MicroRNAs in metabolism and metabolic disorders

Veerle Rottiers[1,2] *and Anders M. Näär*[1,2]

Abstract | MicroRNAs (miRNAs) have recently emerged as key regulators of metabolism. For example, miR-33a and miR-33b have a crucial role in controlling cholesterol and lipid metabolism in concert with their host genes, the sterol-regulatory element-binding protein (SREBP) transcription factors. Other metabolic miRNAs, such as miR-103 and miR-107, regulate insulin and glucose homeostasis, whereas miRNAs such as miR-34a are emerging as key regulators of hepatic lipid homeostasis. The discovery of circulating miRNAs has highlighted their potential as both endocrine signalling molecules and disease markers. Dysregulation of miRNAs may contribute to metabolic abnormalities, suggesting that miRNAs may potentially serve as therapeutic targets for ameliorating cardiometabolic disorders.

P-bodies
Distict cytoplasmic foci that contain a number of enzymes involved in mRNA turnover. They are important for mRNA degradation, storage of mRNA for translation and translational repression by microRNAs.

[1]*Massachusetts General Hospital Cancer Center, Charlestown, Massachusetts 02129, USA.*
[2]*Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115, USA.*
Correspondence to A.M.N.
e-mail: naar@helix.mgh.harvard.edu
doi:10.1038/nrm3313
Corrected online
26 March 2012

Proper control of metabolic homeostasis is crucial to the maintenance of human physiology and health. Accordingly, intricate and interwoven regulatory networks have evolved to monitor and respond to changes in environmental conditions and physiological states. Work over several decades has suggested that much of the orchestration of cellular and physiological responses to altered dietary and metabolic conditions occurs at the level of gene regulation in the cell nucleus. Indeed, a number of key transcription factors, including peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), sterol-regulatory element-binding proteins (SREBPs), carbohydrate response element-binding protein (CHREBP), CCAAT-enhancer-binding protein (C/EBP) and forkhead box protein O1 (FOXO1), respond directly or indirectly to nutrients and metabolic cues such as cholesterol, lipids, glucose and insulin to rapidly alter gene expression programmes governing metabolic homeostasis[1–5].

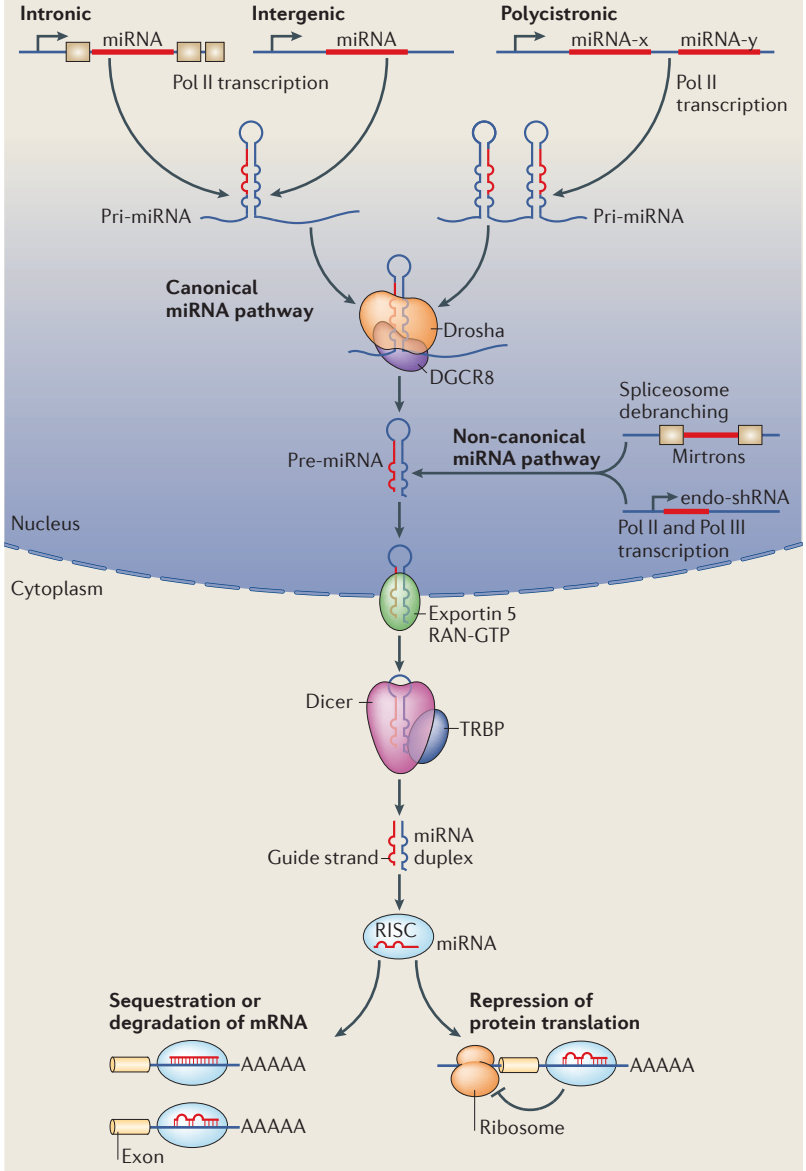
A class of small non-coding RNAs termed microRNAs (miRNAs) has recently been found to represent another crucial regulatory layer overlaying and intersecting with transcriptional control mechanisms in guiding metabolic homeostasis. Initially discovered in the nematode *Caenorhabditis elegans* as regulators of developmental timing, numerous miRNAs have subsequently been found in species from plants to humans, with regulatory roles touching on all aspects of biology. The biogenesis of miRNAs is described in BOX 1 (REFS 6,7).

In contrast to plants, in which miRNAs are often fully complementary to their mRNA targets and promote RNA cleavage and degradation, metazoan miRNAs typically exhibit only partial sequence complementarity to their mRNA targets, and initial studies suggested that they promote translational repression rather than cleavage of the mRNA[8]. However, it has recently become apparent that metazoan miRNAs may also affect mRNA stability by promoting mRNA deadenylation and subsequent sequestration and turnover in P-bodies[9].

Although functional validation is frequently lacking, target prediction databases based primarily on Watson–Crick base-pairing (for example, TargetScan, miRanda and Pictar[10–12]) have suggested that miRNAs may have hundreds of mRNA targets, thereby rivalling transcriptional mechanisms in regulatory output complexity. However, whereas transcription factors may elicit profound changes in mRNA expression levels, single miRNAs typically exert relatively modest effects on individual mRNA targets and are thought to act primarily as 'rheostats' that modulate protein expression in a nuanced fashion[7]. However, single miRNAs can have multiple target sites in the 3′ untranslated regions (UTRs) of a particular mRNA, thereby increasing repression efficiency. Furthermore, mRNAs are predicted to be targets of many distinct miRNAs, suggesting that different miRNAs might act in a concerted manner to regulate mRNA translation and turnover[13]. As discussed below, certain miRNAs have also been shown to affect multiple targets in linear pathways or interconnected nodes in regulatory networks, thereby exerting a larger cumulative effect[14]. miRNAs are also frequently found to act in feedforward and feedback regulations that can amplify or dampen signal output[15], making timing of analysis after miRNA perturbation crucial to an accurate assessment of the regulatory impact.

Box 1 | **The miRNA biogenesis pathway**

MicroRNAs (miRNAs) generated by the canonical biogenesis pathway are transcribed as precursor RNAs from intergenic, intronic or polycistronic genomic loci by RNA polymerase II (Pol II)[6]. The primary miRNA (pri-miRNA) transcript forms a stem–loop structure that is recognized and processed by the Drosha and DGCR8 RNase III complex or the spliceosome apparatus in the nucleus. In the non-canonical miRNA pathway, miRNAs are transcribed directly as endogenous short hairpin RNAs (endo-shRNAs) or derive directly through splicing from introns that can refold into hairpins (mirtrons) (reviewed in REF. 134). The trimmed precursor (pre-miRNA) hairpins from both canonical and non-canonical miRNA pathways are then transported by an exportin 5 and RAN-GTP-dependent process to the cytosol, where they are typically further processed by the Dicer and transactivation-response RNA-binding protein (TRBP) RNase III enzyme complex to form the mature double-stranded ~22-nucleotide miRNA. Argonaute proteins (for example, AGO2, not shown) then unwind the miRNA duplex and facilitate incorporation of the miRNA-targeting strand (also known as the guide strand) into the AGO-containing RNA-induced silencing complex (RISC). The RISC–miRNA assembly is then guided to specific target sequences in mRNAs. The initial recognition of mRNAs by the RISC–miRNA complex is driven primarily by Watson–Crick base-pairing of nucleotides 2 to 8 in the mature miRNA (termed the seed sequence) with specific mRNA target sequences chiefly located in the 3′ untranslated region, and additional base-pairing affords greater affinity and targeting efficiency[7].



Finally, whereas miRNA functions under normal physiological conditions might be integrated into multi-layered control circuits ensuring proper development and homeostasis, dysregulation of miRNA expression or function in response to intrinsic factors (genetic or epigenetic) or extrinsic factors (environmental cues or stress) may contribute to aberrant gene expression patterns underlying abnormal developmental patterning or metabolic dysfunction. Although it is clear that the complex mechanisms of action and impact of miRNAs on animal development, physiology and disease need much further study, progress has been made in elucidating the individual roles of certain miRNAs in specific biological contexts.

In this Review, we discuss recent advances in our understanding of the emerging roles of miRNAs in controlling cholesterol and lipid homeostasis, with particular emphasis on the well-characterized liver-specific miRNA miR-122 and the regulatory circuit comprising miR-33a and miR-33b miRNAs and their SREBP host genes. The role of miRNAs such as the related miR-103 and miR-107 in controlling insulin signalling and glucose homeostasis is also highlighted. The potential pathological functions of miRNAs such as miR-33 and miR-34a in conditions associated with metabolic syndrome (MetS) are discussed, and we cover current efforts to therapeutically target specific metabolic miRNAs. Finally, we briefly discuss circulating miRNAs, with emphasis on new data linking miRNAs with lipoproteins and possible functions of miRNAs as endocrine signalling molecules.

miRNA regulation of lipid homeostasis

Although roles for miRNAs were first described in the regulation of metazoan development, recent studies have revealed that miRNAs also play key parts in controlling metabolic homeostasis. Below, we discuss the functions of the liver-specific miRNA miR-122 and the miR-33–SREBP host gene circuit in cholesterol and lipid homeostasis and highlight new and exciting data pointing to important physiological and therapeutic implications of these miRNAs in metabolic diseases.

Lipids are structural components of cell membranes (for example, cholesterol and phospholipids) and are important for energy storage (for example, triglycerides) but can also act as signalling molecules (for example, steroid hormones). Lipids such as cholesterol and fatty acids are taken up in the diet and are synthesized *de novo*, predominantly in the liver. Regulation of the biosynthesis of cholesterol, fatty acids and phospholipids is mediated by transcription factors such as SREBPs, the activity of which is under tight feedback control to maintain proper homeostasis (reviewed in REF. 16). Cholesterol and other lipids are transported in the blood by association with lipoproteins such as very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). LDL primarily transports cholesterol to peripheral tissues, where the LDL receptor (LDLR) mediates LDL uptake. HDL is important for the removal of cholesterol from peripheral tissues to the liver through a system called reverse cholesterol transport (RCT).

Box 2 | **miR-122 and its function in hepatitis C virus propagation**

Apart from its function in metabolic control, the microRNA miR-122 has also been shown to promote the propagation of hepatitis C virus (HCV) by multiple mechanisms[28–30]. In addition to binding the HCV 5′ untranslated region to facilitate proper folding of the HCV RNA for translation and replication, miR-122-dependent increase in cholesterol and lipid synthesis may stimulate the production of endoplasmic reticulum-associated lipid droplets and cholesterol and lipid-rich membrane domains (termed 'lipid rafts') that are central sites of viral replication and production[135]. Hence, miR-122 antagonism could inhibit HCV both by preventing direct enhancement of viral RNA folding or replication and by lowering required cholesterol or lipid cofactors for viral packaging or extrusion, suggesting that antisense-based targeting of miR-122 could represent a powerful approach for treating chronic HCV infections, which affect up to 3% of the world's population. Accordingly, weekly subcutaneous injections of miR-122-targeting locked nucleic acid (LNA)-based antisense oligonucleotides (miravirsen, Santaris Pharma A/S) have been found efficacious in decreasing HCV titres in chimpanzees[136], and early indications from Phase II studies in humans with chronic HCV infection seem promising[137]. Indeed, a 4-week therapy with miravirsen in treatment-naive patients with chronic HCV genotype 1 infection provides long-lasting suppression of viraemia. This suppressive effect was maintained for more than 4 weeks after the last dose, did not reveal viral resistance and was well tolerated. These studies also showed lowered total cholesterol, and further assessment of miravirsen in humans should provide important information as to whether miR-122 therapeutic targeting might have a positive impact on circulating cholesterol and lipid abnormalities linked to metabolic syndrome. HCV infection is associated with non-alcoholic fatty liver disease[101], and inhibition of viral propagation by miR-122-targeting antisense approaches might also ameliorate HCV-associated fatty liver disease. However, the emerging link of miR-122 to hepatocyte differentiation or cell fate determination[138] may also suggest that attention should be paid to potential deleterious long-term effects, such as the development of hepatocellular carcinoma[26].

Aberrant cholesterol and lipid homeostasis represents a crucial risk factor for cardiometabolic diseases prevalent in the developed world, such as MetS[17]. For example, the risk of atherosclerosis and coronary artery disease increases with a rise in circulating LDL levels, whereas HDL levels are inversely associated with cardiovascular disease risk[17]. Insulin resistance and other metabolic abnormalities can result in excess accumulation of hepatic triglycerides and fatty acids, which is associated with fatty liver diseases (hepatosteatosis) such as non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), which are precursors to more severe liver diseases such as fibrosis and hepatocellular carcinoma[18].

Cholesterol and lipid regulation by miR-122. miR-122 was the first miRNA to be linked to metabolic control. It is expressed primarily in the liver and was initially shown to affect hepatic cholesterol and lipid metabolism but has also been implicated in the maintenance of liver cell differentiation (reviewed in REF. 19). Two early studies showed that antisense targeting of miR-122 results in a vast (~25–30%) reduction in plasma cholesterol levels[20,21]. First, a study showed that injection of miR-122-targeting antagomirs into mice resulted in altered hepatic expression of several genes, including genes involved in cholesterol biosynthesis, such as 3-hydroxy-3-methylglutaryl-CoA reductase (*Hmgcr*), 3-hydroxy-3-methylglutaryl-CoA synthase 1 (*Hmgcs1*) and 7-dehydrocholesterol reductase (*Dhcr7*)[21]. Accordingly, they observed significant lowering of plasma cholesterol levels in response to miR-122 antagonism. In a separate study, antisense-based silencing of miR-122 led to decreased hepatic cholesterol and fatty acid biosynthesis and an increase in fatty acid β-oxidation associated with a reduction in circulating total cholesterol and triglycerides as well as decreased hepatosteatosis in mice on a high-fat diet[20]. In subsequent studies, miR-122 was successfully targeted by antisense inhibitors using locked nucleic acid (LNA) chemistry[22] in non-human primates such as African green monkeys, resulting in lowered circulating cholesterol[23]. Antisense targeting of miR-122 was not associated with hepatotoxicity or adverse liver histopathology in mice or non-human primates, indicating the apparent safety of this therapeutic approach.

Taken together, these pioneering studies pointed to the exciting possibility of miR-122 antisense oligonucleotides as a novel treatment strategy for lowering circulating cholesterol, which would represent the first therapeutic targeting of an miRNA. However, the initial enthusiasm waned after it was found that miR-122 antagonism not only lowers LDL levels but also causes a decline in the levels of HDL, both in mice and in non-human primates[20,23,24]. This presumably deleterious effect has called into question the therapeutic value of miR-122 as a target for the treatment of cholesterol-related disorders. Moreover, although genes involved in cholesterol and lipid metabolism are affected by miR-122 in the liver, these genes do not seem to be direct targets of miR-122 (REFS 19–21,25). This lack of a mechanistic understanding of the effects of miR-122 on cholesterol homeostasis, and the possibilities of other adverse consequences, such as hepatocellular carcinoma[26,27], has also dampened the enthusiasm for the development of miR-122 antisense technologies as a therapeutic approach for the long-term management of cholesterol disorders.

Recently, miR-122 was found to be required for the propagation of hepatitis C virus (HCV), both directly at the level of viral replication and in controlling lipid cofactors required for virus replication and assembly[28–30]. The therapeutic targeting of miR-122 in this context is very intriguing and represents the first miRNA-targeting drug in human clinical trials (BOX 2).

The SREBP–miR-33 regulatory circuit. The SREBP family of basic helix–loop–helix leucine zipper transcription factors controls the expression of numerous genes involved in cholesterol and fatty acid biosynthesis and uptake and in the production of phospholipids and triglycerides. The human *SREBF1* gene encodes SREBP1A and SREBP1C, which are alternative splice variants that primarily regulate lipogenic genes such as fatty acid synthase (*FASN*), acetyl-CoA carboxylase (*ACC*; also known as *ACAC*), and stearoyl-CoA desaturase (*SCD*). The SREBP2 isoform produced by the *SREBF2* gene preferentially controls the expression of cholesterogenic genes, including *HMGCR* and *LDLR*[3].

Intriguingly, both human SREBP-encoding genes were recently found to be host genes to highly conserved miRNAs[31–35]. The *SREBF1* gene on chromosome 17 harbours *mir-33b* in intron 17, whereas *SREBF2* on chromosome 22 contains *mir-33a* in intron 16. Mature miR-33a and miR-33b only differ in two nucleotides and are predicted to have largely overlapping target gene sets. Of note, rodents lack *mir-33b* in the corresponding intron

Metabolic syndrome
(MetS). A combination of metabolic disorders characterized by insulin resistance, obesity, abnormalities in circulating cholesterol and lipid profiles, non-alcoholic fatty liver disease and hypertension. MetS is associated with increased risk of type 2 diabetes and cardiovascular disease (coronary artery disease and stroke).

Endocrine signalling
Hormonal signal, secreted from a specific cell and causing a specific effect in distal target cells. Hormones produced by endocrine cells can travel through the blood to reach all parts of the body.

Triglycerides
Esters of glycerol and fatty acids, used for the storage of energy.

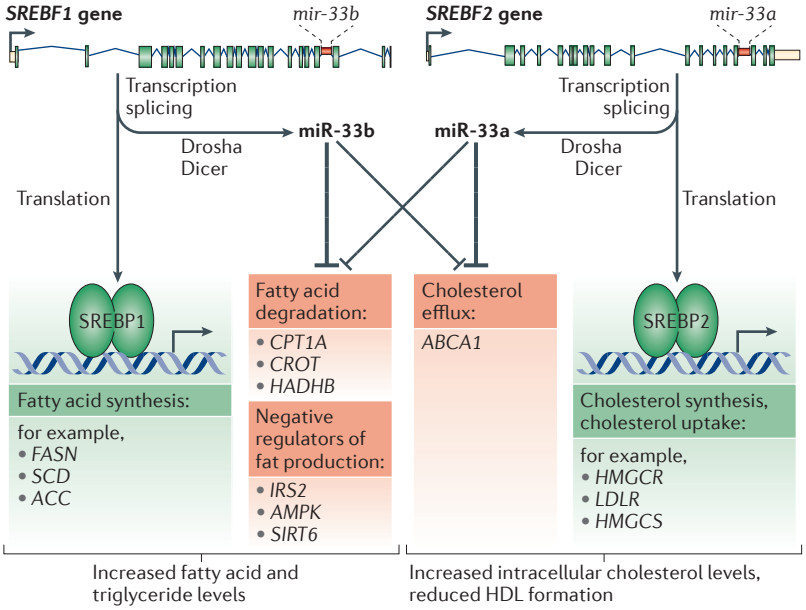


Figure 1 | **Model of the SREBP and miR-33 circuit.** The sterol-regulatory element-binding protein (SREBP) transcription factors act coordinately with their intronic microRNAs (miRNAs) miR-33a and miR-33b to regulate fatty acid, triglyceride and cholesterol homeostasis. Transcription of the *SREBF1* and *SREBF2* loci gives rise to SREBP1 and SREBP2 and to the miRNAs miR-33b and miR-33a. SREBP1 activates genes involved in fatty acid, phospholipid and triglyceride synthesis (for example, *FASN* (fatty acid synthase), *SCD* (stearoyl-CoA desaturase) and *ACC* (acetyl-CoA carboxylase)), whereas SREBP2 activates genes involved in cholesterol synthesis and uptake (such as *HMGCR* (3-hydroxy-3-methylglutaryl-CoA reductase), *LDLR* (low-density lipoprotein receptor) and *HMGCS* (3-hydroxy-3-methylglutaryl-CoA synthase)). miR-33a and miR-33b act to repress genes functioning in fatty acid β-oxidation (for example, *CROT* (carnitine *O*-octanoyltransferase), *HADHB* (hydroxyacyl-CoA dehydrogenase–3-ketoacyl-CoA thiolase–enoyl-CoA hydratase (trifunctional protein) beta subunit) and *CPT1A* (carnitine palmitoyltransferase 1A)), cholesterol efflux (for example, *ABCA1* (ATP-binding cassette subfamily A member 1)) and negative regulators of SREBPs (for example, *IRS2* (insulin receptor substrate 2), *AMPKA1* (AMP-activated protein kinase alpha 1 subunit; also known as *PRKAA1*) and *SIRT6* (sirtuin 6)). HDL, high-density lipoprotein.

in the *Srebp1* gene[35]; the importance of this will be discussed later. In humans, *mir-33a* and *mir-33b* seem to be extensively co-expressed with their host genes in many cells and tissues[34,36], suggesting that they derive from the same primary transcript. This also pointed to a potential coordinated function of the miR-33 isoforms and their host gene products. Indeed, several studies have now revealed extensive collaboration of miR-33a and miR-33b and the SREBP gene regulators in controlling cholesterol and lipid homeostasis (FIG. 1).

miR-33a and SREBP2 control cholesterol homeostasis. Several recent studies reported the discovery of the miR-33a and SREBP2 miRNA–host gene circuit, demonstrating that miR-33a cooperates with the SREBP2 cholesterogenic transcription factor to boost intracellular cholesterol levels[31–35] (FIG. 1). miR-33a and miR-33b were found to have a crucial role in the post-transcriptional repression of ATP-binding cassette transporter subfamily A member 1 (ABCA1), which promotes the efflux of free cholesterol from within the cell to apolipoprotein A1 (APOA1) and is essential for the formation of HDL[37]. Plasma HDL levels show a strong inverse relationship with cardiovascular disease risk, and intensive efforts are ongoing to find effective pharmacological approaches to increase HDL levels for the treatment of cardiovascular disease[38]. ABCA1 is regulated by the miR-33 isoforms through its 3′ UTR, confirming ABCA1 as a direct miR-33 target. In accord with regulation of ABCA1 by miR-33, modulation of miR-33a levels also results in changes in cholesterol efflux in mouse macrophages[32–35], suggesting that miR-33-targeting antisense approaches might promote RCT from atherogenic macrophages and decrease atherosclerosis. Importantly, in mouse models, hepatic and macrophage ABCA1 expression and circulating HDL levels increase following inhibition or knockout ablation of miR-33a[32–35]. Moreover, antisense inhibition of miR-33a in LDLR-knockout mice, a well-validated model for hypercholesterolaemia and cardiovascular disease, caused a reduction in atherosclerotic plaque size and lipid content in mice fed a high-fat diet and also promoted RCT, verifying the therapeutic potential of miR-33 targeting for the treatment of cardiovascular disease[39].

As noted above, rodents lack miR-33b, complicating the translation and relevance of these results to humans. This difference is important because expression of SREBP1C (and *mir-33b* embedded within the *SREBF1* gene) is highly upregulated following insulin stimulation[36]. Under high circulating insulin conditions (such as in insulin resistance), increased SREBP1C and miR-33b levels in the liver could thus potentially contribute to both high VLDL levels and low HDL levels found in individuals suffering from MetS[40]. Recent studies have indeed verified that hepatic miR-33b levels are increased in non-human primates in response to a high-carbohydrate diet and show that antisense oligonucleotides targeting miR-33a and miR-33b are effective in increasing HDL levels and lowering VLDL triglycerides, paving the way for future human clinical trials to evaluate the safety and efficacy of antisense targeting of miR-33a and miR-33b for the treatment of cardiometabolic disorders[41] (V.R. and A.M.N., unpublished observations). It is interesting to note that ABCA1 is also targeted by other miRNAs, such as miR-758 and miR-106b, which were also shown to modulate cellular cholesterol efflux[42,43], indicating that ABCA1 and HDL regulation by miRNAs is likely to be complex.

Other miR-33 targets involved in lipid homeostasis. The discovery of coordinated regulation of cholesterol levels by miR-33a and the SREBP2 host gene product has prompted investigation into whether the SREBP host genes and their intronic miRNAs might act together more broadly to control not only cholesterol but also fatty acid and lipid homeostasis in an integrated manner. Indeed, we and others have now found that miR-33a and miR-33b also regulate intracellular fatty acid and lipid levels in concert with their SREBP host gene products[14,31,36] (FIG. 1).

First, it was shown that miR-33a and miR-33b directly control the expression of several proteins involved in fatty acid β-oxidation, the process by which fatty acids are degraded in mitochondria and peroxisomes to produce acetyl-CoA destined for the citric acid cycle and ATP for energy generation[14,31,36]. These proteins include: carnitine *O*-octanoyltransferase (CROT), which helps to

Antagomirs
Small, synthetic, cholesterol-conjugated DNA oligonucleotides that are complementary to an endogenous microRNA (miRNA) of interest. The cholesterol moiety allows antagomirs to enter most cell types efficiently, where they specifically bind and sequester endogenous miRNAs.

Fatty acid β-oxidation
Degradation process of fatty acids to acetyl-CoA primarily in the mitochondria. Long chain fatty acids need to be transported via binding to carnitine.

break down very long-chain fatty acids (>20 carbons) in peroxisomes; carnitine palmitoyltransferase 1A (CPT1A), which is the rate-limiting transporter of fatty acids into mitochondria; and hydroxyacyl-CoA dehydrogenase–3-ketoacyl-CoA thiolase–enoyl-CoA hydratase (trifunctional protein) β-subunit (HADHB), which is directly involved in mitochondrial fatty acid β-oxidation[14,31,36].

In addition to regulating fatty acid degradation, miR-33a and miR-33b have been shown to control crucial upstream regulators of fatty acid and lipid homeostasis. For example, sirtuin 6 (SIRT6), an NAD^+-dependent histone deacetylase that has been shown to be a crucial regulator of glucose metabolism and stress resistance[44–47], was found to be targeted by miR-33a and miR-33b[14,36]. Interestingly, a recent study of liver-specific *Sirt6*-knockout mice revealed increased hepatic glycolysis, lipogenesis and triglyceride production, resulting in hepatosteatosis[48]. SIRT6 was shown to directly regulate SREBP1 target genes relevant for fatty acid production, such as *Acc1*, *Scd1* and *Fasn*. This suggests that miR-33-mediated inhibition of SIRT6 expression may result in increased chromatin acetylation and derepression of SREBP-dependent fatty acid biosynthesis genes and increased lipogenesis.

The α1 subunit of AMP-activated protein kinase (AMPKα1) has also been found to be targeted by miR-33a and miR-33b[14,36,41]. AMPK is a master regulator of cellular energy levels in response to energy stress[49]. It responds to low cellular energy levels (increased AMP/ATP ratio) and acts to reduce energy-consuming processes such as protein, fatty acid and cholesterol synthesis, in addition to activating mitochondrial biogenesis, fatty acid β-oxidation and glucose uptake to promote ATP synthesis. AMPK directly phosphorylates and deactivates several key SREBP lipogenic and cholesterogenic targets, such as ACC1 and HMGCR. AMPK also inhibits SREBPs themselves both indirectly, through LXR[50], and through direct phosphorylation[51]. Thus, negative regulation of AMPKα1 by miR-33a and miR-33b may relieve AMPK inhibition of both SREBPs and their target genes to coordinately boost intracellular levels of cholesterol, fatty acids and other lipids.

Insulin receptor substrate 2 (IRS2) was also recently shown to be a miR-33 target[14,36]. IRS2 is one of two intracellular adaptor proteins for the insulin receptor (INSR) that relay insulin signalling to downstream effectors (such as phosphoinositide 3-kinases (PI3Ks) and protein kinase B (PKB; also known as AKT)). In mouse models, reduction of IRS2, but not IRS1, has been found to increase SREBP1C expression and activity[52–54]. Interestingly, in mouse NAFLD models, decreased hepatic levels of IRS2 have been shown to result in a compensatory increase in IRS1 levels, which in turn activates SREBP1 (REF. 55). Thus, miR-33 antisense targeting could potentially result in a reversal of the hepatic changes in IRS2 and IRS1 expression, thereby decreasing SREBP1C levels or activity associated with hepatosteatosis.

Taken together, these results reveal an extensive and integrated network of functional interactions between the SREBP transcription factors and their intronic miRNAs miR-33a and miR-33b to regulate cholesterol and lipid homeostasis (FIG. 1).

Locked nucleic acid
(LNA). A modified DNA oligonucleotide analogue that is locked in an 'N-type conformation'. LNA is capable of recognizing DNA and RNA with high affinity and is resistant to degradation.

Apolipoprotein A1
(APOA1). The major protein component of high-density lipoprotein (HDL) in plasma. It promotes the efflux of cholesterol from tissues to the liver, and defects cause low HDL levels, which are often associated with cardiovascular disease.

Atherogenic macrophages
Cholesterol-loaded macrophages that accumulate in the arterial wall and can lead to atherosclerotic lesions.

miRNA regulation of insulin signalling

Several miRNAs have recently been implicated in controlling both insulin signalling and glucose metabolism at multiple levels[56]. Insulin is a hormone synthesized in and secreted by pancreatic β-cells in response to increased nutrient levels (for example, glucose) in the blood. Insulin acts in concert with glucagon (a hormone produced by pancreatic α-cells, with opposite functions to insulin) to maintain glucose homeostasis. Insulin secretion causes uptake of glucose in muscles and adipose cells (through glucose transporters such as GLUT4), blocks *de novo* production of glucose in the liver and increases storage of nutrients in form of fat, glycogen and protein[57]. Binding of insulin to INSR promotes an intracellular signalling cascade involving INSR tyrosine kinase activity, the adaptor proteins IRS1 and IRS2 and a cascade of kinases including PI3Ks, 3-phosphoinositide-dependent protein kinase 1 (PDPK1) and AKT[58]. These kinases affect a complex downstream network of proteins such as glycogen synthase kinase 3β (GSK3β), mammalian target of rapamycin complex 1 (mTORC1) and the FOXO family transcription factors[59]. Insulin also activates *SREBF1* transcription and SREBP1C processing and transport, thereby stimulating lipogenesis[60].

Insulin resistance (a condition where tissues are no longer able to respond adequately to the action of insulin) is one of the most prevalent metabolic abnormalities in the developed world and is strongly associated with obesity, circulating cholesterol and lipid abnormalities and NAFLD, which are all components of MetS[59]. Whereas the mechanisms underlying this complex metabolic disease remain to be fully elucidated, an increasing number of miRNAs have now been implicated in the control of insulin signalling and glucose homeostasis at all steps of regulation, from pancreatic islet development, β-cell differentiation and insulin secretion to target tissue insulin sensitivity and intracellular insulin signalling to downstream effectors (FIG. 2; TABLE 1).

miRNA regulation of pancreatic insulin production. The pancreatic miRNA miR-375 was shown to be required for pancreatic island development in zebrafish[61] and for the maintenance of pancreatic α-cell and β-cell mass in mice[62]. Interestingly, miR-375 may have a dual role, as miR-375 was also found to decrease insulin exocytosis and secretion at least in part through the repression of myotrophin (*Mtpn*), a gene involved in actin depolymerization and, potentially, in vesicular fusion[63]. It may additionally affect downstream insulin signalling through direct repression of the insulin signalling intermediate kinase PDPK1 (REF. 64). The miR-124a miRNA is co-expressed with miR-375 and, at least in cultured cells, also targets *Mtpn*, suggesting coordinated control of *Mtpn* by several miRNAs[65]. miR-124a is also involved in pancreatic islet development, potentially through regulation of the FOXA2 transcription factor, which is involved in β-cell differentiation, and RAB27A, a GTPase required for insulin secretion[66,67]. Other miRNAs have also been implicated in controlling pancreatic insulin exocytosis. For example, miR-9 may regulate insulin secretion through its inhibition of the transcription factor one cut homeobox 2

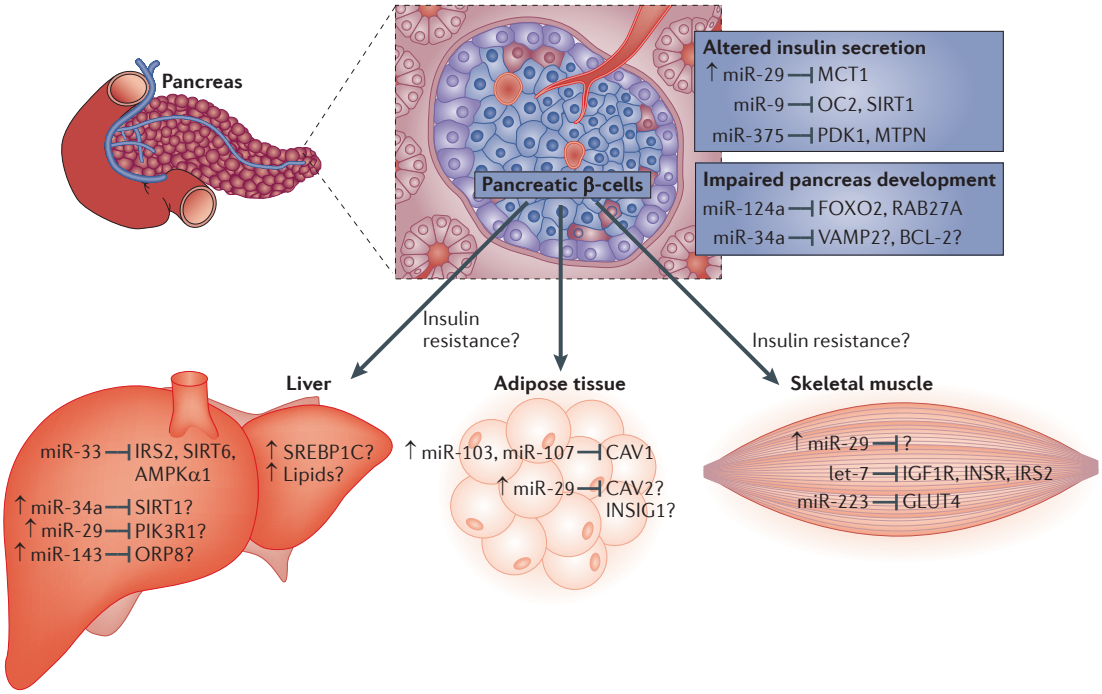


Figure 2 | **miRNA regulation of insulin signalling and glucose homeostasis.** Normally, following feeding, insulin is produced in pancreatic β-cells and after release will reach target tissues such as the muscle, liver and adipose to cause uptake of glucose, reduce the production of glucose and activate fat production and storage. MicroRNAs (miRNAs) that affect diverse parts of insulin signalling in the pancreas, liver, muscle and adipose tissue have been identified. miR-124a and miR-34a are involved in pancreatic development (through effects on FOXO2 (forkhead box protein O2), RAB27A, VAMP2 (vesicle-associated membrane protein 2) and BCL-2 (B cell lymphoma 2)). miR-29, miR-9 and miR-375 are involved in insulin secretion (miR-29 activates insulin secretion, whereas miR-9 and miR-375 inhibit insulin secretion), acting through MCT1 (monocarboxylate transporter 1), OC2 (one cut homeobox 2), SIRT1 (sirtuin 1), PDK1 (phosphoinositide-dependent kinase 1) and MTPN (myotrophin). miR-33, miR-34a, miR-29 and miR-143 act in the liver on targets involved in insulin signalling and its regulation (such as IRS2 (insulin receptor substrate 2), SIRT6, AMPKα1 (AMP-activated protein kinase-α subunit 1), SIRT1, PIK3R1 (phosphatidylinositol 3-kinase subunit-α) and ORP8 (oxysterol-binding protein-related protein 8)). Insulin signalling in adipose tissue is modulated by miR-103 and miR-107 (through CAV1 (caveolin 1)) and miR-29 (through INSIG1 (insulin-induced gene 1) and CAV2). The miR-29, let-7 and miR-223 miRNAs may act in muscle to modulate insulin signalling (IGF1R (insulin-like growth factor receptor 1), INSR (insulin receptor) and IRS2) and glucose uptake (GLUT4 (glucose transporter type 4)). Known and predicted targets that lack *in vivo* evidence are marked with a question mark. In disease conditions, such as impaired insulin secretion or insulin resistance, several miRNAs are upregulated (marked with an arrow).

(OC2; also known as ONECUT2) and through SIRT1 (REFS 68,69), whereas miR-29a and miR-29b, which are highly expressed in the pancreatic islets of diabetic mice, inhibit the expression of monocarboxylate transporter 1 (MCT1) and its function in insulin release[70]. Taken together, these findings indicate that miRNAs have key roles in controlling insulin secretion through effects on both pancreatic development and insulin exocytosis.

miRNAs regulate insulin signalling in target tissues. Other miRNAs act in target tissues to regulate responses to insulin and glucose homeostasis. For example, miR-29a and miR-29b, which are upregulated in the muscle, white adipose tissue (WAT) and the liver of diabetic Goto–Kakizaki rats[71], have been linked to insulin resistance, at least in cell culture experiments. The effects of miR-29a and miR-29b were suggested to be mediated through downregulation of proteins that promote insulin signalling such as caveolin 2 (CAV2), a lipid raft-associated protein that responds to insulin levels[72], insulin-induced gene 1 (INSIG1), a negative regulator of SREBPs, and the insulin signalling intermediate phosphatidylinositol 3-kinase regulatory subunit-α (PIK3R1)[71,73]. Another layer of insulin regulation by miRNAs is provided by miR-126, which promotes insulin resistance through the inhibition of IRS1 (REF. 74). Intracellular glucose levels can also be directly regulated by miRNAs. For example, in skeletal muscle, miR-223 was found to inhibit glucose uptake through targeting GLUT4 (REF. 75). As discussed above, miR-33a and miR-33b may also influence insulin signalling and glucose regulation by targeting IRS2, SIRT6 and AMPKα1 (REFS 14,36). miRNAs are thus acting to control insulin signalling and glucose uptake at multiple levels in target cells and tissues.

A number of miRNAs have also been implicated in metabolic disorders associated with aberrant insulin response, including obesity and NAFLD[76–82]. For example, the related *mir-103* and *mir-107* (which are located in introns in the pantothenate kinase 1 (*Pank1*), *Pank2* and *Pank3* genes)[83] were recently shown to be upregulated in livers of leptin-deficient (*ob/ob*) and diet-induced obese (DIO) mice[81]. Interestingly, antisense-mediated silencing

Lipid raft
Microdomain of the cell plasma membrane with high cholesterol and sphingolipid content that compartmentalizes cellular processes by acting as a platform to colocalize proteins such as signalling molecules and receptors.

Table 1 | **MicroRNAs involved in metabolism and metabolic disorders**

miRNA	Target tissue(s)	Function	Target genes*	Refs
miR-103 and miR-107	Adipose, liver	Insulin and glucose homeostasis, adipogenesis	*CAV1*, *DICER*	81,82,85
miR-122	Liver	Hepatic lipid metabolism	*SLC7A1* (also known as *CAT1*), *ADAM17*	20,139,140
miR-124a	Pancreas	Pancreatic islet development	*FOXA2*, *RAB27A*	66,67
miR-143	Adipose, liver, pancreas	Adipocyte differentiation, insulin resistance	*ERK5* (also known as *BMK1* or *MAPK7*), *OSBPL8* (also known as *ORP8*)	77,80,94
miR-223	Muscle	Glucose uptake, insulin resistance	*SLC2A4* (also known as *GLUT4*)	75
miR-27a	Adipose	Adipogenesis	*(PPARG, CEBPA)*‡	141,142
miR-29	Muscle, adipose, liver	Glucose transport	*INSIG1*, *CAV2*, *SLC16A1* (also known as *MCT1*), *PIK3R1*	70,71,73
miR-33a and miR-33b	Liver, macrophage	Cholesterol, lipid and energy homeostasis	*ABCA1*, *NPC1*, *CPT1A*, *HADHB*, *CROT*, *IRS2*, *SIRT6*, *PRKAA1* (also known as *AMPKA1*)	31–36,39
miR-335	Pancreas, liver, adipose	Insulin production, fatty acid and triglyceride biosynthesis	*STXBP1*	76,79
miR-34a	Liver, pancreas	Lipid metabolism, B cell exocytosis	*SIRT1*, *VAMP2*, *ACSL1*	88,113,143
miR-375	Pancreas	Insulin secretion, pancreatic islet development	*MTPN*, *USP1*, *JAK2*, *ADIPOR2*, *PDPK1*	62–64
miR-378 and miR-378*	Adipose	Adipocyte differentiation, lipid synthesis	*ESRRG*, *GABPA*, (ribosomal genes)‡	144–146
miR-9	Pancreas	Insulin secretion	*ONECUT2*, *SIRT1*	68,69
miR-130	Adipose	Adipogenesis	*PPARG*	147
let-7	Muscle, adipose	Insulin sensitivity	*IGF1R*, *INSR*, *IRS2*, *HMGA2*	91,148

ABCA1, ATP-binding cassette subfamily A member 1; ACSL1, acyl-CoA synthetase 1; ADAM17, ADAM metallopeptidase domain 17; ADIPOR2, adiponectin receptor protein 2; CEBPA, CCAAT-enhancer-binding protein alpha; CAT1, cationic amino acid transporter 1; CAV, caveolin; CPT1A, carnitine palmitoyltransferase 1A; CROT, *O*-octanoyltransferase; ERK5, extracellular signal-regulated kinase 5; ESRRG, oestrogen-related receptor gamma; FOXA2, forkhead box A2; GABPA, GA-binding protein alpha; HADHB, hydroxyacyl-CoA dehydrogenase–3-ketoacyl-CoA thiolase–enoyl-CoA hydratase (trifunctional protein) beta subunit; HMGA2, high-mobility group protein A2; IGF1R, insulin-like growth factor receptor 1; INSIG1, insulin-induced gene 1; INSR, insulin receptor; IRS2, insulin receptor substrate 2; JAK2, Janus activated kinase 2; MTPN, myotrophin; NPC1, Niemann–Pick C1 protein; ONECUT2, one cut homeobox 2; OSBPL8, oxysterol binding protein-like 8; PDPK1, 3-phosphoinositide-dependent protein kinase 1; PIK3R1, phosphatidylinositol 3-kinase subunit p85 alpha; PPARG, peroxisome proliferator-activated receptor gamma; PRKAA1, protein kinase, AMP-activated, alpha 1; SIRT, sirtuin; SLC, solute carrier; STXBP1, syntaxin-binding protein 1; USP1, ubiquitin-specific protease 1; VAMP2, vesicle-associated membrane protein 2.*Human names are used. ‡Genes in parentheses have not been shown to be direct targets.

of miR-103 and miR-107 improved insulin sensitivity and glucose homeostasis, whereas overexpression (predominantly in adipose tissue) was sufficient to cause defects in glucose homeostasis in these mouse models. If confirmed in non-human primates and humans, this suggests that miR-103 and miR-107 may represent therapeutic targets to ameliorate obesity-associated insulin resistance[84]. These miRNAs were proposed to exert their functions on insulin and glucose regulation at least in part through the inhibition of CAV1, which influences lipid raft signalling and affects INSR availability[81]. However, it is also possible that some of the effects are mediated through other miRNAs as miR-103 and miR-107 also strongly inhibit the miRNA-processing enzyme Dicer[85]. Indeed, partial knockdown of Dicer and pancreas-specific knockout experiments have revealed that this enzyme (and, presumably, its target miRNAs) is required for both the development and maintenance of the pancreas and insulin signalling[86,87].

The expression of several additional miRNAs is increased in obesity models, and these miRNAs exert regulatory effects on insulin signalling and glucose homeostasis. Similarly to miR-103 and miR-107, miR-143 is overexpressed in obese leptin receptor-mutant (*db/db*) mice and DIO mice[77]. Overexpression of miR-143 reduces insulin sensitivity, presumably through its target oxysterol-binding protein-related protein 8 (ORP8), a protein involved in AKT activation. miR-34a, an miRNA that may have important roles in cancer development through its function in a network with SIRT1 and p53, has additionally been implicated in diabetes as a target to prevent pancreatic β-cell death[88–90]. Finally, several members of the let-7 miRNA family have been found to be upregulated in *ob/ob* and DIO mice[81]. The tumour suppressor roles of let-7 are well studied in cancer biology, but let-7 was recently also shown to be involved in the regulation of glucose metabolism, as overexpression of let-7 in skeletal muscle resulted in insulin resistance and impaired glucose tolerance[91]. This effect may, at least partially, be mediated by let-7-targeting of insulin-like growth factor receptor 1 (IGF1R), INSR and IRS2.

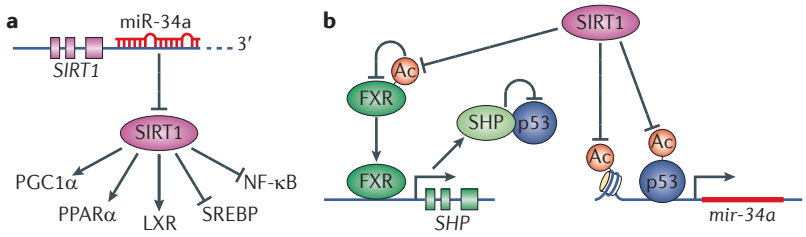


Figure 3 | **The regulatory loop of miR-34a, SIRT1, FXR and p53.** The microRNA miR-34a is highly expressed in patients with non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and type 2 diabetes. At the molecular level, miR-34a has been shown to exert its function through its effect on sirtuin 1 (SIRT1). **a** | miR-34a inhibits SIRT1 expression and reduces its protein levels, thereby preventing the activation of peroxisome proliferator-activated receptor-α (PPARα), PPARγ co-activator 1α (PGC1α) and liver X receptor (LXR) (key regulators of cholesterol, lipid and energy homeostasis). Furthermore, miR-34a-mediated SIRT1 repression results in inhibition of sterol-regulatory element-binding proteins (SREBPs) and nuclear factor-κB (NF-κB) (activators of lipogenesis and cholesterogenesis and of inflammation, respectively). **b** | miR-34a is in turn inhibited by SIRT1 in a regulatory loop that includes miR-34a, SIRT1, farnesoid X receptor (FXR) and p53 to affect cholesterol, lipid and energy homeostasis and inflammation. SIRT1 feedback inhibits miR-34a in several ways: it deacetylates p53 and inhibits p53-dependent transcriptional activation of *mir-34a*. In addition, SIRT1 inhibits the *mir-34a* promoter through histone deacetylation. Finally, SIRT1 deacetylates and activates FXR. FXR transcriptionally activates small heterodimer partner (SHP), which sequesters p53 and thus inhibits miR-34a transcription.

In conclusion, some miRNAs seem to affect multiple aspects of insulin signalling and glucose homeostasis, from pancreatic insulin production to insulin sensitivity and regulation of glucose uptake in target tissues. However, much work remains to elucidate the *in vivo* contribution of specific miRNAs to prevalent pathological conditions, such as obesity-associated insulin resistance, and to evaluate their potential for therapeutic targeting.

miRNAs in obesity and hepatosteatosis

Conditions of nutritional excess and lack of physical activity can result in excessive fat storage manifesting in obesity and NAFLD and strongly increase the risk for cardiovascular disease and type 2 diabetes. Normal adipogenesis involves regulation by transcriptional factors such as peroxisome proliferator-activated receptor-γ (PPARγ) and its co-activators (for example, PPARγ co-activator 1α (PGC1α)) as well as members of the C/EBP family and Krüppel-like factors (KLFs) (reviewed in REF. 92). miRNAs have also recently been implicated in adipogenesis[93]. For instance, miR-143 has been shown to be important in adipocyte differentiation of cultured mouse 3T3-L1 pre-adipocytes, perhaps through its regulation of extracellular signal-regulated kinase 5 (ERK5; also known as MAPK7)[94]. Several other miRNAs, including miR-204, miR-141, miR-200a, miR-200b, miR-200c and miR-429, are involved in early adipocyte cell fate determination, whereas the miR-17–miR-92 miRNA cluster, miR-130, miR-27a and miR-27b as well as miR-378 and miR-378* have been suggested to be involved in terminal differentiation and mature white adipocyte function (recently reviewed in REF. 95). Brown adipose tissue (BAT), which is a highly metabolically active adipose tissue type involved in thermogenesis and energy expenditure, has been suggested to correlate inversely with obesity[96]. A recent study reported a key role for the miR-193b–miR-365 miRNA cluster in brown fat differentiation, in part by repressing myogenesis[97]. The accumulating data thus indicate that miRNAs are acting as central modulators of normal WAT and BAT differentiation and biology. A growing number of studies have also found obesity-associated alterations in expression levels of miRNAs in WAT[82,98–100]. For example, Xie *et al.*[82] showed that certain miRNAs involved in adipogenesis exhibit inverse expression patterns in obesity. Although many miRNAs have been linked to adipogenesis *in vitro*, or have altered expression in WAT in obese individuals and in animal obesity models, evidence for a causative role in obesity-related diseases is still lacking, and much additional detailed *in vivo* work is needed to clarify the regulatory roles of individual miRNAs in modulating energy balance and adipose biology and their potential contribution to obesity.

The hepatic miR-34a and SIRT1 regulatory circuit. As mentioned above, several studies have measured miRNA expression in metabolic tissues in rodent models of obesity, type 2 diabetes and NAFLD[78,81,82]. Interestingly, a number of miRNAs that were dysregulated in these models were also changed in human patients with obesity-related NAFLD and NASH[81,101–103]. Among these, miR-34a may play a particularly relevant part in hepatic metabolic diseases. Patients with NAFLD and NASH exhibit increased hepatic expression of miR-34a[101,102], and miR-34a levels were also higher in subjects with type 2 diabetes as compared to healthy controls[103]. A plausible molecular mechanism for the function (or functions) of miR-34a in metabolic control has recently been suggested (reviewed in REF. 104, summarized in FIG. 3). This involves an intricate regulatory network of miR-34a, SIRT1, farnesoid X receptor (FXR) and p53. SIRT1 is a key sensor and regulator of metabolic states, as it responds to NAD^+ levels in the cell and directly deacetylates and modulates both histone and non-histone targets, including important metabolic regulators such as PGC1α, PPARs, p53, FOXO1, LXR, FXR and SREBPs, to alter the expression of transcriptional programmes governing cholesterol, lipid and energy homeostasis[105–112]. SIRT1 was recently shown to be regulated by miR-34a, causing p53-dependent apoptosis in human colon cancer cells[113]. Subsequent work showed that miR-34a also inhibits SIRT1 expression in the liver[114]. The current data suggest that miR-34a, SIRT1, p53 and FXR constitute a regulatory loop: miR-34a inhibits SIRT1 and is itself regulated by SIRT1 through several mechanisms. First, SIRT1 directly inactivates the *mir-34a* promoter through histone deacetylation. Second, SIRT1 deacetylates and inactivates p53, a transcriptional activator of *mir-34a*[115–118]. Finally, SIRT1 deacetylates FXR, which then activates the liver repressor small heterodimer partner (SHP; also known as NR0B2). SHP sequesters p53 away from the promoter of miR-34a, which results in reduced miR-34a levels[105,114]. Low hepatic levels of SIRT1 and high levels of miR-34a are associated with fatty liver disease. We speculate that miR-34a regulation of SIRT1 could affect other key metabolic targets of SIRT1, such as SREBPs, PPARs, PGC1α, LXR and FOXO1, potentially further contributing to hepatosteatosis (FIG. 3). A crucial

next step is to investigate whether antagonizing increased miR-34a levels in models for NAFLD and obesity has an effect on SIRT1 expression and deacetylation of SIRT1 downstream targets. SIRT1 activators, such as resveratrol and other more potent synthetic inducers, improve hepatosteatosis and insulin resistance in DIO mice[111,119–121]. If verified *in vivo*, activation of SIRT1 through antisense inhibition of miR-34a could potentially represent an alternative therapeutic avenue for the treatment of NAFLD and other obesity-related diseases.

Circulating miRNAs as 'endocrine signals'?

Recent studies have found that miRNAs can be readily detected in human plasma, suggesting possibilities for novel disease biomarker discovery (reviewed in REFS 122,123) and the intriguing notion that some miRNAs (endocrine-miRNAs) may serve regulatory purposes in target cells. Plasma fractionation schemes initially isolated circulating miRNAs as part of microvesicles and exosomes derived from plasma membranes of 'donor' cells[124]. Subsequent findings indicate that miRNAs can also be found in lipid-free protein assemblies, such as in association with the miRNA-processing enzyme Argonaute 2 (AGO2)[125–127]. The discovery of circulating miRNAs represents an important advance for biomarker discovery, and altered circulating miRNA profiles have already been linked to disease states, including NAFLD, NASH and hepatic injury (miR-122)[102], atherosclerosis (miR-223)[128], type 2 diabetes (miR-126)[129] and hypertension (let-7e)[130]. It is likely that additional circulating miRNAs will be identified as biomarkers for metabolic diseases, and this represents a rapidly evolving area of research.

Endocrine miRNAs? Interestingly, circulating miRNAs traffic from donor cells to distant sites and alter the gene expression output of recipient cells[131]. This suggests that circulating miRNAs are not merely 'vesicular passengers' but may rather act as endocrine or paracrine signalling molecules to deliver a regulatory 'message' from donor to target cells. However, in comparison with the highly specific and potent action of classical endocrine hormones such as steroids[132], circulating miRNAs may have only modest effects on mRNA and protein output. In a similar way to mRNAs taken up by cells from microvesicles[133], miRNAs presumably do not require specific receptors for their action and are therefore only limited by the fact that their target mRNAs need to be expressed in target cells for the miRNA to have an impact, and it is unclear whether miRNAs are regulated by intercellular feedback mechanisms. Nevertheless, this intriguing notion should be thoroughly investigated. Could specific classes of miRNAs be enriched in different types of vesicles and lipoprotein particles, with distinct cellular targeting potential depending on surface lipid and protein content, thus allowing selective messaging to specific organs or cell types? If so, might specific miRNAs that are highly abundant in certain vesicles and lipoprotein particles exert 'mass action' effects on gene expression programmes in target cells or tissues, thereby acting in a more potent manner akin to classic endocrine cues?

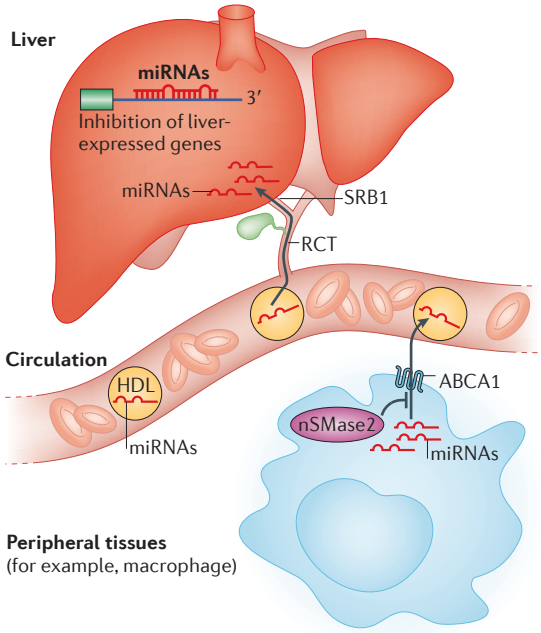


Figure 4 | **Model for the function of circulating miRNAs associated with HDL.** MicroRNAs (miRNAs) such as miR-375 and miR-223 are produced in peripheral tissues and incorporated into high-density lipoproteins (HDLs). This incorporation is controlled by the cholesterol transporter ATP-binding cassette subfamily A member 1 (ABCA1) and inhibited by neutral sphingomyelinase 2 (nSMase2). miRNAs associated with HDL are thought to be trafficked to the liver through the reverse cholesterol transport (RCT) pathway and taken up by a scavenger receptor class B member 1 (SRB1)-dependent mechanism. Once inside target cells, miRNAs can then exert inhibitory effects on a range of target genes.

miRNAs in HDL. An exciting new study revealed that miRNAs, such as miR-375 and miR-223, can also be found in HDL particles, suggesting new potential roles for HDL, and perhaps other lipoprotein assemblies, in transporting not only cholesterol and other lipids and proteins but also regulatory RNAs[128]. Vickers *et al.*[128] found that certain endogenous miRNAs (for example, miR-135a*, miR-375 and miR-223) are exported from peripheral cells in HDL particles. Export of miRNAs to HDL was shown to be controlled by neutral sphingomyelinase 2 (nSMase2; also known as SMPD3), the rate-limiting enzyme in ceramide biosynthesis, suggesting that plasma membrane lipids such as sphingomyelin and ceramides are involved in controlling miRNA incorporation into HDL.

Once miRNAs are embedded in HDL, they are probably trafficked to the liver by the classic RCT pathway for uptake in a scavenger receptor class B member 1 (SRB1; also known as SCARB1)-dependent manner and may exert effects on hepatic gene expression programmes (FIG. 4). Consistent with this notion, Vickers *et al.*[128] showed that treatment of hepatic Huh-7 cells with HDL loaded with exogenous miRNAs (for example, miR-223) could indeed affect target mRNA expression. Interestingly, they also found that HDL miRNA profiles differ between normal subjects and atherosclerotic individuals, both in

Exosomes
Small (30–90 nm) vesicles secreted from the plasma membrane of mammalian cells. They contain proteins, RNA and microRNA molecules and can be transported from cell to cell.

Paracrine signalling
Signalling mediated by secreted molecules that act locally.

mice and humans. This warrants follow-up studies to determine whether circulating miRNAs associated with HDL in patients with heart disease could participate in the disease process, or whether specific miRNAs associated with HDL might constitute part of the protective function against atherosclerosis ascribed to HDL. Finally, plasma HDL–miRNA profiling might serve as a novel biomarker tool for detecting or monitoring cardiovascular disease progression.

Concluding remarks

miRNAs are important regulators of numerous aspects of animal development, physiology and disease and their function in the control of metabolic homeostasis has recently emerged. The best-characterized metabolic role for miRNAs is in the maintenance of normal cholesterol and lipid homeostasis (for example, miR-122 and miR-33). New data reveal that miRNAs also contribute to insulin signalling and glucose homeostasis and highlight the potential pathological roles of aberrant miRNA expression in cardiometabolic disorders such as obesity, NAFLD, insulin resistance, type 2 diabetes and coronary artery disease. The exciting finding that miRNAs can be found circulating in the blood not only points to their potential use as biomarkers for disease states, but also suggests that circulating miRNAs may be actively secreted from donor cells and tissues to act as signalling molecules that affect the gene expression output in distant target organs and cells (for example, in the liver in the case of HDL-associated miRNAs), akin to classical endocrine cues.

The emergence of miRNAs as important regulators of metabolism has garnered much interest not only from a scientific point of view but also from a clinical perspective, with the potential contribution of aberrant miRNA expression and therapeutic implications for the treatment of cardiometabolic diseases raising considerable excitement. Therapeutic efforts to treat metabolic disorders have traditionally centred on the inhibition of what are perceived as 'druggable' targets, such as enzymes (for example, HMGCR targeted by cholesterol-lowering statins). However, the recent discovery by us and others that certain miRNAs may represent crucial regulators of metabolism has provided important insights into metabolic regulatory networks that are already yielding novel types of therapeutic targets and strategies for the treatment of metabolic diseases. For example, the central and concerted role of miR-33a and miR-33b and their SREBP host genes in controlling metabolic homeostasis, together with promising data from mice and non-human primate models, suggest that antisense targeting of miR-33a and miR-33b for the treatment of MetS and cardiovascular disease should be explored further. It is likely that other miRNAs with regulatory roles in human metabolism (for example, miR-103 and miR-107 in insulin resistance) may also serve as suitable candidates for therapeutic intervention.

A number of open questions remain concerning the function of miRNAs in metabolic control. For example, although it is apparent that several miRNAs, including miR-33a and miR-33b, have an important regulatory impact on cholesterol and lipid metabolism *in vivo* by acting in concert with their SREBP host genes, it is unclear how widespread the contribution of individual miRNAs is to metabolic control. miRNAs typically have rather modest effects on target protein levels, and, as is the case for miR-33, combinatorial actions on multiple functionally related targets in linear pathways or key nodes in regulatory networks are probably required for single miRNAs to significantly influence a complex cellular and organismal process such as metabolic homeostasis. As mRNAs have multiple predicted miRNA target sites, it is likely that groups of miRNAs also act in concert to exert more potent effects on specific target mRNAs. New experimental strategies using systems biology methodologies may be required to determine the composition and regulation of such putative miRNA networks and to elucidate how miRNA networks are coordinated with other regulatory systems (for example, transcriptional and translational control circuits). Akin to the restrictions imposed by complex chromatin architectures on accessibility of DNA sequences to regulatory factors, could secondary and tertiary structural features of 3′ UTR folding govern miRNA efficacy? Are other regulators that bind mRNAs — including proteins such as the ubiquitous member of the Hu family of RNA-binding proteins, HUR, and other non-coding RNAs — working together or in opposition to miRNAs in controlling mRNA folding and accessibility, stability and translational output? Additionally, individual miRNAs typically have many targets, which gives rise to the question of whether certain miRNAs may coordinately control metabolism with other cellular and organismal processes, such as cell growth and proliferation, cellular differentiation or organ growth. Moreover, given the likelihood that many miRNAs may regulate targets involved in multiple cellular processes, what are the possible long-term consequences of miRNA-directed therapeutics? These and many other unanswered questions concerning miRNA biology and the impact of miRNAs on metabolic homeostasis will undoubtedly be topics for intensive and exciting research for years to come.

1. Lehrke, M. & Lazar, M. A. The many faces of PPARγ. *Cell* **123**, 993–999 (2005).
2. Zelcer, N. & Tontonoz, P. Liver X receptors as integrators of metabolic and inflammatory signaling. *J. Clin. Invest.* **116**, 607–614 (2006).
3. Horton, J. D., Goldstein, J. L. & Brown, M. S. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **109**, 1125–1131 (2002).
4. Nakae, J., Oki, M. & Cao, Y. The FoxO transcription factors and metabolic regulation. *FEBS Lett.* **582**, 54–67 (2008).
5. Uyeda, K. & Repa, J. J. Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metab.* **4**, 107–110 (2006).
6. Bartel, D. P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281–297 (2004).
7. Bartel, D. P. MicroRNAs: target recognition and regulatory functions. *Cell* **136**, 215–233 (2009).
8. Voinnet, O. Origin, biogenesis, and activity of plant microRNAs. *Cell* **136**, 669–687 (2009).
9. Fabian, M. R. *et al.* Mammalian miRNA RISC recruits CAF1 and PABP to affect PABP-dependent deadenylation. *Mol. Cell* **35**, 868–880 (2009).
10. Grimson, A. *et al.* MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol. Cell* **27**, 91–105 (2007).
11. Betel, D., Wilson, M., Gabow, A., Marks, D. S. & Sander, C. The microRNA.org resource: targets and expression. *Nucleic Acids Res.* **36**, D149–D153 (2008).
12. Krek, A. *et al.* Combinatorial microRNA target predictions. *Nature Genet.* **37**, 495–500 (2005).

13. Hon, L. S. & Zhang, Z. The roles of binding site arrangement and combinatorial targeting in microRNA repression of gene expression. *Genome Biol.* **8**, R166 (2007).
14. Rottiers, V. *et al.* MicroRNAs in metabolism and metabolic diseases. *Cold Spring Harb. Symp. Quant. Biol.* 12 Dec 2011 (doi:10.1101/sqb.2011.76.011049).
15. Tsang, J., Zhu, J. & van Oudenaarden, A. MicroRNA-mediated feedback and feedforward loops are recurrent network motifs in mammals. *Mol. Cell* **26**, 753–767 (2007).
16. Raghow, R., Yellaturu, C., Deng, X., Park, E. A. & Elam, M. B. SREBPs: the crossroads of physiological and pathological lipid homeostasis. *Trends Endocrinol. Metab.* **19**, 65–73 (2008).
17. Cornier, M. A. *et al.* The metabolic syndrome. *Endocr. Rev.* **29**, 777–822 (2008).
18. Angulo, P. Obesity and nonalcoholic fatty liver disease. *Nutr. Rev.* **65**, S57–S63 (2007).
19. Lewis, A. P. & Jopling, C. L. Regulation and biological function of the liver-specific miR-122. *Biochem. Soc. Trans.* **38**, 1553–1557 (2010).
20. Esau, C. *et al.* miR-122 regulation of lipid metabolism revealed by *in vivo* antisense targeting. *Cell Metab.* **3**, 87–98 (2006).
21. Krutzfeldt, J. *et al.* Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature* **438**, 685–689 (2005).
References 20 and 21 report two pioneering studies utilizing antisense approaches to elucidate *in vivo* roles of an miRNA (miR-122).
22. Veedu, R. N. & Wengel, J. Locked nucleic acid as a novel class of therapeutic agents. *RNA Biol.* **6**, 321–323 (2009).
23. Elmen, J. *et al.* LNA-mediated microRNA silencing in non-human primates. *Nature* **452**, 896–899 (2008).
In this study LNA-antisense oligonucleotides were used to inhibit miR-122 in the livers of non-human primates. This is the first study of miRNAs in a primate model.
24. Elmen, J. *et al.* Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. *Nucleic Acids Res.* **36**, 1153–1162 (2008).
25. Iliopoulos, D., Drosatos, K., Hiyama, Y., Goldberg, I. J. & Zannis, V. I. MicroRNA-370 controls the expression of microRNA-122 and Cpt1α and affects lipid metabolism. *J. Lipid Res.* **51**, 1513–1523 (2010).
26. Coulouarn, C., Factor, V. M., Andersen, J. B., Durkin, M. E. & Thorgeirsson, S. S. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene* **28**, 3526–3536 (2009).
27. Bai, S. *et al.* MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. *J. Biol. Chem.* **284**, 32015–32027 (2009).
28. Jopling, C. L., Schutz, S. & Sarnow, P. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. *Cell Host Microbe* **4**, 77–85 (2008).
29. Norman, K. L. & Sarnow, P. Modulation of hepatitis C virus RNA abundance and the isoprenoid biosynthesis pathway by microRNA miR-122 involves distinct mechanisms. *J. Virol.* **84**, 666–670 (2010).
30. Roberts, A. P., Lewis, A. P. & Jopling, C. L. miR-122 activates hepatitis C virus translation by a specialized mechanism requiring particular RNA components. *Nucleic Acids Res.* **39**, 7716–7729 (2011).
31. Gerin, I. *et al.* Expression of miR-33 from an SREBP2 intron inhibits cholesterol export and fatty acid oxidation. *J. Biol. Chem.* **285**, 33652–33661 (2010).
32. Horie, T. *et al.* MicroRNA-33 encoded by an intron of sterol regulatory element-binding protein 2 (*Srebp2*) regulates HDL *in vivo*. *Proc. Natl Acad. Sci. USA* **107**, 17321–17326 (2010).
33. Marquart, T. J., Allen, R. M., Ory, D. S. & Baldan, A. miR-33 links SREBP-2 induction to repression of sterol transporters. *Proc. Natl Acad. Sci. USA* **107**, 12228–12232 (2010).
34. Najafi-Shoushtari, S. H. *et al.* MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis. *Science* **328**, 1566–1569 (2010).
35. Rayner, K. J. *et al.* MiR-33 contributes to the regulation of cholesterol homeostasis. *Science* **328**, 1570–1573 (2010).
This study shows, together with references 31, 32 and 33, that miR-33a and miR-33b within introns in the SREBP genes act in concert with their host genes as crucial regulators of cholesterol and lipid homeostasis.
36. Davalos, A. *et al.* miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. *Proc. Natl Acad. Sci. USA* **108**, 9232–9237 (2011).
37. Tang, C. & Oram, J. F. The cell cholesterol exporter ABCA1 as a protector from cardiovascular disease and diabetes. *Biochim. Biophys. Acta* **1791**, 563–572 (2009).
38. Rye, K. A., Bursill, C. A., Lambert, G., Tabet, F. & Barter, P. J. The metabolism and anti-atherogenic properties of HDL. *J. Lipid Res.* **50**, S195–S200 (2009).
39. Rayner, K. J. *et al.* Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. *J. Clin. Invest.* **121**, 2921–2931 (2011).
40. Brown, M. S., Ye, J. & Goldstein, J. L. Medicine. HDL miR-ed down by SREBP introns. *Science* **328**, 1495–1496 (2010).
41. Rayner, K. J. *et al.* Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. *Nature* **478**, 404–407 (2011).
This paper shows the therapeutic promise of antisense targeting of miR-33a and miR-33b to raise HDL and lower triglyceride levels in a non-human primate model.
42. Ramirez, C. M. *et al.* MicroRNA-758 regulates cholesterol efflux through posttranscriptional repression of ATP-binding cassette transporter A1. *Arterioscler. Thromb. Vasc. Biol.* **31**, 2707–2714 (2011).
43. Kim, J. *et al.* miR-106b impairs cholesterol efflux and increases Aβ levels by repressing ABCA1 expression. *Exp. Neurol.* 18 Nov 2011 (doi:10.1016/j.expneurol.2011.11.010).
44. Mostoslavsky, R. *et al.* Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* **124**, 315–329 (2006).
45. Michishita, E. *et al.* SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. *Nature* **452**, 492–496 (2008).
46. Xiao, C. *et al.* SIRT6 deficiency results in severe hypoglycemia by enhancing both basal and insulin-stimulated glucose uptake in mice. *J. Biol. Chem.* **285**, 36776–36784 (2010).
47. Zhong, L. *et al.* The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1α. *Cell* **140**, 280–293 (2010).
48. Kim, H. S. *et al.* Hepatic-specific disruption of SIRT6 in mice results in fatty liver formation due to enhanced glycolysis and triglyceride synthesis. *Cell Metab.* **12**, 224–236 (2010).
49. Hardie, D. G. AMP-activated protein kinase: a cellular energy sensor with a key role in metabolic disorders and in cancer. *Biochem. Soc. Trans.* **39**, 1–13 (2011).
50. Yap, F., Craddock, L. & Yang, J. Mechanism of AMPK suppression of LXR-dependent Srebp-1c transcription. *Int. J. Biol. Sci.* **7**, 645–650 (2011).
51. Li, Y. *et al.* AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab.* **13**, 376–388 (2011).
52. Matsumoto, M. *et al.* Role of the insulin receptor substrate 1 and phosphatidylinositol 3-kinase signaling pathway in insulin-induced expression of sterol regulatory element binding protein 1c and glucokinase genes in rat hepatocytes. *Diabetes* **51**, 1672–1680 (2002).
53. Taniguchi, C. M., Ueki, K. & Kahn, R. Complementary roles of IRS-1 and IRS-2 in the hepatic regulation of metabolism. *J. Clin. Invest.* **115**, 718–727 (2005).
54. Tobe, K. *et al.* Increased expression of the sterol regulatory element-binding protein-1 gene in insulin receptor substrate-$2^{-/-}$ mouse liver. *J. Biol. Chem.* **276**, 38337–38340 (2001).
55. Kohjima, M. *et al.* SREBP-1c, regulated by the insulin and AMPK signaling pathways, plays a role in nonalcoholic fatty liver disease. *Int. J. Mol. Med.* **21**, 507–511 (2008).
56. Guay, C., Roggli, E., Nesca, V., Jacovetti, C. & Regazzi, R. Diabetes mellitus, a microRNA-related disease? *Transl. Res.* **157**, 253–264 (2011).
57. Herman, M. A. & Kahn, B. B. Glucose transport and sensing in the maintenance of glucose homeostasis and metabolic harmony. *J. Clin. Invest.* **116**, 1767–1775 (2006).
58. Siddle, K. Signalling by insulin and IGF receptors: supporting acts and new players. *J. Mol. Endocrinol.* **47**, R1–R10 (2011).
59. Leavens, K. F. & Birnbaum, M. J. Insulin signaling to hepatic lipid metabolism in health and disease. *Crit. Rev. Biochem. Mol. Biol.* **46**, 200–215 (2011).
60. Osborne, T. F. & Espenshade, P. J. Evolutionary conservation and adaptation in the mechanism that regulates SREBP action: what a long, strange tRIP it's been. *Genes Dev.* **23**, 2578–2591 (2009).
61. Kloosterman, W. P., Lagendijk, A. K., Ketting, R. F., Moulton, J. D. & Plasterk, R. H. Targeted inhibition of miRNA maturation with morpholinos reveals a role for miR-375 in pancreatic islet development. *PLoS Biol.* **5**, e203 (2007).
62. Poy, M. N. *et al.* miR-375 maintains normal pancreatic α- and β-cell mass. *Proc. Natl Acad. Sci. USA* **106**, 5813–5818 (2009).
63. Poy, M. N. *et al.* A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* **432**, 226–230 (2004).
This study provides the first evidence for miRNA regulation of insulin secretion, suggesting that miRNAs may represent important regulators of glucose homeostasis.
64. El Ouaamari, A. *et al.* miR-375 targets 3′-phosphoinositide-dependent protein kinase-1 and regulates glucose-induced biological responses in pancreatic β-cells. *Diabetes* **57**, 2708–2717 (2008).
65. Baroukh, N. N. & Van Obberghen, E. Function of microRNA-375 and microRNA-124a in pancreas and brain. *FEBS J.* **276**, 6509–6521 (2009).
66. Baroukh, N. *et al.* MicroRNA-124a regulates Foxa2 expression and intracellular signaling in pancreatic β-cell lines. *J. Biol. Chem.* **282**, 19575–19588 (2007).
67. Lovis, P., Gattesco, S. & Regazzi, R. Regulation of the expression of components of the exocytotic machinery of insulin-secreting cells by microRNAs. *Biol. Chem.* **389**, 305–312 (2008).
68. Ramachandran, D. *et al.* Sirt1 and mir-9 expression is regulated during glucose-stimulated insulin secretion in pancreatic β-islets. *FEBS J.* **278**, 1167–1174 (2011).
69. Plaisance, V. *et al.* MicroRNA-9 controls the expression of Granuphilin/Slp4 and the secretory response of insulin-producing cells. *J. Biol. Chem.* **281**, 26932–26942 (2006).
70. Pullen, T. J., da Silva Xavier, G., Kelsey, G. & Rutter, G. A. miR-29a and miR-29b contribute to pancreatic β-cell-specific silencing of monocarboxylate transporter 1 (Mct1). *Mol. Cell. Biol.* **31**, 3182–3194 (2011).
71. He, A., Zhu, L., Gupta, N., Chang, Y. & Fang, F. Overexpression of micro ribonucleic acid 29, highly up-regulated in diabetic rats, leads to insulin resistance in 3T3-L1 adipocytes. *Mol. Endocrinol.* **21**, 2785–2794 (2007).
72. Kim, S. & Pak, Y. Caveolin-2 regulation of the cell cycle in response to insulin in Hirc-B fibroblast cells. *Biochem. Biophys. Res. Commun.* **330**, 88–96 (2005).
73. Pandey, A. K. *et al.* miR-29a levels are elevated in the *db/db* mice liver and its overexpression leads to attenuation of insulin action on PEPCK gene expression in HepG2 cells. *Mol. Cell. Endocrinol.* **332**, 125–133 (2011).
74. Ryu, H. S., Park, S. Y., Ma, D., Zhang, J. & Lee, W. The induction of microRNA targeting IRS-1 is involved in the development of insulin resistance under conditions of mitochondrial dysfunction in hepatocytes. *PLoS ONE* **6**, e17343 (2011).
75. Lu, H., Buchan, R. J. & Cook, S. A. MicroRNA-223 regulates Glut4 expression and cardiomyocyte glucose metabolism. *Cardiovasc. Res.* **86**, 410–420 (2010).
76. Esguerra, J. L., Bolmeson, C., Cilio, C. M. & Eliasson, L. Differential glucose-regulation of microRNAs in pancreatic islets of non-obese type 2 diabetes model Goto–Kakizaki rat. *PLoS ONE* **6**, e18613 (2011).
77. Jordan, S. D. *et al.* Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism. *Nature Cell Biol.* **13**, 434–446 (2011).
78. Li, S. *et al.* Differential expression of microRNAs in mouse liver under aberrant energy metabolic status. *J. Lipid Res.* **50**, 1756–1765 (2009).
79. Nakanishi, N. *et al.* The up-regulation of microRNA-335 is associated with lipid metabolism in liver and white adipose tissue of genetically obese mice. *Biochem. Biophys. Res. Commun.* **385**, 492–496 (2009).
80. Takanabe, R. *et al.* Up-regulated expression of microRNA-143 in association with obesity in adipose tissue of mice fed high-fat diet. *Biochem. Biophys. Res. Commun.* **376**, 728–732 (2008).

81. Trajkovski, M. *et al.* MicroRNAs 103 and 107 regulate insulin sensitivity. *Nature* **474**, 649–653 (2011).
82. Xie, H., Lim, B. & Lodish, H. F. MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity. *Diabetes* **58**, 1050–1057 (2009).
83. Wilfred, B. R., Wang, W. X. & Nelson, P. T. Energizing miRNA research: a review of the role of miRNAs in lipid metabolism, with a prediction that miR-103/107 regulates human metabolic pathways. *Mol. Genet. Metab.* **91**, 209–217 (2007).
84. Naar, A. M. MiRs with a sweet tooth. *Cell Metab.* **14**, 149–150 (2011).
85. Martello, G. *et al.* A MicroRNA targeting dicer for metastasis control. *Cell* **141**, 1195–1207 (2010).
86. Morita, S. *et al.* Dicer is required for maintaining adult pancreas. *PLoS ONE* **4**, e4212 (2009).
87. Lynn, F. C. *et al.* MicroRNA expression is required for pancreatic islet cell genesis in the mouse. *Diabetes* **56**, 2938–2945 (2007).
88. Lovis, P. *et al.* Alterations in microRNA expression contribute to fatty acid-induced pancreatic β-cell dysfunction. *Diabetes* **57**, 2728–2736 (2008).
89. Roggli, E. *et al.* Involvement of microRNAs in the cytotoxic effects exerted by proinflammatory cytokines on pancreatic β-cells. *Diabetes* **59**, 978–986 (2010).
90. Yamakuchi, M. & Lowenstein, C. J. MiR-34, SIRT1 and p53: the feedback loop. *Cell Cycle* **8**, 712–715 (2009).
91. Zhu, H. *et al.* The Lin28/let-7 axis regulates glucose metabolism. *Cell* **147**, 81–94 (2011).
This paper, together with references 77 and 81, links aberrant expression of an miRNA in obesity-related disorders to impaired insulin signalling and glucose homeostasis, thereby providing important evidence that dysregulation of miRNA expression may contribute to human metabolic diseases.
92. Lefterova, M. I. & Lazar, M. A. New developments in adipogenesis. *Trends Endocrinol. Metab.* **20**, 107–114 (2009).
93. Alexander, R., Lodish, H. & Sun, L. MicroRNAs in adipogenesis and as therapeutic targets for obesity. *Expert Opin. Ther. Targets* **15**, 623–636 (2011).
94. Esau, C. *et al.* MicroRNA-143 regulates adipocyte differentiation. *J. Biol. Chem.* **279**, 52361–52365 (2004).
The first study linking an miRNA to the differentiation and function of a metabolically important tissue, highlighting the potential for dysregulation of miRNAs in metabolic disorders.
95. Romao, J. M. *et al.* MicroRNA regulation in mammalian adipogenesis. *Exp. Biol. Med. (Maywood)* **236**, 997–1004 (2011).
96. Cypess, A. M. *et al.* Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* **360**, 1509–1517 (2009).
97. Sun, L. *et al.* Mir193b-365 is essential for brown fat differentiation. *Nature Cell Biol.* **13**, 958–965 (2011).
98. Kloting, N. *et al.* MicroRNA expression in human omental and subcutaneous adipose tissue. *PLoS ONE* **4**, e4699 (2009).
99. Heneghan, H. M., Miller, N., McAnena, O. J., O'Brien, T. & Kerin, M. J. Differential miRNA expression in omental adipose tissue and in the circulation of obese patients identifies novel metabolic biomarkers. *J. Clin. Endocrinol. Metab.* **96**, e846–e850 (2011).
100. Ortega, F. J. *et al.* MiRNA expression profile of human subcutaneous adipose and during adipocyte differentiation. *PLoS ONE* **5**, e9022 (2010).
101. Cheung, O. *et al.* Nonalcoholic steatohepatitis is associated with altered hepatic microRNA expression. *Hepatology* **48**, 1810–1820 (2008).
102. Cermelli, S., Ruggieri, A., Marrero, J. A., Ioannou, G. N. & Beretta, L. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. *PLoS ONE* **6**, e23937 (2011).
103. Kong, L. *et al.* Significance of serum microRNAs in pre-diabetes and newly diagnosed type 2 diabetes: a clinical study. *Acta Diabetol.* **48**, 61–69 (2011).
104. Lee, J. & Kemper, J. K. Controlling SIRT1 expression by microRNAs in health and metabolic disease. *Aging (Albany NY)* **2**, 527–534 (2010).
105. Kemper, J. K. *et al.* FXR acetylation is normally dynamically regulated by p300 and SIRT1 but constitutively elevated in metabolic disease states. *Cell Metab.* **10**, 392–404 (2009).
106. Li, X. *et al.* SIRT1 deacetylates and positively regulates the nuclear receptor LXR. *Mol. Cell* **28**, 91–106 (2007).
107. Motta, M. C. *et al.* Mammalian SIRT1 represses forkhead transcription factors. *Cell* **116**, 551–563 (2004).
108. Ponugoti, B. *et al.* SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. *J. Biol. Chem.* **285**, 33959–33970 (2010).
109. Purushotham, A. *et al.* Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell Metab.* **9**, 327–338 (2009).
110. Vaziri, H. *et al.* hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell* **107**, 149–159 (2001).
111. Walker, A. K. *et al.* Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. *Genes Dev.* **24**, 1403–1417 (2010).
112. Yeung, F. *et al.* Modulation of NF-κB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J.* **23**, 2369–2380 (2004).
113. Yamakuchi, M., Ferlito, M. & Lowenstein, C. J. miR-34a repression of SIRT1 regulates apoptosis. *Proc. Natl Acad. Sci. USA* **105**, 13421–13426 (2008).
114. Lee, J. *et al.* A pathway involving farnesoid X receptor and small heterodimer partner positively regulates hepatic sirtuin 1 levels via microRNA-34a inhibition. *J. Biol. Chem.* **285**, 12604–12611 (2010).
115. Chang, T. C. *et al.* Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Mol. Cell* **26**, 745–752 (2007).
116. He, L. *et al.* A microRNA component of the p53 tumour suppressor network. *Nature* **447**, 1130–1134 (2007).
117. Raver-Shapira, N. *et al.* Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol. Cell* **26**, 731–743 (2007).
118. Tarasov, V. *et al.* Differential regulation of microRNAs by p53 revealed by massively parallel sequencing: miR-34a is a p53 target that induces apoptosis and G1-arrest. *Cell Cycle* **6**, 1586–1593 (2007).
119. Milne, J. C. *et al.* Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature* **450**, 712–716 (2007).
120. Baur, J. A. *et al.* Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444**, 337–342 (2006).
121. Feige, J. N. *et al.* Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. *Cell Metab.* **8**, 347–358 (2008).
122. Chen, X., Liang, H., Zhang, J., Zen, K. & Zhang, C. Y. Secreted microRNAs: a new form of intercellular communication. *Trends Cell Biol.* **22**, 125–132 (2012).
123. Creemers, E. E., Tijsen, A. J. & Pinto, Y. M. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular disease? *Circ. Res.* **110**, 483–495 (2012).
124. Hunter, M. P. *et al.* Detection of microRNA expression in human peripheral blood microvesicles. *PLoS ONE* **3**, e3694 (2008).
The first demonstration of the presence of miRNAs in circulating microvesicles.
125. Arroyo, J. D. *et al.* Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc. Natl Acad. Sci. USA* **108**, 5003–5008 (2011).
126. Turchinovich, A., Weiz, L., Langheinz, A. & Burwinkel, B. Characterization of extracellular circulating microRNA. *Nucleic Acids Res.* **39**, 7223–7233 (2011).
127. Wang, K., Zhang, S., Weber, J., Baxter, D. & Galas, D. J. Export of microRNAs and microRNA-protective protein by mammalian cells. *Nucleic Acids Res.* **38**, 7248–7259 (2010).
128. Vickers, K. C., Palmisano, B. T., Shoucri, B. M., Shamburek, R. D. & Remaley, A. T. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nature Cell Biol.* **13**, 423–433 (2011).
The first demonstration that miRNAs are present in circulating HDL, indicating that disease-associated miRNAs may traffic in lipoprotein particles and convey regulatory information to distant target tissues.
129. Zampetaki, A. *et al.* Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. *Circ. Res.* **107**, 810–817 (2010).
130. Li, S. *et al.* Signature microRNA expression profile of essential hypertension and its novel link to human cytomegalovirus infection. *Circulation* **124**, 175–184 (2011).
131. Kosaka, N. *et al.* Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J. Biol. Chem.* **285**, 17442–17452 (2010).
132. Bagamasbad, P. & Denver, R. J. Mechanisms and significance of nuclear receptor auto- and cross-regulation. *Gen. Comp. Endocrinol.* **170**, 3–17 (2011).
133. Valadi, H. *et al.* Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biol.* **9**, 654–659 (2007).
This study identified miRNAs in circulating exosome vesicles and suggested that exosome-associated miRNAs may transmit gene regulatory cues between cells.
134. Yang, J. S. & Lai, E. C. Alternative miRNA biogenesis pathways and the interpretation of core miRNA pathway mutants. *Mol. Cell* **43**, 892–903 (2011).
135. Herker, E. & Ott, M. Unique ties between hepatitis C virus replication and intracellular lipids. *Trends Endocrinol. Metab.* **22**, 241–248 (2011).
136. Lanford, R. E. *et al.* Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* **327**, 198–201 (2010).
137. Stenvang, J., Petri, A., Lindow, M., Obad, S. & Kauppinen, S. Inhibition of microRNA function by antimiR oligonucleotides. *Silence* **3**, 1 (2012).
138. Laudadio, I. *et al.* A feedback loop between the liver-enriched transcription factor network and Mir-122 controls hepatocyte differentiation. *Gastroenterology* **142**, 119–129 (2012).
139. Chang, J. *et al.* miR-122, a mammalian liver-specific microRNA, is processed from hcr mRNA and may downregulate the high affinity cationic amino acid transporter CAT-1. *RNA Biol.* **1**, 106–113 (2004).
140. Tsai, W. C. *et al.* MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. *Hepatology* **49**, 1571–1582 (2009).
141. Lin, Q., Gao, Z., Alarcon, R. M., Ye, J. & Yun, Z. A role of miR-27 in the regulation of adipogenesis. *FEBS J.* **276**, 2348–2358 (2009).
142. Kim, S. Y. *et al.* miR-27a is a negative regulator of adipocyte differentiation via suppressing PPARγ expression. *Biochem. Biophys. Res. Commun.* **392**, 323–328 (2010).
143. Li, W. Q. *et al.* The rno-miR-34 family is upregulated and targets ACSL1 in dimethylnitrosamine-induced hepatic fibrosis in rats. *FEBS J.* **278**, 1522–1532 (2011).
144. Gerin, I. *et al.* Roles for miRNA-378/378* in adipocyte gene expression and lipogenesis. *Am. J. Physiol. Endocrinol. Metab.* **299**, e198–e206 (2010).
145. Jin, W., Dodson, M. V., Moore, S. S., Basarab, J. A. & Guan, L. L. Characterization of microRNA expression in bovine adipose tissues: a potential regulatory mechanism of subcutaneous adipose tissue development. *BMC Mol. Biol.* **11**, 29 (2010).
146. Eichner, L. J. *et al.* miR-378* mediates metabolic shift in breast cancer cells via the PGC-1β/ERRγ transcriptional pathway. *Cell Metab.* **12**, 352–361 (2010).
147. Lee, E. K. *et al.* miR-130 suppresses adipogenesis by inhibiting peroxisome proliferator-activated receptor γ expression. *Mol. Cell. Biol.* **31**, 626–638 (2011).
148. Sun, T., Fu, M., Bookout, A. L., Kliewer, S. A. & Mangelsdorf, D. J. MicroRNA let-7 regulates 3T3-L1 adipogenesis. *Mol. Endocrinol.* **23**, 925–931 (2009).

Acknowledgements
This work was supported by US National Institutes of Health (NIH) grants R21DK084459 and R01DK094184. The authors apologize for their inability to discuss and cite all relevant papers owing to space constraints.

Competing interests statement
The authors declare competing financial interests: see Web version for details.

FURTHER INFORMATION
Anders M. Näär's homepage:
http://www.hms.harvard.edu/dms/bbs/fac/naar.php

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

ERRATUM

MicroRNAs in metabolism and metabolic disorders

Veerle Rottiers and Anders M. Näär

Nature Reviews Molecular Cell Biology **13**, 239–250 (2012)

On page 250 of this article, the competing financial interests statement incorrectly stated that the authors had no competing interests. The authors' statement is reproduced below, and the article has been corrected online. The editors apologize for this omission.

Competing financial interests statement
Anders M. Näär has patents pending on miR-33a and miR-33b antisense targeting for the treatment of cardiometabolic disorders. These patents have been licensed by Santaris Pharma, Denmark. Veerle Rottiers declares no competing financial interests.