

REVIEW

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Decoding metabolic diseases through bacterial extracellular vesicles: mechanisms, diagnostics, and therapeutics

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Abstract

Metabolic diseases represent a class of chronic conditions characterized by disturbances in energy and material metabolism. These diseases primarily include obesity, metabolic dysfunction-associated steatotic liver disease, atherosclerosis, type 2 diabetes, osteoporosis, and polycystic ovary syndrome. A core pathological feature underlying their development is the mutual reinforcement between chronic low-grade inflammation and metabolic imbalance. The gut microbiota has been highlighted by recent research as a key regulator of host metabolism, while bacterial extracellular vesicles (BEVs) are emerging as critical mediators. BEVs transport bioactive molecules (proteins, lipids, nucleic acids) to facilitate microbiota-host crosstalk without direct contact. Pathogen-derived BEVs often exacerbate metabolic dysfunction via inflammation, while commensal/probiotic BEVs protect against disorders by enhancing intestinal barrier function and regulating immunity. Furthermore, owing to their high stability, easy penetration of biological barriers, and capacity to carry abundant cellular information, BEVs demonstrate broad application prospects as non-invasive diagnostic biomarkers for metabolic diseases and as natural or engineered targeted drug delivery vehicles. This review outlines the mechanisms and progress in the application of gut BEVs in metabolic diseases. It aims to deepen our understanding of host-microbiota interaction networks and support the development of novel intervention strategies for metabolic diseases.

Keywords Metabolic diseases, Gut microbiota, Bacterial extracellular vesicles, Drug delivery carriers

Introduction

Metabolic diseases are a group of chronic disorders that are distinguished by abnormalities in energy metabolism, glucose and lipid metabolism, and endocrine homeostasis. The pathogenesis of these diseases involves multiple pathological factors, including genetic susceptibility, environmental factors, lifestyle choices, and inflammatory responses [1, 2]. Common metabolic diseases include metabolic dysfunction-associated steatotic liver disease (MASLD), type 2 diabetes mellitus (T2DM), obesity, osteoporosis, and polycystic ovary syndrome (PCOS). These diseases affect different organs and systems yet share similar metabolic abnormalities [1–4]. The

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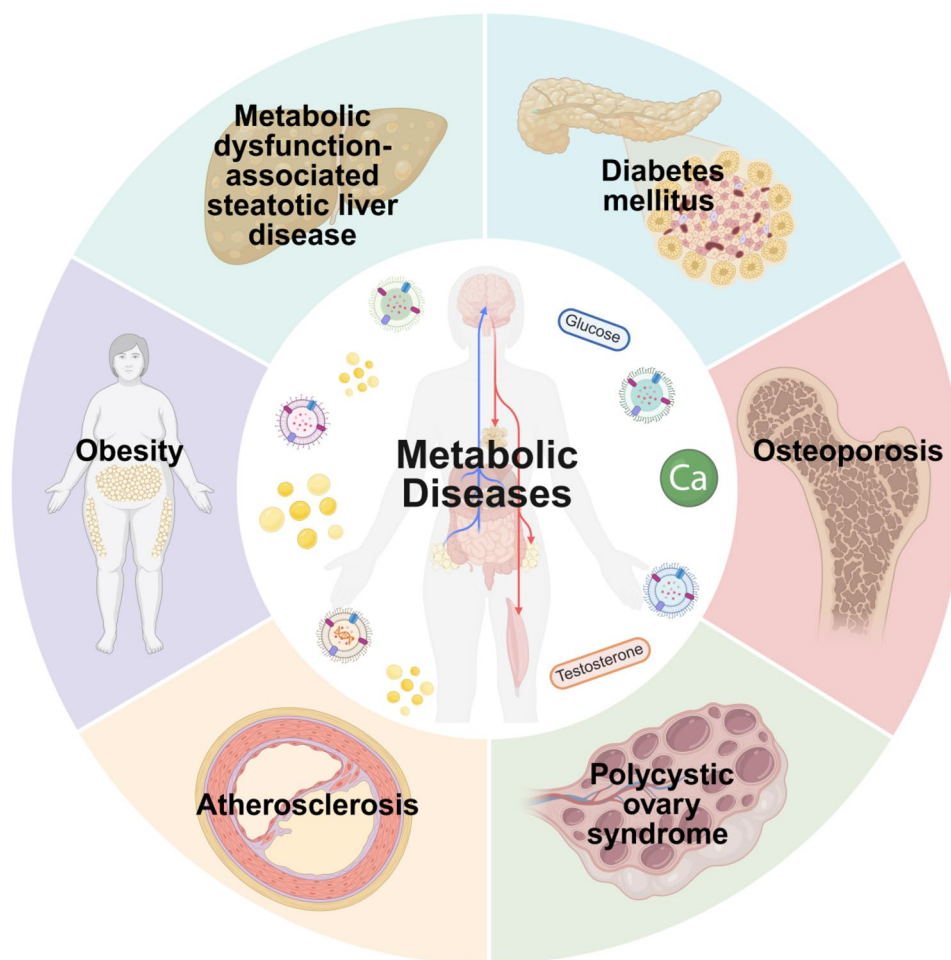
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Graphical abstract



global burden of metabolic diseases has increased rapidly over the past decades, making them a major public health challenge worldwide. Data showed that about 828 million adults were living with diabetes in 2022 worldwide. Despite an increase of more than 600 million since 1990, nearly 60% of affected individuals remained untreated [5]. Obesity has become much more common, with its prevalence having doubled since 1990 and now affecting over one billion people worldwide [6–8]. MASLD is estimated to affect about 38% of the global population. The disease increases the risk of cardiovascular disease and liver cancer [9]. Women with PCOS frequently show insulin resistance and metabolic abnormalities, which are also often observed in their family members, supporting the view that PCOS is a systemic metabolic disorder rather than a disease limited to the reproductive system [4]. Although these metabolic disorders present in various forms, they are interconnected through pathological processes such as chronic low-grade inflammation and disruption of metabolic homeostasis [3, 6, 10]. This interrelation makes

metabolic diseases and their associated complications an important part of the global disease burden. A better understanding of the common mechanisms may support the development of more effective prevention and treatment strategies.

The human gut harbors approximately 100 trillion microorganisms, whose combined genomes exceed those of the host by a hundredfold. Functionally, it is regarded as a vital “virtual organ” that maintains bodily homeostasis [11]. Fermentation of dietary fiber and endogenous substrates by gut microbes produces various metabolites, including short-chain fatty acids (SCFAs). These metabolites subsequently regulate host energy balance, immune responses, and inflammatory states, playing a critical role in nutrient absorption and metabolic health [11, 12]. Changes in the composition and function of the gut microbiota are closely linked to metabolic diseases. Several signaling molecules are involved in this link, such as bile acids, branched-chain amino acids, tryptophan derivatives, and trimethylamine N-oxide (TMAO) [13,

14]. Such molecules not only serve as energy substrates or signaling molecules but also coordinate metabolic communication across multiple organs by activating receptor networks in the intestinal epithelium, liver, and adipose tissue [15]. The gut microbiota and its bioactive products therefore play central roles in host metabolic regulation. Loss of microbial homeostasis can disturb metabolic balance, thus promoting the onset and progression of metabolic diseases and positioning them as key targets for therapeutic intervention.

Recent studies have identified bacterial extracellular vesicles (BEVs) as important mediators of host-microbiota communication [16]. Bioactive substances (including lipids, proteins, metabolites, and nucleic acids) are encapsulated within BEVs, which allows for cross-barrier communication in the absence of direct contact [17]. BEVs not only regulate microbial homeostasis and modulate intestinal barrier function and mucosal immune responses [18], but also traverse the intestinal epithelium into systemic circulation, delivering functional molecules to distant organs such as the adipose tissue and liver, thereby influencing systemic metabolism [19, 20]. Notably, gut-derived BEVs exhibit significant compositional differences from the maternal microbiota and can be regarded as independent functional entities [21]. As a result, understanding the processes of BEVs in metabolic illnesses is critical for exposing unique patterns of host-microbiota interactions, as well as giving fresh perspectives and therapeutic options for the diagnosis and treatment of metabolic disorders.

Recent reviews have summarized the topic from the perspectives of probiotic-derived BEVs and the regulation of BEV signaling pathways, respectively [22, 23]. Unlike previous reviews that adopted a singular perspective, this review introduces innovative additions and expansions from multiple angles. It adopts a broader perspective, in which BEVs from probiotics, commensal bacteria, and opportunistic pathogens are all considered. It also focuses on major metabolic diseases, including obesity, MASLD, diabetes, osteoporosis, and PCOS. The mechanisms through which BEVs contribute to each condition are discussed separately. Furthermore, this review summarizes the clinical applications of BEVs as non-invasive diagnostic biomarkers, engineered drug delivery vehicles, and natural prebiotics, aiming to provide a comprehensive analysis of the role of BEVs in metabolic diseases from three dimensions: mechanisms, diagnosis, and therapeutic strategies.

Overview of gut microbiota and BEVs

Gut microbiota and metabolic health

Gut microbiota play an important role in maintaining metabolic homeostasis in the host [24]. They support nutrient absorption, regulate energy metabolism, and

help preserve immune balance. When this microbial ecosystem is disrupted, however, it can trigger chronic inflammation, impair insulin sensitivity, and disturb lipid metabolism, thereby contributing to various metabolic diseases. This relationship is further supported by fecal microbiota transplantation studies: transplanting gut microbiota from lean, healthy individuals into patients with metabolic syndrome significantly improves insulin sensitivity, suggesting that gut microbiota could be a target for metabolic disease interventions [25].

Different gut microbiota exert distinct and even opposing roles in metabolic regulation. *Akkermansia muciniphila* (*A. muciniphila*) effectively reduces the pathological progression of metabolic syndrome and MASLD by enhancing intestinal barrier function and regulating bile acid and amino acid metabolic pathways [26, 27]. *Bacteroides* species, such as *Bacteroides thetaiotaomicron* (*B. thetaiotaomicron*) and *Bacteroides uniformis* (*B. uniformis*), improve metabolic dysfunction in MAFLD by regulating gut-liver axis metabolism. Their metabolites, including hexadecanedioic acid and 3-succinylated cholic acid, help protect against MAFLD by improving lipid metabolism and reducing oxidative stress [28–30]. *Faecalibacterium prausnitzii* shows beneficial effects in diabetes by reducing inflammatory activity and improving insulin sensitivity [31]. In contrast, some gut microbes contribute to the onset of metabolic diseases. Higher abundance of *Prevotella copri* has been associated with insulin resistance and childhood obesity. The bacterium enhances branched-chain amino acid biosynthesis and disrupts bile acid metabolism, which promotes metabolic abnormalities [32, 33]. *Bacteroides vulgatus* (*B. vulgatus*) can induce insulin resistance and ovarian dysfunction by disrupting the bile acid-interleukin (IL)-22 signaling pathway or activating the farnesoid X receptor (FXR) pathway through its metabolite agmatine [34, 35].

The gut microbiota also play an important role in bone metabolism. *B. vulgatus* abundance shows a negative correlation with bone mineral density (BMD) in human studies [36]. Conversely, probiotics including *Lactobacillus reuteri* have been reported to slow BMD loss in elderly women [37]. Animal studies show that *Bacillus coagulans* improves osteoporosis by enhancing the immunoregulatory function of regulatory B cells [38], while *Bifidobacterium animalis* subsp. *lactis* A6 exerts bone-protective effects by increasing butyrate production [39].

Classification and characteristics of BEVs

Basic characteristics, classification, and composition

BEVs are nanoscale, lipid-bilayer-enclosed particles released by bacteria during their development. Their diameter usually ranges from 20 to 400 nanometers [40]. Based on the Gram staining characteristics of their bacterial sources and membrane structure, BEVs are primarily

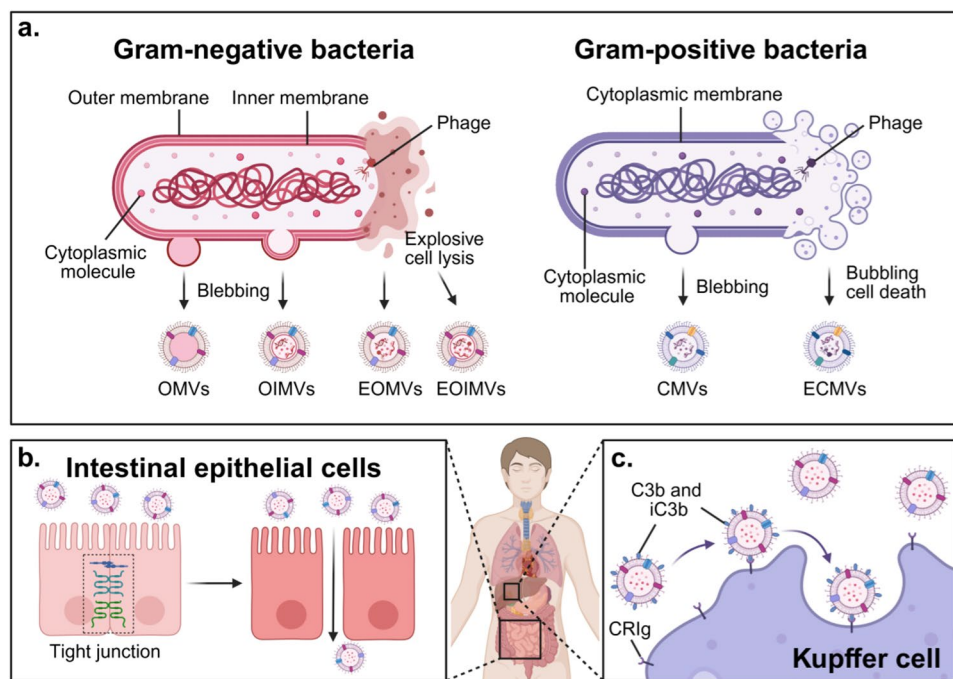


Fig. 1 Biogenesis and hepatic clearance of BEVs. **a.** Gram-negative and Gram-positive bacteria produce BEVs through distinct pathways. **b.** BEVs traverse the intestinal epithelium and enter the systemic circulation. **c.** A subset of BEVs is recognized and subsequently cleared by Kupffer cells in the liver. Abbreviations: OMVs, outer membrane vesicles; OIMVs, outer-inner membrane vesicles; EOMV, explosive outer membrane vesicles; EOIMV, explosive outer-inner membrane vesicles; CMVs, cytoplasmic membrane vesicles; ECMVs, explosive cytoplasmic membrane vesicles; CR1g, complement receptor of the immunoglobulin superfamily

Table 1 Classification and composition of BEVs

Classification	Gram-negative bacteria-derived				Gram-positive bacteria-derived	
	OMVs	OIMVs	EOMV	EOIMV	CMVs	ECMV
Biogenesis	Blebbing		Explosive cell lysis		Blebbing	Bubbling cell death
Membrane composition	Outer membrane	Outer and inner membranes	Outer membrane	Outer and inner membranes	Cytoplasmic membrane	Cytoplasmic membrane
Cytoplasmic content	No	Yes	Yes	Yes	Yes	Yes

classified into two major categories: vesicles derived from Gram-negative bacteria and those from Gram-positive bacteria. Further subclassification occurs within these categories based on their biogenesis pathways. Gram-negative bacteria primarily produce outer membrane vesicles (OMVs) and outer-inner membrane vesicles (OIMVs) through blebbing. Alternatively, they can generate explosive outer membrane vesicles (EOMVs) and explosive outer-inner membrane vesicles (EOIMVs) via explosive cell lysis. In contrast, Gram-positive bacteria generate cytoplasmic membrane vesicles (CMVs) through blebbing and produce explosive cytoplasmic membrane vesicles (ECMV) via bubbling cell death [40, 41]. Based on the differences in the aforementioned biogenesis pathways, different types of vesicles exhibit distinct variations in the composition of their contents. OMVs, formed via blebbing, predominantly enrich periplasmic proteins, outer membrane components (e.g.,

LPS), and hydrophobic molecules, typically lacking cytoplasmic contents. In contrast, vesicles generated by explosive cell lysis, such as EOMVs and EOIMVs, contain cytoplasmic components, including genomic DNA [40, 42] (Fig. 1; Table 1).

Mechanisms of BEV biogenesis

The generation of distinct BEV types arises from the remarkable complexity and diversity of their biogenesis mechanisms. This process is precisely regulated through a multifaceted network involving bacterial structural homeostasis and microenvironmental responses. In Gram-negative bacteria, blebbing can be induced by various factors. These include weakened cross-linking between peptidoglycan and lipoproteins, which compromises outer membrane stability; abnormal accumulation of misfolded intracellular proteins; repulsive interactions among negatively charged lipopolysaccharide (LPS)

molecules that disrupt membrane structural equilibrium; and the insertion of specific signaling molecules, such as *Pseudomonas* quinolone signal, which induces membrane rearrangement [43, 44]. In contrast, the generation of BEVs mediated by explosive cell lysis primarily depends on the activation of prophage-derived endolysins in response to genotoxic stress. This activation degrades the peptidoglycan layer and disrupts cell integrity, thereby driving BEV formation [40]. The biogenesis of BEVs is finely regulated by bacterial growth phase and environmental conditions. For instance, *B. thetaiotaomicron* releases lipoprotein-rich BEVs during early growth stages, whereas late-stage growth predominantly produces lytic vesicles rich in cytoplasmic proteins, suggesting that dynamic vesicle composition changes are closely linked to bacterial physiological states [45]. Beyond these internally programmed dynamic shifts, BEV production is also affected by the surrounding environment. External stresses (such as antibiotic exposure or nutrient deficiency) can significantly alter BEV yield and composition. This adaptive regulation not only facilitates bacterial environmental adaptation but also exerts vital functions in host-microbe interactions and the pathogenic processes mediated by these vesicles [46, 47].

Host factors can also influence BEV production. Recent studies have shown that proteins produced by the host can stimulate BEV release from specific gut commensal bacteria. For instance, APOL9a/b, the human homolog of APOL2, secreted by mouse intestinal epithelial cells, can induce BEV production in *Bacteroidales* by recognizing commensal ceramide-1-phosphate on the surface of these bacteria. Host IFN- γ signaling and MHC-II expression in the intestinal epithelium are enhanced by this interaction, thereby preserving the integrity of the intestinal immune barrier [48]. These results suggest that the host not only receives signals from BEVs but also actively regulates the BEV production process. However, compared with environmental influences on BEV production, host-driven regulation has received much less attention in most studies. It is essential to investigate whether other host factors, such as lipids and nucleic acids, perform similar functions, whether the host regulates the selective packaging of cargo into BEVs, and how the host's ability to regulate BEVs is altered in states of metabolic disease in future studies.

Sources and biological significance of BEV heterogeneity

The properties and functions of BEVs vary substantially depending on their origin and cargo content. This is primarily influenced by factors such as bacterial species, growth conditions, environmental stress, and the surrounding microbial community. However, most current knowledge comes from in vitro experiments focusing

on individual bacterial species, with limited information about BEV behavior in the complex gut environment.

Different bacterial species represent a significant source of heterogeneity in BEVs. BEVs released by different bacteria often differ in size, concentration, and protein content [49]. Taking gut-associated *Bacteroides* as an example, a proteomic analysis of BEVs derived from *Bacteroides fragilis* (*B. fragilis*) and *B. thetaiotaomicron* revealed that, although both species selectively package acid glycosidases and proteases, there are significant differences in the types of enzymes they package. Specifically, the BEVs from *B. fragilis* contain 15 hydrolytic enzymes, whereas those from *B. thetaiotaomicron* contain 25 hydrolytic enzymes [50]. Differences are also present among strains within the same genus. Functional cargo varies across *Bacteroides* strains. For instance, BEVs released by *Bacteroides finegoldii* can contain concentrations of GABA as high as 4 μ M, in contrast to lower levels found in BEVs from other *Bacteroides* strains [51]. Differences in bacterial species are evident not only among commensal bacteria but also between pathogenic and probiotic bacteria. A comparative analysis of the proteomes of BEVs derived from the urinary tract pathogenic *Escherichia coli* (*E. coli*) strain UPEC 536 and the probiotic *E. coli* Nissle 1917 (EcN) revealed that BEVs from *E. coli* UPEC 536 were enriched in ribosome-associated proteins, while those from EcN were predominantly enriched in proteins involved in glycolysis [52]. These observations indicate substantial differences in the physical properties and cargo composition of BEVs, which can be detected at both the species and strain levels.

Both growth conditions and environmental stresses in the gut microenvironment influence the production and composition of BEVs. *B. fragilis* provides a representative example. It can dynamically adjust the protein cargo composition of BEVs by specifically enriching glycoside hydrolases and binding proteins encoded by polysaccharide utilization loci (PULs), depending on the type of dietary or host-derived polysaccharides present in their environment. Greater efficiency in polysaccharide degradation and utilization can result from these changes [53]. The nutritional status of the host also affects BEV cargo. During fasting, BEVs released by the gut commensal *B. thetaiotaomicron* contained lower levels of serine protease family proteins, including DPP-4. Such changes may reflect bacterial adaptation to nutritional stress through selective modification of BEV cargo [54]. Additionally, signaling molecules secreted by the host in the gut participate in the regulation of BEVs. Sodium taurocholate, for example, can enhance the production of BEVs from *Campylobacter jejuni* while simultaneously reducing their volume by influencing the lipid asymmetry pathway [55]. Collectively, these studies show that the growth environment, nutritional status, and host-derived

signaling molecules shape the heterogeneity of BEVs through various mechanisms.

The microbial community background also influences the heterogeneity of BEVs. The bacterial species and their relative abundance detected in BEVs differ from those in fecal samples [21], which suggests that BEV composition is not a simple reflection of the microbial community. Gut microorganisms engage in various interactions, including nutrient sharing, resource competition, and cell-to-cell signaling. Such interactions can influence BEV formation, resulting in different compositional profiles.

BEVs display substantial diversity in particle size, membrane structure, and cargo composition, which affects host cell uptake mechanisms and efficiency. Several cellular uptake pathways have been identified, including caveolin-mediated endocytosis, clathrin-mediated endocytosis, macropinocytosis, and non-clathrin/non-caveolin-mediated endocytosis [47, 56]. BEV size is an important factor during cellular uptake: smaller BEVs (e.g., 20–100 nm) are typically preferentially internalized via caveolin-mediated endocytosis, while larger BEVs (e.g., 90–450 nm), due to their size constraints, tend to enter cells through pathways such as macropinocytosis [57]. Membrane composition also influences uptake behavior, with structural differences in LPS being particularly important. For instance, BEVs lacking O-antigen (a component of LPS) primarily rely on clathrin-mediated endocytosis, whereas BEVs containing intact O-antigen can bypass the clathrin pathway and achieve efficient uptake via lipid raft-mediated endocytosis [58]. Different types of BEVs can enter host cells through different uptake pathways. These pathways help deliver BEVs to specific intracellular locations, where they exert distinct biological effects.

BEVs released by different bacterial species show marked functional diversity. Research on MASLD indicates that BEVs from *Romboutsia hominis* can exacerbate hepatic lipid deposition and inflammatory responses through the TNF- α signaling pathway. In contrast, BEVs derived from *A. muciniphila* (Akk-EVs) exert a protective effect by downregulating genes associated with lipid synthesis [59]. Studies in prediabetes have also linked the biological activity of BEVs to their bacterial source. Vesicles containing high levels of *Lachnospiraceae* and *Oscillospiraceae* DNA exhibit stronger pro-inflammatory activity, whereas those enriched in *Akkermansia* DNA display anti-inflammatory properties [60]. In the context of bone metabolism, BEVs produced by bacteria such as *A. muciniphila* [61], *Roseburia intestinalis* [62], *Lactobacillus salivarius* (*L. salivarius*) [63], and *Christensenellaceae* [64] can mitigate osteoporosis. In contrast, BEVs derived from *Klebsiella variicola* promote bone loss and may worsen osteoporosis progression

while BEVs derived from *Klebsiella variicola* can accelerate bone loss [65].

In summary, multiple factors contribute to the heterogeneity of gut-derived BEVs. Variations among bacterial strains form the intrinsic basis of this heterogeneity, while the gut microenvironment, host status, and interspecies interactions within the microbiota reshape their structure and function externally. Different types of BEVs can also have different effects on intestinal barrier function and immune homeostasis. However, most current studies focus on individual bacterial species or simple co-culture models, so information from complex microbial communities remains limited. As a result, the dynamic changes, underlying mechanisms, and biological consequences of BEV heterogeneity in complex gut ecosystems are not fully understood and require further investigation.

Functions of BEVs in the gut microenvironment

The gut serves as both the primary habitat of the gut microbiota and the main site where BEVs exert local effects by supporting microbial communication and regulating host intestinal functions. These local actions may in turn influence systemic metabolism.

The intestinal barrier represents a key protective interface that prevents the entry of harmful substances and is fundamental for maintaining host homeostasis. BEVs derived from different sources exert varying effects on the intestinal barrier; they can either reinforce it or promote its degradation. BEVs derived from probiotic or commensal bacteria can penetrate the mucus layer, thereby enhancing intestinal barrier integrity and promoting immune tolerance through the upregulation of tight junction proteins (e.g., occludin), stimulation of mucus secretion, or induction of a tolerant phenotype in dendritic cells [18, 66]. BEVs derived from *A. muciniphila* can stimulate goblet cells to secrete mucus through endocytosis. Simultaneously, they activate the AMPK pathway, upregulate the expression of occludin and ZO-1, and strengthen the intestinal barrier [18]. *Lactobacillus murinus*-derived BEVs have been shown to mitigate deoxynivalenol-induced disruption of the intestinal barrier by stimulating TLR2 signaling, inducing M2 macrophage polarization, and enhancing macrophage–epithelial cell crosstalk [67]. These studies suggest that BEVs derived from beneficial bacteria possess significant potential in maintaining microbiota balance, enhancing mucosal immunity, and protecting the intestinal barrier. In contrast, BEVs from pathogenic bacteria often damage the intestinal barrier. A notable example is *Fusobacterium nucleatum*-derived BEVs, which deliver virulence factor DNA into intestinal epithelial cells. This activates the AIM2 inflammasome, induces pyroptosis, and consequently disrupts barrier function [68]. BEVs from this bacterium can also disrupt the integrity of the intestinal

epithelial barrier by activating the receptor-interacting protein kinase 1 (RIPK1) -mediated cell death pathway [69]. Furthermore, BEVs derived from *Listeria monocytogenes* primarily damage the intestinal epithelial barrier via the JAK-STAT3 pathway by inducing M1 polarization of macrophages and activating the AIM2/NLRP3 inflammasome [70]. Moreover, BEVs released by adherent-invasive *E. coli* promote intestinal inflammation and barrier dysfunction through multiple mechanisms. They enhance TLR signaling, promote the release of inflammatory cytokines such as IL-8 and TNF- α , and impair tight junction integrity by altering the levels of proteins including claudins and ZO-1 [71].

Beyond their effects on the epithelial barrier, BEVs contribute to gut homeostasis by shaping the activity of intestinal immune cells. For instance, BEVs derived from EcN (EcN-EVs) promote the polarization of macrophages toward the M1 phenotype, which exhibits antibacterial properties, and reshape their metabolism to defend against pathogen invasion [72]. BEVs released by the commensal bacterium *B. fragilis* also affect immune cells. They drive dendritic cells toward an immature and immunosuppressive phenotype, which promotes immune tolerance to commensal bacteria and dietary antigens and helps maintain gut immune homeostasis [73]. Although BEVs derived from beneficial bacteria generally support immune homeostasis, those released by pathogenic bacteria are more commonly associated with inflammatory activation. Notably, the magnitude and nature of these effects depend largely on the intestinal microenvironment. In an in vitro non-inflammatory setting, BEVs derived from *Fusobacterium nucleatum* can activate the innate immune response in intestinal epithelial cells via the TLR2-dependent NF- κ B pathway. No barrier damage is observed under these conditions [74]. Antibiotic pretreatment changes the response to these vesicles. In mice with depleted gut microbiota, the same BEVs disrupt colonic structure, reduce the mucus layer, and increase TLR4-dependent pro-inflammatory cytokine production. These changes do not occur in mice with an intact gut microbiota [75]. The studies described above indicate that the immune effects of BEVs depend on both their cargo and the gut microbiota. The same BEVs can produce different biological responses under different microbiota conditions. More studies using complex in vivo models are still needed to clarify these effects.

The regulation of the gut microbiome by BEVs is also an important aspect of maintaining host-microbial symbiosis. *Bacteroides* species can utilize BEVs to export glycoside hydrolases, such as the alpha-amylase SusG, which are encoded by polysaccharide utilization loci. These enzymes degrade complex polysaccharides into metabolites that can be utilized by other bacterial species. This

BEV-mediated resource-sharing mechanism facilitates cross-feeding among microorganisms with diverse metabolic phenotypes [76, 77]. Akk-EVs selectively fuse with the membranes of beneficial bacteria, including members of the *Bacteroides* genus. This interaction promotes the growth of these bacteria and reduces the relative abundance of opportunistic pathogens, including members of the *Proteobacteria* phylum. As a result, the richness and diversity of the gut microbiota increase, and sodium dextran sulfate-induced dysbiosis is alleviated [18]. BEVs derived from *E. coli* also shape the gut microenvironment. These vesicles consume oxygen in the gut and restore anaerobic conditions, which suppresses the growth of facultative anaerobic pathogens in the *Enterobacteriaceae* family [78]. Collectively, these findings indicate that BEVs from gut microorganisms have the potential to positively regulate microbial communication, nutrient allocation, and environmental regulation, providing essential support for the maintenance of microbiota balance.

Gut microbiota derived BEVs need to traverse the intestinal epithelial barrier and reach the circulation to influence distant host tissues. BEVs primarily utilize two pathways for this crossing: the paracellular pathway (through intercellular gaps) and the transcellular pathway (through the interior of cells). In the transcellular pathway, BEVs can be internalized by intestinal epithelial cells via various mechanisms, including macropinocytosis and endocytosis mediated by laminins or caveolins [79]. Studies of BEVs from enterohemorrhagic *E. coli* O157 show that these vesicles cross polarized Caco-2 monolayers through both paracellular and transcellular pathways. During paracellular transport, BEVs cause a temporary redistribution of the tight junction protein ZO-1. This change reduces trans-epithelial resistance and briefly increases paracellular permeability. Transcellular transport follows a different route. BEVs enter epithelial cells mainly through kinesin-dependent endocytosis, with a smaller contribution from clathrin-mediated endocytosis. They are then released from the basolateral side to complete epithelial transport [80]. One study injected fluorescently labeled engineered EcN-EVs into the colonic lumen of healthy mice. 8 h after administration, fluorescent signals were detected on the basolateral side of the colon, as well as in the liver and kidneys [81]. This confirms that BEVs can cross the intestinal barrier even under healthy conditions.

The studies discussed above show that BEVs regulate several signaling pathways in the gut, including NF- κ B, AMPK, the AIM2 inflammasome, and RIPK1. These effects involve the intestinal barrier, mucosal immunity, and gut microbiota homeostasis. BEVs can also enter the circulation and carry bioactive molecules, such as LPS, bacterial DNA, enzymes, and metabolites, to peripheral

organs, including the liver, pancreatic islets, adipose tissue and bone. This transport links local gut signals with systemic metabolic regulation. The following sections describe the protective and pathogenic roles of BEVs in metabolic disorders.

Common mechanisms of BEV–host interactions in metabolic diseases

BEVs derived from the gut microbiota function as nanoscale signaling carriers that facilitate cross-organ communication with the host in metabolic diseases. They achieve this by traversing biological barriers, delivering diverse cargo, and activating host-specific signaling pathways. This interaction exhibits several common characteristics across various metabolic diseases.

First, impairment of the intestinal barrier is an essential condition that allows pathogenic BEVs to exert effects on distant organs. Metabolic disorders such as obesity, MASLD, and diabetes are often accompanied by decreased levels of intestinal tight junction proteins, including occludin, ZO-1, and claudin-5, which compromises epithelial barrier integrity and increases mucosal permeability. This alteration allows BEVs carrying LPS, microbial DNA, and other bioactive molecules to escape the intestinal barrier and enter the bloodstream [66, 82, 83]. In MASLD, BEVs isolated from patient fecal samples contribute to intestinal barrier dysfunction through activation of the nmMLCK pathway. This process decreases the levels of tight junction proteins, resulting in disruption of barrier integrity. Simultaneously, these BEVs can increase vascular endothelial permeability and exacerbate inflammation via TLR4 signaling, further promoting the migration of BEVs from the vasculature to peripheral tissues [83] (Fig. 3).

Once in circulation, BEVs are subject to clearance by the hepatic immune system. Liver-resident complement receptor of the immunoglobulin superfamily-positive (CRIg⁺) macrophages (also known as Vsrg⁺ macrophages) efficiently mediate the capture and phagocytic clearance of circulating pathogens in the liver by recognizing and binding to complement opsonins iC3b and C3b deposited on pathogen surfaces via their CRIg receptor [84] (Fig. 1). This characteristic positions CRIg⁺ macrophages as crucial players in clearing complement-opsonized BEVs. Under conditions of obesity, the reduction in this cell population leads to the abnormal accumulation of BEVs in circulation and peripheral tissues, further exacerbating local and systemic inflammation and insulin resistance [85, 86]. In MASLD, loss of hepatic CRIg⁺ macrophages reduces the ability of Kupffer cells to remove these vesicles. As a result, BEVs

accumulate within the liver, which further promotes hepatic steatosis and fibrosis [87].

BEVs can enter the circulation and deliver microbial DNA to host tissues. This DNA activates the cyclic GMP-AMP synthase/stimulator of interferon genes (cGAS/STING) pathway and contributes to the development of metabolic disorders. In the liver, adipose tissue, and skeletal muscle, activation of this pathway promotes local inflammation and increases pro-inflammatory macrophage infiltration. These changes reduce protein kinase B (AKT) phosphorylation, disrupt insulin signaling, and impair insulin sensitivity in these tissues [86]. In the pancreas, BEVs taken up by pancreatic β -cells trigger local inflammation via the cGAS/STING pathway, characterized by CD11c⁺ macrophage infiltration and upregulation of IL-1 β expression. This process decreases the expression of Insulin and glucose transporter 2, which weakens glucose-stimulated insulin secretion and ultimately disrupts normal β -cell function [85]. Microbial DNA-containing BEVs also act on liver cells. By activating the same signaling pathway in hepatocytes and hepatic stellate cells, they induce hepatic inflammation and drive the progression of fibrosis. When the clearance function of CRIg⁺ macrophages in the liver is impaired, these vesicles accumulate abnormally, further exacerbating the degree of steatosis and fibrosis [87] (Figs. 2, 3 and 4). Notably, STING, as the central node of the cGAS/STING pathway, has multiple downstream signaling branches. BEVs derived from specific bacterial strains, such as those from *B. fragilis*, can also induce macrophage polarization toward a pro-calcification M2 phenotype via the STING-Mef2d pathway, further promoting diabetes-related vascular damage [88].

LPS is a significant pathogenic component of pathological BEVs. In the intestines of mice with diet-induced obesity (DIO) from a high-fat diet, the proportion of LPS-carrying BEVs (LPS⁺ BEVs) is elevated. These LPS⁺ BEVs abnormally accumulate in the portal vein and liver tissue. They stimulate TLR4 signaling in hepatic tissue, leading to elevated expression of inflammatory factors such as Cxcl10. This response also promotes the accumulation and activation of hepatic macrophages, which further drives chronic inflammation and immune imbalance in the liver [82]. Patients with MASLD also exhibit an increase in LPS content within fecal-derived BEVs. Once inside the liver, these BEVs can directly activate stellate cells, inducing the expression of pro-fibrotic and pro-inflammatory factors, such as α -SMA and IL-1 β , thereby driving the systemic progression of hepatic inflammation and fibrosis [83] (Figs. 2 and 3). In diabetic nephropathy, LPS carried by BEVs can activate the caspase-11 pathway

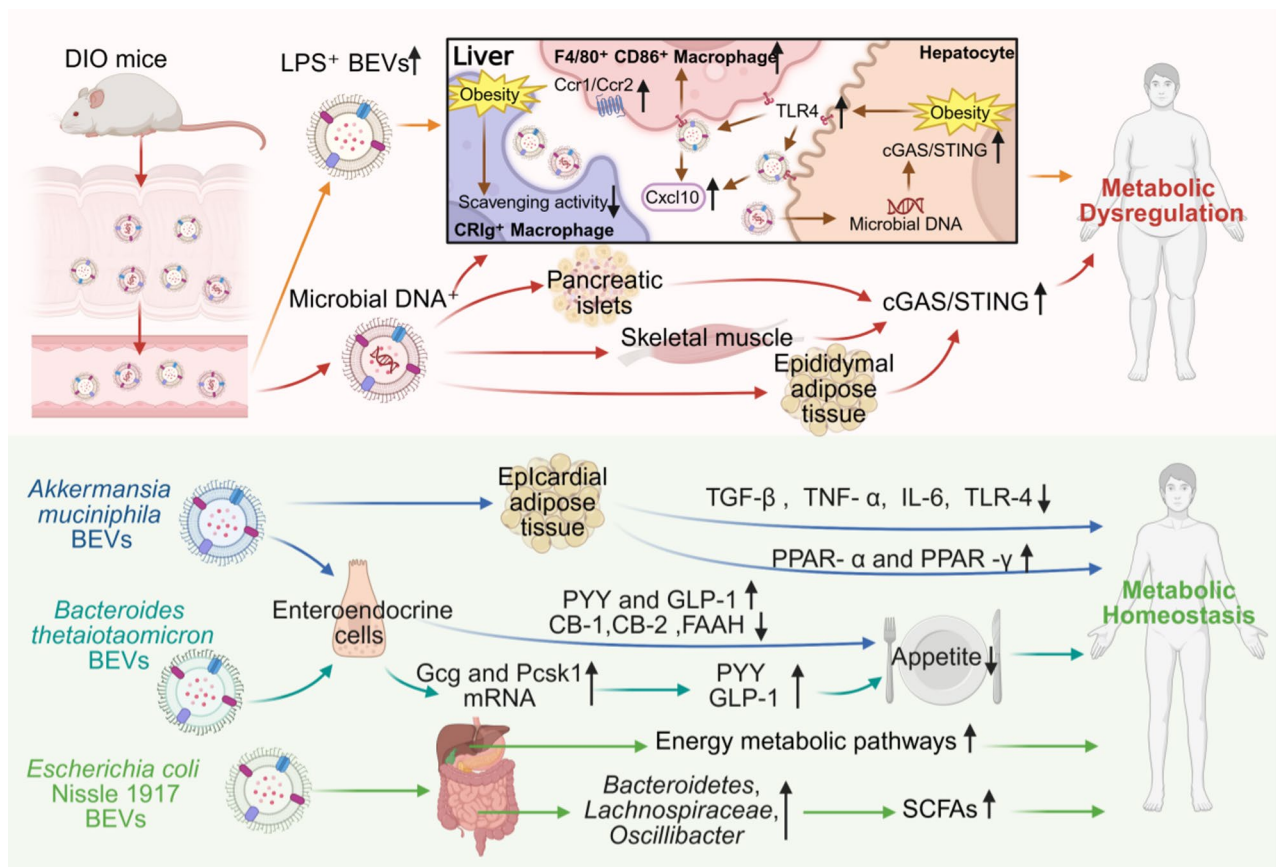


Fig. 2 Mechanisms of BEVs from Different Sources in Obesity. In the DIO mouse model, BEVs produced by the gut microbiota are capable of transporting LPS and microbial DNA into the circulatory system. These detrimental BEVs specifically target the liver, pancreatic islets, skeletal muscle, and adipose tissue, leading to the disruption of immune homeostasis and the onset of metabolic disorders and chronic inflammation. Conversely, beneficial BEVs derived from *Akkermansia muciniphila*, *Bacteroides thetaiotaomicron*, and *Escherichia coli* Nissle 1917 confer protective effects by ameliorating obesity and associated inflammation through various mechanisms. Abbreviations: BEVs, Bacterial extracellular vesicles; DIO, diet-induced obesity; LPS, lipopolysaccharides; SCFAs, Short-Chain Fatty Acids; CRIg, complement receptor of the immunoglobulin superfamily; TLR, toll-like receptor; cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; Ccr, C-C motif chemokine receptor; Cxl10, C-X-C motif chemokine ligand 10; TNF- α , tumor necrosis factor- α ; TGF- β , transforming growth factor- β ; IL-6, interleukin-6; PPAR, peroxisome proliferator-activated receptor; PYY, peptide YY; GLP-1, glucagon-like peptide-1; CB-1, cannabinoid receptor 1; CB-2, cannabinoid receptor 2; FAAH, fatty acid amide hydrolase

in renal tubular epithelial cells, exacerbating local inflammation [89]. In contrast, BEVs released by probiotic bacteria, including Akk-EVs, help reduce inflammatory responses triggered by LPS [90].

The studies discussed above show that gut microbiota-derived BEVs mediate communication between the gut and peripheral organs. They cross the intestinal barrier, avoid immune clearance, and transport bioactive molecules such as microbial DNA and LPS. These molecules activate signaling pathways, including cGAS/STING and TLR4. As a result, BEVs regulate inflammation, immune responses, and metabolic homeostasis in different tissues. Current evidence supports an important role for BEVs in the development of several metabolic diseases, and the following sections review recent studies on their roles in obesity, MASLD, atherosclerosis, type 2 diabetes, and osteoporosis.

Research progress on gut BEVs in specific metabolic diseases

Diseases associated with lipid metabolic disorders

Obesity

BEVs play a complicated and pivotal role in the development and progression of obesity, with mechanisms that involve precise regulation of metabolic inflammation, tissue function, and systemic energy homeostasis [19, 91]. Obesity-related gut microbiota dysbiosis changes the production, composition, and distribution of gut-derived BEVs. These changes activate metabolic inflammation through the gut-liver-adipose axis and promote the progression of obesity and its complications.

In disease states, LPS⁺ BEVs and microbial DNA-containing vesicles promote hepatic inflammation, systemic metabolic disturbances, and insulin resistance across hepatic and peripheral tissues. This occurs through shared mechanisms discussed in Sect. "Common

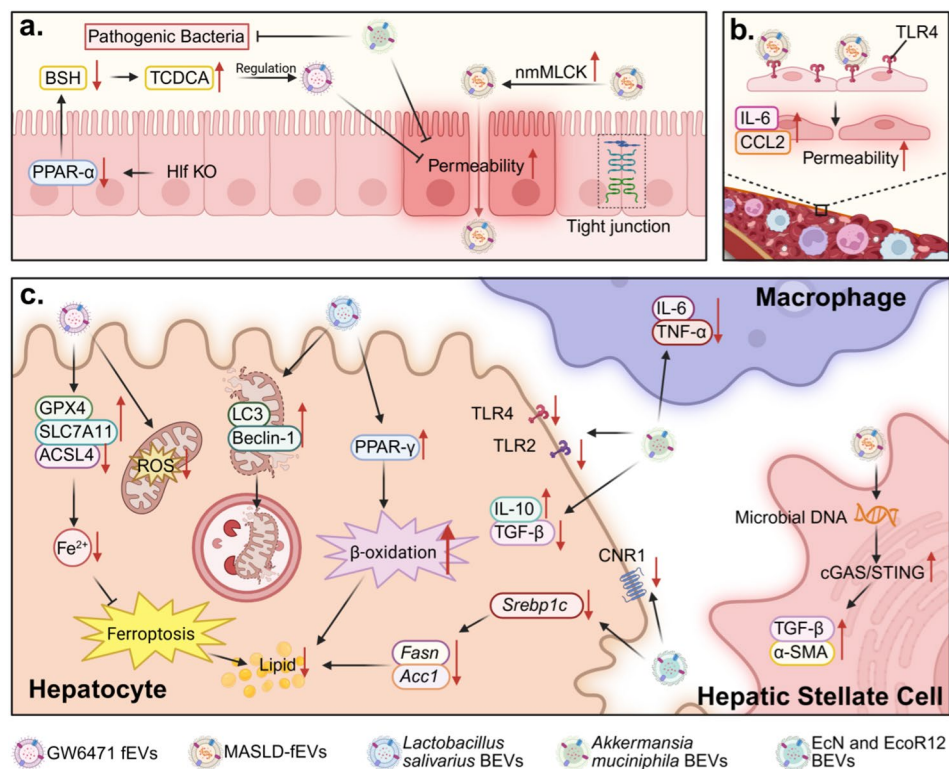


Fig. 3 Mechanisms by which BEVs from various sources contribute to MASLD pathogenesis. **a.** BEVs derived from gut microbiota compromise tight junctions in intestinal epithelial cells, thereby breaching the intestinal barrier and facilitating their entry into the bloodstream. **b.** Upon entering circulation, BEVs activate TLR4, which subsequently triggers an inflammatory response in vascular endothelial cells, further compromising the endothelial barrier. **c.** In the liver, BEVs from diverse origins exert effects on hepatocytes, macrophages, and hepatic stellate cells. Abbreviations: BEVs, Bacterial extracellular vesicles; fEVs, fecal extracellular vesicles; EcN, *Escherichia coli* Nissle 1917; EcoR12, *Escherichia coli* O12:H12; PPAR, peroxisome proliferator-activated receptor; nmMLCK, non-muscular myosin light chain kinase; TCDCa, taurochenodeoxycholic acid; HLF, hepatic leukemia factor; BSH, bile salt hydrolases; TLR, toll-like receptor; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; IL-10, interleukin-10; CCL2, C-C motif chemokine ligand 2; SLC7A11, solute carrier family 7 member 11; GPX4, glutathione peroxidase 4; ACSL4, acyl-CoA synthetase long-chain family member 4; Fasn, fatty acid synthase; Srebp1c, sterol regulatory element-binding protein 1c; Acc1, acetyl-CoA carboxylase 1; α-SMA, α-smooth muscle actin; LC3, microtubule-associated protein 1 A/B-light chain 3; ROS, reactive oxygen species; CNR1, cannabinoid receptor 1

mechanisms of BEV–host interactions in metabolic diseases".

BEVs originating from probiotic and commensal bacteria are generally associated with favorable effects on obesity and related metabolic diseases. Among these, Akk-EVs have been extensively validated for their ability to improve obesity phenotypes and inflammatory states through multiple mechanisms. They reduce gut-derived inflammation by enhancing intestinal barrier function and maintaining mucosal immune homeostasis, while simultaneously suppressing inflammation in adipose tissue and promoting peroxisome proliferator-activated receptor (PPAR)-α/γ-mediated fatty acid oxidation, thereby improving obesity-related metabolic disorders [92]. Additional studies indicate that both inactivated *A. muciniphila* cells and Akk-EVs alleviate obesity-related metabolic disorders by reducing lipid accumulation through the downregulation of genes associated with lipogenesis in the liver, such as *Pparg* and lipoprotein lipase (*Lpl*) [93]. In vitro experimental models confirm

that *A. muciniphila* and Akk-EVs can influence the secretin tumor cell line-1 (STC-1), a mouse intestinal neuro-endocrine cell line, by upregulating the expression of intestinal endocrine hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). This regulation impacts appetite and energy metabolism, inhibits excessive activation of the endogenous cannabinoid system, and improves metabolic inflammatory states [94] (Fig. 2). Despite these encouraging experimental findings, evidence from clinical studies remains limited. A randomized controlled trial involving obese or overweight patients with T2DM revealed that the metabolic benefits of *A. muciniphila* supplementation were highly dependent on individual baseline gut levels of this bacterium. Improvements in body weight, body fat, and glycated hemoglobin were observed only in participants with low baseline levels [95]. These results suggest that patient selection may influence treatment efficacy. Future studies should examine the host factors that influence treatment response, such as genetic background, dietary habits, and

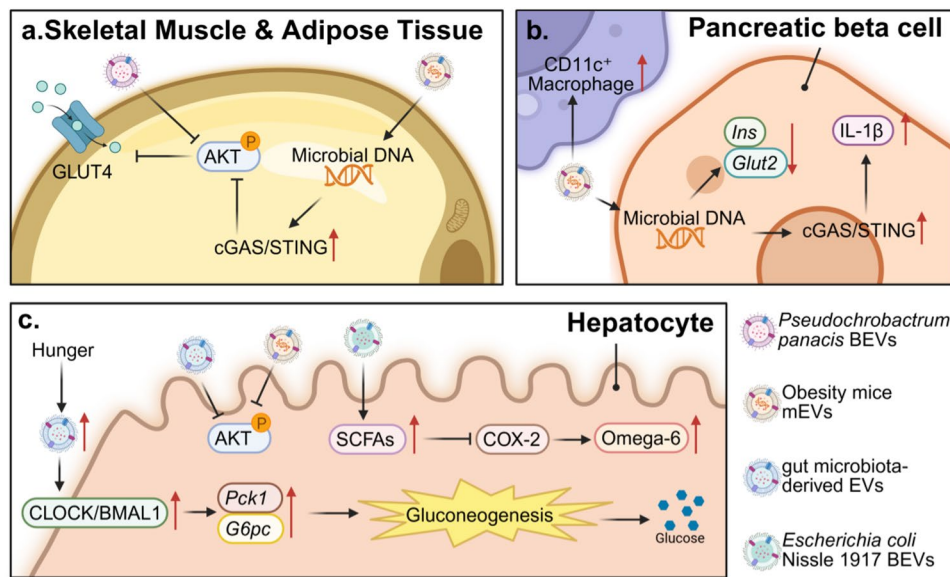


Fig. 4 Mechanisms by which BEVs from different sources contribute to diabetes and insulin resistance. **a.** BEVs from different sources act on skeletal muscle and adipose tissue. **b.** BEVs act on the pancreas, inducing metabolic abnormalities via activation of the cGAS-STING pathway. **c.** BEVs from different sources act on the liver, regulating glucose metabolism by modulating pathways such as gluconeogenesis. Abbreviations: BEVs, Bacterial extracellular vesicles; mEVs, microbial DNA-containing extracellular vesicles; SCFAs, Short-Chain Fatty Acids; AKT, protein kinase B; GLUT4, glucose transporter type 4; GLUT2, glucose transporter type 2; Ins, insulin; G6pc, glucose-6-phosphatase catalytic subunit; cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; SCFAs, short-chain fatty acids; IL-1 β , interleukin-1 beta; COX-2, cyclooxygenase-2; Pck1, phosphoenolpyruvate carboxykinase 1

disease severity. They should also evaluate the optimal administration route, dose, and long-term safety of Akk-EVs before clinical application.

BEVs from other commensal and probiotic bacteria also regulate metabolism through different mechanisms. In vitro experiments demonstrate that derivatives of *B. thetaiotaomicron*, including BEVs, can upregulate *Gcg* and *Pcsk1* mRNA expression in STC-1 cell lines, thereby increasing the secretion of PYY and GLP-1. This mechanism enhances satiety hormone signaling, aiding appetite suppression and improving energy homeostasis [96]. EcN-EVs also show beneficial metabolic effects in obesity and diabetes models. They can alter the gut microbial community and increase the production of short-chain fatty acids (SCFAs), which is associated with reduced hepatic COX-2 expression and decreased metabolism of omega-6 unsaturated fatty acids. In addition, EcN-EVs improve energy metabolism in the liver by modulating multiple metabolic pathways, including oxidative phosphorylation and the tricarboxylic acid cycle, which reduces hepatic steatosis [97] (Fig. 2).

Overall, gut microbiota-derived BEVs exert both detrimental and protective effects in obesity, depending on their microbial source and cargo composition.

MASLD

Recent studies have highlighted the role of gut-derived BEVs in gut-liver communication and MASLD development [98–100]. In the progression of MASLD, LPS+

BEVs and BEVs enriched with microbial DNA transmit pathogenic signals between the gut and the liver. Section "Common mechanisms of BEV–host interactions in metabolic diseases" describes these pathogenic mechanisms in detail.

In contrast, BEVs released by probiotic and commensal bacteria can protect against MASLD. Among them, *A. muciniphila* and Akk-EVs have been studied most extensively [90, 93, 101]. Animal studies showed that treatment with live *A. muciniphila* or Akk-EVs protected mice from HFD-induced MASLD by reducing hepatic lipid accumulation and inflammatory cell infiltration [93]. Furthermore, mechanistic investigations reveal that Akk-EVs demonstrate remarkable structural stability in simulated gastrointestinal environments and can dose-dependently suppress LPS-induced macrophage inflammatory responses, thereby reducing the expression of key pro-inflammatory factors, including IL-6 and TNF- α [90]. These properties suggest that Akk-EVs may be useful as oral delivery vehicles through the gut-liver axis. Akk-EVs also reduce liver fibrosis in an HFD/CCl4 model. They not only enhance intestinal barrier function but also suppress TGF- β -related fibrotic signaling and the activation of hepatic stellate cells. This is achieved through the downregulation of the inflammatory receptors TLR-2 and TLR-4, which leads to a reduction in inflammatory responses and extracellular matrix deposition, ultimately slowing the progression of liver fibrosis

[101]. These studies highlight the therapeutic potential of *A. muciniphila* and Akk-EVs in MASLD (Fig. 3).

Protective effects have also been reported for BEVs derived from other probiotic bacteria. BEVs released by *L. salivarius* act on both the liver and the gut. In the liver, they activate the Beclin-1-dependent mitochondrial autophagy pathway, which promotes the clearance of damaged mitochondria and enhances hepatocyte metabolic homeostasis. These BEVs also regulate hepatic lipid metabolism. They increase the expression of *Ppara* and *Pparg* to promote fatty acid oxidation while reducing fatty acid synthase (*Fasn*) expression to limit lipid synthesis, improving the balance between lipid synthesis and degradation. In the gut, *L. salivarius*-derived BEVs reshape the gut microbiota. They increase the abundance of beneficial *Bacteroides* and reduce potentially harmful *Desulfovibrio*. These changes improve intestinal barrier function and reduce intestinal inflammation [102] (Fig. 3). Similar effects have also been observed for BEVs from the non-pathogenic *E. coli* strains EcN and *E. coli* O12:H12. In healthy newborn rats, they reduced intestinal permeability, improved hepatic antioxidant capacity, and regulated the expression of genes involved in hepatic lipid metabolism. These BEVs upregulate genes associated with fatty acid oxidation, such as *PPARα* and carnitine palmitoyltransferase 1 A (*Cpt1a*), while down-regulating the activity of genes linked to lipid synthesis, including sterol regulatory element-binding protein 1c (*Srebp1c*), acetyl-CoA carboxylase 1 (*Acc1*), and *Fasn* [103]. Some commensal BEVs protect the liver by delivering metabolites. BEVs that carry taurochenodeoxycholic acid (TCDCA) can inhibit oxidative stress and ferroptosis in hepatocytes while enhancing intestinal barrier function, thereby slowing the progression of MASLD. Loss of hepatic leukemia factor (HLF) in the intestinal HLF/PPARα signaling axis increases the TCDCA content of BEVs, further enhancing their ability to suppress hepatocyte ferroptosis [104] (Fig. 3).

Overall, current studies support both harmful and protective roles of gut microbiota-derived BEVs in MASLD. Pathogenic BEVs promote disease progression by delivering inflammatory cargos. Beneficial BEVs help maintain gut-liver homeostasis through multiple mechanisms, including improved barrier function, regulation of lipid metabolism, and reduced inflammation.

Atherosclerosis

Atherosclerosis is a metabolism-related disease characterized by lipid deposition, chronic inflammation, and vascular wall remodeling. Its onset and progression are closely associated with metabolic abnormalities, including obesity, MASLD, type 2 diabetes, and metabolic syndrome [105–107]. Growing evidence also links gut microbiota dysbiosis to atherosclerosis [108]. Clinical

studies have revealed detectable bacterial DNA in the plaques of certain patients with carotid atherosclerosis, alongside clear changes in their gut microbiota composition [109]. These findings suggest that gut bacteria or their products can cross the intestinal barrier and reach the vascular wall. Gut microbiota also influence atherosclerosis through microbial metabolites. For example, TMAO, produced from dietary choline by gut microbiota, promotes the upregulation of macrophage scavenger receptors, foam cell formation, and the progression of atherosclerosis [110]. Recent studies further reveal that the microbially derived metabolite imidazole propionate (Imp) activates the imidazoline-1 receptor (IIR) in myeloid cells, inducing atherosclerosis in mice while maintaining normal lipid levels [111]. The evidence presented indicates that gut-derived bacterial components possess the biological capacity to cross the intestinal barrier, reach the vascular wall, and participate in plaque formation. In this context, gut microbiota-derived BEVs have garnered increasing attention as a class of nanocarriers capable of transporting nucleic acids, proteins, and metabolites while penetrating biological barriers [112].

The protective effects of probiotic BEVs in atherosclerosis have been experimentally validated, suggesting a promising avenue for targeted interventions. BEVs isolated from *Lactobacillus rhamnosus* are preferentially taken up by foam macrophages. After uptake, these BEVs increase the expression of nuclear receptor subfamily 1 group H member 3 (*Nr1h3*), which then upregulates ATP Binding Cassette Subfamily A Member 1 (*Abca1*) and promotes intracellular lipid efflux. They also facilitate macrophage polarization toward the anti-inflammatory M2 phenotype while reducing the M1 phenotype. This change contributes to improvements in both lipid metabolism and inflammatory status. In apolipoprotein E knockout (ApoE^{-/-}) mice, treatment with these BEVs reduced aortic plaque size and alleviated vascular lesions [113].

To date, investigations of BEV-mediated changes in vascular endothelial function and coagulation have mainly been carried out in acute inflammatory settings, especially in sepsis models [114–117]. However, there are relatively few studies addressing the atherosclerosis-specific pathological microenvironment. Disruption of vascular endothelial function and imbalance in the coagulation system are critical components in the development and progression of atherosclerosis. Therefore, there is an urgent need to elucidate the precise molecular mechanisms by which intestinal BEVs regulate vascular endothelial homeostasis and coagulation system activity within atherosclerosis-specific models, as well as to clarify their roles in critical pathological processes such as plaque rupture and thrombosis. This research will provide theoretical support and identify novel intervention

targets for advancing prevention and treatment strategies against atherosclerosis and its associated cardiovascular complications, including myocardial infarction and ischemic stroke.

Disorders associated with impaired glucose metabolism *Insulin resistance and T2DM*

Gut microbiota-derived BEVs are now considered important contributors to diabetes and insulin resistance. They participate in the communication between the gut environment and the mechanisms that maintain metabolic balance in the host [91, 118].

As discussed in Sect. "Common mechanisms of BEV–host interactions in metabolic diseases", pathological conditions allow microbial DNA-containing BEVs to activate the cGAS/STING signaling pathway. Activation of this pathway promotes inflammation and insulin resistance in adipose tissue, the liver, and pancreatic β -cells. Furthermore, BEVs derived from the feces of HFD mice, particularly those from *Pseudomonas panacis*, have been shown to specifically inhibit AKT phosphorylation and block glucose transporter type 4 (GLUT4) transport in skeletal muscle and adipose tissue, thereby directly inducing insulin resistance [119] (Fig. 4). Together, these studies suggest that BEVs carrying different cargos affect multiple metabolic organs through different mechanisms, which ultimately contributes to insulin resistance.

BEV production and function change with the physiological state of the host. For instance, BEV production follows a circadian rhythm and increases during fasting, when energy demand is high. These BEVs can be specifically taken up by the liver, where they activate the circadian clock pathway (CLOCK/BMAL1) within hepatocytes. This activation directly upregulates key gluconeogenesis genes, such as glucose-6-phosphatase catalytic subunit (*G6pc*), thereby enhancing the liver's capacity for glucose production to meet energy challenges [120]. Host metabolism can also influence the composition and function of BEVs. For example, Metformin reshapes the microbial-derived BEV community structure in plasma from patients with polycystic ovary syndrome with insulin resistance (PCOS-IR), increasing its diversity and elevating the abundance of BEVs derived from *Streptococcus salivarius*. This suggests that the drug may improve insulin sensitivity by modulating BEV-mediated gut-metabolic axis signaling [121]. Probiotic BEVs also show metabolic benefits. EcN-EVs improve obesity and diabetes phenotypes by reshaping the gut microbiota and restoring gut-liver metabolic homeostasis [97]. Overall, BEVs respond to changes in host metabolism and therapeutic intervention. This feature makes them potential targets for future metabolic disease therapies (Fig. 4).

Diabetes complications

Chronic diabetic complications involve metabolic disorders, persistent inflammation, and tissue damage in multiple organs. Increasing evidence suggests that gut microbiota-derived BEVs contribute to the development of these complications. In terms of pathogenic mechanisms, these pathological BEVs can cross the impaired intestinal barrier into the circulation, targeting specific peripheral organs to induce damage. In diabetic kidney disease (DKD), gut dysbiosis increases BEV production. These vesicles migrate to the kidneys, where they are internalized by renal tubular epithelial cells via multiple endocytic pathways. The LPS they carry activate the caspase-11 inflammatory signaling pathway within these cells, upregulating the expression of pro-inflammatory factors such as IL-1 β , TNF- α , CCL2, and IL-6. This response promotes tubulointerstitial inflammation and accelerates DKD progression [89]. *B. fragilis*-derived BEVs contribute to the development of diabetic vascular calcification. These vesicles contain double-stranded DNA and activate the STING-myocyte enhancer factor 2D (Mef2d) signaling pathway, which promotes macrophage polarization toward the pro-calcification M2 phenotype and increases Serpine1 expression. Together, these changes accelerate vascular calcification in T2DM [88] (Fig. 5).

BEVs in diabetic complications do not always exert harmful effects, and their functions depend on the bacterial source and host regulation. Studies indicate that BEVs derived from *Lactobacillus reuteri* (LR-BEVs) are enriched with endogenous miR-21a-5p. In diabetic wound models, LR-BEVs, when delivered via subcutaneous injection, are taken up by skin cells. The transferred miR-21a-5p activates the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway. Activation of this pathway enhances angiogenesis and re-epithelialization while limiting inflammatory responses, which helps improve the healing of diabetic wounds [122]. Furthermore, benefits can be achieved through dietary interventions that reshape the microbiota. For instance, intermittent fasting can remodel the gut microbiota by enriching Christensenellaceae, prompting this bacterium to release functional BEVs rich in miR-551b. These BEVs promote osteoblast activity, playing a crucial role in improving diabetic osteoporosis [64] (Fig. 5).

Gut microbiota dysbiosis in diabetes is associated with abnormal production and dissemination of pathological BEVs. These BEVs contribute to complications in distant organs, such as the kidneys and blood vessels. However, the mechanisms of BEVs in diabetic retinopathy and autonomic neuropathy, including gastroparesis and cardiovascular autonomic dysfunction, are still not well understood. Future research is needed to address these gaps and clarify the role of BEVs in systemic diabetic

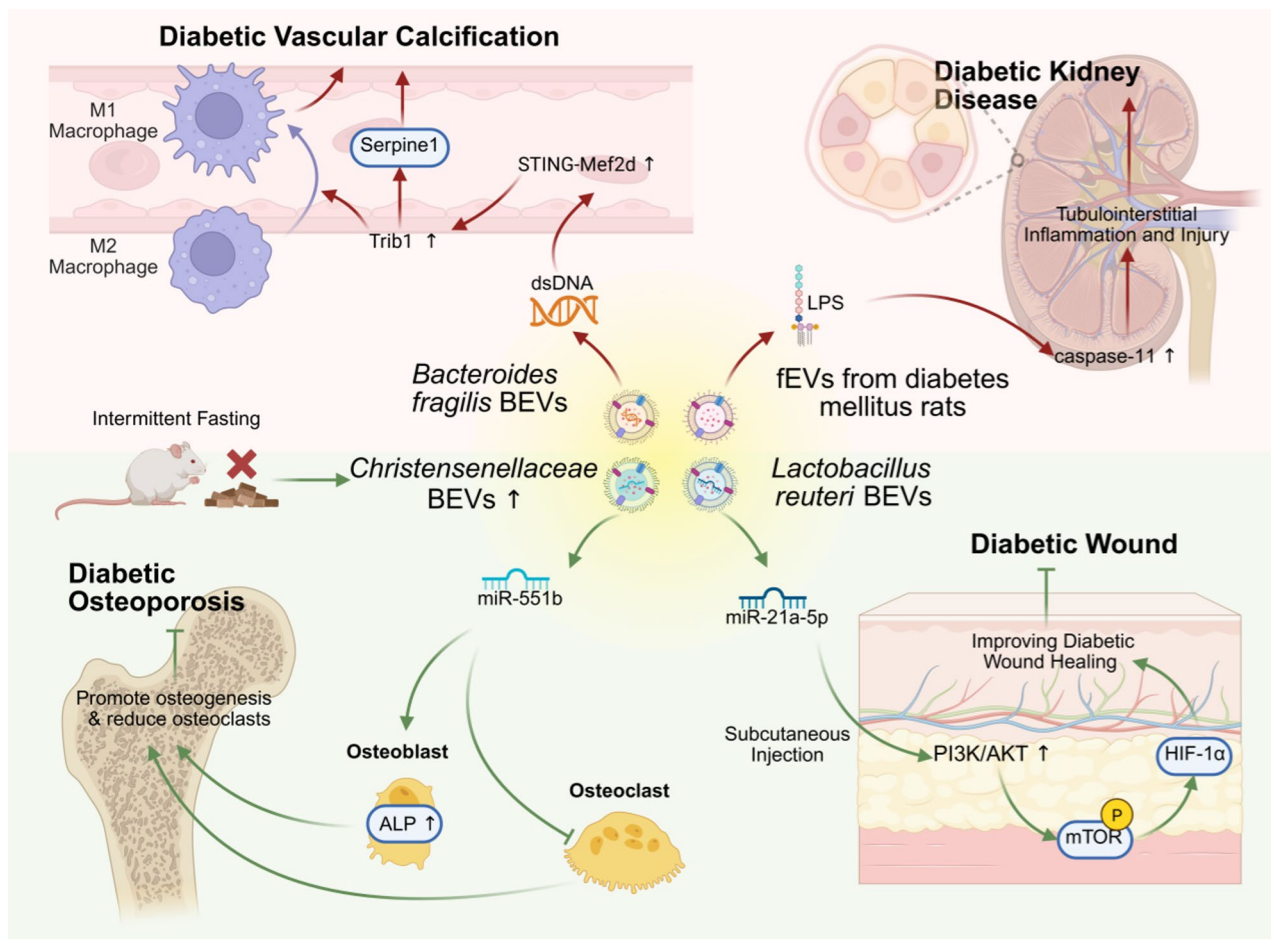


Fig. 5 Mechanisms of BEVs from different sources in diabetic complications. BEVs derived from *Bacteroides fragilis* exacerbate vascular calcification in diabetes by activating the STING-Mef2d signaling pathway. Additionally, LPS carried by EVs from the feces of diabetic rats accelerates the progression of diabetic nephropathy by activating the intracellular caspase-11 inflammatory signaling pathway. Furthermore, BEVs from *Christensenellaceae*, which are rich in miR-551b, contribute to the improvement of diabetic osteoporosis. Lastly, BEVs from *Lactobacillus reuteri*, abundant in miR-21a-5p, enhance the repair process of diabetic wounds. Abbreviations: fEVs, fecal extracellular vesicles; BEVs, Bacterial extracellular vesicles; STING, stimulator of interferon genes; Mef2d, myocyte enhancer factor 2D; Trib1, tribbles pseudokinase 1; AKT, protein kinase B; PI3K, phosphoinositide 3-kinase; ALK, alkaline phosphatase; HIF1- α , hypoxia-inducible factor 1-alpha; mTOR, mammalian target of rapamycin

complications in a more complete way, which will help build a basis for more precise intervention strategies.

Bone metabolism-related diseases—osteoporosis

The gut microbiota regulates the gut–bone axis through the secretion of BEVs. This process has become an important focus in osteoporosis research and its related pathogenesis [123, 124]. Studies show that BEVs from different gut bacteria can act directly on bone tissue by delivering functional molecules, participating in the regulation of bone homeostasis in both directions.

On one hand, substantial evidence supports the protective effect of gut-derived BEVs on bone health. In ovariectomy-induced osteoporotic mice, treatment with BEVs isolated from the pediatric gut microbiota, particularly those released by *A. muciniphila*, preserved both bone mass and bone strength. After crossing the intestinal

barrier, these vesicles accumulated in bone tissue, where they promoted osteoblast activity while limiting osteoclast formation, helping restore the balance of bone remodeling [61]. This finding provides direct experimental support for the gut microbiota-BEVs-bone axis. In an androgen-deficient osteoporosis model, a reduction in *L. salivarius* and its BEVs was associated with the progression of osteoporosis. BEVs derived from this bacterium promote the osteogenic differentiation of bone marrow stromal cells and suppress osteoclast generation, thereby exerting protective effects on bone health [63]. A similar osteogenic effect has also been observed for BEVs derived from *Lactobacillus reuteri* and *Lactobacillus paracasei* after their uptake by bone marrow stromal cells. Co-administration of these two types of vesicles produces stronger osteogenic activity than either treatment alone, leading to a further increase in bone formation [125]. An

in-depth exploration of their mechanisms reveals that the benefits of these probiotic BEVs are closely linked to specific signaling molecules they carry. For instance, the bone-protective effects of *Christensenellaceae* enriched by intermittent fasting depend on their secreted BEVs. The miR-551b abundant in these vesicles has been confirmed as a key mediator that promotes osteoblast activity and improves diabetic osteoporosis [64]. However, beyond beneficial bacteria, BEVs derived from certain pathogenic bacteria also exert protective regulation on bone homeostasis. For instance, BEVs from *Proteus mirabilis* suppress osteoclast formation and activity by downregulating miR-96-5p in osteoclasts. This further upregulates ATP binding cassette subfamily A member 1 (Abca1) expression and triggers mitochondria-mediated apoptosis, indicating a promising anti-osteoporotic effect [126] (Fig. 6).

On the other hand, in the context of gut dysbiosis, BEVs derived from certain pathogenic bacteria may disrupt bone homeostasis. Research indicates that metronidazole-induced dysbiosis results in a significant increase in the pathogenic bacterium *Klebsiella variicola*. The BEVs secreted by this bacterium upregulate the expression of IL-1 β and IL-6 in bone tissue, promote the differentiation of bone macrophages into osteoclasts, and ultimately accelerate inflammatory bone loss [65] (Fig. 6).

In summary, BEVs derived from different microbial sources can exert either beneficial or detrimental effects on bone metabolism, depending on the bioactive molecules they carry. These vesicles serve as an important bridge between gut microbiota alterations and bone homeostasis.

Endocrine-reproductive system metabolic abnormalities—polycystic ovary syndrome

PCOS is a multifactorial endocrine and metabolic disorder that is typically marked by excessive androgen production, impaired ovulation, and polycystic ovarian changes. Insulin resistance and persistent low-grade inflammation are also common features of this condition [127]. Gut microbiota and their metabolites contribute to PCOS-related ovarian dysfunction [128, 129] and insulin resistance through various pathways, including the regulation of sex hormone secretion [130] and metabolism [131], and modulation of glucose metabolism [132, 133]. Gut microbial imbalance is commonly observed in individuals with PCOS, characterized by a reduction in SCFA-producing bacteria (e.g., *Lachnospira*) and an enrichment of pro-inflammatory taxa, including *Escherichia/Shigella* and *Bilophila*. This shift may contribute to PCOS pathogenesis [134].

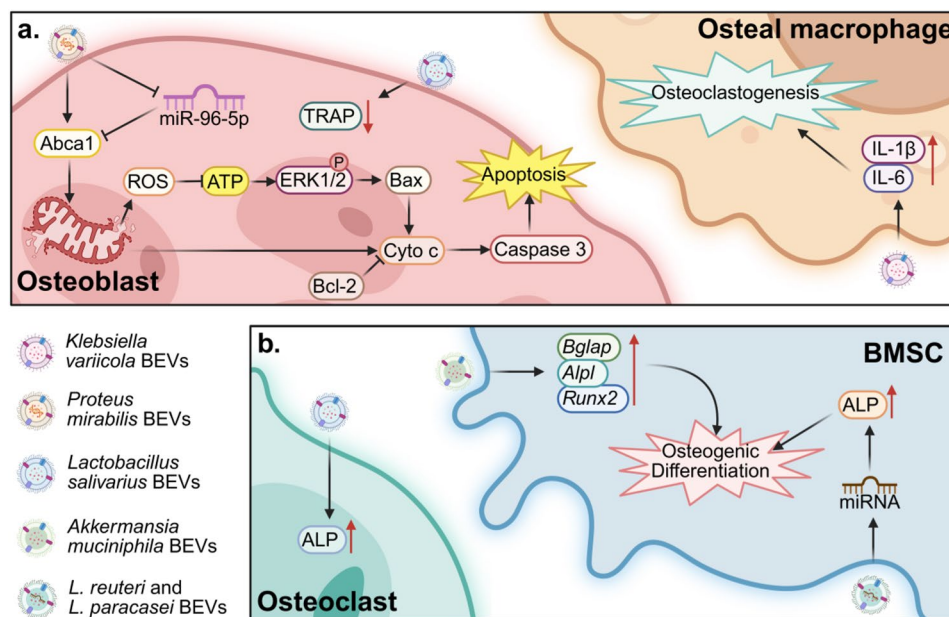


Fig. 6 Mechanisms by which BEVs from different sources regulate bone homeostasis in osteoporosis. BEVs regulate bone homeostasis by acting on key cell types in the bone remodeling network: **a.** In bone resorption, they regulate the differentiation of macrophages into osteoclasts and the activity of mature osteoclasts; **b.** In bone formation, they influence the osteogenic differentiation of bone marrow mesenchymal stem cells and the function of mature osteoblasts. Abbreviations: BEVs, Bacterial extracellular vesicles; BMSC, bone marrow mesenchymal stem cell; *L. reuteri*, *Lactobacillus reuteri*; *L. paracasei*, *Lactobacillus paracasei*; Abca1, ATP binding cassette subfamily A member 1; Cyto c, cytochrome c; ERK, extracellular signal-regulated kinase; Bax, BCL2-associated X protein; Bcl-2, B-cell leukemia/lymphoma 2; TRAP, tartrate-resistant acid phosphatase; Bglap, bone gamma-carboxyglutamate protein; Alpl, alkaline phosphatase, liver/bone/kidney; Runx2, runt-related transcription factor 2; IL-6, interleukin-6; IL-1 β , interleukin-1 beta; ROS, reactive oxygen species

In recent years, BEVs have gained increasing attention for their potential role in PCOS, offering new insights into gut-ovary communication [135]. A recent clinical study examined this link from the BEV perspective and found that metformin treatment for three months increased the microbial diversity of plasma BEVs in PCOS patients with insulin resistance [121]. Specifically, the relative abundance of BEVs derived from *Streptococcus salivarius* increased from 0.03% to 4.33%, suggesting that metformin may improve metabolic status by promoting the generation of probiotic-associated BEVs [121]. This study offers valuable insights into exploring the role of BEVs in the pathogenesis of PCOS and in drug intervention, and it further suggests that the gut-ovary axis may achieve systemic metabolic regulation through BEVs-mediated signaling.

Other metabolic disorders

Metabolic syndrome and hypertension

Metabolic syndrome refers to a group of metabolic abnormalities primarily characterized by insulin resistance, central obesity, dyslipidemia, and hypertension [136–138]. Among these, the mechanisms underlying lipid metabolism and insulin resistance have been discussed in previous sections. Hypertension, as a key phenotype of metabolic syndrome, has a pathogenesis that is closely linked to gut microbiota and the regulatory role of BEVs, which exhibit bidirectional regulatory characteristics in this context.

Recent studies have revealed that specific gut microbiota and BEVs may exert protective effects against hypertension. Akk-EVs have demonstrated significant antihypertensive effects in a spontaneously hypertensive rat model by increasing the proportions of peripheral regulatory T cells (Treg) and suppressing inflammatory responses, thus delaying the progression of hypertension [139]. Further investigations indicate that Akk-EVs upregulate the expression of vasodilatory factors (*At2r*, *Mas1*, *Nos2*) in the kidneys while downregulating vasoconstrictive factors (*Agt* and *At1ar*), thereby maintaining vascular tension balance and exerting antihypertensive effects [140]. However, BEVs derived from intestinal opportunistic pathogens have been shown to drive the development of hypertension. Recent research indicates that BEVs derived from *Klebsiella pneumoniae* suppress the sirtuin 1 (SIRT1) signaling pathway in endothelial cells, leading to a downregulation of phosphorylated endothelial nitric oxide synthase (p-eNOS) while simultaneously upregulating factors such as NADPH oxidase 2 (NOX2), COX-2, endothelin-1 (ET-1), and p53. This dysregulation results in endothelial oxidative stress and dysfunction, ultimately contributing to elevated blood pressure [141]. In contrast, research on obesity-related hypertension indicates that in obese states, gut BEVs

more readily enter the circulation and accumulate microbial DNA in the adrenal medulla. The resulting local inflammation and elevated norepinephrine secretion contribute to higher blood pressure, which is driven in part by reduced CRIg⁺ macrophages and compromised barrier integrity. Restoring these macrophage populations can improve the abnormalities [142]. Overall, gut microbiota and their BEVs appear to regulate blood pressure in a bidirectional manner.

Vascular calcification

Vascular calcification is a common complication of systemic metabolic disorders. This process is characterized by the conversion of vascular smooth muscle cells toward an osteogenic phenotype and the gradual accumulation of calcium deposits within the vascular wall. It occurs more commonly in individuals affected by diabetes and chronic kidney disease. Research indicates that BEVs may play a crucial regulatory role in the development of vascular calcification. For instance, BEVs released by *Lactobacillus rhamnosus* GG have been shown to worsen vascular calcification in a chronic kidney disease model by promoting osteogenic differentiation of vascular smooth muscle cells via the PI3K/AKT signaling pathway [143]. *B. fragilis* and its BEVs activate the STING-Mef2d pathway, which upregulates Trib1 and the pro-calcification factor Serpine1. This leads to M2 polarization of macrophages and further aggravates vascular calcification in patients with type 2 diabetes [88].

Applications of gut microbiota-derived BEVs in the diagnosis of metabolic diseases

Biomarkers are measurable indicators of physiological processes, disease progression, or treatment responses. As key mediators of gut microbiota–host communication, BEVs transport a variety of bioactive molecules from the intestine to distant organs [82], making them promising candidates reflecting the state of the gut microbiota and its systemic effects on the host. On one hand, as vesicles secreted by bacteria, BEVs directly carry and stably transmit crucial molecular information that reflects the functional states of gut microbiota, such as LPS, thereby forming the biological basis for disease detection [144]. On the other hand, these vesicles can enter the bloodstream, where their abundance and specific components (e.g., LPS, lipoteichoic acid) exhibit detectable, disease-specific alterations that are significantly correlated with disease severity or staging. This provides direct and effective clinical evidence for non-invasive diagnosis and disease monitoring [145] (Fig. 7).

In clinical applications for metabolic diseases, the disease-specific features of BEVs have shown promising diagnostic value. In T2DM, for example, BEVs isolated from patient serum, urine, and feces exhibit

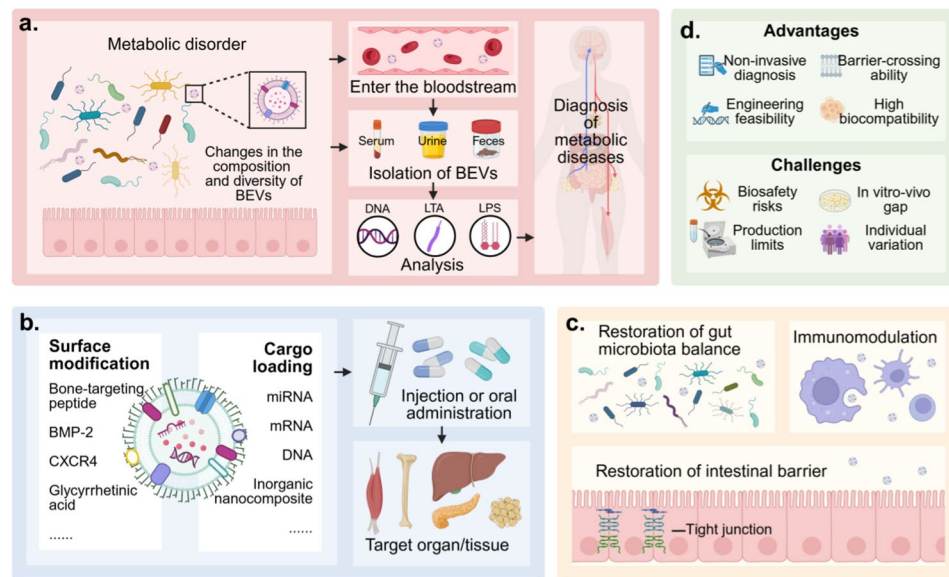


Fig. 7 Diagnostic and therapeutic applications of BEVs in metabolic diseases. **a.** BEVs as biomarkers for disease diagnosis; **b.** Engineered BEVs as drug delivery vehicles for therapeutic applications; **c.** Natural postbiotic functions of BEVs in metabolic disease treatment; **d.** Advantages and challenges in the clinical translation of BEVs. Abbreviations: LTA, lipoteichoic acid; LPS, lipopolysaccharide; BMP-2, bone morphogenetic protein-2; CXCR4, C-X-C chemokine receptor type 4

significantly stronger microbial associations compared with healthy individuals. Such alterations are associated with impaired intestinal barrier function and increased permeability in T2DM, indicating the potential of BEVs as biomarkers for intestinal barrier disruption and systemic inflammation [146]. In gestational diabetes mellitus (GDM), serum BEV microbiomes also display notable shifts, with increased *Firmicutes* and *Actinobacteria* and decreased *Bacteroidetes* and *Proteobacteria*. These alterations appear to correlate with the metabolic disturbances seen in GDM and may help identify high-risk individuals [147]. Furthermore, fEVs show disease-specific compositions across various pathological states. For instance, fEVs from morbidly obese individuals, Crohn's disease patients, and diarrhea sufferers not only display markedly altered metagenomic structures but also increase intestinal epithelial permeability. These findings highlight their potential for early disease screening and for understanding underlying disease mechanisms [148].

Beyond reflecting disease-associated changes in microbial composition, BEVs also show considerable promise because of their strong discriminatory performance. In one study, metadata classification based on BEVs distinguished solid tumors with 100% accuracy, exceeding the 93% accuracy achieved by conventional fecal microbiota analysis [149]. Such results imply that BEVs capture disease-related information with greater specificity and stability than microbial composition alone. Despite this advantage, several barriers remain before BEV-based biomarkers can be implemented in routine clinical settings. Healthy individuals exhibit substantial inter-individual

variation in the microbial composition of fEVs [150]. This heterogeneity may support the development of personalized diagnostic strategies, yet it also complicates the establishment of standardized analytical workflows and broadly applicable clinical criteria Fig. 7.

Therapeutic applications of gut microbiota-derived BEVs in metabolic diseases

Application of BEVs as engineered drug delivery vehicles

BEVs derived from gut microbiota are emerging as a novel drug delivery platform due to their inherent biocompatibility, engineering potential, and capabilities for cross-biological communication [151, 152] (Fig. 7). Experimental evidence supports this application from both mechanistic and functional perspectives.

After being released into the intestinal lumen, BEVs can cross the intestinal barrier and transport bioactive cargo to distant organs, including the liver, brain, and heart. This reveals their potential as natural biological shuttles [20]. Consistent with these observations, BEVs derived from *B. thetaiotaomicron* can be actively taken up by intestinal epithelial cells and subsequently distributed throughout systemic tissues, providing direct evidence of their role as long-range delivery vehicles [153].

Advances in relevant technologies have improved our understanding of BEV biology and expanded the use of BEV-based drug delivery systems. Using genetic engineering together with specific fluorescent probes, researchers established a method that enables real-time visualization and tracking of gut microbiota-derived BEVs under anaerobic conditions. This technique enables

direct visualization of their delivery pathways in the body and demonstrates their therapeutic functions, including antimicrobial effects and barrier repair. Such tools offer strong experimental evidence and technical support for developing BEVs as oral medications [154].

Engineering approaches based on synthetic biology and nanoengineering have transformed BEVs from naturally occurring vesicles into programmable delivery systems with greater therapeutic precision. Current strategies largely center on two objectives: improving tissue targeting and increasing cargo-loading capacity. Surface engineering has received particular attention because the display of specific ligands enables preferential recognition and accumulation within target tissues or cell populations. A representative example is the BEVs-BC system. This system, generated by decorating EcN-EVs with bone morphogenetic protein-2 (BMP-2) and C-X-C motif chemokine receptor 4, integrates bone-targeting capability with osteogenic activity, leading to efficient bone accumulation, enhanced bone formation, and osteoporosis attenuation in vivo [155]. A comparable strategy was applied to BEVs derived from *Lactocaseibacillus paracasei*, where conjugation with the liver-targeting ligand glycyrrhetic acid enabled selective hepatic delivery of fucoxanthin and mitigated the pathological progression of MASLD [156]. Engineering efforts have also focused on expanding the cargo that BEVs can transport. Electroporation enables efficient encapsulation of plasmid DNA, mRNA, miRNA, and other nucleic acids, allowing the engineered vesicles to deliver these molecules into mammalian cells and support functional gene expression [157]. Protein delivery has likewise been demonstrated. BEVs released by the commensal bacterium *B. thetaiotaomicron* successfully transport exogenous proteins and vaccine antigens, highlighting their capacity to function as delivery vehicles in both the respiratory and gastrointestinal tracts [158]. Material engineering has further broadened the potential applications of BEVs. Fusion of *Lactocaseibacillus paracasei*-derived BEVs with MoS₂-ZnO nanocomposites generated biomimetic nanoantibiotics that combine antimicrobial activity with favorable biocompatibility, expanding opportunities for the treatment of infectious diseases [159]. Despite these advances, several obstacles continue to limit clinical translation. Large-scale production remains one of the major challenges for BEV-based therapeutics. Recent work in *B. thetaiotaomicron* identified a family of dual membrane-spanning anti-sigma factors that regulate vesicle biogenesis, providing potential molecular targets for improving vesicle yield through genetic or metabolic engineering [160]. Current targeting strategies also rely heavily on ligand engineering or chemical modification, yet their influence on the intrinsic immunological properties,

biodistribution, and long-term biosafety of BEVs has not been systematically investigated.

At the disease treatment level, engineered BEVs have demonstrated therapeutic efficacy in specific metabolic disease models. In osteoporosis research, in addition to the BEVs-BC system [155], bone-targeting peptide-modified *Lactobacillus rhamnosus* GG extracellular vesicles facilitate targeted delivery of bacterial endogenous microRNAs to bone tissue. This approach promotes osteoblast activity while inhibiting osteoclast differentiation, showcasing superior therapeutic efficacy along with high biosafety [161]. Another study developed a novel targeted delivery system by anchoring bone-targeting peptides to the membrane surface of *Proteus mirabilis* BEVs. This system effectively delivers bioactive molecules to the bone microenvironment and promotes the osteogenic differentiation of bone marrow mesenchymal cells, presenting a new therapeutic strategy for conditions such as osteoporosis [162]. In addition to optimizing carrier targeting, researchers utilized engineered BEVs capable of upregulating host BMP-2 and vascular endothelial growth factor (VEGF). These BEVs were co-loaded with IL-4 into hydrogels, forming a composite material that sequentially modulates the immune microenvironment and promotes bone repair, thereby accelerating osteoporotic fracture healing [163]. In the context of lipid metabolism-related diseases, liver-targeted BEVs derived from *Lactocaseibacillus paracasei* delivered fucoxanthin to exert anti-lipogenic effects. This intervention improved hepatic lipid accumulation and inflammatory responses in MASLD model mice, validating the application potential of engineered extracellular vesicles in hepatic metabolic disorders [156]. In the treatment of hyperuricemia, researchers utilized engineered BEVs constructed from EcN as a novel delivery platform. After loading uricase into the BEVs, oral administration reduced serum uric acid levels in hyperuricemic mice. Compared with conventional protein secretion systems, this approach showed superior efficacy. The enzyme-loaded BEVs also metabolized uric acid efficiently in human serum samples [81].

Overall, research on BEVs derived from gut microbiota as drug carriers is gradually transitioning from the validation of fundamental principles to the exploration of practical applications. With deepening insights into the biogenesis mechanisms and compositional patterns of BEVs [77], along with systematic investigations into their in vivo distribution and immunoregulatory effects [164, 165], the core principles governing BEVs in immune homeostasis regulation, tissue-targeting specificity, and delivery kinetics are gradually becoming clearer.

Despite some advancements in basic research, the clinical translation of engineered BEVs remains in its nascent stages. Most evidence still comes from rodent models,

and clinical studies are lacking. As a result, therapeutic efficacy, pharmacokinetics, and long-term safety remain poorly characterized. Research is also unevenly distributed across metabolic diseases. Most studies focus on bone metabolism, whereas engineered BEVs have been investigated far less in high-burden diseases such as diabetes, obesity, and MASLD. More evidence from these diseases is needed before engineered BEVs can move from experimental studies to clinical application.

Functions and mechanisms of BEVs as natural probiotic sources

In recent years, probiotics and their derived BEVs have gained increasing attention as key effector molecules regulating host health [166]. Compared to traditional live probiotic bacteria, probiotic BEVs exhibit characteristics such as nanoscale particle size, high stability, the ability to traverse mucosal barriers, and precise signal delivery, positioning them as critical mediators of probiotic biological effects [167]. Methodological optimizations have further advanced the isolation and study of probiotic BEVs. For instance, ultracentrifugation facilitates the rapid production of BEVs with higher yields and more uniform particle sizes, thereby providing a reliable experimental foundation for subsequent functional investigations [168]. These discoveries have shifted the paradigm from live-culture intervention to BEV-based microbiome therapy.

Host immune imbalance and chronic low-grade inflammation are key pathological mechanisms in the development of metabolic diseases. BEVs derived from probiotics exert systemic protective effects through immune modulation. In perioperative animal models, BEVs from *Lactobacillus*, *Bifidobacterium*, and *Lactococcus* species migrate to the liver, where they regulate the hepatic immune microenvironment. They reduce the expression of cell adhesion molecules, including intercellular adhesion molecule 2 and vascular cell adhesion molecule 1. As a result, immune cell infiltration decreases and liver tissue recovery is promoted [169]. Furthermore, studies in neonatal and lactating rats indicate that BEVs derived from probiotic and commensal *E. coli* enhance the maturation of the intestinal and immune systems. They elevate plasma levels of IgG, IgA, and IgM while increasing the proportions of cytotoxic T lymphocyte, Natural killer (NK) cell, and NKT cells in the spleen, thereby establishing a foundation for host immune homeostasis [170]. Collectively, these findings demonstrate that probiotic BEVs function not only as local signaling carriers but also as crucial regulators of systemic immune homeostasis.

Intestinal barrier dysfunction serves as a pivotal link connecting disturbances in the intestinal microenvironment to systemic metabolic abnormalities. Its impairment leads to increased intestinal permeability,

facilitating the translocation of endogenous harmful substances (such as certain BEVs) into the bloodstream, which subsequently causes metabolic imbalance. Conversely, certain BEVs derived from probiotics can act as natural prebiotics, exerting beneficial effects by repairing the barrier. BEVs from the probiotic EcN and the commensal ECOR63 can upregulate ZO-1 and claudin-14 while suppressing claudin-2, thereby enhancing intestinal epithelial barrier function [171]. Further studies revealed that EcN BEVs promote intestinal mucosal healing and tight junction reconstruction. They upregulate trefoil factor 3 through TLR2 signaling and downregulate miR-7-5p via the PI3K pathway, highlighting specific molecular mechanisms involved in mucosal repair [172]. In studies of the *Lactobacillus* genus, BEVs derived from *Limosilactobacillus johnsonii* mitigate oxidative stress via activating the nuclear factor erythroid 2-related factor 2/heme oxygenase 1 antioxidant signaling pathway, thereby maintaining intestinal barrier homeostasis and inhibiting inflammatory responses [173]. BEVs released by *Lactobacillus kefirgranum* PRCC-1301 also show protective effects in the intestine. They attenuate inflammation by suppressing NF- κ B signaling and restore intestinal barrier function through the recovery of tight junction proteins, including ZO-1, claudin-1, and occludin [174]. Overall, these studies indicate that probiotic BEVs effectively enhance intestinal epithelial barrier function through different mechanisms, limiting the passage of harmful substances into circulation and highlighting their potential value in metabolic disease prevention and treatment.

Beyond barrier regulation, probiotic BEVs also indirectly enhance metabolic homeostasis by reshaping the balance of the gut microbiome. Studies indicate that BEVs derived from *L. salivarius* and *Lactobacillus muciniferus* directly inhibit the growth and virulence of *Salmonella typhimurium* and *Campylobacter jejuni*. The F3 fraction, which is rich in antibacterial proteins such as lysozyme motifs and peptidoglycan hydrolases, exhibited the strongest inhibitory activity [175]. In contrast, BEVs from *Bacteroides* possess various glycosidases and proteases that decompose complex polysaccharides into public nutrients for commensal bacteria, thereby sustaining gut microbial diversity and metabolic synergy [77]. These findings suggest that probiotic BEVs not only regulate host inflammation and barriers at the molecular level but also reconstruct the intestinal microenvironment at the ecological level. They inhibit pathogen competition and promote the colonization of beneficial bacteria, thereby achieving systemic regulation of microbial balance. However, it is challenging to ascertain whether the alterations in microbiota induced by BEVs are the direct cause of metabolic enhancements or merely a secondary effect stemming from improvements in the host environment.

Furthermore, the majority of studies do not validate their findings using germ-free animals or colonization models, leaving the causal relationship between BEVs and microbiota remodeling yet to be fully clarified.

In summary, probiotic BEVs exhibit precision regulation capabilities that surpass those of traditional live-culture preparations through three synergistic mechanisms: modulating host immunity, repairing the intestinal barrier, and reshaping the microbial ecosystem (Fig. 7). This establishes a solid theoretical and practical foundation for developing next-generation strategies for the prevention and treatment of metabolic diseases based on natural probiotic sources.

Challenges and limitations of BEVs in clinical translation

Although BEVs derived from the gut microbiota have demonstrated significant potential in disease diagnosis and treatment, numerous challenges remain to be addressed before their implementation in clinical settings.

Biosafety concerns represent a primary challenge that limits the clinical translation of BEVs. As derivatives of bacteria, BEVs possess a complex composition. The presence of immunogenic components within these vesicles, such as LPS, outer membrane proteins, and lipoproteins, can trigger excessive inflammatory responses or systemic toxicity [176, 177]. Additionally, BEVs can disseminate throughout the body via the bloodstream and have the ability to traverse physiological barriers, including the blood-brain barrier. While this characteristic confers a distinct advantage for cross-organ therapies, it also raises the risk of abnormal accumulation of BEVs in non-target organs, thereby increasing the potential for autoimmunity [176]. Consequently, developing strategies to preserve essential immunological activity while reducing toxicity has emerged as an urgent issue that requires attention.

The gap between experimental models and the actual *in vivo* microenvironment can impact the validity and practical relevance of research conclusions. Current studies primarily focus on BEVs secreted in pure *in vitro* cultures. Such studies are detached from the complex microbial ecosystem of the gut and cannot replicate the true composition and functions of BEVs under physiological conditions, making it difficult to effectively extrapolate the conclusions of *in vitro* experiments to the *in vivo* environment [49, 178]. Furthermore, high concentrations of BEVs are often employed in experiments to validate their functions, resulting in experimental doses that do not correspond with physiological levels *in vivo*, which may exaggerate the regulatory effects of BEVs [179]. Animal models also contribute to translational uncertainty. Current studies investigating the pathogenic mechanisms

of pathological BEVs in metabolic diseases mainly rely on high-fat diet-induced obese mouse models or chemically induced diabetic models. These models do not adequately simulate the long-term, complex, and multifactorial pathological processes of human metabolic diseases. Such limitations further complicate the determination of the causal role of BEVs. Whether BEVs actively drive the initiation and progression of metabolic diseases or simply reflect metabolic disturbances in the host remains to be determined. Although animal studies have demonstrated that specific BEVs can induce metabolic disorders such as obesity, MASLD, and insulin resistance, most human studies are limited to analyzing correlations between disease status and changes in BEV abundance or composition. Moreover, existing research lacks long-term longitudinal follow-up and direct causal intervention studies, rendering it impossible to establish the true causal role of BEVs in metabolic diseases.

Technical standardization is another major barrier. There is no unified protocol for BEV culture, isolation, purification, or characterization, leading to variation in yield, purity, and composition across studies, which severely impacts the comparability and reproducibility of research findings [177, 180]. Culture conditions, including temperature, pH, incubation time, and medium composition, further influence BEV heterogeneity [181]. After cultivation, BEVs coexist with numerous contaminants such as protein aggregates, flagella, fimbriae, and other extracellular components, making complete separation technically challenging. Ultracentrifugation may compromise vesicle integrity, whereas precipitation-based methods often introduce substantial protein contamination [182]. Different purification methods, including density gradient centrifugation, ultrafiltration, immunoaffinity capture, and microfluidic systems, also produce inconsistent results [181]. In addition, BEV yield remains low, and large-scale production is still difficult [180]. Storage conditions are also not standardized, with freeze-thaw cycles posing a risk to vesicle integrity [182]. Collectively, these technical challenges hinder the standardization of BEV research and impede the progress of clinical translation.

Biological variability introduces another layer of complexity. Significant interindividual differences in fecal BEV composition have been observed in both healthy and diseased populations, complicating efforts to define universal diagnostic criteria [150]. Disease states such as obesity, Crohn's disease, and diarrhea further alter BEV profiles and intestinal permeability, adding additional variability to clinical interpretation [148]. Importantly, BEVs do not strictly mirror the taxonomic composition of the gut microbiota, suggesting that they represent a distinct biological entity with independent functional characteristic [21]. This dissociation limits the ability of microbiota profiling alone to reflect BEV origin or

Table 2 Summary of studies on gut microbiota-derived BEVs in metabolic diseases

Disease	BEVs origin	Active substance	Target tissue/cell	Biological function	Mechanism	Reference
Obesity	Fecal samples from DIO mice	LPS	Liver tissue	Negative	Upregulation of TLR4 expression; Upregulation of macrophage markers F4/80, CD86, CD206 expression; Upregulation of cytokines and their receptors <i>Cxcl10</i> , <i>Il2rg</i> , <i>Il1rn</i> , <i>Ccr1</i> , <i>Ccr2</i> expression	[82]
	<i>Akkermansia muciniphila</i>	-	Adipose tissue; Intestinal epithelial cells	Positive	Upregulation of <i>Ppara</i> and <i>Pparg</i> expression in adipose tissue; Upregulation of <i>Tjp1</i> , <i>Ocln</i> , <i>Cldn1</i> expression in intestinal epithelial cells.	[92]
	<i>Akkermansia muciniphila</i>	-	Adipose tissue; Liver tissue; Gut microbiota	Positive	Upregulation of <i>Ppara</i> expression in adipose tissue; Downregulation of <i>Pparg</i> , <i>Lpl</i> , <i>Tgf-β</i> expression in liver tissue; Decrease in <i>Firmicutes/Bacteroidetes</i> ratio	[93]
	<i>Akkermansia muciniphila</i>	-	Enteroendocrine cells	Positive	Upregulation of GLP-1, PYY expression; Downregulation of endocannabinoid system-related genes CB-1, CB-2, FAAH expression	[94]
	<i>Escherichia coli</i> Nissle 1917	-	Liver tissue; Gut tissue	Positive	Upregulation of multiple energy metabolism pathways in the intestine and liver; Decrease in <i>Firmicutes/Bacteroidetes</i> ratio.	[97]
	<i>Bacteroides thetaiotaomicron</i>	-	Enteroendocrine cells	Condition-dependent	50 µg/mL: Upregulation of <i>Pcsk1</i> and <i>Gcg</i> mRNA expression; Increase the secretion of GLP-1 and PYY 100 µg/mL: Downregulation of <i>Gpr119</i> and <i>Tgr5</i> mRNA expression.	[96]
	<i>Bacteroides fragilis</i>	-	Enteroendocrine cells	Condition-dependent	50 µg/mL: Downregulation of <i>Gpr119</i> and <i>Pcsk1</i> mRNA expression. 100 µg/mL: Downregulation of <i>Gpr120</i> , <i>Tgr5</i> and <i>Gcg</i> mRNA expression.	[96]
MASLD	Feces from NAFLD and NASH patients	LPS	Intestinal epithelial cells; Endothelial Cells; Hepatic Stellate Cells	Negative	Increase in intestinal epithelial cell permeability via nmMLCK-dependent pathway; Increase in endothelial permeability via TLR4 activation; Upregulation of α-SMA, type I collagen (α-1), TGF-β, G-CSF, and IL-1β expression in hepatic stellate cells	[83]
	Feces from NASH mice	Microbial DNA	Hepatocytes; Hepatic Stellate Cells	Negative	Enrichment in hepatocytes and enhancement of hepatocyte inflammatory response; Upregulation of <i>Timp1</i> , <i>Acta2</i> , <i>Des</i> , and <i>Col1a1</i> expression in hepatic stellate cells; Hepatic dysfunction associated with cGAS/STING signaling activation.	[87]

Table 2 (continued)

Disease	BEVs origin	Active substance	Target tissue/cell	Biological function	Mechanism	Reference
	<i>Akkermansia muciniphila</i>	-	Hepatic Stellate Cells; Intestinal epithelial cells; Adipose tissue	Positive	Downregulation of TGF- β , TLR-2, TLR-4, Col1a1, TIMP1 and α -SMA expression in hepatic stellate cells; Upregulation of ZO-1 expression in intestinal epithelial cells; Downregulation of α -SMA and IL-6 expression in adipose tissue, and upregulation of IL-10 expression	[101]
	<i>Akkermansia muciniphila</i>	-	Macrophage	Positive	Downregulation of IL-6 and TNF- α expression.	[90]
	EcN and EcoR12	-	Liver tissue	Positive	Upregulation of <i>Sod1</i> , <i>Cat</i> , and <i>Gpx</i> expression; Upregulation of <i>Cpt1a</i> and <i>Ppara</i> expression; Inhibition of <i>Srebp1c</i> , <i>Acc1</i> , <i>Fasn</i> , and <i>Cnr1</i> expression.	[103]
	<i>Lactobacillus salivarius</i>	-	Liver tissue; Gut microbiota	Positive	Upregulation of Beclin-1-related mitophagy pathway; Upregulation of <i>Ppara</i> and <i>Pparg</i> expression, and downregulation of <i>Fasn</i> expression; Promotion of beneficial <i>Bacteroides</i> growth, and inhibition of harmful <i>Desulfovibrio</i> growth	[102]
	Feces from GW6471 treated mice	TCDCA	Hepatocytes; Intestinal epithelial cells	Positive	Upregulation of mitochondrial number and levels of GSH, SOD, and CAT in hepatocytes; Upregulation of ferroptosis-related proteins SLC7A11 and GPX4 expression in liver, and downregulation of ACSL4 expression; Upregulation of occludin expression in intestinal epithelial cells.	[104]
Atherosclerosis	<i>Lactobacillus rhamnosus</i>	-	Foam cell	Positive	Upregulation of ABCA1 expression in foam macrophages via upregulation of NR1H3; Increase in the proportion of M2-type macrophages	[113]
Type 2 diabetes and insulin resistance	Small intestine lumen contents of HFD mice	Microbial DNA	Pancreatic islets	Negative	Upregulation of CD11c ⁺ macrophages and IL-1 β abundance; Downregulation of <i>Ins</i> and <i>Glut2</i> expression; Activation of cGAS/STING signaling contributes to pancreatic islet inflammatory injury	[85]
	Small intestine lumen contents of HFD mice	Microbial DNA	Liver tissue; Skeletal muscle; Adipose tissue	Negative	Impaired insulin signaling with decreased AKT phosphorylation and sensitivity in hepatic and adipose tissues; Induction of inflammation in liver, adipose tissue and skeletal muscle via activation of the cGAS/STING pathway	[86]
	<i>Pseudochrobactrum panacis</i>	-	Adipose tissue; Skeletal muscle	Negative	Downregulation of insulin-induced pAKT expression in skeletal muscle and adipose tissue; Impaired insulin-induced GLUT4 translocation in myotubes	[119]

Table 2 (continued)

Disease	BEVs origin	Active substance	Target tissue/cell	Biological function	Mechanism	Reference
Diabetic Complications	Cecum content or faecal pellets from mice	-	Liver tissue	Condition-dependent	Activation of HMGB1 signaling contributes to hepatic inflammatory responses; Upregulation of circadian clock-related genes <i>Per1</i> , <i>Rora</i> , and <i>Ciart</i> expression in liver; Upregulation of <i>G6pc</i> expression via activation of the CLOCK/BMAL1 pathway	[120]
	<i>Akkermansia muciniphila</i>	-	Intestinal epithelial cells	Positive	Induction of AMPK phosphorylation in a dose-dependent manner; Upregulation of occludin, zonula occludens, and claudin-5 expression	[66]
	Feces from diabetes mellitus rats	LPS	Renal tubular epithelial cells	Negative	Upregulation of MCP-1, TNF- α , IL-1 β and IL-6 expression; Induction of tubulointerstitial inflammation via activation of the caspase-11 pathway	[89]
	<i>Bacteroides fragilis</i>	dsDNA	Macrophage; Vascular smooth muscle cells	Negative	Promotion of M2 macrophage secretion of Serpine1 through activation of the Sting-Mef2d signaling pathway; Upregulation of Runx2 and Alp expression; Downregulation of smooth muscle contractile markers α -SMA and Sm22 α expression	[88]
	<i>Christensenellaceae</i>	miR-551b	Osteoblasts	Positive	Upregulation of osteoblast activity and ALPase activity; Upregulation of OCN, RUNX2, and ALP expression.	[64]
	<i>Lactobacillus reuteri</i>	miR-21a-5p	Endothelial cells; Keratinocytes	Positive	PI3K/AKT signaling activation enhances endothelial and epidermal cell activity; Upregulation of VEGF and HIF-1 α expression; Upregulation of IL-10 expression and downregulation of TNF- α , IL-6, and IL-1 β expression	[122]
	<i>Klebsiella variicola</i>	-	Osteal macrophages	Negative	Dose-dependent upregulation of IL-1 β and IL-6 expression; Promotion of osteoclast differentiation in bone macrophages.	[65]
	Feces from children	-	Gut microbiota; Osteoblasts; Osteoclast	Positive	Restoration of <i>Verrucomicrobia</i> depletion induced by ovariectomy; Upregulation of osteogenesis-related genes <i>Runx2</i> , <i>Alpl</i> , and <i>Bglap</i> expression; Downregulation of osteoclastogenesis-related genes <i>Oscar</i> , <i>Ctsk</i> , <i>Oc-stamp</i> , <i>Mmp9</i> , and <i>Trap</i> expression	[61]

Table 2 (continued)

Disease	BEVs origin	Active substance	Target tissue/cell	Biological function	Mechanism	Reference
	<i>Lactobacillus salivarius</i>	Differentially Expressed Proteins	Intestinal epithelial cells; Osteoblasts; Osteoclast	Positive	Downregulation of MLCK expression and upregulation of ZO-1 and occludin expression in intestinal epithelial cells; Upregulation of ALP activity in osteoblasts; Decreased TRAP activity and osteoclast abundance.	[63]
	<i>Lactobacillus reuteri</i> and <i>Lactobacillus paracasei</i>	miRNA	BMSCs	Positive	Upregulation of ALP expression in BMSCs and promotion of osteogenic differentiation	[125]
	<i>Proteus mirabilis</i>	miRNA	Osteoclast	Positive	Downregulation of <i>Acp5</i> , <i>Mmp9</i> , <i>Ctsk</i> , and <i>Itgb3</i> expression; Disruption of F-actin ring structure, leading to reduced osteoclast size; Inhibition of miR-96-5p, upregulation of <i>Abca1</i> expression, and involvement in mitochondria-dependent apoptosis	[126]
Hypertension	<i>Klebsiella pneumoniae</i>	-	Endothelial cells	Negative	Inhibition of SIRT1 signaling pathway in endothelial cells; Downregulation of p-eNOS expression; Upregulation of p53, COX-2, NOX2, and ET-1 expression	[141]
	Small intestine lumen contents of HFD mice	Microbial DNA	Adrenal glands	Negative	Induction of adrenal medullary dysfunction via activation of the cGAS/STING pathway; Promotion of norepinephrine secretion from the adrenal medulla	[142]
	<i>Akkermansia muciniphila</i>	-	Treg cells	Positive	Upregulation of peripheral Treg cell proportion; Upregulation of Treg cell-related gene <i>Il-10</i> expression; Downregulation of <i>Il-1β</i> and <i>Il-6</i> expression	[139]
	<i>Akkermansia muciniphila</i>	-	Kidneys	Positive	Upregulation of vasodilatory factors <i>At2r</i> , <i>Mas1</i> , and <i>Nos2</i> expression; Upregulation of vasoconstrictive factors <i>Agt</i> and <i>At1ar</i> expression	[140]
Vascular calcification	<i>Lactobacillus rhamnosus</i> GG	-	Gut microbiota; Vascular smooth muscle cells	Negative	Enrichment of <i>Lactobacillus</i> within the gut microbiota; PI3K/AKT signaling activation promotes osteogenic differentiation of vascular smooth muscle cells	[143]

biological activity. While such heterogeneity may eventually support personalized diagnostic approaches, it simultaneously challenges the establishment of standardized thresholds applicable across populations.

At present, most research on gut-derived BEVs focuses on gastrointestinal diseases, whereas metabolic and endocrine disorders remain relatively underexplored. Conditions such as gout, thyroid dysfunction, PCOS, and atherosclerosis have received limited attention, and

their relationships with BEVs are still poorly defined. A more systematic investigation of these disease contexts is therefore required to fully understand the biological and clinical significance of BEVs.

Conclusions and future perspectives

The present review systematically summarizes the mechanisms and translational potential of gut microbiota-secreted BEVs in metabolic diseases. As crucial signaling

carriers between microbes and hosts, BEVs can traverse the intestinal barrier to deliver functional molecules to distant tissues, including the liver, adipose tissue, bones, blood vessels, and pancreatic islets. This capability enables them to exert regulatory effects in various conditions, such as obesity, MASLD, T2DM, atherosclerosis, osteoporosis, PCOS, and metabolic syndrome (Table 2). Furthermore, BEVs exhibit promising potential as diagnostic biomarkers, engineered drug delivery systems, and natural prebiotic-based intervention strategies. These applications may provide new opportunities for the precise prevention and treatment of metabolic diseases.

Despite the progress made in this field, current research remains in its nascent stages and is still distant from clinical translation. Advances achieved in other disease areas offer valuable insights that can assist in overcoming existing bottlenecks and accelerating the clinical translation of BEVs for metabolic diseases. For instance, the synergistic effects of BEVs with drugs in anti-tumor immunotherapy [183] and their innovative applications in biocompatible nanodelivery systems [184] further highlight the unique advantages of these carriers in cross-organ signaling regulation and precision medicine. These successful experiences offer valuable insights for the field of metabolic diseases.

Future research on gut microbial BEVs in metabolic diseases presents numerous avenues for in-depth exploration. First, it is essential to establish in vitro and animal models that closely mimic the natural intestinal ecosystem to understand the formation, cargo selection, and inter-organ transport characteristics of BEVs under natural conditions. Second, clarifying the biological mechanisms by which gut bacteria generate BEVs is particularly crucial. Advances in technologies such as single-vesicleomics and in vivo imaging will facilitate a more accurate mapping of the spatiotemporal behavior of BEVs within metabolic tissues. Concurrently, engineered BEVs represent a significant breakthrough area. Enhancing their safety and targeting through surface modification or gene editing, coupled with the delivery of active molecules such as SCFAs, miRNAs, or metabolic enzymes, holds promise for intervening in the progression of metabolic disorders. Finally, advancing standardized pre-clinical and early clinical evaluations will be critical in validating the feasibility of BEVs as biomarkers or post-biotic therapies. As these challenges are progressively addressed, BEVs hold promise as a vital tool for precision intervention in metabolic diseases.

Abbreviations

BEVs	Bacterial extracellular vesicles
MASLD	Metabolic dysfunction-associated steatotic liver disease
PCOS	Polycystic ovary syndrome
SCFAs	Short-chain fatty acids

TMAO	Trimethylamine N-oxide
IL-22	Interleukin-22
FXR	Farnesoid X receptor
HAD	Hexadecanedioic acid
3-sucCA	3-succinylated cholic acid
OMVs	Outer membrane vesicles
OIMVs	Outer-inner membrane vesicles
EOMVs	Explosive outer membrane vesicles
EOIMVs	Explosive outer-inner membrane vesicles
CMVs	Cytoplasmic membrane vesicles
ECMV	Explosive cytoplasmic membrane vesicles
PQS	Pseudomonas quinolone signal
LPS	Lipopolysaccharide
PULs	Polysaccharide utilization loci
RIPK1	Receptor-interacting protein kinase 1
DIO	Diet-induced obese
TLR	Toll-like receptor
Cxcl10	C-X-C motif chemokine ligand 10
ZO-1	Zonula occludens-1
cGAS	Cyclic GMP-AMP synthase
STING	Stimulator of interferon genes
CRlg	Complement receptor of the immunoglobulin superfamily
Vsig4	V-set and immunoglobulin domain containing 4
TNF- α	Tumor necrosis factor- α
IL-6	Interleukin-6
PPAR	Peroxisome proliferator-activated receptor
STC-1	Secretin tumor cell line-1
LPL	Lipoprotein lipase
GLP-1	Glucagon-like peptide-1
PYY	Peptide YY
HFD	High-fat diet
COX-2	Cyclooxygenase-2
Ccr	C-C motif chemokine receptor
TGF- β	Transforming growth factor- β
CB-1	Cannabinoid receptor 1
CB-2	Cannabinoid receptor 2
FAAH	Fatty acid amide hydrolase
fEVs	Fecal-derived extracellular vesicles
nmMLCK	Non-muscular myosin light chain kinase
α -SMA	α -smooth muscle actin
IL-1 β	Interleukin-1 beta
Timp1	Tissue inhibitor of metalloproteinases 1
Col1a1	Collagen type I alpha 1 chain
IL-10	Interleukin-10
FASN	Fatty acid synthase
<i>E. Coli</i>	<i>Escherichia coli</i>
CPT1A	Carnitine palmitoyltransferase 1 A
SREBP1C	Sterol regulatory element-binding protein 1c
ACC1	Acetyl-CoA carboxylase 1
TCDCa	Taurochenodeoxycholic acid
GSH	Glutathione
SOD	Superoxide dismutase
CAT	Catalase
SLC7A11	Solute carrier family 7 member 11
GPX4	Glutathione peroxidase 4
ACSL4	Acyl-CoA synthetase long-chain family member 4
HLF	Hepatic leukemia factor
BSH	Bile salt hydrolases
CCL2	C-C motif chemokine ligand 2
LC3	Microtubule-associated protein 1 A/1B-light chain 3
ROS	Reactive oxygen species
CNR1	Cannabinoid receptor 1
EcN	<i>Escherichia coli</i> Nissle 1917
EcN-EVs	BEVs derived from <i>Escherichia coli</i> Nissle 1917
EcoR12	<i>Escherichia coli</i> O12:H12
Akk-EVs	BEVs derived from <i>Akkermansia muciniphila</i>
<i>A. muciniphila</i>	<i>Akkermansia muciniphila</i>
<i>B. vulgatus</i>	<i>Bacteroides vulgatus</i>
<i>B. thetaiotaomicron</i>	<i>Bacteroides thetaiotaomicron</i>
<i>B. uniformis</i>	<i>Bacteroides uniformis</i>
<i>B. fragilis</i>	<i>Bacteroides fragilis</i>

LR-BEVs	BEVs derived from <i>Lactobacillus reuteri</i>
ImP	Imidazole propionate
ITR	Imidazoline-1 receptor
NR1H3	Nuclear receptor subfamily 1 group H member 3
ABCA1	ATP Binding Cassette Subfamily A Member 1
ApoE ^{-/-}	Apolipoprotein E knockout
AKT	Protein kinase B
GLUT4	Glucose transporter type 4
GLUT2	Glucose transporter type 2
Ins	Insulin
G6pc	Glucose-6-phosphatase catalytic subunit
PCK1	Phosphoenolpyruvate carboxykinase 1
DKD	Diabetic kidney disease
VC	Diabetic vascular calcification
Mef2d	Myocyte enhancer factor 2D
PI3K	Phosphoinositide 3-kinase
Trib1	Tribbles pseudokinase 1
ALK	Alkaline phosphatase
HIF1-α	Hypoxia-inducible factor 1-α
mTOR	Mammalian target of rapamycin
Abca1	ATP binding cassette subfamily A member 1
Cyto c	Cytochrome c
ERK	Extracellular signal-regulated kinase
Bax	BCL2-associated X protein
Bcl-2	B-cell leukemia/lymphoma 2
TRAP	Tartrate-resistant acid phosphatase
Bglap	Bone gamma-carboxyglutamate protein
Alpl	Alkaline phosphatase, liver/bone/kidney
Runx2	Runt-related transcription factor 2
At2r	Angiotensin II receptor type 2
Mas1	MAS1 proto-oncogene, G protein-coupled receptor
Nos2	Nitric oxide synthase 2
Agt	Angiotensinogen
At1ar	Angiotensin II receptor type 1a
SIRT1	Sirtuin 1
p-eNOS	Phosphorylated endothelial nitric oxide synthase
NOX2	NADPH oxidase 2
ET-1	Endothelin-1
p53	Tumor protein p53
LTA	Lipoteichoic acid
BMP-2	Bone Morphogenetic Protein-2
CXCR4	C-X-C motif chemokine receptor 4
GA	Glycyrrhetic acid
VEGF	Vascular endothelial growth factor
IL-4	Interleukin-4
VCAM-1	Vascular cell adhesion molecule 1
ICAM-2	Intercellular adhesion molecule 2
NKT	Natural killer T cells
TFF3	Trefoil Factor 3
Nrf2	Nuclear factor erythroid 2-related factor 2
HO-1	Heme Oxygenase 1

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Author contributions

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Data availability

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Declarations

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Competing interests

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this manuscript, the authors used ChatGPT to assist with English translation, grammar correction, and language refinement to improve the clarity and readability of the manuscript. The authors have carefully reviewed and edited all AI-assisted content and take full responsibility for the final version of the manuscript.

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