

REVIEW

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.⁸ 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

multicellular organisms studied to date, demonstrating astonishing evolutionary conservation between vastly dissimilar species (humans, mice, fish and nematodes).²² miRNAs have important molecular roles in normal physiology and development as well as in disease processes such as cancer and obesity.

miRNA biogenesis, post-transcriptional editing and function

In contrast to messenger RNAs (mRNAs), miRNAs are not translated. Mature miRNAs exert biological effects by regulating the post-transcriptional regulation of protein-coding mRNAs via two recognized mechanisms: target transcript degradation/decay and inhibition of target transcript translation. There are an estimated ~45 000 miRNA-targeting sites in the human genome, affecting the expression of ~60% of genes.²³ miRNA genes can be coded by the intronic regions of protein-coding genes (intronic miRNAs) or by sequences outside protein-coding genes (intergenic miRNAs).²⁴ In addition, miRNAs genes are often found in polycistronic clusters (independent of intronic or intergenic genomic organization), with one transcriptional event (RNA polymerase II) resulting in a primary miRNA (pri-miRNA) strand with multiple stem-loop structures, which may be canonically processed into multiple miRNAs. Pri-miRNA transcripts in the nucleus, whether mono or polycistronic, undergo trimming and processing by the Drosha, DGCR8 RNase III complex into pre-miRNAs before export into the cytoplasm by exportin-5/GTP61, as shown in Figure 1.²⁵ In the cytoplasm, the Dicer complex (Dicer, AGO2 and TRBP) cleaves the hairpin loop of the cytoplasmic pre-miRNAs resulting in an miRNA/miRNA* duplex (two distinct complementarily bound RNA strands called the guide and passenger strands (*), respectively) and assists in the loading of either strand into the miRNA-induced silencing complex.²⁶ The miRNA-induced silencing complex is composed of Argonaute 2 (AGO2) and GW182 proteins in association with a single-stranded miRNA, most often the guide strand.²⁷ The miRNA-induced silencing complex is capable of binding to mRNAs that are targeted by 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 the miRNA can also bind mRNA and contribute to gene repression.²⁸

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,^{27,29} we will briefly touch on endogenous RNA editing processes³⁰ and their relevance to metabolic disease. A family of adenosine deaminases (ADARs) edits double-stranded RNA species, proximately 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-miRNAs) and Dicer, TRBP, AGO2 (pre-miRNAs) cleavage may occur depending on the location of A-to-I conversion.^{31,32} A-to-I conversions are best detected by next-generation sequencing, which will be discussed later in this review.³³ 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.³⁴ ADAR-catalyzed conversions occur mainly in noncoding RNA sequences including the introns and untranslated regions of mRNAs and small RNA species such as miRNAs, however, the processes controlling specificity of adenosine conversion is undefined.³⁴ 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.³⁵

Experimental approaches to miRNA research

Systemic and organ-specific knockouts/transgenic mouse strategies are available for some miRNAs/miRNA families (<http://rna.keck.ucsf.edu/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.*³⁶

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.*³⁷ 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 with 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.³⁷

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.*³⁷ 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.^{38–40} Heneghan *et al.*³⁹ confirmed lower than healthy control miR-17-5p expression in human omental adipose tissue as well as in blood from obese patients. Similar findings were also reported in human atherosclerosis by Fichtlscherer *et al.*³⁸ where miR-17 was significantly lower in the plasma of patients with coronary artery disease. Although none of these studies looked specifically at plasma exosomal miRNA expression,

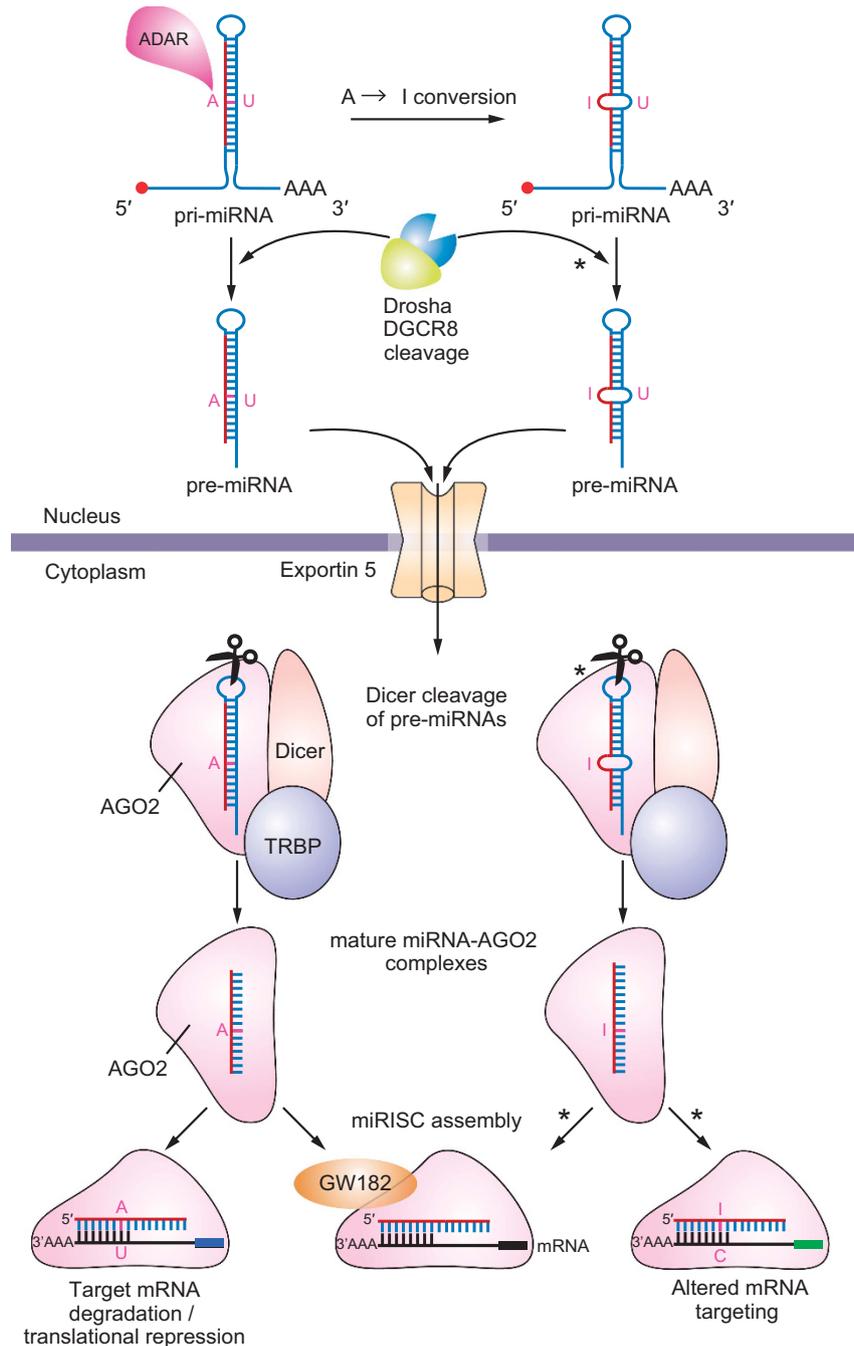


Figure 1. microRNA biogenesis and ADAR-mediated editing during miRNA maturation. ADAR, family of adenosine deaminase; miRISC, miRNA-induced silencing complex.

miR-17 is well known to be associated with the exosomal compartment (Table 2).⁴⁰ Interestingly, immunologic studies by Steiner *et al.*⁴¹ suggest that miR-17/92 family members potentiate T helper cell proliferation. Recently, Li *et al.*⁴² 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,⁴³ and consequently are often studied/reported concomitantly.

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 <i>et al.</i> ³⁷
miR-132, -17-5p, -143, -145	Omental, subQ fat, plasma	Obese vs lean (n = 50 total)	Human	—	Heneghan <i>et al.</i> ³⁹
miR-132, -184, -338-3p	↑, ↓, ↓	Improved beta cell mass and activity	Mouse (<i>db/db</i>), Human and Rat cells	—	Nesca <i>et al.</i> ¹²²
miR-143	↑ Mesenteric fat	DIO-IR, 45% E from fat	Mouse (C57BL/6J)	PPAR γ , aP2, leptin pathways	Takanabe <i>et al.</i> ⁴⁶
miR-143	↑ Insulin resistance, Protected from DIO-IR	Conditional overexpression of miR-143, miR-143 deficient	Mouse	ORP8	Jordan <i>et al.</i> ⁴⁸
miR-27a,	↑ Omental whole adipose, Change not significant	Obese vs lean (n = 15 vs 15)	Human	—	Viesti <i>et al.</i> ¹²³
miR-143, -145	Omental, subQ and liver difference not significant	Obese vs lean (n = 15 vs 15)	Human	—	Viesti <i>et al.</i> ¹²³
Let-7	↑ Insulin resistance, Protected from DIO-IR	Overexpressed—global and pancreas, whole-body inhibition	Mouse	INSR, IRS-2	Frost and Olson ⁵⁹
Let-7-d	↑ Amnion	Obese pregnant women	Human	—	Nardelli <i>et al.</i> ⁶³
miR-222, -27a	↑ Whole adipose	Genetic IR (non-obese)	Rat (GK, WKY, BN)	—	Herrera <i>et al.</i> ⁶⁶
miR-222	↑ Omental whole adipose	Gestational diabetes mellitus	Human	—	Shi <i>et al.</i> ⁶⁸
miR-130	↑ Whole subcutaneous adipose	Obese vs lean (nondiabetic, normotensive)	Human (female)	—	Lee <i>et al.</i> ⁵²
miR-130	↑ Whole subcutaneous adipose	HFD vs SCD	Mouse	—	Kim <i>et al.</i> ¹²⁴
miR-126, -193b	↑ SubQ adipose	Obese, IR	Human (female)	CCL2	Arner <i>et al.</i> ¹²⁵
miR-29	↑ Urine	Normal weight with T2DM, normo- vs albuminuria	Human	—	Peng <i>et al.</i> ⁹³
miR-10a, -200a, -409-5p, -125-3p	↑ Hypothalamus	Perinatal leptin blockade and DIO	Rat (Wistar)	Adiponectin pathway	Benoit <i>et al.</i> ⁷⁵
miR-200a, b, -420	↑ Hypothalamus	Genetic obesity and IR	Mouse (<i>ob/ob</i> , <i>db/db</i>)	Insulin and leptin pathways	Crepin <i>et al.</i> ⁷⁶
miR-34a, -146a, -199a-3p, -203, -210, -383	-	Increased beta cell apoptosis	Mouse (<i>db/db</i>), Human and Rat cells	—	Nesca <i>et al.</i> ¹²²
miR-375	↑ Pancreas	Genetic obesity	Mouse (<i>ob/ob</i>), KO	—	Poy <i>et al.</i> ⁹⁶
miR-375, -802	↑ Serum, ↑ Serum, Liver, epi WAT	T2DM vs NGT, SCD vs HFD	Human plasma, Mouse	—	Higuchi <i>et al.</i> ⁹⁹

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; PPAR γ , peroxisome proliferator-activated receptor gamma; SCD, standard chow diet; T2DM, type 2 diabetes mellitus.

miR-143 was first identified as a positive regulator of human adipocyte differentiation in 2004 via effects on ERK5 signaling.⁴⁴ miR-143 is the only miRNA to date shown to be similarly regulated during human and mouse adipocyte differentiation (Figure 2).⁴⁵ 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,⁴⁶ and tumor necrosis factor alpha treatment decreased miR-143 expression suggesting that obesity-associated inflammation may dysregulate miR-143 expression affecting adipogenesis (Table 1).⁴⁷ Despite miR-143 being a positive regulator of adipogenesis, miR-143-145 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, oxysterol

binding protein-like 8.⁴⁸ 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 emerged in lipolysis. Obesity increased the expression of the miR-143-145 cluster in adipose tissue/adipocytes and liver of humans and mice,^{39,49-51} and overexpression of miR-145-enhanced tumor necrosis factor alpha secretion and lipolysis in human adipocytes *in vitro* via a nuclear factor kappa b mechanism.⁴⁹ A conflicting report, however, showed that miR-145 overexpression suppressed lipolysis.⁵⁰ Although significance of miR-145 in IR is under investigation, the genomic association with miR-143 suggests potential.

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

2011, Lee *et al.*⁵² showed lower miR-130a and -130b expression in the abdominal subcutaneous adipose of nondiabetic, 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.⁵³ 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 <i>et al.</i> ⁵⁴
miR-17-5p	↓ Plasma	Obesity	Human	—	Heneghan <i>et al.</i> ³⁹ Hulsmans <i>et al.</i> ⁴⁰
miR-17-5p	↓ Plasma	Coronary artery disease	Human	—	Fichtschcherer <i>et al.</i> ³⁸
miR-326, Let-7a, f	↑, ↓ Plasma exosomes	Treatment naive diabetic	Human	—	Santovito <i>et al.</i> ⁶²
Let-7b, miR-130a,	↑, ↓ Serum	Lower glycemic index diet	Human	—	McCann <i>et al.</i> ⁶¹
miR-221, -130b, -423-5p	↓ Plasma	Obesity	Human	—	Ortega <i>et al.</i> ^{53,69}
miR-221, let-7g	↑ Plasma	Metabolic syndrome, females	Human (♀)	—	Wang <i>et al.</i> ⁷⁴
miR-222, -142-3p	↑ Plasma	Obesity	Human	—	Ortega <i>et al.</i> ^{53,69}
miR-222	↑ Plasma HDL	Familial hypercholesterolemia vs normo-	Human	—	Vickers <i>et al.</i> ⁷⁰
miR-29b, -223, -126	↓ Plasma	T2DM vs matched controls	Human	—	Zampetaki <i>et al.</i> ⁸¹
miR-15b	↑ Plasma	Obesity/T2DM	Human	—	Pescador <i>et al.</i> ¹⁰⁸
miR-138, -376a, -503	↓ Plasma	Obesity/T2DM	Human	—	Pescador <i>et al.</i> ¹⁰⁸
miR-34a, -375	↑ Plasma	T2DM vs IGT and NGT	Human	—	Kong <i>et al.</i> ⁹²
miR-122	↓ Plasma	Severe NASH	Human	—	Miyaaki <i>et al.</i> ¹⁴¹
miR-375, -802	↑ Serum,	T2DM vs NGT	Human	—	Higuchi <i>et al.</i> ⁹⁹

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 <i>et al.</i> ⁶⁶
miR-302a, -664, -1264, -378	↑	Pioglitazone treatment	Human primary pre-adipocytes from subQ	PPAR γ	Yu <i>et al.</i> ¹²⁶
miR-338-5p, -143, 30b, -378	↑	Pioglitazone treatment	Human primary pre-adipocytes from visceral	PPAR γ	Yu <i>et al.</i> ¹²⁶
miR-378	↑ Adipose, ↓ lipolysis <i>in vitro</i>	Cachexia, inhibited miRNA	Humans, human primary adipocytes	ATGL	Kulyte <i>et al.</i> ¹²⁷
miR-375	↑ Adipogenesis	Overexpressed miRNA	3T3-L1 cells (mouse)	—	Ling <i>et al.</i> ¹⁰⁰
miR-143	↑, Blocked differentiation	Adipogenesis, inhibited miR-143	Human, mouse	ERK5	Esau <i>et al.</i> ⁴⁴ Kajimoto <i>et al.</i> ⁴⁵
miR-143	↑, ↓	Adipogenesis, in obesity models -TNF α treatment	Mouse primary adipocytes (<i>ob/ob</i> , DIO)	—	Xie <i>et al.</i> ¹²⁸
miR-126, -193b, -143, Let-7d	CCL2 secretion decreased	Overexpressed miRNA	Human and mouse adipocytes	CCL2	Arner <i>et al.</i> ¹²⁵
Let-7	↓ Adipogenesis	Overexpressed miRNA	Human and mouse (3T3-L1)	HMGA2	Sun <i>et al.</i> ⁶⁴ Wei <i>et al.</i> ⁶⁵
miR-344	↓ Adipogenesis	Overexpressed miRNA	3T3-L1 cells (mouse)	GSK3 β	Chen <i>et al.</i> ¹²⁹
miR-34a	↓ Adiposity, ↑ Browning of white fat	Lentiviral-mediated downregulation of miR-34a	Mouse	FGFR1, SIRT1	Fu <i>et al.</i> ¹³⁰
miR-130a, -130b	↓ Adipogenesis	Overexpressed miRNA	Human pre-adipocytes	PPAR γ	Lee <i>et al.</i> ⁵²
miR-130b	↓ Fat accumulation	Microvesicles enriched in miR-130b	Porcine adipocytes	PPAR γ	Pan <i>et al.</i> ^{55,56}
miR-130	↑	TNF α treatment	3T3-L1 cells (mouse)	PPAR γ , NF κ B (p65) pathway	Kim <i>et al.</i> ¹²⁴
miR-181a	↑ Lipogenesis	Overexpressed miRNA	Porcine adipocytes	TNF α , lipogenic/lipolytic pathways	Li <i>et al.</i> ¹³¹
miR-17-5p, 106a	↑ Adipogenesis, ↓ Adipogenesis	Overexpressed, inhibited miRNA	Human adipose-derived mesenchymal stem cells	BMP2	Li <i>et al.</i> ⁴²
miR-145, let-7d	↑ Lipolysis and TNF α release, ↓ lipolysis	Overexpressed miRNA	Human adipocytes	ADAM17/NF- κ B and HSL pathways	Lorente-Cebrian <i>et al.</i> ⁴⁹
miR-21	↓, Improved PI3K/Akt signaling	High glucose media, overexpressed miRNA	3T3-L1 cells (mouse)	PTEN	Ling <i>et al.</i> ¹³²
miR-222	↓ Insulin-stimulated glucose uptake	Inhibited miRNA	3T3-L1 cells (mouse)	ER α , GLUT4	Shi <i>et al.</i> ⁶⁸
miR-103, -107	↑ Insulin sensitivity	Inhibited miRNA whole body	Mouse	CAV1/Insulin signaling	Trajkovski <i>et al.</i> ¹³³
miR-145	↓ Lipolysis <i>in vitro</i>	Inhibited miRNA	Mouse primary adipocytes	FOXO1, ABHD5/KSRP pathway	Lin <i>et al.</i> ⁵⁰
miR-34a	Browning of fat	Inhibited miRNA	Mouse	FGF21, SIRT1	Fu <i>et al.</i> ¹³⁰

Abbreviations: BMP2, bone morphogenetic protein 2; miRNA, microRNA; NF- κ B, nuclear factor kappa b; PPAR γ , peroxisome proliferator-activated receptor gamma; PTEN, phosphatase and tensin homolog; TNF α , tumor necrosis factor alpha.

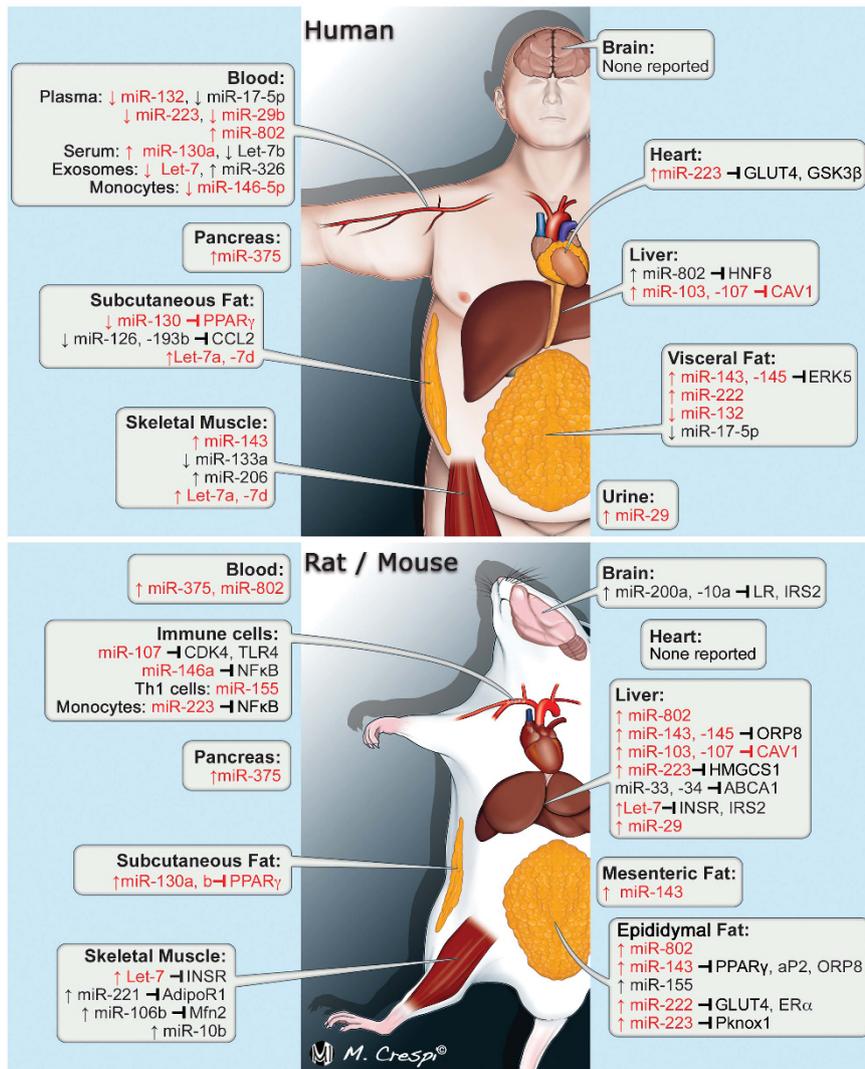


Figure 2. Obesity/insulin resistance-related miRNA expression changes in humans and in murine models. miRNAs in red font were differentially expressed in human *and* murine obesity with arrow indicating up- or downregulation. Gene symbols or conventional gene synonyms are used to show miRNA–mRNA repression. Gene symbol in red font indicates conserved miRNA-targeting between species. The image is published with the permission of Mr Massimiliano Crespi, medical illustrator.

coronary artery disease have lower circulating miR-130a expression.³⁸ 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

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 <i>et al.</i> ¹⁰¹
miR-494	↑, ↑ Insulin resistance	TNF α treatment	Mouse C2C12 cells	Insulin signaling	Lee <i>et al.</i> ¹⁴⁴
miR-221	↑	Genetic IR	Mouse (<i>ob/ob</i>)	AdipoR1/Adiponectin and PTB pathway	Lustig <i>et al.</i> ⁷¹
Let-7	↑ Insulin resistance, protected from DIO-IR	Overexpressed—Global and pancreas, inhibited miRNA—whole body	Mouse	INSR (muscle)	Frost <i>et al.</i> ⁵⁹
Let-7a, d	↑, ↑ Insulin resistance ↓ IL-13 secretion	Overweight (NGT vs T2DM), Overexpressed miRNA	Human, primary human myotubes	IL-13	Jiang <i>et al.</i> ⁶⁰
Let-7	↑ Insulin resistance	Overexpressed miRNA—muscle specific	Mouse	Lin28a, IGF1R, INSR, IRS-2	Zhu <i>et al.</i> ⁵⁸
miR-106b	↑, ↑ Insulin resistance,	TNF α treatment, overexpressed miRNA	Mouse C2C12 myotubes	Mfn2, mitochondrial dysfunction	Zhang <i>et al.</i> ¹⁴⁵
miR-10b	↓ Skeletal muscle	Genetic IR (non-obese)	Rat (GK, WKY, BN)	—	Herrera <i>et al.</i> 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, polypyrimidine tract-binding protein; T2DM, type 2 diabetes mellitus; TNF α , tumor necrosis factor alpha.

Table 5. miRNAs involved in hepatic glucose homeostasis

miRNA	Finding(s)	Intervention/phenotype	Model	Target/pathway	Author
miR-143	↑	Genetic and DIO models	Mouse (<i>db/db</i>)	ORP8	Jordan <i>et al.</i> ⁴⁸
miR-145	↑, ↑ Insulin resistance	SCD fed, Resistin injected (6 days), overexpressed miRNA	Mouse (C57BL/6J), HepG2 cells	—	Wen <i>et al.</i> ⁵¹
miR-130a	↓ Insulin resistance and steatosis	Adenoviral overexpressed	Mouse	Grb10	Xiao <i>et al.</i> 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 ⁵⁹
miR-195, -103	↑	Genetic IR (non-obese)	Rat (GK, WKY, BN)	—	Herrera <i>et al.</i> ⁶⁶
miR-21	↑	Unsaturated fatty acid treatment	Rat hepatocytes	PTEN	Vinciguerra <i>et al.</i> ¹³⁵
miR-802	↑, ↑ Insulin resistance	HFD vs SCD, overexpressed miRNA	Mouse and human	HNF1B	Kornfeld <i>et al.</i> ¹³⁶
miR-181a	↑, ↑ Insulin resistance - ↓ SIRT1	Genetic obesity/IR, overexpressed miRNA	Mouse (<i>db/db</i>), HepG2 cells	SIRT1	Zhou <i>et al.</i> ¹³⁷
miR-103, -107	↑ Insulin resistance, improved glucose tolerance	Hepatic miR-107 overexpression, inhibition of miRNAs	Mouse (C57BL/6J)	CAV1	Trajkovski <i>et al.</i> ¹³³
miR-29	↑, ↓ miRNA expression	HFD vs SCD mice, pioglitazone treatment	Mouse and Rat (<i>fa/fa</i>)	PPARGC1A, HMGCS2, ABHD5/FOXA2	Kurtz <i>et al.</i> ⁹⁰
miR-370	↑ Lipogenesis, ↓ Lipogenesis	Overexpressed miRNA, inhibited	Human HepG2 cells	miR-122, SREBP1c, DGAT2, Cpt1 α	Iliopoulos <i>et al.</i> ¹³⁸
miR-221	↓ AdipoR1	Overexpressed miRNA	Human HepG2 cells <i>in vitro</i>	AdipoR1/Adiponectin and PTB pathway	Lustig <i>et al.</i> ⁷¹
miR-126-3p, -24-3p	↓	Genetic obesity/IR	Mouse (<i>ob/ob</i>)	—	Liang <i>et al.</i> ¹³⁹
miR-200s (miR-200a,b,c)	↓	Genetic obesity/IR	Mouse (<i>db/db</i>)	FOG2, IL6	Dou <i>et al.</i> ⁷⁷
miR-122	↓ Steatosis, ↓ Plasma cholesterol	Inhibited miRNA	Mouse SCD and HFD models	SREBP1c, AMPK pathway	Esau <i>et al.</i> ¹⁴⁰
miR-122	↓ Plasma	Mild vs severe steatosis/NAFLD	Human	—	Miyaaki <i>et al.</i> ¹⁴¹
miR-33	↑ HDL, ↑ Reverse cholesterol transport	Inhibited miRNA	Mouse (LDLr ^{-/-} —western diet)	ABCA1/SREBF2	Rayner <i>et al.</i> ^{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; ORP8, oxysterol binding protein-like 8; PTEN, phosphatase and tensin homolog; SCD, standard chow diet.

anti-inflammatory Th2 cytokine interleukin 13 (IL-13) in myotubes, suggesting that let-7 potentially modulates muscle inflammation via IL-13 repression.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).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.⁶² Interestingly, after receiving treatment, let-7 levels increased in the diabetic patients to levels not significantly different than normal controls. Let-7d was found to be more highly expressed in the amnion of obese pregnant

women (Table 1).⁶³ Finally, let-7 has been reported in adipogenesis, with overexpression of let-7 in pre-adipocytes resulting in reduced adipogenesis.^{64,65} 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.^{66,67} 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).⁶⁸ miR-222 seems to negatively regulate adipose insulin sensitivity via repression of ERα and GLUT4.⁶⁸ miR-222 was found to be significantly higher in the whole plasma of two distinct cohorts of obese human patients.^{53,69} 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).⁷⁰ 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.⁷⁰ It should be mentioned that circulating miR-222 levels may be rapidly altered by insulin administration.⁶⁹ 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-221 may regulate IR via effects on adiponectin expression. In cultured human adipocytes, miR-221 mimics repressed adiponectin expression. Recently, Lustig *et al.*⁷¹ 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.⁷² Conversely, subcutaneous adipose expression of miR-221 was positively correlated with body mass index in nondiabetic Pima Indians.⁷³ Ortega *et al.*⁵³ reported decreased plasma miR-221 levels in obese humans, while Wang *et al.*⁷⁴ 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.*⁷⁵ 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.⁷⁶ 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* mice⁷⁷ and the visceral white adipose of HFD-fed mice,⁶⁷ where it likely has an important role in adipogenesis via regulation of the Wnt pathway.⁷⁸ 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 'polarization' in the context of obesity. Though the most thoroughly investigated function of miR-223, outside of cancer, involves monocyte-macrophage differentiation and macrophage activation,^{79,80} differential miR-223 expression has been linked to both human and murine obesity (Figure 2).^{17,81} Zhuang *et al.*¹⁷ showed that HFD-fed miR-223 knockout mice had worsened IR and that miR-223 deficient macrophages showed increased inflammatory potential compared with miR-223 containing 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.^{70,82,83} In humans, plasma miR-223 was reported to be lower in T2DM patients vs normal multivariable-matched controls.⁸¹ Perhaps conversely, HDL isolated from patients with familial hypercholesterolemia had 3781-fold greater miR-223 than HDL from normal patients.⁷⁰ 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.⁸³ 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^(ref. 84) and miR-146-5p.⁸⁵ miR-155-deficient macrophages had a decreased inflammatory potential,⁸⁶ and miR-155 inhibited adipogenesis in adipocytes.⁸⁷ Finally, it should be mentioned that miR-223 is robustly expressed in the liver and regulates liver cholesterol biosynthesis and HDL uptake through repression of various targets,⁸⁸ 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,⁸⁹ however, a definitive mechanism has not been elucidated. Recently, however, Kurtz *et al.*⁹⁰ 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 PPARGC1A, HMGCS2 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 tissue of offspring lambs, along with decreased markers of insulin signaling, suggesting fetal programming of miR-29 expression.⁹¹ Interestingly, miR-29 in T cells represses both Tbet and Eomes, transcription factors involved with IFN γ expression; consequently miR-29 mimetics suppress Th1/inflammatory potential of T cells.⁴¹ miR-29 upregulation in immune cells may be protective (anti-inflammatory),

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.*⁸¹ 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.⁹² Peng *et al.*⁹³ 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 normoalbuminuric 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.*⁹⁴ 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.^{95,96} 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.⁹⁷ 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.⁹⁸ 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.*¹⁰¹ 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 mRNA 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.¹⁰² 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.*¹⁰³ 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.*¹⁰³ 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.⁹⁸ 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.¹⁰⁴ 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).⁹¹ 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)¹⁰⁵ has resulted in the pursuit of miRNA-based biomarkers (miRNA signatures) for multiple diseases including cardiometabolic 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 stability¹⁰⁶ at room temperature and during multiple freeze–thaw cycles probably owing to association with AGO2 complexes,⁸² lipoproteins⁷⁰ 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.⁶⁹

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,⁸¹ receiver operating characteristic curves¹⁰⁸). 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 pathophysiologic 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.*¹¹⁰ 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 mRNA targets/pathway that may be affected by administration of a potent miRNA mimetic 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 mRNAs 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 miRTarBase¹¹⁹ and Targetscan, as well as databases specifically for circulating miRNAs¹²⁰ 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-221.¹²⁰ 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).¹²¹ 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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