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REVIEW

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From bench to bed: deep insights the tacrolimus-induced diabetes and gut microbiota dysbiosis

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Abstract

The gut–liver axis is particularly vulnerable, and the alterations in gut microbiota composition have been linked to post-transplant complications. Liver transplantation is a life-saving procedure for patients with end-stage liver disease, but post-transplant complications are common and can significantly impact long-term outcomes. Tacrolimus is a widely used immunosuppressive agent in liver transplantation, primarily aimed at preventing graft rejection and promoting long-term graft survival. However, its effects on gut microbiota and the subsequent metabolic consequences remain underexplored. In this review, we will synthesize the latest findings on the effects of tacrolimus on gut microbiota in liver transplant patients and discuss how these changes may contribute to the development of diabetes and insulin resistance. By elucidating the interplay between tacrolimus, gut microbiota, and metabolic health, we aim to provide insights into potential therapeutic strategies for improving post-transplant outcomes.

Highlights

Liver transplantation is the only effective treatment for end-stage liver disease, greatly improving patients' quality of life and survival.

Tacrolimus is the preferred immunosuppressant post-liver transplant, preventing allograft rejection and enhancing liver function and survival.

The intestinal microflora, termed a "special organ," is vital in metabolic diseases, such as obesity, hypertension, type 2 diabetes, and cancer.

New strategies for the prevention, management, and treatment of diabetes can be achieved by regulating the gut flora.

Keywords Tacrolimus, Liver transplantation, Gut microbiota, Metabolites, Diabetes, Insulin resistance

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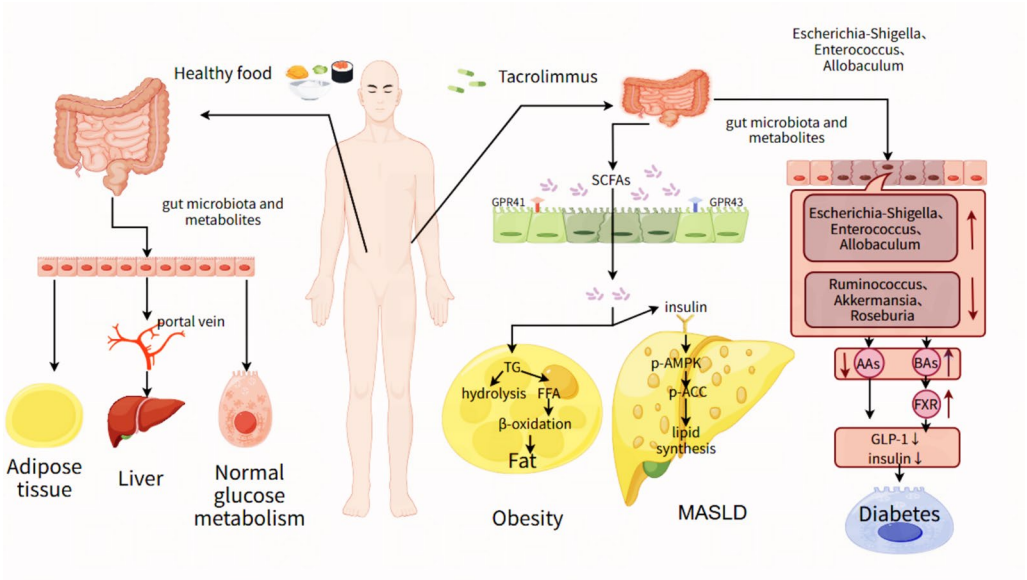
Graphic Abstract



Introduction

Liver transplantation (LT) has emerged as a critical therapeutic option for patients suffering from end-stage liver disease, providing life-saving benefits and improving quality of life. The demand for LT is rising due to the growing number of liver diseases, such as hepatoma, hepatitis C, alcoholic liver disease, and metabolic dysfunction-associated steatotic liver disease. Recent advancements in surgical techniques and post-operative care have significantly improved transplant outcomes, with 5-year survival rates reaching over 70% in many centers [1]. Tacrolimus is an immunosuppressant widely used in liver transplants and other solid organ transplants, crucial for preventing graft rejection and ensuring patient survival. Due to its effectiveness in preventing both acute and chronic rejections, Tacrolimus has become a cornerstone of immunosuppressive therapy [2–4].

In recent years, the gut microbiota has gained attention for its significant impact on metabolic health and its potential role in the pathophysiology of various diseases, including those affecting the liver. The gut microbiota comprises trillions of micro-organisms that inhabit the gastrointestinal tract, influencing nutrient metabolism, immune function, and overall health [5]. Dysbiosis, or an imbalance in the gut microbiota, has been linked to metabolic disorders, including diabetes and insulin resistance, which are critical concerns for liver transplant recipients given their susceptibility to these conditions post-transplant [6].

Diabetes and insulin resistance (IR) represent significant clinical challenges in LT, affecting graft function and patient outcomes [7]. The interplay between the gut microbiota and metabolic health has been increasingly recognized, suggesting that alterations in gut flora may influence glucose metabolism and insulin sensitivity [8]. Understanding this relationship may provide insights into the management of metabolic complications in liver transplant patients, highlighting the importance of maintaining a balanced gut microbiota for optimal health outcomes [9]. The integration of tacrolimus therapy and the modulation of gut microbiota present a promising avenue for enhancing the success of LT and improving the metabolic health of recipients. This review aims to elucidate the current understanding of these interactions and their implications for clinical practice.

Pharmacological mechanisms of tacrolimus

Tacrolimus (Fig. 1) is essential in immunosuppressive therapy for liver transplantation due to its effectiveness in inhibiting calcineurin and suppressing T-cell activation. Here, we will further elaborate on its pharmacological mechanisms.

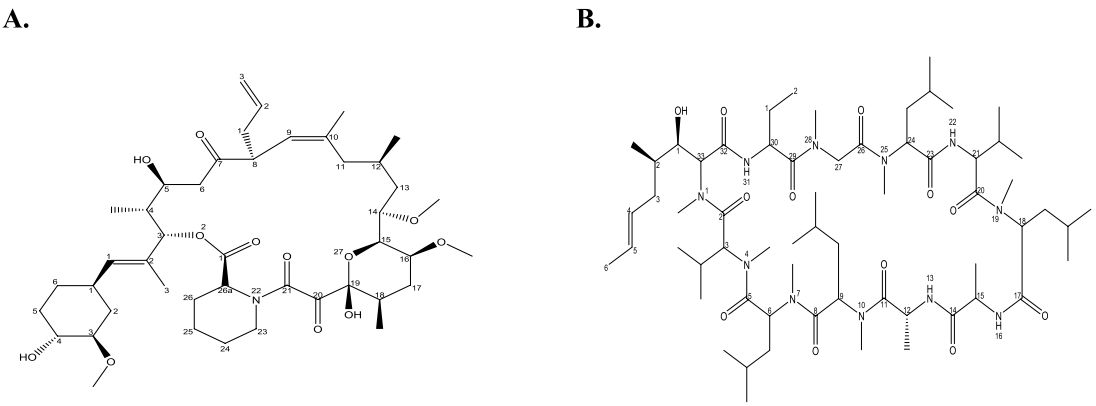


Fig. 1 Chemical formulas for tacrolimus (**A**) and cyclospori (**B**)

Immunosuppressive mechanism of tacrolimus via calcineurin inhibition

Tacrolimus exerts its immunosuppressive effects by binding to the immunophilin FK-binding protein-12 (FKBP-12), forming a complex that inhibits calcineurin [10]. Calcineurin (CaN), also known as protein phosphatase 2B (PP2B), is a calcium/calmodulin (CaM)-dependent serine/threonine phosphatase that plays a critical role in regulating intracellular signaling pathways. It consists of two subunits: a catalytic subunit (CN-A), and a regulatory subunit (CN-B) [11].

CaN becomes active when intracellular calcium levels rise, necessitating calmodulin's binding to its regulatory domain [12, 13]. CaN is involved in many cellular functions. It is mainly known for regulating the immune system, especially in activating T cells. In addition, it affects various physiological processes in other types of cells [14]. A key component of this process involves the elevation of intracellular Ca^{2+} levels, which in turn activates CaN. Specifically, the interaction between the TCR(T-cell receptor) and the antigen–MHC complex on APCs triggers the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) by phospholipase Cγ1 (PLCγ1), resulting in the production inositol-3-phosphate (IP3) and diacylglycerol (DAG) [15], IP_3 binds to IP_3 receptors on the endoplasmic reticulum (ER), leading to the release of Ca^{2+} into the cytoplasm. This rise in intracellular Ca^{2+} further drives the influx of extracellular Ca^{2+} through the Orai1 calcium-release activated calcium (CRAC) channel [15]. Upon activation of the TCR, a series of signaling cascades is triggered, one of the key steps in this process is the activation of CaN, which promotes the nuclear translocation of transcription factors, specifically nuclear factor of activated T cells (NF-ATc) and cAMP response element-binding protein (CREB), by dephosphorylating them. Dephosphorylation of NF-ATc allows it to translocate from the cytoplasm to the nucleus, where it binds to protein dimers (such as NF-ATn) induced by the TCR signaling pathway, forming the NFAT complex [16, 17]. This complex binds to the promoter regions of cytokine genes within the nucleus, significantly enhancing the expression of multiple cytokine genes, such as interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). These cytokines play crucial roles in immune responses, especially IL-2, an important growth factor that promotes the proliferation of effector T cells and memory T cells. Upon binding to its receptor, IL-2 activates signaling pathways, such as JAK–STAT, PI3K/Akt/mTOR, and MAPK/ERK. The activation of these signaling pathways triggers a series of kinase cascades, ultimately promoting the proliferation and differentiation of T cells [18].

Tacrolimus modulating immune cells to exert immunosuppressive effects

Tacrolimus is a potent immunosuppressive agent belonging to the macrolide class of compounds. Tacrolimus exerts its immunosuppressive effects by inhibiting CaN, a crucial enzyme that activates T cells. Specifically, Tacrolimus binds to the cytosolic protein FKBP12, creating a complex that inhibits CaN, which blocks the calcium-dependent signaling pathway in T cells, preventing the dephosphorylation and subsequent nuclear translocation of NFAT. When NFAT is sequestered in the cytoplasm, it inhibits the transcription of pro-inflammatory cytokines, which are essential for T-cell proliferation and differentiation [19]. By preventing NFAT from entering the nucleus, Tacrolimus effectively suppresses the expression of these cytokines, thereby dampening the immune response. It resembles cyclosporin A (Fig. 1) and is particularly effective in reducing Th1 cytokines, such as IL-2 and IFN-γ, and IL-17 [20]. NF-κB, as predominantly heterodimerized by the p50 and p65 excluding p56, is a key transcription factor and plays a potential role in inflammatory

responses. It affects histopathological indexes by modulating downstream inflammatory mediators, including IL-1, IL-6, as well as granulocyte–macrophage colony-stimulating factor (GM-CSF). In its inactive state, NF-κB binds to the endogenous inhibitory protein IκB-α, which prevents it from entering the nucleus [21]. Upon specific stimulation, the IκB kinase (IKK) complex is activated and phosphorylates IκB-α, leading to the degradation of the latter and the release of the NF-κB heterodimer, which allows it to enter the nucleus and regulate the transcription of various genes [21]. Previous studies confirm that CaN inhibitors (CNIs) such as tacrolimus and cyclosporin A can potentiate the expression of inflammatory mediators via NF-κB-related pathways. Meanwhile, Frantz et al. proposed that CaN promotes the transcription of IL-2 via NF-κB, which suggests that the synergistic effects of CNIs and CaN can strengthen the interaction between NF-κB and IκB, thereby suppressing the generation of pro-inflammatory cytokines driven by NF-κB [22]. Recent studies have shown that tacrolimus can block the entry of NF-κB subunits RelA align with p50 into the nucleus[23], which ultimately leads to reduced DNA binding and lower production of IL-1, IL-6, as well as TNF-α [23].Tacrolimus also prevents T-cell activation by blocking the phosphorylation of JNK and p38 MAPK. This action further down-regulates NF-ATc activation and alters the transcription of cytokines, such as IL-2, IFN-γ, and TNF-α, as illustrated in Fig. 2 [24, 25].

T follicular helper (Tfh) cells are a specialized subset of $CD4^+$T cells that play a crucial role in B-cell-mediated humoral immunity. Tfh cells promote the generation and proliferation of B cells into memory B cells via germinal center reactions and extrafollicular antibody responses [26]. Tfh cells provide essential signals to B cells, including IL-21, CD40L, and other cytokines, which support B-cell differentiation, affinity maturation, and the formation of long-lived memory B cells. Tacrolimus has been proven to suppress humoral immunity upon inhibiting Tfh cells [27]. Specifically, studies have demonstrated that Tacrolimus exerts its immunosuppressive effects by targeting Tfh cells, which are critical for B-cell activation, proliferation, and differentiation into plasma cells and memory B cells. Tacrolimus also reduces levels of IL-21 produced through Tfh cells, which is critical for B-cell proliferation, activation, as well as plasma cell generation [28, 29]. Tacrolimus effectively dampens B-cell activation and plasma cell generation by inhibiting IL-21 production. Furthermore, tacrolimus blocks translocation of the cytoplasmic component of NFAT, which down-regulates expression of the CD40L gene, then disrupts the co-stimulatory signals required for B-cell activation, thereby reducing the production of autoantibodies, and preventing B cells from developing into antibody-secreting plasma cells [30]. Thus, the immunosuppressive properties of tacrolimus extend beyond T cells; it also affects B-cell function and reduces antibody production, which

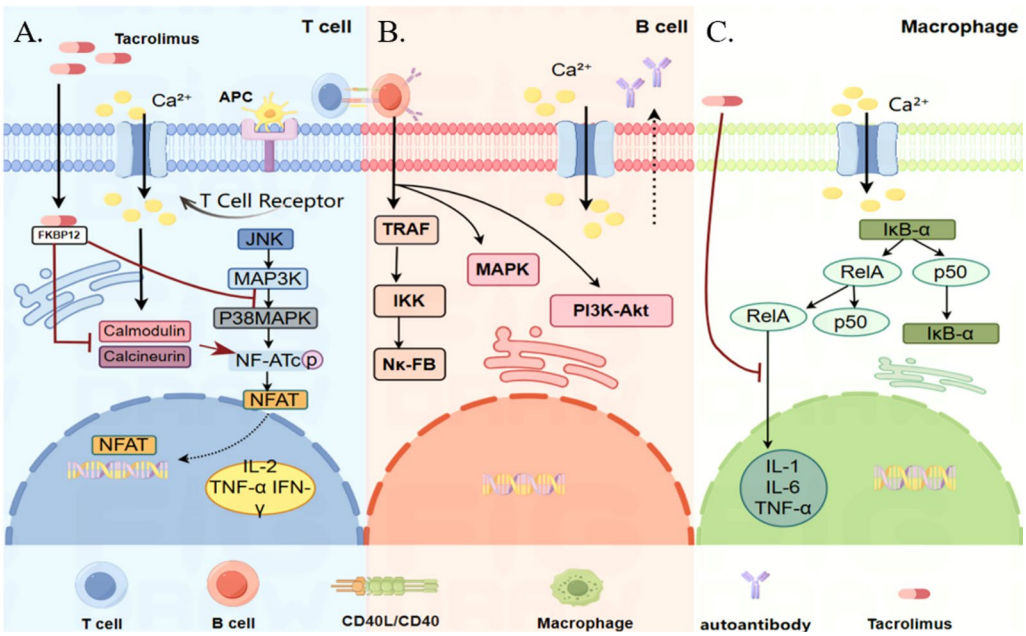


Fig. 2 Immunosuppressive mechanism of Tacrolimus. **A** Tacrolimus binds FKBP12, inhibiting JNK and p38 pathways, suppressing T-cell activation, halting NF-ATc dephosphorylation, and cytokine production (IL-2, TNF-α, and IFN-γ). **B** T cell also blocks CD40L–CD40 binding, reducing B-cell proliferation. **C** In macrophages, tacrolimus prevents NF-κB translocation, lowering cytokines, such as IL-1, IL-6, and TNF-α

is particularly advantageous for preventing allograft rejection [31]. In addition, tacrolimus has been shown to inhibit the maturation of dendritic cells (DCs), which are crucial for initiating immune responses [32]. By blocking the maturation of DCs, Tacrolimus dampens the overall immune response by limiting T-cell activation [33, 34]. However, due to the narrow therapeutic window of Tacrolimus, insufficient drug exposure increases the risk of rejection, while excessive exposure raises the incidence of adverse reactions [35]. The use of Tacrolimus may also lead to additional adverse effects, such as hyperglycemia, hypertension, increased risk of infections, and gastrointestinal discomfort. In addition, drug interactions can affect Tacrolimus blood concentrations. For example, certain drugs, such as azole antifungal agents, can inhibit its metabolism, thereby increasing the risk of toxicity [36, 37].

The hepatoprotective effects of tacrolimus

In addition to its immunosuppressive actions, tacrolimus exhibits hepatoprotective properties by reducing pro-inflammatory cytokines and promoting hepatocyte survival during liver transplantation, as evidenced by multiple research findings [38]. The ability of tacrolimus to modulate immune response in turn helps mitigate liver injury [39]. Tacrolimus can improve hepatic microcirculation to mitigate hepatic ischemia–reperfusion injury (IRI). It achieves this by suppressing endothelin-1 (ET-1), a potent vasoconstrictor that narrows blood vessels, in endothelial cells. This helps maintain blood flow and protects the liver from ischemic damage [40, 41]. During IRI, tacrolimus effectively maintains the level of glutathione (GSH) inside and outside the cells, enabling them to counteract oxidative stress [42–44]. Tacrolimus treatment improves outcomes in liver transplant recipients by preserving liver function and reducing the incidence of acute rejection episodes [45]. The pathophysiology of IRI is multifactorial and involves not only oxidative stress and inflammation but also endothelial dysfunction and microcirculatory disturbances. The hepatoprotective mechanism of tacrolimus may involve modulating oxidative stress and inflammation, as it reduces inflammatory mediators while enhancing antioxidant defenses in liver cells [46]. Moreover, studies suggest that tacrolimus may protect against IRI, a common complication during liver transplantation, by stabilizing mitochondrial function and reducing apoptosis in hepatocytes [47]. First, it reduces oxidative stress and maintains mitochondrial stability to prevent pro-apoptotic protein release, crucial for cell integrity. Second, Tacrolimus enhances mitochondrial metabolism by increasing ATP levels and regulating electron transfer to support cell survival [48–50]. Finally, Tacrolimus reduces apoptosis by preventing cytochrome c release from mitochondria, inhibiting the protease cascade, and may lower pro-apoptotic gene expression while increasing anti-apoptotic proteins like Bcl-2, thus protecting hepatocytes from IRI. These actions enhance liver resilience against IRI, reducing acute rejection risks post-transplant. Tacrolimus also protects the liver by lowering reactive species oxygen (ROS), modulating cytokines, enhancing hepatocyte survival, and improving microcirculation, though its use as a rinse solution is still debated and needs more research [38, 51].

Changes in gut microbiota after liver transplantation

The gut microbiota is a vital and dynamic part of human health that significantly changes after liver transplantation. Several factors drive these changes, such as liver disease, surgical trauma, antibiotic use during surgery, and the start of immunosuppressive therapy. Changes in the composition of gut microbiota can have significant implications for the recipient's health [52].

Analysis of gut microbiota composition before and after transplantation

According to meta-analyses, the total number of bacteria in the gastrointestinal tract of a healthy adult is approximately 10^{14}, which perform essential functions, such as maintaining the integrity of the gut barrier, regulating immune function, and promoting metabolism, among others [53]. The major phyla of gut micro-organisms include *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Clostridia,* and *Verrucomicrobia*, with *Firmicutes* and *Bacteroidetes* accounting for 90% of the gut microbiota [54]. The gut microbiota undergoes significant alterations following liver transplantation, which can have profound implications for the recipient's health. Prior to transplantation, patients often exhibit dysbiosis, characterized by less microbial diversity and an imbalance in beneficial and harmful bacteria. This dysbiosis is further intensified by liver diseases, such as cirrhosis and metabolic dysfunction-associated steatotic liver disease (MASLD), exacerbating systemic inflammation and impairing immune function [55]. After transplantation, the immunosuppressive therapies like CNI further alters the gut microbiota composition, often increasing in pathogenic bacteria and a decreasing beneficial strains [52]. For instance, there is usually a transient rise in potentially pathogenic bacteria, such as *Enterobacteriaceae* and *Enterococcus*, while beneficial bacteria such as *Bifidobacterium* and *Faecalibacterium prausnitzii* tend to decrease.

Wu ZW's team conducted an observational study to evaluate the composition of the gut microbiota in liver transplant recipients. Stool and blood samples were

collected from 28 healthy subjects, 51 patients with cirrhosis, and 111 liver transplant recipients, totaling 190 participants. These samples were used to assess gut flora composition and immune parameters using real-time quantitative PCR (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA). The results showed that the levels of *Eubacteria*, *Bifidobacterium spp.*, *Faecalibacterium prausnitzii*, and *Lactobacillus spp*. were significantly lower in the liver transplantation group compared to healthy control subjects. Conversely, the levels of *Enterobacteriaceae* and *Enterococcus spp*. were significantly higher in the liver transplantation group than in the healthy controls [56].

Studies have demonstrated that the gut microbiota shifts towards a more diverse and stable community after transplantation; however, this recovery is often incomplete and can take months to years [57]. In the early stages after liver transplantation (1–3 months postoperatively), the diversity of the patient's gut microbiota is low and characterized by an increase in potential pathogenic bacteria, such as *Enterobacteriaceae* and *Enterococcus*. The dysbiosis during this stage may be related to the use of perioperative antibiotics and long-term immunosuppressants therapy [58]. As time goes by 3–12 months after surgery, the diversity of gut microbiota gradually increases, and the proportion of beneficial bacteria, such as *Firmicutes* and *Bacteroidetes*, begins to recover [58]. However, this recovery is often incomplete and may take several years to reach the level of a healthy control population [59]. Long-term follow-up studies (more than 1 year after surgery) have shown that although the diversity and composition of gut microbiota have improved, complete recovery to a healthy state may take several years [59]. Of particular concern is that long-term immunosuppressive therapy and potential chronic rejection reactions after transplantation may also continue to affect the stability of gut microbiota [60]. Moreover, the restoration of microbial diversity post-transplant is associated with improved liver function and lowed incidence of post-transplant complications, such as infections and rejection episodes [61]. These findings emphasize the importance of monitoring gut microbiota composition as a potential prognostic marker for liver transplant outcomes and the highlights illustrated in Table 1.

Impact of tacrolimus on gut microbiota

Tacrolimus has been demonstrated to have a major impact on the gut microbiota composition and function. By suppressing the immune system, Tacrolimus can alter the gut environment to favor certain bacterial species. According to research, using tacrolimus is linked to reduced microbial diversity, especially with regard to beneficial bacteria that are essential for preserving gut health and regulating the immune system [52]. Tacrolimus undergoes metabolism by gut microbiota. Studies demonstrate that various commensal bacteria (e.g., *Clostridiales* and *Erysipelotrichales*) metabolize tacrolimus into its derivative M1. This biotransformation may significantly reduce tacrolimus bioavailability through partial intestinal degradation, consequently impairing drug absorption and therapeutic efficacy. High-dose tacrolimus alters gut microbiota composition, thereby modulating immune responses. Mouse models reveal that such microbiota modifications regulate colonic and systemic immunity, ultimately improving xenograft survival rates [63]. Clinical studies indicate an association between gut microbiota diversity and tacrolimus dosing requirements. Notably, patients requiring higher tacrolimus doses exhibit elevated gut microbiota α-diversity [64]. This suggests that microbial composition influences tacrolimus metabolism and clinical effectiveness. Research has demonstrated that following 8 weeks of feeding Tacrolimus, the relative abundance of *Alistipes*, *Allobaculum* and *Bacteroidesroses* is noticeably reduced in comparison with the control group. The tacrolimus-treated group showed a substantial drop in the abundance of *NK4A136*, *UCG-014*, and *Akkermansia* [65].

One of the critical concerns regarding tacrolimus use is its potential to promote the overgrowth of opportunistic pathogens, including *Escherichia coli* and *Enterococcus* species, thereby increasing the risk of infections and complications, such as post-transplant diarrhea and graft rejection [66]. Studies have shown that tacrolimus-induced gut dysbiosis can inhibit the expression of the efflux transporter ATP-binding cassette subfamily B member 1 (ABCB1) in the small intestine [67], which leads to decreased blood levels of tacrolimus and increased metabolism in the liver by the CYP3A enzyme [67], along with disrupting the gut barrier function and promoting inflammation due to altered drug metabolism by gut microbes [57].

The impact of tacrolimus on the gut microbiota is multifaceted, with both beneficial and adverse effects. While it can improve graft survival rates and reduce inflammation, it also poses significant risks of opportunistic infections, metabolic disorders, and drug toxicity. Future research should focus on developing strategies to mitigate these adverse effects, such as probiotic supplementation or dietary interventions, to optimize the use of tacrolimus in clinical practice [68].

Gut microbiota metabolites and their physiological functions

The gut microbiota plays a pivotal role in human health through the production of various metabolites that significantly influence physiological functions. These

Table 1 Comparison of gut microbiota composition before and after liver transplantation

Item	Pre-transplantation (Liver Disease Patients)	Post-transplantation week 1 (LT1W)		Post-transplantation week 2 (LT2W)	Post-transplantation Year 1 (LT1Y)	Healthy controls (CG)	Refs.
Dominant Phyla	Predominantly *Firmicutes* and *Bacteroidetes*, with increased *Proteobacteria* and *Actinobacteria*	Decreased *Firmicutes* and *Bacteroidetes*, significantly increased *Proteobacteria* and *Enterobacteriaceae*		Partial recovery of *Bacteroidetes* and *Firmicutes*, but still lower than healthy controls	Approaching a healthy level: The F/B ratio recovered to 1.2–1.5 (CG: 1.5) The proportion of Proteobacteria decreased to 8–12% (CG: 5–8%)	Dominated by *Firmicutes* and *Bacteroidetes*, with low levels of *Proteobacteria*	[58, 62]
Dominant Genera	Reduced *Bacteroides, Prevotella*, and *Ruminococcus*; increased *Enterococcus, Klebsiella, and Enterobacter*	*Enterococcus, Klebsiella,* and *Enterobacter*	Further increase in Enterobacter and Enterococcus; nearly complete disappearance of Blautia and Bifidobacterium	Decreased *Enterobacter* and *Enterococcus*, but still higher than healthy controls	Significant improvement: Pathogenic bacteria: Enterobacter decrease Symbiotic bacteria: Bacteroides increase *Faecalibacterium* recovery delay	Predominantly *Bacteroides, Prevotella, Blautia,* and *Bifidobacterium*	[58, 62]
Microbial Diversity	Significantly reduced microbial diversity compared to healthy controls	Further decline in microbial diversity, reaching the lowest point at week 1		Partial recovery of microbial diversity by week 2, but still lower than healthy controls	Continuous recovery: Shannon index is close to normal	Highest microbial diversity	[58, 62]
Functional Changes	Metabolic dysfunction, associated with inflammation and immune regulation	Significant metabolic changes at week 1, related to immunosuppressive use and surgical stress		Partial recovery of metabolic function by week 2, but still with significant differences	Key metabolic repair: SCFAs production returns to normal level The gene expression of bile acid metabolism enzymes tends to be normal The pathways related to ammonia metabolism are still impaired	Stable metabolic function, focused on energy metabolism and nutrient absorption	[58, 62]

metabolites, primarily derived from dietary components, can impact metabolic pathways, immune responses, and even neurological functions. Understanding the mechanisms through which these metabolites exert their effects is crucial for developing targeted therapeutic strategies for various diseases.

The role of tacrolimus in modulating short-chain fatty acids

Tacrolimus has a profound impact on the gut microbiota, particularly on short-chain fatty acid (SCFA)-producing bacteria. SCFAs, including acetate, propionate, and butyrate, are essential for maintaining gut health and modulating immune responses. However, tacrolimus-induced gut dysbiosis often leads to a significant reduction in the abundance of SCFA-producing bacteria, such as *Ruminococcaceae* and *Lachnospiraceae*, which are key producers of butyrate [67]. This reduction in butyrate-producing bacteria can disrupt gut barrier integrity, increase inflammation, and contributes to metabolic disturbances.

Butyrate is a critical SCFA that provides energy to colonocytes and has anti-inflammatory properties. Its depletion due to tacrolimus-induced dysbiosis can impair gut barrier function, leading to increased translocation of endotoxins and subsequent activation of inflammatory pathways. This disruption in the gut microbiota also affects the pharmacokinetics of tacrolimus itself. For instance, the altered microbial composition can inhibit the expression of the efflux transporter ABCB1 in the small intestine, resulting in decreased tacrolimus blood levels and increased hepatic metabolism by the CYP3A enzyme [69].

The reduction in SCFA-producing bacteria also has systemic implications. Lower levels of butyrate can lead to decreased production of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), which are essential for glucose homeostasis and appetite regulation. This can contribute to hyperglycemia and dyslipidemia, common side effects observed in patients receiving tacrolimus.

To mitigate these adverse effects, interventions such as probiotic supplementation and dietary modifications have been explored. Probiotics, including strains of *Lactobacillus* and *Bifidobacterium*, help restore microbial balance and enhance SCFA production. Dietary interventions, such as the incorporating prebiotics or fermented foods, also support a healthier gut microbiota and improve metabolic outcomes. Therefore, strategies to restore microbial balance and SCFA production are essential for optimizing tacrolimus therapy and minimizing its adverse effects [70].

There are ongoing clinical trials and research exploring the use of probiotics to counteract tacrolimus-induced gut dysbiosis, as shown in Table 2. These studies aim to restore microbial balance and mitigate the adverse effects associated with tacrolimus treatment, such as immune dysregulation, metabolic disturbances, and increased susceptibility to infections. Several studies have demonstrated the potential benefits of probiotic supplementation in patients receiving tacrolimus. For example, research demonstrates that probiotics help restore microbial balance and enhance gut barrier function, thereby reducing the risk of hyperglycemia and other metabolic complications [71]. Probiotics, such as *Lactobacillus* and *Bifidobacterium*, are known to produce SCFAs like butyrate. By restoring the levels of these beneficial bacteria, probiotics can help counteract the depletion of SCFA-producing microbes caused by tacrolimus. This restoration can improve gut barrier function, reduce inflammation, and enhance overall metabolic health.

Therefore, SCFAs can enhance insulin sensitivity and regulate lipid metabolism, making them potential therapeutic targets for conditions, such as obesity and type 2 diabetes mellitus (T2DM) [74]. The diverse roles of SCFAs underscore their importance in linking gut microbiota composition with host health and disease.

Impact of tacrolimus on other important metabolite: health and disease

The gut microbiota indeed produces a variety of metabolites beyond SCFAs that significantly influence health. One notable example is how gut bacteria metabolize tryptophan to produce indole and its derivatives. These indole-based metabolites play a crucial role in host health by activating the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor [75]. The activation of AhR by indole derivatives enhances gut–brain communication and strengthens the intestinal barrier function. This occurs by increasing the expression of tight junction proteins, which reduce gut permeability and prevent bacterial endotoxins, like lipopolysaccharides (LPS), from entering the bloodstream. This mechanism is vital for reducing endotoxemia, a condition linked to metabolic disorders, such as obesity, T2DM, and cardiovascular diseases.

Moreover, AhR activation helps regulate the immune system by balancing the Th17/Treg cell ratio, which is essential for maintaining immune homeostasis. Dysregulation of this balance is often associated with inflammatory and autoimmune diseases. The anti-inflammatory effects of indole metabolites also contribute to the management of chronic inflammatory conditions, such as inflammatory bowel disease (IBD) [76]. Recent studies have shown that tacrolimus significantly alters the gut and serum metabolome, with notable increases in specific amino acids (e.g., phenylalanine, leucine,

Table 2 Summarizing specific probiotic strains being tested in clinical trials

Probiotic strain	Research background	Potential effects	Related studies/trials	Study subjects	Refs.
Lactobacillus fermentum CECT5716 (LC40)	Preventing endothelial dysfunction and hypertension induced by tacrolimus	Reducing adverse effects of tacrolimus and improving gut health	*LC40* effectively prevents tacrolimus-induced hypertension and vascular dysfunction in animal experiments by modulating gut microbiota	Mouse models	[72]
Lactobacillus spp.	Increased abundance in tacrolimus-treated patients with ulcerative colitis	Anti-inflammatory effects, enhanced gut barrier function	*Lactobacillus* reduce the inflammatory response of colitis by increasing Treg cells and enhancing gut barrier function	Patients and animal models	[67]
Bifidobacterium spp.	Widely studied for SCFA production and gut barrier enhancement	Restoring gut microbiota balance, improving metabolic health	*Bifidobacterium* supplementation can improve gut dysbiosis and alleviate side effects such as hyperglycemia	Clinical and animal studies	[67]
Synbiotics (Probiotics + Prebiotics)	Used to improve gut health in organ transplant recipients	Reducing infectious complications, restoring gut microbiota balance	Randomised controlled trials (RCTs) and quasi-RCTs and cluster RCTs	Organ transplant recipients	[73]
Akkermansia, Alistipes, Muribaculum	Abundance changes observed in tacrolimus-treated mice	Potential to restore microbiota balance targeting these genera	*Akkermansia, Alistipes,* and *Muribaculum* can restore microbiota balance by regulating gut microbiota diversity and metabolic function	Mouse models	[67]

Clinical Trials	Research purpose	Trial number	Study subjects	Research team
	Assessing the clinical effects of probiotics in early enteral nutrition after liver transplantation	ChiCTR1800017180	Liver transplant patient	
	Evaluating the impact of perioperative probiotic administration on gut microbiota in liver transplant recipients	UMIN000024009	Liver transplant patients	

tryptophan, and tyrosine) and dipeptides (e.g., His-Glu and Tyr-Glu) [77]. These changes are time-dependent, with metabolite levels accumulating over the course of treatment. Table 3 demonstrates the alterations are closely linked to shifts in gut microbiota composition, as certain bacterial taxa associated with these metabolites are affected by tacrolimus. For instance, a reduction in beneficial butyrate-producing bacteria such as *Ruminococcaceae* and *Faecalibaculum* is observed [67]. The gut and serum metabolites are highly correlated, indicating a coordinated systemic metabolic response. This metabolic reprogramming may underlie some of tacrolimus's therapeutic effects and side effects, such as changes in glucose and lipid metabolism. Understanding these interactions provides valuable insights into the drug's mechanisms and potential for personalized medicine [67]. We also need to attach great importance to the regulation of tumor prognosis and tumor microenvironment by tacrolimus through its impact on amino acid metabolism in tumors. Tacrolimus suppresses T-cell activation causing new-onset diabetes after transplant (NODAT) in 15–30% of recipients by inhibiting insulin secretion [78, 79]. It can increase cancer risk by suppressing immune cells but may lower hepatocellular carcinoma recurrence with low doses [80, 81]. Tacrolimus alters the tumor microenvironment by reducing anti-tumor immune cells and promoting immunosuppressive ones, while diabetes worsens its pro-tumor effects through hyperglycemia and immune dysfunction [82]. Management involves monitoring tacrolimus levels, screening for diabetes and cancer, maintaining strict glycemic control, and considering mTOR inhibitors for high-risk patients [83]. Studies have shown that Tacrolimus can alter the metabolism of specific amino acids, such as tryptophan and arginine, which are critical in tumor biology [84]. For instance, tryptophan metabolism via the kynurenine pathway is upregulated by tacrolimus, producing immunosuppressive metabolites like kynurenine that inhibit T-cell function and promote tumor growth [84]. Tacrolimus does not directly alter arginine. Instead, it indirectly modulates arginine metabolism and availability within immune cells (primarily T cells and macrophages) by inhibiting signaling pathways that upregulate key enzymes (e.g., iNOS and arginase-1) [77]. Following short-term tacrolimus treatment, arginine metabolic pathways remain active both systemically (serum) and locally (intestinal microenvironment). This preserves intracellular arginine pools in immune cells while concurrently suppressing overall immune activation. The use of tacrolimus may promote the development of diabetes by affecting immune system function. Evidence has also shown that aerobic exercise can regulate the p-STAT3/ROR-γt signaling pathway, inhibit the differentiation of Th17 cells and secret of IL-17A, thereby improving glucose metabolism and vascular endothelial function [85]. Furthermore, cancer-associated fibroblasts (CAFs) play a crucial role in modulating tumor metabolism through amino acid transport. CAFs supply aspartate to cancer cells via SLC1A3, supporting

Table 3 Summarizes the important metabolites produced by the gut microbiota affected by tacrolimus

Metabolite	Mechanism of action	Impact of tacrolimus	Research subjects	Refs.
Amino Acids	Involved in protein synthesis and immune regulation	Tacrolimus treatment increases specific amino acids (Phe, Leu, Trp, Tyr) and dipeptides (His-Glu, Tyr-Glu) in the gut and serum	Mice, clinical studies	[67]
Tryptophan Metabolites	Tryptophan metabolites (indole, serotonin) are involved in immune regulation and gut–brain axis signaling	Tacrolimus upregulates tryptophan metabolism pathways, potentially affecting immune homeostasis	Mice, clinical studies	[67]
Bile Acids (BAs)	Bile acids are involved in lipid digestion and modulate gut microbiota composition	Tacrolimus alters bile acid conjugation, increasing serum levels of glycocholic acid (GCA) and taurocholic acid	Mice, clinical studies	[67]
Histamine	Histamine is an inflammatory mediator derived from histidine metabolism	Tacrolimus increases histidine conversion to histamine, potentially contributing to inflammation	Mice, clinical studies	[67]
Polyamines	Polyamines are involved in cell growth and proliferation	Tacrolimus treatment increases polyamine biosynthesis, reflecting increased cellular growth demand in an immunosuppressed environment	Mice, clinical studies	[67]
LPS	LPS are pro-inflammatory molecules produced by certain gut bacteria	Tacrolimus-induced dysbiosis lead to an overgrowth of LPS-producing bacteria, contributing to systemic inflammation	Mice, clinical studies	[67]
Trimethylamine N-oxide (TMAO)	TMAO is a pro-inflammatory metabolite linked to cardiovascular diseases	Tacrolimus-induced dysbiosis alter TMAO production, affecting cardiovascular health	Mice, clinical studies	[67]

tumor proliferation, while cancer cells reciprocate by providing glutamate to maintain the redox balance in CAFs [86]. This metabolic crosstalk is essential for tumor growth and metastasis, making SLC1A3 a potential therapeutic target [87]. Moreover, gut dysbiosis resulting from Tacrolimus treatment can lead to an overgrowth of potentially harmful microbes that produce proinflammatory metabolites, contributing to systemic inflammation and immune dysregulation [77]. These findings underscore the need for a comprehensive understanding of the interactions between Tacrolimus, host metabolism, and the microbiota to develop more effective cancer therapies and mitigate the adverse effects of immunosuppressive treatments [77].

Mechanisms of diabetes and insulin resistance

Diabetes and insulin resistance are multifactorial conditions. Insulin resistance arises from impaired insulin signaling, inflammation, dysfunctional adipose tissue, endoplasmic reticulum (ER) stress, and mitochondrial dysfunction. Diabetes results from the interplay of insulin resistance and insufficient insulin secretion, influenced by genetic predispositions, autoimmune reactions, and environmental factors, such as diet and lifestyle. Understanding these mechanisms is crucial for developing targeted therapies.

Changes in insulin signaling pathway

The insulin signaling pathway is pivotal for glucose homeostasis and energy metabolism, but it is significantly disrupted by inflammation, oxidative stress, and obesity. In diabetes, this pathway is significantly altered, often due to insulin resistance or impaired insulin secretion. The insulin signaling pathway is essential for maintaining glucose homeostasis. When blood glucose levels rise, insulin is secreted by pancreatic β cells and binds to insulin receptors on target cells, such as muscle and adipose tissue. This binding activates a cascade involving insulin receptor substrates (IRS), phosphatidylinositol 3-kinase (PI3K), and protein kinase B (Akt). These downstream signals promote glucose transporter type 4 (GLUT4) translocation to the cell membrane, facilitating glucose uptake and utilization. In addition, insulin inhibits gluconeogenesis in the liver, reducing glucose production. Together, these actions help maintain stable blood glucose levels. Obesity and over-nutrition lead to chronic inflammation and ectopic lipid deposition in tissues, such as adipose tissue, liver, and muscles, This triggers the release of pro-inflammatory cytokines, such as IL-1β and TNF-α, which impair insulin signaling by downregulating insulin signaling molecules, activating inflammatory pathways, including NF-κB, JAK/STAT, and JNK, and accelerating ceramide synthesis [88]. In addition, oxidative stress, often coexisting with inflammation, further exacerbates insulin resistance by damaging cellular components and interfering with insulin signaling [88]. For instance, the activation of pro-inflammatory cytokines can impair IRS signaling, leading to reduced glucose uptake in peripheral tissues, such as muscle and adipose tissue [89]. ER stress, caused by the accumulation of misfolded proteins, triggers a stress response that interferes with insulin signaling. This is particularly detrimental to pancreatic β cells, where sustained ER stress can lead to apoptosis, further impairing insulin secretion [90].

Recent studies have illuminated the intricate interplay between lipid metabolism, inflammation, and insulin resistance, shedding light on the multifaceted mechanisms underlying metabolic disorders. Specifically, alterations in lipid metabolism, marked by the accumulation of free fatty acids (FFAs), have been shown to precipitate insulin resistance by catalyzing the activation of serine kinases that impair the function of IRS [91]. This metabolic perturbation is further exacerbated by chronic low-level inflammation, a common consequence of obesity. Obesity triggers a surge in inflammatory factors and FFAs, with TNFα playing a pivotal role. TNFα, upon binding to its receptor, activates the JNK signaling pathway in insulin-targeted cells. This activation not only disrupts insulin signaling but also perpetuates a vicious cycle through a positive feedback mechanism, leading to sustained inflammation and worsening insulin resistance. These insights underscore the complexity of metabolic regulation and highlight potential therapeutic targets for combating insulin resistance and related metabolic diseases [92, 93]. Research has confirmed that the activation level of phosphorylated JNK is notably higher in subcutaneous adipose tissue biopsies from obese individuals. This elevated activation not only intensifies the inflammatory response but also disrupts insulin signaling, highlighting a robust connection between JNK pathway activation and obesity-induced insulin resistance. These findings suggest that targeting the JNK pathway could offer novel therapeutic strategies for managing diabetes [89]. Understanding the intricate changes in the insulin signaling pathway is crucial combating insulin resistance and metabolic disorders. Research shows that elevated JNK activation in obese individuals' adipose tissue worsens inflammation and impairs insulin signaling, linking JNK pathway activation to obesity-driven insulin resistance, as illustrated in Fig. 3.

The role of gut microbiota in metabolic diseases

The gut microbiota, a complex community of microorganisms residing in the gastrointestinal tract, plays a crucial role in various aspects of human health, including

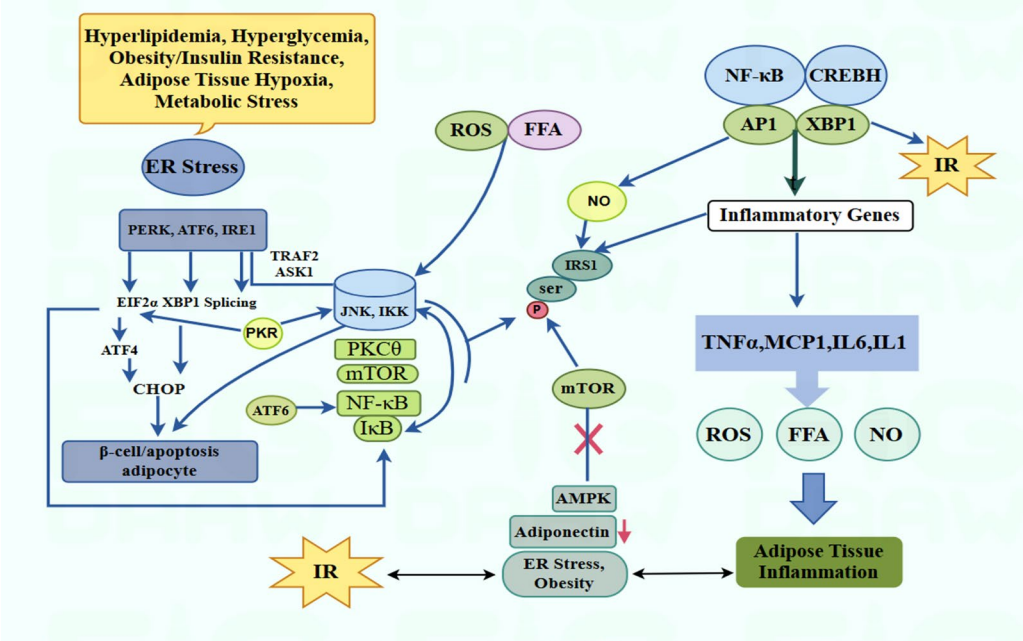


Fig. 3 Association between ER stress in adipocytes and IR. The process is multifactorial and highly interlinked. *ER* endoplasmic reticulum, *IR* insulin resistance, *ROS* reactive oxygen species, *FFA* free fatty acid, *NO* nitric oxide

metabolism and immune function. Recent research has highlighted the significant impact of gut microbiota on metabolic diseases, such as obesity, T2DM, and MASLD [94, 95] and insulin resistance [96]. Certain bacterial populations, such as *Firmicutes* and *Bacteroidetes*, have been linked to variations in body weight and metabolic health. It has been shown that the composition and function of gut micro-organisms in patients with T2DM differ significantly from those of healthy individuals [97]. The relative abundance of *Bifidobacterium, Bacteroides, Akkermansia*, and *Roseburia* was lower than that of healthy individuals in patients with T2DM, whereas the relative abundance of *Ruminococcus*, *Clostridium* and *Brucella* had higher relative abundance than healthy individuals. An increased *Firmicutes/Bacteroidetes* ratio has been observed in obese individuals, suggesting a potential mechanism through which gut microbiota influences energy extraction from the diet [98].

Furthermore, the production of SCFAs by gut bacteria through the fermentation of dietary fibers plays a crucial role in regulating insulin sensitivity. SCFAs promote glucose uptake and utilization through activation of GPR43 and GPR41 receptors and upregulation of AMPK-dependent gene expression, reduce hepatic gluconeogenesis, and indirectly affect blood glucose levels through PPY and GLP-1 hormones [99, 100]. Propionate not only helps to reduce glucose production in the liver, but also has beneficial effects on pancreatic β-cell function, enhancing glucose-stimulated insulin release and increasing β-cell mass through GPR43 activation [101]. Dietary interventions promoting a healthy gut microbiota, such as increased fiber intake and probiotics, show potential in preventing and managing metabolic diseases [102]. However, the relationship between gut microbiota and metabolic health is complex and varies among individuals. For instance, while some studies suggest that certain bacteria like *Alistipes indistinctus* can improve insulin sensitivity, others highlight the need for further research to understand the specific mechanisms and pathways involved [103]. In addition, the effectiveness of dietary interventions can be influenced by individual gut microbiota composition and other lifestyle factor [104]. Therefore, while the gut microbiota is a promising target for therapeutic strategies, more personalized approaches and validation in diverse human cohorts are needed to address insulin resistance and related metabolic disorders as summaries in Table 4.

Relationship between tacrolimus, intestinal flora and diabetes mellitus

The relationship between tacrolimus, the gut microbiota, and diabetes mellitus is multifaceted. While tacrolimus can disrupt the gut microbiota, leading to metabolic complications, strategies to modulate the gut microbiota may offer therapeutic benefits. Further research is needed to elucidate the specific mechanisms and to develop targeted interventions for patients receiving tacrolimus therapy.

Table 4 Mechanism of gut microbiota relative abundance and SCFAs in metabolic diseases

Relative abundance	SCFAs	Metabolic diseases	Mechanism	Probiotics	Prebiotic application	Refs.
Increased *Firmicutes*, decreased *Bacteroidetes*	Acetate, Propionate, Butyrate	Obesity	Altered energy extraction, increased gut permeability, metabolic endotoxemia	*Lactobacillus, Bifidobacterium*	Inulin, fructo-oligosaccharides (FOS)	[105]
Dysbiosis with increased pathogenic bacteria	Reduced SCFA production	T2DM	IR, chronic low-grade inflammation, increased acetogenic potential	*Akkermansia muciniphila*, *Lactobacillus*	Galacto-oligosaccharides (GOS), inulin	[106]
Altered microbiota with increased LPS	Altered SCFA production	MASLD	Inflammation due to LPS, impaired liver function	*Lactobacillus rhamnosus*	Lactulose	[106]

Analysis of clinical research data

Long-term use of tacrolimus is not only associated with glucose metabolic disorders but may also disrupt the stability of the gut microbiota, thereby affecting overall health. As elaborated in Fig. 4, it is generally believed that prolonged oral administration of tacrolimus can lead to dysbiosis of the gut microbiota, characterized by a reduction in beneficial bacteria, such as members of the *Lachnospiraceae* and *Akkermansia*. This alteration in microbial composition can further result in decreased production of important metabolites like butyrate that plays a vital role in gut barrier function and overall metabolic health, as it binds to GPR43/41 receptors in the intestinal crypts, stimulating L cells in the colonic mucosa to secrete GLP-1. When butyrate levels decrease, which in turn disturb the production of gut hormones, such as GLP-1 and PYY, resulting in IR and hyperglycemia [65].

Table 5 shows Tacrolimus' effects on gut microbiota and metabolites across diseases, such as colitis, UC, SLE,

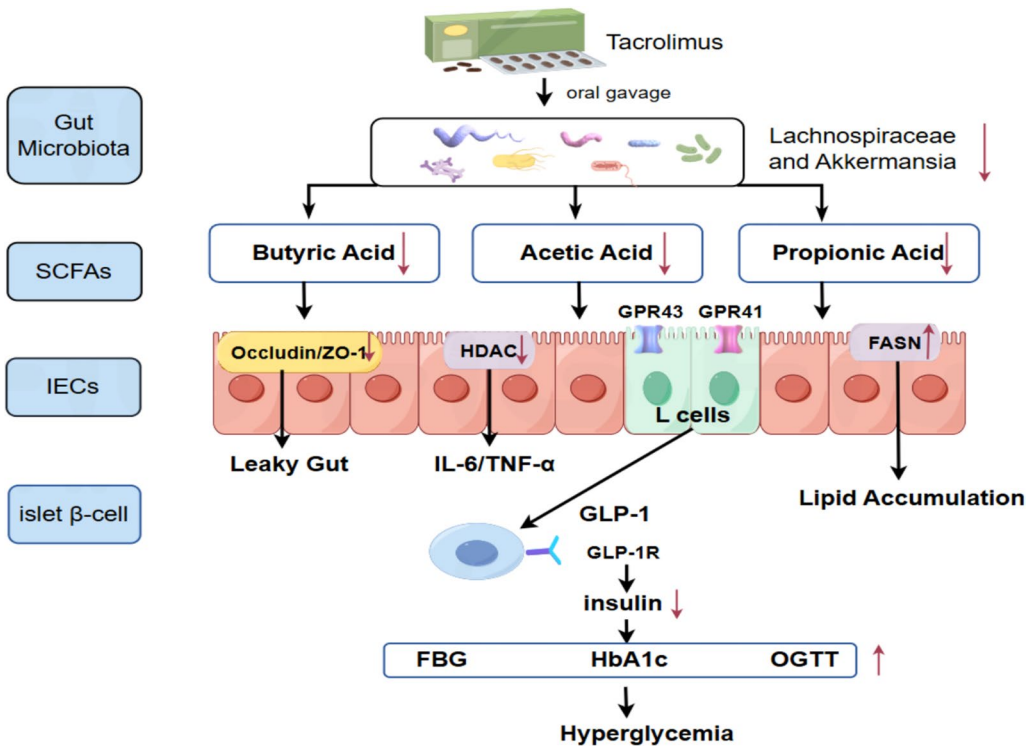


Fig. 4 Tacrolimus disrupts gut microbiota balance, lowers SCFA production, impairs IEC function, and harms pancreatic beta cells, causing hyperglycemia by affecting energy metabolism and hormone secretion. It reduces butyrate-producing bacteria, decreasing butyrate levels, which lowers GLP-1 expression, resulting in decreased insulin secretion and elevated blood glucose levels

Table 5 Comparison of the effects of tacrolimus on gut microbiota, metabolites, mechanisms, and pathways in different diseases

Disease	Gut microbiota changes	Metabolite changes	Mechanisms	Pathways	Refs.
Ulcerative Colitis (UC)	Relative abundance of *Lactobacillus, Allobaculum, Adlercreutzia,* and *Coprobacillus* increased, Relative abundance of *Bilophila, SMB53, Mucispirillum, Turicibacter, Escherichia,* and *Oscillospira* decreased	Increased production of SCFAs such as butyrate, helping in gut barrier function and anti-inflammatory effects	*Lactobacillus* lows intestinal inflammation through its anti-inflammatory and gut barrier protective effects	*Lactobacillus* modulates immune responses through the SIGNR3 pathway	[67]
Systemic Lupus Erythematosus (SLE)	Increased Relative abundance of *Alloprevotella, Clostridium, Enterorhabdus*、*Escherichia and Faecalibaculum*. Decreased relative abundance of *Lactobacillus and Alistipes*	Decreased *Lactobacillus* is associated with reduced immune regulation (Th17/Tregs cell ratio)	Reduced *Lactobacillus* leads to immune dysregulation and increased inflammation	Affects Th17/Tregs cell ratio through the SIGNR3 pathway, modulating immune responses	[67]
Tacrolimus-induced diabetes	Increased Relative abundance of *Listipes, Allobaculum, Bacteroides, Erysipelotrichaceae*、*Erysipelotrichia, Erysipelotrichales, Bacteroidaceae* and *Muribaculum,* Decreased relative abundance of *Ruminococcaceae, Faecalibaculum, Lachnospiraceae* and *Verrucomicrobiae*	Decreased butyrate levels, leading to reduced GLP-1, PYY, and insulin levels	Reduced butyrate-producing bacteria lead to decreased butyrate levels, affecting glucose regulation	Affects glucose and lipid levels through the butyrate–GPCR43–GLP-1 pathway	[67]
Post-heart transplantation	Within the first 3 months exhibited greater gut microbiota α-diversity such as *Subdoligranulum*, LPS but not TNF-α, 8,12-iso-isoprostane F-2alpha-VI	Increased abundance of potential anti-inflammatory short-chain fatty acid producers such as *Subdoligranulum*	Increased gut microbiota diversity and abundance of anti-inflammatory bacteria reduce inflammation and oxidative stress	Modulates inflammation and oxidative stress levels by regulating gut microbiota diversity and function	[64]
Post-kidney transplantation	Increased relative abundance of *Allobaculum*、*Bacteroides* and *Lactobacillus* decreased relative abundance of *Clostridium*、*Rumin ococcus*、*Rikenella*、*Ruminococcac eae* and *Oscillospira*_	Changes in SCFA metabolism, as inferred from PICRUSt analysis of 16S rRNA composition	*Faecalibacterium prausnitzii* and other commensal gut bacteria can directly metabolize tacrolimus to metabolite M1, which lower immunosuppressive activity	M1 is a gut bacteria-specific metabolite, as it is not produced in hepatic microsome incubations and can be detected in fecal samples of kidney transplant patients	[64, 69, 107]

tacrolimus-induced diabetes, and post-heart/kidney transplantation. By altering specific bacteria and metabolite levels, Tacrolimus impacts immune regulation, gut barrier function, and metabolic pathways. These changes act via specific mechanisms (e.g., SIGNR3 pathway, Th17/Tregs ratio, and butyrate–GPR43–GLP-1 pathway) to influence inflammation and immunity. Understanding these mechanisms is key for enhancing Tacrolimus' therapeutic effects and reducing adverse impacts.

Future research directions and challenges

Future research should focus on developing personalized treatment plans by integrating genetic and microbiota profiling to predict individual responses to tacrolimus, optimizing dosing, and minimizing adverse effects [67]. Moreover, it is important to investigate the bidirectional interaction between tacrolimus and the gut microbiota, including how microbial enzymatic activity influences drug metabolism and efficacy. This involves examining how specific bacterial taxa on tacrolimus pharmacokinetics and its subsequent influence on metabolic health [77]. Conduct larger multicenter trials to confirm the findings of smaller studies. These trials should also aim to deepen our understanding of the long-term effects of tacrolimus on both metabolic health and gut microbiota [67]. Investigate the potential of using tacrolimus in combination with probiotics, prebiotics, or other immunosuppressive agents to enhance therapeutic outcomes and mitigate metabolic side effects [67]. Develop novel formulations and delivery systems to improve the bioavailability and stability of tacrolimus, particularly for ocular and other non-traditional applications [108]. This includes exploring nanotechnology and other advanced drug delivery systems to enhance therapeutic efficacy while minimizing side effects [77].

The significant variability in gut microbiota composition among individuals complicates the development of standardized treatment protocols and necessitates personalized approaches [77]. Prolonged use of tacrolimus is associated with an increased risk of developing metabolic disorders, such as diabetes and obesity. Future research must focus on mitigating these side effects while maintaining therapeutic efficacy [77]. The bidirectional interaction between tacrolimus and the gut microbiota leads to variability in drug levels and metabolism. Understanding and managing this interaction is crucial for optimizing treatment [67]. Addressing the challenges of interindividual variability, metabolic side effects, and complex pharmacokinetics will be essential for advancing the use of tacrolimus in clinical practice.

Future research should prioritize understanding how tacrolimus alters gut microbiota and how these changes correlate with metabolic health outcomes. Exploring the use of prebiotics or probiotics to reduce adverse effects, along with the impact of diet, may open new pathways for improving patient care. It is also crucial to understand the genetic and environmental factors that cause individual differences in response to tacrolimus to develop personalized treatment strategies.

Limitation, conclusion and perspective

Limitations of current research

Current studies investigating the impact of tacrolimus on gut microbiota metabolites in liver transplant recipients and its association with diabetes and IR exhibit several major limitations. First, there are significant constraints in research subjects. Existing studies predominantly rely on animal models (particularly murine models), while clinical investigations remain limited in number and generally feature small sample sizes, compromising the extrapolation of findings to human populations and reducing statistical power [109]. Furthermore, the lack of subgroup analyses (e.g., by age or primary etiology) and long-term follow-up data impedes comprehensive assessment of tacrolimus' long-term metabolic effects.

Second, notable methodological deficiencies exist. Although metagenomic technologies have been widely employed, there is a paucity of systematic integration with other omics data (e.g., metabolomics and proteomics), hindering elucidation of the complete "drug–microbiota–host" interaction mechanism. In addition, current analyses fail to adequately control for confounding factors (e.g., concomitant immunosuppressants and antibiotics), making it challenging to delineate tacrolimus-specific effects [69].

Finally, intervention strategies remain oversimplified. Most studies focus solely on tacrolimus monotherapy, neglecting real-world polypharmacy scenarios, and lack investigations into microbiota-targeted interventions (e.g., probiotics and dietary modifications), thereby limiting translational potential. These methodological shortcomings collectively constrain deeper understanding of post-transplant metabolic complications and hinder development of personalized therapeutic strategies [77].

Conclusion from bench to bed

Current evidence suggests tacrolimus-associated metabolic perturbations may involve interactions between gut microbial ecology and host metabolic homeostasis. Preclinical studies have identified potential links between tacrolimus exposure, dysbiosis diabetes pathogenesis, particularly through microbial metabolite-mediated IR [110]. However, the mechanistic details, such as how calcineurin/NFAT signaling interacts with microbial-derived aromatic amino acid metabolites, and pancreatic β-cell dysfunction remains obscure. A critical temporal

limitations, as > 80% of preclinical studies restrict observation windows to < 12 weeks [77], which restricts insights into long-term microbiota–metabolome dynamics under chronic tacrolimus exposure. Furthermore, while this work focues on liver transplant recipients, extrapolation to other solid organ transplants (e.g., kidney, heart, and lung) requires caution due to potential differences in drug metabolism, baseline microbiota, and metabolic risk profiles. Although microbiota-directed interventions (e.g., probiotics and FMT) show promise in mitigating tacrolimus-induced diabetes in animal models[65], clinically applicable protocols encompassing strain-specific probiotics, optimized fecal microbiota transplantation schedules, and metabolite-targeted dietary modifications require rigorous standardization through phase II/III pharmacomicrobiomics trials [111].

Critically, interindividual variability in baseline gut microbiota, pharmacogenomics, and metabolic resilience complicates therapeutic predictions. Current data lack validated stratification models that integrate metagenomic (e.g., *Bacteroidetes/Firmicutes* ratio), pharmacogenomic variants, and metabolomic fingerprints to guide personalized tacrolimus–microbiota modulation [111]. This knowledge gap underscores the imperative for developing machine learning-driven predictive platforms that reconcile multi-omics data with clinical outcomes.

Perspective of research

Future research on the impact of tacrolimus on the intestinal flora metabolites of liver transplant patients, as well as and its relationship with diabetes and insulin resistance has great potential. First, the ongoing development of multi-omics technologies will enable researchers to better assess tacrolimus's impact on intestinal microbiota. By synthesizing information from metagenomics, metabolomics, and proteomics, it becomes possible to elucidate how tacrolimus modifies the composition and functionality of the intestinal flora, thereby affecting the production and metabolic pathways of various metabolites. For example, further research could focus on the specific bacterial taxa influenced by tacrolimus and their corresponding metabolites, including short-chain fatty acids and bile acids. In addition, it could investigate how these metabolites may modulate glucose metabolism and insulin signaling in the host. Moreover, extended longitudinal studies will yield a more nuanced understanding of the temporal effects of tacrolimus on both intestinal flora and their metabolites, ultimately leading to enhanced predictive and intervention strategies for clinical applications.

From a clinical perspective, personalized therapeutic approaches that utilize the intestinal microbiota could improve care for liver transplant patients. Modulation of the intestinal microbiota may mitigate adverse effects, such as hyperglycemia and insulin resistance, associated with tacrolimus therapy. For example, the administration of targeted probiotics or dietary fibers aimed at reshaping the gut microbiota could facilitate improvements in glucose metabolic processes among these patients. Moreover, investigations into the synergistic application of various immunosuppressive agents or antibiotics are likely to yield more refined pharmacological combinations that can be integrated into clinical practice, thereby minimizing the incidence of undesirable side effects. As we better understand how the intestinal microbiota interacts with drug absorption, we expect to develop more effective treatment plans, improving outcomes and quality of life for liver transplant patients [112].

Abbreviations

ABCB1	ATP-binding cassette subfamily B member 1
AhR	Aryl hydrocarbon receptor
Akt	Protein kinase B
APCs	Antigen presenting cells
ARG1	Arginase 1
BAs	Bile acids
CAFs	Cancer-associated fibroblasts
CaM	Calmodulin
CaN	Calcineurin
CN-A	Calcineurin A
CN-B	Calcineurin B
CNIs	Calcineurin inhibitors
CRAC	Calcium-release activated calcium
CREB	CAMP response element-binding protein
DAG	Diacylglycerol
DCs	Dendritic cells
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
FFAs	Free fatty acids
FKBP12	FK506-binding protein 12
ET-1	Endothelin-1
FOS	Fructo-oligosaccharides
GCA	Glycocholic acid
GLP-1	Glucagon-like peptide-1
GM-CSF	Granulocyte–macrophage colony-stimulating factor
GOS	Galacto-oligosaccharides
GPR41	G protein-coupled receptor 41
GPR43	G protein-coupled receptor 43
GSH	Glutathione
GLUT4	Glucose transporter type 4
HFD	High-fat-diet mice
IBD	Inflammatory bowel disease
IFN-γ	Interferon-gamma
IKK	IκB kinase
IκB-α	Inhibitor of κ light polypeptide gene enhancer in B cells, alpha
IL-2	Interleukin-2
IL-21	Interleukin-21
IP3	Inositol-3-phosphate
IR	Insulin resistance
IRI	Ischemia–reperfusion injury
IRS	Insulin receptor substrate
JNK	C-Jun N-terminal kinase
LPS	Lipopolysaccharide
LT	Liver transplantation
MAPK	Mitogen-activated protein kinase
MASLD	Metabolic dysfunction-associated steatotic liver disease
NFAT	Nuclear factor of activated T cells
NF-ATc	Nuclear factor of activated T cells, cytoplasmic
NF-ATn	Nuclear factor of activated T cells in the nucleus

NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide.
p38	MAPK p38 mitogen-activated protein kinase
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PI3K	Phosphoinositide 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PLCγ1	Phospholipase Cγ1
PP2B	Protein phosphatase 2B
PYY	Peptide YY
ROS	Reactive oxygen species
RT-qPCR	Real-time quantitative polymerase chain reaction
SCFAs	Short-chain fatty acids
SLC1A3	Solute carrier family 1 member 3
SLE	Systemic lupus erythematosus
TCR	T-cell receptor
Tfh	T follicular helper
TMAO	Trimethylamine n-oxide
TME	Tumor microenvironment
TNF-α	Tumor necrosis factor-alpha
T2DM	Type 2 diabetes mellitus
UC	Ulcerative colitis

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Y.T.H., Z.Y.B., and J.T.Z. drafted the manuscript. Y.T.H., and W.K.S. polished the figures. H.Y.X., H.Y.W analyzed the data. Y.T.H., J.J.L and F.G created the graphical abstract and conducted literature research. J.T.Z. and W.K.S conducted literature review and compiled the summary table. J.D.X., and C.X supervised the data analysis and revised the manuscript critically. All authors contributed to manuscript editing, read and approved the final manuscript, participated sufficiently in the work, and agreed to be accountable for all aspects of the work.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

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