REVIEW ARTICLE

Exosomes as Crucial Player in Insulin Resistance and Obesity: Potential Therapeutic Approach

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ABSTRACT

Increasing obesity has become a serious health problem worldwide in recent years, both in adults and children. Obesity can lead to numerous diseases such as osteoarthritis, diabetes, cardiovascular and respiratory diseases, and other metabolic disorders. In addition, obesity is considered one of the most common causes of insulin resistance in the body. Moreover, obesity disrupts normal insulin physiology, resulting in impaired insulin signaling and other intrinsic abnormalities such as poor glucose movement, phosphorylation, and decreased glucose oxidation and glycogenesis. Therefore, it is necessary to find an effective treatment for such conditions. Recently, exosomal therapy has been proposed as one of the alternative approaches to treat metabolic diseases. Exosomes are endosome-derived extracellular vesicles that circulate in body fluids such as mucus, blood plasma, urea, etc., transfer molecules and signals from one cell to another, and thus are involved in various normal and pathological processes in the human body. It has been discovered that exosomes play a role in processes related to obesity, such as adipocyte differentiation, angiogenesis, inflammation, etc. The current study reviewed exosomes' role in the development of insulin resistance associated with obesity and possible targets.

Keywords: Adipogenesis, Adipose tissue, Angiogenesis, Exosomes, Inflammation, Insulin, Insulin resistance, Macrophages, Obesity.

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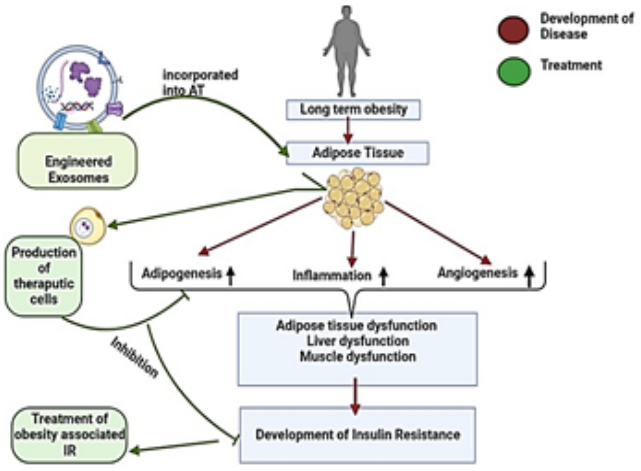
GRAPHICAL ABSTRACT



INTRODUCTION

Obesity or overweight has increased at an alarming rate worldwide, affecting not only industrialized countries but the entire world population[1,2]. Obesity is characterized by a number of metabolic disorders, especially lipid metabolism disorders and their complications. The disruption of lipid synthesis and breakdown processes can lead to dysregulation of lipid metabolism or lipid dysmetabolism, which in turn exacerbates the progression of obesity and associated metabolic diseases such as diabetes or insulin resistance.[1,3] Insulin is an important metabolic function that controls glucose and cholesterol by assisting the uptake of glucose by skeletal muscle, liver, and adipose tissue. Since extra fat makes cells poorly receptive to insulin, this could lead to insulin resistance in the body, where insulin seems unable to perform its usual

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physiological functions. There are several evidence that adipose tissue is more insulin resistant than muscle, or it can even be said that insulin is not as effective in obese people, resulting in constantly elevated blood glucose levels and may lead to diseases such as diabetes (type-II) [4]. Therefore, it is important to find an effective biomarker for the early diagnosis and treatment of such diseases related to obesity and its associated insulin resistance as soon as possible. Recently, exosomes have been recognized as a potential biomarker and therapeutic tool that may be helpful in many metabolic diseases, including obesity and insulin resistance.[5]

Exosomes are endosome-derived extracellular vesicles 30 to 150 nm in diameter, rich in lipids, proteins, and nucleic acids, surrounded by a lipid bilayer that can be transferred from the donating cell to target cells.[6] Because of the transport of exosomal cargo from one cell to another, they thought to be involved in the promotion/inhibition of various diseases. Depending on the cell type from which they are released, i.e., exosomes produced by tumor cells are involved in the spread of the tumor to unaffected cells .[7,8] In recent years, the study of adipocyte-derived exosomes has attracted much attention in metabolic dysfunction and related diseases. White adipose tissue-derived exosomal cargo may have both advantages and disadvantages in the case of insulin resistance.[9–12]

Exosomes have also been shown to be involved in the control of numerous processes such as adipogenesis, angiogenesis, inflammation, etc., associated with obesity-induced insulin resistance.[13,14] Adipogenesis is a process in which adipocyte progenitor cells proliferate and differentiate into adult adipocytes. This contributes to the ability of adipocytes to store excess fat rather than allowing its accumulation in various organs, e.g., heart, liver, muscle, etc.[15] Impairment of adipogenesis can lead to the promotion of insulin resistance by causing a decrease in GLUT4, an important glucose transporter, which decreases the uptake of glucose by cells and organs, leading to increased glucose levels in the blood.[16] Zhang *et al.* 2017 showed that rat adipose tissue-derived exosomes containing miR-450a-5p promote adipogenesis by suppressing the expression of WISP2, a novel adipokine released in obesity that contributes to mediating inflammation.[17] Similarly, Xia *et al.* 2020 found that exosomes released by M1 macrophages reportedly play a role in activating the glitazone receptor for reverse insulin resistance (also known as PPAR-γ) and promote adipogenesis, implying that insulin resistance is less likely in these cases.[18] In contrast, Zhou *et al.* 2019 and 2020 suggested that exosomes derived from fetal bovine serum and the K562 tumor cell line may suppress adipogenesis, increasing the likelihood of developing insulin resistance.[19,20]

Furthermore, several pieces of evidence suggest that inflammation and insulin resistance are very closely related to each other depending upon the exosome released. One study unveiled that adipose tissue-derived mesenchymal cells produce exosomes having a higher amount of STAT3 protein which can improve macrophage-2 anti-inflammatory characteristics. In contrast, Żbikowski *et al.* in 2021 demonstrated that, exosomal miRNAs generated from adipose tissue, such as miR27a, miR34, miR29a, miR223, miR155, etc. can influence cellular metabolism by suppressing of PPAR, IRS1, or polarisation of macrophages (M1) which can cause inflammation and can result in insulin resistance in diverse tissues.[21] Consistent with these findings, another study showed that angiogenesis, which is the process of growth and proliferation of endothelium cells (EC), inside the blood vessel, is also one of the remarkable cause of insulin resistance associated with obesity.[21] Exosomes produced from Mesenchymal stem cells (MSCs) and Adipose tissue-derived stem cells (ADSCs) provoke certain processes such as activation of intracellular signal transduction pathways, transportation of pro-angiogenic molecules and microRNA, inhibition of endothelial cell ageing, and upregulation of expression of vascular endothelial growth factor (VEGF), all of these results in angiogenesis.[22,23] All these studies suggest that the exosomal cargos are the main regulators of these conditions. Therefore, this review emphasizes identifying, targeting, suppressing, or increasing the expression of these exosomal cargos, which can be proved to be a potential therapy for metabolic or many other diseases.

Common Physiological Pathway of Insulin Resistance

Understanding the importance of insulin to a wider range of physiological processes, as well as its biosynthesis, secretion, and activities at the molecular and whole-body levels, has significant implications for much of the chronic disease that occurs in contemporary western society. Insulin is a chemical released by pancreatic islet cells that regulates blood glucose levels by aiding the uptake of glucose into cells and promoting the metabolism of lipids, proteins, carbohydrates, etc. It is produced in β-cells as preproinsulin, which is emptied into the cisternal region of the sarcoplasmic reticulum in less than an instant after production, where it is cleaved into proinsulin by proteolytic enzymes. This results in the formation of two polyprotein chains, A and B, consisting of 21 and 30 amino acids, respectively, linked together by chain C so that microvesicles can transport the proinsulin into the dictyosome, where upon the proinsulin is released into the other compartments and vesicles. In the developing granules, prohormone convertases 2 and 3 and carboxypeptidase continue to convert proinsulin to insulin.[24,25] Under normal physiological conditions, minor postprandial fluctuations in glucose concentration are an important regulator of insulin production, influencing the islet cell response to a number of neurohumoral agonists. Adenylate cyclase (AC) is activated by glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1, whereas cholecystokinin and acetylcholine activate polyphosphoinositide hydrolysis. These two major categories of neurohumoral agents act synergistically to stimulate insulin production when glucose concentration is above 6.0 mM. However, in the absence of a neurohumoral agonist, an increase in plasma glucose concentration from 8 to 10 mM results in an increase in insulin production. Surprisingly, both high glucose and a combination of factors lead to an increase in intracellular signaling.[26,27] In order

to act, insulin must interact with specific receptors on the cytoplasmic membrane. These receptors are glycoproteins consisting of at least two distinct α and β subunits with molecular weights of 135,000 and 95,000, respectively. The insulin-binding properties of these receptors have been determined by their biological activity, which includes high affinity for insulin, rapid and saturable binding, and selectivity for insulin and related compounds relative to their biological activity. Therefore, insulin is considered a critical influencing factor in regulating the concentration of insulin receptors.[4] When cells (such as lymphocytes, hepatocytes, fibroblasts, and adipocytes) are cultured in an insulin-containing medium, the concentration of insulin receptors decreases with time and temperature; this phenomenon is referred to as downregulation. The cause of this phenomenon is unknown, but rapid receptor degradation appears after insulin exposure is involved. This mechanism, in which ambient insulin levels control insulin receptor concentration, is thought to play an important role in developing insulin resistance in various disease settings.

In-vivo and *in-vitro* research has identified dozens of additional receptor concentration and affinity modulators that can alter receptor expression, including hormones, ions, nucleotides, ketones, and autoantibodies to the receptor. Insulin receptor alterations and clinical insulin resistance may be caused by one or more of these receptor modulators in numerous diseases. Insulin is thought to act on several insulin-dependent enzymes by altering their phosphorylation state. In this context, it was recently discovered that insulin receptors can be rapidly phosphorylated after interaction with insulin.[28,29] Given the diversity of insulin's effects on cellular function, no single early biochemical event can be shown to be important for all insulin effects. The lack of understanding in this area makes the identification of the molecular errors that cause insulin resistance in disease even more challenging.

Obesity in Relationship to Insulin Resistance

As per World Health Organization (WHO), obesity is the weight in relation to height (kg/m^2) guideline that is considered most appropriate in describing obesity or overweight. According to this, if a person's body mass index (BMI) is greater than 30 kg/m^2, he or she is considered obese. Obesity can be classified into three categories:

Group I (30.0 to 34.9 kg/m^2),

Group II (35.0 to 39.9 kg/m^2), and

Group III (greater than/equivalent to 40 kg/m^2) [3].

Higher the BMI values greater will be the chances of risks associated with obesity. Obesity has two effects on the body:(a) a rise in the bulk of fatty tissue, (b) the increased release of pathogenetic products by bigger fat cells.

Each disease with an increased risk due to obesity can be divided into two pathophysiological groups. The first group contains disabilities associated to excess fat, while the risks related to metabolic changes fall under the second category. The fat cell's reaction to its hormonal secretions is also dependent on the lipid profile. A variety of factors influence fat accumulation in fat cells. The production of male and female

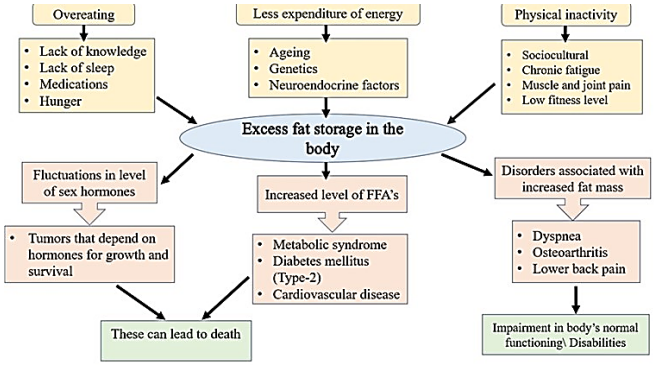

Figure 1: Relationship between excess fat and physiological pathways. This Figure depicts the etiology of health disorders related to obesity.

hormones by different glands, or the periphery transition in fat tissue, promotes body fat distribution. Although gender plays an important role in forming heavy mass around abdominal organs in adults, the impacts of glucocorticoid, decreased growth hormone, and fluctuating levels of other sex hormones are also involved in the deposition of fat associated with aging (Figure 1).[1]

Obesity is also found to be one of the most prominent causes of insulin resistance among all the factors that contribute to it. Insulin resistance is described as the incapability of fat cells and voluntary muscle cells to enhance the absorption of glucose and prevent glycogen breakdown. It also may contribute in the pathogenesis of type II diabetes, pregnancy-induced diabetes, brain disorders like presenile dementia, etc.[30,31] It has been observed that, on the insulin receptors, insulin-stimulated protein kinase activity, which promotes tyrosine autophosphorylation, is lower in fat people. Therefore, insulin signaling is found to be more disrupted in obese people than in non-obese people. Obesity is also linked to various post-receptor binding problems in insulin action, such as altered second messenger production, impaired glucose movement and alterations in certain crucial biochemical pathways involved in the utilization of glucose. Obesity can also affect lipid synthesis and biosynthesis of proteins in the body.[32] Obese people have higher amounts of free fatty acids (FFAs), which contribute to problems with glucose consumption and storage.[33,34] The rate of lipolysis increases as body fat increases, resulting in higher transportation and oxidation of free fatty acids in muscle and hepatocytes. Muscle glucose utilization reduces when FFA are used as an alternative energy source, whereas synthesis of glucose from the liver increases to meet the rising deterioration of FFAs. These aspects induce systemic hyperinsulinemia (raised circulatory insulin concentrations) but lower skeletal insulin sensitivity with decreased glucose absorption. Hyperinsulinemia also leads to several pathophysiological alterations such as impaired pancreatic islet function, increased food consumption, hyperlipidemia, etc.[31,35,36] Therefore, these acts result from hyperglycemia and poor glucose tolerance (Figure 2).[37,38]

In liver, FFAs lead to an increase in gluconeogenesis, while acting on skeletal muscle leads to increased insulin resistance. These ultimately results in hyperglycemia.

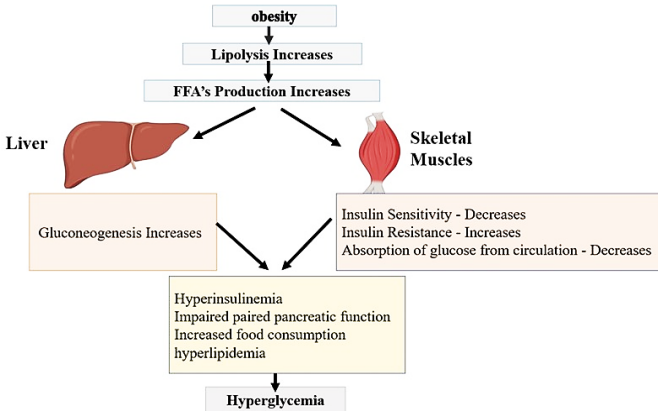

Figure 2: This is the schematic diagram showing FFAs produced during obesity and responsible for insulin resistance and hyperglycemia.

One more cause found to be responsible for insulin resistance is the activation of innate immunity during metabolic dysfunction. There is a link connecting metabolism dysfunction to the immune system; for instance, macrophages are involved in initiating a systemic inflammatory response during obesity, ultimately causing resistance to insulin. M1-polarized macrophages infiltrate adipose tissues as a result of an increased outflow of FFA through subcutaneous and visceral adipose depots. By signaling through pathways like JNK and IKK, macrophages can trigger inflammatory pathways in cells and cause insulin resistance. Even though these large phagocytes, termed as macrophages , act as key component of obesity-related inflammation of adipose tissue, the process of Inflammation is also aided by lymphocytes and mast cells. Thus, immune system stimulation in fat population results in inflammation of adipocytes and resistance to insulin is developed in the body.[39,40]

Exosomes

All cells (nucleated or non-nucleated) secrete membrane vesicles called extracellular vesicles (EVs), both as part of their conventional physiology and in acquired disease. Ectosomes and exosomes are the two types of EVs found in the body. Ectosomes occur as microvesicles, microparticles, and large vesicles ranging in size from 50 to 1 mm and are released outside the cytomembrane by external cleavage.[41,42] Exosomes are endosome-derived EVs with shell-like structures that have a lipid bilayer membrane and a diameter of 30 to 150 nm. Exosomes have been associated with various immunological responses, virulence, pregnancy, chorionic villus sampling (CVS) disease, central nervous system (CNS) disease, and cancer progression. Exosomes carry information molecules such as lipids, proteins, and nucleic acids that are shielded by bilayer phospholipids for protection and can be transferred from mother cells to recipient cells. Chemicals delivered by exosomes can effectively affect the biological response of recipient cells. Therefore, exosome-mediated responses can either promote or inhibit disease. Heat shock molecules such as HSP-60, HSP-70, HSP-90, the Apoptosis-Linked gene-2-interacting protein X (ALIX) protein and the ESCRT-1 complex subunit TSG-101, glucose-regulated protein 94 (Grp-94), etc. are just some of the molecules that can be used to identify exosomes.[8]

The biogenesis of exosomes begins within the endosomal system. They originate as intraluminal vesicles (ILVs), which are formed by reverse bending of the membrane during the conversion of the endosome to its mature state to generate a number of small vesicles known as multivesicular bodies (MVBs).[43] A "transport-related endosomal sorting complex" (ESCRT) is one of the key mechanisms for exosome production. The subunits of the ESCRT are divided into four categories, which are then recruited to perform vesicle sorting of ubiquitinated proteins and direct the packing of exosomes, leading to exosome formation. Exosome formation is also controlled by the ESCRT-independent pathway, which includes proteins such as tetrasapins (CD -9, CD -63, CD -81, CD -82) and the lipid ceramide, once all four ESCRT subunits are depleted. Exosomes contain many of these proteins, which aid in cargo integration and membrane curvature (Figure 3). Multivesicular bodies (MVBs) can then take one of four pathways: (I) degradation of material by fusion with lysosomes, (II) fusion of ILVs with cell membranes, releasing exosomes into the extracellular environment, (III) human leukocyte antigen proteins on the cell surface contributing to antigen presentation, or (IV) recycling, as shown in Figure 3 can be clearly seen [44]. Several proteins regulate the interaction of MVB with the phospholipid bilayer of the cell and, thus, the unloading of exosomes. The Rab GTPases are one of these proteins that act as regulators of the exosomal transport pathway and the transport of cellular cargo across the membrane, implying different Rab proteins can influence the release of exosomes. Soluble N-ethylmaleimide-sensitive factor attachment protein receptors, or SNAP receptors, are also a category of proteins involved in regulating attachment, fusion with the membrane, and releasing exosomes. The metabolic state of the cell, which is controlled by factors such as endoplasmic reticulum stress, autophagy, and Ca^{2+} within the cell, also controls the synthesis and release of exosomes.[43]

Several exosomal membrane proteins, such as phosphatidylserine receptors (PSRs), glucans, integrins, etc., allow receptors on the plasma and endosomal surfaces of the receiving molecules to interact in a specific manner that enables precise targeting to the cell. One of the pathways mentioned below may lead to this type of interaction: (I) uptake of the exosome or fusion of the exosome with the cell membrane, allowing the material contained in the exosome to enter the cytosol of the recipient cell and trigger subsequent actions; (II) direct activation of target cell surface receptors by exosomal membrane proteins, triggering further intracellular signaling cascades without being absorbed; or (III) degradation of exosomes.[44]

Exosomes emerge as intraluminal vesicles by bending the cell membrane inwards, forming early endosomes. Proteins and other genetic material present in cytoplasm is then incorporated into it. This endosome is then matured to form

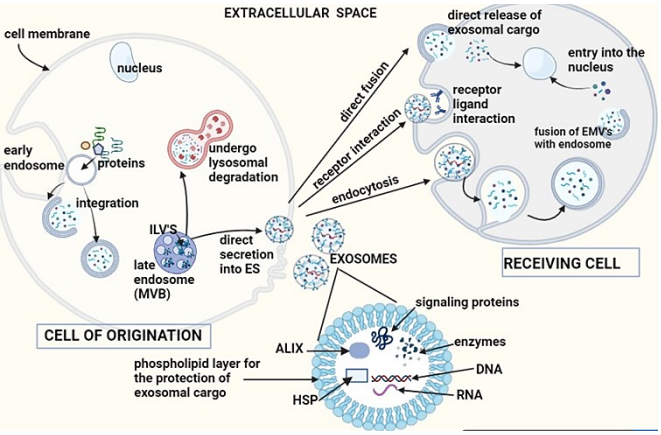

Figure 3: This diagram depicts each step involved in the biogenesis and release of exosomes.

MVBs. These MVBs then either merge with lysosomes for the deterioration of material or they directly fuse with the cell membrane to release exosome into extracellular space. After the release, exosomes targets the other cells in three ways: (I) direct fusion, (II) receptor ligand interaction, and (III) endocytosis. After incorporating exosomes into new target cells, exosomes start releasing the material they carried from previous cells and mediating their action. ILV's: Intraluminal vesicles, MVB's: Multivesicular bodies, ES: Extracellular space, ALIX: Apoptosis Linked-gene 2 Interacting Protein, Heat-shock proteins (HSP).

Exosomes are released by various cells, including hepatocytes, myocytes, fat cells, and somatic cells. They are also found in blood, sputum, mammary gland milk, sweat, tears, urine, and other body fluids. The production of exosomes by endothelial cells and skeletal muscle cells encoding the cell surface protein T-cadherin is triggered by adiponectin, a protein produced exclusively by adipocytes, whereas glucagon modulates exosome secretion by endothelial cells in adipose tissue. In summary, exosome synthesis and biotransformation function bidirectionally, with the former influenced by exosomes and the latter by metabolic regulation.[45,46]

Adipose Tissue-derived Exosomes

Adipose tissue, commonly referred to as "fat," is a type of areolar tissue composed of cells (adipocytes) filled with lipids and surrounded by fibrous tissue, arteries, veins, leukocytes, white blood cells, and so on. This adipose tissue can store triglycerides in almost unlimited amounts.[47–50] Based on color and function, adipose tissue is divided into two main types: white adipose tissue (WAT) and brown adipose tissue (BAT). Brown adipose tissue is used to generate heat at low temperatures, while white adipose tissue is designed to store large amounts of fats.[51] Each of these tissues can release a variety of proteins and intermediates that interact with other cells and help regulate the body's energy and glucose. Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) are two other important white adipose tissue storage sites. Subcutaneous adipose tissue and visceral adipose tissue have different adipocyte populations in terms of volume and physiological functions. Subcutaneous adipose tissue is the largest fat depot in the human body and is important for glucose and lipid regulation throughout the body through the action of insulin. It is thought to act as an "expansion buffer" and protect adjacent tissues and organs from the deleterious effects of excessive fat accumulation, whereas VAT appears to be more susceptible to catecholamine-stimulated lipolysis than subcutaneous fat depots and has a much higher reactivity for free fatty acid release when stimulated by adrenergic agents.[52–55] VAT because of the direct portal connection of visceral adipose tissue compartments with the liver, obesity is more prone than SAT obesity to the buildup of liver fat and the occurrence of insulin resistance.[56,57] In addition, VAT adipocytes have lower insulin receptor sensitivity than SAT adipocytes, making them less susceptible to insulin-stimulated lipogenesis. As a result, metabolic problems associated with the liver are more likely to be associated with visceral adipose tissue enlargement than with SAT. Several events that occur during obesity may affect the development and function of WAT. The study of adipocyte-derived exosomes has become very important in the field of metabolic disorder-related diseases in recent years. White adipose tissue-derived exosomal cargo, consisting of DNA, RNA, snRNA, tRNA, miRNA, lipids, and proteins, can positively and negatively affect insulin sensitivity. Remarkably, this exosome-mediated insulin resistance's positive or unfavorable consequences are closely related to the type of exosome-releasing cells.[21]

Exosomes in the Regulation of Adipogenesis

Adipocyte hypertrophy, commonly referred to as adipogenesis, is the phenomenon in which mesenchymal stem cells divide, proliferate, and develop from adipocyte progenitor cells into adult adipocytes.[58] It is a crucial step in determining the number of adipocytes, which occurs mainly in childhood and adolescence, and the ability of adipose tissue to store lipids and fat accumulation also increases in adults (Table 1). Adipogenesis is influenced by a number of transcriptional regulators and signaling cascades, including CCAAT/enhancer binding proteins (CEBPS), STAT proteins, glitazone reverse insulin resistance receptor and Kruppel-like factor (KLF) proteins, etc. They have been found to have an important function in adipocyte formation, differentiation, and physiology.[59] Therefore, regulation of adipose tissue diameter and fullness may be a promising treatment for obesity.[60,61] In a number of target tissues, active lipid synthesis mediators such as protein kinase C (PKC) and ceramide molecules increase lipid accumulation. The ability of SAT to collect excess lipid rather than accumulate it in fat stores such as hepatocytes (as in NAFLD), heart, muscle tissue, etc., which contribute to the diseases of obesity, may serve as a key indicator of metabolic health.[16]

In 2021, Al-mansoori *et al.* demonstrated that the link between classical BMP4 signaling and WNT signaling is responsible for the early recruitment of new adipocytes; BMP4 initiates their commitment to the adipogenic lineage, whereas WNT promotes their undifferentiated and proliferating state.

When energy expenditure increases, the storage capacity of SAT decreases, causing excess fat to be deposited around internal tissues and organs, which also leads to dysregulation of WNT and BMP4 signaling pathways SAT hypertrophy, ultimately resulting in adipose tissue dysfunction.[62] Impaired adipogenesis and loss of the ability of SAT to store excess fat promote insulin resistance by causing a decrease in GLUT4, which decreases the uptake of glucose by skeletal muscle.[16] Therefore, adipogenesis dysfunction may play an important role in the development of insulin resistance in the body.

Recent research suggests that exosomes are involved in the regulation of adipogenic differentiation. They transfer genes or proteins related to fat accumulation to promote or inhibit fat synthesis.[17] Exosomes comprise a variety of proteins and ribonucleic acids that are endocytosed by cells and serve as regulators of adipogenesis. Some of these functions include rat adipose tissue exosomes containing miR-450a-5p, which stimulates adipogenic differentiation by suppressing WISP -2 activity.[17] In contrast, exosomes released by pro-inflammatory M1 cytokines upregulate PPAR-γ gene activity and stimulate lipid droplet formation, ultimately leading to increased adipogenesis.[18] Exosomes produced by macrophages such as M0, M1, and M2 have differential effects on MSC proliferation and differentiation. MiR1246 exosomes produced in fetal bovine serum (FBS) prevent the development of fat production by targeting EBF1.[20] ciRS-133 transported by exosomes in gastric cancer contributes to adipogenesis by decreasing miR-133 activity and increasing PRDM16 expression.[63] Exosomes produced from adenocarcinomatous human alveolar basal epithelial cells suppress adipogenesis *via* the TGF pathway .[64] In the K562 cell line, miR-92a-3p reduces ADSC adipogenesis via decreasing C/EBP expression after transcription.[20] Thus, the use of exosomes from different cells to treat adipose tissue dysfunction-related insulin resistance has many therapeutic implications.

Role of Exosomes as a Regulator of Enlargement

An increase in the diameter of fat cells is the main symptom of obesity, which increases the risk of fat cell death due to lack of oxygen during the enlargement of fat tissue. In response to this mechanism, toll-like receptor 4 (TLR -4) is activated in native fat cells, triggering an immune response. Via the MyD88- and TRIF-mediated downstream cascades, fatty acids stimulate NF-B and P38 MAPK signaling, which increases oxidative stress in ER, generates free radicals, and stimulates macrophages to release cell signaling proteins, i.e., interleukin 1β, interleukin 6, and tumor necrosis factor α (TNFα).[65,66] Insulin response is also affected by hormones produced by fat cells known as adipokines (Table 2). They are divided into two classes: (a) adipokines that increase insulin response and (b) adipokines that inhibit insulin response. Leptin and adiponectin are some adipokines released by adipocytes that have been shown to be involved in regulating inflammation. These locally released chemokines recruit pro-inflammatory macrophages to the AT. adipose tissue macrophages (ATMs) are the most abundant type of immune cells in adipose tissue and are involved in a variety of biological processes, including tissue regeneration and insulin response. ATMs play an important role in mediating inflammation and insulin resistance, leading to adipocyte dysfunction as obesity progresses. The inflammatory process can be enhanced or attenuated depending on macrophage activation, in which there are two types of M1 and M2. Anti-inflammatory cytokines such as IL -10 are released by M2 macrophages, which serve to suppress immunological reactivity and increase insulin sensitivity.[67] M1 macrophages, on the other hand, generate cytokines, induce inflammatory signals and stress within cells that communicate via JKK and IKK cascades, and secrete cytokines that, together with inflammation, lead to worsening insulin resistance.[68,69] Overall, insulin resistance is largely caused by adipose tissue inflammation.

Adipose tissue macrophage polarization is regulated by a number of processes, one of which is an exosome-dependent process. Adipose tissue macrophages can use white AT cells to change their properties from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophage properties. Exosomes from adipose cells contain a high amount of miRNA 34a, which promotes M1 properties, whereas exosomes from adipose stem cells contain a higher amount of STAT -3 protein, which enhances M2 properties. Remarkably, M2 macrophages release exosomes loaded with miRNA690; these increase insulin sensitivity in animal models with HFD (high fat diet).

Table 1: Exosomes involved in the regulation of adipogenesis by targeting different genes and signaling pathways involved in the process

Source of exosome	*Exosomal cargo (RNA/ Protein)*	*Target gene or signaling pathway*	*Adipogenesis (promotion or suppression)*	*Development of insulin resistance*
Rat adipose tissue	miR-450a-5p	suppressing the expression of WISP2	Adipogenesis Increases	Fewer chances of IR development
M1 macrophages		Upregulation of expression of PPAR-gamma gene	Adipogenesis Increases	Fewer chances of IR development
Fetal bovine serum	miR-1246	Targeting EBF1	Prevention of adipogenesis	Increased chances of IR
Gastric cancer cells	ciRS-133	Decreasing miR-133 activity and increasing PRDM16 expression	Promotion of adipogenesis	Less chances of IR development
Lung tumor cell A549		Suppression of TGF signaling pathway	Suppression of adipogenesis	Increased chances of IR
Tumor cell line K562	miR-92a-3p	Suppression of C/EBP expression	Suppression of adipogenesis	Increased chances of IR

Table 2: The cytokines and signaling pathways that connect inflammation to obesity-related resistance to insulin.

S.no	*Cytokine or signalling pathway*	*Source*	*Cause for the development of resistance of insulin during obesity*	*References*
1.	TNF α	Adipose tissue derived pro-inflammatory cytokine	*via* boosting adipocyte lipolysis and raising the IRS-1. It activates JNK1/2, which causes insulin resistance in visceral adipocytes	98,99
2.	Interleukin-1*β* (IL-1*β*)	Inflammasome activity controls secretion	Insulin signaling is disrupted in peripheral tissues and macrophages, resulting in diminished insulin sensitivity and possibly altered insulin production in β cells	100
3.	IL-6	Many tissues, including adipose tissue, secrete IL6	IL-6 stimulates JAK-STAT pathway and enhances activity of suppressor of cytokine signaling-3(SCOS-3), As a result, the activity of IRS-1 and Glucose transporter 4 (GLUT 4) decreases	101
4.	Leptin	White adipose tissue-derived protein	Leptin seems important for regulating bodily energy homeostasis. They are in higher concentration in obese people. According to one study, Leptin affects the signaling of insulin via enhancing the phosphorylation of IRS-1 at S318 (Serine 318). While leptin is found to suppress insulin-mediated expression of PEPCK and also reduces phosphorylation of IRS-1 tyrosine in response to insulin in Hep G2 cell line	102,103
5.	Adiponectin	Secreted from white adipose tissue (WAT)	With the exception from others, adiponectin is found to enhance insulin sensitivity by stimulating Serine/threonine kinase 11/AMP activated TSC1/2 signaling. But TNF-α and IL-6 are inflammatory cytokines that inhibit adiponectin, which can result in resistance to insulin. The adiponectin concentration is lower in obese people.	104,105
6.	IKK*β*/NF-*κ*B Pathway	In normal circumstances, NF-κB is retained in the cytoplasm and bound to IκB proteins, preventing nuclear localization of NF κB.	In IR-related illnesses like overweight and Type II diabetes, activation of IKKβ limits the activity of anti-inflammatory mediators; similarly, NF-κB is a crucial mediator that links IR to the pro-inflammatory cytokine IL-1. Therefore, deletion or inhibition of IKK*β and* NF-*κ*B can result in enhanced tolerance to glucose and insulin sensitivity.	106
7.	JNK Pathway	extracellular (e.g., inflammatory signals, pathogens, developmental factors, and neurotransmitters) as well as intracellular (e.g., oxidative stress, and DNA damage)	Through influencing the generation of inflammation causing cytokines, karyomitosis, and cascade mediated cell death, JNK promotes inflammation and metabolic disorders, like obesity, and Insulin resistance. JNK induced from endoplasmic reticulum stress can also cause serine phosphorylation of IRS-1	107

This suggests that ADSC and macrophages communicate *via* exosomes, which improves immunological and metabolic balance in white adipose tissue.[17] When adipose tissue secretes exosomes in vigorous animals such as mice, they are taken up by polymorphonuclear leukocytes, which promotes their development into functional macrophages with higher levels of cytokines. Exosome-mediated activation of TLR4/TRIF signaling may also be a critical component in the development of insulin resistance associated with obesity.[70] Exosomes derived from adipocytes of higher body weight individuals consist of varying numbers of miRNA-55 that largely influence TGF-β and Wnt/β-catenin signaling. The interplay of these signaling pathways appears to be critical in developing and maintaining long-term inflammation and functioning as a major feature of obesity.[71,72]

Adipose tissue-derived exosomal miRNAs such as miRNA29a, miRNA27a, miRNA34, miRNA155, miRNA223, etc., affect biochemical responses by suppressing insulin receptor substrate 1, polarizing M1 macrophages, and activating peroxisome proliferator-activated receptor, leading to insulin resistance in a variety of cells.[21] The key regulator involved in the polarization of M1 macrophages *via* Ptch/PI3K signaling has been identified as Sonic Hedgehog (Shh) from IRADEs (insulin resistance-derived exosomes). It was found that IRADE macrophages further contribute to insulin resistance by reducing the expression of IRS 1 and hormone-sensitive lipase (HSL). Therefore, Shh was suggested as a promising approach to prevent insulin resistance. Activated immune cell-derived lipopolysaccharide (LPS) exosomes have been shown to affect inflammation-related gene expression of adipocytes. M2 macrophage polarization is also blocked by exosomal miR-34a released by adipocytes. As mentioned earlier, it enhances M1 properties by targeting Krueppel-like factor 4 (Klf4), which is responsible for white blood cell

differentiation and promotes the anti-inflammatory activity of M2 macrophages.[73] In short, adipocyte-derived exosomes can trigger a bidirectional mechanism, as they can increase the number of M1 immune cells (macrophages) that produce inflammatory cytokines leading to the progression of insulin resistance. In contrast, alternatively, they can promote insulin sensitivity by increasing the M2 profile, which in contrast, has an anti-inflammatory property, depending on the source from which it originates and its target gene or receptor.

Role of Exosomes in Angiogenesis

Changes in angiogenesis and insulin resistance are closely related.[57] When the endothelial cells (EC) present on the inner lining of blood arteries migrate, proliferate, and differentiate, this process is called angiogenesis.[37] The ability of adipose tissue to exhibit plasticity or expansion during the development and progression of obesity is associated with its angiogenic capabilities.[74] Hypoxia, inflammation, and structural changes in blood vessels accelerate the spread of adipose tissue. VEGF also known as vascular permeability factor, is a signal peptide produced by numerous cells responsible for the development of arteries and blood vessels.[75] Therefore, VEGF is considered an important regulator of angiogenesis in adipose tissue.[76-78] The vascular permeability factor system includes several receptors such as vascular permeability factor-A, vascular permeability factor-B, vascular permeability factor-C, and vascular permeability factor-D, which different family members activate. VEGFR-1 and VEGFR-2 are two tyrosine kinase receptors with which vascular permeability factor interacts to exert various biological activities. By binding to these receptors, vascular permeability mediates most of the cellular responses that cause endothelial cells to migrate, survive, and proliferate. According to various studies, vascular permeability factor-A appears important for healthy and pathological blood vessel development. It can bind to both VEGF-R1 and VEGF-R2, but inhibition of only VEGF-R2 can suppress diet-induced adipose tissue growth by reducing angiogenesis and adipogenesis.[79] Other members of the VEGF family that may be involved in adipose tissue angiogenesis include vascular permeability factor-B, which has been observed to be involved in ECM degradation by modulating plasminogen activation. Both vascular permeability factor-C and vascular permeability factor-D have been found to promote lymphangiogenesis.[77,78]

Exosomes produced by various cell types such as cord blood-derived mesenchymal stem cells, malignant cells, endogenous cardiac stem cells, etc., have been found to have an important function in angiogenesis. They trigger angiogenesis through a number of processes, including activation of key intracellular signal-regulated kinases, transfer of pro-angiogenic molecules, and prevention of endothelial cell senescence.[80,81] Appropriate vascularization is required to promote blood flow to new cells during healthy adipose tissue development; however, in several cases, undesired adipose tissue growth, local oxygen starvation leads to persistent inflammation, adipocyte dysfunction, and so on.[82,83] It has been reported that exosomes from AT-derived stem cells contain a number of miRNAs that enhance endothelial cell vascularization. MiR-21 from ADSC exosomes stimulates the development of new vessels from existing ones by increasing the activity of transcription factors that respond in the state of hypoxia, MAPK/ERK, stomatal cell-derived factor-1, PI3K/AKT, etc., all of which contribute to the development of angiogenesis.[84] MiR125a, a miRNA produced by adipose-derived stem cells, stimulates endothelial cell revascularization by inhibiting DLL4 gene expression *via* its 3'-UTR before differentiation. In the presence of vascular permeability factor-C, AT-derived stem cells release exosomes containing miRNA-132, which help to promote lymphangiogenesis in lymphoid endothelial cells *in-vitro* models by modulating the TGF/Smad signaling pathway.[85] A reduction in the amount of angiogenic molecules such as vascular permeability factor may lead to a reduced revascularization capacity of exosomes in obesity. Because obesity is an important factor in insulin resistance, we can theorize that exosomes that promote angiogenesis are also involved in the development of insulin resistance.

Exosomes Generated from Cells and Organs apart from Adipose Tissue that can cause Resistance of Insulin During Obesity

In obesity, other tissues besides adipose tissue can produce exosomes and play an important role in regulating insulin secretion and its physiology, such as exosomes from the liver, exosomes from skeletal muscle, exosomes from the pancreas, and so on. In general, exosomes released from liver cells increase insulin sensitivity in both in vitro and in vivo models. These beneficial effects are due to the abundance of miRNAs such as miRNA3075 in liver cell exosomes. It has been shown that miRNA3075 primarily targets the FA2H gene, which is present in fat cells, liver cells, muscle tissue, etc., and has also been associated with the progression of insulin resistance in obese individuals, so that lowering FA2H levels in these cells by a small interfering RNA leads to improved insulin sensitivity. However, in the chronic obesity studies (high-fat diet for 17–19 weeks), exosomes from hepatocytes of obese patients were found to be responsible for enhancing insulin resistance. Upon further investigation, the scientists found that exosomes from hepatocytes of people with long-term obesity enhance the activation of inflammatory cytokines but do not affect insulin signaling. *In-vitro*, these 17–19 week HFD-induced hepatic cell-derived exosomes do not alter insulin signaling but enhance inflammation-inducing macrophage activity.[86] In 2020, Wu *et al.* discovered that miRNA130a3p, which is produced by hepatic exosomes, plays a critical role in the biotransformation of glucose and triglycerides by targeting adipocytes.[87] In experiments using miRNA130a3p KO mice compared with overexpressed miRNA130a3p mice and wild-type mice, the miRNA130a3p KO mice were found to exhibit a sharp increase in plasma concentrations of LDL, HDL, and blood glucose levels in animals consuming a high-fat diet. The researchers discovered that downregulation of enzymes such as fatty acid synthase and peroxisome proliferator-activated

receptor γ gene by miRNA130ap from liver cells can suppress adipogenic development. In addition, miRNA130a3p was found to increase the levels of pAKT and pAS160 protein and enhance the activity of GLUT -4 transporter by targeting PH domain leucine-rich repeat protein phosphatase-2 (PHLPP-2).[87] The exosomes and soluble factors released by mesenchymal stem cells (MSCs) are among the biomolecules in established media that are now being extensively studied. Exosomes produced by MSCs have been found to retain the properties of their parent cells. Sun *et al.* (2018) investigated that exosomes produced from human umbilical cord MSCs (hucMSCex), as the primary paracrine approach of MSCs, may have a therapeutic impact on insulin-resistant cases by reducing hyperglycemia. Exosomes produced from huMSCs have been shown to increase glucose biotransformation by restoring insulin receptor substrate-1 and protein kinase B phosphorylation, promoting transcription and phospholipid migration from GLUT-4 into myocytes, and increasing glycogen uptake and storage by hepatocytes while maintaining glucose balance in the body.[88] While, Su *et al.* in 2019 showed that exosomal miR-29b-3p produced by bone marrow MSCs can control senescence-induced insulin resistance and may be a viable therapeutic focus for age-related insulin resistance.[89] Therefore, it can be said that exosomes produced by mesenchymal stem cells can help in the treatment of insulin resistance by enhancing the biotransformation of glucose, triglycerides, and lipids.

Potential Diagnostic and Therapeutic Aspects of Exosome

Liquid biopsy is an extremely useful test that provides information about a person's overall health. Because body fluids contain a wide range of chemicals, selecting appropriate and accurate biomarkers is critical.[90] Biomarkers are important for prognosis, diagnosis, and monitoring the efficacy of treatments for common complicated multifactorial metabolic diseases. According to numerous studies in recent years, exosomes produced by endosomal compartments in a variety of cell types are considered to be one of the most important categories for biomarker identification in many diseases such as cancer, neurological, viral, and metabolic disorders. The collection of exosome-like extracellular vesicles, particularly those composed of β-cells, and the study of their intravascular contents can provide information about cellular activity in people with diabetes and help in assessing the efficacy of antidiabetic drugs to improve clinical outcomes.[91] Exosomes are released from the cell under physiological or pathological conditions and are commonly accessible in physiological fluids such as urine, blood, or saliva, suggesting considerable potential for the discovery of novel and readily available biomarkers.[92] Exosomes are less convoluted than total physiological fluids and have high content stability, allowing long-term storage compared with standard biomarkers.[44] According to several findings, exosomes may also serve as an effective therapeutic tool. Since exosomes can be readily taken up by cells and the risk of immunological resistance is very low, this has sparked interest in their use as natural drug delivery tools. Electropermeabilization, liposome transfection, ultrasound treatment, and treatment with $CaCl_2$ are some of the procedures that can be used to deliver curative nucleotides, peptides, or antagonists into the exosomes of donor cells. This exosomal cargo is then taken up by recipient cells, where it becomes functionally active and produces the desired effects.[93,94]

Exosomes can likely be used to improve insulin resistance. Exosomes produced by insulin-resistant adipocytes have been found to contain proteins such as calregulin, S100 calcium-binding protein A6, osteoglycine, DJ1, peptidyl-prolyl cis-trans isomerase-B, and extracellular matrix glycoproteins that are significantly associated with insulin resistance.[95] Eguchi *et al.* 2016 pointed out that perilipin A serves as an indicator of adipose tissue-derived exosomes in a mouse model of obesity, the concentration of which increases as obesity progresses. In this regard, insulin resistance is linked to both perilipin A and exosome expression.[96] Another potential exosomal biomarker discovered during the course is cystatin C, an insulin resistance indicator linked to metabolic problems associated with obesity.[97] Using exosomes, once we find the cause or mediators involved in the development of disease, we can easily target them and improve the condition. All these studies suggest that exosomes are a valuable tool to detect and cure insulin resistance associated with obesity and other metabolic disorders. Not withstanding the fact that exosomes are not currently widely used as a detection method, further in-depth studies on exosomes will help to better understand their superiority as a liquid biopsy tool.

Conclusion and Future Perspectives

Obesity is considered one of the most common causes of insulin resistance, and insulin receptors cannot recognize insulin, reducing glucose uptake *via* hepatocytes, skeletal muscle, and fat cells. Many researchers have recently proposed numerous therapies to treat this condition. Exosomes were one of these proposals that attracted the attention of many scientists. Much of the literature indicates that one or probably the most important biological benefit of exosomes is their potential use as biomarkers in clinical diagnostics. Because exosomes are released from the cell under physiological or pathological conditions, they are generally accessible in physiological fluids such as urine, blood, or saliva and exhibit high content stability that allows long-term preservation compared to standard biomarkers. Exosomes are also associated with the modulation of various phenomena such as adipogenesis, angiogenesis, inflammation, etc., which, depending on the type of cells from which they are released, may contribute to the development of insulin resistance associated with obesity. Identification and modification of exosomal cargo may serve as a potential therapy in the treatment of such diseases. This article highlights recent discoveries about exosomes and their possible function in obesity-related insulin resistance, suggesting that they have immense potential for diagnosing and ameliorating insulin resistance. Although the identification and treatment of this disease using exosomes has not been as widespread as yet, the use of new technologies and approaches will make it possible in the future to use exosomes to identify and cure many diseases, such as insulin resistance.

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