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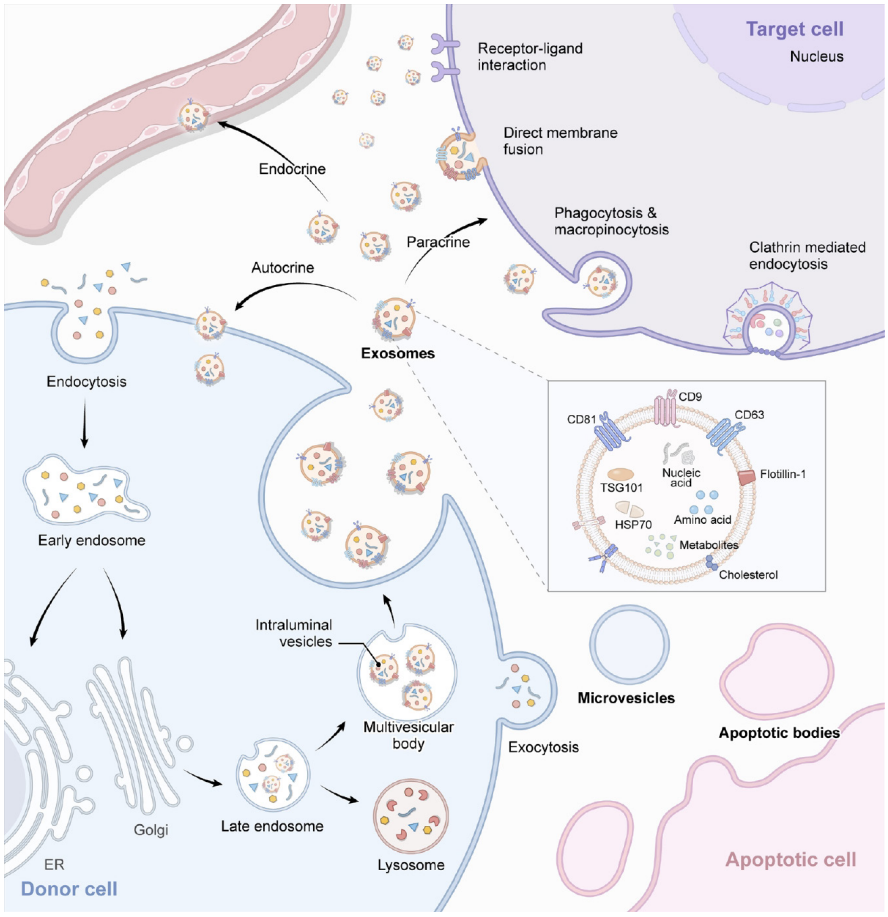
Diabetes Metab J 2025;49:348-367 | *https://doi.org/10.4093/dmj.2025.0184*



Highlights

- **EVs are key modulators in various physiological and pathological processes.**
- **EV-mediated interorgan crosstalk contributes to cardiometabolic disease progression.**
- **EV-based biomarkers offer non-invasive tools for early disease prediction.**
- **Therapeutic EVs enable targeted delivery of cargos for disease treatment.**

How to cite this article:
Lee J, Choi WG, Rhee M, Lee SH. Extracellular Vesicle-Mediated Network in the Pathogenesis of Obesity, Diabetes, Steatotic Liver Disease, and Cardiovascular Disease. Diabetes Metab J 2025;49:348-367. https://doi.org/10.4093/dmj.2025.0184

Review
Basic and Translational Research
Diabetes Metab J 2025;49:348-367
https://doi.org/10.4093/dmj.2025.0184
pISSN 2233-6079 · eISSN 2233-6087

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Extracellular Vesicle-Mediated Network in the Pathogenesis of Obesity, Diabetes, Steatotic Liver Disease, and Cardiovascular Disease

Joonyub Lee[1,2,*], Won Gun Choi[1,2,*], Marie Rhee[1,2], Seung-Hwan Lee[1,2,3]

[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul,
[2]Institute of Biomedical Industry, [3]Department of Medical Informatics, College of Medicine, The Catholic University of Korea, Seoul, Korea

Extracellular vesicles (EVs) are lipid bilayer-enclosed particles carrying bioactive cargo, including nucleic acids, proteins, and lipids, facilitating intercellular and interorgan communication. In addition to traditional mediators such as hormones, metabolites, and cytokines, increasing evidence suggests that EVs are key modulators in various physiological and pathological processes, particularly influencing metabolic homeostasis and contributing to the progression of cardiometabolic diseases. This review provides an overview of the most recent insights into EV-mediated mechanisms involved in the pathogenesis of obesity, insulin resistance, diabetes mellitus, steatotic liver disease, atherosclerosis, and cardiovascular disease. EVs play a critical role in modulating insulin sensitivity, glucose homeostasis, systemic inflammation, and vascular health by transferring functional molecules to target cells. Understanding the EV-mediated network offers potential for identifying novel biomarkers and therapeutic targets, providing opportunities for EV-based interventions in cardiometabolic disease management. Although many challenges remain, this evolving field highlights the need for further research into EV biology and its translational applications in cardiovascular and metabolic health.

Keywords: Biomarkers; Cardiovascular diseases; Diabetes mellitus; Extracellular vesicles; Fatty liver; Insulin resistance; Obesity

INTRODUCTION

Extracellular vesicles (EVs) are non-self-replicating, lipid bilayer-enclosed particles that contain bioactive substances [1]. EVs have gained increasing attention in biomedical research due to their pivotal roles in interorgan and intercellular crosstalk, which are important components of metabolic homeostasis and the development of various diseases [2-5]. Initially identified as platelet-derived microparticles and reticulocyte-released vesicles [6,7], EVs are now recognized as a diverse group of membranous vesicles released into the extracellular space by virtually all cell types. They carry cargo of bioactive molecules, including proteins, lipids, and nucleic acids, capable of modulating recipient cell function and phenotype. Over the past few years, the study of EVs has expanded rapidly, highlighting their role in diverse physiological and pathological processes. In addition to traditional mediators such as hormones and metabolites, EVs have emerged as key mediators in a wide range of biological processes, including immune regulation, tissue repair, angiogenesis, and metabolic control. Dysregulation of EV biogenesis, release, or cargo content has been implicated in numerous diseases, notably cancer, neurodegenerative disorders, and cardiometabolic diseases [5]. In the context of metabolic disorders, EVs derived from metabolically stressed tissues such as adipose tissue, liver, skeletal muscle, and pancreas have been shown to carry inflammatory signals,

Corresponding author: Seung-Hwan Lee https://orcid.org/0000-0002-3964-3877
Division of Endocrinology and Metabolism, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Korea
E-mail: hwanx2@catholic.ac.kr

*Joonyub Lee and Won Gun Choi contributed equally to this study as first authors.

Received: Mar. 6, 2025; Accepted: Apr. 16, 2025

lipotoxic mediators, and regulatory RNAs that propagate systemic insulin resistance, endothelial dysfunction, and organ fibrosis [4]. Therefore, we provide a comprehensive overview of the current understanding of the role of EVs in cardiometabolic diseases. Understanding the EV-mediated network in the pathophysiological context of cardiometabolic diseases holds immense potential for identifying novel biomarkers and therapeutic targets.

NOMENCLATURE, SUBTYPES, AND BIOLOGICAL FEATURES OF EVs

The nomenclature surrounding EVs has evolved over time, reflecting advances in our understanding of their biogenesis and function. Traditionally, EVs were widely categorized into three subtypes based on their size, biogenesis, and molecular composition: exosomes, microvesicles (also known as microparticles or ectosomes), and apoptotic bodies [8]. The smallest subtype, exosomes, are derived from the endosomal pathway and have a diameter ranging from 30 to 150 nanometers. Exosome formation begins with the inward budding of the endosomal membrane, leading to the formation of multivesicular bodies containing intraluminal vesicles. These multivesicular bodies can either fuse with lysosomes for degradation or travel to the cell surface, where they integrate to the plasma membrane and release exosomes into the extracellular milieu under the regulation of several Rab-GTPases [9]. Microvesicles, with diameters ranging from 100 to 1,000 nanometers, originate through direct outward budding and shedding from the plasma membrane. Apoptotic bodies, the largest subtype, are formed during programmed cell death and typically range from 500 to 2,000 nanometers in size (Fig. 1). The molecular composition of EVs is not random but reflects selective sorting mechanisms. Several pathways have been implicated in cargo loading, including the endosomal sorting complex required for transport (ESCRT)-dependent machinery, tetraspanins, and lipid raft-associated mechanisms [10]. Recent insights have revealed that certain microRNAs (miRs) contain specific sequence motifs that direct their preferential secretion into EVs or retention within the parent cell. RNA-binding proteins such as Alyref and Fus recognize these motifs and mediate the selective packaging of miRs into EVs, influencing intercellular gene regulation at distant sites. Because different cell types use specific sorting sequences, this miR code provides information on the tissues of origin of circulating small EVs [11]. Upon release, EVs interact with recipient cells through diverse mechanisms, including receptor-ligand interactions, membrane fusion, and different forms of endocytosis such as clathrin-mediated endocytosis, macropinocytosis, or phagocytosis (Fig. 1). Surface molecules on EVs, such as integrins, tetraspanins, and lectins, play crucial roles in determining target cell specificity and uptake efficiency. Following internalization, EV cargo can modulate cellular signaling pathways, gene expression, and metabolic functions of recipient cells, thereby contributing to homeostasis or disease progression.

EVs carry a diverse range of cytosolic proteins originating from their parent cells. They are notably enriched with proteins such as integrins, major histocompatibility complex molecules and components of the cytoskeleton [5]. In addition, EVs display certain proteins that are relatively specific to vesicles, including tetraspanins like tumor susceptibility gene 101 (TSG101) and CD63, which are typically associated with exosomes and used as representative markers. Technological advances have significantly enhanced the ability to isolate, characterize, and analyze EVs. Techniques such as differential ultracentrifugation, size-exclusion chromatography, immunoaffinity capture, and nanoparticle tracking analysis, combined with omics approaches have allowed a more detailed understating of EV heterogeneity and function [12]. However, the 'minimal information for studies of extracellular vesicles' produced by the International Society for Extracellular Vesicles recommends using the generic term EV and discourages using exosomes or ectosomes unless subcellular origin can be demonstrated [1]. Exosomes and ectosomes are biogenesis-related terms indicating origin from the endosomal system and plasma membrane, respectively, which are difficult to characterize with most EV separation techniques. Furthermore, universal molecular markers of exosomes, ectosomes, or other EV subtypes are unknown. Although most earlier research studied a broad population of EVs, the terms EV and exosome are both used without exact distinction. In this article, we followed the terminology of the reference paper to avoid any confusion.

THE ROLE OF EVs IN THE PATHOGENESIS OF OBESITY AND INSULIN RESISTANCE

Insulin resistance is the pivotal pathogenic component of metabolic syndrome and related diseases, although its underlying mechanisms are not fully understood [13]. Excess adiposity and the accompanying disruption in insulin signaling, inflammation, and

Table 1. Representative studies on the role of EVs in obesity, insulin resistance, and metabolic syndrome

Source	Cargo	Target	Main findings	Reference
miRNA				
Adipocyte	miR-34a	ATM	Adipocyte-secreted exosomes transport miR-34a into macrophages, and suppress M2 polarization by repressing KLF4 expression Adipose-specific miR-34a knockout mice were resistant to obesity-induced metabolic derangement	[14]
ATM	miR-155	Liver, muscle, adipose tissue	Treatment of lean mice with obese ATM exosomes causes insulin resistance Treatment of obese mice with lean ATM exosomes improves insulin resistance miR-155 in obese ATM exosomes impairs cellular insulin action by targeting PPARγ	[15]
ATM	miR-690	Liver, muscle, adipose tissue	IL-4/IL-13 induced M2 macrophages produce miR-690 containing exosomes miR-690-*Nadk* axis regulates inflammation and insulin sensitivity	[16]
Hepatocyte	miR-3075	Liver, muscle, adipose tissue	In early obesity, hepatocytes produce exosomes highly expressing insulin sensitizing miR-3075, which down-regulate fatty-acid 2-hydroxylase in adipocytes, myocytes and hepatocytes In chronic obesity, this compensatory effect is lost and hepatocyte-derived exosomes promote insulin resistance	[18]
ADSC	miR-193b/328/378a	Adipocyte, adipose tissue, liver	miR-193b, miR-328, and miR-378a were enriched in the BD-EV Treatments of BD-EV attenuate diet-induced obesity through browning of adipose tissue in mice HFD-induced hepatic steatosis and glucose tolerance are improved by BD-EV treatment	[20]
Adipocyte	miR-27a	Skeletal muscle cell	Adipocyte-derived miR-27a induce insulin resistance in C2C12 skeletal muscle cells through repression of PPARγ Serum miR-27a level is positively correlated with obesity and insulin resistance	[22]
Serum	miR-20b-5p	Skeletal muscle cell	miR-20b-5p is highly abundant in serum exosomes of patients with type 2 diabetes mellitus miR-20b-5p alters skeletal muscle cell glucose metabolism and suppresses STAT3 and AKT signaling pathway	[23]
Plasma	miR-191-5p	Adipocyte	miR-191-5p is found to be lowly expressed in the EVs from mice with long-term exercise EVs from mice with long-term exercise promote WAT browning by silencing miR-191-5p The lowly expressed miR-191-5p in EVs promotes the browning of WAT by negatively targeting the PRDM16-3′UTR	[25]
Macrophage (human THP-1)	miR-21/99a/146b/378a	Adipose tissue, liver	IL-4 polarized human macrophage increased lipophagy, mitochondrial activity, and oxidative phosphorylation in macrophages and adipocytes Treatment of exosomes derived from IL-4 polarized human macrophage improved hepatic steatosis, glucose tolerance, and insulin sensitivity	[26]
Protein				
Adipocyte	Insulinotropic protein	β-Cell	Adipocyte-derived EVs inform pancreatic β-cells about insulin resistance to compensate increased insulin demand and amplify glucose-stimulated insulin secretion	[17]
Adipocyte	Adiponectin	Adipose tissue, liver	Oligomeric forms of adiponectin is enriched in small EVs Adiponectin-enriched EVs reduce HFD-induced weight gain, insulin resistance, and tissue inflammation	[19]
ADSC	STAT3	Macrophage	ADSC-derived exosomes are transferred into macrophages to induce M2 macrophage polarization through transactivation of arginase-1 by exosomal STAT3 Treatment of obese mice with ADSC-derived exosomes improve insulin sensitivity, induce beiging of WAT, and alleviate hepatic steatosis	[21]
Lipid				
Intestinal epithelial cell	Phosphatidylcholine	Hepatocyte, macrophage	HFD alters lipid composition of intestinal exosomes from phosphatidylethanolamine to phosphatidylcholine Intestinal exosomes taken up by hepatocytes and macrophages induce insulin resistance via an AhR-mediated pathway	[24]

EV, extracellular vesicle; miR, microRNA; ATM, adipose tissue macrophage; KLF4, Krüppel-like factor 4; PPARγ, peroxisome proliferator-activated receptor gamma; IL, interleukin; *Nadk*, NAD+ kinase; ADSC, adipose-derived stem cell; BD, beige-adipogenic differentiating; HFD, high-fat diet; STAT3, signal transducer and activator of transcription 3; AKT, protein kinase B; WAT, white adipose tissue; PRDM16, PR-domain containing 16; UTR, untranslated region; AhR, aryl hydrocarbon receptor.

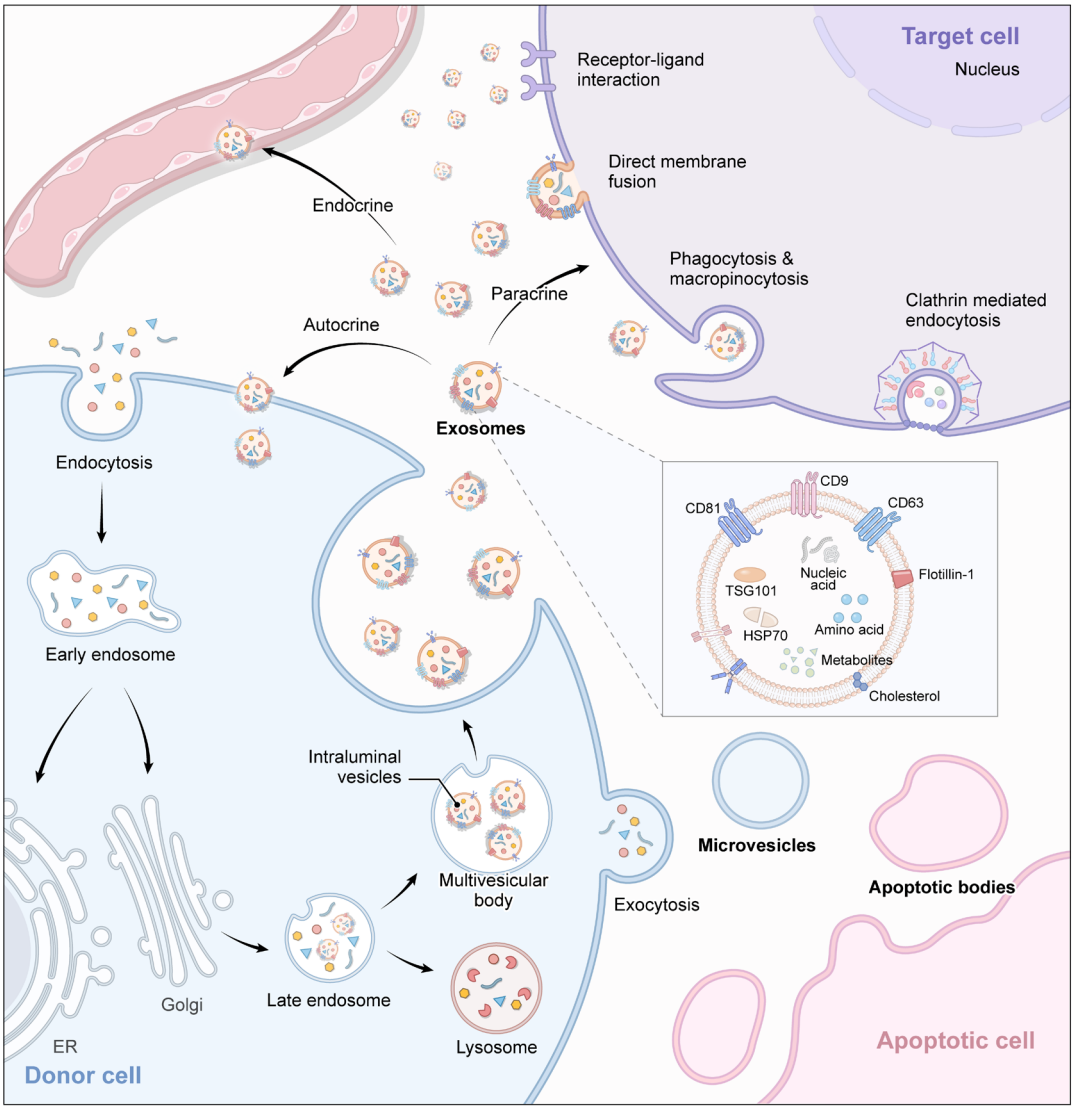


Fig. 1. Biogenesis, secretion, and action of extracellular vesicles. Exosomes are extracellular vesicles generated by virtually all cells, carrying nucleic acids, proteins, lipids, and metabolites as cargo. They originate through the inward budding of endosomal membranes, incorporating various molecules. Their formation occurs within multivesicular bodies, which eventually fuse with the plasma membrane, releasing exosomes into the extracellular environment. In contrast, microvesicles and apoptotic bodies are shed directly from the plasma membrane. Exosomes function in an autocrine, paracrine, or endocrine manner. They are taken up by nearby or distant cells through phagocytosis/macropinocytosis, direct membrane fusion, receptor-ligand interactions, or clathrin-mediated endocytosis. TSG101, tumor susceptibility gene 101; HSP70, heat shock protein 70; ER, endoplasmic reticulum.

endoplasmic reticulum (ER) stress are suggested to be the main drivers of insulin resistance. Recent research has demonstrated that EVs may contribute to these processes (Table 1).

EVs in adipose tissue: mediators of metabolic communication and insulin sensitivity

Adipose tissue depots are composed of heterogeneous cells that mediate key functions of metabolism, including energy storage and release, adipokine secretion, non-shivering thermogenesis, and immune responses [27,28]. Beyond mature adipocytes and their progenitors, various immune and endothelial cells orchestrate the function of adipose tissue as an endocrine organ. Adipocyte-secreted exosomes transport miR-34a into macrophages and suppress M2 polarization by re-

pressing Krüppel-like factor 4 (KLF4) expression in a paracrine manner. Adipose-specific miR-34a knockout mice were protected from obesity-induced metabolic impairment [14]. Adipose tissue macrophages (ATMs) also secrete and transfer exosomes to insulin sensitive cells. Ying et al. [15] showed that the treatment of lean mice with obese ATM exosomes caused glucose intolerance and insulin resistance, whereas treatment of obese mice with lean ATM exosomes restored glucose tolerance and insulin sensitivity. miR-155 was overexpressed in obese ATM exosomes and impaired cellular insulin action by targeting peroxisome proliferator-activated receptor gamma (PPARγ) [15]. Similarly, when the exosome-like vesicles from obese adipose tissue were injected intravenously (IV), peripheral blood monocytes differentiated into activated macrophages by taking up these vesicles and secreted inflammatory cytokines. This caused insulin resistance in wild-type mice, although the effect was significantly reduced in toll-like receptor 4 (TLR4) knockout mice [29]. Alternatively activated, anti-inflammatory M2 macrophages are important in maintaining metabolic homeostasis opposed to inflammatory M1 macrophages. M2 polarized bone marrow-derived macrophages produced exosomes enriched with miR-690, which improved glucose tolerance and insulin sensitivity. *Nadk*, a gene encoding NAD+ kinase, was a target mRNA of miR-690 and regulated cellular insulin activity [16]. Small EVs derived from ATMs of rosiglitazone-treated mice improved glucose intolerance and insulin sensitivity in obese mice, with miR-690 identified as a key mediator of these beneficial effects. Notably, common adverse effects associated with thiazolidinedione therapy, such as weight gain and hemodilution, were not observed following EV treatment, in contrast to rosiglitazone administration via diet [30]. In individuals with prediabetes or early type 2 diabetes mellitus (T2DM), insulin secretion is increased to cope with insulin resistance. Adipocyte-derived EVs from diet-induced obese mice conveying insulinotropic protein cargo enhanced first phase glucose-stimulated insulin secretion, which was not observed by EVs from lean mice. Therefore, the status of insulin resistance in adipose tissue is communicated to pancreatic beta-cells by EVs to meet the increased insulin demand [17]. Similarly, hepatocytes produce different exosomes depending on the duration of obesity to control insulin sensitivity. In early obesity, miR-3075 is enriched in exosomes and increases insulin sensitivity by down-regulating fatty-acid 2-hydroxylase in adipocytes, myocytes, and hepatocytes. However, this compensatory action is lost in chronic obesity and insulin resistance is aggravated by proinflammatory macrophage activation [18]. Liver-derived EV secretion is also increased in response to hyperglycemia, enhancing glucose effectiveness in skeletal muscle and insulin secretion from pancreas through endocrine signaling [31].

Among various adipokines, oligomeric forms of adiponectin were enriched in adipose tissue-derived small EVs and were mainly distributed at the external surface of EVs. The transfer of these EVs to high-fat diet (HFD)-fed mice prevented obesity, insulin resistance, and tissue inflammation [19]. Additionally, adiponectin is known to regulate exosome biogenesis and secretion through binding with T-cadherin [32]. In adipose tissue, EVs carrying proteins and lipids are transferred between cell types to influence cellular signaling pathways, a process regulated by fasting, refeeding, and obesity. This indicates that EVs play a role in the tissue response to shifts in systemic nutrient status [33].

EVs from thermogenic adipocytes: roles in energy homeostasis

Brown and beige adipocytes are thermogenic fat cells involved in the regulation of systemic energy metabolism and glucose homeostasis [34]. Biomodulation of these thermogenic fat cells can be a promising approach to improve energy homeostasis, and various inducers have been identified. Brown adipocytes release exosomes and this is increased by brown adipose tissue (BAT) activation. Serum concentrations of exosomal miR-92a were inversely correlated with human BAT activity in cohorts of healthy individuals [35]. EVs isolated from human adipose-derived stem cells during beige-adipogenic differentiation attenuated diet-induced obesity, hepatic steatosis, and glucose intolerance through adipose tissue browning. miR-193b, miR-328, and miR-378a enriched in EVs were responsible for the observed effect and suggest a role of EVs in cellular reprogramming [20]. In another study, exosomes secreted from adipose-derived stem cells were transferred into macrophages to induce M2 polarization, inflammation alleviation, and white adipose tissue browning in diet-induced obese mice. Arginase-1 was activated by exosome-carried phosphorylated signal transducer and activator of transcription 3 (STAT3) resulting in the alternative activation of macrophages [21]. Interestingly, cellular components can be loaded in EVs. Oxidatively damaged components of mitochondria were ejected via EVs from thermogenically stressed brown adipocytes. Clearance of extracellular mitochondria by BAT-resident macrophages was

instrumental in maintaining the thermogenic program [36]. Similarly, small EVs from stressed adipocytes carried respiration-competent, but oxidatively damaged mitochondria to cardiomyocytes, triggering a burst of reactive oxygen species (ROS). This pro-oxidant signal stimulates compensatory antioxidant signaling and protects cardiomyocytes from ischemia/reperfusion injury showing an example of interorgan mitohormesis [37].

EV-mediated brain-adipose tissue crosstalk: implications of cognition and obesity

The brain is an insulin-responsive organ, and brain insulin resistance is linked to memory impairment and cognitive dysfunction [38]. EVs and their cargo miRNAs have been shown to regulate adipose tissue-brain interorgan communication, resulting in cognitive impairment related to insulin resistance. miR-9-3p in adipose tissue-derived EVs from HFD-fed mice suppressed brain-derived neurotrophic factor and induced remarkable synaptic damage, especially in the hippocampus [39]. The loss of function of AMP-activated protein kinase alpha 1 (AMPKα1) in steroidogenic factor 1 (SF1) neurons within the ventromedial nucleus of the hypothalamus leads to resistance to obesity. Central delivery or IV injection of small EVs carrying a plasmid encoding a dominant-negative mutant of AMPKα1, targeted to SF1 neurons, resulted in sympathetic nerve activation and uncoupling protein 1 (UCP1)-dependent thermogenesis in BAT [40,41]. These findings highlight the potential of small EV-based technologies to selectively modulate brain-adipose tissue crosstalk as a therapeutic approach against obesity.

EVs in skeletal muscle and intestine: regulators of insulin sensitivity

Skeletal muscle cells also secrete EVs and their contents are altered in various conditions such as aging, neuromuscular disorders, HFD, diabetes, and acute or chronic exercise [42,43]. When exosome-like vesicles from quadriceps muscle of palmitate-enriched diet-fed mice were isolated and injected *in vivo*, the size of the islet was increased suggesting that EVs may mediate beta-cell adaptation during insulin resistance [44]. Adipocyte-derived serum exosomal miR-27a was suggested as a biomarker of obesity and insulin resistance. Overexpression of miR-27a in C2C12 skeletal muscle cells induced insulin resistance via repression of PPARγ and downstream genes [22]. Similarly, circulating exosomal miR-20b-5p, which was highly abundant in individuals with T2DM compared to those with normal glucose tolerance, modulated insulin-stimulated glucose metabolism via protein kinase B (AKT) signaling in primary human skeletal muscle cells [23]. Intestinal epithelial cells were also shown to participate in the regulation of systemic metabolism. The composition of lipids in exosomes isolated from feces was changed from phosphatidylethanolamine to phosphatidylcholine by HFD. These intestinal exosomes were taken up by insulin sensitive tissues and induced insulin resistance via aryl hydrocarbon receptor (AhR)-activation and inhibition of the insulin signaling pathway including insulin receptor substrate 2 (IRS-2), phosphoinositide 3-kinase (PI3K), and Akt [24]. Therefore, EV-mediated intercellular or interorgan crosstalk is a fundamental component of insulin sensitivity and energy metabolism.

EVs IN DIABETES MELLITUS: BIOMARKERS, PATHOGENIC MEDIATORS, AND THERAPEUTIC OPPORTUNITIES

Chronic disturbances in glucose homeostasis and metabolic perturbations caused by diabetes mellitus (DM) lead to multiple organ dysfunction and premature mortality [45]. Emerging evidence suggests that EVs may play a key role in the development and progression of DM (Table 2) [46,47].

EVs in type 1 diabetes mellitus: early indicators and mediators of autoimmunity

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder caused by the destruction of pancreatic β-cells by T-cells. Current insights suggest the involvement of EVs in the early stages of T1DM development. A study showed that cytokine treatment to mimic the inflammatory milieu in T1DM increased miR-21-5p expression in EVs from rodent and human islets (MIN6 cells, EndoC-βH1 cells, and human islets). Moreover, serum EV miR-21-5p was elevated in newly diagnosed T1DM patients compared to that in healthy controls and in non-obese diabetic (NOD) mice prior to the onset of diabetes [48]. Interferon (IFN)-α and IFN-γ are pivotal cytokines involved in the pathogenesis of T1DM, and islet IFN signaling is known to enhance programmed death-ligand 1 (PD-L1) expression in β-cell. Exposure to IFN also increased PD-L1 on the surface of β-cell-derived EVs, which bind to programmed death protein 1 (PD1) and suppress $CD8^+$ T-cell proliferation and cytotoxicity. Thus, EVs carrying PD-L1 may represent a potential strategy

Table 2. Representative studies on the role of EVs in diabetes

Source	Cargo	Target	Main findings	Reference
miRNA				
T-lymphocyte	miR-142-3p, miR-142-5p, miR-155	β-Cell	Exosomes from T-lymphocytes can trigger chemokine expression and apoptosis of rodent and human pancreatic β-cells miR-142-3p, miR-142-5p, and miR-155 are enriched in T-lymphocyte released exosomes Bocking microRNAs (miR-142-3p, miR-142-5p, and miR-155) can decrease β-cell destruction and diabetes incidence in NOD mice	[50]
M1 macrophage	miR-212-5p	β-Cell	M1 macrophages are enriched in HFD-fed mice pancreatic islets Exosomes from M1 macrophages impaired glucose-stimulated insulin secretion in β-cells miR-212-5p was the main contributor in the M1-exosome-induced β-cell dysfunction which is thought to be driven by targeting SIRT2-Akt-GSK-3β-β-catenin pathway	[56]
β-Cell, serum	miR-26a	β-Cell, hepatocyte	miR-26a is decreased in overweight humans and inversely correlate with T2DM features (HOMA-IR, fasting insulin) miR-26a modulates insulin secretion in β-cells and alleviates insulin resistance in hepatocytes	[54]
Bone marrow cell	miR-106b-5p, miR-222-3p	β-Cell	miR-106b-5p, miR-222-3p is increased in serum exosomes after bone marrow transplantation in mice Intravenous delivery of the miRNAs improved hyperglycemia in STZ injected mice by inducing β-cell proliferation through Cip/Kip family downregulation	[68]
β-Cell	miR-29s	Hepatocyte	Pancreatic β-cells secrete miR-29s in response to high levels of free fatty acids of HFD feeding miR-29s inhibit insulin signaling in the liver and increase hepatic glucose production	[60]
MIA PaCa-2 cell (human pancreatic cancer cell line)	miR-6796-3p, miR-6763-5p, miR-4750-3p, miR-197-3p	STC-1 cell (mouse enteroendocrine cell)	Exosomes from MIA PaCa-2 cells decreased the production of GIP and GLP-1 in STC-1 cells MIA PaCa-2 cell-derived exosomes were enriched in miR-6796-3p, miR-6763-5p, miR-4750-3p, and miR-197-3p which is thought to decrease GIP and GLP-1 in STC-1 cells by down-regulating PCSK1/3 leading to pancreatic cancer-associated diabetes	[65]
Protein				
β-Cell	GAD65, IA-2, proinsulin	Dendritic cell	Exosomes derived from primary human and rat islets carry the autoantigens GAD65, IA-2, and proinsulin Cytokine induced ER stress promotes release of exosomes containing both autoantigens and immunogenic chaperones which have potential to initiate β-cell autoimmunity	[51]
PANC-1 cell line, pancreatic cancer patient-derived cell line, plasma (peripheral, portal vein)	Adrenomedullin, CA 19-9	INS-1 cell line, human islet	Pancreatic cancer patient-derived exosome decreases glucose-stimulated insulin secretion in INS-1 cells and human islets Adrenomedullin and CA 19-9 is enriched in pancreatic cancer patient-derived exosomes which can increase ER stress and reactive oxygen/nitrogen species leading to paraneoplastic β-cell dysfunction	[66]
Others				
β-Cell, serum	circGlis3	β-Cell, islet endothelial cell	Exosomal circular RNA (circGlis3) was increased in MIN6 cells under lipotoxic conditions and serum of HFD-fed mice and T2DM patients CircGlis3 mediate lipotoxicity-induced β-cell dysfunction and drive islet endothelial dysfunction by GMEB1/MIB2/HSP27 pathway	[55]
HucMSC	Unknown	β-Cell, muscle, liver	HucMSC-derived exosomes alleviate hyperglycemia in T2DM rat model (STZ+HFD) when delivered intravenously HucMSC-derived exosomes enhance insulin sensitivity by decreasing glycogen storage in liver and increasing GLUT4 translocation in muscle HucMSC-derived exosomes decrease STZ mediated β-cell	[67]

EV, extracellular vesicle; miR, microRNA; NOD, non-obese diabetic; HFD, high-fat diet; SIRT2, sirtuin 2; Akt, protein kinase B; GSK-3β, glycogen synthase kinase-3β; T2DM, type2 diabetes mellitus; HOMA-IR, homeostatic model assessment of insulin resistance; STZ, streptozotocin; Cip, CDK interacting protein; Kip, kinase inhibitory protein; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; PCSK, proprotein convertase subtilisin/kexin; GAD65, glutamate decarboxylase 65; IA-2, islet antigen 2; ER, endoplasmic reticulum; Glis3, Gli-similar 3; GMEB1, glucocorticoid modulatory element-binding protein 1; MIB2, mindbomb E3 ubiquitin protein ligase 2; HSP27, heat shock protein 27; HucMSC, human umbilical cord mesenchymal stem cell; GLUT4, glucose transporter 4.

to inhibit immune-mediated β-cell destruction [49]. A study by Guay et al. [50] demonstrated that T-lymphocytes derived EVs can induce chemokine expression in pancreatic β-cells, potentially leading to apoptosis. These EVs were enriched in miR-142-3p, miR-142-5p, and miR-155. Furthermore, inhibiting these miRNAs in NOD mice reduced β-cell destruction and diabetes development [50]. In the initiation of autoimmunity in T1DM, protein-loaded exosomes originating from islets were taken up by dendritic cells and boosted antigen presentation and T-cell activation. Intracellular β-cell autoantigens (glutamate decarboxylase 65 [GAD65], islet antigen 2 [IA-2], and proinsulin) and immunostimulatory chaperones (calreticulin, Gp96, and 150-kDa oxygen-regulated protein [ORP150]) were the main cargos of these exosomes [51].

EVs in T2DM: modulators of β-cell function and systemic insulin resistance

The pathophysiology of T2DM involves an interplay among multiple organs [45]. Enhanced adiposity leads to increased free fatty acid uptake in muscles and liver, resulting in peripheral insulin resistance [52]. This drives hepatic gluconeogenesis and increases systemic insulin demand. In the face of failing compensatory responses from β-cells, progression towards β-cell failure begins. Reduced functional β-cell mass combined with increased hepatic gluconeogenesis contributes to hyperglycemia and T2DM. A growing body of literature suggests the potential role of EVs in this pathophysiological cascade. EV characteristics differ between T2DM and T1DM patients [53]. Earlier studies illustrated T2DM distinct EVs which play a role in T2DM pathogenesis. For example, serum EV miR-26a levels were decreased in overweight individuals and were inversely associated with T2DM characteristics in humans. In a rodent model, miR-26a was demonstrated to modulate insulin secretion and β-cell replication in an autocrine manner, as well as to alleviate insulin resistance in hepatocytes [54]. Lipotoxicity is one of the important metabolic stimuli that can cause β-cells failure. The level of circular Gli-similar 3 (circGlis3), a circular RNA, was elevated in EVs from MIN6 cells under lipotoxic conditions which was further validated in the serum of HFD-fed mice and T2DM patients. CircGlis3 was suggested to mediate lipotoxicity-induced β-cell dysfunction and trigger islet endothelial dysfunction through the glucocorticoid modulatory element-binding protein 1 (GMEB1)/mindbomb E3 ubiquitin protein ligase 2 (MIB2)/heat shock protein 27 (HSP27) pathway [55]. Pancreatic islets of mice fed with a HFD are enriched with M1 macrophages, whose exosomes impaired glucose-stimulated insulin secretion in β-cells. miR-212-5p was the main contributor in this process which is thought to be driven by targeting sirtuin 2 (SIRT2) and inhibiting the Akt/glycogen synthase kinase-3β (GSK-3β)/β-catenin pathway [56]. Similarly, β-cell-macrophage crosstalk mediated by miR-29/TNF-receptor-associated factor 3 (TRAF3) axis aggravates systemic inflammation and glucose intolerance in the process of developing diabetes from prediabetes [57]. Islet amyloid polypeptide (IAPP) is a hormone co-secreted with insulin in β-cells, and its deposition in the islet contributes to β-cell dysfunction in patients with T2DM [58]. One study demonstrated that EVs from healthy donor pancreatic islets attenuated IAPP amyloid deposition by peptide scavenging while this function was impaired in EVs from T2DM donors [59]. β-cells release exosomal miR-29s in conditions of high free fatty acid levels, which target insulin signaling in the liver and blunt hepatic insulin sensitivity [60]. This finding suggests pancreas-liver crosstalk mediated by EVs in controlling glucose homeostasis. As such, EVs participate in the pathophysiologic process of T2DM by modulating β-cell function and peripheral insulin resistance in an endocrine and paracrine manner.

EVs in gestational diabetes mellitus and latent autoimmune diabetes in adults

Gestational diabetes mellitus (GDM) is an important metabolic disorder during pregnancy, significantly raising the risk of perinatal complications and contributing to a higher chance of obesity, T2DM, and cardiovascular risk in both mothers and their offspring [61]. Exosomes from women with GDM were enriched in miR-423-5p, while miR-122-5p, miR-148a-3p, miR-192-5p, and miR-99a-5p were downregulated compared to those from normal glucose tolerant (NGT) pregnant women. These miRNAs were associated with insulin and AMPK signaling and were useful in the early prediction of GDM [62]. Furthermore, placenta-derived exosome concentrations were more markedly elevated in patients with GDM than those from NGT pregnant women, suggesting their potential role in the proinflammatory process [63]. EVs could serve as early diagnostic biomarkers for GDM and as mediators of metabolic physiology in pregnant women. Latent autoimmune diabetes in adults (LADA) is a unique subgroup of the disease with patients who develop autoimmune destruction of β-cells within a few years after their initial clinical manifestation of T2DM. A small study suggested that miR-146a-5p, miR-223-3p, and miR-21-5p were

upregulated in plasma-derived exosomes from the LADA group compared with those in the T2DM group [64].

EVs in pancreatic cancer-associated diabetes

Pancreatic cancer is linked to an elevated risk of diabetes, although the mechanisms remain unclear. Studies suggest the involvement of EVs in pancreatic cancer-related diabetes. Exosomes from pancreatic cancer cell lines (MIA PaCa-2 cells) decreased glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) production in neuroendocrine cells (STC-1 cells). miR-6796-3p, miR-6763-5p, miR-4750-3p, and miR-197-3p were highly expressed in MIA PaCa-2 cell-derived exosomes, which downregulated proprotein convertase subtilisin/kexin (PCSK) type 1/3 in STC-1 cells and reduced GIP and GLP-1 production [65]. Another study demonstrated that exosomes from pancreatic cancer patients reduced glucose-stimulated INS-1 cells and human islets. Adrenomedullin was suggested to mediate exosome induced β-cell dysfunction in pancreatic cancer patients by increasing ER stress and reactive oxygen/nitrogen species [66].

Therapeutic potential of EVs in diabetes management: preclinical insights

As described above, EVs participate in various processes of diabetes pathogenesis and offer potential therapeutic promise. IV delivery of human mesenchymal stem cell-derived exosomes alleviated hyperglycemia by decreasing glycogen storage in the liver, increasing glucose transporter 4 (GLUT4) translocation in muscle, and decreasing streptozotocin (STZ) mediated β-cell apoptosis in a T2DM rat model [67]. Similarly, IV delivery of miR-106b-5p and miR-222-3p improved hyperglycemia by promoting post-injury β-cell proliferation mediated by downregulation of the CDK interacting protein/kinase inhibitory protein (Cip/Kip) family proteins [68]. As these studies were primarily validated in rodent models, the clinical applicability remains limited. Nevertheless, the results are promising and strongly indicate encouraging prospects for leveraging EVs in future diabetes treatments.

EVs IN METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE: KEY MEDIATORS OF LIPID METABOLISM, INFLAMMATION, AND FIBROSIS

Metabolic dysfunction-associated steatotic liver disease (MASLD) is one of the most prevalent chronic liver diseases globally, affecting over 30% of the population [69-71]. MASLD not only impacts liver health leading to liver fibrosis, cirrhosis, and carcinoma but also contributes to systemic complications, including cardiovascular diseases (CVD), T2DM, and mortality [69, 72-74]. Resulting from toxic lipid accumulation, damaged cells drive the formation and release of EVs/exosomes, which have pivotal roles in advancing MASLD. EVs from metabolically stressed cells carry a diverse cargo, disseminating it through autocrine, paracrine, and systemic pathways. Of note, intrahepatic and extrahepatic cellular components communicating via EVs are emerging as critical factors in the pathogenesis of MASLD [75,76].

miRNA cargo in EVs: key regulators of MASLD progression and pathogenesis

EVs harboring miRNA are internalized by hepatocytes, hepatic stellate cells (HSCs) and macrophages, orchestrating a regulatory role in the development and progression of MASLD. In obese mice with metabolic dysfunction-associated steatohepatitis (MASH), neutrophils infiltrate around lipotoxic hepatocytes communicating with each other. The elevated levels of miR-223 in hepatocytes result from the selective absorption of miR-223-enriched EVs originating from neutrophils in an apolipoprotein E/low-density lipoprotein receptor (LDLR)-dependent manner. miR-223 inhibits inflammatory and fibrotic gene expression, and augmentation of EV transfer by upregulated LDLR with PCSK9 inhibitor ameliorated MASH in mice [77]. Another study also elucidated the antifibrotic effect of exosomal miR-223 transferred from macrophages to hepatocyte under interleukin 6 (IL-6) signaling [78]. miR-199a-5p expression was abundant in the adipose tissue of HFD-fed mice. Exosomal miR-199a-5p aggravated hepatic lipid accumulation through its interaction with mammalian sterile 20-like kinase 1 (MST1) and modulation of the downstream pathway of lipogenesis and lipolysis [79]. In the context of lipotoxicity, several miRNAs act as messengers between hepatocytes and HSCs to accelerate the progression of MASLD. Exosomal miR-1297 promoted HSC activation and proliferation through the PTEN/PI3K/AKT signaling pathway [80]. Furthermore, hepatocytes damaged by lipotoxicity release exosomes that carry and transmit miR-27a to HSCs, thereby triggering liver fibrosis associated with MASLD through inhibition of PTEN-induced kinase 1 (PINK1)-mediated mitophagy. Serum exosomal miR-27a levels were positively associated with the de-

gree of hepatic fibrosis in both mice and humans [81]. In similar conditions, EVs originating from lipotoxic hepatocytes shuttled miR-128-3p to HSCs and induced profibrogenic activation by inhibiting the expression of PPARγ [82]. miR-122 has undergone extensive study and is recognized for its significant role in orchestrating gene expression within the liver. In the context of MASLD, miR-122 exhibited a close association with the development and progression of the disease [83]. Furthermore, it garnered attention as a prospective biomarker for MASLD. In mice subjected to methionine choline-deficient diet, the transference of proinflammatory miR-122 from hepatocytes to resident liver macrophage cells was demonstrated to hinge on matrix metalloprotease 2 (MMP2) [84]. Lipotoxic hepatocytes release exosomal miR-192-5p, which triggers M1 macrophage activation and induce hepatic inflammation through the rapamycin-insensitive companion of mammalian target of rapamycin (Rictor)/Akt/forkhead box O1 (FoxO1) signaling pathway [85].

Protein cargo in EVs: drivers of lipid accumulation and inflammation in MASLD

Proteins are also loaded in the EVs to mediate interorgan or intercellular crosstalk in the progression of MASLD. Aldo-keto-reductase 1b7 (Akr1b7) and scavenger receptor class B (CD36) are protein cargos in the adipocyte-derived exosomes inducing lipid accumulation in hepatocytes. Obesity-triggered ER stress within adipose tissue facilitated the secretion of exosomes from adipocytes containing Akr1b7, contributing to the development of hepatic steatosis, inflammation, and fibrosis [86]. In addition, obesity-associated inactivation of AMPKα1 in white adipose tissue contributed to increased exosome biogenesis and release by elevating TSG101 and facilitating CD36 sorting into exosomes. This led to hepatic lipid accumulation and inflammation, which was mitigated by metformin-induced AMPK activation [87]. Hepatocytes exposed to lipotoxicity also transfer EVs containing protein cargos to macrophages or monocytes initiating chemotaxis. Integrin β1 (ITGβ1)-enriched EVs intensify the adherence of monocytes to liver sinusoidal endothelial cells, consequently initiating hepatic inflammation. Of note, ITGβ1 antibody treatment significantly ameliorated liver injury and fibrosis [88]. Hepatocyte-derived EVs induced by palmitate or lysophosphatidylcholine were discovered to encompass both C-X-C motif chemokine 10 (CXCL10) and tumor necrosis factor-like apoptosis-inducing ligand (TRAIL), which induce chemotaxis and proinflammatory cascade in macrophages [89]. The death receptor 5 signaling cascade activated rho-associated coiled-coil-containing protein kinase 1 (ROCK1) and promoted the release of TRAIL-bearing EVs, which subsequently activated macrophages via the receptor interacting protein kinase 1 (RIP1)-nuclear factor kappa-B (NF-κB) signaling pathway. Thereby, treatment of ROCK1 inhibitor fasudil effectively decreased liver inflammation and fibrosis [90].

Lipid cargo in EVs: mediators of liver injury and fibrosis in MASLD

EVs containing lipids can potentially induce liver injury by directly affecting macrophages and endothelial cells. Hepatocytes damaged by lipotoxic ER stress stimulate inositol-requiring enzyme 1α (IRE1α)/X-box binding protein 1 (XBP1), leading to the release of ceramide-enriched EVs, some of which contain sphingosine-1-phosphate, a ceramide metabolite. These EVs recruit monocyte-derived macrophages to the liver, causing inflammation and injury [91,92]. Lipotoxic stress triggers hepatocytes to release small EVs enriched in palmitic (C16:0) and stearic (C18:0) saturated fatty acids. These small EVs target Kupffer cells and induce inflammation via TLR4. Treatment of conditioned media from macrophages/Kupffer cells loaded with lipotoxic small EVs impaired hepatocyte insulin signaling making a hepatocyte-macrophage-hepatocyte crosstalk in a paracrine manner [93]. Iron homeostasis in the liver is also associated with steatosis and fibrosis. Hepatocyte-secreted iron-containing EVs lead to hepatocyte iron deficiency and HSC iron overload. Iron accumulation promotes ROS overproduction and fibrogenic activation of HSCs, and this effect is mitigated by blocking EV secretion or depleting EV iron cargo [94]. In summary, EVs originating from various cells under metabolic stress contribute to various facets of MASLD pathogenesis, including hepatic inflammation, angiogenesis, and fibrosis, through the transfer of various cargos within the complex network (Table 3).

EVs IN ATHEROSCLEROSIS: LOCAL AND SYSTEMIC CROSSTALK DRIVING CVD

In the process of atherosclerosis development and progression, EVs from various cells are emerging as key players and have both favorable and detrimental effects by controlling endothelial dysfunction, vascular calcification, plaque stability and thrombus formation [95,96]. An analysis of plaque micropar-

Table 3. Representative studies on the role of EVs in metabolic dysfunction-associated steatotic liver disease

Source	Cargo	Target	Main findings	Reference
miRNA				
Neutrophil	miR-223	Hepatocyte	miR-223-enriched EVs derived from neutrophils are taken up by hepatocytes in an APOE-LDLR dependent manner miR-223 inhibits hepatic inflammatory and fibrogenic gene expression and ameliorates NASH	[77]
Macrophage	miR-223	Hepatocyte	IL-6 promotes miR-223-enriched exosome production in macrophages and reduces profibrotic TAZ expression in hepatocytes by exosomal transfer HFD-fed IL-6 knockout mice had worse liver injury and fibrosis, and hepatocyte-specific IL-6 receptor knockout mice had more steatosis and liver injury compared to those in wild-type mice	[78]
Adipose tissue	miR-199a-5p	Hepatocyte	miR-199a is increased in the HFD-fed mice, especially in the adipose tissue miR-199a suppresses MST1 expression and modulates hepatic lipogenesis and lipolysis in hepatocytes aggravating liver lipid accumulation	[79]
Hepatocyte	miR-1297	HSC	miR-1297 is highly expressed in lipotoxic hepatocyte-derived exosomes miR-1297 promotes activation and proliferation of HSCs through the PTEN/PI3K/AKT signaling pathway and accelerates the progression of MAFLD	[80]
Hepatocyte	miR-27a	HSC	Serum exosomal miR-27a level is positively correlated with liver fibrosis in MAFLD patients and mice Lipotoxic hepatocyte-exosomal miR-27a inhibits mitophagy and promotes MAFLD-related liver fibrosis by negatively regulating PINK1 expression	[81]
Hepatocyte	miR-128-3p	HSC	Hepatocyte-derived EVs are released as a response to lipotoxicity, and internalized by HSC, leading to the activation of HSC The EVs carry miR-128-3p which suppresses PPARγ expression, thereby contributing to the HSC activation	[82]
Hepatocyte	miR-122	Macrophage	MMP2 is essential for transfer of EVs and their miRNA content from hepatic to non-hepatic cells The transfer of proinflammatory miR-122 from hepatocytes to liver resident macrophage cells is dependent on MMP2 in MCD diet-fed mice liver	[84]
Hepatocyte	miR-192-5p	Macrophage	Lipotoxic hepatocytes release exosomes enriched with miR-192-5p, which induce M1 macrophage activation miR-192-5p inhibits Rictor/Akt/FoxO pathway which induce inflammatory response Serum miR-192-5p levels positively correlate with hepatic inflammation in NAFLD patients	[85]
Protein				
Adipocyte	Aldo-keto-reductase 1B7	Hepatocyte	ER stress-induced adipocyte exosomes trigger NASH by delivering aldo-keto-reductase 1B7 Aldo-keto-reductase 1B7 leads to accumulation of glycerol and triglycerides in hepatocytes	[86]
Adipocyte	CD36	Hepatocyte	HFD-mediated AMPKα1 inhibition increases adipocyte exosome release CD36-containing exosomes are endocytosed by hepatocytes to induce lipid accumulation and inflammation promoting NAFLD	[87]
Hepatocyte	Integrin β1	Monocyte	Lipotoxic stressed hepatocytes release integrin β1 enriched EVs Integrin β1 enriched EVs enhance monocyte adhesion to liver sinusoidal endothelial cells which lead to hepatic inflammation	[88]
Hepatocyte	CXCL10	Macrophage	MLK3 mediates the release of EVs laden with CXCL10 from lipotoxic hepatocytes, which induce macrophage chemotaxis The protective effect against liver injury is conferred through genetic or chemical inhibition of MLK3	[89]
Hepatocyte	TRAIL	Macrophage	Lipotoxic hepatocytes release inflammatory EVs via DR5-ROCK1 signaling TRAIL-bearing EVs stimulate proinflammatory cascade in macrophages	[90]
Lipid				
Hepatocyte	Ceramide	Macrophage	Activated IRE1α promotes transcription of serine palmitoyltransferase genes via XBP1 in hepatocytes, resulting in ceramide biosynthesis and release of EVs These EVs recruit macrophages to the liver, resulting in inflammation and injury	[91]
Hepatocyte	C16:0 Ceramide	Macrophage	C16:0 ceramide-enriched proinflammatory EVs are released from lipotoxic hepatocytes in an IRE1α-dependent manner These EVs activated macrophage chemotaxis via formation of S1P from C16:0 ceramide	[92]
Hepatocyte	SFA (palmitic, stearic)	Macrophage	Hepatocyte-derived small EVs transport SFA to macrophages/Kupffer cells which activate TLR4-mediated inflammatory response Macrophage inflammation subsequently induces hepatic insulin resistance	[93]
Others				
Hepatocyte	Iron	HSC	Hepatocyte-derived iron-containing EVs lead to hepatocyte iron deficiency and HSC iron overload Iron accumulation results in reactive oxygen species overproduction and HSC activation leading to liver steatosis and fibrosis	[94]

EV, extracellular vesicle; miR, microRNA; APOE, apolipoprotein E; LDLR, low-density lipoprotein receptor; NASH, nonalcoholic steatohepatitis; IL, interleukin; TAZ, transcriptional activator with PDZ-binding motif; HFD, high-fat diet; MST1, macrophage stimulating 1; HSC, hepatic stellate cell; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; MAFLD, metabolic dysfunction-associated fatty liver disease; PINK1, PTEN-induced kinase 1; PPARγ, peroxisome proliferator-activated receptor gamma; MMP2, matrix metalloproteinase 2; MCD, methionine/choline-deficient; Rictor, rapamycin-insensitive companion of mammalian target of rapamycin; FoxO, forkhead box O; NAFLD, nonalcoholic fatty liver disease; ER, endoplasmic reticulum; AMPK, AMP-activated protein kinase; CXCL10, C-X-C motif chemokine ligand 10; MLK3, mixed lineage kinase 3; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; DR5, death receptor 5; ROCK1, rho-associated coiled-coil-containing protein kinase 1; IRE1α, inositol-requiring enzyme 1α; XBP1, X-box binding protein 1; S1P, sphingosine 1-phosphate; SFA, saturated fatty acid; TLR4, toll-like receptor 4.

ticles from carotid endarterectomy samples revealed that EVs involved in atherogenesis originated from leukocytes, macrophages, granulocytes, erythrocytes, smooth muscle cells, and endothelial cells [97].

Local crosstalk by EVs among diverse cells in the atherogenic milieu

A complex network between diverse immune cells and endothelial cells mediated by EVs has been identified in the atherogenic milieu. Exosomes from immune-activated monocytes carry inflammatory miRNA cargo and upregulate intercellular adhesion molecule-1 (ICAM-1), chemokine ligand 2 (CCL-2), and IL-6 via TLR4 and NF-κB pathways in endothelial cells [98]. Plaque EVs also promote inflammatory cell recruitment by transferring ICAM-1 to endothelial cells which contributes to plaque instability [99]. Enrichment of several miRNAs, particularly miR-146a, was found in EVs from oxidized low-density lipoprotein-treated atherogenic macrophages. These EVs are delivered to naïve recipient macrophages and reduce migratory capacity promoting macrophage entrapment in the vessel wall and the development of atherosclerosis [100]. Vascular smooth muscle cell (VSMC)-derived exosomes from diabetic sources induce vascular inflammation, proinflammatory polarization of monocytes, and atherosclerotic plaque formation by a paracrine signaling of transferring miR-221/222 [101]. EVs isolated from the red blood cells of patients with T2DM impair endothelial function by delivering arginase-1 and inducing oxidative stress [102].

Perivascular adipose tissue also plays a role in vascular homeostasis. Perivascular adipose tissue-derived exosomes from patients with coronary atherosclerotic heart disease showed a lower miR-382-5p expression level than those from healthy individuals. miR-382-5p reduces macrophage foam cell formation through the upregulation of the cholesterol efflux transporters adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) [103]. Exosomes from adipose-derived mesenchymal stem cells transfer miR-324-5p to endothelial cells, which protects endothelial cells against atherosclerosis by inhibiting protein phosphatase 1 regulatory subunit 12B (PPP1R12B)-mediated apoptosis [104]. Exosomes secreted from dendritic cells containing miR-203-3p target cathepsin S in bone marrow-derived macrophages attenuating atherosclerosis at both the cellular and mouse levels [105]. These examples reveal local crosstalk among various components in the vessel.

Distant organ-derived EV signals driving atherosclerosis and CVD

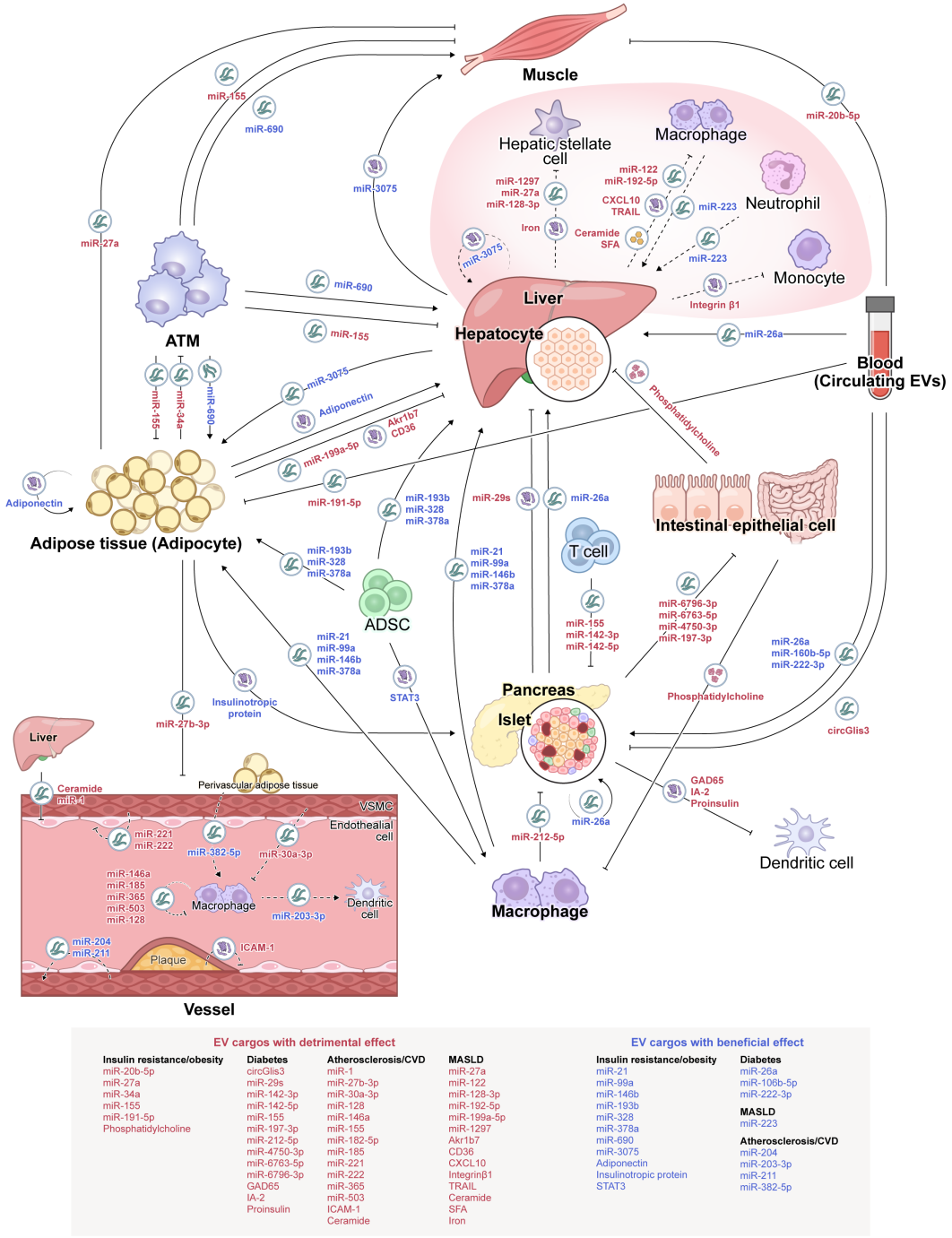
Signals from distant organs also drive atherosclerosis and CVD. Epidemiologic studies demonstrated MASLD as an independent risk factor for CVD, although underlying molecular mechanisms are not fully understood [69]. Recent studies suggest that EVs may play a role in mediating this association. EVs derived from steatotic hepatocytes showed inducing of endothelial inflammation and promoting atherosclerosis. miR-1 was identified as a key cargo within these EVs, contributing to this effect through the suppression of KLF4 and activation of NF-κB. miR-1 inhibition attenuated atherogenesis in $ApoE^{-/-}$ mice [106]. Similarly, miR-30a-3p from steatotic hepatocyte-derived small EVs inhibits ABCA1-mediated cholesterol efflux, thereby promoting foam cell formation in atherosclerosis. Treatment of antagomir-30a-3p attenuated diet-induced atherosclerosis in $ApoE^{-/-}$ mice [107]. Liver-specific deletion of the acid ceramidase gene N-acylsphingosine amidohydrolase 1 (*Asah1*) enhanced ceramide levels and liver lipid deposition in HFD-fed mice accompanied by increased EV release. This led to Nod-like receptor pyrin domain 3 (NLRP3) inflammasome activation and neointimal hyperplasia explaining the mechanism of MASLD-associated vascular endothelial dysfunction [108]. Exosomal miR-27b-3p transferred from the visceral adipocytes to vascular endothelial cells also activates NF-κB pathway by degrading PPARα mRNA which eventually induces endothelial inflammation and atherogenesis. Administration of miR-27b-3p mimic accelerated atherosclerotic plaque formation in $ApoE^{-/-}$ mice [109]. Another study showed that exosomes from visceral adipose tissue of HFD-fed mice facilitated macrophage foam cell formation, M1 polarization, and proinflammatory cytokine secretion. These findings were accompanied by the downregulation of ATP-binding cassette transporter-mediated cholesterol efflux and increased phosphorylation of NF-κB-p65. Systemic injection of these exosomes for 6 weeks exacerbated atherosclerosis in HFD-fed $ApoE^{-/-}$ mice [110].

Insufficient or disrupted sleep is also associated with cardiovascular disease risk. Melatonin plays important roles in sleep, circadian rhythms, and cardiovascular systems. Melatonin treatment attenuated osteogenic differentiation and senescence of VSMCs, and therefore delaying vascular calcification and aging. These effects were mediated by miR-204 and miR-211 from VSMC-derived exosomes through paracrine mechanism [111]. Circulating exosomes from sleep-deprived human and

Table 4. Representative studies on the role of EVs in atherosclerosis and cardiovascular disease

Source	Cargo	Target	Main findings	Reference
miRNA				
Macrophage	miR-146a, miR-128, miR-185, miR-365, miR-503	Macrophage	OxLDL-treated macrophage-derived EVs transfer miRNA to naïve recipient macrophages and reduce migratory capacity promoting macrophage entrapment in the vessel wall	[100]
VSMC	miR-221/222	Endothelial cell	ICAM-1 expression and monocyte adhesion were increased in endothelial cells exposed to VSMC-derived exosomes from diabetic sources VSMC-derived exosomes from diabetic sources promoted proinflammatory polarization of monocyte in a miR-221/222 dependent manner *In vivo* administration of VSMC-derived exosomes from diabetic sources increased atherosclerotic plaque development	[101]
Perivascular adipose tissue	miR-382-5p	Macrophage	Exosomes released from perivascular adipose tissue reduce macrophage foam cell formation through miR-382-5p-mediated upregulation of cholesterol efflux transporters	[103]
Dendritic cell	miR-203-3p	Macrophage	Transfer of miR-203-3p by dendritic cell-derived exosomes target cathepsin S in bone marrow-derived macrophages and attenuate atherosclerosis progression	[105]
Hepatocyte	miR-1	Endothelial cell	EVs derived from steatotic hepatocytes induce endothelial inflammation via miR-1 The effect of miR-1 is mediated by KLF4 suppression and NF-κB activation Inhibition of miR-1 attenuates atherogenesis	[106]
Hepatocyte	miR-30a-3p	Macrophage	Small EVs from steatotic hepatocytes promote foam cell formation and atherosclerosis progression via inhibition of ABCA1-mediated cholesterol efflux miR-30a-3p is enriched in EVs which inhibits ABCA1 expression and cholesterol efflux	[107]
Visceral adipocyte	miR-27b-3p	Endothelial cell	Visceral fat-derived exosomal miR-27b-3p enters into the vascular endothelial cells and activates the NF-κB pathway by down-regulating PPARα Administration of miR-27b-3p mimic increased inflammation and atherogenesis in ApoE-deficient mice	[109]
VSMC	miR-204, miR-211	VSMC	Exosomes secreted by melatonin-treated VSMCs attenuate the osteogenic differentiation and senescence of VSMCs in a paracrine manner mediated by miR-204/miR-211	[111]
Small intestinal epithelium	miR-182-5p	Endothelial cell	Sleep deprivation or reduction of melatonin decreased the synthesis of miR-182-5p in small intestinal epitheium Plasma exosomes from sleep-deprived mice or human induced endothelial inflammation and atherogenesis through miR-182-5p – MYD88 – NF-κB/NLRP3 pathway	[112]
Protein				
Plaque	ICAM-1	Endothelial cell	Microparticles isolated from human atherosclerotic plaques transfer ICAM-1 to endothelial cell membrane Plaque microparticles promote atherosclerotic plaque progression by recruiting inflammatory cells	[99]
Lipids				
Hepatocyte	Ceramide	Endothelial cell	Acid ceramidase/ceramide signaling pathway controls EV release from the liver Deficiency of acid ceramidase gene Asah1 aggravates NAFLD and increases hepatic EV release promoting endothelial NLRP3 inflammasome activation and carotid neointima hyperplasia	[108]

EV, extracellular vesicle; miR, microRNA; OxLDL, oxidized low-density lipoprotein; VSMC, vascular smooth muscle cell; ICAM-1, intercellular adhesion molecule-1; KLF4, Krüppel-like factor 4; NF-κB, nuclear factor kappa-B; ABCA1, ATP-binding cassette transporter A1; PPARα, peroxisome proliferator-activated receptor alpha; MYD88, myeloid differentiation factor 88; NLRP3, Nod-like receptor pyrin domain 3; Asah1, N-acylsphingosine amidohydrolase 1; NAFLD, nonalcoholic fatty liver disease.



EV cargos with detrimental effect

Insulin resistance/obesity	Diabetes	Atherosclerosis/CVD	MASLD
miR-20b-5p	circGlis3	miR-1	miR-27a
miR-27a	miR-29s	miR-27b-3p	miR-122
miR-34a	miR-142-3p	miR-30a-3p	miR-128-3p
miR-155	miR-142-5p	miR-128	miR-192-5p
miR-191-5p	miR-155	miR-146a	miR-199a-5p
Phosphatidylcholine	miR-197-3p	miR-155	miR-1297
	miR-212-5p	miR-182-5p	Akr1b7
	miR-4750-3p	miR-185	CD36
	miR-6763-5p	miR-221	CXCL10
	miR-6796-3p	miR-222	Integrinβ1
	GAD65	miR-365	TRAIL
	IA-2	miR-503	Ceramide
	Proinsulin	ICAM-1	SFA
		Ceramide	Iron

EV cargos with beneficial effect

Insulin resistance/obesity	Diabetes	MASLD	Atherosclerosis/CVD
miR-21	miR-26a	miR-223	miR-204
miR-99a	miR-106b-5p		miR-203-3p
miR-146b	miR-222-3p		miR-211
miR-193b			miR-382-5p
miR-328			
miR-378a			
miR-690			
miR-3075			
Adiponectin			
Insulinotropic protein			
STAT3			

Fig. 2. Extracellular vesicle (EV)-mediated network in the pathogenesis of cardiometabolic diseases. EVs carry miRNAs, proteins, and lipids that mediate intercellular or interorgan communication, contributing to the development of obesity, insulin resistance, diabetes mellitus, metabolic dysfunction-associated liver disease, and cardiovascular disease. Blue characters indicate EV cargos with beneficial effects, while red characters indicate EV cargos with detrimental effects. Dashed lines represent intraorgan crosstalk, and solid lines denote interorgan or distant crosstalk. CXCL10, C-X-C motif chemokine 10; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; SFA, saturated fatty acid; ATM, adipose tissue macrophage; ADSC, adipose-derived stem cell; STAT3, signal transducer and activator of transcription 3; VSMC, vascular smooth muscle cell; ICAM-1, intercellular adhesion molecule-1; GAD65, glutamate decarboxylase 65; IA-2, islet antigen 2.

mice were a potent inducer of atherogenesis and endothelial inflammation. In this condition, exosomal miR-182-5p secreted from small intestinal epithelium was decreased, upregulating myeloid differentiation factor 88 (MYD88)—NF-κB/NLRP3 pathway in endothelial cells [112]. Collectively, complex interplay among various cells and organs via EVs mediate atherogenesis and CVD (Table 4).

THERAPEUTIC POTENTIALS OF EV

EVs have potential as a drug-delivery platform or as therapeutics to target specific cells and transfer functional molecules. Incorporation of cargos into or onto isolated EVs (exogenous loading) by manipulations including co-incubation, electroporation, and sonication can utilize EVs as natural delivery vectors. Alternatively, the parental cell can be genetically modified to overexpress desired RNA or protein of interest, which will be loaded during the EV biogenesis (endogenous loading) [113]. According to a literature review by Fusco et al. [114], 40 studies examining EVs as human therapeutics were published up to the end of 2023. Most of these studies were small-sized non-randomized trials without control group. Treated diseases varied including malignant tumor, coronavirus disease-19 infection, skin diseases, neurologic diseases, graft versus host disease, ulcers and delayed wound healing, skin aging, hair loss, etc. Approximately two thirds used mesenchymal stem cells as an EV source, and all were non-engineered EVs. Based on these observations, it seems that the application of EV formulations for human therapeutics is still in the early stage and multiple challenges remain. Defining optimal cellular sources for specific diseases, standardization of methods for isolation, characterization and large-scale production of EVs, development of efficient storing procedure, establishment of appropriate dosing and route of administration for efficacy and safety, and evaluation of biodistribution, clearance, and the long-term risks of EVs are important aspects that need to be further clarified [113-115].

CONCLUSIONS AND FUTURE PERSPECTIVES

Cardiometabolic diseases are systemic disorders with complex interorgan networks in their pathophysiology. Despite rapid expansion and meaningful progress in EV research, it is still underrepresented in the field of cardiovascular or metabolic diseases. However, emerging evidence suggests that EVs play a significant role in these processes as described in this study (Fig. 2). Therefore, research into EVs holds immense promise for advancing our understanding of cardiovascular and metabolic diseases. Advances in EV isolation methods and characterization techniques may enable better disease prediction and monitoring by utilizing EVs as early and non-invasive biomarkers. Novel techniques for profiling EV surface markers, such as the proximity-dependent barcoding assay or immunomagnetic bead-based methods, are being applied to separate EVs originating from specific cells in mixed samples [116,117]. In addition, EVs have therapeutic potential with their ability to serve as targeted delivery systems for drugs, RNAs, and other bioactive compounds. Engineering EVs for loading specific cargo and targeting specific receptor cells could lead to breakthroughs in treating diseases by modulating pathological signaling pathways. However, many challenges remain, including standardization of EV isolation methods, understanding the heterogeneity of EV populations, establishing accurate differentiation and separation techniques, and elucidating the precise mechanisms through which EVs contribute to disease progression. The efficacy, safety, and scalability of EVs also need to be addressed because of potential immunogenicity, unintended off-target effects, and variability in EV production during large-scale manufacturing. Regulatory guidelines for EV-based therapies need to evolve to ensure clinical applicability. Addressing these challenges will require interdisciplinary collaboration and innovative technologies, ultimately opening the way for EV-based diagnostics and therapies in cardiometabolic disease management.

CONFLICTS OF INTEREST

Seung-Hwan Lee has been an associate editor of the *Diabetes & Metabolism Journal* since 2022. He was not involved in the review process of this article. The authors declare that they have no competing interests.

ORCID

Joonyub Lee *https://doi.org/0000-0003-0533-9786*
Won Gun Choi *https://doi.org/0000-0002-5628-5581*
Seung-Hwan Lee *https://doi.org/0000-0002-3964-3877*

FUNDING

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2023R1A2C2004341 to Seung-Hwan Lee and RS-2025-00555223 to Joonyub Lee) and the research fund of Seoul St. Mary's Hospital, The Catholic University of Korea (to Seung-Hwan Lee).

ACKNOWLEDGMENTS

None

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