MicroRNAs as regulators of metabolic disease: pathophysiologic significance and emerging role as biomarkers and therapeutics

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The prevalence of overweight and obesity in developed and developing countries has greatly increased the risk of insulin resistance and type 2 diabetes mellitus. It is evident from human and animal studies that obesity alters microRNA (miRNA) expression in metabolically important organs, and that miRNAs are involved in changes to normal physiology, acting as mediators of disease. miRNAs regulate multiple pathways including insulin signaling, immune-mediated inflammation, adipokine expression, adipogenesis, lipid metabolism, and food intake regulation. Thus, miRNA-based therapeutics represent an innovative and attractive treatment modality, with non-human primate studies showing great promise. In addition, miRNA measures in plasma or bodily fluids may be used as disease biomarkers and predictors of metabolic disease in humans. This review analyzes the role of miRNAs in obesity and insulin resistance, focusing on the miR-17/92, miR-143-145, miR-130, let-7, miR-221/222, miR-200, miR-223, miR-29 and miR-375 families, as well as miRNA changes by relevant tissue (adipose, liver and skeletal muscle). Further, the current and future applications of miRNA-based therapeutics and diagnostics in metabolic disease are discussed.

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INTRODUCTION

In 2013, the American Medical Association recognized obesity as a disease, highlighting the importance in public health. Of particular concern is the incidence of overweight and obesity in children, with an estimated one-third of children and adolescents affected in the United States.1 In addition, metabolic and food intake programming may result in transgenerational metabolic dysfunction, with parental obesity potentially affecting the metabolic health of offspring and potentially future generations.2–4 Over the last decade, the nutrition–immunity theory has been hypothesized, suggesting that starvation leads to immunosuppression and that overnutrition/obesity promotes inflammation.5 During overnutrition, inflammation typically occurs in visceral adipose depots, where changes include increased immune cell infiltration, proliferation and activation, along with adipocyte hypertrophy, impaired adipogenesis, and inflammatory peptide production by immune cells and adipocytes.6,7 Insulin-resistant adipocytes exhibit abnormal lipolysis (increasing circulating free fatty acids levels), which promotes ectopic lipid storage (liver, muscle).8 Abnormal hepatic function in metabolic disease (dysregulated gluconeogenesis and lipogenesis/fatty acid esterification) is likely a result of hepatocellular insulin resistance (IR), as well as insulin signaling independent mechanisms and circulating fatty acid (substrate)-dependent mechanisms.9 In the liver of organisms with metabolic disease, it is common to find activation of resident immune cell populations (Kupffer cells), lipid accumulation (fatty liver disease/nonalcoholic steatohepatitis, hepatic steatosis) and inflammatory chemokine production.9,10 Skeletal muscle, however, tends to be a target of inflammatory cytokines originating from inflamed visceral adipose and liver tissue and is often characterized by ectopic lipid accumulation.11 The kidney and vasculature are similarly affected by circulating pro-inflammatory and pro-atherogenic compounds,11 providing a link between metabolic and cardiovascular disease. Finally, obesity and IR involve neurologic (hypothalamic) changes, which may increase food intake and the defense of increased body fat levels.12

Insulin resistance (IR), at a cellular level, is the result of blunted insulin-stimulated tyrosine phosphorylation of the insulin receptor (IRS-1/IRS-2)13 and associated downstream signaling events (translocation of glucose transporters to cell membranes) in glucose-metabolizing cells.14 Stress and inflammatory signaling events (JNK activation) result in serine phosphorylation (instead of tyrosine) of insulin receptor proteins, inhibiting insulin signaling (cellular IR).15 The current, prevailing theory is that inflammatory/stress signaling is the common causal event leading to cellular IR in energy homeostatic tissues.15 Briefly, cellular abnormalities that result in inflammation impaired insulin signaling. Impaired insulin signaling in turn contributes to the metabolic abnormalities specific to the cell type affected (adipocyte, hepatocyte, myocyte). Reviews on the role of inflammation in metabolic disease are available.15,16 However, models of IR and obesity demonstrate abnormalities in a myriad of processes, often concomitantly, including inflammation and abnormal microRNA (miRNA) expression in various tissues and cell types.17–19 Determining if and how miRNA changes are causal in the development of IR is the main challenge of obesity-related miRNA research.

Mature miRNAs are small noncoding single-stranded RNAs (~21 nucleotides) that negatively regulate or 'repress' target gene expression. The first miRNA was described in Caenorhabditis elegans, in 1993.20,21 MiRNAs have since been identified in all...
Mature miRNAs to the 3′ end of a miRNA hairpin result in the release of a small RNA species (miRNA) which can be translated. Mature miRNAs exert biological effects by regulating the expression of ~60% of genes. miRNA genes can be accomplished by the binding of the seed sequence of the matured miRNA guide strand. This is accomplished by the binding of the seed sequence (at the 5′ end of a mature miRNA) to the 3′ untranslated region of target mRNA transcripts. In addition, the middle and 3′ end of a miRNA can also bind miRNA and contribute to gene repression.

Clearly, miRNA maturation depends on multiple pathways that regulate the biogenesis/stability/formation of miRNA-protein complexes which modulate gene expression. Though these processes have been thoroughly reviewed elsewhere, we will briefly touch on endogenous RNA editing processes and their relevance to metabolic disease. A family of adenosine deaminases (ADARs) edits double-stranded RNA species, proportionally responsible for the conversion of adenosine residues to inosine. Ultimately, ADARs-mediated A-to-I conversions may result in guide strand base alteration(s) and a change in targeting efficiency or complete mRNA re-targeting. Also, inhibition of Drosha (pri-miRNA) and Dicer, TRBP, AGO2 (pre-miRNA) cleavage may occur depending on the location of A-to-I conversion. A-to-I conversions are best detected by next-generation sequencing, which will be discussed later in this review. The physiological ramifications of ADAR activity in normal and pathophysiology are still being examined, though it is likely that a majority of RNAs in a cell are edited by ADARs to some degree. ADAR-catalyzed conversions occur mainly in noncoding RNA sequences including the introns and untranslated regions of miRNAs and small RNA species such as miRNAs, however, the processes controlling specificity of adenosine conversion is undefined. It is clear, though, that ADARs affect miRNAs in humans, mice and rats and thus their role in miRNA-mediated metabolic disease should be considered when using these models. Glucose and JNK signaling regulate ADAR2 expression in the pancreatic beta cells of mice suggesting that ADARs may have a role in pancreatic adaptations in overnutrition.

Experimental approaches to miRNA research

Systemic and organ-specific knockouts/transgenic mouse strategies are available for some miRNA/subfamily families (https:// crumblingmiRNA.org/mirKO-DB); this often depends on the genomic organization of the miRNA/miRNA family of interest and on embryonic lethality of miRNA knockout. Gain-of-function strategies include injection/transfection with synthetic miRNA mimics and vector-mediated miRNA overexpression by lentivirus or AAV infection. Other loss-of-function strategies include injection/transfection with anti-miRNA oligonucleotide or miR-sponges (primary cells and drosophila). miR-sponges are transgenes that harbor multiple miRNA binding sites, effectively depleting endogenous miRNA levels. In contrast, anti-miRs bind to mature miRNAs canceling out (derepressing) their effect on-target genes in a dose-dependent manner. Anti-miR approaches are thoroughly reviewed by Stenvang et al.

There is a substantial body of literature characterizing miRNA expression in lean and obese mice and humans. However, the clarity of many publications is affected by changes in and nonstandard uses of miRNA nomenclature; miRBase.org can be used to research nomenclature. Furthermore, many publications do not cite miRNA sequences or fail to identify particular family members when reporting miRNAs with multiple sequence variants (for example, Let-7). Given their central importance in regulation of pleiotropic pathways that cut across organ systems involved in IR, miRNAs may act in concert in determining susceptibility/severity of defects associated with obesity/IR.

The Pubmed database was searched for relevant studies published between 15 September 2008 and 23 January 2015. The search terms ‘obesity’ and microRNA’ identified 344 articles, ‘(insulin resistance) AND microRNA’ identified 225 articles. The literature was screened for seminal findings and for the most reported and mechanistically studied miRNAs in obesity and metabolic disease. Focus was placed on in vivo interventional (especially non-human primate) studies and human plasma biomarker research. Additional searches for specific miRNAs and ‘obesity’ or ‘insulin resistance’ (for example, ‘miR-221’ and obesity’ identified eight articles) were performed. Reviews, commentaries and non-original research articles were excluded.

The initial report (2009) of miRNA expression in human obesity by Kloting et al. examined a small cohort (n=15; 53–73 years; body mass index 25.4–38.1 kg m⁻²) of overweight and obese subjects with preserved glucose tolerance compared to those of type 2 diabetes mellitus (T2DM). Many of the miRNAs first identified in this study (miR-17-5p, -145, -34a, -132, -181a) have been confirmed independently in human studies and rodent models of obesity/IR.

The miR-17/92 family

The family contains three polycistronic miRNA genes producing 15 mature miRNA species (miR-17, 18a, 18b, 20a, 20b, 93, 106a, 106b). Kloting et al. reported significantly lower expression of miR-17-5p in the omental adipose tissue of T2DM patients compared with normal glucose tolerance (NGT) and a negative correlation with visceral fat area (Table 1). Since these initial findings, there have been multiple reports linking plasma levels of miR-17-5p with cardiometabolic disease, suggesting usefulness as a biomarker in multiple diseases.

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miR-17 is well known to be associated with the exosomal compartment (Table 2). Interestingly, immunologic studies by Steiner et al. suggest that miR-17/92 family members potentiate T helper cell proliferation. Recently, Li et al. reported that miR-17-5p was increased during human adipose-derived mesenchymal stem cell adipogenesis in vitro and that miR-17-5p mimic transfection resulted in enhanced adipogenesis in the same cell population via repression of bone morphogenetic protein 2 and increased CCAAT/enhancer-binding protein alpha and peroxisome proliferator-activated receptor gamma expression. Taken together, these findings suggest that the miR-17/92 family may have a role in adipogenesis and cardiometabolic disease, and be a useful positive control for plasma/exosome-based studies of obese and/or cardiovascular disease patients.

The miR-143-145 cluster

The role of miRNAs in adipocytes/adipogenesis is the most studied area of miRNA biology as it relates to obesity and IR, with 148 citations under ‘(microRNA) AND adipogenesis’. Of the adipogenesis-related miRNAs, miR-143 and miR-130 are the best studied (Table 3). miR-143 and miR-145 are located in close genomic proximity, may be transcribed in a bicistronic fashion, and consequently are often studied/reported concomitantly.

Figure 1. microRNA biogenesis and ADAR-mediated editing during miRNA maturation. ADAR, family of adenosine deaminase; miRISC, miRNA-induced silencing complex.
miR-143 was first identified as a positive regulator of human adipocyte differentiation in 2004 via effects on ERK5 signaling. miR-143 is the only miRNA to date shown to be similarly regulated during human and mouse adipocyte differentiation (Figure 2). Figure 2 illustrates obesity/IR-related miRNA expression changes in humans and rodents, comparing and contrasting major findings. miR-143 expression was increased in the mesenteric adipose of high-fat diet (HFD)-fed mice, and tumor necrosis factor alpha treatment decreased miR-143 expression suggesting that obesity-associated inflammation may dysregulate miR-143 expression affecting adipogenesis (Table 1). Despite miR-143 being a positive regulator of adipogenesis, miR-143 cluster knockout mice were protected from obesity-induced IR, while conditional overexpression of miR-143 results in worsened IR in diet-induced obesity (DIO). miR-143 may increase IR by increasing the degradation of positive regulator of AKT signaling, oysterol binding protein-like 8. Additional mechanisms for miR-143 in metabolic disease have not been reported. The role for miR-145 in obesity is less clear, though a putative role has been suggested in tumor necrosis factor alpha secretion and lipolysis in human adipocytes.  

The miR-130 family

The miR-130 family has four members in humans: miR-130a, miR-130b, miR-301a and miR-301b (on three chromosomes). In

### Table 1. miRNAs involved in obesity: insights from in vivo models and whole adipose

| miRNA     | Finding(s)                                      | Intervention/phenotype         | Model          | Target/pathway          | Author            |
|-----------|------------------------------------------------|-------------------------------|----------------|-------------------------|-------------------|
| miR-132, -181a, -17-5p, -155 | Omental, subQ fat, correlation with glucose metabolism, macrophage infiltration | NGT vs T2DM (n = 15 total) | Human | —                       | Kloting et al. 

| miR-132, -17-5p, -143, -145 | Omental, subQ fat, plasma | Obese vs lean (n = 50 total) | Human | —                       | Heneghan et al. |
| miR-132, -184, -338-3p | ↑, ↓ | Improved beta cell mass and activity | Mouse (db/db), Human and Rat cells | — | Nesca et al. |
| miR-143 | ↑ Mesenteric fat | DIO-IR, 45% E from fat | Mouse (C57BL/6J) | PPARγ, aP2, leptin pathways | Takanabe et al. |
| miR-143 | ↑ Insulin resistance, Protected from DIO-IR | Conditional overexpression of miR-143, miR-143 deficient | Mouse | ORP8 | Jordan et al. |
| miR-27a | ↑ Omental whole adipose, Change not significant | Obese vs lean (n = 15 vs 15) | Human | — | Viesti et al. |
| miR-143, -145 | Omental, subQ and liver difference not significant | Obese vs lean (n = 15 vs 15) | Human | — | Viesti et al. |
| Let-7 | ↑ Insulin resistance, Protected from DIO-IR | Overexpressed—global and pancreas, whole-body inhibition | Mouse | INSR, IRS-2 | Frost and Olson |
| Let-7-d | ↑ Amnion | Obese pregnant women Genetic IR (non-obese) | Human | — | Nardelli et al. |
| miR-222 | ↑ Whole adipose | Gestational diabetes mellitus | Human | — | Herrera et al. |
| miR-130 | ↑ Omental whole adipose | Gestational diabetes mellitus | Human (female) | — | Lee et al. |
| miR-130, -126, -193b | ↑ SubQ adipose | Gestational diabetes mellitus | Human (female) | — | Kim et al. |
| miR-129 | ↑ Urine | Normal weight with T2DM, normo- vs albuminaria | Human | — | Arner et al. |
| miR-10a, -200a, -409-5p, -125-3p | ↑ Hypothalamus | Perinatal leptin blockade and DIO | Rat (Wistar) | Adiponectin pathway | Benoit et al. |
| miR-200a, b, -420 | ↑ Hypothalamus | Genetic obesity and IR | Mouse (ob/ob, db/db) | Insulin and leptin pathways | Crepin et al. |
| miR-34a, -146a, -199a-3p, -203, -210, -383 | ↑ SubQ adipose | Increased beta cell apoptosis | Mouse (db/db), Human and Rat cells | — | Nesca et al. |
| miR-375 | ↑ Pancreas | Genetic obesity | Mouse (ob/ob), KO | — | Poy et al. |
| miR-375, -802 | ↑ Serum, ↑ Serum, Liver, epi WAT | T2DM vs NGT, SCD vs HFD | Human plasma, Mouse | — | Higuchi et al. |

Abbreviations: CCL2, chemokine (C-C motif) ligand 2; DIO, diet-induced obesity; HFD, high-fat diet; IR, insulin resistance; miRNA, microRNA; NGT, normal glucose tolerance; PPARγ, peroxisome proliferator-activated receptor gamma; SCD, standard chow diet; T2DM, type 2 diabetes mellitus.
2011, Lee et al. showed lower miR-130a and miR-130b expression in the abdominal subcutaneous adipose tissue of non-diabetic, obese women compared with lean subjects, although murine models have conflicting findings (Table 1). Plasma miR-130 expression was found to be lower in obese humans. miR-130a is highly expressed in human endothelial cells. Interestingly, patients with

### Table 2. Circulating miRNAs in human cardiometabolic disease

| miRNA     | Finding(s) | Intervention/phenotype | Model                  | Target/pathway | Author     |
|-----------|------------|------------------------|------------------------|----------------|------------|
| miR-221, -130b, -142-3p | ↓, ↑ Plasma | Obesity                | Human (children)       | —              | Prats-Puig et al. |
| miR-17-5p | ↓ Plasma    | Obesity                | Human                  | —              | Heneghan et al. |
| miR-17-5p | ↓ Plasma    | Coronary artery disease | Human                  | —              | Fichtlscherer et al. |
| miR-326, Let-7a, f | ↑, ↓ Plasma exosomes | Treatment naive diabetic | Human                  | —              | Santovito et al. |
| Let-7b, miR-130a, | ↑, ↓ Serum | Lower glycemic index diet | Human                  | —              | McCann et al. |
| miR-221, -130b, -423-5p | ↓ Plasma | Obesity                | Human                  | —              | Ortega et al. |
| miR-221, let-7g | ↓ Plasma | Metabolic syndrome, females | Human (♀)              | —              | Wang et al. |
| miR-222, -142-3p | ↓ Plasma | Obesity                | Human                  | —              | Ortega et al. |
| miR-222 | ↓ Plasma HDL | Familial hypercholesterolemia vs normo- | Human              | —              | Vickers et al. |
| miR-29b, -223, -126 | ↓ Plasma | T2DM vs matched controls | Human                  | —              | Zampetaki et al. |
| miR-15b | ↓ Plasma | Obesity/T2DM            | Human                  | —              | Pescador et al. |
| miR-138, -376a, -503 | ↓ Plasma | Obesity/T2DM            | Human                  | —              | Pescador et al. |
| miR-34a, -375 | ↓ Plasma | T2DM vs IGT and NGT    | Human                  | —              | Kong et al. |
| miR-122 | ↓ Plasma | Severe NASH            | Human                  | —              | Miyaiya et al. |
| miR-375, -802 | ↓ Serum, | T2DM vs NGT            | Human                  | —              | Higuchi et al. |

Abbreviations: HDL, high-density lipoprotein; IGT, impaired glucose tolerance; miRNA, microRNA; NASH, nonalcoholic steatohepatitis; NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus.

### Table 3. Adipocytes: miRNAs involved in adipogenesis, lipogenesis and drug effects

| miRNA     | Finding(s) | Intervention/Phenotype | Model                  | Target/pathway | Author     |
|-----------|------------|------------------------|------------------------|----------------|------------|
| miR-222, -29a, -27a | ↑          | High glucose media    | 3T3-L1 cells (mouse)   | —              | Herrera et al. |
| miR-302a, -664, -1264, -378 | ↑          | Pioglitazone treatment | Human primary pre-adipocytes from subQ | PPARγ | Yu et al. |
| miR-338-5p, -143, 30b, -378 | ↑          | Pioglitazone treatment | Human primary pre-adipocytes from visceral adipocytes | — | Yu et al. |
| miR-378 | ↑ Adipose, ↓ lipolysis in vitro | Cachexia, inhibited miRNA | Human, mouse           | ERK5           | Esau et al. |
| miR-375 | ↑ Adipogenesis | Overexpressed miRNA | 3T3-L1 cells (mouse)   | —              | Li et al. |
| miR-143 | ↑, Blocked differentiation | Overexpressed miRNA | Human and mouse adipocytes | CCL2 | Xie et al. |
| miR-143 | ↑, ↓ Adipogenesis, in obesity models -TNFα treatment | Overexpressed miRNA | Human and mouse adipocytes | CCL2 | Arner et al. |
| miR-126, -193b, -143, Let-7d | CCL2 secretion decreased | Overexpressed miRNA | Human and mouse adipocytes | CCL2 | Arner et al. |
| Let-7 | ↑ Adipogenesis | Overexpressed miRNA | Human and mouse (3T3-L1) | HMGA2 | Sun et al. |
| miR-344 | ↑ Adipogenesis | Overexpressed miRNA | 3T3-L1 cells (mouse)   | —              | Wei et al. |
| miR-34a | ↑ Adiposity, ↑ Browning of white fat | Overexpressed miRNA | Mouse                  | GSK3βi | Chen et al. |
| miR-130a, -130b | ↑ Adipogenesis | Overexpressed miRNA | Human pre-adipocytes   | PPARγ | Lee et al. |
| miR-130b | ↑ Fat accumulation | Overexpressed miRNA | Porcine adipocytes     | PPARγ | Pan et al. |
| miR-130 | ↑ | TNFα treatment | 3T3-L1 cells (mouse)   | PPARγ, NfxB (p65) pathway | Kim et al. |
| miR-181a | ↑ Lipogenesis | Overexpressed miRNA | Porcine adipocytes     | TNFα, lipogenic/ lipolytic pathways BMP2 | Li et al. |
| miR-17-5p, 106a | ↑ Adipogenesis, ↓ Adipogenesis | Overexpressed, inhibited miRNA | Human adipose-derived mesenchymal stem cells | — | Li et al. |
| miR-145, let-7d | ↑ Lipolysis and TNFα release, ↓ lipolysis | Overexpressed miRNA | Human adipocytes        | ADAM17/NF-κB and HSL pathways | Lorente-Cebrian et al. |
| miR-21 | ↑, Improved PI3K/Akt signaling | High glucose media, overexpressed miRNA | 3T3-L1 cells (mouse)   | PTEN | Ling et al. |
| miR-222 | ↑ Insulin-stimulated glucose uptake | Inhibited miRNA | 3T3-L1 cells (mouse)   | ERx, GLUT4 | Shi et al. |
| miR-103, -107 | ↑ Insulin sensitivity | Inhibited miRNA whole body | Mouse                  | CAV1/Insulin signaling | Trajkovski et al. |
| miR-145 | ↑ Lipolysis in vitro | Inhibited miRNA | Mouse primary adipocytes | FOXO1, 1ABHD5/ KSRP pathway | Lin et al. |
| miR-34a | ↑ Browning of fat | Inhibited miRNA | Mouse                  | PPARγ | Fu et al. |

Abbreviations: BMP2, bone morphogenetic protein 2; miRNA, microRNA; NF-κB, nuclear factor kappa b; PPARγ, peroxisome proliferator-activated receptor gamma; PTEN, phosphatase and tensin homolog; TNFα, tumor necrosis factor alpha.
coronary artery disease have lower circulating miR-130a expression. However, these lower levels of miR-130a may be due to increased cardiovascular risk, metabolic disease or both. In contrast, it should be mentioned that miR-130b was found to be higher in the plasma of obese children (9 years of age, Table 2).54 Bioactive miR-130 in the plasma may affect distal tissue when transported by and taken up from the circulation. Microvesicular transport of plasma miRNA is a putative mechanism by which miRNAs could be mediators of local and systemic IR, similar to cytokines. Pan et al.55,56 reported that HeLa cells overexpressing miR-130b produced microvesicles capable of decreasing adipogenesis and peroxisome proliferator-activated receptor gamma expression in cultured porcine adipocytes. Although these experiments do not provide in vivo relevance, they support the concept of microvesicle-mediated miRNA transport by the plasma.

The let-7 family
Let-7 is well-conserved across species, has a substantial role in developmental processes, is considered an 'oncogenic' miRNA in vertebrates and has a substantial role in metabolic disease. Let-7 was the first human miRNA discovered,57 and the family contains 11 members on three chromosomes in humans. Transgenic mouse experiments have shown that let-7 is a potent regulator of glucose metabolism and peripheral IR, by targeting IGF1R, insulin receptor (INSR) and insulin receptor substrate-2 (IRS-2) in skeletal muscle (Table 4) and liver tissues.58 Let-7 and the RNA binding protein Lin28 form a regulatory axis affecting insulin production and sensitivity. Lin28 overexpressing mice fed a HFD had dramatically improved glucose metabolism via inhibition of let-7 (as well as let-7-independent mechanisms). Similarly, let-7 overexpressing mice demonstrated glucose intolerance and peripheral IR on a normal or high-fat diet, despite increased insulin production and secretion. Frost and Olson59 reported complementary findings with global and pancreas-specific let-7 overexpression. Let-7 anti-miR administration partially ameliorated the effect of HFD on IR measures and liver TG accumulation in mice through anti-let-7-mediated insulin receptor derepression (Table 5). Let-7 also has a role in other tissues including skeletal muscle, where Let-7a and -7d were higher in the skeletal muscle tissue of T2DM patients compared with body mass index-matched NGT controls.60 In addition, let-7 may repress the
anti-inflammatory Th2 cytokine interleukin 13 (IL-13) in myotubes, suggesting that let-7 potentially modulates muscle inflammation via IL-13 repression.\textsuperscript{60} Let-7 has substantial potential as a biomarker of metabolic disease. In a human interventional study reducing the glycemic load in the diet of healthy premenstrual women, let-7b was the most dramatically altered microRNA, with nearly an eightfold increase of plasma let-7b after 12 months (Table 2).\textsuperscript{61} Similarly, when comparing plasma exosomes from obese diabetic patients naive to treatment and normal patients, Let-7a and -7f were found to be lower in the obese cohort.\textsuperscript{62} Interestingly, after receiving naive to treatment and normal patients, Let-7a and -7f were found when comparing plasma exosomes from obese diabetic patients to be more highly expressed in the amnion of obese pregnant women.\textsuperscript{62}

### Table 4. Skeletal muscle: miRNAs in peripheral IR

| miRNA   | Finding(s) | Intervention/phenotype | Model | Target/pathway                     | Author                  |
|---------|------------|------------------------|-------|------------------------------------|-------------------------|
| miR-133a, -143, -144 | ↑, ↑ | Obese (NGT, IGT, T2DM) | Human (n = 118 total) | — | Gallagher et al.\textsuperscript{101} |
| miR-494 | ↑, ↑ | Insulin resistance | TNFα treatment | Mouse C2C12 cells (ob/ob) | Insulin signaling AdipoR1/Adiponectin and PTB pathway INSR (muscle) | Lee et al.\textsuperscript{144} |
| miR-221 | ↑ | Genetic IR | Mouse (ob/ob) | — | Lustig et al.\textsuperscript{71} |
| Let-7   | ↑ | Insulin resistance, protected from DIO-IR | Overexpressed—Global and pancreas, inhibited miRNA—whole body | Mouse | Frost et al.\textsuperscript{29} |
| Let-7a, d | ↑, ↑ | Insulin resistance ↓ IL-13 secretion | Overweight (NGT vs T2DM), Overexpressed miRNA | Human, primary human myotubes | IL-13 | Jiang et al.\textsuperscript{30} |
| Let-7   | ↑ | Insulin resistance | Overexpressed miRNA—muscle specific | Mouse | Lin28a, IGF1R, INSR, IRS-2, Mfn2, mitochondrial dysfunction | Zhu et al.\textsuperscript{58} |
| miR-106b | ↑, ↑ | Insulin resistance, | TNFα treatment, overexpressed miRNA | Mouse C2C12 myotubes | — | Zhang et al.\textsuperscript{145} |
| miR-10b | ↓ | Skeletal muscle | Genetic IR (non-obese) | Rat (GK, WKY, BN) | — | Herrera et al.\textsuperscript{66} |

Abbreviations: AdipoR1, adiponectin receptor 1; DIO, diet-induced obesity; IGT, impaired glucose tolerance; INSR, insulin receptor; IR, insulin resistance; miRNA, microRNA; NGT, normal glucose tolerance; PTB, polyomaviridae tract-binding protein; T2DM, type 2 diabetes mellitus; TNFα, tumor necrosis factor alpha.

### Table 5. miRNAs involved in hepatic glucose homeostasis

| miRNA   | Finding(s) | Intervention/phenotype | Model | Target/pathway | Author                  |
|---------|------------|------------------------|-------|----------------|-------------------------|
| miR-143 | ↑ | Insulin resistance | Genetic and DIO models | Mouse (db/db) | ORP8 | Jordan et al.\textsuperscript{58} |
| miR-145 | ↑ | Insulin resistance | SCD fed, Resistin injected (6 days), overexpressed miRNA | Mouse (C57BL/6J), HepG2 cells | Grb10 | Wen et al.\textsuperscript{51} |
| miR-130a | ↓ | Insulin resistance and steatosis | Adenoviral overexpressed | Grb10 | Xiao et al.\textsuperscript{134} |
| Let-7   | ↑ | Insulin resistance, protected from DIO-IR and fatty liver | Overexpressed—Global and pancreas, whole-body miRNA inhibition | Mouse | INSR, IRS-2 (liver) | Frost and Olson\textsuperscript{59} |
| miR-195, -103 | ↑ | Insulin resistance | Genetic IR (non-obese) | Rat (GK, WKY, BN) | — | Herrera et al.\textsuperscript{56} |
| miR-21  | ↑ | Insulin resistance | Unsaturated fatty acid treatment | Rat hepatocytes | PTEN | Vinciguerra et al.\textsuperscript{135} |
| miR-802 | ↑, ↑ | Insulin resistance | HFD vs SCD, overexpressed miRNA | Mouse and human | HNF1B | Kornfeld et al.\textsuperscript{136} |
| miR-181a | ↑, ↑ | Insulin resistance - SIRT1 | Genetic obesity/IR, overexpressed miRNA | Mouse (db/db), HepG2 cells | SIRT1 | Zhou et al.\textsuperscript{137} |
| miR-103, -107 | ↑, ↑ | Insulin resistance, improved glucose tolerance | Hepatic miR-107, overexpression, inhibition of miRNAs | Mouse (C57BL/6J) | CAV1 | Trajkovski et al.\textsuperscript{133} |
| miR-29 | ↑, ↓ | miRNA expression | HFD vs SCD mice, pioglitazone treatment | Mouse and Rat (fa/fa) | PPARGC1A, HMGC2, SREBP1c, DGAT2, Cpt1α | Kurtz et al.\textsuperscript{90} |
| miR-370 | ↑ | Lipogenesis, Lipogenesis | Overexpressed miRNA, inhibited | Human HepG2 cells | — | Liopoulos et al.\textsuperscript{138} |
| miR-221 | ↓ | AdipoR1 | Overexpressed miRNA | Human HepG2 cells | — | Lustig et al.\textsuperscript{71} |
| miR-126-3p, -24-3p | ↓ | Genetic obesity/IR | Genetic obesity/IR | Mouse (ob/ob) | AdipoR1/Adiponectin and PTB pathway | Liang et al.\textsuperscript{139} |
| miR-200s (miR-200a,b,c) | ↓ | Genetic obesity/IR | Genetic obesity/IR | Mouse (ob/ob) | — | Dou et al.\textsuperscript{27} |
| miR-122 | ↓, ↓ | Steatosis, Plasma cholesterol | Inhibited miRNA | Mouse (ob/ob) | FOG2, IL6 | Esau et al.\textsuperscript{40} |
| miR-122 | ↓ | Plasma, HDL, Reverse cholesterol transport | Mild vs severe steatosis/NAFLD Inhibited miRNA | Human (Ldlr<sup>−/−</sup> — western diet) | ABCA1/SREBF2 | Miyaaki et al.\textsuperscript{141} |
| miR-22 | ↓ | Plasma | — | — | — | Rayner et al.\textsuperscript{142,143} |

Abbreviations: AdipoR1, adiponectin receptor 1; DIO, diet-induced obesity; HDL, high-density lipoprotein; HFD, high-fat diet; INSR, insulin receptor; IR, insulin resistance; miRNA, microRNA; NGT, normal glucose tolerance; PTB, polyomaviridae tract-binding protein; T2DM, type 2 diabetes mellitus; TNFα, tumor necrosis factor alpha.
women (Table 1). Finally, let-7 has been reported in adipogenesis, with overexpression of let-7 in pre-adipocytes resulting in reduced adipogenesis. Taken together, the data strongly suggest that members of the let-7 family modulate systemic insulin sensitivity and glucose metabolism by effects on the insulin signaling/Pi3K and mTOR pathways and may have significant potential as a blood biomarker of glycemic control and metabolic disease.

The miR-221/222 family

The miR-221 family in mice and humans consists of two members, miR-221 and miR-222, both located in close proximity on the X chromosome and both linked to metabolic disease. With the most references in plasma miRNA-based reports, miR-222 shows arguably the greatest promise as a clinical biomarker of metabolic disease. Functionally, miR-222 is a negative regulator of adipocyte insulin sensitivity in humans and rodents. Adipose levels of miR-222 are elevated in murine models of diabetes. Human data from Shi et al. also reported higher miR-222 expression in the omental adipose tissue of women with gestational diabetes at the time of cesarean delivery compared with pregnant women with NGT (Table 1). MiR-222 seems to negatively regulate adipocyte insulin sensitivity via repression of ERα and GLUT4. MiR-222 was found to be significantly higher in the whole plasma of two distinct cohorts of obese human patients. Similarly, high-density lipoproteins (HDL) isolated from patients with familial hypercholesterolemia had 8.2-fold higher levels of miR-222 than HDL from normal patients (Table 2). The functional significance of this differential in circulating miR-222 expression is unknown, however, plasma transport of this miRNA to adipose tissues may affect glucose metabolism. It should be mentioned that circulating miR-222 levels may be rapidly altered by insulin administration. These reports suggest a role for miR-222 in adipocyte insulin sensitivity, hormone-induced IR in white adipose tissue and as a putative circulating marker for diabetes and cardiovascular disease.

miR-225 may regulate IR via effects on adiponectin expression. In cultured human adipocytes, miR-225 mimics repressed adiponectin expression. Recently, Lustig et al. showed that miR-221 was selectively increased in the livers of ob/ob mice, but not in muscle, even though miR-221 repressed adiponectin receptor 1 in liver and muscle cells in vitro. In vitro miR-221 levels of human adipocytes were negatively correlated with tumor necrosis factor alpha mRNA levels and body mass index of donors. Conversely, subcutaneous adipose expression of miR-221 was positively correlated with body mass index in non-diabetic Pima Indians. Ortega et al. reported decreased plasma miR-221 levels in obese humans, while Wang et al. reported increased miR-221 in non-obese females with metabolic syndrome vs controls. The disparity in these findings may be owed to methodological differences or undefined variables in the cohorts. Despite the conflicting human findings, miR-221 may potentiate the development of IR via suppression of adiponectin signaling and shows potential as a plasma marker.

The miR-200 family (miR-8/miR-200 family)

Studies of obesity on hypothalamic leptin/food intake mechanisms have revealed a putative role for miR-200a in food intake regulation and body mass accumulation. Benoit et al. administered a leptin antagonist to Wistar rats shortly after birth. Leptin antagonism resulted in increased IR and increased miR-200a in the hypothalamus (interestingly, hypothalamic miR-222 was also upregulated). Rats were then fed a HFD. The leptin-antagonized group exhibited significantly higher hypothalamic miR-200a levels than control rats on the same diet. Interestingly, when fed a chow diet, leptin-antagonized rats had lower miR-200a levels than control rats. In a subsequent study using ob/ob, db/db mouse models, hypothalamic miR-200a was increased in obesity, with leptin treatment resulting in normalization/downregulation of miRNA expression. Importantly, miR-200a inhibition in the hypothalamus of ob/ob mice increased leptin receptor expression, reduced body weight and improved markers of whole-body insulin sensitivity. Differential miR-200 expression has also been reported in the livers of db/db mice where the visceral white adipose of HFD-fed mice where it likely has an important role in adipogenesis via regulation of the Wnt pathway. These data present a strong case for miRNAs, especially miR-200a in food intake and appetite regulation making miR-200 a significant candidate for future investigation.

miR-223

miR-223 is the lone member of the family and resides on the X chromosome. Although immune cell-mediated (innate and adaptive) inflammation is known to have a significant role in obesity and IR, there are few miRNAs linked to macrophage ‘polarisation’ in the context of obesity. Though the most thoroughly investigated function of miR-223, outside of cancer, involves monocyte–macrophage differentiation and macrophage activation, differential miR-223 expression has been linked to both human and murine obesity (Figure 2). Zhuang et al. showed that HFD-fed miR-223 knockout mice had worsened IR and that miR-223 deficient macrophages showed increased inflammatory potential compared with wild type macrophages. The increased inflammatory stress in knockout animals was hypothesized to exacerbate obesity-related metabolic disease through derepression of PBX/knotted 1 homeobox 1 (Pknx1). miR-223 is associated in human circulation with vesicles/exosomes, lipoproteins and AGO2 complexes. In humans, plasma miR-223 was reported to be lower in T2DM patients vs normal multivariable-matched controls. Perhaps conversely, HDL isolated from patients with familial hypercholesterolemia had 3781-fold greater miR-223 than HDL from normal patients. The functional significance of differential plasma miRNA expression in metabolic disease is unknown at this time, though a repressive effect by miR-223 delivered by an exosomal fraction has been demonstrated experimentally. Other miRNAs implicated in adaptive and innate immune activation/polarization with the potential to have a role in obesity-mediated inflammation are: miR-155, miR-107 and miR-146-5p. These miRNAs functioned through regulation of the transcription factor DIO mice and in Zucker Diabetic Fatty (fa/fa) rats. In this model, miR-29 functioned through regulation of the transcription factor FOXA2, (FOXA2-mediated regulation of PPARC1A, HMGC52 and ABHD5). Interestingly, pioglitazone treatment normalized miR-29 levels in both murine models. Obesity in pregnant sheep leads to increased miR-29 expression in the liver and regulates liver cholesterol biosynthesis and HDL uptake through repression of various targets, suggesting a role in cardiovascular implications of obesity.

The miR-29 family

The miR-29 family has four members, miR-29a, b-1, b-2 and c on two chromosomes. Since 2007, miR-29 has been known to negatively regulate insulin signaling in adipocytes, however, a definitive mechanism has not been elucidated. Recently, however, Kurtz et al. showed that miR-29 was upregulated in the livers of DIO mice and in Zucker Diabetic Fatty (fa/fa) rats. In this model, miR-29 functioned through regulation of the transcription factor FOXA2 (FOXA2-mediated regulation of PPARC1A, HMGC52 and ABHD5). Interestingly, pioglitazone treatment normalized miR-29 levels in both murine models. Obesity in pregnant sheep leads to increased miR-29 expression in the liver and regulates liver cholesterol biosynthesis and HDL uptake through repression of various targets, suggesting a role in cardiovascular implications of obesity.
whereas upregulation in metabolic tissues may impair insulin signaling.

Perhaps more importantly, miR-29b shows greatest promise as a biomarker for T2DM and atherosclerotic disease. Zampetaki et al. examined plasma miRNA expression from a relatively large prospective human cohort (n = 822). They reported lower plasma miR-29b (and miR-223 expression) in T2DM patients vs controls matched for multiple variables in a smaller cohort (Table 2). Others have reported increased circulating miR-29a in T2DM. Peng et al. examined urinary miRNA expression in patients with T2DM, with a focus on diabetic nephropathy and atherosclerotic measures. miR-29a (but not b or c) was higher in patients with albuminuria, a measure of diabetic nephropathy, compared with normoalbuminurimic patients (Table 1). miR-29b was also positively correlated with intimal thickness in patients. miR-29 may be a useful marker for cardiometabolic disease, especially the atherogenic risk associated with obesity in humans.

miR-375

The role of miRNAs in the pancreas is well-studied, see Plaisance et al. for a current review. One miRNA in particular, miR-375, has an important role in multiple tissues including the pancreas during obesity. miR-375 is highly expressed in pancreatic beta cells and is important in B cell maintenance, potentiating increased insulin production during murine IR.59,96 Mice lacking miR-375 exhibit hyperglycemia owing to decreased insulin production (lack of beta cell expansion), while ob/ob mice have increased miR-375 expression.96 Interestingly, miR-375 and miR-184 form a network with AGO2, which likely regulates pancreatic beta cell expansion in human and murine IR. miR-184 represses AGO2 which in turn modulated miR-375 functionality. Processes that regulate the RNA-induced silencing complex, in this case differential expression of another miRNA, have the potential to affect gene and protein expression via modulation of miRNA function. There is also evidence that pancreatic miR-375 is involved with inherited metabolic abnormalities in rats.96 miR-375 was significantly increased in the plasma of T2DM humans vs control groups in two studies (Table 2),92,99 and miR-375 promotes adipogenesis in mouse pre-adipocytes via regulation of ERK1/2 signaling upstream of peroxisome proliferator-activated receptor gamma (Table 3),100 The role of miR-375 in obesity and IR warrants continued attention.

Skeletal muscle miRNAs

The role of miRNAs in human skeletal muscle glucose metabolism is potentially important, though not well investigated (Table 4). Gallagher et al. published the only comprehensive profiling study of skeletal muscle miRNA expression in IR/T2DM. The authors examined three groups with relatively robust sample sizes (T2DM n = 45, IGT n = 26, NGT n = 47) by percutaneous needle biopsy. All the patients were characterized by oral glucose tolerance testing. Profiling identified 29 miRNAs, some of which are known to be important in obesity-related mechanism in other tissues. Significantly upregulated miRNAs include miR-143 and miR-144, and downregulated miRNAs include miR-133a and miR-126-5p. miR-133a was most consistently inversely correlated with fasting glucose, HbA1c, and 2-h glucose tolerance. It should be noted that there were no significant differences in miRNA expression. miR-126 was shown in another study to be lower in patients with T2DM vs a normal glucose tolerance group.81 It seems that multiple, muscle-specific miRNAs (‘myomiRs’) are regulated in diabetes though the pathophysiological consequences of this differential are not currently understood and suggest that further investigation is warranted.

**DISCUSSION OF MICRORNAS AND MICRORNA-BASED TECHNOLOGIES IN TRANSLATIONAL MEDICINE AND HUMAN HEALTH**

miRNAs have both a compensatory and pathophysiological role in metabolic tissues during obesity, the development of IR, and T2DM. In addition, circulating and biofluid-based miRNAs measures can lend insight into distal tissue miRNA changes, microvesicle and lipoprotein miRNA enrichment and can be used as biomarkers or ‘miRNA signatures’ of disease.

miRNAs have a role in transgenerational obesity and metabolic dysfunction

The development of obesity in children is a complex process involving genetic and epigenetic predisposition and various external influences. The exposure of the developing fetus to abnormal metabolic conditions (hyperglycemic, hyperinsulinemic, low-nitrogen and so on) alters genomic methylation affecting gene expression in childhood, adulthood and in subsequent generations, creating a predisposition for metabolic disease and/ or obesity. There is strong evidence that epigenetic mechanisms affect metabolic fitness, with certain genomic regions (imprinted loci) prone to altered methylation. It is estimated that 7% of miRNA coding regions are located in imprinted loci in humans. Epigenetics of obesity as it relates miRNAs is little studied, though it offers great potential to expand our understanding of transgenerational predisposition to metabolic disease. A Pubmed search for ‘(microRNA AND (epigenetic))’ AND obesity’ resulted in 26 publications and ‘(miRNA promoter methylation) AND obesity’ resulted in only three. Kameswaran et al. showed that the MEG3-DLK1 locus was hypermethylated in the beta cells of T2DM patients, significantly decreasing the expression of miRNAs coded by this locus. Although Kameswaran et al. are the only researchers to demonstrate altered miRNA promoter methylation in T2DM, there is evidence to suggest that miRNA expression in offspring may be affected by maternal nutrition. HFD feeding in mouse dams resulted in altered hepatic miR-122 and miR-370 expression in offspring, though this was not proven to be because of epigenetic mechanisms. Nevertheless, miR-122 and miR-370 have been shown to have a causal role in hepatic lipid metabolism. Similarly, maternal obesity in ewes increased offspring hepatic expression of miRNAs well known to be associated with metabolic disease in humans and rodents (miR-29b, -103, -107, -143). miRNAs likely mediate the phenotypes observed in obesity-related, transgenerational metabolic disturbances.

miRNAs as biomarkers

The presence of miRNAs in human biofluids (plasma, serum, urine, tears, saliva, colostrum, amniotic, cerebrospinal and seminal fluid) has resulted in the pursuit of miRNA-based biomarkers (miRNA signatures) for multiple diseases including cardiometa-bolic disease (Table 2). With the proper development, miRNA-based biomarkers have the potential to identify metabolic problems during disease latency (preclinical), assess severity of disease, identify patients with a predisposition to metabolic disease (assess risk), address disease etiology, confirm diagnosis/ reduce misdiagnosis on the basis of current clinical markers and monitor response to interventions. The relative postprandial stability of some plasma miRNAs (vs other measures such as blood glucose or insulin) as well as the unique kinetic responses to therapy are potentially attractive aspects of their implementation in a clinical setting that have not been explored. There is also potential utility in subgroups of patients not optimally served by current clinical measures (overnight fasting) such as the elderly, children, persons of a non-Caucasian background, pre-diabetic patients with normal fasting glucose who have prolonged
postprandial hyperglycemia, or otherwise healthy people consuming a high glycemic diet.

Plasma miRNAs have great potential as biomarkers as they have been shown to have excellent stability\(^{106}\) at room temperature and during multiple freeze–thaw cycles probably owing to association with AGO2 complexes,\(^{82}\) lipoproteins\(^{70}\) and enrichment in circulating vesicles. The first step in developing a biomarker, showing a statistically relevant difference between a healthy and metabolic disease cohort, has clearly been met by multiple studies identifying numerous plasma miRNAs that are associated with metabolic disease in a range of demographics (children, women, during pregnancy, IR at low body mass). However, it is also clear that there are marked inconsistencies in the findings for specific plasma miRNAs from different groups (for example, miR-223). This is, in part, likely owing to differences in RNA isolation, detection and normalization methodologies; a recent methods publication compares platforms.\(^{107}\) This highlights the need for increased standardization of plasma-based miRNA measures to optimize comparison of findings by different groups and to address stability of putative biomarker over short time periods and in the presence of various stimuli. For instance, plasma miR-222 appears to be a top biomarker candidate based on multiple reports, but its use may be problematic because of rapidly changing levels of expression dependent on variables such as insulin.\(^{59}\)

For the most part, plasma-based research has not furthered the development of plasma miRNA biomarkers of metabolic disease, including necessary risk prediction analysis such as odds ratio and risk ratio calculations or diagnostic analysis such as receiver operating characteristic curve analysis and likelihood ratios (positive/negative LHRs); only two publications performed these analyses (odds ratio,\(^{83}\) receiver operating characteristic curves\(^{108}\)). Furthermore, analysis to show the value of plasma miRNA biomarkers compared with current clinical markers in patient care is absent from the literature, but is needed. The proper statistical handling and development of clinical biomarkers is vital to the advancement of this area.\(^{109}\) It is important to note that there are no reports comparing plasma miRNAs in humans with allelic variants associated with T2DM. Longitudinal studies in patients with and without known genomic variants and the development of metabolic disease have the potential to yield important information regarding prognostic and predictive value of plasma miRNAs compared with the penetrance of known genomic variants.

miRNAs as therapeutics

Given the role of miRNAs in multiple pathways with pathophysiological relevance to IR/obesity, there is great interest in miRNA-based therapeutic strategies. However, at the time of writing, there were 10 registered clinical trials at clinicaltrials.gov (search: ‘(microRNA AND obesity)’ none of which aimed to test miRNA-based therapeutics in humans; all studies aim to measure miRNA expression in a disease state or in response to weight or exercise. Currently, anti-miRs are the most common in vivo approach when designing miRNA-based therapeutics.\(^{110}\) Anti-miRs sequester endogenous mature miRs, rescuing (derepressing) target gene expression. In 2012, van Rooij et al.\(^{10}^{10}\) published an exhaustive review on miRNA therapeutics covering the discovery, therapeutic development, main private industry players and patent issues associated with miRNA-based therapeutics. According to van Rooij, ’The key requirements for an anti-miR are that the chemistry must be cell permeable, cannot be rapidly excreted, must be stable in vivo and should bind to the miRNA of interest with high specificity and affinity.’ Anti-miRNAs chemistry (modifications to the sugar moiety, the nucleobase or the internucleotide linkages) is a topic of much interest, with the goal of increased stability (nuclease resistance), increased binding affinity and optimized in vivo functionality.\(^{36}\) Stenvang et al.\(^{36}\) published an excellent review of the available anti-miRNA oligonucleotides that have been developed.

Early evidence from preclinical trials in non-human primates shows encouraging pharmacokinetic properties of naked modified oligonucleotides including low toxicity. In 2008, Elmen et al.\(^{111}\) published findings in African green monkeys using intravenous injection of naked/unconjugated 15-mer locked nucleic acid (LNA)-anti-miR-122. The authors reported no detectable toxicity and long-lasting on-target effects. Rayner et al.\(^{112}\) administered 2′-fluoro/methoxyethyl modified anti-miR-33 (5 mg kg\(^{-1}\); Regulus Therapeutics, San Diego, CA, USA) to African green monkeys via subcutaneous injection 2× per week for 2 weeks, then once per week for 10 weeks. After 8 weeks, they reported a 50% increase in HDL cholesterol in anti-miR treated monkeys.\(^{112}\) Pleiotropic effects of miRNA therapy, however, are of concern owing to the number of possible miRNA targets/pathway that may be affected by administration of a potent miRNA mimic or inhibitor, especially with long-term administration. For instance, short-term anti-miR-33 in mice resulted in decreased atherosclerosis with no observed side effects,\(^{113}\) while long-term use led to increased circulating TG levels and hepatosteatosis suggesting that off-target/pleiotropic effects were significant. Thorough monitoring of multiple pathways are prudent when investigating the in vivo use of miRNA-based therapeutics.\(^{114}\)

Though currently not investigated in non-human primate models, ‘Tiny LNA’ technology, which uses 8-mer LNA-anti-miRs, shows particular promise in mice as a potential next step in in vivo anti-miR therapeutics. Obad et al.\(^{115}\) showed that tiny LNAs targeted the seed sequence of mature miRNAs and were able to derepress target genes as well as 15-mer LNA-anti-miRs. Theoretically full-length anti-miRs may have increased potential to bind nonspecifically in the translated portion of an mRNA, inducing an siRNA-like, knockdown effect. The authors showed that there were no significant off-target effects with tiny LNA technology when examining miRNAs with tiny LNA binding sites in the 5′ translated area, making tiny LNAs potentially superior to full-length LNA anti-miRs for in vivo studies, though additional comparisons are needed. The authors also showed that binding multiple mature miRNAs at non-seed sequences had little to no effect on miRNA activity. Interestingly, 8-mer anti-miR-21 modified by 2′-O-Me (instead of LNA technology) was not effective at blocking miRNA activity; the mechanism behind this is unknown. Technological advancements in miRNA research

Ultra-deep RNA-Seq technology is a major advancement in studying noncoding RNA species. Though small RNA-Seq is costly and analysis heavy compared with other detection platforms, it has the potential to identify novel obesity-associated change(s) in RNA species unavailable from current microarray and PCR-based platforms, such as miRNA isomers. miRNA ‘genes’ may produce miRNA transcript variants (‘isomiRs’) owing to processing or post-transcriptional modifications, such as the activity of ADARs as discussed in the introduction.\(^{116}\) Two novel 5′-shifted isomiRs of miR-375 have been identified in mouse insulinoma cells that have distinctly different targeting than ‘canonical’ miR-375.\(^{117}\) The authors also found that isomiRs had potential targets in genes associated with the development of T2DM.\(^{117}\)

Software-based tools are numerous with many online and propriety sources. Valuable online resources for miRNA-target interactions such as mirWalk,\(^{118}\) miR TAR Base\(^{119}\) and Targetscan, as well as databases specifically for circulating miRNAs\(^{120}\) that attempt to incorporate information from various online resources (PubMed, miRBase, miRO, DAVID and so on) and associate them with disease. However, annotation of these resources for obesity researches is limited. For instance, miRWalk yield no results for ‘obesity’ or ‘metabolic disease’. MiRandola search under ‘Diseases
and Malignant Cell Line”—term ‘obesity’ yielded three miRNAs such as miR-138, miR-15b, miR-376a; while term ‘metabolic syndrome’ yielded two miRNAs such as let-7g and miR-221. Advancements in online miRNA analytics specifically optimized for metabolic disease, in addition to journal-mandated submission of findings to an online database of miRNAs profiling (for example, miRandola) will significantly contribute to information processing as the field expands (biomarkers, off-target effects of mimetics/anti-miRs and so on).

CONCLUSION
Understanding the role of miRNAs in whole-body metabolism and the pathophysiology of metabolic disease has been challenged in part by the complexity of miRNA–mRNA interactions as well as methodologic and technical limitations, especially in vivo. Future studies utilizing available miRNA profiling data from human disease, combined with miRNA detection techniques that are more sensitive, accurate and flexible (small RNA sequencing, quantitative PCR methods for blood miRNAs), and advancing bioinformatics/biostatistical analyses of complex data sets will undoubtedly result in further clarification of miRNA-mediated pathways in cardiometabolic disease. Experimentally, the development of conditional and tissue-specific miRNA knockout mice will be especially valuable in studying miRNAs that are cell/tissue specific or whose deletions are embryonically lethal (for example, miR-133a). Reports of miRNA–mRNA relationships based on in vitro data may have limited physiologic relevance and must be properly vetted in vivo. From a therapeutics standpoint, advancements in miRNA-based mimics and inhibitors, with increased specificity and in vivo stability, will likely potentiate efficacy and support preclinical testing of additional miRNAs in non-human primates. In the near future, we will likely see the maturation of circulating miRNAs as biomarkers of metabolic disease with their eventual clinical use in metabolic disease testing, risk assessment, and/or grading of disease. Future advances will further clarify the role of miRNAs in (1) the circulation (vesicular, nonvesicular, lipoprotein-associated) and effects in distal tissues, (2) innate and adaptive immune cell-mediated inflammation during overnutrition (for example, visceral adipose), (3) central regulation of appetite and food intake, (4) lipoprotein metabolism and fibrosis in the liver during overnutrition, (5) beta cell expansion in overnutrition and failure in T2DM and (6) cross-generational effects of obesity, when well-defined miRNA–mRNA pathways are established.

CONFLICT OF INTEREST
The author declares no conflict of interest.

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