

Original Research Article

EXTRACELLULAR VESICLES (EXOSOMES) AS EMERGING BIOMARKERS IN METABOLIC SYNDROME AND DIABETIC CARDIOVASCULAR RISK

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ABSTRACT

Background: Metabolic syndrome now affects an estimated 1.54 billion adults worldwide, and diabetes-associated cardiovascular disease remains the leading cause of death in this population. Conventional biomarkers — fasting glucose, HbA1c, lipid panels, and high-sensitivity C-reactive protein — capture only part of the underlying pathophysiology and often detect cardiovascular risk only after subclinical damage has occurred. Extracellular vesicles (EVs), particularly exosomes, have emerged as nanoscale carriers of tissue-specific molecular cargo that may close this gap. The objective is to systematically review evidence for EVs/exosomes as biomarkers of metabolic syndrome and diabetic cardiovascular risk, appraise their merits and demerits relative to conventional biomarkers, and outline a translational research agenda suited to a medical physiology–biotechnology collaboration.

Materials and Methods: A PRISMA-informed search of PubMed/MEDLINE, Scopus, Web of Science, and the Cochrane Library, supplemented by ISEV/MISEV methodological archives and ClinicalTrials.gov, was conducted for records to July 2026. Human biomarker/cohort studies, mechanistic studies with human validation, EV isolation/characterisation papers, and therapeutic EV studies were included; 87 sources met eligibility and were synthesised narratively across four domains.

Results: Adipose, hepatic, skeletal-muscle, and endothelial cell-derived EVs act as genuine endocrine mediators, transferring miRNA and protein cargo that modulates insulin signalling, inflammation, and vascular tone in recipient tissues. Circulating EV numbers and cargo correlate with HOMA-IR and respond to interventions such as insulin infusion. In diabetic cardiovascular disease, exosomal miRNA panels — including miR-155-5p (AUC 0.901) and a four-miRNA panel (AUC 0.833, validated at 0.816) — showed strong diagnostic performance for subclinical carotid atherosclerosis, consistently outperforming unfractionated plasma markers.

Conclusion: EV-based biomarkers offer mechanistically grounded advantages over conventional markers, particularly earlier detection and tissue-of-origin specificity, but face standardisation, cost, and validation barriers. Pairing physiological cohort access with biotechnological EV analysis capability represents an under-exploited research opportunity.

Keywords: extracellular vesicles; exosomes; metabolic syndrome; insulin resistance; type 2 diabetes; cardiovascular risk; biomarkers; microRNA; MISEV; systematic review.

INTRODUCTION

The Scale of the Problem: Metabolic Syndrome and Diabetic Cardiovascular Risk

Metabolic syndrome — the clustering of central obesity, insulin resistance, dyslipidaemia, and hypertension — has become one of the defining chronic disease burdens of the twenty-first century. A

2025 Bayesian modelling analysis spanning more than 45 million adults across multiple countries estimated that global metabolic syndrome prevalence rose from 14.7% among women and 9.0% among men in 2000 to 31.0% and 25.7% respectively by 2023, corresponding to approximately 1.54 billion affected adults worldwide.^[1] Type 2 diabetes mellitus (T2DM), both a component and a consequence of this cluster, affected an estimated 506 million people in 2021 and is projected by the International Diabetes Federation's 11th edition atlas to reach a global prevalence of 12.96% — roughly 853 million people — by 2050.^[2]

The clinical significance of this burden lies substantially in its cardiovascular consequences. Cardiovascular disease remains the leading cause of death among individuals with T2DM, and diabetes independently confers a roughly two-fold increase in cardiovascular risk even after adjusting for conventional risk factors. Among people already diagnosed with T2DM,^[3] pooled estimates suggest metabolic syndrome itself is present in well over half of patients depending on the diagnostic criteria applied, compounding cardiovascular risk through overlapping mechanisms of insulin resistance, chronic low-grade inflammation, dyslipidaemia, and endothelial dysfunction that begin years before any clinical event.^[4]

The Limits of Conventional Biomarkers

Current clinical practice relies on a well-established but fundamentally limited panel of biomarkers: fasting plasma glucose, glycated haemoglobin (HbA1c), fasting lipid profile, the homeostatic model assessment of insulin resistance (HOMA-IR), and, for cardiovascular risk stratification, high-sensitivity C-reactive protein, natriuretic peptides, and cardiac troponins. These markers are inexpensive, standardised, and clinically actionable, but they share two structural limitations relevant to this review. First, they are largely tissue-non-specific — an elevated HbA1c reflects average glycaemia but says nothing about which organ (liver, muscle, adipose, endothelium) is driving a given patient's insulin resistance, information that could meaningfully guide individualised management.^[5] Second, most of these markers become abnormal only after substantial pathophysiological change has already occurred; hs-CRP and troponin, for instance, typically rise only once vascular inflammation or myocardial injury is already underway, offering limited lead time for the truly early, subclinical intervention window that would do the best.^[6]

The clinical void that this strategy aims to bridge is to address by a combination of a sizable and expanding at-risk population with biomarkers that report tissue-specificity and/or consistently at inception.

Extracellular Vesicles: Biology at a Glance

Extracellular vesicles are lipid-bilayer-enclosed particles released by essentially all cell types into biological fluids, incapable of self-replication since they lack a functional nucleus. Once dismissed after

their initial description in 1983 as a mechanism for discarding unwanted cellular material,^[7] EVs are now recognised as active, [Figure 1] bidirectional carriers of biological information — proteins, lipids, and nucleic acids (mRNA, miRNA, and long non-coding RNA) — that are transferred between cells and can functionally reprogram the behaviour of recipient tissue.^[8]

Two broad EV subpopulations are conventionally distinguished by biogenesis route. Exosomes originate from the endosomal system: inward budding of the limiting membrane of a late endosome generates intraluminal vesicles (ILVs) within a multivesicular body (MVB), through either ESCRT-dependent machinery or ESCRT-independent, ceramide-driven pathways; the MVB then either fuses with the lysosome for degradation or traffics to and fuses with the plasma membrane, releasing its ILVs into the extracellular space as exosomes,^[9] typically 30-150 nm in diameter. Ectosomes (often loosely termed microvesicles), by contrast, form by direct outward budding and fission from the plasma membrane itself, and are generally larger (100-1,000 nm). Because these two biogenesis routes cannot be reliably distinguished by size or density alone using standard isolation methods, the field's 2023 consensus guidance (MISEV2023, discussed in Section 3.2) now recommends the operational terms 'small EVs' (<200 nm) and 'large EVs' (>200 nm) rather than exosome/microvesicle terminology unless endosomal origin has been specifically confirmed. Figure 1 summarises this biogenesis pathway and the cargo carried by released vesicles.^[10]

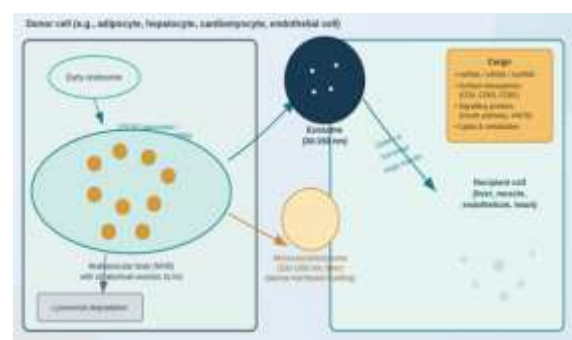


Figure 1: Biogenesis, cargo, and intercellular transfer of extracellular vesicles (exosomes and ectosomes).

Rationale: Why Extracellular Vesicles as Biomarkers

EVs offer several theoretical advantages over free-circulating analytes as biomarkers. Because they combine biological specificity with useful benefits in terms of cost, ease of sample extraction, transportation, storage, and diagnostic performance. Extracellular vesicles (EVs), such as exosomes and microvesicles, are becoming more viable biomarkers.^[11] EVs can be non-invasively separated from easily accessible bodily fluids such as urine, saliva; while in more serious cases minimally invasive methods of sample extraction may be implemented such as blood and cerebrospinal fluid:

which lowers procedure risks and medical expenses in contrast to traditional tissue biopsies.

The stability of EVs is offered by their phospholipid bilayer that ensures higher molecular stability than free-circulating analytes during sample handling, transportation, and long-term storage by shielding encapsulated proteins, lipids, metabolites, and especially delicate RNA species, like miRNAs, from degradation by circulating RNases. EV-containing samples are therefore ideal for biobanking, multicenter research, and centralized diagnostic testing since they may be transferred over great distances and kept at -80°C or under suitable cryopreservation settings with little loss of biomarker integrity. Crucially, EVs maintain surface proteins that are typical of their parent cells, such as tetraspanins (CD9, CD63, and CD81) and tissue-specific markers.^[12] This allows for the identification of the EVs' original tissue, such as hepatocytes, adipocytes, cardiomyocytes, or endothelial cells, a degree of specificity that is rarely attained by traditional serum biomarkers. Furthermore, EVs are active mediators of intercellular communication and pathophysiological processes rather than just passive markers of illness. alterations in their molecular cargo frequently take place prior to observable biochemical or clinical alterations, offering a chance for early illness monitoring and identification. These combined advantages have already established EV-based liquid biopsy in oncology and are driving its rapid development as a cost-effective and highly informative diagnostic platform for cardiometabolic and other complex diseases.^[13]

Objectives of This Review

This review had four objectives: (i) to summarise EV biology, isolation, and characterisation as relevant to biomarker research; (ii) to synthesise evidence on EVs as biomarkers and mediators in metabolic syndrome and insulin resistance; (iii) to synthesise evidence on EVs as biomarkers in diabetic cardiovascular disease, including a structured merits/demerits appraisal against conventional biomarkers; and (iv) to outline a concrete, collaborative physiology-biotechnology research agenda in this space.

MATERIALS AND METHODS

Search Strategy: A structured search of PubMed/MEDLINE, Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials was conducted for records to July 2026, supplemented by the International Society for Extracellular Vesicles (ISEV) MISEV methodological archive and ClinicalTrials.gov. Search terms combined 'extracellular vesicle', 'exosome', 'microvesicle', 'metabolic syndrome', 'insulin resistance', 'type 2 diabetes', 'cardiovascular disease', 'atherosclerosis', 'diabetic cardiomyopathy', 'biomarker', and 'microRNA' using standard Boolean operators.

2.2 Eligibility Criteria

Eligible study types included human biomarker or cohort studies reporting EV-derived analytes in relation to metabolic or cardiovascular outcomes; mechanistic or preclinical studies that included a human validation component; EV isolation and characterisation methodology papers, including the MISEV2018 and MISEV2023 position papers; and therapeutic or mesenchymal stem cell (MSC)-EV studies relevant to metabolic-cardiovascular disease. Animal-only or in-vitro-only studies without any human correlate, conference abstracts without full data, duplicate cohort reporting, and studies not reporting EV-specific isolation or characterisation methods were excluded.

2.3 Study Selection

Titles and abstracts were screened for relevance, followed by full-text assessment of potentially eligible records against the criteria above. The selection process, summarised in Figure 2, yielded 87 included studies grouped into four domains: EV biology and isolation methodology, EVs in metabolic syndrome/insulin resistance, EVs in diabetic cardiovascular risk, and EV-based therapeutics.

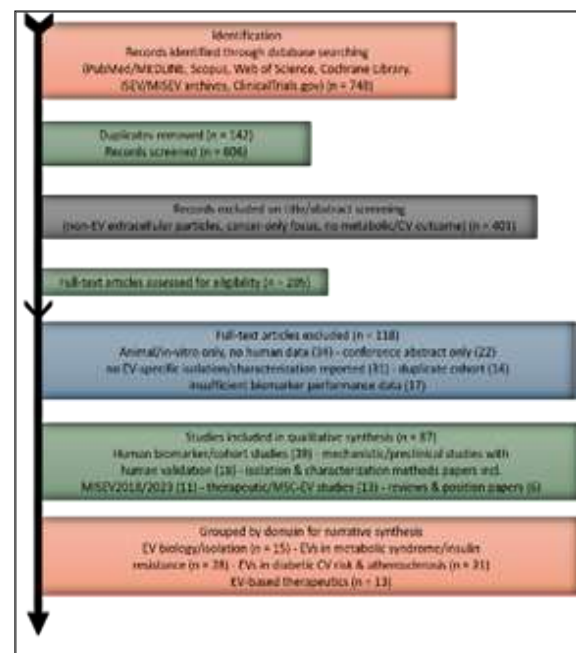


Figure 2: PRISMA-style flow diagram of study identification and selection for this review.

Quality Appraisal: Human biomarker and cohort studies were appraised informally for sample size adequacy, use of validation cohorts, and adherence to MISEV reporting standards for EV isolation and characterisation, since inadequate EV characterisation is a well-recognised source of false-positive biomarker findings in this field. Diagnostic accuracy studies were additionally assessed for whether receiver operating characteristic (ROC) analysis was performed in an independent validation

cohort rather than the discovery cohort alone. Mechanistic/preclinical studies were weighted as hypothesis-generating unless they included a human validation arm. No formal GRADE certainty rating was applied, consistent with this review's narrative rather than meta-analytic design (Section 2.5).

Synthesis Approach: Given the heterogeneity of isolation methods, cargo analytes, and outcome definitions across included studies, a narrative synthesis organised by physiological domain (metabolic syndrome/insulin resistance; diabetic cardiovascular disease) was used in preference to formal meta-analysis. Where multiple studies reported diagnostic accuracy (AUC) for the same or overlapping analytes, results are presented together for comparison (Figure 3) while explicitly noting that cross-study AUC comparisons are indicative rather than pooled effect estimates, given differing patient populations, EV isolation protocols, and reference standards across studies.

RESULTS

EV Subtypes, Isolation, and Characterisation: The MISEV Framework

Reproducibility has historically been the extracellular vesicle field's central methodological challenge, prompting the International Society for Extracellular Vesicles to issue consensus 'Minimal Information for Studies of Extracellular Vesicles' (MISEV) guidelines in 2014,^[15] updated in 2018 and most recently in 2023 (MISEV2023).^[16,17] These guidelines specify minimum requirements for EV

production, separation, and characterisation across cell culture, body fluid, and tissue sources, and are now the de facto standard against which biomarker studies in this field are judged for methodological rigour.^[18]

Isolation methods vary substantially in their trade-off between purity and yield. Differential ultracentrifugation, historically the most widely used approach, separates EV subtypes by size and density but co-isolates non-vesicular extracellular particles (protein aggregates, lipoproteins) at high centrifugal speeds. Size-exclusion chromatography (SEC) separates EVs from many, though not all, non-vesicular particles while keeping all EV subtypes together, and MISEV2023 rates its specificity as broadly comparable to ultracentrifugation.¹⁸ Density-gradient ultracentrifugation offers higher specificity at the cost of lower yield and longer processing time.^[19] Precipitation-based commercial kits, widely used for their convenience, are explicitly flagged in MISEV2023 as typically isolating a mixture of EVs and non-vesicular extracellular particles rather than pure EV preparations, a limitation with direct implications for biomarker specificity discussed further in Section 4.2. Immunoaffinity capture, using antibodies against surface tetraspanins or tissue-specific markers, offers the highest specificity and is particularly relevant for isolating tissue-of-origin-specific EV subpopulations (for example, adipocyte- or cardiomyocyte-derived EVs) but at correspondingly lower yield and higher cost. [Table 1] summarises these methods alongside recommended characterisation techniques.

Table 1: Extracellular vesicle isolation and characterisation methods per MISEV2023 recommendations.

Method	Principle	Specificity	Yield	Key limitation
Differential ultracentrifugation	Sequential centrifugation at increasing speeds pellets EVs by size/density	Moderate	Moderate-High	Co-isolates non-vesicular particles at high speed; time-intensive
Density-gradient ultracentrifugation	EVs separated by buoyant density in sucrose/iodixanol gradient	High	Low-Moderate	Long processing time; specialised equipment
Size-exclusion chromatography (SEC)	Particles separated by hydrodynamic size through porous resin	Moderate-High	Moderate	Co-elutes similarly sized lipoproteins; dilutes sample
Precipitation-based kits	Polymer-induced EV aggregation and low-speed pelleting	Low	High	Co-precipitates non-EV proteins; not pure EV isolation per MISEV2023
Immunoaffinity capture	Antibody-bead capture of surface tetraspanins or tissue-specific markers	Very High	Low	High cost; requires known target marker; low throughput

For characterisation, MISEV2023 emphasises that no single technique is sufficient and recommends combining at least one bulk-level technique (protein quantification, Western blot for positive markers such as tetraspanins and negative markers such as intracellular organelle proteins to exclude cellular contamination) with at least one single-particle-level technique (nanoparticle tracking analysis, nanoflow cytometry, or transmission/immuno-electron microscopy) to confirm both the presence of bona fide EVs and their approximate size distribution and concentration.^[20]

Extracellular Vesicles in Metabolic Syndrome and Insulin Resistance

Adipose Tissue-Derived EVs as Endocrine Mediators: Adipose tissue is now recognised as a

major source of circulating exosomal miRNA,^[21] and adipose-derived EVs have been established as genuine endocrine mediators capable of regulating gene expression in distant tissues including the liver.^[22] In murine models of obesity, adipose tissue macrophage-derived exosomal miRNAs directly modulate glucose and lipid metabolism in recipient hepatocytes and myocytes, providing a mechanistic explanation for the long-observed but poorly understood metabolic crosstalk between visceral adiposity and systemic insulin resistance.^[23] Human studies corroborate this: obesity-associated EVs ('obesomes') isolated from patients with obesity promote pro-inflammatory cytokine secretion when co-cultured with macrophages and impair insulin sensitivity — evidenced by reduced Akt and S6

phosphorylation — when co-cultured with hepatocytes, directly altering lipid and glucose metabolism in the recipient cells.^[24]

Hepatic and Skeletal Muscle EVs: The liver contributes its own EV population to this crosstalk network. EVs secreted by steatotic hepatocytes ('steatosomes') induce insulin resistance and disrupt lipid and glucose metabolism when transferred to healthy hepatocytes *in vitro*, in part through decreased expression of PPAR α , PPAR β , and perilipin 3. (PPAR α , PPAR β , and perilipin 3 are the master lipid sensors),^[25] Proteomic profiling of steatosomes has identified liver-disease-associated proteins with potential utility as early biomarkers of hepatic steatosis, a common and often clinically silent component of metabolic syndrome.^[26] Skeletal muscle, the primary site of insulin-stimulated glucose disposal, also releases EVs whose cargo shifts with exercise, disuse, and metabolic status, though human mechanistic data here remain less mature than for adipose- and liver-derived EVs.^[27]

Circulating EV Number and Insulin Sensitivity: Direct Human Evidence: Beyond cargo-specific mechanistic studies, direct human physiological evidence links circulating EV burden to insulin

sensitivity. In adults with metabolic syndrome, a controlled insulin infusion (hyperinsulinaemic-euglycaemic clamp) produced a measurable decrease in circulating medium-sized EVs, one of the first studies to directly demonstrate that acute changes in insulin action are reflected in real time by circulating EV populations,^[28] and supporting a mechanistic role for EVs in modulating fuel substrate utilisation — EVs have separately been shown to increase reactive oxygen species production, which in turn suppresses mitochondrial fat oxidation.^[29] EVs also independently elevate reactive oxygen species (ROS) production within recipient cells; this oxidative burden suppresses mitochondrial fatty acid oxidation, impairing lipid clearance and further promoting metabolic dysfunction and insulin resistance in target tissues. A pilot proteomics comparison of serum exosome-enriched EVs from individuals with obesity-related insulin resistance (HOMA-IR > 2.0) against insulin-sensitive controls identified a distinct EV protein signature associated with the insulin-resistant state, reinforcing the concept that EV cargo, not merely EV number, tracks metabolic phenotype.^[30]

Table 2: summarises key EV-derived cargo and their reported metabolic associations across the studies reviewed here.

EV source	Key cargo	Metabolic association	Study design
Adipose-derived EVs ('obesomes')	miRNAs regulating hepatic/muscle gene expression; pro-inflammatory cytokines	Insulin resistance; macrophage inflammatory activation; correlates with central adiposity	Murine models + human obesity cohorts
Hepatocyte-derived EVs ('steatosomes')	Liver-disease-associated proteins; reduced PPAR α /PPAR β /perilipin 3 expression	Hepatic steatosis; impaired glucose/lipid metabolism in recipient hepatocytes	In-vitro human hepatocyte co-culture
Circulating medium/large EVs (mixed origin)	Total EV particle number/concentration	Decreases acutely with insulin infusion in metabolic syndrome adults	Hyperinsulinaemic-euglycaemic clamp study
Serum exosome proteome	Distinct protein signature (obesity-related IR vs insulin-sensitive)	Differentiates HOMA-IR > 2.0 from insulin-sensitive controls	Pilot proteomics comparison
Postprandial/diet-modulated EVs	Lipid and miRNA cargo reshaped by dietary fat quantity/quality	Endothelial dysfunction and thrombo-inflammation risk modulation	Narrative synthesis of omics-based studies

Emerging Directions: Diet, the Postprandial State, and Non-Mammalian EVs

A 2025 narrative review highlighted that EV abundance, cellular origin, and cargo are dynamically reshaped by the postprandial state, with the quantity and quality of dietary fat reprogramming EV lipid and miRNA content in ways that may modulate endothelial dysfunction, insulin resistance, and thrombo-inflammation risk after meals — a potentially important and still under-explored source of pre-analytical variability in fasting-state EV biomarker studies (Section 4.2).³¹ The same period has also seen growing interest in plant-derived nanoparticles and bacterial outer-membrane vesicles as scalable, potentially lower-cost alternatives to mammalian-cell-derived EVs for both diagnostic and therapeutic applications, though substantial interspecies differences in cargo composition and clearance kinetics currently limit direct translation of preclinical findings using these sources.

Merits and Demerits of EVs as Metabolic Syndrome Biomarkers

- **Merit:** Tissue-of-origin specificity allows, in principle, distinguishing whether a given patient's insulin resistance is predominantly adipose-, hepatic-, or muscle-driven — information no single conventional serum marker can provide.
- **Merit:** EVs are mechanistically causal, not merely correlative, mediators of insulin resistance (demonstrated via hepatocyte/macrophage co-culture transfer experiments), strengthening their biological plausibility as biomarkers over purely statistical associations.
- **Merit:** Circulating EV number responds acutely and measurably to changes in insulin action (e.g., clamp studies), suggesting potential utility as a dynamic, real-time functional marker rather than only a static risk indicator.
- **Demerit:** Postprandial and dietary-fat-dependent fluctuation in EV cargo introduces substantial

pre-analytical variability that most published studies have not systematically controlled for.

- **Demerit:** Most mechanistic evidence for adipose/hepatic EV-mediated insulin resistance derives from murine models or in-vitro co-culture systems; large, prospective human cohort validation with standardised isolation protocols remains comparatively limited.
- **Demerit:** No single EV cargo analyte or panel has yet been validated across multiple independent cohorts as a standardised clinical test for insulin resistance, unlike HOMA-IR or HbA1c.

Extracellular Vesicles in Diabetic Cardiovascular Risk

EVs, Endothelial Dysfunction, and the Vascular-Metabolic Interface: Endothelial dysfunction is now understood to often precede overt impairment of insulin-stimulated glucose uptake, positioning the vascular endothelium as both a contributor to and an early casualty of the insulin-resistant state. EVs sit directly at this interface: they carry insulin-signalling proteins, endothelial nitric oxide synthase (eNOS)-related cargo, and miRNA capable of modulating vascular tone and inflammation, and elevated circulating endothelial-derived microparticles are a well-established marker of vascular injury across cardiometabolic disease.^[32] Mechanistically, exosomes derived from mature dendritic cells have been shown to increase endothelial inflammation and accelerate atherosclerosis via membrane TNF- α -mediated NF- κ B pathway activation, directly linking EV-mediated intercellular signalling to a core step in atherogenesis.^[33]

Exosomal miRNA Panels as Diagnostic Biomarkers: The Evidence Base

The strongest and most clinically translatable evidence for EV-based biomarkers in diabetic cardiovascular disease comes from exosomal miRNA diagnostic panels. In type 2 diabetic patients, exosomal miR-155-5p achieved an AUC of 0.901 and miR-15a-3p an AUC of 0.874 for ischaemic heart disease,^[19] while a four-miRNA panel (miR-433-3p, let-7b, miR-30-5p, and miR-122-5p) achieved an AUC of 0.833 in a discovery cohort for carotid atherosclerosis,^[34] with diagnostic performance maintained at AUC 0.816 in an independent validation cohort — a methodologically important detail, since validation-cohort persistence is precisely what distinguishes a robust biomarker panel from an overfit discovery finding. This same panel remained useful for detecting early-stage carotid atherosclerosis (carotid intima-media thickness 1.0–1.5 mm, AUC 0.79),^[35] suggesting potential for subclinical screening before overt plaque burden develops. Individual miRNAs in the panel also correlated with relevant metabolic parameters: miR-433-3p, let-7b, and miR-30-5p correlated with HbA1c, while miR-122-5p correlated with LDL-cholesterol, indicating these exosomal markers capture dimensions of cardiovascular risk not fully reflected by standard metabolic biomarkers alone.

Beyond diabetes-specific studies, exosomal miRNA panels have shown comparable diagnostic promise in stable coronary artery disease more broadly: a three-miRNA panel (let-7c-5p, miR-335-3p, and miR-652-3p)³⁶ and a separate three-miRNA panel (miR-942-5p, miR-149-5p, miR-32-5p, individual AUCs 0.69–0.70)³⁷ have each demonstrated significant discriminative capacity for coronary artery disease from plasma-derived exosomes isolated by ultracentrifugation and validated by qRT-PCR. Comparative work in large-artery atherosclerosis stroke has additionally found that exosomal miRNA panels achieve superior diagnostic efficiency compared with the same miRNAs measured in unfractionated plasma — direct evidence for the EV-compartmentalisation advantage proposed in Section 1.4. [Figure 3] summarises the diagnostic performance of the key exosomal miRNA biomarkers identified in this review.

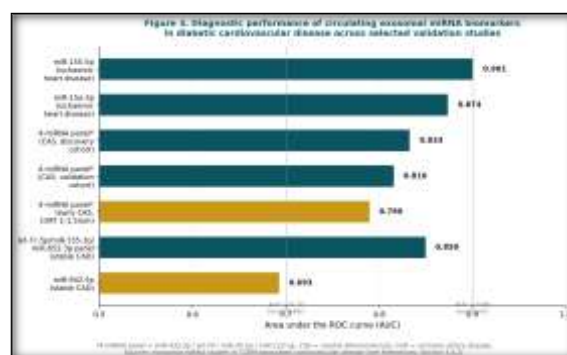


Figure 3: Diagnostic performance of circulating exosomal miRNA biomarkers in diabetic cardiovascular disease across selected validation studies.

Diabetic Cardiomyopathy and Cardiosome-Mediated Mechanisms: Diabetes independently increases cardiovascular disease risk roughly two-fold, and the diabetic heart shows a distinctive pattern of exosome-mediated dysfunction.³⁸ Diabetic cardiomyocyte-derived exosomes ('cardiosomes') have been shown to carry an anti-angiogenic signal via transfer of miR-320 into endothelial cells, directly impairing the coronary microvascular angiogenic response that would otherwise compensate for ischaemic injury in diabetic hearts. Conversely, exercise-conditioned cardiosomes carrying miR-29b and miR-455 modulate matrix metalloproteinase-9 (MMP9) activity in the diabetic heart, illustrating that cardiosome cargo is not fixed but is dynamically responsive to physiological state and represents a potential mechanism through which exercise confers cardioprotection in diabetes.^[34] Serum microRNA-1 and microRNA-133a have separately been shown to reflect myocardial steatosis even in uncomplicated (subclinical) type 2 diabetes, and circulating miR-30 has been proposed as a biomarker of coronary microvascular dysfunction — a particularly difficult-to-diagnose entity that underlies anginal symptoms in diabetic patients without obstructive epicardial

coronary disease, an area where conventional angiographic and biomarker approaches are least effective and where an EV-based biomarker could offer genuine added clinical value.

Related Microvascular Complications: A Brief Note

Although this review's primary focus is macrovascular/cardiac risk, it is worth noting that the same exosomal miRNA biomarker logic extends to diabetic microvascular complications. Urinary exosomal miRNAs, including miR-4534 (AUC 0.786) and miR-136-5p (sensitivity 72.2%, specificity 78.4%),^[26] have shown significant diagnostic performance for diabetic nephropathy, with a meta-analysis of circulating miRNAs in diabetic kidney disease reporting a pooled AUC of 0.79 — figures broadly comparable to the cardiovascular panels discussed above, and consistent with shared mechanistic pathways (fibrosis, inflammation, oxidative stress, endothelial dysfunction) linking miR-21, miR-192,^[27] and the anti-fibrotic miR-29 family across both renal and cardiac diabetic complications. Given the well-established bidirectional worsening relationship between diabetic kidney disease and cardiovascular outcomes (cardiorenal syndrome), a combined renal-cardiac exosomal miRNA panel may ultimately prove more clinically useful than either domain assessed in isolation — a specific, testable hypothesis for future collaborative research (Section 4.5).

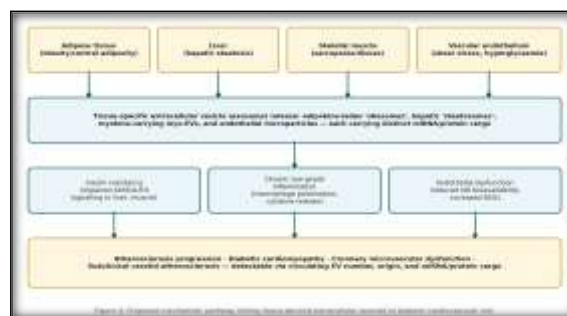
Merits and Demerits of EVs as Diabetic Cardiovascular Biomarkers

- **Merit:** Individual and panel exosomal miRNA biomarkers have achieved AUCs of 0.79-0.90 for ischaemic heart disease and subclinical carotid atherosclerosis in type 2 diabetes, comparable to or exceeding many conventional cardiovascular biomarkers at equivalent disease stages.
- **Merit:** Exosomal miRNA panels have demonstrated superior diagnostic efficiency compared with the same miRNAs measured in unfractionated plasma across multiple disease contexts, providing direct evidence of a genuine EV-compartmentalisation advantage rather than a redundant measurement.
- **Merit:** Some panels remain informative at early, subclinical disease stages (e.g., carotid intima-media thickness 1.0-1.5 mm), offering a potential lead-time advantage over biomarkers that only become abnormal with overt disease.

- **Merit:** EV cargo correlates with, and may mechanistically explain, dimensions of cardiovascular risk (HbA1c, LDL-cholesterol associations) not fully captured by either marker alone, suggesting integrative diagnostic value.
- **Demerit:** No exosomal miRNA panel discussed in this review has yet undergone large-scale, multi-centre, prospective outcome validation of the kind required for clinical guideline adoption; most evidence remains cross-sectional, case-control, or single-centre.
- **Demerit:** Substantial heterogeneity in reported miRNA panels across studies (no two reviewed studies used an identical panel) suggests either genuine biological heterogeneity across cohorts or methodological/pre-analytical inconsistency that has not yet been resolved (Section 4.2).
- **Demerit:** Exosomal miRNA quantification (typically qRT-PCR following ultracentrifugation-based isolation) remains substantially more labour-intensive, costly, and technically demanding than a standard lipid panel or hs-CRP assay, a real barrier to near-term clinical adoption outside specialised centres.
- **Demerit:** As with metabolic syndrome biomarkers, isolation-method inconsistency across studies (ultracentrifugation versus precipitation-based kits versus SEC) limits confident cross-study comparison of the AUC values summarised in Figure 3.

EVs Versus Conventional Biomarkers: A Comparative Summary

[Figure 4] consolidates the mechanistic threads from Sections 3.2 and 3.3 into a single pathway view, tracing how tissue-derived EVs link early metabolic dysfunction to overt diabetic cardiovascular disease.



Mechanistic/causal insight	Largely correlative	Often directly causal/mechanistic (demonstrated via cargo-transfer experiments)
Regulatory/clinical validation status	Fully validated; embedded in clinical guidelines	Not yet validated in large multi-centre prospective outcome studies
Sample/analyte stability	Generally stable; established storage protocols	Lipid bilayer protects RNA cargo from degradation better than free plasma RNA

Therapeutic and Theranostic Potential

Beyond their diagnostic role, EVs — particularly mesenchymal stem cell-derived exosomes (MSC-EVs) — are simultaneously being developed as cell-free therapeutics, a dual diagnostic-therapeutic ('theranostic') potential that is relatively unusual among biomarker classes and directly relevant to a physiology-biotechnology collaboration. As of January 2025, 64 registered clinical trials of MSC-EVs were listed on ClinicalTrials.gov across a range of indications, including an ongoing trial (NCT02138331),^[28] evaluating repeated intravenous MSC-derived exosome infusions in type 1 diabetes. In cardiovascular applications specifically, MSC-derived exosomes have shown cardioprotective effects in preclinical and early clinical work through modulation of cardiomyocyte viability, inflammation, angiogenesis, and post-infarction ventricular remodelling; mechanistically, exosomal miR-25-3p has been shown to protect ischaemic myocardium via EZH2/H3K27me3-mediated de-repression of the cardioprotective gene eNOS and the anti-inflammatory gene SOCS3, while adipose-derived regenerative cell exosomes enriched in miR-214 inhibit cardiomyocyte apoptosis under hypoxic stress. Engineering approaches — surface modification with aptamers, antibodies, or targeting peptides — are increasingly used to improve MSC-EV targeting and therapeutic potency, and represent a natural extension of diagnostic EV characterisation work into a therapeutic development pipeline within the same research programme.

DISCUSSION

EVs as Mediators, Not Merely Markers: Why This Matters for Biomarker Development

A recurring and clinically important theme across Section 3 is that EVs are not simply passive correlates of metabolic and cardiovascular disease but active mechanistic participants — adipose- and hepatic-derived EVs directly impair insulin signalling in recipient cells; cardiosomal miR-320 directly suppresses angiogenesis in the diabetic heart; dendritic-cell exosomes directly activate endothelial NF-κB signalling to accelerate atherosclerosis. This distinguishes EV biomarker development conceptually from most conventional biomarker discovery, which typically identifies statistical association without direct evidence of causal involvement. A biomarker that is also a validated causal mediator carries two practical advantages: first, greater biological plausibility and cross-cohort reproducibility, since a true causal signal is less likely to be a cohort-specific statistical artefact; and second, a natural bridge to therapeutic development, since a

cargo molecule shown to cause pathology in one context becomes a candidate therapeutic target, or its delivery vehicle a candidate treatment platform, in another — precisely the diagnostic-to-therapeutic pipeline outlined in Section 3.5.

Standardisation and Pre-Analytical Challenges

The single largest barrier to clinical translation identified across this review is standardisation. Three specific issues recur throughout the literature. First, isolation-method heterogeneity: as detailed in Section 3.1, ultracentrifugation, SEC, density-gradient centrifugation, precipitation kits, and immunoaffinity capture each recover different, only partially overlapping EV (and non-EV particle) populations, meaning that biomarker findings generated with one isolation method may not reproduce with another — a plausible explanation for the panel heterogeneity noted in Section 3.3.5. Second, pre-analytical variability: the postprandial-state findings summarised in Section 3.2.4 indicate that even fasting-status timing, let alone diet composition in preceding days, may materially affect EV cargo, yet few reviewed studies reported detailed pre-analytical protocols to this level of granularity. Third, reference standard and cohort definition variability: studies define 'metabolic syndrome', 'insulin resistance', and 'diabetic cardiovascular disease' using different criteria (NCEP-ATP III, IDF, WHO for metabolic syndrome; HOMA-IR thresholds; angiographic versus symptom-based versus imaging-based cardiovascular disease definitions), complicating direct comparison of biomarker performance across studies. The MISEV2023 guidelines directly address the first of these three problems and are increasingly treated as a mandatory reporting standard by major journals in the field; the second and third problems remain comparatively under-addressed and represent a clear opportunity for methodologically rigorous new research. Moreover, in recent times, researchers and scientists are scavenging new ways to standardise EV isolation and purification. Scientists are exploring antibody-specific magnetic pull-down approaches to selectively isolate extracellular vehicles (EVs), where they are very specifically targeting- EV surface-enriched markers such as CD9, CD63, CD81, or tissue-specific membrane proteins using antibody-conjugated magnetic beads. These new strategies are slowly yielding positive results that indicates them to be strong candidates for screening test. This strategy is not only providing greater specificity than conventional bulk EV isolation methods but also enable specificity/enrichment of disease- or tissue-associated EV subpopulations. But, this extensive research has also indicated the absence of universally specific surface markers which makes it the option

for a single procedure obsolete. Moreover, in certain cases, epitope accessibility also turns out to be a question due the 3-dimensional structure of the EVs.

Analytical Platforms and Technological Advances

Beyond isolation, the characterisation and quantification side of EV research has advanced substantially in ways directly relevant to future biomarker work. Nanoparticle tracking analysis (NTA) and, increasingly, nanoflow cytometry (nFCM) allow single-particle-level size and concentration measurement, while bead-based multiplex flow cytometry platforms (e.g., MACSPlex) enable simultaneous surface-marker profiling of EV subpopulations from a single sample, offering a practical route toward the tissue-of-origin specificity discussed in Section 1.4. Small RNA sequencing, as used in several of the diagnostic panel discovery studies reviewed here, allows unbiased identification of differentially expressed exosomal miRNA candidates ahead of targeted qRT-PCR validation, the two-stage discovery-then-validation design that most of the strongest studies in Section 3.3.2 employed. Looking forward, single-EV, multi-parametric analysis (combining size, surface marker, and cargo detection on individual vesicles rather than bulk populations) and machine-learning-based integration of multi-omic EV cargo data represent the technological frontier most likely to yield the next generation of clinically robust panels, and are precisely the kind of specialised capability a biotechnology-focused collaborator would be expected to contribute to a joint research programme.

Clinical Translation Pathway and Regulatory Considerations

No EV-based diagnostic test for metabolic syndrome or diabetic cardiovascular risk has yet achieved the kind of regulatory clearance or clinical-guideline inclusion seen for EV-based liquid biopsy tests in oncology. The pathway to that point would likely require, at minimum: (i) a single, pre-specified, MISEV-compliant isolation and quantification protocol locked in advance of a large prospective cohort study; (ii) demonstration of diagnostic accuracy in an independent validation cohort, ideally multi-centre and multi-ethnic given known population differences in EV cargo composition; (iii) demonstration of incremental value over, not merely correlation with, existing biomarkers (HbA1c, lipid panel, hs-CRP) in appropriately adjusted statistical models; and (iv) cost-effectiveness analysis given the currently higher per-test cost of EV isolation and miRNA quantification relative to routine biochemistry. None of the studies reviewed here has completed all four steps, which is the central reason this review characterises EV biomarkers as promising and mechanistically well-supported, but not yet clinically ready — an honest characterisation that should temper enthusiasm without diminishing the genuine scientific opportunity the field represents.

The Physiology-Biotechnology Collaboration Opportunity:

The gaps identified throughout this

Discussion map unusually well onto a natural division of expertise between a medical physiologist and a biotechnology scientist, making this a genuinely well-suited area for the kind of collaborative research programme this review was commissioned to inform. A physiologist's contribution would typically include access to well-phenotyped patient cohorts across the metabolic syndrome-to-cardiovascular-disease spectrum, rigorous standardised clinical and biochemical characterisation (HOMA-IR, oral glucose tolerance testing, carotid intima-media thickness imaging, echocardiographic diastolic function assessment relevant to early diabetic cardiomyopathy), and the physiological framework needed to interpret EV cargo findings mechanistically rather than as isolated statistical associations. A biotechnology scientist's contribution would typically include MISEV-compliant EV isolation and multi-platform characterisation (NTA/nFCM, bead-based multiplex flow cytometry, small RNA sequencing), bioinformatic and machine-learning analysis of multi-omic cargo data, and — if the programme extends into Section 3.5's therapeutic direction — EV engineering and functional validation capability. Three concrete, testable research questions emerge directly from the gaps identified in this review: first, whether a combined cardiac-renal exosomal miRNA panel (Section 3.3.4) offers superior discrimination for early diabetic cardiovascular-renal syndrome compared with either domain's panel alone; second, whether standardising pre-analytical fasting/postprandial timing (Section 3.2.4) materially changes the diagnostic performance of the existing four-miRNA carotid atherosclerosis panel when re-validated in a new cohort; and third, whether serial, longitudinal EV profiling across an insulin-sensitisation intervention (lifestyle, metformin, or GLP-1 receptor agonist therapy) can establish EV cargo change as an early pharmacodynamic marker of treatment response, ahead of conventional biomarker improvement — a study design directly extending the acute insulin-clamp findings summarised in Section 3.2.3 into a chronic intervention setting.

Future Directions: Beyond the specific research questions above, several broader trends are likely to shape the field over the coming years. Single-EV, multi-parametric analytical platforms will likely supersede bulk population-level measurement as their cost and throughput improve, enabling far more precise tissue-of-origin attribution than current bead-based multiplex approaches allow. Plant-derived and bacterial-derived EVs, while still early-stage, may offer a scalable, lower-cost production route for both diagnostic reference standards and therapeutic applications if interspecies cargo-translation questions can be resolved. And the dual diagnostic-therapeutic (theranostic) nature of MSC-EVs, already reflected in 64 active clinical trials as of January 2025, suggests that the most successful future research programmes in this space may be those that,

from the outset, design their biomarker discovery work with a clear eye toward eventual therapeutic application, rather than treating diagnosis and therapy as separate downstream tracks.

Limitations of This Review: Several limitations apply. First, as a narrative rather than quantitative meta-analytic synthesis, this review does not provide pooled effect estimates or a formal GRADE certainty rating, and the AUC comparisons presented in Figure 3 and Section 3.3 should be read as indicative rather than statistically pooled, given differing patient populations, isolation protocols, and reference standards across the underlying studies (Section 2.5). Second, this review relies on published literature and did not access primary study datasets, and therefore inherits any reporting limitations, including possible publication bias favouring studies reporting statistically significant, high-AUC biomarker findings over null results. Third, much of the mechanistic evidence for EV-mediated insulin resistance (Section 3.2.1-3.2.2) derives from murine models or in-vitro co-culture systems rather than direct human intervention studies, and the extent to which these mechanisms fully generalise to human physiology remains incompletely established. Finally, given the pace of publication in this field — several of the studies cited in this review were published within the twelve months preceding its compilation — some very recent findings may not yet be reflected here, and readers conducting further work in this area should verify currency against original sources before finalising any study design.

CONCLUSION

Extracellular vesicles have moved, within roughly a decade, from a peripheral curiosity in cell biology to a mechanistically well-supported and diagnostically promising biomarker class in metabolic syndrome and diabetic cardiovascular disease. The evidence reviewed here demonstrates that EVs are genuine, tissue-specific endocrine mediators of insulin resistance and vascular dysfunction, and that exosomal miRNA panels can achieve diagnostic accuracy (AUC 0.79-0.90) for subclinical and overt diabetic cardiovascular disease that consistently exceeds the same analytes measured in unfractionated plasma. At the same time, standardisation gaps — inconsistent isolation methods, unresolved pre-analytical variability, and the absence of any panel validated across multiple independent cohorts to guideline-ready standards — mean EV biomarkers remain a research tool rather than a clinical one today. This combination of strong mechanistic promise and unresolved methodological challenge is precisely the profile of a research area well suited to a focused collaboration between a medical physiologist, contributing rigorous clinical phenotyping and mechanistic interpretation, and a biotechnology scientist, contributing standardised EV isolation, multi-omic characterisation, and

engineering capability. Executed well, such a collaboration is genuinely positioned to produce internationally competitive, high-impact original research rather than a further incremental review of an already crowded field.

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