Potential Roles of Adipocyte Extracellular Vesicle–Derived miRNAs in Obesity-Mediated Insulin Resistance

Yujeong Kim1 and Ok-Kyun Kim1,2
1Division of Food and Nutrition, Chonnam National University, Gwangju, Republic of Korea; and 2Human Ecology Research Institute, Chonnam National University, Gwangju, Republic of Korea

ABSTRACT

Recently, extracellular microRNAs (miRNAs) from adipose tissue have been shown to be involved in the development of insulin resistance. Here, we summarize several mechanisms explaining the pathogenesis of obesity-induced insulin resistance and associated changes in the expression of obesity-associated extracellular miRNAs. We discuss how miRNAs, particularly miR-27a, miR-34a, miR-141-3p, miR-155, miR210, and miR-222, in extracellular vesicles secreted from the adipose tissue can affect the insulin signaling pathway in metabolic tissue. Understanding the role of these miRNAs will further support the development of therapeutics for obesity and metabolic disorders such as type 2 diabetes. Adv Nutr 2020;00:1–9.

Keywords: extracellular vesicle, miRNAs, obesity, insulin resistance, insulin signaling

Introduction

Insulin is a hormone that is secreted by pancreatic B cells in response to increased circulating glucose concentrations. It reduces blood glucose concentrations by binding to the insulin receptor (IR) in the cell membrane of tissues such as the liver, skeletal muscles, and in adipose tissue. Insulin stimulates the synthesis of glycogen, lipids, and protein and inhibits glucose production in the liver (1, 2). Although insulin plays many physiological roles, its key function is to regulate glucose homeostasis as it is the only hormone that can lower blood glucose concentrations. Thus, insulin resistance results in systemic hyperglycemia by impairing the ability of insulin to stimulate glycolysis and inhibit gluconeogenesis, which is closely linked to the pathogenesis of metabolic disorders such as type 2 diabetes, nonalcoholic fatty liver disease, and other metabolic syndromes (3, 4).

Obesity is directly or indirectly associated with the development of insulin resistance. Numerous studies have investigated several molecular mechanisms linking the pathogenesis of obesity to insulin resistance (5, 6). Here, we present a review of several such mechanisms to explain the pathogenesis of obesity-induced insulin resistance, including inflammation and cellular stress. In addition, we provide an overview of the role of extracellular vesicle (EV) microRNAs (miRNAs) as novel metabolic regulators during the development of obesity-induced insulin resistance. In particular, this review covers the effects of EV miRNAs secreted from adipose tissue on the insulin signaling pathway in metabolic tissues including the adipose tissue, muscle, and liver. This review investigates this novel mechanism underlying the development of obesity-mediated insulin resistance and how this could provide a potential avenue for miRNA-based therapeutics for obesity and type 2 diabetes.

Obesity-Associated Insulin Resistance Development

Insulin signaling pathway

Insulin acts by binding to the IR, which is a transmembrane protein comprising 2 extracellular α-subunits and
2 intracellular β-subunits. The tyrosine kinase activity of the β-subunits is induced when insulin binds to the α-subunits. This triggers autophosphorylation in the β-subunits as well as activation of docking and tyrosine phosphorylation of intracellular proteins known as insulin receptor substrates (IRSs). Phosphorylation of IRS-1 at a tyrosine residue activates phosphoinositide 3 kinase (PI3K), which comprises a heterodimer consisting of a p110 catalytic subunit and a p85 regulatory subunit. The p85 regulatory subunit is responsible for PI3K activity and is an important factor regulating the insulin signaling pathway (7). Activated PI3K converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3), which then activates Akt [also known as protein kinase B (PKB)]. The downstream substrates of Akt include Akt substrate of 160 kDa (AS160), glycogen synthase kinase 3 (GSK3), mammalian target of rapamycin (mTOR), and forkhead box protein O1 (FoxO1) (8–10). AS160 promotes glucose uptake into the muscle and adipose tissues via the translocation of glucose transporter 4 (GLUT4)–containing storage vesicles to the plasma membrane (11). Deactivation of GSK3 by Akt-mediated phosphorylation stimulates glycogen synthesis by dephosphorylating and activating glycogen synthase. mTOR stimulates protein synthesis and inhibits the translocation of the transcription factor FoxO1 into the nucleus via Akt-mediated phosphorylation; it also inhibits the expression of enzymes involved in gluconeogenesis such as phosphoenolpyruvate carboxykinase (PEPCK) (8–10). Thus, this series of intermediary steps, following insulin binding, provides an integrated set of signals for balancing nutrient availability, particularly the promotion of anabolic metabolism. However, when IR is negatively regulated by dephosphorylation, it can lead to insulin resistance via a negative feedback mechanism.

**Negative regulators of insulin activity in obesity**

In this article, we have comprehensively summarized the negative regulators of insulin activity in obesity-mediated insulin resistance, focusing on inflammatory mediators, cellular stress, and adipocyte factors. Obesity is characterized by chronic low-grade inflammation in white adipose tissue. In low-fat adipose tissue, most adipose tissue–resident macrophages are polarized to M2 macrophages, which contribute to insulin sensitivity by secreting IL-10 and arginine substrates. However, in adipose tissue, adipocytes release monocyte chemoattractant protein 1 (MCP-1) and proinflammatory cytokines, which induce monocyte infiltration and M1 macrophage polarization with increased production of inducible NO synthase (iNOS) and proinflammatory cytokines, further promoting local inflammatory responses in adipose tissue. This obesity-associated inflammation is closely related to insulin resistance and other well-known metabolic abnormalities (12, 13). Kamei et al. (14) reported that, in mice, MCP-1 overexpression caused macrophage accumulation and increased proinflammatory cytokine mRNA levels in the adipose tissue. In addition, suppression of insulin signaling has been observed in both the skeletal muscle and liver of mice with MCP-1 overexpression, suggesting that MCP-1–induced inflammation in adipose tissue contributes to the development of insulin resistance.

The proinflammatory cytokines, TNF-α, IL-6, and IL-1β are overproduced in adipose tissue and polarized M1 macrophages in conditions of obesity; these cytokines stimulate the c-Jun N-terminal kinase (JNK) and IκB kinase β (IKKβ)/NF-κB pathways not only in adipose but also in other tissues (12, 13, 15). The JNK and IKKβ/NF-κB pathways play an important role in inflammation-induced insulin resistance in obesity. When the proinflammatory cytokines bind the receptors, the JNK and IKKβ/NF-κB pathways trigger the upregulation of target genes and inflammatory mediators such as TNF-α, IL-6, and IL-1β, and this induces the blocking of IR signaling (13, 15). Aguirre et al. (15) showed that anisomycin, a strong activator of JNK, inhibits insulin-stimulated tyrosine phosphorylation of IRS-1 in vitro. Cai et al. (16) reported that a high-fat diet induced insulin resistance and inflammation, whereas inhibitors of IKKβ and NF-κB reversed insulin resistance. Consequently, we can surmise that the activation of JNK and IKKβ/NF-κB pathways by circulating inflammatory mediators contributes to the progression from inflammation to insulin resistance in obesity.

Obesity-induced insulin resistance is associated with cellular stress signaling such as oxidative stress and endoplasmic reticulum (ER) stress (17, 18). When excess nutrient intake causes an oversupply of fatty acids and glucose in mitochondria, reactive oxygen species (ROS) are overproduced via mitochondrial oxidation. The level of ROS overproduction reaches beyond the threshold, and when the balance between ROS production and the antioxidant defense system is disturbed, oxidative stress occurs (18). Under conditions of oxidative stress, ROS can inhibit insulin signaling by activating the JNK and IKKβ/NF-κB pathways, similar to that in an inflammatory response (19). Wen et al. (20) demonstrated that palmiitrate induces the activation of the NLRP3-ASC inflammasome for IL-1β production that is involved in mitochondrial ROS and AMP-activated protein kinase (AMPK) activation. Han (18) reported that ROS is the key regulator of early events in obesity-induced inflammation and that mitochondria-derived ROS lead to the development of insulin resistance as well as inflammation in the late stages of obesity. Therefore, ROS not only inhibits insulin signaling but also induces an inflammatory response, thereby adversely affecting insulin resistance.

Many recent studies have reported that obesity-mediated inflammation is associated with ER stress and affects insulin signaling. The ER plays an essential role in calcium storage, lipid synthesis, and protein folding. Unfolded proteins induce the dissociation of chaperone proteins from each ER transmembrane protein, thereby activating transcription factor 6 (ATF6) and inositol requiring enzyme 1 (IRE1). The activation of ER transmembrane proteins leads to chaperone production to promote protein maturation; this response is termed the unfolded protein response (UPR). When an imbalance occurs between the cellular demand
for protein folding and the ability of the ER to promote protein maturation, accumulation of unfolded proteins is promoted in the ER lumen; this is defined as ER stress (17). Several studies have demonstrated that ER stress leads to JNK and IKKβ/NF-κB pathway activation and ROS accumulation, which may subsequently induce inflammatory mediator production, and these inflammatory mediators can, in turn, induce ER stress. Many studies have demonstrated that obesity-associated insulin resistance is associated with inflammation and oxidative stress as well as ER stress both in vitro and in vivo (21). Taken together, oxidative stress and ER stress affect each other and lead to mitochondrial dysfunction, thereby adversely affecting insulin resistance.

Adipocytes can secrete proinflammatory cytokines as well as metabolically active proteins such as leptin and adiponectin, the absence of which leads to dramatic metabolic disturbance (22). Leptin is a hormone that regulates appetite and energy balance by acting on receptors in the hypothalamus. In addition, leptin binds the receptor Ob-Rb and phosphorylates Janus kinase (JAK) 2, which leads to IRS and Akt activation, thereby affecting insulin signaling. Leptin in normal muscle tissue stimulates fatty acid oxidation by inhibiting acetyl-CoA carboxylase (ACC) activation by activating AMPK, which helps improve insulin sensitivity. However, in conditions of obesity, leptin resistance develops, and this signal does not function effectively, leading to an increase in serum leptin concentrations. Obesity-induced leptin resistance adversely affects insulin signals, resulting in decreased glucose uptake and glycolysis in muscles and increased gluconeogenesis in the liver, leading to the development of insulin resistance (22, 23).

Adipocyte-secreted adiponectin also plays a key role in obesity-related insulin resistance. Achari and Jain (24) reported that the adiponectin signaling pathway directly interacts with insulin receptor substrates. When adiponectin binds to its receptor, it activates protein phosphatase 2A, resulting in the activation of AMPK and dephosphorylation and inactivation of protein kinase C (PKC), which phosphorylates and inhibits the insulin receptor. Thus, adiponectin plays a major role in improving insulin sensitivity. Several studies have shown that a high-fat diet inhibits adiponectin activity and decreases serum adiponectin concentrations relative to those observed in response to a normal diet. Reduced adiponectin concentration may be a major factor as it is an important physiological regulator of obesity-induced insulin resistance (22, 25). Therefore, adiponectin replacement therapy may be effective at treating obesity and insulin resistance.

**miRNAs Released from Adipocytes through EVs in Obesity**

**EVs and miRNA loading**

Almost all cell types can produce EVs that act as mediators of cell–cell communication (26). Cells can release different types of EVs, exosomes, microvesicles (MV), and apoptotic vesicles, which differ in size, biogenesis pathway, and biological function. Exosomes (40–100 nm in diameter) are secreted from normal or diseased cells and are formed upon the fusion of the multivesicular bodies (MVBs) with the plasma membrane. MVs (100–1000 nm in diameter) are also secreted from normal or diseased cells but differ from exosomes in that they are formed at the cell membrane surface directly by budding. In apoptotic cells (1–4 μm in diameter), cells shed apoptotic vesicles by outward blebbing of the membrane that can be removed by phagocytosis. Most body fluids, such as urine, blood, breast milk, and saliva, contain MVs or exosomes, which are structurally similar but induce different extrinsic signals (26, 27).

miRNAs are a major class of small noncoding RNAs that regulate gene expression by post-transcriptional gene silencing via binding to specific target miRNAs. A single miRNA can regulate several mRNA targets, so the abnormal expression of 1 miRNA can affect a wide range of biological processes in both normal and pathological conditions. Although most miRNAs regulate gene expression inside cells, extracellular miRNAs can affect gene expression in recipient cells after loading into and circulating within EVs or exosomes (28, 29). miRNAs packaged within EVs are protected from RNase degradation and can be effectively delivered to recipient cells. In addition, circulating miRNAs in EVs contain attractive candidate biomarkers for the diagnosis of disease (28, 29). Chen et al. (30) analyzed miRNA profiles in cell lysates and EVs, including MVs and exosome, from the isogenic colorectal cancer cell line, and demonstrated the selective packaging of cellular miRNAs into MVs and exosomes. In addition, they discovered that the profiles of several miRNAs in MVs and exosomes differ. This result suggests that the sorting of miRNAs into EVs in cells occurs selectively, and that the mechanism by which miRNAs are loaded into MVs and exosomes is different. The mechanism that determines the specific loading of miRNA into EVs is yet to be understood completely. Nevertheless, Villarroya-Beltri et al. (31) have demonstrated major advances in how miRNAs are loaded into exosomes and secreted from cells. They determined that the sumoylated heterogeneous nuclear ribonucleoprotein A2B1 (hnRNP A2B1) specifically binds exosomal miRNAs and plays an important role in determining the content of exosomal miRNAs. In addition, some studies have suggested that protein argonaute-2 (AGO2) phosphorylation and neutral sphingomyelinase 2 (nSMase2) regulate not only exosome secretion but also secretion of miRNAs (32). However, the research on mechanisms of miRNA loading into EVs and secretion remains challenging.

**Expression patterns of extracellular miRNA in obesity**

Recently, circulating miRNAs were identified as novel adipokines that can affect pathophysiological mechanisms involved in obesity-induced metabolic syndromes, and thus serve as attractive potential biomarkers. Several studies have demonstrated different extracellular miRNA expression patterns between the normal and obese states and examined the effects of miRNAs secreted from obesity-induced adipocytes.
in metabolic disease. Most studies on extracellular miRNA expression patterns in obesity involve isolation of miRNAs from blood, and only a few have studied EVs; however, extracellular miRNAs are abundant in plasma-derived EVs (33–36, 32, 37–61). In this review, we summarize in Table 1 the obesity-associated changes in circulating miRNAs and their expression patterns.

Expression patterns of circulating miRNAs in obesity.
In 2011, Heneghan et al. (33) investigated blood miRNAs in specimens from individuals with obesity versus those from normal individuals for the first time. They showed that the concentrations of blood-carried miR-17-5p and miR-132 were decreased in omental fat tissue from individuals with obesity compared with those from normal individuals and suggested that extracellular miRNAs are potentially important players in metabolic pathways. Pescador et al. (34) found that serum miR-138, miR-376a, and miR-503 concentrations were decreased and serum miR-15b concentration was increased in obesity [BMI (kg/m²): 42.73 ± 4.67] compared with that in controls (BMI: 22.7 ± 2.43); they further suggested these miRNAs are potential biomarkers in obesity. Wang et al. (35) found that the concentrations of circulating miR-130b were increased in ob/ob mice as well as in Chinese individuals with obesity; in addition, they found that adipocytes secrete miR-130b, which regulates muscle metabolism by targeting peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α). Prats-Puig et al. (36) also showed increased concentrations of circulating miR-130b in individuals with obesity compared with those in lean individuals. However, Ortega et al. (32) and Thomé et al. (37) found an opposite result. Ortega et al. (32) showed decreased concentrations of miR-130b in morbidly patients with obesity, and Thomé et al. (37) showed decreased concentrations of miR-130b in patients with obesity and heart failure compared with those in lean patients with heart failure and controls. Additionally, miR-21, miR-423-5p, and miR-532-5p were found in both the increased miRNAs and decreased miRNAs. Therefore, these miRNAs have not yet proven to be biomarkers of obesity. However, the concentrations of circulating miR-122, miR-222, and miR-320a followed the same trend in these 3 papers (36, 32, 37). Increased circulating miR-122 concentrations were associated with childhood obesity, young-adult obesity, and mouse obesity, while increased circulating miR-222 concentrations were associated with childhood obesity and patients with morbid obesity and were correlated with BMI; decreased circulating miR-320a concentrations were also associated with childhood obesity and adult obesity (36, 32, 37). Thus, these 3 miRNAs may be suitable for use as biomarkers for diagnosing obesity.

Expression patterns of EV miRNAs in obesity.
In 2011, Müller et al. (38) showed that the concentrations of miR-16, miR-27a, miR-146b, and miR-222 were increased in MVs from large primary rat adipocytes and older rats compared with those in small adipocytes and younger rats. Although these results were identified for miRNAs from MVs, this study suggested that obesity affects the secretion of specific extracellular miRNAs. Increased miR-155 concentrations have been identified in MVs from mice, in which obesity was induced by a high-fat diet, and caused M1 macrophage polarization by targeting suppressor of cytokine signaling 1 (SOCS1) and JAK/signal transducers and activators of transcription (STAT) signaling (39).

Exosomes have also shown different patterns of specific miRNAs in obesity. During MVB formation, pre-miRNAs or mature miRNAs can be selected and sorted into the intraluminal vesicles in MVBs, and can then be secreted from cells as exosomes, in the form of secreted intraluminal vesicles. Secreted exosomal miRNA is delivered to target cells, and the physiological functions of exosomes can be largely deduced based on the content of these miRNAs. Exosomes and exosomal miRNAs have been demonstrated to be involved in multiple processes during the development of several diseases. Therefore, exosome-carried miRNAs may play an important role in the therapy and diagnoses of such conditions, and consequently, this has resulted in an expansion of interest in exosomes during recent years (28, 29).

Ferrante et al. (40) demonstrated altered expression of exosomal miRNAs between obesity and lean subjects. They demonstrated upregulation of miR-23b and miR-4429 and downregulation of miR-148b and miR-4269, which are correlated with transforming growth factor β (TGF-β) and Wnt/β-catenin signaling pathways. We show in Table 1 that miR-122 in exosomes and miR-222 in MVs are both increased in the obese state. This trend matches that of circulating miRNAs in other obesity studies. Obesity leads to changes in exosomal miRNAs from not only adipose tissue but also from adipose tissue macrophages. Ying et al. (41) demonstrated obesity-induced changes in the expression of exosomal miRNAs from adipose tissue macrophages by deep sequencing of small RNAs. In particular, the increase in exosome miR-155 from obese adipose tissue macrophages in this study displays a similar trend as the increase in miR-155 in MVs in individuals with obesity (39, 41).

Most of the studies that examined changes in exosomal miRNAs in obesity have demonstrated that these affect metabolic regulation, including obesity-mediated insulin resistance, by delivering miRNAs to target cells. This supports the hypothesis that obesity-mediated insulin resistance develops in response to exosomal miRNAs that act as novel adipokines. In this review, we summarize the findings on the potential role played by EV miRNAs released from adipocytes in obesity-mediated insulin resistance.

Function of Adipocyte-Derived Extracellular miRNAs in Insulin Resistance
In the recent paper by Thomou et al. (60), circulating exosomal miRNAs from the adipose tissue of distant tissues were demonstrated to act as regulators of glucose tolerance and expression of hepatic fibroblast growth factor 21 (FGF21). Extracellular miRNAs from adipose tissue have
TABLE 1 Extracellular miRNA profiles differ between obese and normal conditions

| miRNAs     | Increased miRNAs in obesity                          | Decreased miRNAs in obesity                      |
|------------|------------------------------------------------------|--------------------------------------------------|
| Blood      |                                                      |                                                  |
| miR-15b    | Pescador 2013 (34), Cui 2018 (51)                    | miR-15a                                          |
| miR-16-1   | Prats-Puig 2013 (36)                                 | Ortega 2013 (32)                                 |
| miR-20a    | Cui 2018 (51)                                        | miR-17                                           |
| miR-21-5p  | Thompson 2017 (49)                                   | Williams 2019 (54)                               |
| miR-21$^2$ | Yang 2020 (55)                                       | miR-21$^2$                                      |
| miR-26b    | Cui 2018 (51)                                        | Murri 2013 (42), Ghorbani 2018 (53)               |
| miR-26b-5p | Iacomino 2016 (46)                                   | miR-23-3p                                        |
| miR-27     | Can 2016 (47)                                        | Goguet-Rubio 2017 (50)                           |
| miR-29a-3p | Thompson 2017 (49)                                   | miR-27a-3p                                      |
| miR-31-5p  | Iacomino 2016 (46)                                   | Murri 2013 (42)                                  |
| miR-34a    | Yang 2020 (55)                                       | miR-28-3p                                        |
| miR-103a-5p| Thompson 2017 (49)                                   | Prats-Puig 2013 (36)                             |
| miR-122    | Prats-Puig 2013 (36), Wang 2015 (44), Jones 2017 (48) | miR-103                                          |
| miR-126    | Yang 2020 (55)                                       | Murri 2013 (42), Mahdavi 2018 (52)               |
| miR-130b$^2$| Prats-Puig 2013 (36), Wang 2013 (35)                 | miR-197                                          |
| miR-140-5p | Prats-Puig 2013 (36), Ortega 2013 (32)              | Murri 2013 (32), Thomé 2015 (37)                 |
| miR-142-3p | Prats-Puig 2013 (36), Ortega 2013 (32)              | miR-130                                          |
| miR-146a   | Cui 2018 (51)                                        | miR-155                                          |
| miR-146b   | Cui 2018 (51)                                        | Cui 2018 (51)                                    |
| miR-148a   | Yang 2020 (55)                                       | Murri 2013 (32), Goguet-Rubio 2017 (50)          |
| miR-150-5p | Thompson 2017 (49)                                   | miR-206                                          |
| miR-192    | Jones 2017 (48)                                      | Iacomino 2016 (46)                               |
| miR-222    | Prats-Puig 2013 (36), Ortega 2013 (32), Cui 2018 (51) | miR-223                                          |
| miR-223-3p | Thompson 2017 (49)                                   | Iacomino 2016 (46), Goguet-Rubio 2017 (50), Yang |
| miR-363    | Prats-Puig 2013 (36)                                 | 2020 (55)                                        |
| miR-370    | Can 2016 (47)                                        | miR-328                                          |
| miR-378    | Can 2016 (47)                                        | miR-335                                          |
| miR-423-5p$^2$| Prats-Puig 2013 (36), Thomé 2015 (37)              | miR-370                                          |
| miR-486-3p | Prats-Puig 2013 (36)                                 | miR-150                                          |
| miR-532-5p$^2$| Prats-Puig 2013 (36)                                | miR-192                                          |
| miR-2355-5p| Iacomino 2016 (46)                                   | miR-210                                          |
| miR-363    | Prats-Puig 2013 (36)                                 | miR-1928                                         |
| miR-370    | Can 2016 (47)                                        | miR-1948b                                        |
| miR-378    | Can 2016 (47)                                        | miR-3968                                         |
| miR-423-5p$^2$| Prats-Puig 2013 (36)                                | miR-4269                                        |
| miR-486-5p | Prats-Puig 2013 (36)                                 | miR-5098                                         |
| miR-532-5p$^2$| Prats-Puig 2013 (36)                                | miR-7054                                         |
| miR-2355-5p| Iacomino 2016 (46)                                   | miR-7070                                         |
| miR-363    | Prats-Puig 2013 (36)                                 |                                                  |
| Microvesicle |                                                  |                                                  |
| miR-16     | Müller 2011 (38)                                     |                                                  |
| miR-27a    | Müller 2011 (38)                                     |                                                  |
| miR-146b   | Müller 2011 (38)                                     |                                                  |
| miR-155    | Zhang 2015 (39)                                      |                                                  |
| miR-222    | Müller 2011 (38)                                     |                                                  |
| Exosome    |                                                      |                                                  |
| miR-23b    | Ferrante 2015 (40)                                   | miR-15b-5p                                       |
| miR-27a    | Yu 2018 (36)                                         | miR-141-3p                                       |
| miR-27a-3p | Castaño 2018 (57)                                    | miR-148b                                         |
| miR-27b-3p | Castaño 2018 (57)                                    | miR-151-5p                                       |
| miR-34a    | Pan 2019 (59)                                        | miR-351-5p                                       |
| miR-122    | Castaño 2018 (57)                                    | miR-365-2                                        |
| miR-149    | Ying 2017 (41)                                       | miR-431-5p                                       |
| miR-155    | Ying 2017 (41)                                       | miR-449a-5p                                      |
| miR-181a-1 | Ying 2017 (41)                                       | miR-511                                          |
| miR-181a-2 | Ying 2017 (41)                                       | miR-682                                          |
| miR-181b-1 | Ying 2017 (41)                                       | miR-690                                          |
| miR-181b-2 | Ying 2017 (41)                                       | miR-692-2                                        |
| miR-192    | Castaño 2018 (57)                                    | miR-692-3                                        |
| miR-210    | Ying 2017 (41)                                       | miR-874-3                                        |
| miR-299a-5p| Ying 2017 (41)                                       | miR-1928                                         |
| miR-1945   | Ying 2017 (41)                                       | miR-1948b                                        |
| miR-4429   | Ferrante 2015 (40)                                   | miR-3968                                         |
| miR-150-5p | Prats-Puig 2013 (36), Ortega 2013 (32)              | miR-4269                                         |

$^1$miRNA, microRNA.

$^2$miRNAs found in both the increased miRNAs and decreased miRNAs list.
been demonstrated to affect the control of gene expression in other tissues that can induce insulin resistance (60–72). Thus, several important extracellular miRNAs play a key role in the development of insulin resistance during obesity.

**Extracellular miR-27a**

miR-27a is an important extracellular miRNA in the development of obesity-mediated insulin resistance. An increase in extracellular miR-27a in response to obesity results in promotion of insulin resistance via inhibition of insulin signaling. Obesity-induced extracellular miR-27a can accumulate in skeletal muscle cells and adipose tissue cells and inhibits the expression of the peroxisome proliferator-activated receptor γ (PPARγ) transcription factor. PPARγ regulates the expression of genes involved in lipid and glucose metabolism and plays an important role in insulin sensitization (56). miR-27a inhibits PPARγ/PI3K/Akt/GLUT4 signaling, leading to insulin resistance (61, 62). In addition, miR-27a leads to macrophage infiltration and activation via PPARγ inhibition and via the activation of NF-κB-mediated transcription (63). miR-27a also targets mitogen-activated protein kinase 14 (MAPK-14), which is mostly expressed in the skeletal muscles and nervous system and activates GLUT4 translocation via IRS-1/Akt/signaling (62, 64). These results show that adipocyte-derived extracellular miR-27a can induce inflammation and insulin resistance and acts as a messenger between adipose tissue and other tissues.

**Extracellular miR-34a**

Pan et al. (59) demonstrated that the concentrations of exosomal miR-34a were elevated in obesity, and that ablation of adipocyte-derived miR-34a protected against obesity-induced insulin resistance and inflammation. Exosomal miR-34a from adipocytes can be transported to macrophages and targets Kruppel-like factor 4 (KLF4), which acts as a regulator of insulin signaling and cell proliferation, differentiation, and apoptosis. miR-34a–mediated downregulation of KLF4 in macrophages leads to inhibition of M2 polarization, which causes obesity-induced inflammation and insulin resistance (65). In the skeletal muscle, miR-34a can target ceramide kinase (CERK). CERK activation induces the phosphorylation of ceramide to produce ceramide-1-phosphate that stimulates PI3K/Akt, extracellular signal-regulated kinase 1/2 (ERK1/2), and mTOR in skeletal muscle cells. miR-34a–mediated suppression of CERK can induce increased ceramide concentrations that activate the JNK pathways and inhibit the insulin signaling pathway (66). Thus, exosomal miR-34a from adipocytes can serve as a key mediator of obesity-induced inflammation and insulin resistance.

**Extracellular miR-141-3p**

Exosomes from adipose tissue in obesity can also deliver to, and induce insulin resistance in, hepatocytes. Exosomal miR-141-3p can inhibit insulin sensitivity and glucose uptake of hepatocytes, but its concentrations are decreased in exosomes from obesity-induced mice compared with those from normal mice (58). miR-141-3p directly targets phosphatase and tensin homolog (PTEN), which acts as a negative regulator of the PI3K/Akt signaling pathway and GLUT4 translocation (67, 68). Thus, obesity-induced suppression of exosomal miR-141-3p led to PTEN overexpression in hepatocyte and skeletal muscle cells, and ultimately to insulin resistance.

**Extracellular miR-155**

Increased miR-155 concentrations in conditions of obesity were found in both MVs and exosomes. The increased miR-155 concentration in adipocyte-derived MVs from obesity-induced mice induced M1 macrophage polarization by targeting the suppressor of SOCS1, which is involved in anti-inflammatory actions. Moreover, obesity caused an increase in the release of exosomal miR-155 from adipose tissue macrophages, which targets PPARγ and inhibits insulin signaling in adipocytes, muscle cells, and hepatocytes (39, 41). These results suggest a mechanism in which obesity-mediated increases in extracellular miR-155 affect the development of inflammation as well as insulin resistance.

**Extracellular miR-210 and miR-222**

Although miR-210 in exosome and miR-222 in MVs are increased in conditions of obesity, there have been no published articles showing that the miR-210 and miR-222 from adipocytes directly affect insulin signaling in obesity. However, studies have indicated that extracellular miR-210 and miR-222 may act as factors regulating the development of obesity-mediated insulin resistance in the insulin signaling pathway (38, 41). Recently, Tian et al. (69) demonstrated that exosomal miR-210 from high-glucose–induced macrophages inhibited NADH dehydrogenase (ubiquinone) 1a subcomplex 4 (NDUFA4) expression and induced the suppression of glucose uptake in adipocytes. NDUFA4 is one of the subunits of mitochondrial respiratory chain complex IV, and thus it is involved in mitochondrial function (70). Therefore, we suggest that the downregulation of NDUFA4 expression by miR-210 can lead to mitochondrial dysfunction that affects insulin signaling during obesity. Overexpression of miR-222 inhibits the expression of IRS-1 protein, a key molecule in the insulin signaling pathway, by directly binding to IRS-1 untranslated regions (71). Ono et al. (72) found that the concentrations of miR-222 are increased in the livers of individuals fed a high-fat/high-sucrose diet, and that this increase is associated with inhibition of insulin signaling. These findings suggest the role of extracellular miR-222 in obesity-induced insulin resistance by targeting IRS-1.

**Conclusions**

In this review, we summarized changes in the concentrations of obesity-associated circulating miRNAs and discussed the potential mechanisms involved in the development of...
Excess energy intake

Extracellular miRNA-associated insulin resistance during obesity. This article discussed how miRNAs, particularly miR-27a, miR-34a, miR-141-3p, miR-155, miR210, and miR-222, in EVs secreted from the adipose tissue can affect the insulin signaling pathway in metabolic tissue (Figure 1). The roles of exosomes and miRNAs in obesity-mediated insulin resistance require further investigation. This review assessed the role played by extracellular miRNAs derived from adipocytes in the development of insulin resistance and potential strategies for the development of therapeutics aimed at obesity and metabolic disorders such as type 2 diabetes.

Acknowledgments

The authors’ responsibilities were as follows: OK-K: was involved in the study design and wrote the manuscript; YK: performed the literature search; both authors: were involved in reviewing and editing and read and approved the final manuscript.

Reference

1. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell 2000;6(1):87–97.
2. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001;414(6865):799–806.
3. Boucher J, Kleinridders A, Kahn CR. Insulin receptor signaling in normal and insulin-resistant states. Cold Spring Harb Perspect Biol 2014;6(1):a009191.
4. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G. Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). J Clin Invest 1997;100(5):1166–73.
5. Oakes ND, Cooney GJ, Camilleri S, Chisholm DJ, Kraegen EW. Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. Diabetes 1997;46(11):1768–74.
6. Kahn SE, Hull RL, Utschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444(7121):840–6.
7. Sun XJ, Rothenberg P, Kahn CR, Backer JM, Araki E, Wilden PA, Cahill DA, Goldstein BJ, White MF. Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 1991;352(6330):73–7.
8. Delcommenne M, Tan C, Gray V, Rue L, Woodgett J, Dedhar S. Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. Proc Natl Acad Sci 1998;95(19):11211–6.
9. Nakae J, Kitamura T, Silver DL, Accili D. The forkhead transcription factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. J Clin Invest 2001;108(9):1359–67.
10. Gonzalez E, McGraw TE. Insulin-modulated Akt subcellular localization determines Akt isomform-specific signaling. Proc Natl Acad Sci 2009;106(17):7004–9.
11. Eguez L, Lee A, Chavez JA, Miinea CP, Kane S, Lienhard GE, McGraw TE. Full intracellular retention of GLUT4 requires AS160 Rab GTPase activating protein. Cell Metab 2005;2(4):263–72.
12. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, et al. Chronic inflammation in fat plays a
crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112(12):1821–30.

13. Hotamisligil GS, Perdahl P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α-negative and obesity-induced insulin resistance. Science 1996;271(5249):665–8.

14. Kamei N, Tobe K, Suzuki R, Ohsugi M, Watanabe T, Kubota N, Ohtsuka-Kowatari N, Kumagai K, Sakamoto K, Kobayashi M, et al. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. J Biol Chem 2006;281(36):26602–14.

15. Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). J Biol Chem 2003;278(4):2907–15.

16. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE. J. Mechanism of ER stress and inflammation for hepatic insulin resistance in obesity. Ann Nutr Metab 2015;67(4):218–27.

17. Han CY. Roles of reactive oxygen species on insulin resistance in adipose tissue. Diabetes Metab J 2016;40(4):272–9.

18. Sadeghi A, Rostamirad A, Seyyedehabadi S, Meshkani R, Curcumin ameliorates palmitate-induced inflammation in skeletal muscle cells by regulating JNK/NF-kB pathway and ROS production. Inflammopharmacology 2018;26(5):1265–72.

19. Wang H, Gris D, Lei Y, Jha S, Zhang L, Huang MT, Brickey WJ, Ting JP. Fatty acid-induced NLRP3-ASC inflammasome activation interacts with insulin signaling. Nat Immunol 2011;12(5):408–15.

20. Chaudhari N, Talwar P, Parimisetty A, Lefebvre d’Hellencourt JP. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat Immunol 2011;12(5):408–15.

21. Pérez C, Fernández-Galaz C, Fernández-Agulló T, Arribas C, Andrés A, Ros M, Carrascosa JM. Leptin impairs insulin signaling in rat adipocytes. Diabetes Metab 2003;29(4):347–54.

22. Achari AE, Jain SK. Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. Int J Mol Sci 2017;18(6):1321.

23. de Oliveira C, de Mattos AB, Biz C, Oyama LM, Ribeiro EB, do Nascimento CM. High-fat diet and glucocorticoid treatment cause hyperglycemia associated with adiponectin receptor alterations. Lipois Health Dis 2011;10:11.

24. Ratjczak MZ, Ratjczak D, Pedziwiatr D. Extracellular microvesicles (ExMVs) in cell to cell communication: a role of telocytes. Adv Exp Med Biol 2016;913:41–9.

25. Sluijter JP, Verhage V, Deddens JC, van den Akker F, Doevendans PA. Microvesicles and exosomes for intracardiac communication. Cardiovasc Res 2014;102(2):302–11.

26. Michael A, Bajracharya SD, Yuen PS, Zhou H, Star RA, Illei GG, Alevizos I. Exosomes from human saliva as a source of microRNA biomarkers. Oral Dis 2010;16(1):34–8.

27. Corrado C, Raimondo S, Chiesi A, Ciccia F, De Leo G, Alessandro R. Exosomes as intercellular signaling organelles involved in health and disease: basic science and clinical applications. Int J Mol Sci 2013;14(3):5336–66.

28. Chen M, Xu R, Rai A, Suwakuliri W, Izumikawa K, Ishikawa H, Greening DW, Takahashi N, Simpson RJ. Distinct shed microvesicle and exosome microRNA signatures reveal diagnostic markers for colorectal cancer. PLoS One 2019;14(1):e0210003.

29. Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F, Pérez-Hernández D, Vázquez J, Martin-Cofores N, Martínez-Herrera DJ, Pascual-Montano A, Millébrunn N, Sánchez-Marid F. Sumoylated hnrRNPAB21 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun 2013;4:2980.

30. Ortega FJ, Mercader JM, Catalán V, Moreno-Navarrete JM, Pueyo N, Sabater M, Gómez-Ambroisi J, Anglada R, Fernández-Fornoso JA, Ricart W, et al. Targeting the circulating microRNA signature of obesity. Clin Chem 2013;59(5):781–92.

31. Heneghan HM, Miller N, McAnena OJ, O’Brien T, Kerin MJ. Differential miRNA expression in omental adipose tissue and in the circulation of obese patients identifies novel metabolic biomarkers. J Clin Endocrinol Metab 2011;96(5):846–50.

32. Pescador N, Pérez-Barba M, Ibarra JM, Corbatón A, Martínez-Larrad MT, Serrano-Ríos M. Serum circulating microRNA profiling for identification of potential type 2 diabetes and obesity biomarkers. PLoS One 2013;8(10):e77251.

33. Wang YC, Li Y, Wang XY, Zhang D, Zhang H, Wu Q, He YQ, Wang YJ, Zhang L, Xia H, et al. Circulating miR-130b mediates metabolic crosstalk between fat and muscle in overweight/obesity. Diabetologia 2013;56(10):2275–85.

34. Prats-Puig A, Ortega FJ, Mercader JM, Moreno-Navarrete JM, Moreno M, Bonet N, Ricart W, López-Bermejo A, Fernández-Real JM. Changes in circulating microRNAs are associated with childhood obesity. J Clin Endocrinol Metab 2013;98(10):1655–60.

35. Thömé JG, Mendoza MR, Cheuiche AV, La Porta VL, Silvello D, Dos Santos KG, Andrades ME, Claussell N, Rohde LE, Biolo A. Circulating microRNAs in obese and lean heart failure patients: a case-control study with computational target prediction analysis. Gene 2015;574(1):1–10.

36. Müller G, Schneider M, Biemer-Daub G, Wied S. Microvesicles released from rat adipocytes and harboring glycosylphosphatidylinositol-anchored proteins transfer RNA stimulating lipid synthesis. Cell Signal 2011;23(7):1207–23.

37. Zhang Y, Mei H, Chang X, Chen F, Zhu Y, Han X. Adipocyte-derived microvesicles from obese mice induce M1 macrophage phenotype through secreted mir-155. J Mol Cell Biol 2016;8(6):505–17.

38. Ferrante SC, Nadler EP, Piliak DK, Hubal MJ, Wang Z, Wang JM, Gordoish-Dressman H, Koeck E, Sevilla S, Wiles AA, et al. Adipocyte-derived exosomal miRNAs: a novel mechanism for obesity-related disease. Pediatr Res 2018;73(3):447–54.

39. Ying W, Riopel M, Bandyopadhyay G, Dong Y, Birmingham A, Seo JB, Ofrecio JM, Wollam J, Fernandez-Carretero A, Fu W, et al. Adipose tissue macrophage-derived exosomal miRNAs can modulate in vivo and in vitro insulin sensitivity. Cell 2017;171(2):372–84.

40. Murri M, Insenser M, Fernández-Durán E, San-Millán JL, Escobar-Morreale HF. Effects of polycystic ovary syndrome (PCOS), sex hormones, and obesity on circulating miRNA-21, miRNA-27b, miRNA-103, and miRNA-155 expression. J Clin Endocrinol Metab 2013;98(11):1835–44.

41. Wen D, Qiao P, Wang L. Circulating microRNA-223 as a potential biomarker for obesity. Obes Res Clin Pract 2013;9(4):398–404.

42. Wang R, Hong J, Cao Y, Shi J, Gu W, Ning G, Zhang Y, Wang W. Elevated circulating microRNA-122 is associated with obesity and insulin resistance in young adults. Eur J Endocrinol 2015;172(3):227–33.

43. Pek SL, Sum CF, Lin MX, Cheng AK, Wong MT, Lim SC, Tavintharan S. Circulating and visceral adipose miR-100 is down-regulated in patients with obesity and Type 2 diabetes. Mol Cell Endocrinol 2016;427:112–23.

44. Iacomino G, Russo P, Stillitano I, Lauria F, Marena P, Ahrens W, De Luca P, Siani A. Circulating microRNAs are deregulated in overweight/obese children: preliminary results of the I.Family study. Genes Nutr 2016;11:7.

45. Can U, Buyukinan M, Yerlikaya FH. The investigation of circulating microRNAs associated with lipid metabolism in childhood obesity. Pediatr Obes 2016;11(3):228–34.

46. Jones A, Danielson KM, Benton MC, Ziegler O, Shah R, Stubbs RS, Das S, Macartney-Coxson D. miRNA signatures of insulin resistance in obesity. Obesity 2017;25(10):1734–44.

47. Thompson MD, Cimowski MJ, Serpico M, Pusateri A, Briggstock DR. Elevation of circulating microRNA levels in obese children compared to healthy controls. Clin Obes 2017;7(4):216–21.

48. Goguet-Rubio P, Klug RL, Sharma DL, Srikanthan K, Puri N, Lakhani YH, Nichols A, O’Hanlon KM, Abraham NG, Shapiro JL, et al. Existence of a strong correlation of biomarkers and miRNA in females with
metabolic syndrome and obesity in a population of West Virginia. Int J Med Sci 2017;14(6):543–53.

51. Cui X, You L, Zhu L, Wang X, Zhou Y, Li Y, Wen J, Xia Y, Wang X, Ji C, et al. Change in circulating microRNA profile of obese children indicates future risk of adult diabetes. Metabolism 2018;78:95–105.

52. Mahdavi R, Ghorbani S, Alipoor B, Panahi G, Khodabandehloo H, Esfahani EN, Razi F, Meshkani R. Decreased serum level of miR-155 is associated with obesity and its related metabolic traits. Clin Lab 2018;64(1):77–84.

53. Ghorbani S, Mahdavi R, Alipoor B, Panahi G, Nasli Esfahani E, Razi F, Taghikhani M, Meshkani R. Decreased serum microRNA-21 level is associated with obesity in healthy and type 2 diabetic subjects. Arch Physiol Biochem 2018;124(4):300–5.

54. Williams A, Dougal DM, Jenkins W, Greene N, Williams-DeVane C, Kimbro KS. Serum miR-17 levels are downregulated in obese, African American women with elevated HbA1c. J Diabetes Metab Disord 2019;18(1):173–9.

55. Yang P, Dong X, Zhang Y. MicroRNA profiles in plasma samples from young metabolically healthy obese patients and miRNA-21 are associated with diastolic dysfunction via TGF-β1/Smad pathway. J Clin Lab Anal 2020;28:e23246.

56. Yu Y, Du H, Wei S, Feng L, Li J, Yao F, Zhang M, Hatch GM, Chen L. Adipocyte-derived exosomal MiR-27a induces insulin resistance in skeletal muscle through repression of PPARγ. Theranostics 2018;8(8):2171–88.

57. Castaño C, Kalko S, Novials A, Párrizas M. Obesity-associated exosomal miRNAs modulate glucose and lipid metabolism in mice. Proc Natl Acad Sci USA 2018;115(48):12158–63.

58. Dang SY, Leng Y, Wang ZX, Xiao X, Zhang X, Wen T, Góng HZ, Hong A, Ma Y. Exosomal transfer of obesity adipose tissue for decreased miR-141-3p mediate insulin resistance of hepatocytes. Int J Biol Sci 2019;15(2):351–68.

59. Pan Y, Hui X, Hoo RLC, Ye D, Chan CYC, Feng T, Wang Y, Lam KSL, Xu A. Adipocyte-secreted exosomal microRNA-434a inhibits M2 macrophage polarization to promote obesity-induced adipose inflammation. J Clin Invest 2019;129(2):834–49.

60. Thomou T, Mori MA, Dreyfuss JM, Konishi M, Sakaguchi M, Wolfrum C, Rao TN, Winnay JN, Garcia-Martin R, Grinspoon SK, et al. Adipose-derived circulating miRNAs regulate gene expression in other tissues. Nature 2017;542(7642):450–5.

61. Chen T, Zhang Y, Liu Y, Zhu D, Yu J, Li G, Sun Z, Wang W, Jiang H, Hong Z. MiR-27a promotes insulin resistance and mediates glucose metabolism by targeting PPAR-γ-mediated PI3K/AKT signaling. Aging 2019;11(18):7510–24.

62. Zhou T, Meng X, Che H, Shen N, Xiao D, Song X, Liang M, Fu X, Ju J, Li Y, et al. Regulation of insulin resistance by multiple MiRNAs via targeting the GLUT4 signalling pathway. Cell Physiol Biochem 2016;38(5):2063–78.

63. Yao F, Yu Y, Feng L, Li J, Zhang M, Lan X, Yan X, Liu Y, Guan F, Zhang M, et al. Adipogenetic miR-27a in adipose tissue upregulates macrophage activation via inhibiting PPARγ of insulin resistance induced by high-fat diet-associated obesity. Exp Cell Res 2017;355(2):105–12.

64. Wang S, Ai H, Liu L, Zhang X, Gao F, Zheng L, Yi J, Sun L, Yu C, Zhao H, et al. MicroRNA-27a/b negatively regulates hepatic gluconeogenesis by targeting FOXO1. Am J Physiol Endocrinol Metab 2019;317(5):911–24.

65. Muthiah A, Angulo MS, Walker NN, Keller SR, Lee JK. Biologically anchored knowledge expansion approach uncovers KLF4 as a novel insulin signaling regulator. PLoS One 2018;13(9):e0204100.

66. Kukreti H, Amuthavalli K. MicroRNA-34a causes ceramide accumulation and effects insulin signaling pathway by targeting ceramide kinase (CERK) in aging skeletal muscle. J Cell Biochem 2020;121:3070.

67. Tian F, Tang P, Sun Z, Zhang R, Zhu D, He J, Liao J, Wan Q, Shen J. miR-210 in exosomes derived from macrophages under high glucose promotes mouse diabetic obesity pathogenesis by suppressing NDUF4 expression. J Diabetes Res 2020;2020:6894684.

68. Li A, Qiu M, Zhou H, Wang T, Guo W. PTEN, insulin resistance and cancer. Curr Pharm Des 2017;23(25):3667–76.

69. de Mendonça M, de Sousa É, da Paixão AO, Araújo Dos Santos B, Roveratti Spagnol A, Murata GM, Araújo IN, Imamura de Lima T, Passos Simões Fróes Guimarães DS, Silveira LR, et al. MicroRNA miR-222 mediates pioglitazone beneficial effects on skeletal muscle of diet-induced obese mice. Mol Cell Endocrinol 2020;501:110661.

70. Ono K, Igata M, Kondo T, Kitano Y, Takaki T, Hanatani S, Sakaguchi M, Goto R, Senokuchi T, Kawashima J, et al. Identification of microRNA that represses IRS-1 expression in liver. PLoS One 2018;13(1):e0191553.