The role of exosomes in the pathophysiology of diabetes and their potential clinical implications

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Thesis mentor: Professor Dijana Detel, MD, PhD

The graduation thesis was graded on 10.06.2024 in Rijeka before the

Committee composed of the following members:

1. Associate Professor Sanja Klobučar, MD, PhD (Committee Head)
2. Associate Professor Lara Batičić, PhD
3. Asistant Professor Sunčica Buljević, PhD

The graduation thesis contains 45 (forty-five) pages, 3 (three) figures, 5 (five) tables, and 52 (fifty-two) references.

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1. List of abbreviations

AMBP - α_1-microglobulin/bikunin precursor

ATP - Adenosine triphosphate

BAT - Brown adipose tissue

BeAT - Beige adipose tissue

BCCAs - Branched-chain amino acids

BMI - Body mass index

circRNAs - circular RNAs

CRP - C-Reactive Protein

DAG - Diacylglycerol

DCCT - Diabetes Control and Complications Trial

DCM - Diabetic Cardiomyopathy

DME - Diabetic macular edema

DM - Diabetes mellitus

DN - Diabetic Nephropathy

DPN - Diabetic polyneuropathy

DR - Diabetic Retinopathy

DWH - Diabetic wound healing

DNA - Deoxyribonucleic acid

ESE - early-sorting endosome

ER - Endoplasmic reticulum

ERK1/2 - Extracellular signal-regulated protein kinases 1 and 2

ESCRT proteins - Endosomal sorting complex required for transport proteins

EndoMT - Endothelial-mesenchymal transition

EVs - Extracellular vesicles

FA - Fatty acids

FPG - Fasting plasma glucose

HbA1C - Hemoglobin A1C

HDF - Human dermal fibroblast

HF - Heart Failure

HRMECs - Human retinal microvascular endothelial cells

HSPs - Heat shock proteins

IL - Interleukin

ILVs - Intraluminal vesicles

IRS1 - Insulin receptor substrate 1

IR - Insulin Resistance

KLF4 - Krüppel-like factor 4

LAMP1 - Lysosomal associated membrane protein 1

lncRNA - long non-coding RNA

LPS - lipopolysaccharide

MLL3 - lysine-specific methyltransferase C2

MiRs - microRNAs

MNT - Medical nutrition therapy

mRNA - messenger RNA

MSCs - Mesenchymal stem cells

MVs - Macrovesicles

ncRNA - non-coding RNA

NEFAs - non-esterified fatty acids

OGTT - oral glucose tolerance test

PDR - proliferative diabetic retinopathy

PI3K - phosphatidylinositol-3 kinase

PKCε - protein kinase C epsilon

PPARγ - peroxisome proliferator-activated receptor γ

PTCH1 gene - protein patched homolog 1 gene

Rab - Ras-associated binding

RNA - Ribonucleic acid

ROS - Reactive oxygen species

SNAREs - soluble N-ethylmaleimide-sensitive factor activating protein receptor

TAG - triglycerides

T1DM - Type 1 diabetes mellitus

T2DM - Type 2 diabetes mellitus

TGFβ - Transforming growth factor β

TLR4 - Toll-like receptor R

TNF-α - tumor necrosis factor alfa

USCs - urine-derived stem cells

VDAC1 - the voltage-dependent anion channel 1

WAT - White adipose tissue

2. Introduction

The thesis entitled 'The Role of Exosomes in the Pathophysiology of Diabetes and Their Potential Clinical Implications' aims to emphasize the significance and function of exosomes in the pathophysiology of type 2 diabetes mellitus (T2DM) and its complications, as well as their prospective applications in clinical diagnostics and therapeutics.

Initially, this thesis will first provide an overview of diabetes mellitus, including its types. It then delves into the pathophysiology of T2DM and the role of obesity in the pathophysiology. The thesis also addresses the current epidemiology of T2DM, its clinical features, current treatment, and chronic complications.

Moreover, the thesis targets extracellular vesicles (EVs), focusing on exosomes and their role in the pathophysiology of T2DM. The various types of EVs are described in detail, with a particular emphasis on exosomes. Additionally, the potential of exosomes in clinical practice, including their applications in diagnostics and therapy, will be discussed.

The thesis will conclude by exploring future possibilities and addressing current challenges associated with exosomes.

3. Aims and Objectives

This research explores the multifaceted role of exosomes in the pathophysiology of diabetes mellitus (DM), focusing mainly on type 2 diabetes (T2DM) and its complications while also considering their clinical implications. It examines the complex mechanisms underlying T2DM, which remain partially understood in current literature.

The study investigates the relationship between T2DM and obesity and the role of exosomes, a type of extracellular vesicles (EVs), in the progression of T2DM and the development of DM complications. Additionally, the potential of exosomes as diagnostic biomarkers for early detection and monitoring of diabetes and its complications is examined. Furthermore, the study delves into the therapeutic potential of exosomes in managing T2DM and its associated complications, aiming to elucidate the mechanisms of their efficacy.

The research identifies and analyzes current challenges and limitations in exosome research, including isolation techniques, assay standardization, and clinical translation hurdles, as well as therapeutic challenges like administration methods, storage, and early-phase clinical trials. By addressing these aims, this study seeks to provide significant insights into diabetes and exosome biology, aiming to inform current practices and shape future strategies for better diagnosis and treatment of T2DM.

4. Literature Review

4.1 What is Diabetes Mellitus, and how is it classified?

Diabetes Mellitus is a multifactorial condition marked by elevated glucose levels in the bloodstream over a prolonged period caused by impaired insulin secretion and resistance. Nowadays, it is a significant worldwide health burden due to its high prevalence. Classification of DM based on etiology (1):

1. Type 1 DM (immune-mediated β cell destruction of pancreatic cells)
2. Type 2 DM (insulin insensitivity and in later stages of the disease, there can be decreased insulin secretion)
3. Specific types of diabetes (monogenic or maturity-onset diabetes of the young)
    - Genetic defects of β cell development or function
    - Neonatal diabetes
    - Diseases of the exocrine pancreas (acute or chronic pancreatitis, pancreatectomy, neoplasia of the pancreas, cystic fibrosis, etc.)
    - Genetic defects in insulin (insulin type A resistance, Leprechaunism, Rabson-Mendenhall syndrome, lipodystrophy syndromes)
    - Endocrinopathies (acromegaly, Cushing's syndrome, glucagonoma, heochromocytoma, etc.)
    - Drug-induced (glucocorticoids, calcineurin, and mTOR inhibitors, protease inhibitors, antipsychotics)
    - Infectious (congenital rubella, cytomegalovirus, coxsackievirus)
    - Rare forms of diabetes – stiff person syndrome, anti-insulin receptor antibodies
    - Genetic syndromes associated with diabetes – Down syndrome, Klinefelter's syndrome, Turner's syndrome, Wolfram syndrome.
4. Gestational diabetes

Type 1 diabetes mellitus (T1DM) and T2DM are the most prevalent types worldwide, with T2DM being the most common.

Type 1 diabetes is triggered by an immune-mediated reaction against β cells of the pancreas due to genetic, environmental, and immunological factors (1).

4.2 Type 2 diabetes mellitus

T2DM is considered a polygenic and multifactorial disease with insulin resistance in its core pathophysiology. T2DM is strongly connected to genetic components. The risk of T2DM in monozygotic twins, ranging between 70% and 90%, strongly indicates a significant genetic component in developing the disease. Persons with a parental history of T2DM are at a higher risk of developing diabetes. In a situation when both parents have been diagnosed with T2DM, the risk increases to almost 70%. It is interesting to note that the in-utero environment can also impact the risk of type 2 diabetes in adulthood. Increased or reduced birth weight has been shown to increase the risk of developing T2DM later in life. Additionally, children born to mothers with gestational hyperglycemia also have an increased risk of developing T2DM (1).

4.2.1 Pathophysiology

In T2DM, insulin secretion is impaired, while liver glucose synthesis is increased. It is characterized by mild systemic inflammation and insulin resistance (IR) (1). According to studies, individuals with T2DM experience a decrease in insulin sensitivity about five years before the diagnosis. Additionally, about three to four years before the diagnosis, there is an increase in pancreatic β cell function, which means an increase in insulin secretion. Until the diagnosis, β cell function is thought to decline, and less insulin is secreted (2). The reduction in insulin production results in a rise in glucose secretion by the liver. Decreased glucose utilization from insulin-sensitive tissues such as skeletal muscles and the liver promotes hepatic glucose secretion, resulting in increased fasting plasma glucose levels due to increased IR. When glucose utilization from the peripheral tissues decreases, it increases glucose levels after a meal (1, 26).

The mechanism of IR development is still unknown despite extensive research. In T2DM, it is observed that the insulin receptor expression and tyrosine kinase activity in skeletal muscles decrease. This reduction is believed to be a result of hyperinsulinemia, but the underlying cause of IR is still unknown. It is believed to be a post-receptor defect that causes IR. Impaired oxidation of fatty acids in skeletal muscles and the accumulation of lipids in them are crucial factors driving insulin resistance. These can generate reactive oxygen species, which cause myocyte damage and lead to a low inflammatory state (1).

An increase in IR proportionally increases with age and weight, significantly increasing an increase in weight. Being overweight or obese, which accompanies people with type 2 diabetes, is part of a pathogenic process. Increased adipocyte mass due to obesity leads to increased free fatty acids and substances secreted by adipocytes known as adipokines. Adipokines include leptin, adiponectin, tumor necrosis factor α, and resistin (2). For example, adipokines affect weight, appetite, and energy

expenditure. They also have a role in insulin sensitivity modulation. Moreover, the products of adipocytes and adipokines lead to an inflammatory state, which is thought to cause elevated CRP (C-reactive protein) and IL-6 (interleukin-6) levels. Fatty acids (FA) decrease glucose utilization by skeletal muscles, promote glucose synthesis in the liver, and impair the function of β-pancreatic cells (1).

Insulin secretion and sensitivity are interdependent. As IR increases, the body tries to compensate by increasing insulin secretion. However, with time, insulin secretion becomes impaired. Initially, this leads to a mild deficiency in insulin secretion that primarily affects glucose-stimulated insulin release, particularly the first secretion phase. Although responsiveness to alternative non-glucose secretagogues like arginine remains intact, overall β cell functionality is decreased by up to 50% upon the onset of T2DM (1). In T2DM, there is either impaired insulin processing by β cells or, because of increased insulin secretion, there is a lack of time for granules in β cells to mature perfectly, so they secrete high amounts of proinsulin (2).

Another unknown fact about T2DM pathophysiology is, what is the reason for the decline in insulin secretion. One hypothesis is that there is a secondary genetic defect superimposed by IR, which leads to decreased secretory functions of β cells of the pancreas (1). It is currently understood that individuals with T2DM have higher levels of amylin, a protein that forms deposits known as amyloid fibrils. Amylin is typically stored in the insulin granules of β cells and released alongside insulin. High amylin concentrations can reduce glucose signaling in pancreatic islet cells and inhibit endogenous insulin secretion (2). This suggests that amylin could have a direct role in the development of T2DM (1, 26).

4.2.2 Role of obesity in insulin resistance and type 2 diabetes

It is known that obesity and diabetes are well interconnected with one another. Increased body mass and obesity are significant risk factors for the development of T2DM. Furthermore, being obese increases the risk of developing IR, which is a crucial factor in the development of T2DM. This was proven by several studies. It's interesting to note that a study of offspring without diabetes of two parents with T2DM found that their insulin sensitivity was similar to that of normal subjects with no first-degree relatives with T2DM at near-ideal body weight. However, with increasing degrees of obesity, the progressive decrease in insulin sensitivity was much more pronounced in those with a family history of T2DM, indicating the importance of the combination of genetic and environmental factors in the development of the disease (2).

The allocation of body fat significantly influences insulin sensitivity. While IR is often linked with obesity, even lean individuals can show different levels of insulin sensitivity based on their fat distribution. Those with a greater amount of peripheral body fat tend to possess higher insulin sensitivity than individuals with a higher concentration of central fat, such as in the abdomen and chest (2).

Obesity involves an excess of adipose tissue. When adipose tissue accumulates in non-adipose areas, it triggers a state of low-grade inflammation. Adipocytes release adipokines that interfere with the insulin signaling pathway, thereby contributing to IR (3, 4).

There are three subtypes of adipocytes, which form three subtypes of adipose tissue.

- White adipose tissue (WAT)
- Brown adipose tissue (BAT)
- Beige adipose tissue (BeAT)

WAT represents the predominant subtype of adipose tissue in the body. These adipocytes are primarily situated beneath the skin, known as subcutaneous adipose tissue, and serve as crucial energy reservoirs. Additionally, white adipocytes function as energy producers, aiding in the storage and release of energy as needed by the body. Moreover, white adipocytes play a crucial role in metabolic regulation by secreting various signaling molecules known as adipokines, which contribute to the regulation of metabolic processes and overall physiological homeostasis. There is much less BAT than WAT and is mainly located in supraventricular, paravertebral, and mediastinal areas. Adipocytes in the BAT are activated during exposure to cold to produce heat and have a thermogenic effect. Beige adipocytes, or brown-white adipocytes, are between the WAT. They can transform into brown adipocytes when required. These beige adipocytes can produce heat and help regulate body temperature when the body is subjected to cold, exercise, or endocrine signaling. Brown and beige adipocytes play significant roles in maintaining energy balance, while dysfunctional white adipocytes in obesity contribute to various metabolic disorders. At the cellular level, white adipocytes, in cases of metabolic disorders, are undergoing some changes. They have changes in their extracellular matrix, size, number, release of reactive oxygen species (ROS) and adipokines, and infiltration of immune cells. These changes in white adipocytes cause IR, as seen in T2DM (4).

4.2.2.1 Fatty acids

FAs, both endogenous and exogenous, are in excess in the case of obesity. However, some FAs have a worse impact on the development of T2DM, especially saturated FAs. Saturated FAs reduce insulin sensitivity by activating specific inflammatory signals (4).

DAG is another lipid molecule that has been associated with T2DM. Lipid molecules like DAG, which serves as a direct precursor to triglycerides (TAG), demonstrate elevated concentrations in T2DM. These heightened levels have been correlated with diminished insulin sensitivity, attributable to aberrant alterations in insulin signaling molecules. Similarly, increased concentrations of ceramides with specific chain lengths and saturated FAs, categorized as sphingolipids, have been documented to

hinder insulin sensitivity initially by reducing adiponectin levels and exacerbating inflammation due to IR (4).

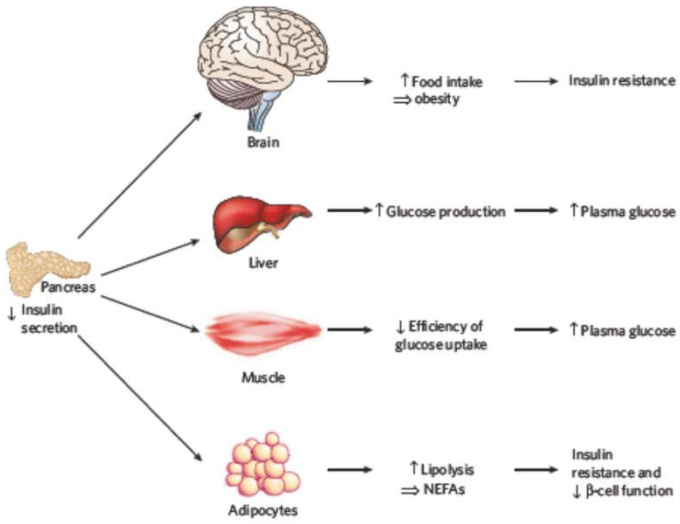

Figure 1: Illustration of the pivotal role of impaired insulin secretion in establishing the connection between obesity, insulin resistance, and type 2 diabetes.

Impaired insulin secretion precipitates a decline in insulin levels and signaling within the hypothalamus. Consequently, this leads to heightened food intake and subsequent weight gain, reduced inhibition of hepatic glucose production, diminished efficiency of glucose uptake in muscle tissue, and elevated lipolysis in adipocytes, culminating in increased plasma non-esterified fatty acid (NEFA) concentrations. The escalation in body weight and NEFAs contributes to insulin resistance, with elevated NEFA levels further dampening the beta-cell's adaptive response to insulin resistance. Elevated glucose and NEFA levels synergize to exacerbate beta-cell dysfunction and insulin action impairment, commonly termed as 'glucolipotoxicity' (3).

Specifically, the expansion of WAT within skeletal muscles and the liver results in elevated DAG levels. This increase in DAG activates protein kinase C epsilon (PKCε), which leads to a decrease in the uptake of glucose by muscle cells and interferes with insulin-triggered activation of glycogen synthesis while preventing the liver from effectively suppressing glucose production. These processes contribute to the development of hyperglycemia. Another vital factor is mitochondrial dysfunction, which is mainly caused by lipotoxicity. Lipotoxicity causes mitochondrial dysfunction; by generating ROS, adenosine triphosphate (ATP) production will be decreased, and the mitochondrial mass will be reduced. As a result, lipotoxicity triggers mitochondrial dysfunction characterized by decreased mitochondrial mass and ATP production alongside an excess generation of ROS. This impairment is detrimental to insulin sensitivity as mitochondria play a crucial role in regulating adipocyte functions. Furthermore, elevated concentration of free FAs resulting from heightened breakdown of lipids may exacerbate endoplasmic reticulum (ER) stress and compromise the function of β cells (4).

Increased lipolysis will increase free FAs and glycerol, contributing to systemic inflammation. Therefore, IR prompts heightened lipolysis, augmenting lipid accumulation and trafficking in both muscles and the liver. Consequently, this amplifies gluconeogenesis in the liver and muscles, exacerbating insulin sensitivity and elevating glycemic levels (4).

Other metabolites known to cause IR and are usually elevated in obese people are non-esterified fatty acids (NEFAs). It is documented that insulin resistance and impaired β-cell function are induced by NEFAs, making them a probable cause of metabolic diseases. Adipose tissue releases NEFAs, glycerol, leptin, adiponectin, and cytokines, which affect metabolism. Among these factors, NEFAs play a pivotal role in modulating insulin sensitivity. Increased levels of NEFAs in obesity and T2DM have been linked to the IR observed in both conditions. IR can occur within hours of an acute increase in plasma NEFA levels in humans. On the other hand, an acute decrease in NEFA levels after treatment with the antilipolytic agent acipimox has been shown to improve insulin-mediated glucose uptake and glucose tolerance (3).

4.2.2.2 Amino Acids

Changes in amino acid metabolism are also known in patients with obesity and the pathophysiology of T2DM. These changes serve predominantly as markers in differentiating the stages of DM. It has been shown that branched-chain amino acids (BCCAs) are correlated with obesity-related metabolic complications and increased cardiovascular risk. In individuals with obesity, IR, and T2DM, the BCCAs increase due to the reduced expression of catabolic enzymes and suppressed oxidation in adipose tissue and liver. This accumulation of lipids results in impaired insulin signaling and the development of T2DM (4).

4.2.3 Systemic inflammation in obesity

Elevated levels of lipopolysaccharide (LPS), hypoxia, and fibrosis can trigger systemic inflammation, which plays a contributing role in IR, insulin deficiency, and dysregulation of energy homeostasis in obesity. Low-grade inflammation caused by obesity can have a negative impact on multiple organs and trigger the innate immune system, ultimately disrupting metabolic balance and causing tissue damage through increased fibrosis and necrosis. Adipocytes and macrophages within adipose tissue can secrete pro-inflammatory cytokines, including IL-6, IL-8, and leptin, leading to systemic inflammation that impairs insulin signaling. This effect is further compounded by reduced levels of IL-10 as anti-inflammatory cytokine and adipokines such as and adiponectin, which can occur due to tissue remodeling caused by adipocyte apoptosis (4).

Furthermore, the liver's insulin sensitivity is impaired due to the increased infiltration of macrophages, which works in conjunction with inflammatory cytokines and chemokines. Inflammation not only exacerbates IR in distant areas such as the liver and skeletal muscle but also induces lipolysis, which increases the delivery of FA flux to the liver. This process enhances glucose synthesis, cooperating the function of alfa and β-pancreatic cells and worsening the glucolipotoxic effects, hyperglycemia, and inflammation. Chronic inflammation and excessive growth of fat tissue over a prolonged period can lead to the onset and advancement of T2DM, which occurs due to the promotion of IR, cell death, adipocyte dysfunction, and fibrosis (4).

4.3 Epidemiology of T2DM

T2DM is a chronic disease affecting many worldwide. The number of affected people has increased massively in the last two decades (5). In 2019, it was estimated that the number of affected people globally was around 463 million. This surge is mainly attributed to the changes in lifestyle, free trade agreements, and urbanization in regions that previously had low numbers of T2DM. It has been recognized that diabetes presents differently among various ethnic groups. According to the Global Burden of Disease data, the prevalence of T2DM has increased significantly since 1990. In 2019, the age-standardized global prevalence of T2DM was approximately 6.0% in men and 5.0% in women, a considerable rise from the estimated 3.9% in men and 3.5% in women in 1990 (5). T2DM incidence is increasing with age, the highest prevalence being in the 55 to 59 years age group . There is an increase in the prevalence of diabetes mellitus in the Middle East, North Africa, and South Asia. Epidemiological data showed one interesting fact: the population of South Asian descent has a higher chance of developing T2DM at a younger age and with a lower BMI (body mass index) compared to other populations (5).

4.4 Clinical presentation and diagnosis

4.4.1 Clinical presentation

The typical symptoms of hyperglycemia are 3P: polyuria and nocturia, polydipsia, and polyphagia. If the disease is in progress, then the patient might present with blurred vision, neuropathy, and difficulties in wound healing, but nowadays, this is rare. Even rarer nowadays is the presentation of patients with hyperosmolar hyperglycemic states. The hyperosmolar hyperglycemic state is a hyperglycemic crisis characterized by hyperglycemia, severe dehydration, and obtundation. Nowadays, most of the patients with T2DM are asymptomatic because of routine tests and increased efforts to diagnose it early. If serum levels exceed 10 mmol/L (180 mg/dL), the patient might experience polyuria, increasing water loss. To compensate for this, the patients drink more fluids, causing polydipsia. Due to IR, cells cannot fully utilize glucose, leading to a lack of cellular energy. The body compensates by increasing hunger, which causes polyphagia (6).

4.4.2 Diagnosis

4.4.2.1 Diagnostic tests

The tests used to diagnose DM are fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), and hemoglobin A1C (HbA1C). OGTT diagnoses more people with diabetes and prediabetes, but due to its impracticality, this test is least preferred. FPG and HbA1C are the most used diagnostic tests in clinical practice. It is important to note that for FPG tests, patients must fast for at least 8 hours. The OGTT is considered the best method for pregnancy screening to detect gestational diabetes.

The development of the diagnostic criteria was based on the correlation between the glycemic thresholds and the probability of developing retinopathy. An important factor in diagnosis is the presence of symptoms; if symptoms are present, diagnosis is easier (6).

The symptomatic patient presents with previously mentioned classic symptoms of diabetes (polyuria, nocturia, polydipsia, polyphagia). On a random blood glucose measurement, the glucose value is 11.1 mmol/L (200 mg/dL) or higher than the diagnosis is established. This is primarily seen in T1DM, but it is not uncommon also for patients with T2DM (6).

Asymptomatic hyperglycemia is common in individuals with T2DM. This condition can be diagnosed based on several criteria, including FPG values of 126 mg/dL (7.0 mmol/L) or higher after at least eight hours of fasting, two-hour plasma glucose levels of 200 mg/dL (11.1 mmol/L) or higher during a 75 g OGTT, or HbA1C levels of 6.5 percent (48 mmol/mol) or greater. Confirming a diabetes diagnosis in the absence of clear symptomatic hyperglycemia requires repeating the same test on a subsequent day. Additional tests are unneeded if two or more tests show concordant levels for diabetes diagnosis.

It is essential to consider factors that may affect test results, such as improper sample handling, inadequate fasting (< 8h), recent illnesses, medications, and hemoglobinopathies that can interfere with HbA1C levels (6).

4.4.2.2 Prediabetes

Individuals with prediabetes are individuals who are at high risk of developing DM. The same diagnostic tests are used to diagnose prediabetes. The American Diabetes Association's criteria for diagnosing prediabetes are:

- Impaired fasting glucose – FPG levels 5.6-6.9 mmol/L (100-125 mg/dL)
- Impaired glucose tolerance – Two-hour plasma glucose value during a 75g OGTT between 7.8-11.0 mmol/L (140-199 mg/dL).

HbA1C – 5.7 - < 6.5 % (39 – 48 mmol/mol) (6).

4.5 Management and Treatment of T2DM

4.5.1 Initial management of type 2 Diabetes Mellitus

Treatment of patients with T2DM involves educating patients, assessing micro and macrovascular complications, and striving to achieve optimal glycemic control based on individual factors such as age, life expectancy, and comorbidities.

Treatment options to improve blood glucose levels are variable (7):

- Lifestyle changes, modifications in diet and exercise
- Increasing insulin availability
    - Direct administration of insulin
    - Through mediators that promote insulin secretion
- Improving insulin sensitivity

- Delaying the delivery and absorption of carbohydrates from the GI tract
- Increasing urinary glucose excretion

Lifestyle modifications, particularly diet and exercise, are essential to maintain healthy glycemic levels (7).

Glycemic values, which are recommended, are presented in Table 1 (8).

Table 1. Recommended glycemic values (8).

Index of glycemic control	**Goal in (non-pregnant adults)**	**Goal in older/high-risk adults**
HbA1C	< 7.0% (53 mmol/mol)	< 8.0% (64 mol/mmol)
Pre-prandial capillary blood glucose	4.4–7.2 mmol/L (80–130 mg/dL)	5.0–7.8 mmol/L (90–140 mg/dL)
Post-prandial capillary blood glucose	<10.0 mmol/L (<180 mg/dL)	<11.1 mmol/L (200 mg/dL)

4.5.2 Lifestyle, diet, and exercise modifications

Patients with T1DM and T2DM should get education about nutrition, physical activity, social support, and medications, especially those that lower plasma glucose levels (7).

4.5.2.1 Education of the patient(s)

Monitoring blood glucose levels through self-monitoring of blood glucose or continuous glucose monitoring is one of the key issues for optimal diabetes self-care. Knowing how to administer medications, especially those that can cause hypoglycemia, such as insulin, is also important. In addition, one should be aware of diabetes management guidelines for illness, prevention and hypoglycemia, foot and skin care, diabetes management before, during, and after exercise, and risk factor-modifying activities. The focus should be on providing patient-centered, individualized education. Frequent communication between the patient and the diabetes management team through electronic, telephone, or video channels can improve glycemic control (7, 8).

4.5.2.2 Diet/Nutritional therapy

Medical nutrition therapy (MNT) involves coordinating caloric intake with other aspects of diabetes therapy based on medical, lifestyle, and personal factors. MNT aims to prevent or delay the onset of T2DM in individuals at high risk, such as those who are overweight or obese with BMI > 30 kg/m^2 or have prediabetes, by promoting weight loss. However, MNT also focuses on managing diabetes-related complications, such as cardiovascular disease and nephropathy, by limiting carbohydrate intake and avoiding simple sugars and fructose. Medical treatment of obesity, including weight loss medications and metabolic surgery, may be recommended for certain patients. The goals of MNT in type 2 DM should focus on weight loss and address the significantly increased prevalence of cardiovascular disease in this population. The majority of people who fall under this category are overweight, and it is highly

recommended for them to lose weight. If individuals with recently diagnosed T2DM follow very low-carbohydrate diets, it may lead to a significant and speedy reduction in their glucose levels. MNT for T2DM should emphasize modest caloric reduction, increased physical activity, and weight loss (goal of at least 5–10% loss) (7, 9).

4.5.2.3 Exercises

Exercise is beneficial in patients with T2DM, independently of weight loss. It has multiple benefits, such as reduction of cardiovascular risk, blood pressure benefits, maintenance of muscle mass, and lowering of stored fat.

The American Diabetes Association recommends that adults with diabetes perform 30 – 60 minutes of moderate-intensity aerobic activity per day or at least 150 minutes of moderate-intensity aerobic exercise per week, spread over at least three days per week, with no more than two consecutive days without exercise (7).

4.5.3 Pharmacological therapy

It is crucial to start pharmacological treatment together with making lifestyle changes. Patients should be aware that weight loss and maintaining it is essential for controlling blood sugar levels. Pharmacological therapy can be initiated along with lifestyle changes when HbA1C levels are higher than 7.5-8%. However, if patients are confident that they can attain optimal blood glucose levels solely through lifestyle changes, a three-month trial may be initiated (7, 8).

Table 2. Glucose-lowering drugs (9).

Group of Glucose lowering agents	**Examples**	**Administration**	**Mechanism of action**	**HbA1C reduction (%)**	**Advantages**	**Side effects**
Biguanides	Metformin	Oral	↓Hepatic glucose production, ↑ insulin sensitivity, influence gut function	1-2	Weight neutral, No hypoglycemia, cheap	Diarrhea, nausea, lactoacidosis, and long-term usage can cause vitamin B12 deficiency.
αGlucosidase inhibitors	Acarbose	Oral	↓ GI glucose absorption	0.5-0.8	Reduces post-prandial glycemia	GI flatulence, elevated liver function tests
Insulin secretagogues: Sulfonylureas	Glibenclamide, gliclazide, glimepiride, gliquidone	Oral	↑ Insulin secretion	1-2	Short onset of action, lower postprandial glucose, cheap	Hypoglycemia, weight gain
Insulin secretagogues: Nonsulfonylurea sc	Mitiglinide, nateglinide, repaglinide	Oral	↑ Insulin secretion	0.5-1	Short onset of action, lower postprandial glucose,	Hypoglycemia
Sodiumglucose cotransporter 2 inhibitors	Dapagliflozin, empagliflozin, canagliflozin, ertugliflozin	Oral	↑ Renal glucose excretion	0.5-1	No hypoglycemia, ↓ weight and BP, reno protective, ↓ CV events	Urinary and genital infections, polyuria, dehydration,
Thiazolidinediones	Pioglitazone	Oral	↓ Insulin resistance, ↑ glucose utilization	0.5-1.4	Lower insulin requirements	Peripheral edema, CHF, weight gain, fractures, macular edema
Dipeptidyl peptidase IV inhibitors	Sitagliptin, vildagliptin, saxagliptin, linagliptin, alogliptin	Oral	Prolong endogenous GLP1 action; ↑ Insulin, ↓ glucagon	0.5-0.8	Well tolerated, do not cause hypoglycemia	Angioedema/urticarial and immune-mediated dermatologic effects
Glucagon-like peptide 1	exenatide, liraglutide,	Parenteral/Oral	↑ Insulin, ↓ glucagon,	0.5-1	Weight loss, no	Nausea, pancreatitis

receptor agonists	dulaglutide semaglutide		slow gastric emptying, satiety		hypoglycemia, ↓ CV events	
Insulin	lyspro, aspart, glulisine, regular human insulin, insulin isophane (NPH), glargine, detemir, degludec	Parenteral	↑ Glucose utilization, ↓ hepatic glucose production, and other insulin actions	Not limited	Known safety profile	Hypoglycemia, weight gain

4.6 Chronic diabetes mellitus complications

Chronic diabetes mellitus complications are relatively common, especially in people whose glucose levels are not well regulated. Common chronic complications of diabetes mellitus are (20):

- Diabetic Retinopathy
- Diabetic Nephropathy
- Diabetic Neuropathy
- Diabetic Cardiomyopathy
- Diabetic Foot

4.6.1 Diabetic Retinopathy

Diabetic retinopathy (DR) stands as a prominent contributor to global vision impairment and ranks as the leading cause of vision deterioration among individuals aged 25 to 74 years. Vision loss attributed to DR typically advances due to the condition's progression and can manifest as macular edema (involving swelling and thickening of the retina around the macula), emergence of hemorrhages from newly formed blood vessels, retinal detachment, or neovascular glaucoma.

The etiology and progression of DR predominantly arise from the detrimental effects of chronic hyperglycemia on tissue integrity, instigating a complex interaction of multiple pathways. These pathways precipitate two primary changes within retinal vasculature: abnormal permeability and occlusion, resulting in ischemia and subsequent neovascularization. These changes give rise to complications such as proliferative diabetic retinopathy (PDR), characterized by abnormal growth of retinal vessels, and diabetic macular edema (DME), defined by increased permeability of vessels leading to fluid accumulation in the macula. Both PDR and DME can lead to substantial and enduring vision impairment (20, 21).

Both the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study revealed that persistent hyperglycemia serves as the primary instigator of diabetic retinal damage. In the DCCT, rigorous insulin therapy among individuals with T1DM, maintaining an average HbA1C level of 7.0 percent, notably decreased the onset of new retinopathy cases by up to 76 percent compared to conventional treatment methods. Presently, effective medical and ophthalmologic interventions have the capacity to prevent over 90% of severe vision loss attributed to PDR. Moreover, approximately 50% of eyes experiencing visual impairment from DME can potentially resolve retinal thickening and/or regain visual acuity of 20/20 or better through diligent medical and ophthalmic management (20, 21).

4.6.2 Diabetic Nephropathy

Diabetic nephropathy (DN) continues to be a significant contributor to illness and mortality among individuals with either T1DM or T2DM. In Western nations, diabetes stands as the foremost solitary factor leading to end-stage renal disease. The onset of renal injury necessitates hyperglycemia, as individuals without diabetes do not experience this form of nephropathy. Furthermore, rigorous therapy

aimed at enhancing glycemic regulation can mitigate the progression of nephropathy, as indicated by urinary albumin excretion, although it cannot completely avert it. Nevertheless, it is evident that additional factors play a role, as sustained intense hyperglycemia is not always essential for the onset of diabetic hyperfiltration and kidney enlargement. In fact, individuals with T1DM may continue to experience glomerular hyperfiltration and tubular hypertrophy even after achieving normal blood glucose levels through intensive insulin treatment. In essence, changes in glomerular blood flow, inflammation, and fibrosis are the key factors that drive kidney tissue injury (20, 22).

4.6.3 Diabetic Neuropathy

The peripheral and autonomic nervous systems are frequently affected by diabetes, constituting one of its most prevalent complications. Diabetic neuropathy clinically manifests in different syndromes based on the neurological distribution, often overlapping. Both T1DM and T2DM exhibit varied prevalence rates of neuropathy, influenced by the intensity and duration of hyperglycemia. The classification of these syndromes predominantly falls into two main categories: diffuse and focal neuropathies. Within the diffuse diabetic neuropathies, distal symmetric polyneuropathy holds the highest prevalence, succeeded by various autonomic neuropathies. Focal neuropathies are less common than diffuse neuropathies (20, 23). There are several types of diabetic neuropathies, but the most common are symmetric polyneuropathy and autonomic polyneuropathy.

Symmetric polyneuropathy, particularly distal symmetric sensorimotor polyneuropathy, is the most common type of diabetic neuropathy. It's characterized by progressive sensory loss in the extremities due to a decline in sensory axons. In severe cases, it can lead to muscle weakness and loss of motor axons, presenting as a "stocking-glove" distribution of sensory loss.

Diabetic autonomic neuropathy, a frequent complication of diabetes, is often overlooked due to its gradual onset and involvement of multiple organs. However, it can lead to significant dysfunction in individual organs. Symptoms may include postural hypotension, gastroparesis, and gastrointestinal issues like constipation or diarrhea (20, 23).

4.6.4 Diabetic Cardiomyopathy

In the latest position statement by the European Society of Cardiology regarding the management of DM and heart failure (HF), the authors state that diabetic cardiomyopathy (DCM) is typically defined as cardiac dysfunction seen in diabetic patients without concurrent cardiovascular conditions such as coronary artery disease, uncontrolled hypertension, significant valvular heart disease, or congenital heart disease. Important to mention is the lack of a universally recognized definition for DCM, posing challenges for studies on its epidemiology, pathophysiology, and clinical features and outcomes. The primary metabolic disturbances contributing to cardiac dysfunction encompass IR in cardiac tissue, compensatory hyperinsulinemia, and escalating hyperglycemia. These abnormalities are further exacerbated by diabetes-induced cardiomyocyte dysfunction, impaired microvascular perfusion due to defective endothelial function, increased collagen deposition resulting in fibrosis, and maladaptive

remodeling following myocardial infarction. Collectively, these factors lead to both diastolic and systolic HF (24).

4.6.5 Diabetic Foot

Foot complications such as ulceration and amputation are linked to high mortality rates; however, diabetic foot ulcers are among the most preventable late-stage adverse outcomes of diabetes. Risk factors associated with foot wounds in diabetic individuals include diminished protective sensation due to neuropathy, prior instances of ulcers or amputations, foot deformities leading to heightened pressure, external injuries, infections, and chronic ischemia, frequently stemming from peripheral artery disease. Individuals with diabetes face an elevated susceptibility to non-healing wounds due to both mechanical and cytogenic factors, alongside a notable prevalence of peripheral artery disease. The development of diabetic foot ulcers is often driven by a combination of two or more risk factors, with the triad of neuropathy, deformity, and trauma present in 63% of new cases. Additionally, edema and ischemia frequently contribute as component causes (20, 25).

Table 3: Wagner Diabetic foot ulcer classification system (20)

Grade	Description
0	No ulcer, but high-risk foot (e.g., deformity, callus, insensitivity)
1	Superficial full-thickness ulcer
2	Deeper ulcer, penetrating tendons, no bone involvement
3	Deeper ulcer with bone involvement, osteitis
4	Partial gangrene (e.g., toes, forefoot)
5	Gangrene of whole foot

4.7 Extracellular vesicles

EVs function is unclear yet, but they are known to have a role in intercellular communication. They are formed and released by a variety of prokaryote or eukaryote cells into the extracellular space as part of their regular function and in response to various abnormalities. The three main subtypes of (EVs) are macrovesicles (MVs), exosomes, and apoptotic bodies. These subtypes are differentiated based on their biogenesis, release pathways, size, content, and function. The composition of EVs is highly diverse, as they can carry various substances such as lipids, ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and a range of proteins. Initially, EVs were suggested to be part of eliminating cellular waste. However, according to recent research, EVs are essential for regulating cell homeostasis and play a vital role in cell communication by transmitting protective or injury signals to nearby or distant cells. Recipient cells can take up EVs to induce cell-cell crosstalk by various mechanisms, including endocytosis, pinocytosis, phagocytosis, and membrane fusion, or signals by directly activating cell surface receptors via ligands or presenting antigens. The process by which a specific EV is absorbed may rely on the presence of proteins and glycoproteins on the surface of the EVs and the cells they target (11, 12).

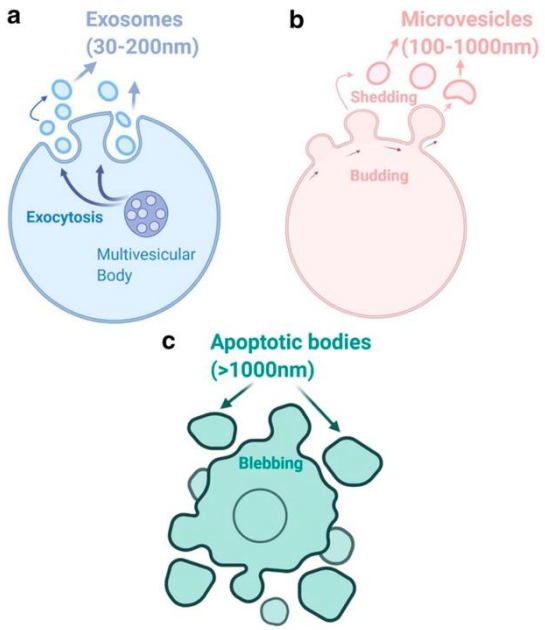

Figure 2: [43] Types of extracellular vesicles (13):

a. Exosomes are released by exocytosis from multivesicular bodies (MVBs). Depending on the literature, their size can range from 30 to 150 nm, sometimes even 200nm.
b. Microvesicles are released via budding from the plasma membrane. They are larger than 100-1000 nm.
c. Apoptotic bodies are released via the blebbing of cells that undergo apoptosis (cell death), and they are the largest of EVs at 50 – 5000 nm.

Based on the size of their diameter, extracellular vesicles can be grouped into (11, 12):

- Exosomes: 30 – 150 nm
- Microvesicles: 100 – 1000 nm
- Apoptotic bodies: 50 – 5000 nm

4.8 Exosomes

Exosomes, also known as intraluminal vesicles (ILVs), are secreted by various cells and can be found in different body fluids like plasma, serum, urine, cerebral spinal fluid, breast milk, etc. Since they are produced in various cells, they reflect their cellular origin and the physiological state of the cells; they have a single outer membrane layer and are a subtype of EVs with a 30-150 nm size range. The endosomal route forms these vesicles and serves various functions such as protein storing, recycling, storage, transport, and release (12).

4.8.1 Biogenies of Exosomes

Exosomes are produced through a process that includes the plasma membrane undergoing double invagination and the creation of intracellular multivesicular bodies (MVBs) that house ILVs. The ILVs are eventually released as exosomes, ranging in size from approximately 40 to 160 nm in diameter, through the fusion of MVBs with the plasma membrane and exocytosis.

The plasma membrane undergoes its first invagination, creating a structure that is shaped like a cup. This structure contains cell-surface and soluble proteins that are associated with the extracellular environment. As a result, an early-sorting endosome (ESE) is formed de novo, and in some instances, it may directly merge with a preexisting ESE. Besides, the trans-Golgi network and ER can shape and provide content to these ESEs. MVBs are created through the inward folding of the endosomal membrane, essentially a double invagination of the plasma membrane. This leads to the formation of MVBs that encompass numerous ILVs, which will eventually become exosomes (10).

4.8.2 Composition of Exosomes

The main proteins found in exosomes are tetraspanin family members (such as CD9, CD81, CD63), ESCRT (endosomal sorting complex required for transport) proteins, actin, integrins, heat shock proteins (HSP), and flotillins. While specific proteins like HSP, ESCRT proteins, and cytoskeletal components are commonly found in all exosomes, others, like major histocompatibility complex class I and II proteins, are specific to the type of cell from which they are derived. Exosome membranes are composed of simple and complex lipids, such as ceramides, sphingomyelin, and cholesterol, which play roles in cargo sorting, secretion, structure, and signaling. Additionally, exosomes contain a complex mixture of nucleic acids, including DNA, messenger RNA (mRNA), and non-coding RNA (ncRNA) species. Among these, microRNAs (miRs) are particularly abundant and are involved in various physiological processes such as hematopoiesis, exocytosis, and angiogenesis, contributing to cellular communication mediated by exosomes. Other RNA species present in exosomes include ribosomal RNA, long non-coding RNA (lncRNA), transfer RNA, circular RNAs (circRNAs), small nuclear RNA, small nucleolar RNA, and piwi-interacting RNA, all of which play roles in various biological pathways and processes, especially in tumor development (13).

4.8.3 Intercellular interaction

When exosomes reach the target cell, they can initiate signaling pathways either directly with extracellular receptors or by being taken up by direct fusion with the plasma membrane or internalization (13).

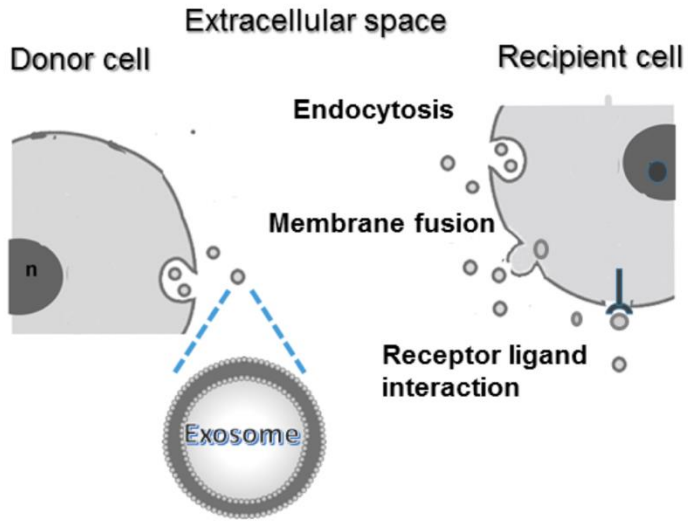

Figure 3: Absorption and excretion mechanism of exosomes (19)

Illustration of how exosomes are absorbed: exosomes are discharged from donor cells into the extracellular environment, subsequently infiltrating target cells through three distinct mechanisms—direct fusion with the plasma membrane, endocytosis, and interactions facilitated by

Direct interaction

Transmembrane ligands located on the surface of exosomes can directly bind to receptors found on the cell surface of recipient cells, thereby initiating downstream signaling cascades that activate the target cell. This pathway is frequently utilized to modulate immune responses and induce apoptotic functions (13).

Fusion with plasma membrane

Exosomes also have the ability to fuse with the plasma membrane. Therefore, they can release their cargo into the cytosol of the target cells. This condensation process involves the formation of a fusion stalk between the hydrophobic lipid bilayers of the exosome and the plasma membrane, followed by expansion to form a single continuous structure. Families of SNAREs (Soluble N-ethylmaleimide-sensitive factor activating protein receptor) and Rab (Ras-associated binding) proteins are thought to play a role in mediating this fusion process. Lipid raft-like domains, adhesion molecules, and integrins on the exosome surface facilitate interaction, attachment, and membrane fusion with the target cell (13).

Internalization

Exosomes are mainly internalized by recipient cells, after which they release their cargo. This uptake process occurs rapidly and is sensitive to temperature changes. Well-known endocytic pathways are typically included in the internalization of exosomes.

Clathrin-mediated endocytosis is another common way of exosome interaction with recipient cells. This model of exosome entry is observed in various cell types, including ovarian and colon tumor cells, cardiomyocytes, macrophages, hepatocytes, neural cells, and epithelial cells (13, 27).

Phagocytosis and micropinocytosis

Phagocytosis is a process known for engulfing large particles such as bacteria and dead cells, but it can also internalize smaller particles like exosomes. Notably, immune cells such as macrophages and dendritic cells predominantly utilize this route of exosome uptake, as evidenced by their reliance on phosphatidylinositol-3 kinase (PI3K) and actin cytoskeleton activity. Also, phagocytosis is a ubiquitous process used by these cells (13, 28).

Micropinocytosis

Macropinocytosis involves the use of actin-driven lamellipodia to induce inward plasma membrane invaginations, which then pinch off to form compartments in the cell known as macropinosomes.

Upon fusion, exosomes deliver their contents into the cytosol. Alternatively, when exosomes directly interact with receptors present on the recipient cell surface, they trigger downstream signaling cascades. Post-internalization, exosomes follow the well-known conventional endosomal pathway, starting with early endosomes serving as sorting compartments and progressing to acidic vesicles like late endosomes and MVBs. Ultimately, these MVBs merge with lysosomes, leading to degradation (13, 29).

Exosomes have developed various mechanisms to evade lysosomal degradation. The ER, a hub for translation initiation, may represent an escape route for exosomes. This pathway is particularly beneficial for exosomes containing mRNA and miRNAs, as it facilitates their release into the ER for rapid translation and modulation of gene expression. Additionally, the nucleoplasmic reticulum, which is characterized by nuclear-associated invaginations, enables the transfer of exosomes into the nucleus. Furthermore, nuclear envelope-associated invaginations associated with late endosomes potentially facilitate the delivery of exosome components into the nucleoplasm and enable the transport of nuclear cargo. Exosomes also employ strategies resembling viruses to skip lysosomal degradation; for instance, in dendritic cells (13, 30).

4.9 Linking exosomes and diabetes

Much research is being carried out in this field, aiming to elucidate the significance and function of exosomes in the pathophysiology of T2DM. This thesis not only explores their involvement in the disease process but also investigates their potential utility as clinical markers for early diabetes diagnosis and their therapeutic benefits in its management. Additionally, they might have a great potential for the clinical diagnosis and treatment of T2DM (14).

4.9.1 Role of exosomes in the development of T2DM

As mentioned earlier, exosomes play a role in the development of T2DM, although their exact role still needs to be fully understood. In the pathophysiology of T2DM, it is mentioned that obesity plays a crucial role in the development of insulin resistance. Excess adipose tissue is a significant source of exosomes, particularly exosomal miRNAs that can regulate gene expression in distant tissues such as the liver. Within exosomes, miRNAs have been identified as impeccable contributors to DM

progression and related complications, primarily characterized by pancreatic β-cell injury and IR. The control of miRNA processing within adipose tissue is vital for both longevity and the organism's resilience to environmental challenges. This regulatory process has a direct impact on the elevated occurrence of diabetes and other metabolic disorders among the elderly population. Exosomal miRNAs released by adipocytes, such as miR-34a, miR-222, miR-27a, and miR-802-5p, have been identified as contributors to IR by modulating critical regulatory factors including Krüppel-like factor 4 (KLF4), insulin receptor substrate 1 (IRS1), peroxisome proliferator-activated receptor γ (PPARγ), and HSP60 (14, 15).

Additionally, the uptake of adipocyte-derived exosomes by hepatocytes in obese mice results in reduced levels of miR-141-3p compared to those observed in healthy counterparts. This decrease in miR-141-3p uptake correlates with impaired glucose uptake by hepatocytes (14, 15). As previously mentioned, obesity often accompanies a state of persistent, low-grade inflammation within adipose tissue, which can eventually result in systemic IR. Notably, EVs may facilitate the communication between adipocytes and macrophages, thereby promoting inflammation. EVs derived from adipocytes prompt the differentiation of monocytes into macrophages, which in turn disrupt insulin signaling within human adipocytes, thus perpetuating a detrimental cycle (14, 16). Macrophages obtained from the adipose tissue of obese mice secrete exosomes that induce glucose intolerance and IR when administered to healthy mice. The impact of these exosomes was driven by elevated levels of miR-155, which subsequently acts on peroxisome PPARγ, reducing insulin sensitivity in various tissues like the liver. It has been documented that exosomes isolated from the plasma of obese mice have the capability to induce glucose intolerance and dyslipidemia when introduced into control mice over a four-week period. Remarkably, exosomes derived from adipose explants collected from obese patients exhibit the capability to penetrate primary hepatocytes in vitro, thereby inducing dysregulation of the transforming growth factor β (TGFβ) pathway (14, 15, 16).

Conversely, repeated administration of exosome-like particles released by hepatic immature myeloid cells from obese mice leads to the accumulation of CD11b cells in the livers of recipient mice. Subsequently, these cells release pro-inflammatory cytokines and induce apoptosis of natural killer T cells. Furthermore, heightened circulating levels of miR-122 are significantly associated with the likelihood of developing IR, obesity, diabetes, and an unfavorable lipid profile among individuals. Similarly, elevated serum levels of miR-130b are observed in overweight and obese individuals, displaying a positive correlation with BMI (14, 15, 16).

Exosomes might have a role in maintaining pancreatic beta cell mass. Specifically, miR-16 was found to be upregulated in these exosomes and capable of modulating protein patched homolog 1 gene (PTCH1 gene), a gene associated with pancreas development. Recent investigations have revealed that the augmentation of specific exosomal miRNAs exerts regulatory control over pertinent genes crucial for maintaining pancreatic β-cell homeostasis during the initial phases of DM. Conversely, prolonged

exposure to elevated concentrations of glucose and FAs suppresses the expression of these exosomal miRNAs. Consequently, the enrichment of these exosome-specific miRNAs is implicated in β-cell dysfunction or injury in DM (14, 15).

Recent studies have shown that exosomes are critical in developing complications associated with T2DM. Notably, pancreatic β-cells release exosomal miR-15a, which, upon uptake by Müller cells, triggers oxidative stress, leading to retinal injury and apoptotic cell death, particularly in T2DM conditions (15, 31). Moreover, recent research has highlighted that endothelial cells stimulated by high glucose release exosomes containing circRNA-0077930, promoting vascular smooth muscle cell senescence and contributing to diabetic vascular complications (15, 32). Furthermore, distinct profiles of circRNAs in serum exosomes have been observed among patients with diabetic foot ulcers, non-foot ulcer diabetes, and healthy individuals. Additionally, studies have shown that high expression of cPWWP2A attenuates diabetes-induced retinal vascular dysfunction in vivo (15, 33). At the same time, serum exosomal circRNA DLGAP4 is elevated in patients with diabetic nephropathy and rat models compared to T2DM individuals without nephropathy and normal rats (15).

4.9.2 Exosomes in Obesity

Several studies have investigated changes in exosomes as a result of obesity (15, 34).. Emerging evidence suggests that exosomes, acting as carriers of ncRNAs between cells, significantly contribute to the onset and progression of obesity-related metabolic conditions. Obese individuals have excess adipose tissue, which plays a role in developing low-grade chronic inflammation, as seen in T2DM. Adipose tissue functions as an actively secreting endocrine organ, releasing diverse cytokines like adiponectin, interleukins, tumor necrosis factor alfa (TNF-α), and other pro-inflammatory molecules like miRNAs. This secretion contributes to metabolic dysregulation, notably IR. Exosomal miR-34a derived from obese mice has been identified as a factor that promotes inflammation by modulating the ratio of M1-to-M2 macrophages (15, 34).

Conversely, obesity can exacerbate insulin resistance by transporting exosomal miRNAs to target cells and tissues, thereby influencing various pathophysiological pathways, including inflammatory responses and insulin signaling. Specifically, miR-192, miR-122, miR-27a-3p, and miR-27b-3p are elevated in plasma exosomes of obese mice. Administration of exosomes containing these elevated miRNAs induces central obesity in lean mice by targeting PPARα (15).

4.10 Clinical potential of exosomes in diagnostics and treatment of T2DM

As discussed thus far, exosomes have emerged as key players in the pathophysiology of various diseases, showcasing immense potential in both diagnostics and therapeutics. While extensively studied in oncology, their application extends to the realm of T2DM diagnostics and treatment. With their ability to carry biomolecules and genetic material, exosomes offer promising avenues for understanding

disease mechanisms and developing targeted interventions. As research continues to unveil their diverse roles, exosomes stand poised as valuable tools in the quest for improved healthcare outcomes (14, 15, 16).

4.10.1 Exosomes as diagnostic markers in T2DM

Numerous studies have highlighted variations in exosome levels between healthy individuals and those with T2DM (17, 31, 35, 37). Certain exosomes exhibit promising potential as clinical markers, particularly in preclinical diagnostics and monitoring of both the disease and its associated complications. These findings underscore the significance of exosomes as potential indicators of T2DM progression and prognosis, paving the way for enhanced clinical management strategies. Numerous exosomal miRNAs, detectable in both serum and urine samples, have demonstrated correlations—either inversely or directly—with T2DM (17).

Table 3: exosomal components with a potential diagnostic purpose in T2DM (17, 31, 35, 36, 37).

Exosomal component	**Source**	**Species**	**Location**
miR-15a	Pancreas (β-cells)	Human	Serum
lncRNA-p3134	Pancreas (β-cells)	Human	Serum
miR-375-3p	Pancreas (β-cells)	Human	Serum
PEPCK (phosphoenolpyruvate carboxykinase)	Not reported	Human/Rat	Urine
miR-144-5p, miR-532-5p, miR-34a	Not reported	Human	Serum
miR-20b-5p	Not reported	Human	Serum
miR-1307-3p, miR-491-5p miR-298	Not reported	Human	Serum

4.10.2 Exosomes as markers of complications of DM

DCM refers to the development of HF in diabetics without identifiable causes, such as hypertension or coronary artery disease. Approximately half of T2DM patients without apparent cardiac symptoms exhibit diastolic dysfunction, indicating a potentially significant underdiagnosis of DCM. The inaugural assessment of miRNA biomarkers in humans for DCM was conducted to evaluate their practical clinical utility. The study revealed a tangible correlation between certain miRNAs and DCM, indicating promising prospects for the clinical application of these biomarkers (17, 43). Another study also explored the potential of miRNAs as possible biomarkers, although their investigation differed by examining EVs comprehensively rather than focusing solely on exosomes (44). Intriguingly, the aforementioned miR-30e-5p and miR-30d-5p exhibited upregulation exclusively in the left ventricle compared to 6 other organs, hinting at the existence of highly potential miRNA biomarkers for DCM that precede symptomatic manifestation (17, 44.).

DN is a complication of diabetes, which is usually present ten to twenty years after diagnosis with T2DM. Three exosomal proteins present in urine emerged as potential biomarkers for patients with DN,

including α-1-microglobulin/bikunin precursor (AMBP), lysine-specific methyltransferase 2C (MLL3), and the voltage-dependent anion channel 1 (VDAC1). AMBP is an interesting glycoprotein since it inhibits protease activity and, therefore, plays a multifaceted role in inflammation. Furthermore, it is described that it has antioxidant effects and modulates the activity of cytokines and immune cell function. Notably, increased levels of AMBP have also been reported in the urine of patients with T2DM. MLL3 in DN patients is upregulated, functioning as a histone methyltransferase, and it is shown that it modulates chromatin and is finally involved in the regulation of gene expression. Given the observed mitochondrial dysfunction in DN, alterations in VDAC1 activity or tissue/organ expression could potentially contribute to the initiation and progression of this pathological condition. miRNAs have also shown promise as biomarkers for the early diagnosis of DN (16, 17, 38).

DR is one of the most frequent complications of DM and is the primary cause of visual impairment and blindness worldwide. In patients with T2DM, elevated levels of IgG-laden exosomes lead to increased exosome-induced complement activation, which subsequently contributes to retinal vascular damage. Exosome miRNAs can be used as markers of diabetic retinopathy development and severity (17, 52). In response to high-glucose conditions, pancreatic β-cells release exosomal miR-15a, while levels of this exosomal miRNA were notably higher in the retina and serum of diabetic patients. Moreover, the extent of this elevation was observed to correspond to the severity of DR. The potential of exosomal miR-15a as a biomarker for early detection of DR before clinical symptoms manifest was investigated. They assessed ganglionic cell complex thickness in three groups (T2DM, impaired glucose tolerance, and healthy controls), finding that thickness reduction preceded overt retinopathy or T2DM onset. This reduction correlated inversely with serum exosomal miR-15a concentration, suggesting its role as an early biomarker for DR, even in preclinical T2DM (16, 17, 45).

Table 4: Exosome components in some of T2DM complications (17).

Exosome component	**Location**	**Association with T2DM**	**Complications that they are related with**	**Species**
miR-1, miR-133a	Serum	Increased	DCM	Mouse Human
AMBP, MLL3, VDAC1	Urine	Increased, increased, decreased	DN	Human
miR-320c, miR- 30d-5p, miR-6068, , miR-1227-5p, miR-6133, miR-4270, miR-4739, miR-371b-5p, miR-638, miR-572, miR-6126, miR-1915-5p miR-4778-5p, miR-2861, miR-30e-5p	Urine	Increased (miR-30d-5p) Decreased (miR-30e-5p)	DN	Human
miR-15b-5p, miR-29c-5p, let-7c-5p	Urine	Decreased, decreased, increased	DN	Human
miR-362-3p, miR-15a-5p miR-877-3p, miR-150- 5p,	Urine	Increased, increased, increased, decreased	DN	Human
miR-133b, miR-342, miR-30	Urine	Increased	DN	Human
miR-15b, miR-34a, miR-636	Urine	Increased	DN	Human
miR-21-5p, miR-30b-5p	Urine	Increased, decreased	DN	Human
miR-15a	Serum	Increased	DR	Human
miR-150-5p, miR-21-3p	Serum	Decreased, increased, increased	DR	Human

Diabetic cardiomyopathy (DCM); Diabetic nephropathy (DN; Diabetic retinopathy (DR); α_1-microglobulin/bikunin precursor (AMBP); lysine-specific methyltransferase 2C (MLL3); Voltage-dependent anion channel 1 (VDAC1).

4.10.3 Exosomes potential in the treatment of T2DM

Exosomes have great potential as therapeutic agents, and there is ongoing research exploring their therapeutic applications. Exosomes represent a novel form of nanomaterial capable of delivering miRNA inhibitors and agonists for the treatment of DM, predominantly in the treatment of complications of diabetes. Injected exosomes exhibit high efficacy in cell entry and can deliver functional cargo with minimal immune clearance upon exogenous administration in mice. Furthermore, their therapeutic potential appears promising as they were shown to be well tolerated. Studies demonstrate that exosomes derived from both mesenchymal and epithelial cells do not induce toxicity upon repeated injection in mice. Naturally released exosomes offer therapeutic potential by influencing

disease development, making them promising for therapeutic delivery. They act as carriers for various biomolecules, ensuring stability as they circulate through the body. Some studies have shown that exosomes, released by mesenchymal stem cells, can facilitate cytoprotective, angiogenic, and regenerative responses (10, 16, 18).

Various methods exist for loading exosomes with therapeutic cargo. These methods encompass loading exosomes isolated from parent cells directly, introducing DNA encoding therapeutic compounds into parent cells for subsequent release in exosomes, and loading parent cells with specific drugs that are later released within exosomes. One of the initial studies demonstrated the therapeutic capability of exosomes. The research illustrates that exosomes effectively transport and deliver the anti-inflammatory agent curcumin, indicating the synergistic effect of curcumin and exosomes in enhancing functional responses, particularly in addressing inflammation (18, 51).

4.11 Exosomes in treatment of diabetic complications

4.11.1 Diabetic Cardiomyopathy

As mentioned above, DCM in diabetic patients develops without any other risk factors, such as hypertension or coronary heart disease. In a diabetic rat model, the activation of Hsp-70, specifically extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), was observed to be diminished despite the presence of exosomal Hsp-70. However, upon isolation of exosomes from healty rats and subsequent injection into diabetic rats, the cardioprotective paths were reinstated. These observations imply that exosomal Hsp-70 may undergo glycation during the onset of diabetes, leading to the loss of its capacity to activate cardioprotective ERK1/2 (17, 39).

The elevation of Hsp-20, p-Akt, superoxide dismutase 1, and survivin within these exosomes results in diminished oxidative stress, reduced fibrosis, and enhanced angiogenesis, indicating potential alleviation of DCM (17, 40).

Exosomes derived from cardiac progenitor cells carry miR-21, which can mitigate oxidative stress-induced apoptosis in H9C2 cardiac cells by suppressing the expression of the target gene, programmed cell death 4. This suppression shields myocardial cells from apoptosis triggered by oxidative stress. These results suggest that exosomes harboring miR-21 hold potential as a novel therapeutic approach for ischemic cardiac diseases associated with diabetic cardiomyopathy (16).

4.11.2 Diabetic Nephropathy

Prevention is of great benefit not only for diabetic patients but also for the healthcare system. MiR-486 causes autophagy and reduction of podocytes. MiR-125a has led to the restoration of the integrity of glomerular tissue and renal function. Research has indicated the involvement of miR-92a-1-5p in the pathophysiology of DN. Inhibition of miR-92a-1-5p demonstrated rescue of nephropathic features both *in vitro* and in a mouse experimental model of DN (17)

4.11.3 Diabetic Retinopathy

The administration of exosomes overexpressing miR-126 successfully reversed the concentration of inflammatory markers and suppressed the high mobility group box 1 signaling pathway. This finding aligns with a prior study where miR-126 was identified as a potential factor in ameliorating DR (17, 41).

LncRNA SNHG7 in DR development was explored by isolating exosomes from mesenchymal stem cells (MSCs) (46). They exposed human retinal microvascular endothelial cells (HRMECs) to increased glucose levels, observing endothelial damage and endothelial-mesenchymal transition (EndoMT), along with reduced lncRNA SNHG7 levels and increased miR-34a-5p expression. Treating HRMECs with exosomes containing overexpressed lncRNA SNHG7 significantly decreased EndoMT and tube formation by suppressing miR-34a-5p. These findings underscore the potential therapeutic value of exosomal lncRNA SNHG7 in alleviating DR. MSC-derived exosomal miR- 222 was closely associated with degenerative changes in the retina in a rabbit model of DR, which suggests that exosome- mal miR-222 plays a vital role in the process of retinal tissue repair (16, 17, 46).

4.11.4 Diabetic Neuropathy

Diabetic neuropathy commonly manifests with characteristic symptoms of nerve-related pathological pain, which encompass spontaneous pain, allodynia (pain triggered by typically non-painful stimuli), and hyperalgesia (heightened sensitivity to painful stimuli).

Studies have shown that certain miRNAs found in exosomes derived from healthy Schwann cells and MSCs have the potential to improve diabetic polyneuropathy (DPN) in T2DM mice. For example, miR-21, miR-27a, and miR-146a from Schwann cell-derived exosomes were found to enhance the growth of nerve fibers in diabetic dorsal root ganglion neurons. Similarly, exosomes released by MSCs were observed to repair damaged neurons and astrocytes, consequently reversing neurological impairments associated with DPN. Exosomes derived from mesenchymal stromal cells containing miR-17, miR-23a, and miR-125b were found to significantly reduce sensitivity to thermal and mechanical stimuli while enhancing nerve conduction velocity in diabetic mice (17, 42). Significantly, while these findings indicate a notable improvement, it's important to note that the restoration of nerve function did not reach the baseline level observed in the non-DPN group (17, 42). These effects were attributed to the inhibition of the TLR4/NF-κB signaling pathway and the suppression of proinflammatory protein expression, ultimately alleviating neurovascular dysfunction in DPN-afflicted mice. These findings collectively suggest that exosomal miRNAs hold promise as an effective therapeutic strategy for treating diabetic nerve injuries (16, 17).

Many studies have been conducted on the potential of exosomes in the therapy of DPN. Although many studies have shown improvement, none have resulted in a return to baseline function (16, 17).

4.11.5 Diabetic Wound Healing

Delayed wound healing (DWH) encompasses several cellular processes, including cell migration, proliferation, fibrogenesis, angiogenesis, and reepithelialization. However, individuals with T2DM experience substantial impairment in this process, resulting in the development of chronic non-healing ulcers and, in severe cases, lower extremity amputation. Therapeutic approaches primarily revolve around conservative measures such as infection control, and glycemic management. However, these interventions are predominantly supportive rather than curative and frequently yield unsatisfactory outcomes (16, 17).

There have been several studies that revealed that some exosomes can be used as therapeutic in DWH. Some exosomes have shown that they have promoted wound healing. The therapeutic potential of exosomes derived from human adipose-derived MSCs in a mouse model of diabetes has been explored. They observed a notable acceleration in wound healing rate in the group treated with exosomes. Subsequent functional assessments unveiled that these exosomes facilitated DWH through various mechanisms (17, 47).

It was revealed that prolonged exposure to elevated glucose levels precipitated senescence in human dermal fibroblasts (HDFs) cultured in vitro, concurrently impeding the proliferation and migration rate of HDFs (Bian et al.). On the other hand, the introduction of exosomes derived from decidual MSCs successfully mitigated the deleterious outcomes of heightened glucose levels. Topical administration of these exosomes to wounds in a mouse experimental model of diabetes notably expedited wound closure, underscoring the potential of exosomes as a promising therapeutic avenue for promoting DWH (17, 48).

Circ-Astn1 was observed to suppress miR-138-5p, leading to increased levels of SIRT1. Elevated levels of circ-Astn1 expedited the healing process of full-thickness cutaneous wounds in diabetic mice (17).

An additional therapeutic alternative, obviating the need for invasive biopsy for adipose-derived MSCs acquisition, involves urine-derived stem cells (USCs). The therapeutic efficacy of exosomes derived from human USCs in managing DWH was investigated (29). Following the demonstration of the therapeutic potential of these exosomes both in laboratory settings and within living organisms, the researchers pinpointed the primary mechanism underlying the enhancement of DWH. This mechanism primarily entails an augmentation in reepithelialization rates, collagen accumulation, and vascular formation (17, 49).

Engineered exosomes have the potential to be used in DWH therapy. A method involving engineered exosomes to target wound recovery was devised (50). Initially, they analyzed miRNA expression in diabetic chronic wounds using patient samples. Upon discovering a notable decrease in miR-31-5p levels in such wounds, they demonstrated that miR-31-5p expression in vitro facilitated fibrogenesis, epithelialization, and angiogenesis (17, 50).

Table 5: Therapeutic potential of miRs in the therapy of diabetic complications (17).

Exosomal component	**Species**	**Potential application**
miR-455, miR-29b	Mouse	DCM
Mst1	Mouse	DCM
SOD1, Hsp-20, p-Akt, , survivin	Mouse	DCM
Hsp-70	Rat	DCM
miR-486	Mouse	DN
miR-125a	Rat	DN
miR-92a-1-5p	Mouse	DN
miR-28 miR-31a miR-130a	Mouse	DPN
miR-17 miR-23a miR-125b	Mouse	DPN
miR-146a	Mouse	DPN
miR-125a-5p	Mouse	DPN
miR-126	Rat	DR
miR-31-5p	Rat	DWH
miR-146a	Mouse	DWH
circ-Astn1	Mouse	DWH

Diabetic cardiomyopathy (DCM); Diabetic nephropathy (DN); Diabetic polyneuropathy (DPN); Diabetic retinopathy (DR); Diabetic wound healing (DWH).

5. Discussion

As time passes, there is a growing body of scientific research and understanding of the role of EVs, particularly exosomes. For many years, exosomes were thought to be cellular waste products that were obtained from the shedding of the plasma membrane. With the advancements in imaging and isolation techniques in subcellular bodies, researchers have discovered that exosomes play a significant role in normal human physiology and pathophysiology. There is a lot of ongoing research being conducted on the role of exosomes in various diseases, especially in oncology, but not limited to it. Exosomes also play a significant role in the pathophysiology of T2DM, as was mentioned above (12, 19).

Exosomes have great potential for use as biological nanoparticles in theranostics for various diseases. Due to their natural source, exosomes exhibit favorable biocompatibility and minimal toxicity. They possess increased stability in the bloodstream by evading immune detection and have low immunogenicity coupled with biodegradability. These characteristics render them superior to synthetic carriers or nanoparticles. Exosomes have the capacity to target specific cell populations by crossing physiological barriers like the blood-brain barrier. Additionally, the intrinsic components within exosomes can synergize with encapsulated drugs, enhancing their therapeutic effects (12, 16, 19).

One of the main challenges in the field of EVs is their isolation and classification. Various techniques are employed for the characterization of isolated EVs. These include conventional methods such as flow cytometry (bead-coupled), Western blotting, mass spectrometry, and microfluidics chips. Additionally, polymerase chain reaction and nuclear magnetic resonance are also utilized. Conventional and emerging methodologies may, at times, prove insufficient for thoroughly characterizing vesicles and obtaining comprehensive qualitative and quantitative data. At the moment, electron microscopy stands as the sole technique capable of simultaneously assessing their dimensions, configuration, structural integrity, particle interaction, and spatial proximity to tissues and cells. Despite the utilization of novel detection methods and advanced analytical approaches, the analysis of EVs remains challenging due to the absence of a purification method capable of strictly segregating them based on size. Additionally, there is currently a lack of consensus regarding specific markers that can definitively differentiate the origin of vesicles once they have exited the cell. Particularly, the ability to discriminate between exosomes and MVs remains a subject of considerable debate. The board members of the International Society for Extracellular Vesicles suggested adopting the term "extracellular vesicles" for: “all particles naturally released from the cell that are delimited by a lipid bilayer and cannot replicate” (12, 19).

Important to mention is that the quantity of exosome production varies among cells based on their conditions. Cellular stress and signaling activation influence their release. Increasing evidence suggests that cancer cells release a greater quantity of exosomes compared to healthy cells. Exosomes originating from tumors can promote cancer progression by triggering anti-apoptotic and oncogenic pathways, including invasion, metastasis, and angiogenesis. Various investigations have indicated that certain cell types are better suited for producing EVs for therapeutic applications, and not all vesicles derived from

cells are suitable as drug carriers. The ability to carry drugs effectively depends on factors such as size, quantity, internal content, and surface proteins, which reflect the characteristics of the originating cell and tissue. The majority of exosomes used for theranostics are derived from dendritic cells, macrophages, and mesenchymal stem cells. Exosomes originating from these cell lines have shown the most potential because of their characteristics. Dendritic cells are utilized for their minimal immunogenicity, and intriguingly, their exosomes retain this immunomodulatory capability. Exosomes derived from dendritic cells have the capacity to bypass biological obstacles, including the blood-brain barrier. Exosomes originating from macrophages express active immune proteins and possess the capability to engage with endothelial cells of brain vessels, facilitating their passage through the blood-brain barrier. This ability is attributed, at least in part, to specific surface components. Furthermore, these exosomes can transport substances such as cytokines and demonstrate potent anti-inflammatory and anti-tumor properties. Mesenchymal stem cells, sourced from various human tissues like bone marrow, dental pulp, and adipose tissue, are widely favored for cell therapy due to their self-renewal ability and immune-modulating properties. EV derived from these cells plays a significant role in enhancing wound healing and tissue repair, particularly in skin and cardiac tissues (16, 17, 18, 19).

Another source of exosomes, which in some studies has shown to have good promise, are extracellular bodies derived from plant cells. Exosome-like nanoparticles, resembling exosomes, sourced from tissues, organs, apoplastic fluid, and extracts of certain plants like ginger, lemon, grapefruits, and carrots, possess diverse favorable characteristics, rendering them appropriate for clinical use. Although exosome-like nanoparticles share similar morphology and composition with mammalian exosomes, and despite the numerous advantages of vesicles derived from food and vegetables, further research is needed. Information regarding their biogenesis is currently insufficient, and it is crucial to identify markers of plant EVs. A comprehensive understanding of their mechanism of action is necessary before they can be widely utilized in medical applications. Exosomes originating from both healthy and cancerous cells, as well as from individuals with chronic ailments such as type 1 and type 2 diabetes, Alzheimer's disease, or chronic inflammatory conditions, exhibit variations in their nucleic acid content and membrane composition. These distinctions hold diagnostic significance across a spectrum of diseases, offering predictive value for even tumor detection. For example, certain exosomal long noncoding RNAs such as UCA1 and exosomal miRNA found in human serum can function as diagnostic indicators for cancer risk (19).

Exosomes offer significant advantages in drug delivery due to their unique characteristics. They serve as effective vehicles for delivering not only chemotherapeutic agents but also other substances to targeted cells and tissues, eliciting specific phenotypic changes. Exosomes exhibit lower immunogenicity compared to liposomes, making them attractive candidates for therapeutic applications. Their lipid bilayer structure gives them an amphiphilic nature that enables them to encapsulate and transport both hydrophilic and hydrophobic compounds. Studies have demonstrated that exosomes loaded with chemotherapeutic drugs display enhanced efficacy compared to free drug

formulations. With advances in medicine and technology and with more research and a better understanding of the physiology and characteristics of exosomes and their usage in clinical medicine (12, 17, 19).

With the progression of medical science and technological innovation, coupled with deeper investigations and enhanced comprehension of exosome physiology and attributes, the potential for their integration into clinical medicine is increasingly promising. Nevertheless, prior to harnessing the complete therapeutic efficacy of these applications, a thorough and meticulous exploration of cytobiology is imperative. Safety assessments are indispensable in certain scenarios, as EVs might exhibit toxicity and immunogenicity influenced by various factors, including their source, experimental model, or composition. Enhanced utilization of systematic in vivo models, along with advanced imaging techniques for monitoring the biogenesis and destinies of EVs, will facilitate a comprehensive comprehension of their fundamental roles and the transition of research findings into clinical practices. Furthermore, addressing primary challenges and technical barriers is imperative to attain ample quantities of these vesicles for clinical trials and to enhance their efficacy in personalized medicine, particularly within the oncological domain. Varied techniques for isolating exosomes, a lack of standardized protocols for engineered exosome preparations, uncertainties regarding the optimal cell sources for generating these vesicles, and the influence of environmental factors on the cargo of EVs pose challenges. To address the issue of heterogeneity in applications involving exosomes, further research with an extensive array of additional EV markers is necessary (16, 19).

6. Conclusion

- T2DM is a widespread chronic disease with rising global incidence.
- T2DM is directly linked to obesity and characterized by insulin resistance.
- IR leads to decreased glucose uptake by the liver, muscles, and adipose tissue, resulting in elevated blood glucose levels.
- The pathophysiology of T2DM is complex and not entirely understood.
- Recent research has focused on the role of extracellular bodies, particularly exosomes, in various diseases, including T2DM.
- Exosomes are small vesicles that facilitate intercellular communication and contribute to the pathophysiology of T2DM and its complications.
- Exosomes hold great promise in theranostics, combining therapeutic and diagnostic approaches.
- Particularly in T2DM, exosomes could play a crucial role in early diagnosis and managing complications.
- Continued research into exosomes is essential to unlock their full potential.
- Advancements in exosome research could lead to novel diagnostic tools and therapeutic strategies for various diseases, including T2DM.
- These developments have the potential to transform the management and treatment of T2DM, with emphasis on T2DM complications.
- Improved understanding and application of exosome-based therapies could significantly enhance patient outcomes.

7. Summary

T2DM stands as one of the most prevalent chronic diseases globally, with an increasing incidence, notably associated with obesity. While its pathophysiology remains incompletely understood, it is primarily linked to insulin resistance, resulting in reduced glucose uptake by the liver, muscles, and adipose tissue, consequently leading to elevated blood glucose levels. Classical clinical features of T2DM include polydipsia, polyuria, and polyphagia, yet many patients are asymptomatic, underscoring the importance of screening, particularly in obese individuals with a positive family history. Diagnosis of T2DM currently relies on assessing levels of FPG, OGTT, and HbA1c. A myriad of glucose-lowering medications are available in the market today. Poorly controlled T2DM is associated with numerous complications, such as diabetic retinopathy, neuropathy, nephropathy, cardiomyopathy, and impaired wound healing.

EVs are released by various cells and categorized into three types based on size: apoptotic bodies, microvesicles, and exosomes, in ascending order. Recent research has underscored the pivotal role of extracellular vesicles, particularly exosomes, in the physiology and pathophysiology of various diseases, including T2DM. Exosomes, small vesicles facilitating intercellular communication, have been shown to contribute to the pathophysiology of T2DM and its complications. Despite ongoing investigations, much remains to be elucidated regarding exosomes. However, they hold immense promise in the realm of theranostics—integrated therapeutic and diagnostic approaches—particularly concerning T2DM complications. Continued research into exosomes has the potential to unveil novel diagnostic tools and therapeutic strategies, revolutionizing the management and treatment of T2DM and enhancing patient outcomes.

Keywords

Type 2 diabetes mellitus, obesity, extracellular vesicles, exosomes, clinical and therapeutic potential

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9. Curriculum Vitae (CV)

Art Sefedini was born on 07 October 2000, in Prishtina, Republic of Kosova. He started his educational journey in 2005 in primary school. In 2018, he finished high school, and in October of the same year, he started studying Medicine at the Faculty of Medicine, University of Rijeka, Croatia. After six years of studies, he finished his undergraduated studies in 2024.