance, a

defining feature of type 2 diabetes and associated metabolic disorders, is largely caused by these disturbances in GLUT4 trafficking (van Gerwen et al., 2023).

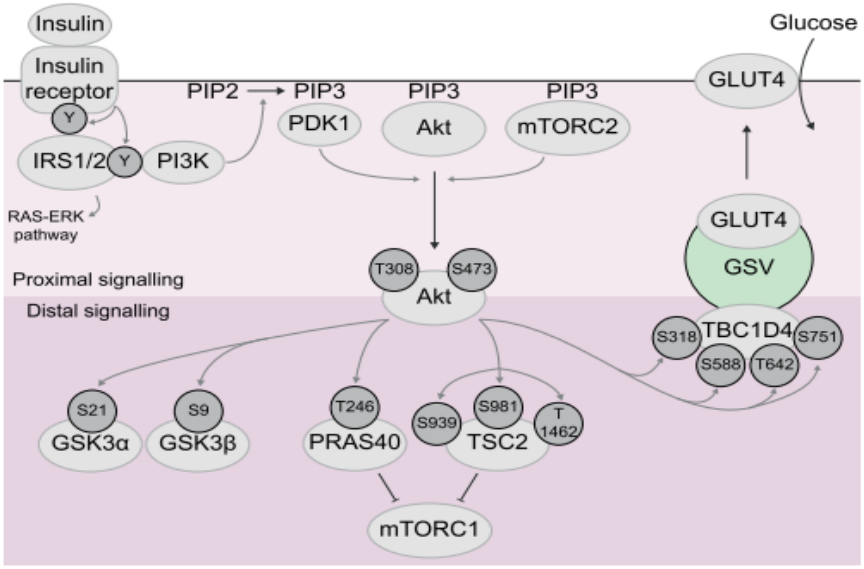

Figure 5. Insulin triggers a cascade of protein phosphorylation to modulate cellular metabolism, with the kinase Akt serving as a pivotal component in this process. The sequence of events that leads to the activation of Akt commences with the binding of insulin to its receptor, which activates the receptor's tyrosine kinase activity and initiates its auto-phosphorylation.

GLUT4 Trafficking Mechanism

Under normal conditions, GLUT4 is mainly stored in intracellular compartments residing in intracellular vesicles and must be translocated to the cell surface for glucose uptake. Insulin promotes its translocation to the plasma membrane through a complex trafficking process involving various proteins and cytoskeletal elements. Recent studies indicate that microtubule-mediated trafficking is essential for GLUT4 movement (Knudsen, *et al.*, 2023). According to this study, the microtubule network is essential for the movement of intramyocellular GLUT4 in adult skeletal muscle fibers. It probably helps to maintain an insulin-responsive pool of recruitable GLUT4 at the cell surface via kinesin-1-mediated transport. It was discovered that GLUT4 was located on the microtubules in the muscle fibers of both mice and humans. Nocodazole (Noco) inhibited long-distance GLUT4 trafficking and caused depletion by pharmacologically disrupting microtubules (Knudsen, et al., 2023). Completely reversible GLUT4-rich structures were seen at microtubule nucleation sites. Skeletal

muscle insulin resistance is a result of GLUT4 translocation being impeded by the disruption of the microtubule network (Knudsen, et al., 2023).

Dynamic Retention Model

The Dynamic Retention Model (DRM) for GLUT4 provides a comprehensive understanding of mechanisms by which the glucose transporter GLUT4 is conserved intracellularly and translocated to the plasma membrane in response to insulin (Wang, *et al*., 2020). This model emphasizes the dynamic nature of GLUT4 retention and trafficking within cells, particularly in adipose and muscle tissues. This model suggests that GLUT4 is in constant equilibrium between intracellular compartments and the plasma membrane. Insulin enhances this equilibrium by increasing the docking and fusion rates of GLUT4-containing vesicles with the plasma membrane (Wang, *et al*., 2020). By integrating insights into its intracellular retention mechanisms, the role of insulin, and the dynamics of trafficking, this model highlights potential targets for therapeutic interventions aimed at improving glucose uptake in conditions like type 2 diabetes.

Implications of Impaired GLUT4 Trafficking

In insulin resistance, there is a notable depletion in insulin-stimulated GLUT4 translocation, rather reduced cell surface GLUT4**.** Studies have shown that insulin-resistant tissues exhibit lower levels of GLUT4 at the plasma membrane despite normal total levels of GLUT4 within cells. This indicates that the defect lies primarily in the trafficking process rather than in GLUT4 synthesis (van Gerwen, *et al*., 2023). The failure to properly translocate GLUT4 contributes significantly to elevated blood glucose levels, leading to further metabolic complications such as obesity and type 2 diabetes. Understanding the interplay between insulin signaling and GLUT4 trafficking is crucial for developing therapeutic strategies targeting insulin resistance. By elucidating the molecular mechanisms involved, researchers can identify potential drug targets aimed at enhancing GLUT4 translocation and improving insulin sensitivity (Alam, et al., 2016).

Potential Therapeutic Targets

Targeting the gut microbiome offers a potential therapeutic avenue for regulating hormones involved in T2DM. Interventions with probiotics and prebiotics aid in the

restoration of the balance in gut microbes and enhance the production of beneficial metabolites like SCFAs, potentially improving hormonal regulation and metabolic health (Wu *et al., 2023*). Dietary modifications like increasing the dietary fiber intake can promote the growth of SCFA-producing bacteria, enhance GLP-1 secretion and improves insulin sensitivity (Crudele et al., 2023).

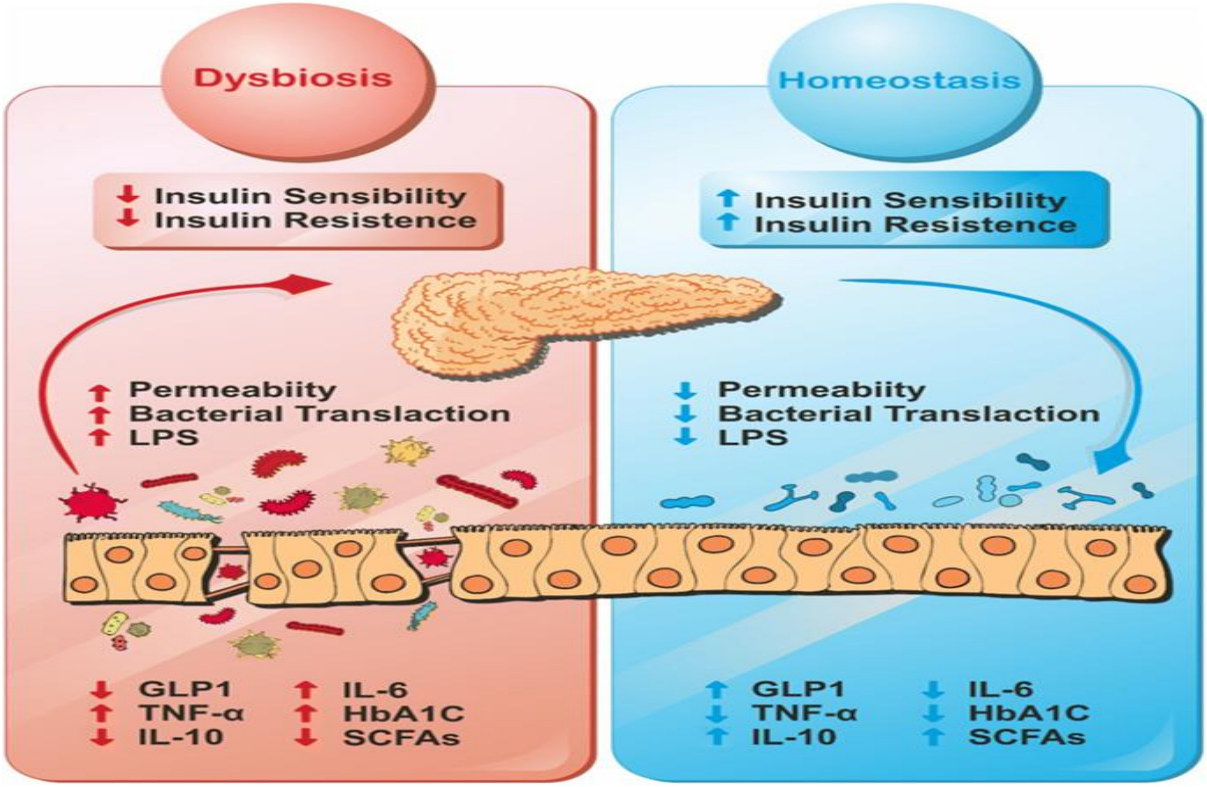

Figure 6: Dysbiosis vs Homeostasis (Salgaço et al., 2019)

Interventions: Microbiome-Targeted Therapies

Microbiome-focused treatments (refer to Fig. 7) include dietary modifications, particular dietary components, probiotics, prebiotics, and synbiotics (BMJ 2023). The effects of these interventions on a range of health conditions, such as immunological function, age-related problems, mental health, and cardiometabolic diseases, are being investigated (Panaitescu *et al*., 2024).

Probiotics

Probiotics are live microorganisms, such as yeasts and bacteria, improve the digestive system of the host when consumed in adequate amounts (Kim et al., 2019). Numerous studies have associated probiotics with the enhancement of a healthy gut

bacteria balance, as well as a broader spectrum of health advantages. These advantages encompass weight loss, digestive health, immune function, and additional benefits (Kim et al., 2019). The positive effects of probiotics may stem from their capacity to restore the natural equilibrium of gut bacteria (Markowiak and Śliżewska 2017). An imbalance in gut bacteria can arise from various factors, including illness, the use of medications such as antibiotics, and an unhealthy diet, potentially leading to digestive problems, allergies, mental health issues, and obesity (Roman et al., 2018). Additionally, probiotics may have anti-inflammatory qualities that could lessen the symptoms of a variety of illnesses (Ferrarese *et al*., 2018).

Some probiotic strains have demonstrated efficacy in treating diarrhea, including diarrhea brought on by antibiotics and C. difficile infection (Goldenberg *et al*., 2017, Yang *et al*., 2019). Numerous studies have associated probiotics supplementation with improved symptoms of mental health disorders like depression, anxiety, and stress (Steenbergen *et al*., 2015, Wang *et al*., 2016). Probiotics improve heart health by lowering blood pressure and LDL ("bad") cholesterol. It is known that certain lactic acid bacteria lower cholesterol by breaking down bile in the digestive system (Wang et al., 2022). Bile, a fluid predominantly composed of cholesterol, plays a crucial role in the digestion of fats. By metabolizing bile, probiotics can inhibit its reabsorption in the gut, thereby preventing its entry into the bloodstream as cholesterol (Kota et al., 2108; Wang et al., 2022). Probiotics may also help maintain urinary and vaginal health as well as treat ailments like colitis, inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS) (Dale et al., 2019). They may also help prevent allergies and colds and improve oral health.

Prebiotics

Prebiotics are indigestible plant fibers that the gut's beneficial bacteria selectively use to support their growth. By serving as food for these microorganisms, prebiotics effectively feed the gut's diverse flora, thereby promoting the growth and activity of these advantageous microorganisms and helping to maintain a healthy digestive system. (Al Bander *et al*., 2020). SCFAs, such as acetate, propionate, and butyrate, are produced during the fermentation of prebiotics and are essential for gut and metabolic health. SCFAs also provide energy for colonocytes and are involved in

production of mucus and intestinal pH regulation (Davani-Davari *et al*., 2019). By preserving the integrity of the gut wall, which serves as a barrier to dangerous molecules, prebiotics are also known to improve the intestinal absorption of minerals like calcium from food, strengthen immune systems, and lower inflammation (Blaak *et al*., 2020, Silva, *et al*., 2020).

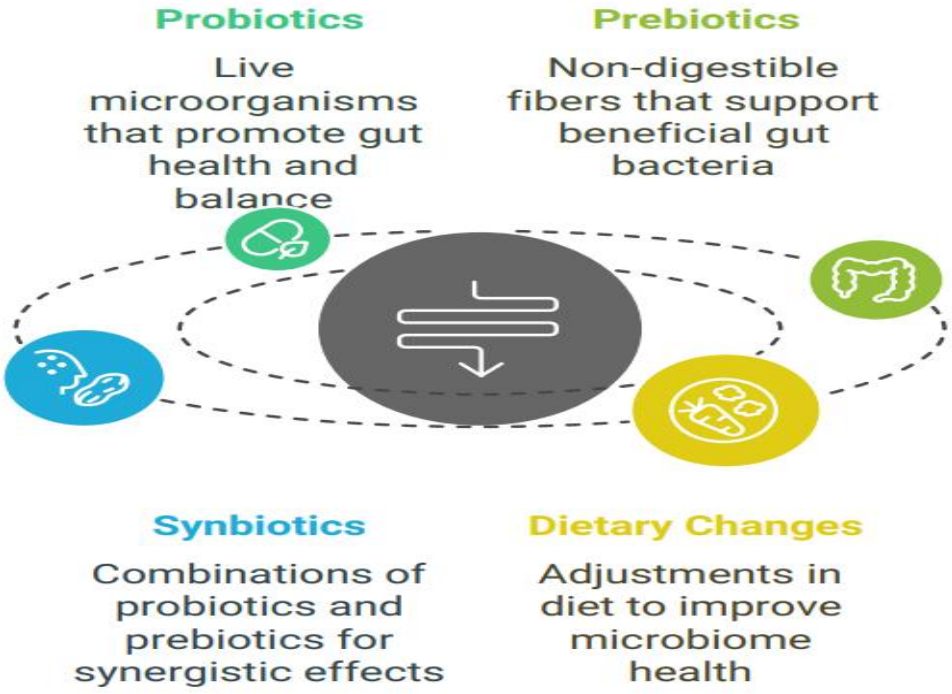

Figure 5: Exploring the microbiome targeted interventions

Synbiotics

Synbiotics are supplements or foods that combine probiotics (beneficial gut bacteria) and prebiotics (BMJ 2023). The term "synbiotic" refers to the synergistic interaction between probiotics and prebiotics, which collectively have a positive impact on the gut microbiome. Synbiotics are available as supplements or in foods like pasta, beverages, candy, and yogurt (BMJ 2023).

A synbiotic is defined by a panel of scientists as "a combination of live microorganisms and substrate(s) that are selectively utilized by host microorganisms, providing a health advantage to the host." (Swanson et al 2020; Kleerebezem & Führen, 2024). Complementary and synergistic synbiotics are the two recognized types. The term "synergistic synbiotic" describes a synbiotic in which the substrate is

specially designed for the co-administered microorganism or microorganisms to use selectively. Conversely, a "complementary synbiotic" that supports autochthonous microorganisms is made up of a probiotic and a prebiotic. Minimum requirements for the current probiotic and prebiotic must be met for both components of a complementary synbiotic. (Swanson et al 2020; Kleerebezem & Führen, 2024). It is important to note that the microorganisms present in a synbiotic product may not directly utilize the substrate contained within that product; rather, the substrate may facilitate the proliferation of other beneficial bacteria that are already present in the host's gastrointestinal tract (Swanson et al 2020).

Health Benefit of Synbiotics

Synbiotics enhance immune function by increasing the levels of beneficial gut-bacteria and decreasing inflammation, increase digestive function, and promote heart health by reducing risk factors of heart diseases. Supplementation with synbiotics can improve insulin metabolism and "good" HDL cholesterol levels. Synbiotics may also reduce gut inflammation and relieve symptoms in people with IBD.

Effects on gut microbiome Specific Conditions

The impact of gut microbiome-focused clinical interventions on cardiometabolic outcomes is being assessed. Specific bacteria, such as *Bacteroides*, *Alistipes sp, Bilophila, Desulfovibrio*, and *Ruminococcus gnavus*, are associated with an increased risk of these diseases (BMJ 2023). Anti-aging effect of Lactobacillus is being studied as a therapeutic intervention to improve aging via microbiota-targeted approaches (Ishaq *et al*., 2021).

Microbiota-focused interventions, such as probiotics, prebiotics, synbiotics, and fecal microbiota transplantation, are gaining recognition as potential methods for addressing mental health issues (Berding and Cryan 2022). Therapies targeting the microbiome (pre-, pro-, and synbiotics) are currently being assessed for their effects on anthropometric measurements and weight loss when combined with traditional weight loss techniques in overweight or obese people. Neuroinflammatory and motor function outcomes have been consistently improved by non-dietary interventions that target the gut microbiome (Moszak et al., 2021). Nutrition interventions that target

the gut microbiome have been investigated for their influence on growth outcomes in children aged 0 to 59 months (Hills et al., 2019).

The personalized nature of gut-targeted interventions is a critical consideration, including the availability of microbial enzymatic repertoire. The choice of intervention, dosage, and duration of treatment need to be carefully considered (Berding and Cryan 2022).

Challenges in Translating Microbiome Research into Clinical Practice

Several challenges in translating microbiome research into clinical practice exist; these challenges stems from methodological variability to an incomplete understanding of bioactive molecules, the complexity of microbial communities, and finally, the difficulty of establishing causality (Isali et al., 2024). Standardization of procedures, enhanced experimental models, and sophisticated analytical techniques are needed to overcome these obstacles. A thorough grasp of the mechanistic action, consideration of the microbe's metabolites and pharmacokinetics is also required for safer and efficient usage of microbiome research in human trials (Xavier 2021, Isali et al., 2024).

Variability in methods

Methodological obstacles in translating microbiome research into clinical practice are multifaceted and impact various stages of the research process. Understanding and comparing results are made more difficult by the differences in sample collection, processing, and analytical techniques used in various studies (EUC 2024).

Different methods for collecting, storing, and stabilizing samples (e.g., using OMNIgene. GUT vs. immediate freezing) can affect the integrity of microbial DNA, leading to inconsistent results across studies. Both 16S rRNA gene sequencing and whole-genome sequencing (WGS) have limitations. For example, 16S sequencing can be less robust than WGS and is affected by preservatives, sample storage, and PCR protocols. Stool samples are not homogenous, restricting sample splitting for different extraction methods without introducing variability (Panek et al., 2018).

Different DNA extraction kits (e.g., QIAGEN vs. MO BIO) have varying efficiencies for extracting DNA from bacterial lineages, particularly Gram-positive bacteria (Panek et al., 2018). Ineffective or vigorous lysis can miss entire genera or degrade DNA from easily lysed cells (Chapman and Stewart 2023). The choice of sequencing platform (e.g., Illumina MiSeq vs. Ion Torrent PGM) affects data quality and comparability across studies (Panek et al., 2018). Analyzing large multivariate datasets requires sophisticated tools like Kraken or Diamond algorithms with appropriate pipelines (e.g., Megan), which can be challenging due to the complexity of microbial data structures (Bharti and Grimm 2021). Standardized implementation of protocols across similar sample types is crucial to reducing between-study bias and enhancing reproducibility. However, a single universal protocol may not suit all microbial communities due to their diversity. Addressing these methodological issues systematically can improve the reliability and comparability of microbiome studies, ultimately facilitating their translation

Causality

Demonstrating a direct causative relationship between specific microorganisms or microbial patterns and health outcomes is challenging due to the complexity of microbial interactions with host biology. Current models, like fecal microbiota transplantation (FMT) or animal models have limitations in accurately replicating human conditions (Wade and Hall 2020). The identification of persistent patterns is further complicated by the intrinsic variability of the microbiome across individuals, influenced by genetic, epigenetic, and environmental factors. Differences between humans and animal models, like mice, hinder the precise replication of human microbial communities (Govender and Ghai 2025).

Epidemiological studies often cannot control for lifestyle, behavioral factors, or reverse causation, hindering the establishment of a causal relationship between microbiome changes and health outcomes (Wade and Hall 2020). Many studies rely on observational data without adequate control groups, complicating the determination of cause-and-effect relationships. While human microbiota-associated (HMA) rodents have been used extensively to study causal relationships,

extrapolating findings from animal models to humans can be problematic due to interspecies differences (Walter *et al*., 2020).

The therapeutic success of FMT in conditions like recurrent *Clostridioides difficile* infection provides strong evidence for a causal role of the microbiome in disease. However, its application is limited by safety concerns and regulatory hurdles. This method uses genetic variants as proxies for traits associated with the gut microbiome. It helps infer causality without expensive randomized controlled trials (RCTs) but requires careful interpretation (Wade and Hall 2020).

Moving to Clinical Application

An incomplete understanding of microbiome derived bioactive molecules challenges the translation of research into clinical applications. Consideration of up-regulated and down-regulated microbiota alone may not result in novel treatments because this approach does not elucidate the mechanisms of action (Isali *et al.*, 2024). The absence of an easily identifiable biomarkers in gut microbiome research makes establishing links with medically relevant endpoints difficult. Knowledge of disease-specific biomarkers, causality, and redundancy of observed changes needs to be expanded and supported by clinical trials. The efficacy of interventions relies on the microbiome's plasticity, which can present a challenge. Dietary changes may alter the microbiota, which changes the response to different foods (Chen *et al*., 2023).

Innovations in Microbiome Therapeutics

Emerging Trends

Emerging trends in microbiome therapeutics include expansion beyond the digestive system, personalized approaches and multi-omics data integration to enhance the understanding and treatment of various conditions (Yadav and Chauhan 2021, Muller *et al*., 2024). Microbiome therapeutics are increasingly being applied to areas beyond the digestive system, such as immunity boosting, vaginal health, and skin health, and dietary supplements for lifestyle conditions. A growing trend in microbiome therapeutics is the engineering of the gut microbiome through additive, subtractive,

or modulatory therapies using either native or engineered microbes, antibiotics, bacteriophages, and bacteriocins (Yadav and Chauhan 2021).

The identification of biomarkers and therapeutic targets through multi-omics initiatives has great potential to enable the development of novel microbe-based personalized approaches for diagnosis, prevention, and/or treatment in the future (Logotheti et al., 2021). By providing a comprehensive understanding of biological mechanisms, the integration of multi-omics data which includes genomics, transcriptomics, proteomics, and metabolomics is revolutionizing personalized medicine, thereby integrating DNA, RNA, proteins, and metabolites within intricate living systems (Arıkan, and Muth, 2023). Investigating the human gut microbiome through multi-omic studies is essential for elucidating its contribution to disease and individual health profiles. This understanding paves the way for more accurate and customized therapeutic strategies (Molla, and Bitew, 2024).

Recent advancements in technology are facilitating targeted microbial manipulation as a new therapeutic strategy (Gulliver et al., 2022). Advances in high-throughput sequencing, machine learning, and data analytics are enhancing the integration of multi-omics data, increasing the effectiveness and accessibility of personalized medicine (Molla, and Bitew, 2024). These technological advancements are are enabling the potential for precise microbial manipulation as a new therapeutic strategy (Gulliver et al., 2022). Prebiotics, synbiotics, antibiotics, fecal microbiota transplantation (FMT), phage therapy, live biotherapeutics, microbiome mimetics, and probiotic engineering are some of the techniques used to treat dysbiosis-related disorders (Yadav and Chauhan 2021, Molla, and Bitew, 2024).

Innovations

Latest innovations in microbiome therapeutics are expanding beyond the digestive system, with novel approaches in targeted delivery, cardiovascular disease prevention, and cancer treatment.

A novel technology developed at the University of Arizona facilitates the precise delivery of the chemotherapy agent 5-Fluorouracil directly within the colon, thereby minimizing systemic exposure and associated adverse effects. This advancement

holds significant promise for the treatment of colorectal and gastric cancers, in addition to inflammatory bowel disease (Chen *et al*., 2024).

Researchers at the Baker Heart and Diabetes Institute have created biotherapeutics that utilize either a single strain or a blend of bacteria to prevent and manage atherosclerosis, the underlying cause of most cardiovascular diseases (Hasani *et al*., 2021) . Furthermore, scientists at Innovate Calgary have found that certain gut bacteria, including *Bifidobacterium pseudolongum, Lactobacillus johnsonii,* and *Olsenella* species, when used in conjunction with immune checkpoint inhibitors (ICI), can significantly decrease tumor size in mice, presenting opportunities for innovative cancer treatments (Chen et al., 2024). Furthermore, researchers at the Universitat Politècnica de Valaña have created a biodegradable polymeric apparatus for the controlled release of intestinal bacteria. By restoring the microbiome following intestinal resection procedures, this device seeks to improve the quality of life for individuals with inflammatory bowel disease (IBD) and other digestive disorders (Mandsberg *et al*., 2020).

Researchers at Cornell University developed a pipeline that can efficiently identify gene transfer methods and build genetic manipulation tools for non-model human gut bacteria communities. This increases the variety of gut microbes that could be used as live biotherapeutics by enabling precise regulation of microbiome molecular output to investigate its impact on host biology (Jin and Guo 2024). Research and development efforts concerning phage therapies are accelerating, with an increasing number of companies now creating bacteriophage-based products aimed at modulating the human microbiome for therapeutic applications (Cui, et al., 2024).

Addressing Challenges through Innovations in Microbiome Research

The application of microbiome research findings to clinical practice encounters significant challenges, such as methodological variability, difficulties in establishing causality, and the complexity of host-microbe interactions. However, recent innovations are paving the way to overcome these obstacles, offering promising solutions to bridge the gap between research and therapeutic applications.

Methodological Variability

One of the primary obstacles in microbiome research is the absence of a standardized protocols for sample collection, DNA extraction, and sequencing. These variabilities often leads to inconsistent results in these methods, hindering reproducibility and comparability across studies (Panek et al., 2018). To address this, multi-omics approaches are being increasingly adopted. For example, integrating metagenomics, metabolomics, and proteomics provides a deeper understanding of microbial communities and their functional outputs (Arıkan & Muth, 2023). Advanced bioinformatics tools, such as Kraken2 and MetaPhlAn4, are also being developed to enhance the accuracy and efficiency of microbiome data analysis (Bharti & Grimm, 2021). These innovations enable researchers to account for methodological biases and generate more reliable datasets.

Establishing Causality

Demonstrating a direct causative connection between distinct microbial changes and health outcomes remains a significant hurdle. Traditional observational studies are often confounded by factors like diet, lifestyle, and reverse causation (Wade & Hall, 2020). Mendelian randomization (MR) has surfaced as a robust method to tackle this issue. MR employs genetic variants as instrumental variables to deduce causal relationships, thereby minimizing the impact of confounding factors. For example, a recent MR investigation uncovered causal associations between gut microbiota composition and insulin resistance, providing robust evidence for microbiome-based interventions in T2DM (Sanna et al., 2019). Additionally, human microbiota-associated (HMA) rodent models are being refined to better replicate human conditions, enabling more accurate mechanistic studies (Walter et al., 2020).

Complexity of Host-Microbe Interactions

The complex interaction between the gut microbiome and host physiology complicates the identification of therapeutic targets. To tackle this, engineered probiotics and microbiome mimetics are being developed. For example, The hormone glucagon-like peptide-1 (GLP-1), which enhances insulin sensitivity and glucose homeostasis, is produced by genetically modifying *Escherichia coli*

Nissle (Duan et al., 2020). In a similar vein, studies are being conducted to determine whether synthetic metabolites that mimic short-chain fatty acids (SCFAs) can lower systemic inflammation and restore gut barrier function (Chen et al., 2024). These innovations allow for precise modulation of microbial functions, offering targeted therapeutic strategies.

Ethical and Regulatory Hurdles

Ethical and regulatory challenges further complicate the microbiome therapies translation into clinical practice. For instance, fecal microbiota transplantation (FMT) has shown promise in treating metabolic disorders but raises concerns about safety and long-term effects (Cui et al., 2024). To address these issues, biodegradable delivery systems are being developed to safely administer live biotherapeutics. In order to minimize systemic exposure and negative effects, a recent study showed how to use a polymeric device for the controlled release of bacteria in the intestine (Mandsberg et al., 2020). Regulatory frameworks are also evolving to accommodate microbiome-based therapies, with agencies like the FDA issuing guidelines for the development and approval of live biotherapeutic products (FDA, 2021).

Personalized Approaches

The heterogeneity of the human microbiome necessitates personalized therapeutic strategies. Advances in machine learning and artificial intelligence (AI) are enabling the development of predictive models that tailor interventions to individual microbial profiles. For example, AI-driven platforms like MicrobiomeHD analyze large datasets to identify patient-specific microbial signatures and predict treatment outcomes (Pasolli et al., 2020). These tools are being integrated into clinical trials to optimize the efficacy of microbiome-targeted therapies.

Conclusion

In the pathophysiology of type 2 diabetes, dysbiosis of the gut microbiota causes metabolic dysfunction, insulin resistance, and chronic inflammation recognizing the mechanistic connections between the pathophysiology of type 2 diabetes and gut microbiome dysbiosis, providing insights into potential therapeutic interventions.

Microbiome-targeted therapies, including probiotics, prebiotics, and synbiotics, present encouraging opportunities for the management of T2DM. However, issues like methodological variability and establishing causality must be addressed in order to translate microbiome research into clinical practice. Emerging trends and innovations in microbiome therapeutics hold the potential to revolutionize T2DM treatment of and various metabolic disorders.

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