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Exosomal Secretion of Adipose Tissue during Various Physiological States

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Abstract

Exosomes are secreted extracellular vesicles containing a wide array of biologically active components. Recent studies have demonstrated that exosomes serve as an important vehicle for extracellular communication and exert systemic effects on the physiology of organisms. Adipose tissues (ATs) play a key role in balancing systemic energy homeostasis as a central hub for fatty acid metabolism. At the same time, proper endocrine function of ATs has also been shown to be crucial for regulating physiological and metabolic health. The endocrine function of ATs is partially mediated by AT-derived exosomes that regulate metabolic homeostasis, such as insulin signaling, lipolysis, and inflammation. During the pathogenesis of obesity, metabolic syndrome, and cancer, exosomes shed by the resident cells in ATs may also have a role in regulating the progression of these diseases along with associated pathologies. In this review, we summarize the contents of AT-derived exosomes and their effects on various cell populations along with possible underlying molecular mechanisms. We further discuss the potential applications of exosomes as a drug delivery tool and therapeutic target.

Keywords

adipocyte; extracellular vesicles; obesity; metabolism; drug delivery

INTRODUCTION

Adipose tissue (AT) was initially characterized as an energy storage organ but after the discovery of leptin, its endocrine function has become a focus of research. Many studies have demonstrated the critical role of AT-derived factors in regulating metabolic homeostasis and immune function. AT contains a highly diverse population of resident cells consisting of mature adipocytes, macrophages, endothelial cells, and a resident stem cell population named adipose-derived stem cells (ADSC), adipogenic progenitors, or preadipocytes (1). Also highly diverse is the AT secretome, dozens of cytokines designated as adipokines are secreted along with exosomes and extracellular vesicles (EVs) (2,3). Processes such as inflammation, apoptosis, differentiation, insulin resistance, and metabolism have been

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shown to be regulated by adipokines, highlighting the endocrine function of AT (4). Furthermore, alterations in adipokine secretion, most notably adiponectin, leptin, and TNF α have been associated with disease severity in cases of obesity, metabolic syndrome, and various cancers (2). Exosomes derived from AT also have a role in this organ's extracellular signaling function (3). The AT-exosomes (AT-Exos) are known to contain various RNAs, proteins, and lipids that affect a host of processes in target cells (5–7). These effects range from regulating macrophage behavior and stimulating angiogenesis to modulating autophagy in hepatocytes (1,8,9). AT-Exos signaling contributes to the diverse extracellular signaling profile of ATs. In the clinical setting, the contents of these AT-Exos are being considered as candidates for disease markers due to the wide body of evidence showing the composition of these AT-Exos are altered under various physiological states (10,11). In addition, there is a growing interest in using AT-Exos as a therapeutic agent or modifying them to become drug delivery vehicles since they have been demonstrated to have low immunogenicity and cytotoxicity when administered *in vivo* (12).

Exosomes are a class of EVs that range in size from 30 to 150 nm and are formed from multivesicular bodies along the endocytic pathway (13). Exosomes were initially reported through two studies that explored EV secretion of reticulocytes (14,15). The membranes of these vesicles are characterized by their enrichment of certain lipids and proteins, including cholesterol, sphingomyelin, ceramide, and phosphatidylserine along with tetraspanins (CD9, CD63, CD81), integrins, and cell adhesion molecules (16,17). Exosomes can be distinguished from other EVs by their enrichment of endosomal components due to the nature of their biogenesis yet there is no universal marker that has been established to identify exosomes (18). The isolation of exosomes from various body fluids and cell culture media has been performed with a plethora of methods including immunoprecipitation targeting exosome-associated markers, various kits designed to precipitate EVs, flow cytometry, and differential ultracentrifugation, with the last being considered the most reliable for isolating pure exosome samples (19,20).

These isolated exosomes have been demonstrated to contain miRNA, lncRNA, mRNA, circRNA, proteins, and lipids that have been implicated in regulating various processes of their cellular targets (Fig. 1a) (5,21–26). MiRNAs are the most well-studied cargos found in exosomes, as they are a potent regulator of gene expression. MiRNAs such as miR-16, miR-27a, miR-146b, and miR-222 have all been demonstrated to be secreted in exosomes and have the effect of promoting adipogenesis (27). Exosomal lncRNAs display a wide range of regulatory functions. For example, GAS5 targets miR-223 to upregulate the PI3K/Akt pathway. RNCR3 found in HUVEC exosomes increases KLF2 expression to affect proliferation and migration, while TUC339/H19 from hepatocarcinoma exosomes affected M1 to M2 macrophage conversion and angiogenesis. In addition, the levels of exosomal lncRNAs are altered by aging and obesity, suggesting a physiological role of these exosomal lncRNAs (22). The presence of functional mRNAs has not only been demonstrated with sequencing of exosomal RNA content but also through studies utilizing CRE recombinase to “turn on” a reporter in remote tissues, showing long-distance transfer of translatable mRNA *in vivo* (28). Studies of exosomal proteins have been performed to identify cancer disease markers (17,29). In a proteomics analysis comparing exosomes secreted by breast cancer cells to those of non-cancer cells, it was shown that the cancer

exosomes had significantly higher levels of metabolic enzymes, signaling, and chaperone proteins, suggesting that these proteins may have a role in tumor-healthy cell crosstalk (30). In this review, the contents of AT-Exos will be discussed along with their functions during healthy AT function, obesity/metabolic syndrome, and cancer.

AT EXOSOMAL SECRETION UNDER NORMAL PHYSIOLOGICAL CONDITIONS

In response to an increased need for lipid storage, there are two primary routes for AT expansion. These are adipocyte hypertrophy and hyperplasia, with the latter generally being correlated with healthy AT expansion. Efficient adipogenesis in AT is also necessary for the process of healthy lipid deposition, and ectopic deposition of lipids in non-adipocytes is heavily associated with organ injury and metabolic diseases (31). Because the expansion of preadipocytes and supporting cell populations is necessary for hyperplasia to occur, exosomal regulation of cell proliferation, apoptosis and adipogenesis can be an important area of research for AT health. AT derived exosomes have been shown to promote adipogenesis in ADSCs (32). Furthermore, ADSC-derived exosomes were shown to promote adipogenesis through miR-450a-5p mediated repression of WISP2 expression (33). These studies indicate that the crosstalk between ADSCs and mature adipocytes may regulate adipogenesis.

The population of AT resident stem cells directly contributes to the development and function of AT. In addition, ADSCs function through their extracellular communication dynamics with other cells in the AT. Many studies have been performed to analyze the content and effects of ADSC-derived exosomes on other cell types and populations (34). During healthy expansion of AT, proper vascularization is needed to facilitate blood flow to new cells; but in many cases of unhealthy AT expansion, local hypoxia leads to chronic inflammation and adipocyte dysfunction (31). ADSCs have been shown to secrete various miRNAs in exosomes that improve vascularization of endothelial cells (35). ADSC exosome derived miR-21 promotes angiogenesis by increasing the expression of HIF-1 α , Akt, ERK, and SDF-1 (36). Interestingly, miR-21 is heavily associated with tumor angiogenesis (37). In a proteomics analysis exploring the secretome of ADSCs overexpressing telomerase and myocardin, exosomes from these cells improved the tube formation of HUVEC cells (38). MiR-125a, a miRNA that is expressed by ADSCs prior to differentiation, has also been found to promote angiogenesis in endothelial cells by repressing expression of *DLL4* through targeting its 3' UTR (9). Upon VEGF-C treatment, ADSC exosome secretion is enriched in miR-132 and promotes lymphoangiogenesis by targeting *Smad-7* and regulating TGF- β /Smad signaling (39). This pro-angiogenic effect is not only observed *in vitro* but also *in vivo*; EVs from ADSCs have been shown to induce healthy vascularized AT formation when injected into mice (32). These represent several mechanisms by which ADSC exosomes regulate endothelial cell angiogenesis, a crucial process for healthy AT expansion.

The proper regulation of inflammation is also a major factor in AT health. AT is highly heterogeneous with a resident macrophage population that not only regulates local inflammatory responses but also influences systemic inflammation (40). Regulation of these

resident macrophages by neighboring cells can occur through a variety of mechanisms with exosomes having a major role. A study demonstrated that miR-34a containing exosomes from adipocytes affected resident macrophages by repressing KLF4, which in turn promoted high fat diet-induced insulin resistance, glucose intolerance, and systemic inflammation (41). The inflammatory profile of macrophages can also be modified via metabolic reprogramming, with M1 (pro-inflammatory) macrophages being more glycolytic and M2 (anti-inflammatory) having a fatty acid oxidation dependent metabolism (42). Adipocytes have been demonstrated to secrete exosomes containing lipids that serve as a major energy source for resident macrophages. Adipocytes from lean mice secreted exosomes with higher lipid content compared to those from obese mice, suggesting exosomal lipids and miRNA from healthy adipocytes are regulators of inflammation (43).

A role of the ADSC population being actively researched is in regulating inflammation. A study demonstrated that the immunosuppressive potential of ADSCs-secreted exosomes was increased in the presence of pro-inflammatory cytokines. Higher production of exosomal IL-10, IL-8, IL-6, and CCL-2 was observed along with upregulation of miR-34a-5p, miR-156a-5p, miR-21-5p, miR-135b-5p, miR-127-3p, and miR-155-5p (44). MiR-30d-5p from ADSC exosomes reversed ischemic stroke-induced, autophagy-mediated injury by promoting M2 macrophage polarization and resolution of the inflammatory response (45). ADSC exosomes have also been demonstrated to promote M1 to M2 macrophage polarization via STAT3 activation and upregulation of ARG1 (46). In a rat model of sepsis-induced overwhelming systemic inflammation, ADSCs cultured in hypoxic conditions were able to reduce the level of inflammation and improved outcomes (47). Another study demonstrated 3T3-L1 preadipocytes secrete exosomes enriched in sonic hedgehog (SHH) when cultured in a high glucose/insulin environment (48). Macrophages exposed to these exosomes exhibited M1 polarization through the Ptch/PI3K pathway. ADSC derived exosomes were also given to neutrophils, leading to a decrease in apoptosis and increased functional capacity (49). Although ADSC regulation of macrophages is not fully understood, preliminary evidence suggests that exosomal communication between the two cell types is an important component of the inflammatory response.

Likewise, resident macrophage exosomal secretion is an important regulatory function for other cell populations within AT. Obese AT derived macrophages secreted miR-29 enriched exosomes that were taken up by adipocytes from lean mice to cause insulin resistance through targeting of PPAR δ (50). Song *et al.* demonstrated that macrophages treated with 3T3-L1 exosomes enriched in SHH reciprocally secreted exosomes that reduced resistant-substrate-1 (IRS1) and hormone-sensitive lipase (HSL) in adipocytes (48). The exosomal crosstalk between resident AT macrophages and neighboring cells shows that exosomes have a key role in regulating local AT inflammation, which in turn can regulate systemic inflammation.

The AT secretome is critical not only for local metabolic homeostasis, but also for regulation of many distant organs and tissues (Fig. 1b). ADSC-derived exosomes enriched in miR-20b-3p were successfully used to treat hyperoxaluria in rat kidneys by reducing oxalate-induced autophagy and inflammatory responses (51). Evidence suggests that liver tumorigenesis and fibrosis are regulated by miR-122, during the development of NAFLD,

AT-Exos are suspected to be a significant source of compensatory miR-122 production for the failing liver (52). Another study showed that miRNA-181-5p from ADSC-derived exosomes downregulated STAT3/Bcl-2/Beclin1, a fibrosis associated pathway in liver cirrhosis (53). Exosomal resistin from AT was also shown to repress phosphorylation of pAMPKa-Thr-172 to trigger ER stress and led to liver damage (54). As obesity is associated with an increased risk of both kidney disease and nonalcoholic fatty liver disease, disruption of the exosomal regulatory functions in obese individuals may be a factor in both pathologies (55).

Brown adipose tissue (BAT) is a form of fat that is metabolically active and has a significant impact on regulating overall energy expenditure (56). The miRNAs of BAT derived exosomes, especially miR-99b, were shown to accumulate in hepatocytes and reduce hepatic FGF21 levels, a metabolic hormone that enhances BAT function (57,58). In another study, BAT derived exosomes of healthy mice were able to improve metabolism, hepatic and cardiac function in a mouse model of diet-induced obesity. These exosomes were also found to preferentially accumulate in the liver and were enriched in functional mitochondrial proteins that altered hepatocyte metabolism (59). These results together suggest that many of the beneficial effects associated with increased BAT mass and activity may be due to the secretion of exosomes and their systemic effects on organs such as the liver. Despite there being evidence that the secretomes of BAT and WAT adipocytes are distinct, a comprehensive analysis identifying differences in EVs or AT-exos secretion has not yet been conducted (60).

Neuronal cells may also be affected by exosomes from AT as ADSC-derived exosomes were used to reduce apoptosis in an *in vitro* model of ALS by increasing Bcl-2 expression (61,62). In addition, EVs from differentiated 3T3-L1 cells were shown to be taken up by POMC neurons *in vitro* and regulate mTOR signaling via MALAT1, a lncRNA upregulated in AT of *ob/ob* mice (63). MALAT1-containing exosomes from *ob/ob* mice injected into lean mice in the same study increased both appetite and weight correlated with hypothalamic mTOR signaling. In another study, differentiated ADSC exosomal RNA was able to trigger neurite outgrowth (64). These studies suggest that exosomal communication is an important method of regulation between AT resident cells and neurons.

Cardiac cells have been shown to respond to ADSC exosomal treatment *in vivo* with reduced apoptosis in a rat ischemia/reperfusion injury model through activation of the Wnt/ β -catenin pathway (65). After a high-calorie meal, serum-derived CD36 exosomes contained free fatty acids that were taken up by cardiac cells (7). Interestingly, adiponectin associated T-cadherin increased exosome biogenesis of aorta cells, leading to a decrease in cellular ceramide (66). As cardiovascular disease is well-known to be associated with obesity, the impact of AT-Exos in the development of the disease warrants future exploration.

ADSCs and their exosomes have been explored as a potential route to enhance wound healing due to their low immunogenicity and ease of harvest (12,67). The exosomes of ADSCs are suspected to play a role in regulating many of the cells involved with the wound healing process. Fibroblasts are a crucial cell type mediating wound healing, their role being the creation of new extracellular matrix and secretion of collagen (68). Exosomes from

ADSCs containing MALAT1 enhanced fibroblast migration and improved wound recovery in an ischemic rat model (69). Another study analyzing the effect of ADSC exosomes on fibroblasts saw an improvement in proliferation, migration, and collagen deposition via upregulation of the PI3K/Akt signaling pathway, leading to increased MMP1, bFGF, TGF- β 1, collagen I and III expression (70). This result was corroborated in another study that analyzed the effect of ADSC exosomes on vaginal fibroblasts and found increased expression of collagen 1 and TIMP-1/3 (regulators of MMPs) (71). Keratinocytes treated with adipocyte-derived exosomes containing hsa-circ-0075932 displayed increased apoptosis and inflammation, mediated by the circRNA binding to PUM2, which upregulates Aurora A kinase and activates NF κ B (72). Notably, this circRNA is overexpressed in the burned skin of obese patients, suggesting another mechanism by which AT-Exos may influence tissue regeneration. Because ADSC exosomes have already been shown to facilitate wound and organ repair in clinical settings, research is being conducted to explore possible ways to optimize their effectiveness. In this regard, additives such as hyaluronic acid have been shown to retain exosomes in a target area for a longer time, improving re-epithelialization and vascularization of the wound area (35).

It is evident that AT is involved in complex extracellular communication dynamics locally and remotely with tissues throughout the body. Proper regulation of functions in these remote tissues is influenced by the AT secretome, and exosomes appear to be a notable component involved in these processes and general homeostasis. Further research into this communication method may be critical in understanding the complex endocrine functions of AT.

AT EXOSOMAL SECRETION DURING OBESITY AND METABOLIC SYNDROME

Obesity is a multifaceted disease continually growing in prevalence and is associated with many other morbidities (4,73). During the onset and progression of obesity, a phenomenon observed is the altered state of AT, displaying insulin resistance, a pro-inflammatory secretome, and dysregulated metabolic profile (40). Among the many aspects of AT altered during obesity, the exosomal secretion profile is modified (Fig. 2) (74). There is increasing interest in using exosomal contents as markers for associated diseases, some of which are suspected to have a major role in disease development. During obesity, the level of plasma EVs, including exosomes, increases by 10-fold and does not return to normal levels after sustained weight loss, suggesting that obesity causes permanent changes in the secretome of AT that may contribute to the development of obesity-related diseases (75).

One of the hallmarks of obesity is a chronic increase in inflammation, not only within AT marked by excessive macrophage accumulation, but also throughout the entire body (76). This inflammation is correlated with the development of obesity-related diseases such as cancer, diabetes, and heart disease (73,77,78). AT-Exos contained a high amount of neutral lipids that induce an AT macrophage-like phenotype in bone marrow cells, potentially contributing to the macrophage population number during obesity (79). Macrophages derived from the white AT of obese mice secrete exosomes that induce insulin resistance in

lean mice, indicating that macrophage dysregulation during obesity may lead to the development of diabetes (50). Injection of *ob/ob* AT-Exos into WT mice caused TLR4-dependent macrophage-induced insulin resistance. Uptake of these exosomes from AT by peripheral blood monocytes caused differentiation into activated macrophages that secreted proinflammatory TNF α and IL-6 (8). Expression of miR-223 was found to be upregulated in the visceral AT of obese mice and patients, specifically in the stromal vascular fraction free of mature adipocytes (80). This microRNA is known to modulate macrophage phenotype and activation state via the TLR4/FBXW7 axis. Regulation of miR-223 expression by the exosomes of preadipocytes has been displayed, implying a possible communication dynamic between the two cell populations to regulate inflammation (81). Upregulated during obesity, miR-34a is found in adipocyte exosomes and suppresses M2 macrophage polarization through its repression of KLF4, leading to obesity induced adipose inflammation (41). MiR-155, another miRNA found in obese adipocyte exosomes, induced the proinflammatory M1-macrophage phenotype by activating STAT1 and repressing STAT6 expression (82). Intercellular communication via exosomes has been consistently demonstrated to be an important regulator of macrophage behavior and may have a role in causing the systemic inflammation heavily associated with obesity and metabolic syndrome.

A common symptom that is found in patients with obesity and metabolic syndrome is systemic insulin resistance, which leads to further complications in metabolism and health (Fig. 2). The contents of exosomes are currently being studied as a possible mechanism by which this symptom develops, as evidence shows that exosomes derived from obese subjects can induce insulin resistance in lean subjects (8). Analysis of the serum exosomal miRNA of nondiabetic obese patients that underwent bariatric surgery identified nine surgery responsive miRNAs (miR-1246, -1290, -193b-5p, -378c/d/g, -424-5p, -4449, and -6126), whose target genes are correlated with insulin signaling (83). Another study analyzing exosomal miRNA from obese children found that miR-146b, miR-15b, and miR-486 were upregulated in the obese group. MiR-486 had been shown to decrease myotube glucose tolerance, while miR-146b and miR-15b suppressed glucose-induced pancreas insulin secretion (84). Notably, another study found a similar result of miR-15b being upregulated in obese women (85). MiR-27a, which was found to be elevated in the exosomal RNA of obese children and mice, was able to induce insulin resistance in C2C12 myocytes through repression of PPAR γ . Macrophages treated with obese AT extracellular vesicles were also able to induce insulin resistance in C2C12 myocytes, indicating that AT may have direct and indirect effects on the progression of skeletal muscle insulin resistance (86,87). Mir-29a and miR-122 were found to be upregulated in another analysis of obese children's exosomes and these two are heavily correlated with insulin resistance and diabetes (50,88). The exosomes of obese AT when given to hepatocytes induced insulin resistance and glucose intolerance possibly due to reduced exosomal miR-141-3p content that affects Akt phosphorylation (89). A proteomic analysis of exosomes released by adipocytes of Otsuka Long-Evans Tokushima Fatty rats showed that one of the exosomal proteins, catalase, was downregulated in obese samples. This protein is associated with hepatocyte insulin resistance by protecting against oxidative stress (90). Lastly, analysis of the exosomal proteome of women with gestational diabetes mellitus (GDM), during which placental hormones cause insulin resistance, found that AT of patients with GDM display higher numbers of exosomes containing proteins that

increased glycolysis and gluconeogenesis, implying that AT-Exos directly regulate glycemic levels (6). This body of evidence demonstrates AT-Exos can have a systemic effect on the development of insulin resistance during obesity.

Obesity and metabolic syndrome are heavily associated with dysfunctional AT, this dysfunction can manifest in several ways. Adipocytes display severe metabolic dysfunction and are unable to perform lipogenesis or lipolysis, leading to improper lipid accumulation in the body (91). The secretome of AT has been shown to be altered during obesity and metabolic syndrome and the contents of AT-Exos may have a paracrine role in the gradual decline of function in AT (75,92). Patients with metabolic syndrome displayed increased levels of miR-31 in exosomal RNA, which has been shown to reduce adipogenic markers: PPAR γ , aP2, and CEBP- α (93). Obese patients had decreased levels of miR-100 in circulation and in visceral AT, miR-100 modulates adipogenesis by reducing mTOR and IGFR (94). In an analysis of circulating miRNAs from overweight patients that responded to exercise mediated weight loss, miR-140 was found to be decreased in responsive patients. This miRNA targets FNDC5 in muscle, therefore reducing the production of irisin, a cleavage product of FNDC5 that promotes lipolysis (95). This result suggests that miR-140 may be an important regulator of myocyte-adipocyte crosstalk. A panel of miRNAs differentially expressed in obese children showed miR-551a and -501-5p were upregulated while miR-10b-5p and -215-5p were downregulated, with the upregulated genes targeting BCAA-1A, typically upregulated in obesity, while downregulated miRNAs target genes were related to proper adipogenesis (96). Patients that underwent bariatric surgery saw a decline in exosomal FABP4, a fatty acid binding protein that is a key regulator of lipid metabolism and is associated with obesity-related diseases (97). Exosomes of obese women contain increased levels of miRNAs that modulated lipolysis and adipogenesis (miR-17 family and miR-15b, respectively) (98). Treatment of adipocytes with these exosomes led to reductions in lipid biosynthesis and increased IRS1. Together, these studies show that obese AT releases exosomes with cargo that deregulates adipogenesis and lipid metabolism, perhaps contributing significantly to AT dysfunction.

Another characteristic of unhealthy AT is decreased vascular function that leads to hypoxia. Dysfunctional vasculature is commonly observed in obese individuals and those with metabolic syndrome. In women with metabolic syndrome, circulating miR-21-5p is downregulated and is predicted to affect endothelial cell migration (99). Morbidly obese patients treated for sleep apnea demonstrated a downregulation of exosomal miR-16-5p, miR-320a, miR-21-5p, miR-126-5p, miR-146a-5p, and miR-320a-3p, all of these having target genes related to cardiovascular disease progression and endothelial cell dysfunction (100). Downregulation of miR-126 was also observed in another study with obese adolescents successfully undergoing weight loss (101). In a study that analyzed the effects of obese perivascular AT-derived exosomes, exosomal miR-221-3p was shown to cause vascular smooth muscle cell dysfunction by suppressing contractile genes (102). In summary, the regulation of vascular function is compromised during obesity and metabolic syndrome and the exosomes secreted by AT during these conditions may have a role.

It is known that obesity and metabolic syndrome cause a plethora of physiological issues in affected patients with their AT often showing severe dysregulation. This dysregulation of AT

affects the secretome leading to systemic effects on the body. One portion of the AT secretome that has been proven to be affected is exosomes, and their cargo have been shown to be implicated in many of the pathologies that are associated with obesity and metabolic syndrome. Therefore, AT-Exos and their targets may be important therapeutic pathways to help alleviate health complications associated with obesity and metabolic syndrome.

EXOSOMES, AT, AND CANCER

In many cases of cancer, one of the phenomena commonly observed is cachexia or wasting of skeletal muscle and AT, often induced by secreted factors from cancer cells (103). Conversely, obesity and secreted factors from adipocytes have been shown to regulate cancer cell behavior *in vitro* and *in vivo*. One of the proposed mechanisms by which these phenomena occur is exosomal communication between tumor cells and AT (Fig. 3) (104). Cancer-associated adipocytes have altered behavior displaying smaller, dispersed lipid droplets and are derived from normal mature adipocytes possibly exposed to oncogenic miRNAs such as miR-144, miR-126, and miR-155. These cancer-associated adipocytes are suspected to have a significant role in the progression of tumors through their secretome (105). Adipocyte-derived exosomes increased MMP3 production in lung cancer while exosomal miR-21 inhibits cancer cell apoptosis by binding to APAF1 (106). Another study found that adipocyte exosomal circ-DB, upregulated during obesity, stimulated the growth of hepatocellular carcinoma by targeting USP7 deubiquitination (23). Fatty acid oxidation enzymes delivered by adipocyte exosomes to cancer led to a more aggressive phenotype (105). The ADSC population of AT may also have a role in cancer progression with their exosomal contents promoting proliferation, invasion, and migration of endothelial cells, possibly for the purpose of tumor supporting angiogenesis (34). A proteomic analysis of immortalized ADSC exosomes (overexpressing telomerase and myocardin) showed an increase of MMP2 and TIMP2, suggesting a possible mechanism by which this increased angiogenesis occurs (38). ADSC exosomes have also been shown to stimulate stem cell behavior of certain tumor cells, allowing for a more metastatic phenotype (107). However, other cancer cells treated with ADSC exosomes showed decreased proliferation via miR-503-3p, a stemness attenuating factor (34). As we can see, AT-Exos contain a variety of cargo that regulates various aspects of cancer cell biology and tumor progression (Fig. 3a), highlighting the complexity of this communication dynamic dependent upon cell type and context.

On the tumor side of cancer-AT crosstalk, the exosomes from tumors have been shown to have a profound impact on the resident cells of AT (Fig. 3b), playing a role in perpetuating the fat-mass loss observed during cachexia due to increased lipolysis (103). Much of this fat-mass loss is suspected to be caused by browning of white adipose tissue (WAT). During WAT browning, increased UCP-1 expression shifts mitochondrial respiration towards thermogenesis, resulting in increased lipolysis, metabolic activity, and contributing to reduction in adipocyte mass (103,104). Pancreatic cancer exosomes contain adrenomedullin (AM), which activates ERK1/2 and p38 MAPK pathways while also increasing the expression of phosphorylated hormone-sensitive lipase – effects that can be ameliorated with AM receptor blockade (108). Tumor cell exosomes contained miR-144, which induced browning through downregulation of MAP3K8, ERK1/2, and PPAR γ , while miR-126

remodeled adipocyte metabolism by disrupting IRS/GLUT-4 to activate autophagy and stabilizing HIF-1A expression (104). Gastric tumor exosomes were able to promote the differentiation of pre-adipocytes into brown adipocyte-like cells by activating PRDM16 and sponging miR-133 through circRNA-133 (24). Dedifferentiated liposarcoma secretes MDM2 DNA copies that were taken up by preadipocytes to increase proliferation and migration through the production of MMP2 (109). Multiple studies have shown that exosomes of cancer cells have a variety of effects on AT cell populations by inducing phenotypes related to cachexia or reprogramming the behavior of adipocytes and ADSCs. The exosomal crosstalk between tumor and AT cells may be an important aspect of tumor progression, with the purpose being to provide tumor cells with metabolites and reprogramming surrounding cells to be more supportive of tumor growth. These results imply therapeutic targeting of exosomal communication between tumor cells and niche supporting cells may be a promising area of research.

EXOSOMAL THERAPEUTICS

Although analysis of endogenous exosomal contents may provide crucial information about extracellular communication dynamics, the use of exogenous exosomes is a promising avenue for drug delivery. Studies have shown therapeutics (miRNA, protein, drugs) packaged into exosomes may be more effective than drugs delivered by traditional delivery methods. The improved effectiveness of therapeutics packed in exosomes is due to the low immunogenic response and higher bioavailability of exosomes (110). Similarly, in the area of stem cell therapy research, stem cell-derived exosomes not only provide the positive effects of direct cell transplantation but have the benefit of being a cell-free therapy. This aspect is of great interest as there is a concern of tumorigenic potential inherent in implanted cells.

Exosomes have been shown to modulate lipid metabolism, adipogenesis, insulin resistance, and secretion, all processes are heavily correlated with the morbidity of obesity and metabolic syndrome (3,111). Due to this regulatory property, there is great potential for using or targeting these exosomes as a treatment for these diseases and others involving dysregulated metabolic processes. Exosomal production is permanently increased during obesity and is correlated with the onset of obesity-related diseases (75). In an adipocyte specific *Sirt1 knockout* mouse model that resulted in obesity, treatment with the exosome production inhibitor, GW4869, resulted in reduced body weight and improved insulin sensitivity, suggesting that ablation of adipocyte exosome release may be a possible treatment for obesity (112). The delivery of miRNA sponge-containing exosomes that target miRNAs responsible for disease progression may also be a viable method that avoids possible off-target effects of reducing total exosome production. Exosomes from cancer cells have been demonstrated to significantly increase the metabolic activity of adipocytes, mostly by increasing lipolysis (103). While this may be highly detrimental during cachexia, it may be possible to use exosomes with similar effects as a method for inducing browning and fat mass loss in obesity treatment. A potentially useful cargo for this purpose would be a new category of secreted molecules deemed “exerkines”, these are released by various tissues during exercise and are demonstrated to be highly beneficial for regulating metabolic homeostasis (113). Exerkines are speculated to be a crucial aspect of organ crosstalk and

responsible for many of the health benefits associated with exercise (114). Adiponectin, an exerkine primarily released by adipocytes, was demonstrated to alter exosome production when associated with T-cadherin, suggesting that exerkine release may play a role in regulating exosome production (66). In addition, there is growing evidence that exosomes and other extracellular vesicles are a major vehicle for these factors (115). Apelin, an exerkine, was demonstrated to be able to prevent obesity and even increased AT browning (116). Irisin, another myokine that is released during exercise is also capable of inducing a similar effect in adipocytes and is negatively correlated with obesity-associated NAFLD and insulin resistance (114). There are countless other exerkines with similarly beneficial properties, thus, creation of an efficient method for producing exosomes containing these factors may be an effective method for combating much of the adipocyte metabolic dysregulation associated with obesity.

Although exosomes have great therapeutic potentials, several key issues still must be addressed before further utilization. Optimizing cells for therapeutic exosomal production is an undeveloped area of research. The exosomal secretion profile of cells is still highly dynamic and the underlying molecular regulation is poorly understood (117). For example, the process of exactly how cells select cargo for packing into these extracellular vesicles is largely unknown. Several groups have tried to culture cells optimized for therapeutic exosomal production by altering growth conditions or increasing cytoplasmic concentration of desired therapeutics, yet the effect on therapeutic viability remains to be seen (118–120). Despite there being certain post-translational modifications and nucleotide sequences that appear to increase exosomal incorporation, utilizing these in therapeutic cargo changes their inherent properties and may also affect the efficiency of production. Further research into this area would greatly improve their utility in a clinical setting, allowing for optimized production and avoidance of undesired cargo being incorporated. Another issue is the general lack of specificity in exosomal targeting. Exosomes do not appear to exhibit target cell selectivity, raising a concern of off-target effects and the necessity for much higher dosages to ensure that target cells will receive an effective dosage. In order to address these issues, incorporation of targeting molecules or peptides into exosomal membranes may be a viable strategy as these tend to preferentially target desired cells (121).

Therefore, elucidating exosomal targeting mechanisms and/or discovering effective targeting modifications would greatly increase potency.

SUMMARY AND FUTURE DIRECTIONS

AT is a multifaceted organ that has critical roles in metabolic homeostasis through its energy storage and endocrine functions. The secretome of AT is complex and consists of countless components with exosomes being one of the most diverse. These exosomes are capable of targeting a wide variety of tissues and cells throughout the body to regulate countless numbers of processes through many mechanisms. As with many other AT functions, exosomal secretion is one that is dependent upon physiological context and is heavily altered during periods of adipose dysfunction such as obesity and metabolic syndrome. This altered exosomal secretion may be responsible for many of the associated maladies that accompany obesity, thus representing a possible opportunity for research into therapeutic avenues to

alleviate consequences of the prevalent obesity pandemic. In the context of cancer, exosomal communication between adipose and tumor tissue may also be a critical component of disease progression and initiation, again opening a wide area of research. However, there are still critical aspects of exosomal secretion that must be investigated further in order to facilitate subsequent applications. The mechanisms by which cells regulate exosomal production is largely unknown, further research into this area would greatly enhance the ability to viably produce exosomal therapeutics and provide avenues of treatment for exosome related pathologies. In addition, the cell type or culture strategy optimal for consistent and efficient exosome production has still not yet been identified, leading to an increased possibility of variable results for exosomal research. Lastly, methods for targeted delivery of exosomes to specific cell types are urgently needed. In conclusion, exosomes are an aspect of extracellular communication that have great potential for further study and manipulation to improve both our understanding and treatment of AT-related diseases such as obesity, cancer, and type 2 diabetes.

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ABBREVIATIONS

ADSC	Adipose-derived stem cells
AKT	Protein kinase B
AM	Adrenomedullin
AMPK	AMP-activated protein kinase
APAF	Apoptotic protease activating factor
AT	Adipose tissue
AT-exos	Adipose tissue derived exosomes
ARG	Arginase
BAT	Brown adipose tissue
BCAA	Branched-chain amino acid transaminase
FABP	fatty acid binding protein
CCL	Chemokine (C-C motif) ligand
CEBP	CCAAT/ enhancer-binding protein
DLL	Delta like canonical Notch ligand
ERK	Extracellular signal regulated kinase
EVs	Extracellular vesicles

FBXW7	F-Box and WD repeat domain containing
FGF	Fibroblast growth factor
FNDC	Fibronectin type III domain-containing protein
GDM	Gestational diabetes mellitus
aP	adipocyte protein
GLUT	Glucose transporter
HSL	Hormone sensitive lipase
HUVEC	Human umbilical vein endothelial cells
IGFR	Insulin-like growth factor receptor
IL	Interleukin
IRS	Insulin receptor substrate
KLF	Kruppel-like factor
MALAT	Metastasis associated lung adenocarcinoma transcript
MAPK	Mitogen-activated protein kinase
MDM	Mouse double minute homolog
MMP	Matrix metalloproteinase
mTOR	Mammalian target of rapamycin
NAFLD	Nonalcoholic fatty liver disease
Bel	B cell lymphoma
ob	Leptin
PI3K	Phosphoinositide 3-kinase
POMC	Pro-opiomelanocortin
PPAR	Peroxisome proliferator-activated receptor
PRDM	PR domain containing
Ptch	Patched-1 protein
PUM	Pumilio RNA binding family member
RNCR3	Retinal noncoding RNA3
SDF	Stromal cell derived factor
SHH	Sonic hedgehog

SIRT	Sirtuin
STAT	Signal transducer and activator of transcription
TGF	Transforming growth factor
TIMP	Tissue inhibitor of metalloproteinases
TLR	Toll-like receptor
TNF	Tumor necrosis factor
UCP	Uncoupling protein
VEGF	Vascular endothelial growth factor
WAT	White adipose tissue
WISP	WNT1 inducible signaling pathway protein
HIF	hypoxia induced factor

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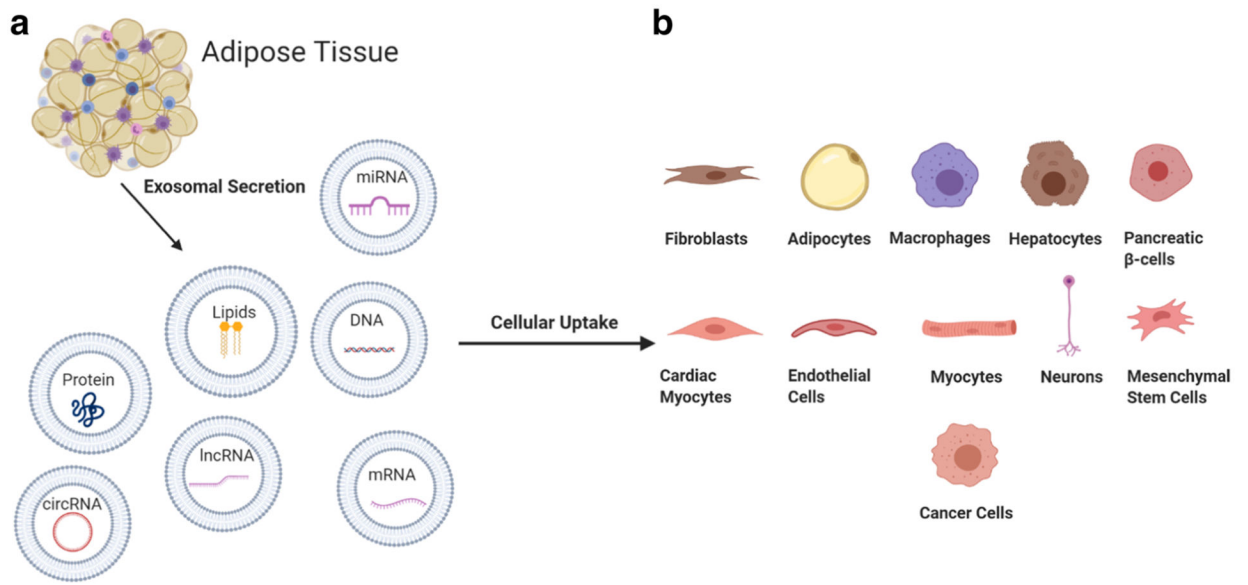


Fig. 1. Adipose tissue exosome contents and targets.

(a) Various cells of adipose tissue have been demonstrated to secrete exosomes containing miRNA, mRNA, lncRNA, circRNA, proteins, lipids, and DNA with active biological function. (b) Reported targets of adipose tissue exosomes, which have been shown to regulate processes such as endothelial cell vascularization (via HIF-1 α , Akt, ERK, and SDF-1), myocyte insulin resistance (through PPARG expression), macrophage inflammatory profile (through regulating gene expression and cell metabolism), etc. Furthermore, evidence suggests that systemic regulation by exosomes is common due to their ability to remain functional *in vivo* after traveling significant distances from donor cells. Created with [BioRender.com](https://www.biorender.com).

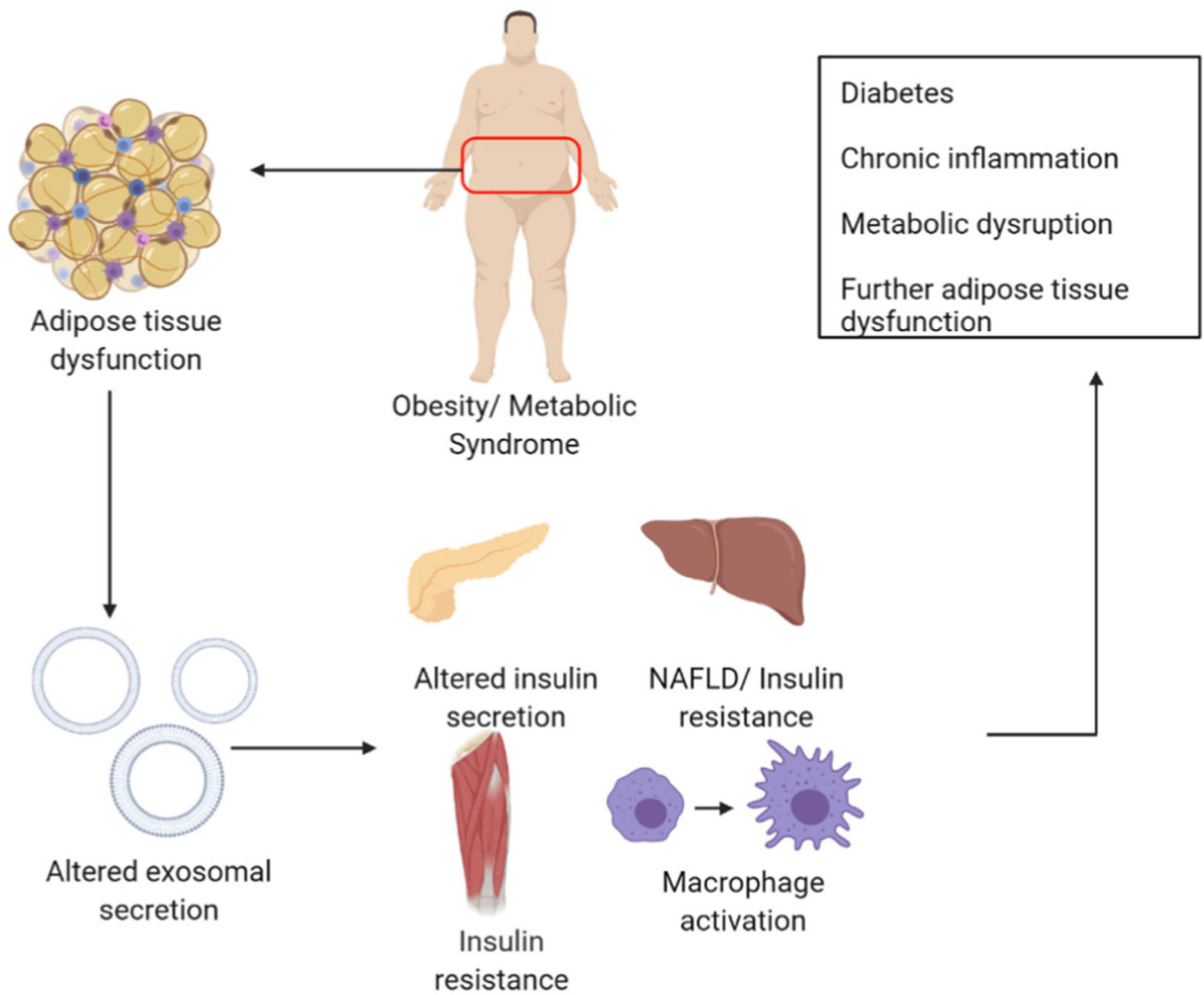


Fig. 2. Proposed impact of AT-Exos on the progression of obesity, metabolic syndrome, and associated diseases.

The dysfunctional state of adipose tissue leads to altered exosomal production that has systemic effects leading to altered pancreatic insulin secretion (via miR-146b/15b), hepatocyte insulin resistance (through regulation of AKT phosphorylation, skeletal muscle insulin resistance (downregulation of PPAR γ) and macrophage activation. Combined, these effects may have an impact on the development of associated diabetes, metabolic dysregulation, and chronic inflammation. Created with [BioRender.com](https://www.biorender.com).

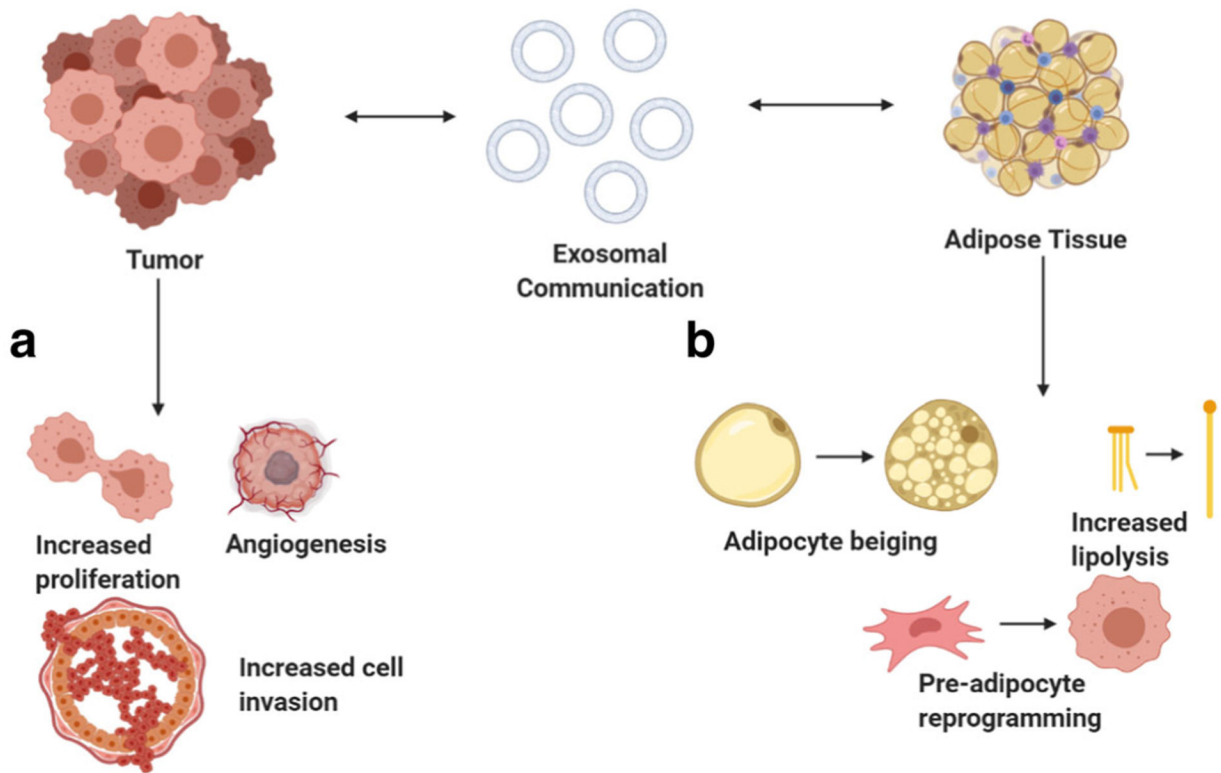


Fig. 3. Adipose and tumor tissue have both been demonstrated to secrete exosomes that affect each other mutually, as well as other tissue and cell types.

(a) Tumor cells exposed to AT-Exos display increased proliferation and a more aggressive phenotype (possibly through delivery of fatty acid oxidation enzymes). Tumor angiogenesis, a vital factor for tumor growth, is theorized to be enhanced by ADSC that affects endothelial cells in the tumor microenvironment (via miR-21). **(b)** Resident cells of adipose tissue treated with cancer cell-derived exosomes display significant changes in behavior. Adipocytes undergo increased lipolysis (via delivery of adrenomedullin), display increased beiging (activation of PRDM16). This adipose-tumor crosstalk via exosomes may be important for regulating cancer disease progression and associated fat-mass loss during cachexia. Created with [BioRender.com](https://www.biorender.com).