Journal of Translational Medicine

REVIEW

Open Access

Impact of monotherapy and combination therapy with glucagon-like peptide-1 receptor agonists on exosomal and non-exosomal MicroRNA signatures in type 2 diabetes mellitus: a systematic review

Haifeng Yu[1], Maryam Davoudi[2], Sahar Sadegh-Nejadi[3], Xiaolei Miao[1*], Molood Bagherieh[4*] and Reza Afrisham[2*]

Abstract

Background Glucagon-like peptide-1 receptor agonist (GLP-1RAs) is a potent therapy for type 2 diabetes mellitus (T2DM) and obesity, especially in patients who are resistant to long-term insulin therapy. Although microRNAs have been linked to GLP-1 signaling, their role in GLP-1RA monotherapy and combination therapy remains unclear. This review synthesizes current evidence of GLP-1RA-induced exosomal and non-exosomal miRNA changes in human and animal models of T2DM.

Methods Scopus, PubMed/Medline, Web of Science, and Google Scholar searches returned 83 studies, of which 11 met the study eligibility criteria (PROSPERO No: CRD42024586000).

Results Human studies showed GLP-1RA combined with metformin modulated non-exosomal *miR-27b*, *miR-130a*, and *miR-210a*, which were linked to cardiovascular health. In T2DM patients on metformin, higher baseline *miR-378-3p* or *miR-126-3p* correlated with greater HbA1c improvement after one year of GLP-1RA therapy. Notably, > 5% weight loss correlated with higher baseline levels of *miR-15a-5p*. Preclinical findings suggested GLP-1RA monotherapy increased cardiovascular action via non-exosomal *miR-29b-3p*, *miR-34a-5p*, *miR-26a-5p*, *miR-181a-5p*, and *miR-93-5p*. Silencing non-exosomal *miR-204*, *miR-375*, or *miR-139-5p* augmented exendin-4/liraglutide monotherapy-induced glucose-stimulated insulin secretion. Interestingly, GLP-1RA monotherapy reduced hepatic lipid accumulation in T2DM models with comorbid NAFLD via *ABHD6* mRNA modulation by non-exosomal *miR-5120*. No clinical studies reported exosomal miRNAs, but a preclinical study linked GLP-1RA-induced exosomal *let-7c-2-3p/miR-322-3p* to bone protection in estrogen-deficient T2DM models.

*Correspondence:
Xiaolei Miao
345701500@qq.com
Molood Bagherieh
Baqeriyeh@gmail.com
Reza Afrisham
rafrisham@sina.tums.ac.ir

Full list of author information is available at the end of the article

Conclusion GLP-1RAs, both as first-line and second-line therapies, are beneficial for T2DM complicated by obesity, NAFLD, cardio-metabolic disease, and postmenopausal osteoporosis. Longitudinal trials that incorporate innovative multi-omics approaches are essential for distinguishing their miRNA expression pattern from other anti-diabetics.

Keywords Glucagon-like peptide-1 receptor agonists, MicroRNAs, Exosomes, Type 2 diabetes mellitus, Drug therapy, combination, Monotherapy

Introduction

Type 2 diabetes mellitus (T2DM) is a growing global health crisis, projected to afflict 783 million by 2045 [1, 2]. Obesity is a significant risk factor for T2DM, and is caused by complex interactions between genetic predisposition and environmental exposures, such as physical inactivity and a diet rich in fat [3–6]. These external factors can induce epigenetic alterations, such as microRNA (miRNA) expression alterations, that are necessary for posttranscriptional gene regulation. These modulations affect key metabolic pathways and lead to T2DM complications such as atherogenic dyslipidemia, hypertension, and cardiovascular disease [3–5].

Although some of the metabolic dysfunctions are lessened with weight loss, individuals who lose considerable amounts of weight continue to be at very high risk for the development of the disease [7–9]. In addition to weight management, the percentage of glycated hemoglobin (HbA1c), a marker for glycemic control, is a strong predictor of disease progression. It has been demonstrated that overt diabetes will develop in no more than 50% of those with impaired glucose tolerance [7–9]. Most importantly, perhaps, high HbA1c levels (> 8%) were linked with a higher risk of death when compared with < 6.5%, indicating how vital it is to maintain good glycemic control [10].

Successful management of T2DM requires a combination of weight management, lifestyle modification, and individualized drug therapies. Metformin is the first-line treatment of choice among the available oral antidiabetic medications (OADs) due to its favorable impact on weight loss and glycemic control [11]. Other treatments include sulfonylureas, dipeptidyl peptidase-4 inhibitors (DPP-4Is), thiazolidinediones (TZDs), sodium-glucose cotransporter 2 inhibitors (SGLT-2Is), and glucagon-like peptide-1 receptor agonists (GLP-1RAs) [12, 13]. Notably, some drugs, such as GLP-1RAs and SGLT-2Is, lead to weight loss, whereas others, such as TZDs and insulin secretagogues, result in weight gain [14]. Considering these distinctions, the American Diabetes Association (ADA) advises that in individualized treatment regimens for the patient, the weight effect of antihyperglycemic therapy should be taken into account [11].

The introduction of GLP-1RAs has revolutionized the treatment of T2DM since they not only enhance glucose-stimulated insulin release but also reduce the risk of hypoglycemia and obesity [15–18]. Furthermore, therapy with GLP-1RA has resulted in a mean loss of 2.9 kg of body weight among T2DM patients compared to the placebo group [19, 20]. In response to food ingestion, glucagon-like peptide-1 (GLP-1) is secreted by intestinal L-cells in a biphasic pattern of initial rapid secretion that stimulates insulin secretion in a glucose-dependent manner [21]. However, in T2DM, impaired incretin function necessitates pharmacological GLP-1 intervention to restore insulin secretion [21, 22]. For this purpose, GLP-1RAs have demonstrated significant efficacy in lowering HbA1c levels, promoting weight loss, and improving lipid profiles, ultimately reducing the risk of T2DM comorbidities such as cardiovascular complications and hepatic steatosis [15–18]. These agents also improve β-cell survival by promoting proliferation and suppressing apoptosis [23, 24]. As incretin mimetics that are resistant to DPP-4, GLP-1RAs possess improved metabolic stability and longer duration of action [15–18]. In addition to glucose lowering, GLP-1RAs also lower total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), and triglycerides, which could be beneficial in comorbid conditions such as obesity, non-alcoholic fatty liver disease (NAFLD), and non-alcoholic steatohepatitis (NASH) [15, 25].

Despite their efficacy, GLP-1RAs also exhibit high interindividual variation in clinical response, which is likely to be moderated by genetic factors [26–28]. Distinct miRNA signatures have already been shown to predict the response of patients to antidiabetic agents such as metformin, sitagliptin, and TZDs [29–31]. In addition to being drug response predictors, new evidence also suggests that miRNA mimics or anti-miRs can impact glucose and lipid metabolism and even trigger β-cell regeneration, highlighting their potential as drug targets in T2DM [32]. The revolution of precision medicine has shed light on the potential of miRNAs as novel biomarkers of outcomes of diabetes treatment as well [33–35]. These small non-coding RNAs serve here to modulate gene expression by binding to the 3′ untranslated region (UTR) of target mRNAs and promoting mRNA degradation or inhibition of translation [36, 37]. The guide miRNA directs the RNA-induced silencing complex (RISC) to mRNAs with a regulatory impact on GLP-1R signaling, glucose homeostasis, and β-cell function [36–40].

Despite being rich in potential, the diagnostic and therapeutic capabilities of miRNA markers are compromised

by the ambiguity of their origin and lack of control during their travel, something that can be addressed by using their exosomal forms [41]. Exosomes are part of the family of small extracellular vesicles (sEVs), with a size range of approximately 40–160 nm, and are derived from almost all types of human cells [41, 42]. Exosomes are present in great abundance in all types of human body fluids such as saliva, ascites, breast milk, cerebrospinal fluid, urine, and semen [42]. These vesicles can transport RNAs, DNAs, lipids, proteins, and metabolites because they have a vital function in the transfer of information from one cell to another [43]. The potential of exosomes in precision medicine is supported by their ubiquity in the body and accessibility for procurement [44]. Interestingly, exosomal miRNAs have some advantages over their non-exosomal counterparts, including enhanced stability in biological fluids since they are being packaged in lipid bilayers, resistance to enzymatic degradation, and their ability to serve as highly reliable biomarkers for disease monitoring and response to treatment [45].

Earlier review articles have evaluated the interaction between incretins, GLP-1, and miRNAs, explaining their role in insulin secretion, cardiovascular protection, and lipid metabolism [37, 40]. To the best of our knowledge, however, no study has evaluated the impact of monotherapy and combination therapy with GLP-1RAs on exosomal and non-exosomal miRNA profiles in T2DM. Therefore, the purpose of this systematic review was to summarize existing knowledge about miRNA signatures and GLP-1RA therapeutic approaches. Although this finding is currently relevant to second-line treatment, it may have implications for first-line treatment regimens in the future, especially in individuals with higher cardiometabolic risk, class III obesity, non-alcoholic fatty liver disease, and postmenopausal osteoporosis in women. Determining miRNA signatures to identify a good metabolic response can aid patient categorization, drug selection, and developing a more personalized treatment regimen for type 2 diabetes.

Methods

Eligibility criteria

This systematic review contrasted studies with GLP-1RA-treated animal models and human T2DM patients, with or without obesity. Both exosomal and non-exosomal miRNA studies in the treatment of T2DM with GLP-1RAs were included if they met the following PICO criteria (*Note*: All inclusion criteria must be met for study inclusion):

- *Populations*: Human T2DM subjects, with or without obesity OR in vivo animal models of T2DM, with or without obesity (in all species and both sexes);
- *Interventions*: All GLP-1RAs approved for use in preclinical/clinical studies, listed as Liraglutide (Victoza), Dulaglutide (Trulicity™), Short-acting Exenatide (Byetta; BID), Long-acting Exenatide (LA-exenatide), Semaglutide, Albiglutide, and Lixisenatide.
  - Comparison:
    - In both clinical and animal studies: GLP-1RA treated diabetic groups vs. untreated diabetic groups.
    - In animal models, studies with healthy controls, streptozotocin (STZ)-treated or vehicle-treated animal models were also preferable.
  - Outcomes:
    - Primary outcome: Changes in miRNA expression profiles in GLP-1RA treated diabetic groups vs. untreated diabetic groups.
    - Secondary outcomes: The impact of GLP-1RA treatment on T2DM-related conditions, such as hyperglycemia, lipid profiles, loss of beta-cell mass, body weight, liver fibrosis, and heart tissue.

Exclusion Criteria were as follows:

- Commentaries, editorials, review articles, meta-analyses, case series, and letters;
- Poorly controlled studies;
- Studies lacking well-defined control groups.
- In vitro experiments.
- Non-experimental methods of miRNA expression analysis such as ex vivo experiments, computational analysis, and in silico models;
- Studies focusing on long noncoding RNAs (lncRNAs) instead of miRNAs.
- Studies examining comorbidities related to either obesity or T2DM without properly defined control groups;
- Existence of clinically significant, striking comorbidities that have no relation with T2DM or obesity (e.g., cancer, autoimmune disease, etc.).

Notably, GLP-1RAs are often prescribed as second-line therapy for T2DM; therefore, in clinical trials, their effect is typically assessed in patients already receiving first-line therapies, e.g., metformin. As a result, studies comparing GLP-1RA treatment with background metformin therapy are common in the literature, such as in earlier research by Nikolic et al. (2017), Gaborit et al. (2019), Giglio et al. (2020), and Formichi et al. (2021). In view of such routine

clinical practice, exclusion of these types of studies would have significantly limited data to analyze. For purposes of guaranteeing scientific rigor as well as appropriate representation of relevant findings, we considered including those studies involving administration of GLP-1RAs with other antidiabetic drugs given on a regular basis. However, in animal models, monotherapy only was used so as to allow direct isolation of GLP-1RAs' effect from confounding interference of other antidiabetic agents. This approach allows us to compare results of monotherapy and combination therapy in a greater picture of the clinical relevance of GLP-1RAs. The employed protocol is listed on the International prospective register of systematic reviews (https://www.crd.york.ac.uk/prospero/; PROSPERO No. CRD42024586000).

Search strategy

A PRISMA-style systematic review was performed for this study [46]. The authors have systematically searched Scopus, PubMed/Medline, Web of Science, and Google Scholar for articles on microRNAs, Type 2 diabetes mellitus, and Glucagon-Like Peptide-1 Receptor Agonists up to September 1, 2024. A customized search strategy has been described in Table 1. We included only original research published in the English language. A search of all relevant references was carried out to identify further papers that met the criteria. Titles, abstracts, and/or full texts were examined for eligibility.

Table 1 Search strategy

Databases	[+]Search strategy	Results
PubMed	((((((((((Glucagon-Like Peptide-1 Receptor Agonists[MeSH Term) OR (Glucagon-Like Peptide-1 Receptor Agonists[Title/Abstract])) OR (GLP-1 analogs[Title/Abstract])) OR (GLP-1DAs[Title/Abstract])) OR (incretin mimetics[Title/Abstract])) OR (GLP-1RAs[Title/Abstract]))) AND ((((((((((Diabetes Mellitus, Type 2[MeSH Terms]) OR (Diabetes mellitus type 2[Title/Abstract])) OR (adult-onset diabetes[Title/Abstract])) OR (noninsulin-dependent diabetes mellitus[Title/Abstract])) OR (NIDDM[Title/Abstract])) OR (Type 2 diabetes mellitus[Title/Abstract])) OR (Type 2 diabetes[Title/Abstract])) OR (T2DM[Title/Abstract])) OR (T2D[Title/Abstract])) OR (Late-onset diabetes[Title/Abstract]))))) AND (((((((MicroRNAs[MeSH Terms]) OR (miRNA[Title/Abstract])) OR (MicroRNAs[Title/Abstract])) OR (miR[Title/Abstract])) OR (MicroRNA[Title/Abstract])) AND (MicroRNAs))))	6
Web of Sciences		15
Scopus		48
Google Scholar		14

[+] The PubMed search strategy was tailored to each specific database

Data extraction

We tried to extract data from tables, text, figures, or graphs. In an effort to enhance the robustness of our systematic review, we employed the use of structured data extraction forms in order to ensure consistency across studies by type.

For clinical trials, the following data were abstracted: (i) study characteristics (authors, year, and type of study); (ii) demographic information (age, gender, inclusion/exclusion criteria, and duration of diabetes); (iii) intervention information (type of GLP-1RA, dose, route of administration, and duration of treatment); and (iv) outcomes (metabolic parameter changes such as glucose, lipid profile, and β-cell function, and miRNA expression changes).

For animal studies, information retrieved was: (i) study features (authors, year, animal model, grouping, and method of diabetes induction and diagnosis); (ii) intervention information (type of GLP-1RA, dose, and treatment duration); and (iii) outcomes (effect on metabolic parameters, β-cell function, and alterations in miRNA expression).

The below information were drawn for studies that examine exosomal miRNAs that act against GLP-1RAs in type 2 diabetes: (i) study description (e.g., study type and type of exosome); (ii) intervention-related information (e.g., type of GLP-1RA, dose, and duration); and (iii) results (influence on exosomal miRNA expression and involved T2DM-associated pathways.

For precision, data extraction was conducted by two separate reviewers (R.A. and M.D.), with discrepancies resolved by a third reviewer (X.M.). In cases of unclear reported data, we contacted the corresponding authors (with up to two follow-ups). These methodological advancements enhance the transparency and stability of our systematic review.

Study risk of Bias assessment

To address potential bias, the results from studies involving humans, animals, and laboratory models were presented independently. Two authors, R.A. and M.D., independently reviewed the records; after the selection process, the third author (X.M.) resolved any potentially conflicting instances. The risk of bias assessment was performed using SYRCLE's Risk of Bias tool for animal studies, ROBINS-I for non-randomized observational clinical studies, and Cochrane's Risk of Bias 2 (RoB 2) for randomized controlled trials.

Results

Outcomes of the study selection process

The PRISMA flow diagram illustrates the study selection process (Fig. 1) [46]. Across the electronic database searches, 83 articles were found: PubMed/Medline ($n=6$), Google Scholar ($n=14$), Scopus ($n=48$), and

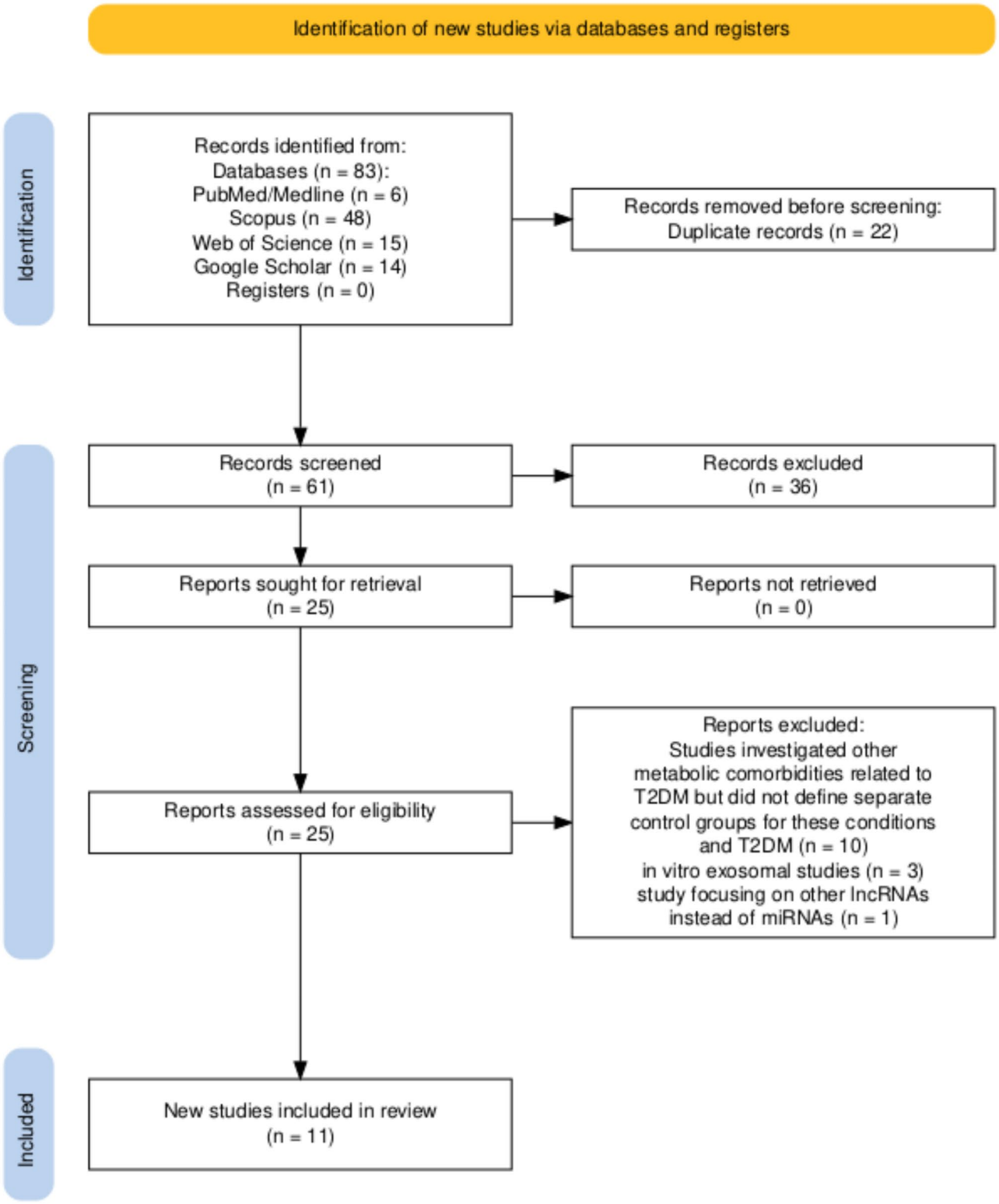

Fig. 1 This PRISMA flow diagram illustrates the rigorous methodology employed in the systematic screening of miRNA research pertaining to the therapeutic management of T2DM using GLP-1 RAs

Web of Science ($n = 15$). Duplicate records ($n = 22$) were excluded, and 61 articles were screened at the title and abstract level using pre-specified inclusion and exclusion criteria.

Of these, 25 studies were deemed to meet the inclusion criteria and were used in the final full-text analysis. Clinical and animal model studies were our primary interest; thus, only in vivo and human studies were included. Ten studies of metabolic comorbidities in T2DM were identified that did not include well-defined diabetic controls and thus may have problematic direct comparisons. However, properly designed comparator trials such as Li et al. (2017), Li et al. (2022), and Fang et al. (2023) were identified as being eligible. At first, we considered including all in vitro studies to attempt to identify the limited data available on both exosomal and non-exosomal miRNAs in GLP-1RA-treated T2DM patients and models. Nevertheless, the large number of eligible in vitro studies made a systematic review impracticable and less specific. An additional attempt to restrict the inclusion to only exosomal in vitro research was also deemed unsuitable because it undermined the inclusion criteria of the study. Because of this, three in vitro exosomal studies were excluded at this stage. One study that was lncRNA-based rather than miRNA-based was also excluded in order to maintain the scope of the review. After applying the eligibility criteria, 11 studies were included in the final review.

Clinical trials on human subjects studying the effects of GLP-1RA treatment in T2DM individuals were conducted by Nikolic et al. (2017) [47], Gaborit et al., (2019) [48], Giglio et al., (2020) [49], and Formichi et al. (2021) [35]. Major findings related to patients treated with GLP-1RA, including treatment forms, standard doses, and treatment periods, are included in Table 2. Among the studied GLP-1RAs, liraglutide [35, 48, 49], dulaglutide [35], and LA-exenatide [47] were assessed for their impact on miRNA signatures in T2DM patients.

In addition, in vivo studies were conducted by Song et al., (2017) [50], Li et al., (2017) [51], Jo et al., (2018) [52], Zhang et al., (2019) [53], Li et al., (2022) [54], Fang et al., and (2023) [55]. Various animal models were utilized in the studies, including C57BL/6J mice, Balb/c mice, 204 knockout (204KO) mice, and Sprague-Dawley rats (see Table 3 for further details). Up to now, in vivo experiments have investigated the effect of exendin-4, liraglutide, semaglutide, and LA-exenatide-loaded microspheres on miRNA profiles in T2DM animal models [50–55]. When analyzing miRNA expression profiles of T2DM patients following GLP-1RA treatment, an in vivo study enlightened us on the bone-protective effect of liraglutide, focusing particularly on BM-derived exosomal miRNAs in animal models simulating postmenopausal women with T2DM (Data are presented in Table 4) [56].

Outcomes of risk of bias assessment

The overall assessment is presented in Fig. 2. Most animal studies had a low risk of bias in several domains. Random sequence generation (D1) and reporting bias (D9) had always been assigned a low risk rating in all the studies. However, other domains, including allocation concealment (D3), random housing (D4), and blinding of investigators (D5), were consistently reported as unclear. Total risk of bias was mostly unclear due to these concerns.

The assessment of non-randomized observational clinical trials revealed various degrees of bias. For instance, Giglio et al. (2020) showed low risk of bias in all domains, while Formichi et al. (2021) revealed moderate risk in most domains, namely in participant selection (D2) and missing data bias (D5). Nikolic et al. (2017), which was only available as an abstract, had a serious risk of bias due to confounding (D1) and an overall serious risk rating. The bias assessment for this study is based on only one abstract provided, and our results could have been affected by this limitation. Finally, the randomized open-label trial by Gaborit et al. (2019) carried a high risk of bias in deviations from the intended intervention (D2) and selection of the reported result (D5). The remaining domains had some problems, contributing to a total high-risk score.

The results indicate that while some studies had a low risk of bias, others exhibited methodological concerns that may affect the validity of their findings.

Clinical and preclinical evaluation of GLP-1RA therapy

This section presents clinical and preclinical findings of the influence of GLP-1RAs on metabolic and miRNA outcomes in T2DM. Importantly, all clinical findings focus on the combination therapy of GLP-1RAs with other anti-diabetic medications, particularly metformin. Conversely, animal models primarily study the impact of GLP-1RA monotherapy and explain mechanistic functions of miRNA regulation and the potential significance for diabetes treatment.

Effects of combination therapy with GLP-1RAs as A Second-Line treatment

Role of MiRNAs in cardiovascular health A number of studies have established the cardiovascular protective effects of GLP-1RAs, which are attributed to miRNAs' impacts on endothelial function. For instance, in a prospective study of an eight-month duration, the effect of LA-exenatide on T2DM patients on metformin with no prior incretin-based therapy was evaluated. Through comparison of the pre-treatment and post-treatment blood parameters, they observed that anthropometric indices, FBS, HbA1c, TC, LDL-C, and carotid intima-media thickness test (CIMT) were decreased significantly. LA-exenatide also increased HDL-C and brachial artery

Table 2 Comprehensive overview of combination therapy of GLP1-RAs with other Anti-Diabetic agents in clinical studies

Reviewed articles		Demographic data					GLP-1RAs		Finding	
Authors (year)	**Study Type**	**Age (years)**	**Gender**	**Inclusion Criteria**	**Exclusion Criteria**	**Duration of Diabetes (years)**	**Dosages and Administration Routes**	**Treatment Durations**	**Changes in Other Parameters** (Tests vs. controls)	**Changes in miRNAs**
Nikolic et al., (2017) [47]	8-month prospective study ($n=60$; NCT02380521)	60 ± 10	F: M: 19:41	T2DM, age ≥ 18, on metformin, no prior incretin-based therapy	Severe liver/renal disorders, major cardiovascular diseases	-	Exenatide (2 mg/kg; subcutaneously); Metformin (from 1500 to 3000 mg/day)	Once weekly for 8 months	↓FBS, HbA1c, TC, LDL-C, and CIMT; ↑HDL-C and FMD	↑miR-27b (correlated with adipokines: adiponectin, leptin, L-selectin)
Gaborit et al., (2019) [48]	Randomized trial (Test: $n=28$; Control: $n=21$; NCT02686177)	≥ 18	-	T2DM (ADA criteria), age ≥ 18, BMI ≥ 25, HbA1c > 6.5%, on standard treatment, effective contraception (women)	Recent incretin therapy, T1DM, ketoacidosis, pregnancy/lactation, acute/infectious diseases, pancreatitis, chronic renal failure (eGFR ≤ 50), recent cardiovascular events, cancer, hepatic insufficiency, gastroparesis, IBD, no social security affiliation	Tests: 3; Controls: 9	Liraglutide (1.2 mg/day SC) + standard treatment	4 weeks	↓Glucose, ↓triglycerides; No significant changes in hemodynamic, liver, and angiogenic parameters	No effect on 43 circulating angio-miRNAs [57]

Table 2 (continued)

Reviewed articles		Demographic data					GLP-1RAs		Finding	
Giglio et al., (2020) [49];	Observational trial ($n=25$)	64.6 ± 8.4	-	T2DM, age ≥ 18, on metformin, no prior incretin-based or obesity treatments	Renal, liver, cardiovascular failure, hepatitis C, cancer	9.6 ± 7.1	Liraglutide (0.6 mg/day → 1.2 mg/day SC) + Metformin (1500 mg/day)	4 months	↓FBS, HbA1c, TC, triglycerides, LDL-C; No significant effect on anthropometric parameters and HDL-C	↑miR-27b, miR-130a, miR-210a (Serum Levels)
Formichi et al., (2021) [35];	Observational pilot study ($n=26$)	35–79	Females ($n=9$) and males ($n=17$)	T2DM, prior metformin use but failed glycemic control (HbA1c ~ 7.7%), eligible for GLP-1RA therapy	Not specified	10.2	Liraglutide (0.6 → 1.8 mg/day) or Dulaglutide (1.5 mg/week)	6 & 12 months	↓Weight, BMI, HbA1c for both drugs; No difference in outcomes between Dulaglutide & Liraglutide	↑miR-21-5p, miR-24-3p, miR-223-3p, miR-375-5p at baseline (linked to better glycemic response); miR-375-5p predicted HbA1c levels; miR-378-3p & miR-126-3p linked to HbA1c/FPG reduction; miR-15a-5p predicted weight loss

ADA, American diabetes association; BMI, body mass index; CIMT, carotid intima-media thickness; DR, diabetic retinopathy; FBS, fasting blood sugar; FMD, flow-mediated dilation; FPG, fasting plasma glucose; GLP1-RA, glucagon-like peptide-1 receptor agonist; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MDRO eGFR, modification of diet in renal disease estimated glomerular filtration rate; miR/miRNAs, micro ribonucleic acid; NYHA, New York heart association functional classification; TC, total cholesterol; T2DM, type 2 diabetes mellitus; VEGF, vascular endothelial growth factor

flow-mediated dilation (FMD), which is indicative of better endothelial function. More significantly, GLP-1RA treatment increased miR-27b, a pro-angiogenic endothelial cell marker, suggesting an epigenetic regulatory mechanism for T2DM [47]. Similarly, liraglutide therapy for metformin-treated T2DM patients could also decrease FBS, HbA1c, TC, triglycerides, and LDL-C within four months of therapy but didn't have a significant impact on anthropometric measures and HDL-C. The treatment also significantly enhanced the expression of *miR-27b*, *miR-130a*, and *miR-210a*, which play key roles as endothelial cell homeostatic regulators, independently of metabolic regulation. These findings suggest that GLP-1RAs offer endothelial protective effects by direct epigenetic modification [49]. In contrast, a randomized open-label trial was also published in obese T2DM patients on standard antidiabetic therapy (metformin and/or sulfonylurea) with or without GLP-1RAs. The liraglutide therapy had positive effects on glycemic control and reduced triglycerides but failed to alter hemodynamic, hepatic, or angiogenic markers significantly. Besides, there was no significant effect on the levels of circulating angio-miRNAs in diabetic retinopathy (DR) [48, 57]. These angio-miRNAs were 43 pro-angiogenic circulating miRNAs [57].

Predictive role of MiRNAs in GLP-1RA treatment response An observational pilot study was performed to investigate the predictive value of certain circulating miRNAs for response to GLP-1RA therapy in patients with T2DM who had not attained glycemic targets on metformin monotherapy. The cohort within this study was classified into low-expressing and high-expressing categories based on eight miRNAs at baseline concentrations: *miR-24-3p*, *miR-126-3p*, *miR-21-5p*, *miR-15a-5p*, *miR-223-3p*, *miR-378-3p*, *miR-375-3p*, and *miR-146-5p*. The study further compared therapeutic efficacies between dulaglutide and liraglutide, which ultimately concluded the two agents were not statistically different. Treatment using these GLP-1RA drugs cut the body weight, BMI, and HbA1c content significantly in favor of those having received these interventions for either 6 months or 12 months compared with the baseline value. Despite there being no significant differences in baseline

Table 3 Comprehensive overview of monotherapy with GLP-1RAs in animal models of T2DM

Authors (year)	Animal testing			GLP-1RA		Findings Regarding GLP-1RA Therapy vs. T2DM	
	Animal Model	Grouping	DM Induction & Diagnosis	Type & Dose	Duration	Other parameters	miRNAs
Song et al., (2017) [50]	Balb/c mice (9–11-week-old; male)	STZ-treated groups, PPAG-treated, Exendin-4-treated	200 mg/kg STZ (IP); glucose monitoring via tail vein/cardiac puncture	Exendin-4, 10 μg/kg/day (IP)	2 days	STZ-treated groups showed a biphasic rise in blood glucose (4 and 24–30 h post-injection) with ~60% beta-cell mass loss. PPAG and Exendin-4 reduced hyperglycemia and preserved beta-cell mass.	miR-375 levels remained stable initially but increased between 6–30 h post-STZ; Both PPAG and Exendin-4 prevented this increase
Li et al., (2017) [51]	Sprague Dawley rats (51 weeks, male)	DM, DM + Liraglutide	HFD from 2 weeks old, 30 mg/kg STZ (IP) at 10 weeks; diagnosis at > 16.7 mmol/L glucose	Liraglutide, 200 μg/kg/day	~10 weeks	↓ pancreatic beta-cell death	Downregulation of miR-139-5p; Upregulation of IRS1 (target of miR-139-5p)
Jo et al., (2018) [52]	miR-204 KO mice	WT, 204KO, TXNIP KO, DM, DM + Exendin-4	**Diabetes Induction**: Multiple low-dose STZ (40 mg/kg/day, IP) **Inhibition of GLP1R Signaling**: GLP1R antagonist exendin(9–39) (0.6 mg/kg.day; intraperitoneally)	Exendin-4, 10 nmol/kg	16 h fasting, injected 30 min before glucose challenge	-	miR-204 deletion enhanced GLP1R expression & responsiveness
Zhang et al., (2019) [53];	Sprague Dawley rats (5 weeks, male)	Control, DM + Saline, DM + LL, DM + HL	4-week HFD, 30 mg/kg STZ (IP); diagnosis at > 11.1 mmol/L glucose	Liraglutide, 0.2–0.4 mg/kg/day (SC)	12 weeks	-Both dosages: ↓body weight, FBS, AUC level in oral glucose tolerance test, fasting serum insulin, HOMA-IR value, and Pten gene and protein expressions; -Ameliorative impact on the impaired endothelium-dependent vasodilation caused by T2DM; ↑ *CREB-1*, *Bcl-2*, and *Sirt1* gene expression in aorta; ↑ protein expression of Bcl-2 in aorta	HL vs. DM: 33 differentially expressed miRNAs (20 upregulated, 13 downregulated); miRNAs linked to endothelial function: miR-26a-5p, miR-34a-5p, miR-93-5p, and miR-181a-5p

Table 3 (continued)

Authors (year)	Animal testing			GLP-1RA		Findings Regarding GLP-1RA Therapy vs. T2DM	
	Animal Model	Grouping	DM Induction & Diagnosis	Type & Dose	Duration	Other parameters	miRNAs
Li et al., (2022) [54];	C57BL/6J mice (8 weeks, male)	Control, T2DM + NAFLD, T2DM + NAFLD + Semaglutide	4-week HFD, 100 mg/kg STZ (IP); diagnosis at > 11.1 mmol/L glucose	Semaglutide, 0.42 mg/kg/week (SC)	12 weeks	↓ blood tests (glucose, body weight, serum TG, TC, FFA, LDL-C, AST, ALT); ↓ hepatic parameters (steatosis, TG and FFA, and pathological changes); -No significant difference in serum HDL-C and hepatic TC -Improved glucose tolerance -ameliorative impact on liver fibrosis induced by T2DM;	Upregulation of miR-5120; Downregulation of ABHD6 expression
Fang et al., (2023) [55]	C57BL/6J mice (8 weeks, male)	Control, DCM, DCM + GLP-1RA	4-week HFD, 100 mg/kg STZ (IP); diagnosis at > 11.1 mmol/L glucose	LA-exenatide (microsphere), 10 mg/kg/week (IP)	6 weeks	↓ blood glucose, body weight, cardiomyocyte abnormalities, lipid accumulation in heart tissues, myocardial expression of SLMAP, and serum BNP level; -No significance difference in heart weight, IVSD, LVPWD, EF, FS, and serum CK-MB level; -The inhibition of myocardial fibrosis induced by DM; -↑ LVEDd, LVESd, LVEDv and LVESv; -Reversed DM-induced E/A value reduction	- Upregulation of miR-29b-3p in ventricular myocardium; - GLP-1RA regulated the expression of SLMAP via miR-29b-3p;

ABHD61: alpha/beta-hydrolase domain-6; ALT: alanine aminotransferase; AST: aspartate aminotransferase; AUC: area under the curve; Bcl-2: B-cell lymphoma 2; BNP: brain natriuretic peptide; CK-MB: creatine kinase-MB; Creb1: cAMP response element-binding protein 1; DCM: diabetic cardiomyopathy; DM: diabetes mellitus; EF: ejection fraction; FBS: fasting blood sugar; FFAs: free fatty acids; GLP-1RA: glucagon-like peptide-1 receptor agonist; GLP1R: glucagon-like peptide-1 receptor; HL: high liraglutide dose; HFD: high-fat diet; HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostatic model assessment for insulin resistance; IRS1: insulin receptor substrate-1; IVSD: interventricular septal thickness; LL: low liraglutide dose; LDL-C: low-density lipoprotein cholesterol; LVEDd: left ventricular end-diastolic diameter; LVEDv: left ventricular end-diastolic volume; LVPWD: left ventricular posterior wall thickness; LVESd: left ventricular end-systolic diameter; LVESv: left ventricular end-systolic volume; NaCl: sodium chloride; NAFLD: non-alcoholic fatty liver disease; PPAG: peroxisome proliferator-activated receptor-γ (PPARγ) agonist; Pten: phosphatase and tensin homolog; Sirt1: sirtuin 1; SLMAP: sarcolemmal membrane-associated protein; STZ: streptozotocin; TG: triglycerides; TC: total cholesterol; TXNIP: thioredoxin-interacting protein; T2DM: type 2 diabetes mellitus; miR/miRNAs: micro ribonucleic acid; WT: wild-type

metabolic or anthropometric characteristics between the low-expression and high-expression groups, the latter demonstrated superior response with greater attainment of glycemic targets at 12 months following initiation. Surprisingly, patients with higher baseline circulating levels of *miR-21-5p*, *miR-24-3p*, *miR-223-3p*, and *miR-375-5p* were significantly more likely to attain glycemic control at 12 months. *MiR-375-5p* was a predictor of reduction

Table 4 Comprehensive overview of Exosomal MiRNAs generated by animal models of T2DM following GLP-1RA therapy

Study	Exosomal Source	Procedure		GLP-1RA			Findings Regarding GLP-1RA Therapy	
		T2DM Model	Protocol	Type	Dose	Treatment Duration	Other T2DM-Related Pathway	miRNA Signature
Li et al., (2017) [56]	**BM**	Sprague-Dawley rats (*n* = 27; 6-month-old females; Groups: OVX control; OVX + T2DM; OVX + T2DM + Liraglutide)	**Induction of T2DM**: 2-month HFD, 35 mg/kg STZ (IP); A second STZ injection was also administered if blood glucose remained below 16.7 mmol/L.	Liraglutide	2 mg/kg twice a day; i.h.	2 months	↓ blood glucose	**BM-Derived Exosomal miRNA Expression**: ***T2DM Group***: ↑ miR-1-3p, let-7i-5p, let-7f-5p, miR-148a-3p, let-7 g-5p, miR-3571, miR-1b, miR-126a-3p, miR-206-3p, and miR-143-3p; ***Liraglutide Group***: Similar miRNA profile as T2DM but with additional upregulation of miR-21-5p. ***OVX vs. Liraglutide***: OVX group showed higher expression of let-7c-2-3p and miR-322-3p (Wnt signaling pathway). ***Liraglutide vs. DM***: Liraglutide treatment led to upregulating miR-322-3p (Wnt signaling).

BM: bone marrow; EVs: extracellular vesicles; HK-2: human renal tubular epithelial cell line; HFD: high-fat diet; miR/miRNAs: micro ribonucleic acid; OVX: ovariectomized; STZ: streptozotocin; T2DM: type 2 diabetes mellitus

in HbA1c by receiver operating characteristic (ROC) curve analysis. Additionally, during the course of one year of GLP-1RA therapy, higher baseline expression of *miR-378-3p* and *miR-126-3p* predicted more significant reductions in HbA1c and FBS. Notably, baseline *miR-15a-5p* concentrations were elevated in individuals who lost more than 5% of their body weight, implying that it could serve as a predictive biomarker for weight loss after GLP-1RA therapy [35].

Effects of monotherapy with GLP-1RAs as A primary treatment

Role of MiRNAs in cardiovascular health Cardioprotective action of LA-exenatide microspheres was explored in GLP-1RA-treated diabetic cardiomyopathy (DCM) C57BL/6J mice compared to healthy and DCM groups. Treatment reduced blood glucose, serum brain natriuretic peptide (BNP), body weight, heart lipid composition, myocardial sarcolemmal membrane-associated protein (SLMAP) expression, while it increased cardiomyocyte integrity and reduced myocardial fibrosis. However, there were no differences in interventricular septal thickness (IVSD), left ventricular posterior wall thickness (LVPWD), ejection fraction (EF), fractional shortening (FS), and serum creatine kinase-MB level. Notably, LA-exenatide improved diabetes-impaired diastolic function by increasing the E/A ratio, an important indicator of left ventricular filling capacity. Ventricular myocardial *miR-29b-3p* upregulation suggested as a possible mechanism of action of GLP-1RA therapy, which could potentially control cardiac SLMAP expression [55, 58].

Zhang et al. (2019) examined the effect of low-dose and high-dose liraglutide on endothelial function in diabetic Sprague Dawley rats. Both treatments had significant reductions in body weight, FBS, oral glucose tolerance test's area under the curve (AUC), fasting serum insulin, and homeostatic model assessment for insulin resistance (HOMA-IR). Besides, liraglutide treatment downregulated phosphatase and tensin homolog (PTEN) gene and protein expression, contributing to an increase in endothelium-dependent vasodilation. Of special note, various miRNAs in the high-dose liraglutide treatment were differentially expressed, including *miR-297*, *miR-592*, *miR-671*, *miR-214-3p*, *miR-1843-3p*, *miR-6334*, *miR-103-1-5p*, *miR-466b-5p*, *miR-96-5p*, *miR-190a-5p*, *let-7c-5p*, *miR-22*, *miR-190a*, *miR-568*, *miR-3586-3p*, *miR-675-3p*, *miR-214*, *miR-134-5p*, *miR-26a-5p*, *miR-488-3p*, and *miR-188*. On the other hand, downregulated miRNAs were *miR-93-5p*, *miR-34a-5p*, *miR-544-3p*, *miR-349*, *miR-547-5p*, *miR-3571*, *let-7b-5p*, *miR-344b-1*, *miR-541-3p*, *miR-879-5p*, *miR-181a-5p*, and *miR-126a-5p*. Of these, miRNAs specifically related to endothelial function were *miR-26a-5p*, *miR-34a-5p*, *miR-93-5p*, and *miR-181a-5p*, suggesting that liraglutide might exert its vascular protective effects through epigenetic regulation of endothelial-related miRNA expression [53].

Role of MiRNAs in Beta-Cell function and insulin regulation According to an in vivo study, *miR-375* is a biomarker that can be used to determine the cytoprotective

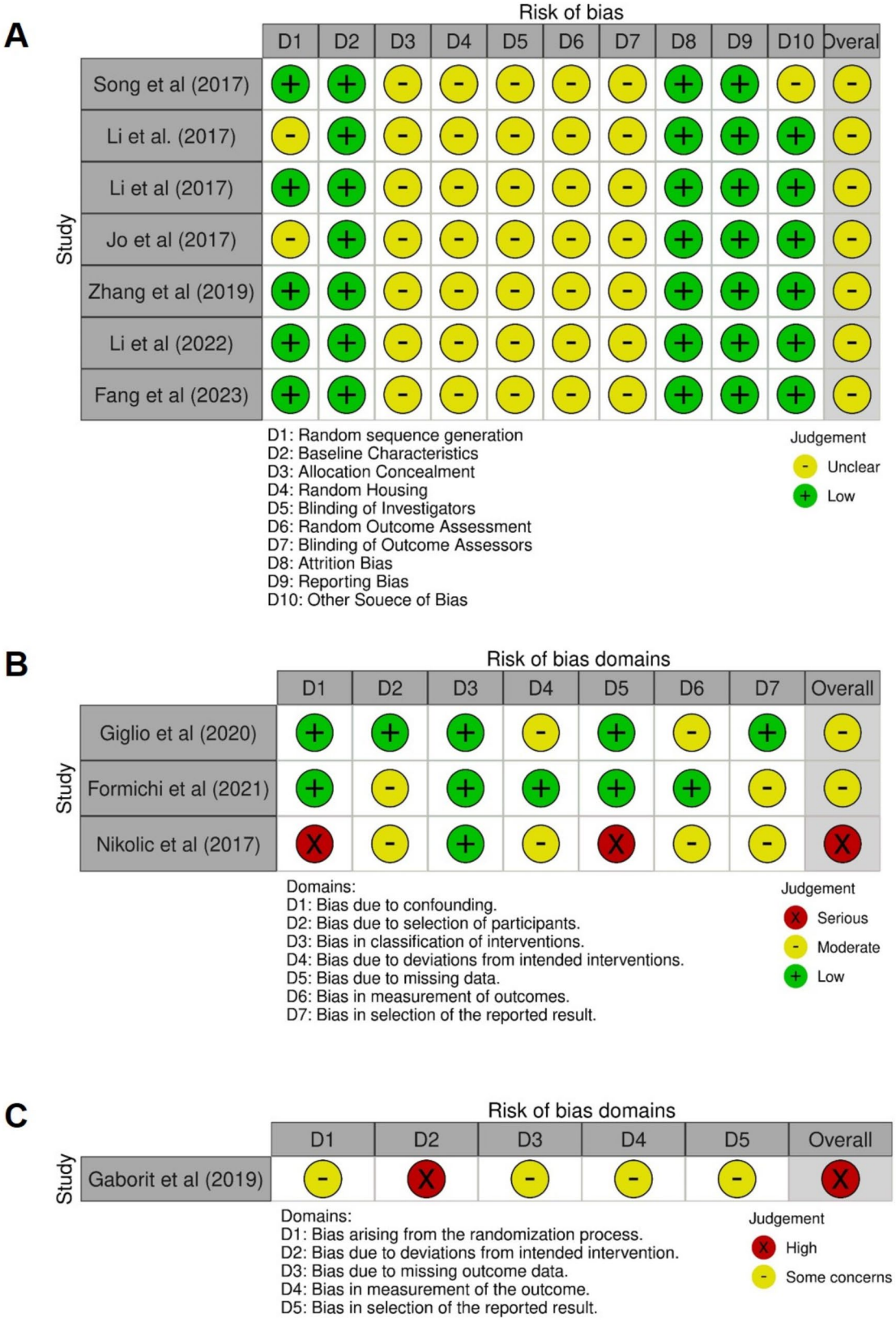

Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 Risk of Bias Assessment for Included Studies. **(A)** SYRCLE's Risk of Bias Tool for animal studies. The studies were assessed in ten domains (D1–D10), and their assessment is depicted as low risk (green), unclear risk (yellow), or high risk (red). **(B)** ROBINS-I assessment for non-randomized observational clinical studies, in which bias was evaluated in seven domains (D1–D7) using the same color coding. Nikolic et al. (2017) arrived in the form of an abstract, and bias assessment was limited to partial data, which might have affected grading. **(C)** Cochrane Risk of Bias 2 (RoB 2) assessment of a randomized open-label trial, demonstrating high risk (red) for key domains. The results demonstrate that although some studies had low bias, there were others with methodological issues that might influence study outcomes

effect of antidiabetic agents such as exendin-4 and phenylpropenoic acid glucoside (PPAG). The study used a two-stage model experiment: diabetic Balb/c mice were used to evaluate the effect of streptozotocin (STZ)-induced beta-cell injury from 4 to 30 h, and the effect of PPAG and exendin-4 treatment was compared with controls. The results depicted biphasic increase in blood glucose levels in STZ-treated mice at 4 h and again at 24–30-hour post-injection along with a ~60% reduction in beta-cell mass at 30 h. Monotherapy with PPAG or exendin-4, however, abrogated STZ-induced hyperglycemia along with maintaining beta-cell mass. Notably, *miR-375* levels were maintained at baseline in untreated and 4-hour post-treatment STZ, while it increased threefold at 6 and 30 h following STZ. Notably, PPAG and exendin-4 prevented this rise in plasma levels of *miR-375*, which would suggest a possible protective mechanism of preventing the release of miR-375 into circulation [50].

In addition, Jo et al. (2018) investigated the role of *miR-204* in GLP-1 receptor (GLP-1R) expression via a comparison of wild-type (WT) and miR-204 knockout (204KO) mice. According to their results, *miR-204* suppresses GLP-1R expression. Interestingly, genetic knockdown of *miR-204* or its upstream regulator, thioredoxin-interacting protein (TXNIP), was dramatically overexpressed GLP-1R on islets, which promoted glucose-stimulated insulin release and glucose tolerance after GLP-1RAs [52].

Lastly, liraglutide-induced pancreatic protection was also linked to the downregulation of *miR-139-5p*, which further upregulated *insulin receptor substrate-1* (*IRS1*) expression. This implicated a mechanistic role for miRNA regulation in pancreatic beta-cell survival, additionally highlighting the therapeutic value of GLP-1RAs in sustaining insulin signaling pathways [51].

Role of MiRNAs in NAFLD and hepatic function NAFLD is present in 70–90% of T2DM patients, mainly due to insulin resistance, elevated liver enzymes, and vascular endothelial inflammation. As miRNAs have been increasingly implicated in these pathologies, Li et al. (2022) analyzed the influence of semaglutide on T2DM models with comorbid NAFLD. The GLP-1RA therapy lowered various metabolic markers including body weight, blood glucose, serum lipids, TC, FFA, LDL-C, aspartate aminotransferase (AST), and alanine transaminase (ALT) to significant extents. However, the levels of HDL-C, as well as hepatic TC, were not affected by treatment. Hepatic TC content, as a reflection of the liver's content of cholesterol, is typically quantified by liver biopsy or approximated with imaging techniques such as computed tomography (CT) for the volumetric liver fat fraction (VLFF). In addition to metabolic effects, semaglutide had the ability to enhance glucose tolerance and minimize T2DM-induced liver fibrosis. Therapy was also associated with upregulation of circulating and hepatic *miR-5120* expression. α/β hydrolase domain-6 (ABHD6), a 32 kDa membrane protein with a metabolic disorder association, was markedly downregulated in mRNA and protein expression by *miR-5120*. According to the findings, semaglutide may possess hepatoprotective activity through the modulation of miR-5120 and ABHD [54].

Bone-Protective effects of bone marrow-derived exosomal MiRNAs An in vivo investigation was conducted to explore the mechanism behind the bone-protective effect of liraglutide, with a particular focus on BM-derived exosomal miRNAs in animal models mimicking postmenopausal women with T2DM. The study borrowed its hypothesis from the fact that an estrogen deficiency and hyperglycemia synergistically contribute to bone destruction, enhancing the osteoporosis risk in postmenopausal women with T2DM. To determine miRNAs with new functions, a comprehensive miRNA profiling analysis detected 460, 431, and 459 exosomal miRNAs in the ovariectomized (OVX), T2DM + OVX, and liraglutide-treated T2DM + OVX groups, respectively. Of these, 39 exosomal miRNAs showed differential expression between the T2DM and liraglutide-treated groups. The most upregulated miRNAs in the T2DM group were *miR-1-3p*, *let-7i-5p*, *let-7f-5p*, *miR-148a-3p*, *let-7 g-5p*, *miR-3571*, *miR-1b*, *miR-126a-3p*, *miR-206-3p*, and *miR-143-3p*. However, *miR-1-3p*, *let-7f-5p*, *miR-148a-3p*, *let-7i-5p*, *miR-3571*, *miR-126a-3p*, *miR-21-5p*, *let-7 g-5p*, *miR-206-3p*, and *miR-1b* were upregulated in the liraglutide treatment group. *Let-7c-2-3p* and *miR-322-3p* were significantly up-regulated when OVX and liraglutide groups were compared. Furthermore, the comparison of the T2DM group and the liraglutide group revealed that the T2DM group had greater expression of *miR-322-3p* against the Wnt pathway. The results demonstrate the promising role of BM-derived exosomal miRNAs in mediating liraglutide's protective effects on the bone via modu-

lation of the bone remodeling-related pathways and the glucose metabolism-related pathways [56].

Discussion

The effects of GLP-1RA medicine either as a monotherapy or in combination with other treatment modalities on miRNA profiles of T2DM patients and animal models were comprehensively evaluated in this systematic review. Besides blood glucose normalization and weight loss, our observation reveals the regulating function of GLP-1RAs towards modulating the expression of both exosomal and non-exosomal miRNAs. The review is of special significance in respect to the effects of GLP-1RAs, i.e., semaglutide, liraglutide, dulaglutide, and LA-exenatide, on miRNA expression and thus how this could affect their potential use in the treatment of type 2 diabetes. Although there have been some fascinating preclinical and clinical study parallels, our results also speak to some significant differences, illustrating the complexities of GLP-1RA-mediated miRNA regulation.

The crosstalk of incretin hormones, such as GLP-1, and miRNA regulatory network is of utmost importance in understanding GLP-1RAs' therapeutic actions. Many previous studies, e.g., those conducted by Radbakhsh et al. (2020), have suggested that many miRNAs have critical roles in modulating incretin hormone regulation. While *miR-155-5p* and *miR-33* promote higher GLP-1 expression, *miR-192*, *miR-6763*, etc., suppress incretin expression and thereby disrupt insulin secretion pathways. Besides, miRNAs such *as miR-204* and *miR-665* control the expression of GLP-1R, which in turn affects insulin signaling. GLP-1 also regulates miRNAs, including *miR-132* and *miR-212*, which amplify insulin secretion by pancreatic β-cells [37]. Certain of these miRNAs have also been found to contribute significantly towards the regulation of GLP-1R signaling by AL-Noshokaty et al. (2025). These comprise significant regulators of GLP-1R signaling, e.g., miR-7, miR-132, miR-204, and miR-212, which regulate insulin secretion, β-cell differentiation, and metabolic homeostasis [40]. Our review builds upon this knowledge by discussing the activity of GLP-1RAs on exosomal and non-exosomal miRNAs, yet another indication of their potential to control T2DM. While clinical studies have failed to identify exosomal miRNAs, preclinical data demonstrate that GLP-1RAs can modify both exosomal and non-exosomal miRNA profiles, revealing mechanistic evidence for their broader physiological actions.

Human experiments demonstrated that combination therapy with GLP-1RAs, such as LA-exenatide and Liraglutide, regulates non-exosomal *miR-27b*, *miR-130a*, and *miR-210* associated with cardiovascular disease and endothelial function [47–49, 57]. Furthermore, elevated levels of miR-27b are detected in circulating exosomes of patients with atherosclerotic coronary artery stenosis (ACAS) [59]. These heightened circulatory *miR-27b* levels serve as predictors of ACAS occurrence and are linked to subsequent cardiovascular events [60]. Essentially, *miRNA-27b* serves as a key regulator in the transcriptional network controlling beige and brown adipogenesis [61], which are crucial in metabolic disorders influencing obesity's nature and its cardiovascular risk implications [62]. Similarly, inhibition of *miR-130a* in hyperglycemia has been linked to endothelial dysfunction, hence hypothesizing to act as a biomarker for cardiovascular disease in T2DM patients [63]. Furthermore, *miR-210* is accountable for regulating oxidative stress responses through the hypoxia-inducible factor (HIF) pathway, indicating that it is a vasculoprotector [64].

Preclinical studies also emphasize the cardioprotective effects of GLP-1RAs through the modulation of *miR-29b-3p*, *miR-34a-5p*, *miR-26a-5p*, *miR-181a-5p*, and *miR-93-5p* [53, 55, 58]. It is noteworthy that *miR-29* has been found to be implicated in the regulation of fibrosis by modulating collagen expression. Indeed, its overexpression has been attributed to antifibrotic activity [65]. However, according to a study by Shi et al. (2019), *miR-29a* promoted cardiac hypertrophy and pathological heart remodeling through the targeting of the PTEN/AKT/ mammalian target of rapamycin (mTOR) pathway, Wnt signaling, and suppression of autophagy [66, 67]. In vivo genetic deficiency of *miR-29* in mouse models decreased cardiac hypertrophy and fibrosis, suggesting therapeutic efficacy through the targeting of this pathway [66, 67]. In addition, liraglutide monotherapy increased the expression of the cAMP response element-binding protein (CREB-1), a transcription factor of the cellular stress response, by repressing *miR-181a-5p*. The viability and function of endothelial cells were also influenced by the modulation of the *miR-181a-5p*/CREB-1 pathway [53]. Finally, by suppressing *miR-93-5p*, liraglutide could increase Sirtuin 1 (SIRT1) protein levels. By deacetylating major transcription factors, such as p53 and Forkhead Box O3 (FOXO3), Sirt1 suppressed the endothelial cell-harming effect of hyperglycemia pathway [53].

GLP-1RAs also have a greater hepatoprotective effect in animal models with T2DM and NAFLD. The molecular pathway of semaglutide-induced treatment hepatoprotective effect in patients with type 2 diabetes and NAFLD, particularly with HFD-induced obesity, is illustrated in Fig. 3. Briefly, semaglutide stimulates insulin release and production through the activation of the GLP-1 receptor on pancreatic β-cells with a series of signaling pathways. When the GLP-1 receptor is stimulated, the associated G-protein, resulting in the formation of cyclic AMP (cAMP). Higher levels of cAMP activate protein kinase A (PKA) and calcium/calmodulin-dependent protein kinase IV (CaMKIV), which phosphorylate

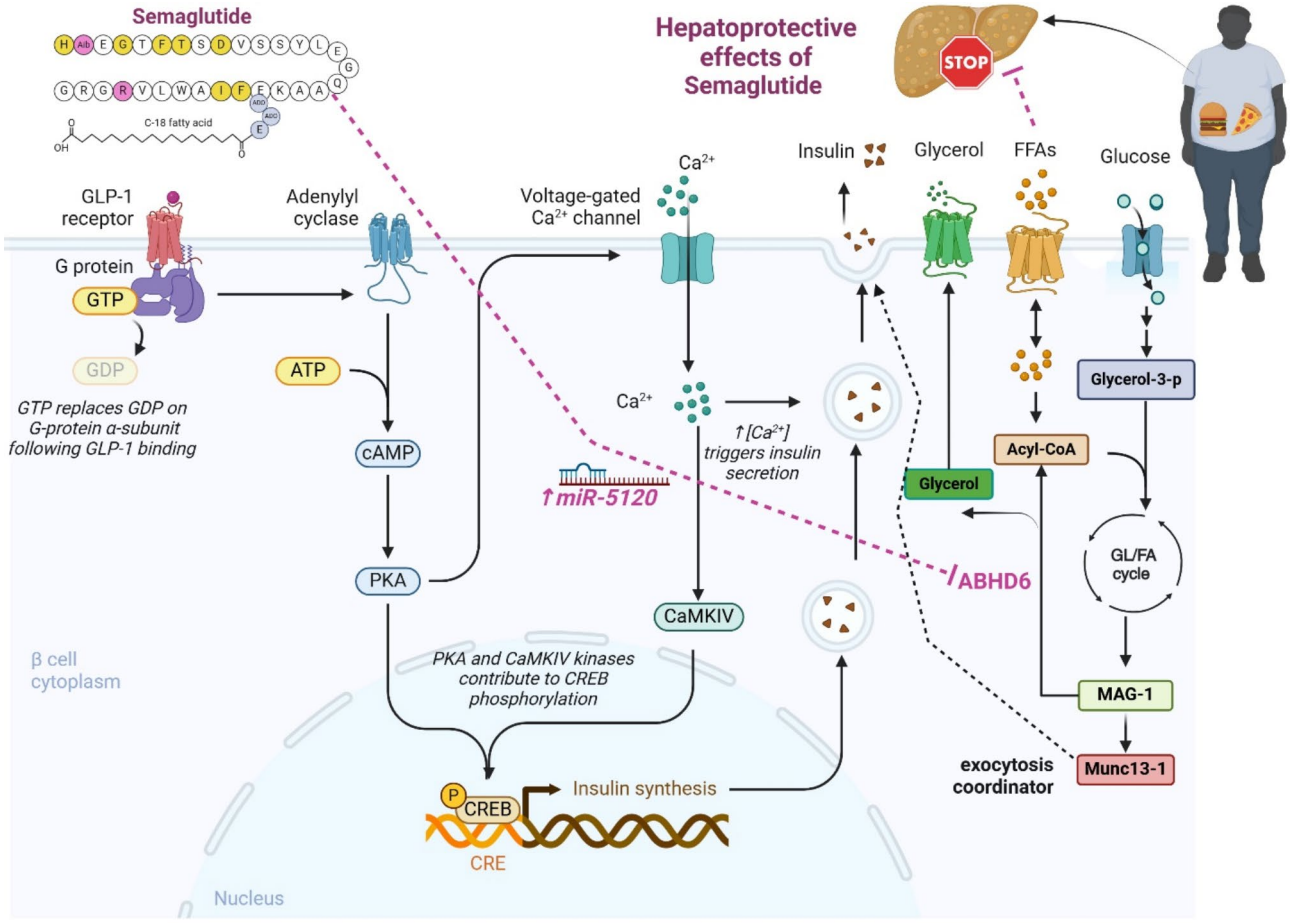

Fig. 3 Proposed Mechanism of Semaglutide-Mediated Hepatocellular Protection against High-Fat Diet-Induced Obesity in T2DM Patients with comorbid NAFLD through *miR-5120* Overexpression and α/β-Hydrolase Domain-6 (ABHD61) Repression. Semaglutide activates pancreatic β-cell GLP-1 receptor, which triggers a cascade signaling mechanism that enhances cyclic AMP (cAMP) synthesis, protein kinase A (PKA) activation, and calcium/calmodulin-dependent protein kinase IV (CaMKIV) signaling, leading to enhanced insulin synthesis and secretion. The illustration also shows the regulatory role of *miR-5120* in suppressing ABHD6 expression, thereby suppressing glycerol and free fatty acid (FFA) release, modulating the glycerol-lipid/fatty acid (GL/FA) cycle, and suppressing lipid accumulation and hepatocellular damage. Through these effects, semaglutide dampens lipotoxicity and metabolic dysfunction, a promising therapeutic strategy for NAFLD treatment in T2DM patients (Created in https://BioRender.com; MAG, Monoacylglycerols; Munc13-1, Mammalian Uncoordinated-13-1)

CREB-1 to enhance insulin gene expression. Semaglutide also has the effect of controlling calcium homeostasis to augment intracellular Ca^{2+} levels via voltage-gated calcium channels and additionally enhance Ca^{2+}-dependent exocytosis of insulin [68, 69]. Interestingly, the upregulation of *miR-5120* with negative feedback on ABHD6, a lipase that is involved in lipid metabolism dysregulation in obesity and NAFLD, is one of the pivotal hepatoprotective effects of semaglutide [54]. ABHD6 blockade disrupts inappropriate lipid storage through inhibition of the release of glycerol and fatty acids, preventing hepatocellular injury resulting from lipotoxicity. Through glycerol-lipid/fatty acid cycle and ABHD6 blockade, semaglutide inhibits hepatic steatosis and lipid toxicity, thus imparting protection against NAFLD development in patients with T2DM [68, 69]. The proposed mechanism highlights the multi-faceted mode of action of semaglutide in metabolic regulation, especially the favorable effect in preventing obesity-related liver disease.

Finally, new evidence suggests that GLP-1RAs influence bone metabolism, thus elucidating the paradoxically increased fracture risk in T2DM patients with normal or high bone mineral density (BMD) [70, 71]. New studies, such as that of Leanza et al. (2024), have elucidated the mechanisms. Specifically, the faulty expression of key bone formation genes, like SOST and RUNX2, and the accumulation of advanced glycation end-products (AGEs) are responsible for defective bone matrix integrity and increased fragility. AGEs aggravate the structural weakening of the bone tissue, thereby explaining the high fracture risk in T2DM patients [71]. These findings highlight the need for therapeutic interventions against the diabetes-induced effects on bone, e.g., the application of GLP-1RAs. Specifically, liraglutide promotes bone

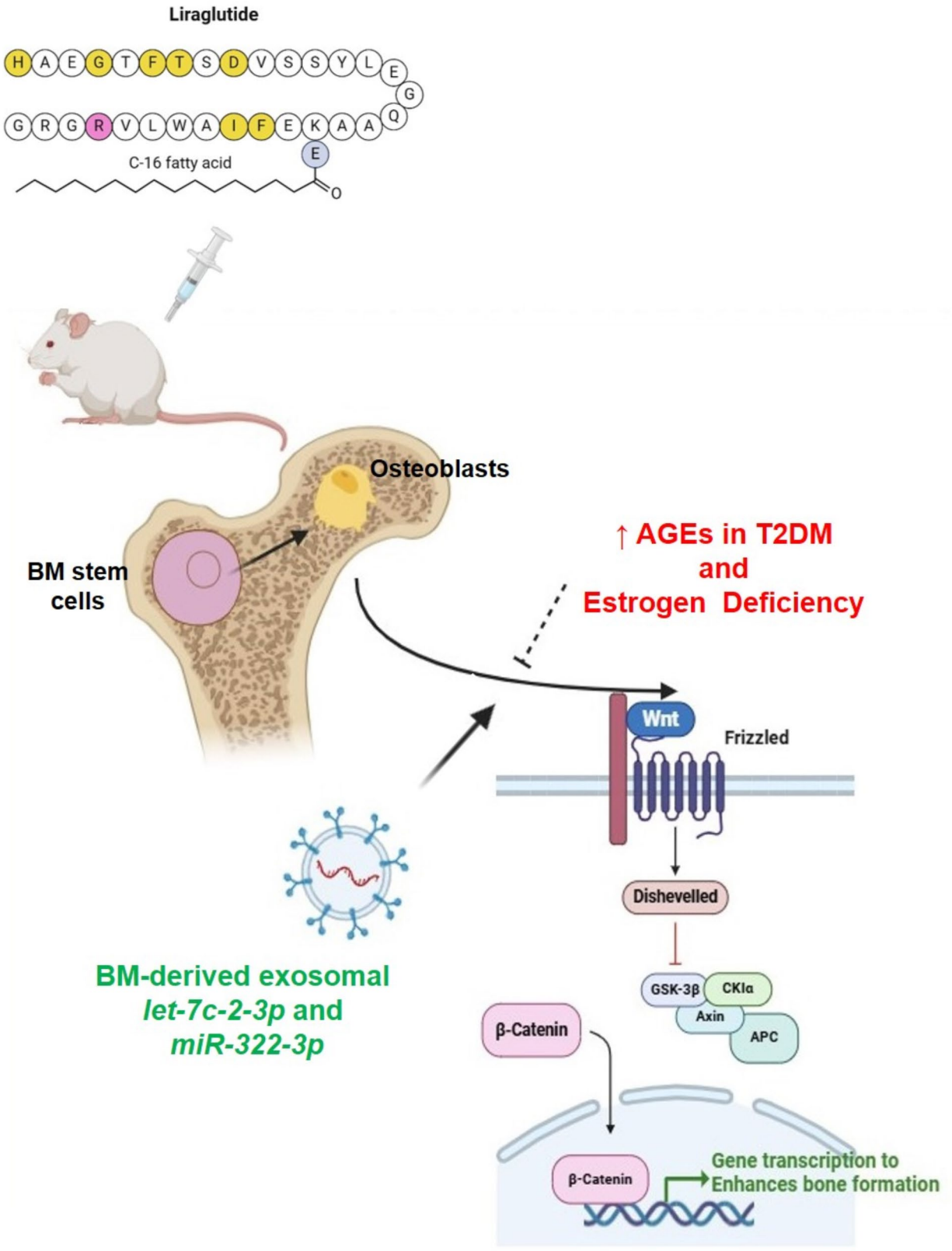

Fig. 4 (See legend on next page.)

(See figure on previous page.)

Fig. 4 Liraglutide Monotherapy Mediates Modulation of Bone Marrow (BM)-Derived Exosomal miRNAs and Wnt Signaling in Diabetic Bone Disease. It illustrates the mechanism of action of Liraglutide in increasing bone formation and counteracting the harmful effects of T2DM and estrogen deficiency on bone. In T2DM, hyperglycemia-induced high levels of advanced glycation end-products (AGEs) impair Wnt signaling, leading to impaired osteoblast function and increased fracture risk. Liraglutide regulates bone metabolism by upregulating BM-derived exosomal *let-7c-2-3p* and *miR-322-3p* that facilitate β-catenin stabilization and nuclear translocation, thereby inducing osteogenic gene transcriptional activation. Such a mechanism inhibits the negative effects of AGEs and estrogen deficiency and offers a likely therapeutic strategy towards improving bone stability in diabetic patients, particularly in postmenopausal women with T2DM (Created in https://BioRender.com)

formation by modulating the BM-derived exosomal *let-7c-2-3p* and *miR-322-3p*. These miRNAs induce Wnt signaling by stabilizing and translocating β-catenin, which leads to bone gene transcription [56]. In diabetic patients with poor bone quality, this pathoprotective mechanism turns around the adverse effect of AGEs on bone quality and has a unique therapeutic potential for fracture prevention [70, 71]. Figure 4 illustrates the complex molecular mechanisms interconnecting diabetes, estrogen deficiency, and osteoporosis and the potential of GLP-1RAs to restore bone homeostasis. These interactions suggest that GLP-1RAs, through their control of miRNAs and Wnt signaling, may mitigate the deleterious effects of estrogen deficiency on bone in diabetic individuals [56, 70–72]. Further work is justified in this area because past research has indicated that *let-7c-2-3p*, a miRNA that is typically downregulated in older bone, appears to play a protective function in bone metabolism [72]. In addition, *miR-322-3p* upregulation strikingly increased the mRNA expression of *Sp7*, a transcription factor needed for osteoblast differentiation and bone formation [73]. The findings indicate the therapeutic use of exosomal *let-7c-2-3p* and *miR-322-3p* regulation in diabetic bone disease.

The ability of circulating miRNAs to serve as therapeutic response predictive biomarkers of GLP-1RA therapy is a fast-rising topic of interest. Observational analysis of baseline expression levels of miRNAs such as *miR-21-5p*, *miR-24-3p*, and *miR-375-5p* in T2DM patients who were metformin monotherapy non-responders found that increased baseline expression levels of certain miRNAs indicated better therapeutic responses [35]. These findings suggest that miRNAs could be useful biomarkers to classify patients at higher risk of responding to GLP-1RA therapy. At the level of β-cell function, GLP-1RAs monotherapy influences miRNAs involved in insulin regulation. Finally, genetic downregulation of *miR-204*, *miR-375*, or *miR-139-5p* promoted glucose-stimulated insulin secretion in monotherapy with exendin-4 and liraglutide; therefore, future studies can be focused more on them [50–52].

Methodological limitations and considerations

The importance of GLP-1RA monotherapy and combination therapy in the modulation of exosomal and non-exosomal miRNAs in type 2 diabetes is highlighted in this systematic review. Exosomal miRNAs are not referred to in any clinical investigations. Indeed, there are technical restrictions of sEV biogenesis and origin, miRNA separation and quantification heterogeneities, and the types of normalization techniques that limit translation their applications to the clinic in spite of promise of the therapy. Multi-omics profiling, digital PCR, and standardized practices should be employed if clinical utility as well as replicability are to be expanded.

Moreover, as GLP-1RA is increasingly utilized in combination with other antidiabetic medications, of interest in future research will be to investigate their additive effect on miRNA levels, particularly in patients at high cardiometabolic risk, obesity, and NAFLD. Future longitudinal studies with standardized workflows and multi-omics approaches are needed to identify which miRNAs are specific to GLP-1RAs and which may also be associated with other antidiabetic drugs. Examination of miRNA changes in heterogeneous patients and prospective monitoring of their expression in concert with clinical markers (e.g., HbA1c, fasting glucose, body mass index parameters, and age-related differences) might provide insights into long-term treatment response.

Although GLP-1RA therapy is currently a second-line standard of diabetes care, its full potential can extend much further. As more studies are revealed, these results might reshape first-line treatment strategies, particularly in those with higher cardiometabolic risk, severe obesity, NAFLD, or postmenopausal osteoporosis. Unlocking miRNA signatures for optimal metabolic responses might pave the way for precision medicine.

Lastly, the study did not touch on practical concerns (e.g., circulating miRNA variability, sample collection standardization, inter-individual variability) due to fewer available studies. Standardizing miRNA extraction methods is a priority that can increase reliability as their potential hurdles to clinical translation. Standardized methods and well-defined treatment regimens are what future studies should emphasize in order to raise reproducibility and offer better conclusions.

Conclusion

In addition to showing promise for the treatment of obesity and type 2 diabetes, especially in those with insulin resistance, GLP-1RAs have also shown promise for the treatment of osteoporosis, NAFLD, and cardiovascular disease. Regulation of exosomal and non-exosomal miRNAs following GLP-1RA treatment and their contribution to tissue-specific effects, lipid metabolism, and

glycemic control have been highlighted in this review. However, current evidence is limited, particularly in human studies, and exosomal miRNA information in the clinic is an enormous gap to be filled. Longitudinal investigations combining cutting-edge multi-omics approaches are essential to implicate the precise molecular mechanisms of GLP-1RAs and compare their miRNA signature to that of other antidiabetic therapies.

Acknowledgements
This project is supported by the Doctoral Research Fund of Hubei University of Science and Technology, project No. Q201810. Therefore, we express our gratitude to them for their assistance.

Author contributions
Study leadership and manuscript revision (HY, XM and RA), review conception and manuscript writing (HY, XM, MD, MB and SS), and figure design (HY and RA). All authors have read and approved the final manuscript.

Funding
This project supported by the Doctoral Research Fund of Hubei University of Science and Technology, project No. Q201810.

Availability of supporting data
Not Applicable.

Declarations

Ethics approval and consent to participate
Not Applicable.

Consent of publication
Not Applicable.

Competing interests
The authors have no conflict of interest.

Disclosure
Declaration of AI-assisted technologies in the writing process: during the preparation of this review, the authors used ChatGPT to check the grammar and improve readability. After using the tools, we reviewed and edited the content as needed and took full responsibility for the content presented in the publication.

Author details
[1]Hubei Key Laboratory of Diabetes and Angiopathy, School of Pharmacy, Xianning Medical College, Hubei University of Science and Technology, Xianning 437100, Hubei Province, China
[2]Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Clinical Laboratory, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
[4]Ramsar Campus, Mazandaran University of Medical Sciences, Ramsar, Iran

Received: 13 January 2025 / Accepted: 6 April 2025
Published online: 25 April 2025

References

1. Ruze R, Liu T, Zou X, Song J, Chen Y, Xu R, et al. Obesity and type 2 diabetes mellitus: connections in epidemiology, pathogenesis, and treatments. Front Endocrinol. 2023;14:1161521.
2. Cavan D, da Rocha Fernandes J, Makaroff L, Ogurtsova K, Webber S. IDF diabetes atlas, Brussels: International Diabetes Federation. ISBN 978-2-930229-81-2; 2015.
3. Arpón A, Milagro FI, Santos JL, García-Granero M, Riezu-Boj J-I, Martínez JA. Interaction among sex, aging, and epigenetic processes concerning visceral fat, insulin resistance, and dyslipidaemia. Front Endocrinol. 2019;10:496.
4. Chambers JC, Loh M, Lehne B, Drong A, Kriebel J, Motta V, et al. Epigenome-wide association of DNA methylation markers in peripheral blood from Indian Asians and Europeans with incident type 2 diabetes: a nested case-control study. Lancet Diabetes Endocrinol. 2015;3(7):526–34.
5. Mansour A, Mousa M, Abdelmannan D, Tay G, Hassoun A, Alsafar H. Microvascular and macrovascular complications of type 2 diabetes mellitus: exome wide association analyses. Front Endocrinol. 2023;14:1143067.
6. Blüher M. Obesity: global epidemiology and pathogenesis. Nat Reviews Endocrinol. 2019;15(5):288–98.
7. Sarma S, Sockalingam S, Dash S. Obesity as a multisystem disease: trends in obesity rates and obesity-related complications. Diabetes Obes Metabolism. 2021;23:3–16.
8. Blüher M, Ceriello A, Davies M, Rodbard H, Sattar N, Schnell O, et al. Managing weight and glycaemic targets in people with type 2 diabetes—How Far have we come? Endocrinology. Diabetes Metab. 2022;5(3):e00330.
9. Weerakiet S, Srisombut C, Bunnag P, Sangtong S, Chuangsoongnoen N, Rojanasakul A. Prevalence of type 2 diabetes mellitus and impaired glucose tolerance in Asian women with polycystic ovary syndrome. Int J Gynecol Obstet. 2001;75(2):177–84.
10. Palta P, Huang ES, Kalyani RR, Golden SH, Yeh H-C. Hemoglobin A1c and mortality in older adults with and without diabetes: results from the National health and nutrition examination surveys (1988–2011). Diabetes Care. 2017;40(4):453–60.
11. Rothberg AE, Ard JD, Gudzune KA, Herman WH. Obesity management for the treatment of type 2 diabetes. Diabetes in America [Internet]. 2024.
12. Owen MR, Doran E, Halestrap AP. Evidence that Metformin exerts its anti-diabetic effects through Inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J. 2000;348(3):607–14.
13. Malboosbaf R, Hatami N. Treatment Approaches and Challenges. Nanoscience Applications in Diabetes Treatment. 2023:31.
14. American Diabetes Association. Obesity management for the treatment of type 2 diabetes: standards of medical care in diabetes—2021. Diabetes Care. 2021;44(Supplement1):S100–10.
15. Capuani B, Pacifici F, Della-Morte D, Lauro D. Glucagon like peptide 1 and MicroRNA in metabolic diseases: focusing on GLP1 action on MiRNAs. Front Endocrinol. 2018;9:719.
16. Garber AJ. Long-acting glucagon-like peptide 1 receptor agonists: a review of their efficacy and tolerability. Diabetes Care. 2011;34(Suppl 2):S279.
17. Gallwitz B. GLP-1 agonists and dipeptidyl-peptidase IV inhibitors. Diabetes-Perspectives Drug Therapy. 2011:53–74.
18. Okerson T, Chilton RJ. The cardiovascular effects of GLP-1 receptor agonists. Cardiovasc Ther. 2012;30(3):e146–55.
19. Zheng SL, Roddick AJ, Aghar-Jaffar R, Shun-Shin MJ, Francis D, Oliver N, et al. Association between use of sodium-glucose cotransporter 2 inhibitors, glucagon-like peptide 1 agonists, and dipeptidyl peptidase 4 inhibitors with all-cause mortality in patients with type 2 diabetes: a systematic review and meta-analysis. JAMA. 2018;319(15):1580–91.
20. Monami M, Marchionni N, Mannucci E. Glucagon-like peptide-1 receptor agonists in type 2 diabetes: a meta-analysis of randomized clinical trials. Eur J Endocrinol. 2009;160(6):909–17.
21. Movahednasab M, Dianat-Moghadam H, Khodadad S, Nedaeinia R, Safabakhsh S, Ferns G, et al. GLP-1-based therapies for type 2 diabetes: from single, dual and triple agonists to endogenous GLP-1 production and L-cell differentiation. Diabetol Metab Syndr. 2025;17(1):60.
22. Miller L-A, Burudpakdee C, Zagar A, Bhosle M, Reaney M, Schabert VF, et al. Exenatide BID and liraglutide QD treatment patterns among type 2 diabetes patients in Germany. J Med Econ. 2012;15(4):746–57.
23. He X, Zhao W, Li P, Zhang Y, Li G, Su H, et al. Research progress of GLP-1RAs in the treatment of type 2 diabetes mellitus. Front Pharmacol. 2025;15:1483792.
24. Miao X, Davoudi M, Alitotonchi Z, Ahmadi ES, Amraee F, Alemi A, et al. Managing cardiovascular events, hyperglycemia, and obesity in type 2 diabetes through MicroRNA regulation linked to glucagon-like peptide-1 receptor agonists. Diabetol Metab Syndr. 2025;17(1):13.
25. Collins L, Costello RA. Glucagon-like peptide-1 receptor agonists. 2019.
26. El Eid L, Reynolds CA, Tomas A, Jones B. Biased agonism and polymorphic variation at the GLP-1 receptor: implications for the development of personalised therapeutics. Pharmacol Res. 2022;184:106411.
27. Jensterle M, Pirš B, Goričar K, Dolžan V, Janež A. Genetic variability in GLP-1 receptor is associated with inter-individual differences in weight Lowering

potential of liraglutide in obese women with PCOS: a pilot study. Eur J Clin Pharmacol. 2015;71:817–24.
28. Luo P, Fan Y, Xiong Y, Feng H, Yang Z, Zhang C, et al. Genetic variants of the GLP-1R gene affect the susceptibility and glucose metabolism of gestational diabetes mellitus: a two-center nested case–control study. Diabetol Metab Syndr. 2022;14(1):190.
29. Demirsoy İH, Ertural DY, Balci Ş, Çınkır Ü, Sezer K, Tamer L, et al. Profiles of Circulating MiRNAs following Metformin treatment in patients with type 2 diabetes. J Med Biochem. 2018;37(4):499.
30. Catanzaro G, Besharat ZM, Chiacchiarini M, Abballe L, Sabato C, Vacca A et al. Circulating microRNAs in elderly type 2 diabetic patients. International Journal of Endocrinology. 2018;2018.
31. Flowers E, Aouizerat BE, Abbasi F, Lamendola C, Grove KM, Fukuoka Y, et al. Circulating microRNA-320a and microRNA-486 predict Thiazolidinedione response: moving towards precision health for diabetes prevention. Metabolism. 2015;64(9):1051–9.
32. Palihaderu P, Mendis B, Premarathne J, Dias W, Yeap SK, Ho WY, et al. Therapeutic potential of MiRNAs for type 2 diabetes mellitus: an overview. Epigenetics Insights. 2022;15:25168657221130041.
33. Lu F, Gong H, Duan L, Yan Y, Chen L, Peng P, et al. miR-29 regulates metabolism by inhibiting JNK-1 expression in non-obese patients with type 2 diabetes mellitus and NAFLD. Open Med. 2023;18(1):20230873.
34. Joglekar MV, Januszewski AS, Jenkins AJ, Hardikar AA. Circulating MicroRNA biomarkers of diabetic retinopathy. Diabetes. 2016;65(1):22–4.
35. Formichi C, Fignani D, Nigi L, Grieco GE, Brusco N, Licata G, et al. Circulating MicroRNAs signature for predicting response to GLP1-RA therapy in type 2 diabetic patients: a pilot study. Int J Mol Sci. 2021;22(17):9454.
36. Yadav P, Tamilselvan R, Harita M, Singh KK. MicroRNA-mediated regulation of nonsense-mediated mRNA decay factors: Insights into microRNA prediction tools and profiling techniques. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms. 2024:195022.
37. Radbakhsh S, Sathyapalan T, Banach M, Sahebkar A. Incretins and MicroRNAs: interactions and physiological relevance. Pharmacol Res. 2020;153:104662.
38. Catalanotto C, Cogoni C, Zardo G. MicroRNA in control of gene expression: an overview of nuclear functions. Int J Mol Sci. 2016;17(10):1712.
39. Agbu P, Carthew RW. MicroRNA-mediated regulation of glucose and lipid metabolism. Nat Rev Mol Cell Biol. 2021;22(6):425–38.
40. Al-Noshokaty TM, Abdelhamid R, Abdelmaksoud NM, Khaled A, Hossam M, Ahmed R et al. Unlocking the multifaceted roles of GLP-1: physiological functions and therapeutic potential. Toxicol Rep. 2025:101895.
41. He X, Kuang G, Wu Y, Ou C. Emerging roles of Exosomal MiRNAs in diabetes mellitus. Clin Translational Med. 2021;11(6):e468.
42. Zhou R, Chen KK, Zhang J, Xiao B, Huang Z, Ju C, et al. The decade of Exosomal long RNA species: an emerging cancer antagonist. Mol Cancer. 2018;17:1–14.
43. Lu T, Zheng Y, Chen X, Lin Z, Liu C, Yuan C. The role of exosome derived MiRNAs in inter-cell crosstalk among insulin-related organs in type 2 diabetes mellitus. J Physiol Biochem. 2024;80(3):501–10.
44. Wang Y, Nie H, He X, Liao Z, Zhou Y, Zhou J, et al. The emerging role of super enhancer-derived noncoding RNAs in human cancer. Theranostics. 2020;10(24):11049.
45. Sou YL, Chilian WM, Ratnam W, Zain SM, Syed Abdul Kadir SZ, Pan Y, et al. Exosomal MiRNAs and IsomiRs: potential biomarkers for type 2 diabetes mellitus. Precision Clin Med. 2024;7(3):pbae021.
46. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Int J Surg. 2021;88:105906.
47. Nikolic D, Giglio R, Chianetta R, Castracani CC, Sardo V, Castellino G, et al. editors. The GLP-1RA exenatide LAR increases circulating miR-27b in patients with type 2 diabetes: an 8-month prospective study of cardiometabolic and epigenetic response. DIABETOLOGIA; 2017: SPRINGER 233 SPRING ST, NEW YORK, NY 10013 USA.
48. Gaborit B, Julla J-B, Besbes S, Proust M, Vincentelli C, Alos B, et al. Glucagon-like peptide 1 receptor agonists, diabetic retinopathy and angiogenesis: the AngioSafe type 2 diabetes study. J Clin Endocrinol Metabolism. 2020;105(4):e1549–60.
49. Rosaria Vincenza Giglio DN, Giovanni Li Volti AP, Stoian. Liraglutide increases serum levels of MicroRNA-27b, -130a and– 210 in patients with type 2 diabetes mellitus: A novel epigenetic effect. Metabolites. 2020;10(10):391. https://doi.org/10.3390/metabo10100391.
50. Song I, Roels S, Martens GA, Bouwens L. Circulating microRNA-375 as biomarker of pancreatic beta cell death and protection of beta cell mass by cytoprotective compounds. PLoS ONE. 2017;12(10):e0186480.
51. Li J, Su L, Gong Y-y, Ding M-l, Hong S-b, Yu S, et al. Downregulation of miR-139-5p contributes to the antiapoptotic effect of liraglutide on the diabetic rat pancreas and INS-1 cells by targeting IRS1. PLoS ONE. 2017;12(3):e0173576.
52. Jo S, Chen J, Xu G, Grayson TB, Thielen LA, Shalev A. miR-204 controls glucagon-like peptide 1 receptor expression and agonist function. Diabetes. 2018;67(2):256–64.
53. Zhang Q, Xiao X, Zheng J, Li M. A glucagon-like peptide-1 analog, Liraglutide, ameliorates endothelial dysfunction through MiRNAs to inhibit apoptosis in rats. PeerJ Biochem Biophys Molec Biol. 2019;7:e6567.
54. Li R, Ye Z, She D, Fang P, Zong G, Hu K et al. Semaglutide may alleviate hepatic steatosis in T2DM combined with NFALD mice via miR-5120/ABHD6. Drug Design, Development and Therapy. 2023:3557-72.
55. Fang P, Ye Z, Li R, She D, Zong G, Zhang L et al. Glucagon-like peptide-1 receptor agonist protects against diabetic cardiomyopathy by modulating microRNA-29b-3p/SLMAP. Drug Design, Development and Therapy. 2023:791–806.
56. Li J, Fu L-Z, Liu L, Xie F, Dai R-C. Glucagon-like peptide-1 (GLP-1) receptor agonist liraglutide alters bone marrow exosome-mediated MiRNA signal pathways in ovariectomized rats with type 2 diabetes. Med Sci Monitor: Int Med J Experimental Clin Res. 2017;23:5410.
57. Gaborit BJJ-B, Besbes S et al. Supplemental data from: glucagon-like peptide 1 receptor agonists, diabetic retinopathy and angiogenesis: safe or not? The AngioSafe type 2 diabetes study. 2019.
58. Redfield MM. Heart failure with preserved ejection fraction. N Engl J Med. 2016;375(19):1868–77.
59. Dolz S, Górriz D, Tembl JI, Sánchez D, Fortea G, Parkhutik V, et al. Circulating MicroRNAs as novel biomarkers of stenosis progression in asymptomatic carotid stenosis. Stroke. 2017;48(1):10–6.
60. Lu T, Li X, Long C, Ji W, Jiang L, Tian J. Circulating miR-27b as a biomarker of the development and progression of carotid artery stenosis. Clin Appl Thromb Hemost. 2021;27:10760296211057903.
61. Sun L, Trajkovski M. MiR-27 orchestrates the transcriptional regulation of brown adipogenesis. Metabolism. 2014;63(2):272–82.
62. Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. Nat Med. 2013;19(10):1252–63.
63. Jia Q-W, Chen Z-H, Ding X-Q, Liu J-Y, Ge P-C, An F-H, et al. Predictive effects of Circulating miR-221, miR-130a and miR-155 for coronary heart disease: a multi-ethnic study in China. Cell Physiol Biochem. 2017;42(2):808–23.
64. Devlin C, Greco S, Martelli F, Ivan M. miR-210: more than a silent player in hypoxia. IUBMB Life. 2011;63(2):94–100.
65. Van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proceedings of the National Academy of Sciences. 2008;105(35):13027-32.
66. Shi Jy, Chen C, Xu X, Lu Q. miR-29a promotes pathological cardiac hypertrophy by targeting the PTEN/AKT/mTOR signalling pathway and suppressing autophagy. Acta Physiol. 2019;227(2):e13323.
67. Sassi Y, Avramopoulos P, Ramanujam D, Grüter L, Werfel S, Giosele S, et al. Cardiac myocyte miR-29 promotes pathological remodeling of the heart by activating Wnt signaling. Nat Commun. 2017;8(1):1–11.
68. Zhao S, Poursharifi P, Mugabo Y, Levens EJ, Vivot K, Attane C, et al. α/β-Hydrolase domain-6 and saturated long chain monoacylglycerol regulate insulin secretion promoted by both fuel and non-fuel stimuli. Mol Metabolism. 2015;4(12):940–50.
69. Pusch L-M, Riegler-Berket L, Oberer M, Zimmermann R, Taschler U. α/β-Hydrolase Domain-Containing 6 (ABHD6)—A multifunctional lipid hydrolase. Metabolites. 2022;12(8):761.
70. Bao K, Jiao Y, Xing L, Zhang F, Tian F. The role of Wnt signaling in diabetes-induced osteoporosis. Diabetol Metab Syndr. 2023;15(1):84.
71. Leanza G, Cannata F, Faraj M, Pedone C, Viola V, Tramontana F, et al. Bone canonical Wnt signaling is downregulated in type 2 diabetes and associates with higher advanced glycation end-products (AGEs) content and reduced bone strength. Elife. 2024;12:RP90437.
72. He X, Zhang W, Liao L, Fu X, Yu Q, Jin Y. Identification and characterization of MicroRNAs by high through-put sequencing in mesenchymal stem cells and bone tissue from mice of age-related osteoporosis. PLoS ONE. 2013;8(8):e71895.

73. Minami S, Fujii Y, Yoshioka Y, Hatori A, Kaneko K, Ochiya T, et al. Extracellular vesicles from mouse bone marrow macrophages-derived osteoclasts treated with Zoledronic acid contain miR-146a-5p and miR-322-3p, which inhibit osteoclast function. Bone. 2025;190:117323.

Publisher's note