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HIGH FAT DIET AND ASSOCIATED CHANGES IN THE EXPRESSION OF MICRO-RNAS IN TISSUE: LESSONS LEARNED FROM ANIMAL STUDIES

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Abbreviations:

ACADVL	gene that encodes very long-chain acyl-CoA dehydrogenase
ACVR1B	activin A receptor, type IB isoform c precursor
Akt	also known as protein kinase B

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AMPK	adenosine monophosphate-activated protein kinase
aP2	adipocyte fatty acid-binding protein
ApoE	apolipoprotein E
Bmp-4	bone morphogenetic protein 4
Cab-39	calcium binding protein 39
CAT1	cationic amino acid transporter 1
C/EBP β	CCAAT-enhancer-binding proteins
CPT1a	carnitine palmitoyltransferase 1A
Crhr1	corticotropin Releasing Hormone Receptor 1
CVD	cardiovascular diseases
ERK	extracellular signal regulated kinase
Esrra	estrogen-related receptor alpha
FABP	fatty acid binding protein
FASN	fatty acid synthase
Fgf10	fibroblast growth factor 10
GATA4	transcription factor that belongs to GATA family
Hif-1 α	hypoxia-inducible factor 1-alpha
HMG2	high-mobility group AT-hook 2
IGF-1	insulin-like growth factor-1
Ihh	indian hedgehog
LDLR	low density lipoprotein receptor
LDLRAP1	low density lipoprotein receptor adapter protein 1
LIPA	lipase A precursor
LKB1	liver kinase B1 also known as serine/threonine kinase 11
LPL	lipoprotein lipase
MAPK	mitogen-activated protein kinases
miRNA or miR	microRNA

MLCK	myosin light chain kinase
MT1	melatonin receptor type 1
mTOR	mechanistic target of rapamycin
MYC	V-Myc Avian Myelocytomatosis Viral Oncogene Homolog
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steato hepatitis
Pdk1	pyruvate dehydrogenase lipoamide kinase isozyme 1
PI3K	phosphatidylinositol-4,5-bisphosphate 3-kinase
PPAR- α	peroxisome proliferator-activated receptor- alpha
PPAR- γ	peroxisome proliferator-activated receptor- gamma
Pref-1	pre-adipocyte factor 1
PTEN	phosphatase and tensin homolog
RISC	ribonucleoprotein miRNA induced silencing complex
RNA	ribonucleic Acids
TENC1	tensin-like C1 domain containing phosphatase
TGF- β	transforming growth factor beta
TNF- α	tumour necrosis factor alpha
UCP-1	uncoupling protein 1
VEGF	vascular endothelial growth factor
Zeb1 and 2	zinc finger E-Box binding homeobox 1 and 2

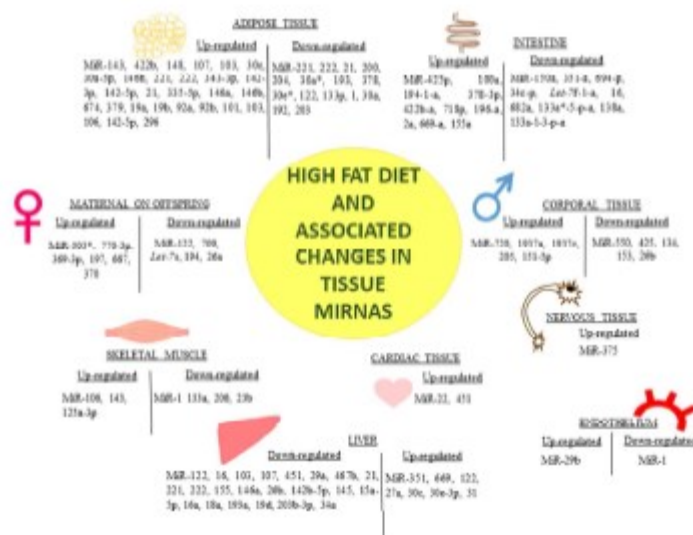
Key words: *gene regulation, high fat diet, disease, miRNA, obesity.*

ABSTRACT

Environment and genetic factors play an important role in the development of obesity, and diet is one of the main contributing factors to this disease. High fat intake is associated with body weight gain, leading to obesity and other metabolic diseases. MicroRNAs (miRNAs) are a group of small, non-coding RNAs that are important regulators of gene expression at post transcriptional level. Studies have shown that high fat intake, independent of body weight status, can significantly impact both negatively and positively the expression of miRNAs and thus the biological function of tissues such as adipose, skeletal and cardiac muscle, liver, neuronal and endothelial. This review will summarise the effects of high calorie diet in the form of high fat intake on miRNA expression in various tissues of animal models and of high fat fed offspring. We will also briefly review the impact of different dietary lipids on miRNA expression. Given changes in miRNA expression have been associated with the development of many diseases including obesity, understanding their biological role could have important clinical implications and offer tangible therapeutic targets for the prevention, management and/or treatment of obesity and other lifestyle related disorders.

High fat intake can impact a variety of tissues independent of body weight status. Small, non-coding RNAs called microRNAs play an important role in the diet-induced changes in tissue function.

Identifying microRNAs that are impacted by diet and understanding their biological role could have important clinical implications and offer tangible therapeutic targets for the prevention, management and/or treatment of obesity and other diet related disorders.



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INTRODUCTION

Lifestyle has radically changed over the last few decades in both developed and developing countries. The consumption of energy dense foods (rich in fat and sugar) has increased owing to its higher palatability and easy availability. On the other hand, daily physical activity has decreased with the advancement in technology, as most professions are now less labour intensive. Over time, this change in lifestyle can shift an individual's overall energy balance to positive, resulting in weight gain due to extra energy accumulation in adipose tissue. Adipose tissue can secrete various peptide hormones, adipokines and non-peptide bioactive molecules that can impact appetite and overall energy homeostasis [1]. During times of overfeeding, the size of adipocytes typically increases due to the storage of extra triglycerides. However as adiposity continues to increase, the normal lipolytic and secretory functions of the adipose tissue become hampered, leading to macrophage accumulation, increased inflammation [1, 2] and possible insulin resistance [3]. Furthermore, altered lipolytic activity within the adipose tissue can result in greater release of free fatty acids into the blood and lead to ectopic accumulation of fats in the liver and muscle [4]. Excessive accumulation of triglycerides in liver can lead to non-alcoholic fatty liver disease (NAFLD), which is associated with hepatic insulin resistance, altered glucose, fatty acid and lipoprotein metabolism, and production of inflammatory cytokines [5]. In the skeletal muscle, studies have reported close association between intramuscular triglyceride accumulation and insulin resistance [6]. Other comorbidities associated with excessive weight gain and obesity include: various types of cancers [7], neuronal damage [8], endothelial dysfunction [9], cardiac hypertrophy [10], and erectile dysfunction [11]. These manifestations of high calorie intake and fat accumulation involve a large panoply of cellular signalling, which itself is driven by various epigenetic modifications. Epigenetics is the heritable change in gene expression without changes in the DNA sequence. Epigenetic changes include DNA methylation, histone modification, chromatin remodelling and most recently discovered, miRNA expression regulation [12-14].

MiRNAs (Micro Ribonucleic Acids or miRNAs or miR) are a group of small, non-coding RNAs which regulate gene expression at the post transcriptional level, by modifying mRNA availability and thus, regulate cell growth, differentiation, development and apoptosis [15, 16]. These small RNA molecules have also been reported to regulate proliferation and differentiation of skeletal muscle [17], adipose tissue [18] and play an important role in the functioning of the liver, pancreas and brain [19]. For more detail regarding the biogenesis and function of miRNAs, which is beyond the scope of this review, please refer to the following papers [20, 21]. Recent studies have suggested that diet alone can alter the expression of miRNAs [22], resulting in changes to normal cellular function and possible tissue dysfunction [23]. High fat diets in particular can alter the expression of miRNAs involved in weight gain and obesity, but also development of obesity-related comorbidities in a variety of tissues including: adipose, liver, skeletal muscle, intestine, nervous, heart, endothelium and corporal tissue. Despite the many diet-induced changes in miRNAs expression being reported, the majority of these studies have compared the miRNA levels of an obese model to

a non-obese model and thus identification of early miRNA changes as a result of the diet, typically high fat, rather than the obesity phenotype *per se* is often difficult to determine.

The objective of this review paper is to summarise the short- and long-term changes in miRNA expression as a result of high fat diet and diet-induced obese phenotype and how these subsequent changes may contribute to the development of not only obesity, but also other lifestyle-related diseases. Importantly, this review will discuss how high fat diet alone can significantly impact the expression of miRNAs in various tissues, independent of body weight changes. The impact of maternal diet on offspring miRNA expression and intake of different dietary lipids will also be briefly discussed. It should be noted that this review will focus specifically on miRNA changes in tissue, rather than circulation, with a number of extensive reviews already published in this area [4, 24]. It is evident that the origin of circulating miRNAs is often debatable with the expression profile of circulating miRNAs similar or different from those in pathological tissues [25] and between tissue and circulation. Although a number of miRNAs that play important roles in the pathophysiology of obesity and associated comorbidities have been identified in both tissue and circulation, very few studies have reported changes in systemic miRNAs following high fat diet. Therefore, a list of miRNAs identified in both tissue and circulation and their expression changes following high fat diet have been reported (See Table 1). Additionally, their link to human pathological conditions have also been noted to demonstrate their clinical relevance and potential as a therapeutic target.

HIGH FAT DIET-INDUCED CHANGES IN miRNA EXPRESSION IN VARIOUS TISSUES

A summary of miRNA changes in adipose, liver, skeletal muscle, intestine, nervous, heart, endothelium and corporal tissue following high fat diet and/or obesity development are depicted in Figure 1. Livers from maternal high fat fed offspring were also included to demonstrate the impact that diet and obesity can have on the intrauterine environment during pregnancy. In order to minimise other extraneous factors, this review will only focus on animal models of high fat feeding to induce obesity and thus exclude diets that used other macronutrients to increase weight such as simple sugar, or genetically modified animal models and human models (See Table 2 for a summary of papers included in this review).

Adipose tissue

Adipose tissue is an endocrine organ and regulator of whole-body energy homeostasis through the storage and release of triglycerides [26, 27]. In response to over-feeding, excess energy stores partition into adipocyte. Adipose tissue grows by two mechanisms: hyperplasia (cell number increase) and hypertrophy (cell size increase) [15]. Expansion of adipocytes, specifically large hypertrophic adipocytes, is limited by multiple factors, including hypoxia and differential matrix mechanics [28]. A significant function of the differentially expressed miRNA targets is cell differentiation and its regulation, which is a reflection of their role in both hypertrophy (enlargement of the existing adipocytes) and hyperplasia (adipogenesis or adipocyte differentiation) during obesity

[29]. If left untreated, continue expansion of adipocytes can result in adipose tissue dysfunction, metabolic syndrome and comorbidities such as insulin resistance, diabetes and cardiovascular diseases [18, 27]. This section (and other sections throughout this review) will focus specifically on the *in vivo* study details and outcomes, and use support from *in vitro* findings if applicable. Using the criteria of high fat feeding, 5 studies were identified in the literature to date. Two of those studies investigated a specific miRNA (miR-21 and miR-143, respectively) [27, 30], while the others examined global changes in miRNA expression [29, 31, 32].

MiR-143 was originally thought to play a key role in cardiac morphogenesis and tumour suppression [33]. However, recent research suggests that it may also be important in the development of obesity [30]. Takanabe and colleagues examined the expression of miR-143 in mesenteric adipose tissue of mice fed either a high fat diet (45% of total calories from fat) or standard chow for 8 weeks [30]. Results showed a significant 3.3 fold increase in miR-143 expression following the consumption of high fat diet compared to standard chow fed mice [30]. In addition, the expression of miR-143 was positively correlated with body weight, mesenteric fat weight, plasma leptin concentration levels, peroxisome proliferator-activated receptor- γ (PPAR- γ) expression and adipocyte fatty acid-binding protein (aP2). PPAR- γ is a nuclear receptor which controls adipocyte differentiation and hypertrophy, while aP2 regulates systemic glucose and lipid metabolism and is highly expressed in obesity [34]. Both are important differentiation markers of adipocytes [35, 36]. The positive correlation could indicate some type of regulatory relationship due to the high fat intake or could be a secondary consequence due to the increased weight. MiR-143 has been linked to the regulation of adipocyte differentiation by targeting extracellular signal-regulated kinase 5 (ERK5) [37], however, no correlation between miR-143 expression levels and ERK5 protein expression was found [30]. Notwithstanding, the study by Takanabe et al. [30] indicates that miR-143 is somehow involved in the development of obesity, possibly via the regulation of adipocyte differentiation through PPAR- γ and its downstream target, ERK5, or other unidentified downstream targets.

Similar to miR-143, MiR-21 has also been targeted for investigation due to its involvement in adipogenic differentiation in human adipose tissue derived mesenchymal stem cells via the modulation of endogenous TGF- β signalling pathway [38]. In 2012, Kim and colleagues [27] investigated the expression levels of miR-21 in epididymal adipose tissue of high fat diet-induced obese mice. Interestingly, a bi-phasic change in miRNA expression was observed during the 10 week high fat dietary intervention (60% of total calories from fat), with a decrease in miR-21 shown as early as week 1, followed by an increase at week 10 [27]. Adipocyte numbers were also increased, with significant changes observed at 3 weeks in the high fat fed group and 7 weeks in the standard chow fed group; although the changes in the standard chow fed mice were most likely due to age dependent increases [39]. The expression of pre-adipocyte factor 1 (Pref-1), a pre-adipocyte marker, also reached its highest level at 3 weeks during high fat feeding before declining thereafter, whereas the expression of fatty acid binding protein 4 (Fabp4), a mature adipocyte marker, continued to increase after the 3 weeks, which corresponded to the increase in size, volume and number of adipocytes in the epididymal fat. In standard chow fed mice, changes in miR-21 expression were in contrast to the high fat fed group with an increase reported at week 5 followed by a decrease at

week 10. Although the changes in standard chow fed mice demonstrated the normal changes with growth development, the down regulation of miR-21 in the early phase of high fat feeding suggests that the miR-21-induced increase of adipogenic differentiation may not contribute to the early stages of obesity [27]. In fact, miR-21 may actually be controlling the proliferation of adipocyte precursors, as evident by the early increases in Pref-1. Taken together, the results suggest that miR-21's role (via down-regulation) is to induce proliferation of adipocyte precursors in the early stages of obesity development and increase the number of adipocytes (via adipogenic differentiation) in the later stages [27].

A pioneering study investigating the global expression of miRNA was recently published in the Journal of Diabetes [29]. The researchers used miRNA microarrays to profile over 370 miRNAs during adipogenesis of pre-adipocytes in both cell and animal models. In the animal model, global miRNA expression was examined in epididymal fat pads of c57BL/6J mice following 12 weeks high fat feeding (55% of total calories from fat). Results showed that ten miRNAs were differentially expressed, with eight miRNAs (miR-422b, miR-148, miR-107, miR-103, miR-30c, miR-30a-5p, miR-146b, and miR-143) increasing their expression and 2 miRNAs (miR-221 and miR-222) decreasing their expression [29]. The study also used a leptin deficient B6.V- *Lepob/J(ob/ob)* obese mice to compare findings and determine if the leptin hormone played any role in subsequent changes in adipose tissue miRNA expression profiles. The expression profiles of *ob/ob* and diet-induced obese mice were similar, suggesting a lack of hormonal involvement in the miRNA changes. Several miRNAs, including miR-103 and miR-143, exhibited inverse patterns of regulation, with up-regulation in pre-adipocytes (cell culture model) and down-regulation in mature adipocytes (animal model). The authors suggested that the contrasting changes in miRNAs, specifically the down-regulation in later stages, could be due the chronic local inflammation environment and enhanced TNF- α levels often observed in adipose tissue of an obese phenotype [29]. Indeed, similar changes in miRNA expression were observed after TNF- α treatment of differentiated adipocytes in the same study [29]. The findings from the Xie et al.[29] study have important implications in the understanding of adipose tissue biology during the development of obesity, with miR-143 (again) and miR-103 identified as key regulators. Furthermore, the dysfunction of miRNAs in adipocytes of an obese phenotype, is likely associated with the chronic inflammatory environment, rather than hormonal.

MiR-103 has also been reported to be up-regulated in steers following 14 weeks of high fat diet (5.85% of total from fat) [32]. In addition, the study demonstrated an increase in Let-7 and miR-27b, both of which have contrasting roles, with Let-7 demonstrating a pro-adipogenic effect by regulating high-mobility group AT-hook 2 (HMGA2), and miR-27b demonstrating a anti-adipogenic effect by regulating PPAR γ [29, 40, 41]. The researchers also showed miR-17-92 cluster (miR-19a and miR-19b) to be highly expressed in adipose tissue. MiR-17-92 cluster is involved in enhanced adipocyte differentiation and triglyceride accumulation through inhibition of Rb2/p130, a regulator of cell cycle and tumour suppressor [42]. An interesting finding was the location specific increases in miRNAs, with increased expression of miR-196a observed in visceral adipose tissue, whereas increased expression of miR-2454 was observed in subcutaneous adipose tissue. This suggests that changes in miRNA can differ within the same tissue at different locations [43, 44]. In addition to the

aforementioned miRNAs, five other miRNAs involved in lipid metabolism and/or adipogenesis were also found to be differentially expressed, and of those, miR-101 and miR-2368*, were shown to target PPAR- γ , a key regulator of energy homeostasis in white adipose tissue [45].

It has been suggested that 3 months or less of a high fat diet may not be long enough to induce the same changes often seen in adipocytes from *ob/ob* mice [30], a model suggested to be a true representation of an obese individual. With this in mind, Chartoumpekis et al., [31] examined the long term impact of high fat feeding on global expression of miRNAs. C57BL/6J mice were fed a high fat diet (60% of total calories from fat) for a period of 5 months, with expression of miRNAs in the epididymal fat pads examined pre- and post-dietary intervention. Researchers reported an up-regulation of miR-221, miR-222, miR-21, miR-343-3p, miR-142-3p, miR-142-5p, miR-146a, miR-146b, miR-674, miR-379 and down-regulation of the miR-200 family, miR-204, miR-30a*, miR-193, miR-378, miR-30e, miR-122, miR-133p, miR-1, miR-30a, miR-192, and miR-203 [31]. Changes in some of these miRNAs are in accordance with the previous reports [29, 46]. Although the target gene analysis of these miRNAs was not performed, the use of bioinformatics tools and literature were used to predict the changes in target genes and thus subsequent function. MiR-142-5p and miR-142-3p are shown to target bone morphogenetic protein 4 (Bmp-4), fibroblast growth factor 10 (Fgf10) and protein kinase B (Akt1/PKB), all of which are involved in the regulation of adipogenesis [47, 48]. MiR-21 [27] and miR-146-a are reported to target CCAAT-enhancer-binding proteins (C/EBP β) and apolipoprotein E (ApoE) and thus regulate adipocyte differentiation [49, 50]. MiR-146a has also been implicated in the inflammatory response and target of interferon- γ [51]. miR-222 targets hypoxia-inducible factor 1- α (Hif-1 α) and tumour necrosis factor α (TNF- α), which regulate cell differentiation and may also be linked to inflammatory processes [23, 46, 52, 53]. MiR-200b and 200c and miR-203 and 192 are predicted to target zinc finger E-box binding homeobox 1 and 2 (Zeb1 and Zeb2), which have been implicated in adipogenesis and obesity [54]. MiR-1, a skeletal muscle specific miRNA, is reported to target uncoupling protein 1 (UCP-1), which uncouples oxidative phosphorylation from ATP production and induces dissipation of energy as heat [55]. Changes in miR-1 could be important for metabolic adaptation following high fat diet feeding [55, 56]. MiR-204, implicated in cell differentiation, and miR-133b have a common target, Runx2, which stimulates osteogenesis and represses adipogenesis [57] indicating that down-regulation of miR-204 and 133b may result in non-inhibition of Runx2 and stimulation of adipogenesis. No plausible targets were mentioned for miR-30a*, miR-30e*, miR-193, miR-378, miR-122, miR-130a, miR-142, miR-21, miR-146a, miR-146b, miR-342-3p, miR-203, and miR-192.

A finding with significant implications for analytical validation techniques was observed by Romao et al. [32] who demonstrated a decreased miR-16 expression within adipose tissue following a high fat diet. This is of particular importance given this miRNA is widely used as an endogenous control miRNA for quantitative RT-PCR analysis. MiR-16 has pro-apoptotic function and given it is influenced by high fat feeding [58], future studies should reconsider using miR-16 as an endogenous control for high fat feeding studies. Data from the aforementioned studies provide evidence that miRNAs within adipose tissue are responsive to high fat feeding, and these changes are linked to adipose cell

development, obesity predisposition and possible adipose tissue dysfunction with obesity. Specifically, miR-143 and miR-21 have been reported by numerous studies to be up-regulated and are suggested to manifest their effects by targeting PPAR γ /ERK5 and TGF β , respectively. Additionally, changes in the expression of miR-142-5p and miR-221/222 have also been reported multiple times, with miR-142-5p targeting Bmp4 and Fgf10 and miR-221/222 associated with arterial smooth muscle angiogenesis [59]. Finally, miR-103 and miR-146b appear to be important in adipose tissue physiology and dysregulation, however, further studies are needed to validate their reported effects.

Liver Tissue

The liver performs various biochemical functions important for systemic homeostasis and is considered a metabolic workhorse [60]. High calorie and fat diets significantly impact the liver with excessive fats in the form of triglycerides accumulating in hepatocytes which can lead to a reversible pathological condition called non-alcoholic fatty liver disease (NAFLD) [61, 62]. Growing evidence suggests an association between conditions or risk factors of metabolic syndrome (i.e. obesity, insulin resistance and hyperlipidaemia) and NAFLD [61, 63]. However, the molecular mechanisms behind the development and progression of NAFLD remains elusive. Notwithstanding, it has been shown that dietary-induced hepatic dysfunction is accompanied by alterations in miRNA expression [64]. To date, 5 studies have been published investigating the effects of high fat diet on liver physiology, development of hepatic steatosis and associated changes in miRNA expression [61, 63, 65-67]. Three of those studies investigated a specific miRNA: miR-122 [65], miR-467b [61] and miR-21 [63], while the other studies performed global changes in miRNA expression [66, 67].

MiR-467b is as an important regulator of lipid metabolism by affecting hepatic levels of lipoprotein lipase (LPL) [61]. MiR-467b is bound to LPL mRNA in the 3' untranslated region. Down-regulation of miR-467b has been reported in high fat diet-induced obese C57BL/6 mice accompanied by non-inhibition of LPL and hepatic steatosis [61]. Suppression of MiR-467b is also linked to a decrease in phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and its downstream target Akt, potentially leading to insulin resistance in hepatocytes. Insulin resistance normally precedes accumulation of fat in hepatic cells [68] and hence contributes to the development of hepatic steatosis. In another study by the same group [63], 8 weeks of high fat feeding (60% of total calories from fat) induced a down-regulation of miR-21 in the liver [63]. Target gene analysis revealed fatty acid binding protein 7 (FABP7) as one of the targets of miR-21 in the liver [69, 70]. High fat diet-induced down-regulation of miR-21 and up-regulation of FABP7 increases fatty acid uptake in the liver which, when excessive, can ultimately lead to hepatic steatosis. Taken together, these studies indicate that miR-467b and miR-21 are important regulators of lipid metabolism and contributors to the development of hepatic steatosis via different pathways in the liver.

To further understand the impact of high fat diet on miRNA expression and also identify any novel changes, Park and colleagues [67] used miRNA microarray and quantitative RT-PCR to investigate miRNA changes in murine liver tissue following high fat (energy percentage from fat not reported) feeding for 6 weeks. More than 50 hepatic miRNAs were dysregulated in the diet-induced obese

mice model. Some of the changes were already known to be involved in obesity and other diseases. For example, miR-451 was reported to down-regulated; a miRNA already known to be associated with non-alcoholic steato-hepatitis (NASH) [67]. Others of note included the down-regulation of miR-16, miR-103, miR-107, miR-143 and miR-148a and up-regulation of miR-351 and miR-669 [67]. The down-regulation of miR-103, miR-143 and miR-148a are consistent with previous findings in genetically modified obese mice [29]. Target gene analysis of these miRNA's show that estrogen-related receptor alpha (Esrra), Indian hedgehog (Ihh), corticotropin releasing hormone receptor 1 (Crhr1) are the direct targets of miR-16, miR-103/107 and miR-669, respectively, with miR-107 and miR-669 manifesting their effects by inhibiting translation of fatty acid synthase (FASN) and V-Myc avian myelocytomatosis viral oncogene homolog (MYC), respectively, rather than mRNA degradation [67]. MiR-107 partially inhibits the expression of FASN, a central player in *de novo* synthesis of fatty acids [71]. Interestingly, the miRNAs shown to be differentially regulated following high fat feeding (miR-16, miR-29a and miR-103) have also been shown to be differentially regulated in osteoarthritis patients, providing support to the hypothesis that miRNA dysregulation may mediate the relationship between obesity and osteoarthritis [72].

MiR-122 is another miRNA of interest due to its involvement in lipid metabolism, high abundance in liver and association with serum cholesterol level in both murine and primate models [37, 73]. However, conflicting observations have been documented with miR-122, with one study reporting a down-regulation [65], while another study reported an up-regulation following high fat diet [66]. The differences could be due to the animal models utilised and duration and composition of the diet given by the researchers. Cirera et al., [65] examined the effects of a 11 week high fat diet (2% cholesterol and 22.7% fat) in Gottingen mini pigs, whereas Karere et al., examined the effects of a 7 week high fat diet (40% from calories), high cholesterol (1.7mg/kcal) in baboons (*Papio hamadryas*) [66]. Interestingly, in the same study [66], differential expression of miRNAs were demonstrated when baboons were separated by their LDL-C serum levels (low and high LDL-C responders), which may indicate that expression of some miRNAs may be dependent on the threshold levels of serum cholesterol. The primary target of miR-122 is cationic amino acid transporter 1 (CAT1). However, despite a decrease in miR-122 expression in one study [66], there was no significant change in CAT1 gene expression, which is in contrast to previous *in vitro* findings demonstrating an inverse relationship between miR-122 and CAT1 expression [74]. Similar to miR-143 in adipose tissue, the aforementioned observations provide another example of miRNA changes *in vitro* not being replicated in an *in vivo* model [30], and demonstrates the complexity of miRNA regulation between tissues, species, methods of analysis and interventions.

Finally, the down-regulation of MiR-155 and miR-146-a following high fat feeding has been associated with enhanced accumulation of oxidized LDL cholesterol in monocytes, a key event in development of atherosclerosis [75, 76]. The genes targeted by these miRNAs include: low density lipoprotein receptor (LDLR), very low density lipoprotein receptor (VLDLR), lipase A precursor (LIPA), phosphatase and tensin homolog (PTEN), tensin-like C1 domain containing phosphatase (TENC1), and activin A receptor, type IB isoform c precursor (ACVR1B) [66]. These gene targets play important

roles in receptor-mediated endocytosis of LDL-C, VLDL triglyceride metabolism, hydrolysis of cholesterol esters and triglycerides in lysosomes and possible tumour suppression, and thus have been identified as potential therapeutic targets for prevention and treatment of atherosclerosis [75, 76].

Although changes in liver specific miRNAs appear to be diverse and unpredictable following high fat diets, it is evident that these miRNAs are intimately involved in the regulation of liver physiology and pathophysiology. With the exception of miR-122, which has been reported in two different studies [65, 66], no other miRNAs have been repeatedly identified in the aforementioned studies. On another note, miR-target prediction techniques have revealed that certain miRNAs have the same gene targets, and thus complementary expression of miRNAs may help fine tune the expression of target genes. For example, miR-27a and miR-26a which were up-regulated and down-regulated, respectively in liver following a high fat diet target low density lipoprotein receptor adapter protein 1 (LDLRAP1) which interacts with LDL cholesterol receptors.

Skeletal Muscle Tissue

The development of type 2 diabetes is linked to the accumulation of fat within skeletal muscle, inducing insulin resistance [77]. During the past 10 years, numerous miRNAs have been identified for their involvement in skeletal muscle proliferation, differentiation and apoptosis [17]. There is accumulating evidence that miRNAs are also associated with the development of diabetic complications [78]. However, how muscle specific miRNAs respond to high fat feeding and their link to diet-induced insulin resistance has not been fully elucidated, with only a few studies examining this link [77]. Using global miRNA analysis, 30 miRNAs in gastrocnemius muscle were found to be differentially expressed following 12 weeks of high fat diet (58% of total calories from fat) compared to the standard chow diet in C57BL/6 mice [77]. MiR-1, miR-133a, miR-133b and miR-206, known as the myomiRs, were down-regulated [79-85], whereas miR-144 and miR-106b were up-regulated. Gene annotation and signalling enrichment analysis of predicted targets of the differentially expressed miRNAs revealed that high fat-induced insulin resistance involves a complex genetic cellular response such as those involved in cellular metabolic processes, cell death, and regulation of transcription [77]. Moreover, subsequent signalling mapping revealed 19 significant pathways related to insulin resistance. These included signal transduction pathways related to cell proliferation [(Mitogen-activated protein kinases (MAPK), Wnt, neurotrophic, hedgehog, vascular endothelial growth factor (VEGF), and transforming growth factor beta (TGF- β)], metabolism (lysine degradation, heparin sulphate biosynthesis and glycan degradation), protein degradation (ubiquitin mediated proteolysis) and oncogenic (colorectal) pathways. Some of these pathways such as MAPK have already been implicated in skeletal muscle insulin resistance [86] and key proteins within the MAPK pathway such as p38 and pJUK have been previously found to be up-regulated in high fat diet-induced diabetic mice [77].

In a more recent study, Frias et al. [87] used a similar model of dietary-induced obesity and also reported a down-regulation of miR-1 expression, but in contrast to Chen et al., [77], demonstrated an up-regulation of miR-133a, and no change in miR-206 [87]. The discrepancy in observed changes

could be due to the different muscle group and fibre type analysed with the Chen et al., [77] study using the gastrocnemius which is predominantly type IIB and IIDB fibres, whereas the soleus muscle used by Frias et al. [87] study is predominantly IIB fibres [88]. Notwithstanding, the down-regulation of miR-1, miR-206, and miR-133 levels has been reported in white adipocytes in high fat diet-induced obese models [31] and in the *vastus lateralis* of type 2 diabetic patients [80]. Of particular importance therapeutically could be miR-1, with its change in expression negatively correlated with glycemia in high fat diet-induced diabetic mice [87] and thus could be a potential target for treatment of skeletal muscle insulin resistance.

Intestine Tissue

Obesity is considered a risk factor for various types of cancers and high fat diets are associated with colorectal cancer [7, 89]. A recent pilot study [7] used an inducible mouse model of colon carcinogenesis to determine whether feeding a high fat diet (45% of total calories from fat) for 20 weeks would enhance tumorigenesis. Researchers identified eight miRNAs that were up-regulated in the colon namely miR-425-p, miR-100a, miR-194-1-a, miR-378-3p/422b-a, miR-718p, miR-196a, miR-669-a, and miR-155. MiR-155 and miR-196a have been previously associated with ulcerative colitis [90] and colon metastasis [91]. The pro-oncogenic influence of miR-196a is thought to act via activation of the Akt signaling pathway [91]. Although the Akt levels were not determined in the Olivo-Marston et al., [7] study, others have shown increased Akt phosphorylation and subsequent cell proliferation following high fat feeding [92]. Moreover, Akt and mammalian target of rapamycin (mTOR) which are downstream pathways of insulin-like growth factor-1 (IGF-1) and other cell-surface receptors are suggested to be modulated by disturbances in dietary energy balance (i.e. high fat diet); reinforcing the link between obesity and cancer [93, 94]. Olivo-Marston et al., [7] also identified 10 miRNAs that were down-regulated including miR-150-a, miR-351-a, miR-16-2-a, miR-694-p, let7f-1-a, miR-682a, miR-133a*-5p-a, miR-34c-p, miR-138-a, miR-133a-1-3p-a. MiR-150a in particular has been confirmed in previous *in vitro* studies to suppress tumour growth by increasing apoptosis and decreasing proliferation [95]. Other members of tumour suppressing miRNAs, namely miR-34c, let-7 and miR-16 have also been down-regulated following high fat diet [96]. These findings indicate that under high fat fed conditions, miRNAs such as miR-196, miR-155 and miR-150 appear to be important in the progression of cancer by enhancing cell proliferation and reducing apoptosis

An emerging area of research is the relationship between consumption of high fat diet, gut dysbiosis and intestinal carcinogenesis [97, 98]. There is growing evidence that diet can affect the composition of intestinal microbiota resulting in dysbiosis, which may lead to cancer development. Shulz and colleagues [97] first demonstrated enhanced tumour progression in the small intestine of genetically susceptible, K-ras(G12Dint) mice, independent of obesity following high fat diet (diet composition not reported) and secondly, the disease from the high fat fed mice with intestinal tumours could be transferred by faecal samples to healthy adult K-ras (G12Dint) mice in the absence of high fat feeding [97]. Interestingly, treatment with antibiotics completely blocked the high fat diet-induced tumour progression, with the authors suggesting that distinct shifts in the microbiota may play a pivotal role in aggravating the disease [97]. These results and others [99, 100], highlight the

importance of a healthy microbiota and relationship between dysbiosis and carcinogenesis. This is an important area of future research in diet-induced obesity and risk factors for gastrointestinal cancers.

Nervous Tissue

High fat diet-induced hyperlipidemia can cause serious digestive problems, including dysmotility syndromes such as altered gastric emptying and constipation [101]. Hyperlipidemia can also damage neurons of the central nervous system by activating apoptotic pathways and disrupting normal cell proliferation [102]. Identifying miRNAs and subsequent function may help understand the mechanisms of high fat diet-induced enteric neuronal cell damage and the pathophysiology of high fat diet-induced gastrointestinal dysmotility in humans [103]. The role of miRNAs in high fat diet-induced damage to the enteric nervous system has been investigated in one study to date [8]. Following 11 weeks of high fat feeding, (60% of total calories from fat), obese mice demonstrated apoptosis of enteric neuronal cells, increased mitochondrial dysfunction and endoplasmic reticulum stress. These diet-induced changes coincided with increased expression of miR-375 in enteric ganglia and decreased levels of the target mRNAs including a family of regulatory proteins conserved in eukaryotes (14-3-3 ζ), pyruvate dehydrogenase lipoamide kinase isozyme 1 (Pdk1), and myotrophin [8]. However, contrasting results have been observed when compared to genetically modified obese mice [8]. This is not surprising given other studies have also demonstrated conflicting changes in mRNA expression between diet-induced and genetically-induced obese models; albeit in different tissues [102]. A finding of possible therapeutic application was also demonstrated in the same study [8], with systemic injections of a mir375 inhibitor preventing high fat diet-induced delays in intestinal transit and

morphologic changes [8]. Although these pre-clinical findings are acute, it does suggest a potential target for clinical gastrointestinal dysmotility.

Endothelium Tissue

The endothelium, which forms the inner cellular lining of blood vessels and lymphatics, is a highly metabolically active organ that is involved in many pathological conditions, including cardiovascular diseases (such as atherosclerosis, and hypertension) and diabetes [104]. There are recent reviews summarising the role of miRNAs on the onset and development of cardiovascular diseases (CVD) [105], however few studies have investigated the impact of high fat diet on miRNAs relating to CVD. High fat diets have been reported to be associated with CVD which is implicated in atherosclerosis [9]. Recently, miRNAs have been associated with the development of atherosclerosis by influencing endothelial permeability and apoptosis [106]. Using a mouse model that advances the progression of atherosclerosis, apoE knock out mice (apoE^{-/-}) were fed a high fat diet (2% cholesterol and 5% lard oil) for a period of 16 weeks [105]. High fat feeding caused a down-regulation of miR-1 within the endothelium, subsequent enhancement of myosin light chain kinase (MLCK) expression and reduced extracellular signal regulated kinase (ERK) phosphorylation. MLCK is essential for endothelial cell function with its phosphorylation affecting endothelial permeability [106]. ERK/MAPK is an important signal transduction pathway and is linked to many inflammatory disorders [107]. The results by Wang et al., [106] suggest that miR-1 contributes to endothelial permeability by changes in MLCK expression and ERK phosphorylation. Findings from the same group demonstrated an up-regulation of another miRNA following high fat diet; miR-29b [108]. MiR-29b has shown to alter the integrity of arterial walls and thus, similar to miR-1, influence endothelial

permeability [108]. In addition, changes in miR-29b expression is linked to apoptosis, with an increase or decrease in miR-29b expression leading to an increase or reduction in apoptosis, respectively [108]. These effects could be driven by melatonin receptor type 1 (MT1) [100], an important hormone that improves endothelial function [109], however, further studies are needed to confirm this. MT1 mediates some physiological effects of melatonin. Finally, miR-155 is involved in the prevention of atherosclerotic lesion development and progression, and is typically up-regulated in both arteries and mononuclear cells in apoE^{-/-} mice [110]. MiR-155 has been shown to be up-regulated in the aorta of apoE^{-/-} mice fed on a high-fat diet for 3–10 months [111, 112]. A major mechanism underlying the anti-atherogenic effects of miR-155 is likely to be the inhibition of inflammation via the MAPK pathway [110]. Taken together, despite limited studies in this area, miR-1, miR-29b and MiR-155 appears to play important roles in the development of atherosclerosis following high fat feeding, and could offer possible therapeutic targets for treatment and/or prevention of such a disease.

Cardiac Tissue

Long term high fat diet feeding induces cardiac remodelling and dysfunction which can lead to obesity-induced heart failure [10, 113]; whether miRNAs play a role in these changes have not been fully elucidated. Recently it has been reported that cardiac myocytes from high fat (45% of total calories from fat) fed C57BL/6 mice exhibited increased expression of miR-451 compared to standard chow fed mice [114]. Increased expression of miR-451 was accompanied by elevated expression of GATA binding protein 4 (GATA4), a protein involved in cardiac hypertrophy and possible gene target of miR-451 [115]. Calcium binding protein 39 (Cab-39) has also been identified as a direct target of miR-451 in the heart by *in vitro* and knock out mice studies [114]. Cab-39 is a scaffold protein of liver kinase B1 (LKB1), and an upstream kinase of 5' AMP-activated protein kinase (AMPK). Indeed, enhanced AMPK phosphorylation has been demonstrated in miR-451 knock out models [114]. It has been suggested that suppression of Cab-39 through miR-451 up-regulation may be partially responsible for the high fat diet-induced cardiac hypertrophy [114]. Targeting miR-451 and reducing the suppression of LKB1/AMPK pathway could be a promising strategy for treating diet-induced cardiac hypertrophy. Finally, miR-22 has also been recently shown to be up-regulated in cardiac hypertrophy following ten weeks of high fat (60% of total calories from fat) feeding in C57BL/6 mice [10]. However, given no details were reported on the mRNA targets and signalling

pathways that we affected by the increased miR-22 expression, it is difficult to comment on the significance of such a finding.

Corporal Tissue

Erectile dysfunction is closely associated with high fat diet-induced obesity [116]. Recently, a pilot study examined the involvement of miRNAs in high fat diet associated erectile dysfunction in C57Bl/6 mice [11]. Following 22 weeks of high fat feeding (60% of total calories from fat), corporal tissue exhibited increased expression of miR-720, miR-1937a, miR-1937c, miR-205, miR-151-5p and decreased expression of miR-550, miR-425, miR-134, miR-153, and miR-26b. Identification of target mRNA and genes for these differentially expressed miRNAs were not investigated in this study [11]. However, the target genes of these miRNAs have been shown in previous literature [11, 117]. For example, miR-425 negatively regulates natriuretic peptide, which is a potent vasodilator and has been suggested to play a role in smooth muscle tone in corpus cavernosum [11]. It is evident that research in this area is still in its infancy and that further studies are needed to validate such findings and investigate the role of other miRNAs in the physiology and pathophysiology of erectile tissue.

Maternal high fat diet and its impact on offspring

Maternal nutrition and obesity status of the mother predisposes the offspring to a number of pathological conditions including metabolic syndrome, adipose tissue dysfunction, appetite dysregulation, cardiovascular disease and altered glucose/insulin homeostasis [118]. The involvement of miRNAs in eliciting such changes has been reported in mouse liver [119, 120]. In a study by Zhang et al. [120], dams were fed a high fat diet (22.6% of total calories from fat), for 4 weeks prior to conception, during pregnancy and during the lactation period. Results showed that livers from maternal high fat fed offspring displayed alterations in approximately 5.7% of miRNAs measured (579 in total) compared to livers from maternal standard chow fed offspring [120]. MiRNAs of interest were the reduced expression of Let-7c which regulates developmental timing and miR-122 which regulates fat metabolism. The change in let-7c could be due to the up-regulation of PPAR α , as PPAR α have previously been reported to induce hepatocellular proliferation by inhibiting Let-7c [121, 122]. The study by Zhang and colleagues [120] suggest that offspring dams fed high fat display co-ordinated changes in key metabolic genes and miRNAs that regulate early fetal growth and fat metabolism [120]. Similarly, Benatti and colleagues [120] demonstrated reduced expression of Mir-122 as well as carnitine palmitoyl-transferase 1alpha (CPT1 α) in the offspring livers of high fat fed dams. In addition, MiR-370 was increased whereas Acyl-CoA Dehydrogenase, Very Long Chain (ACADVL) protein, which catalyzes the first step of the mitochondrial fatty acid beta-oxidation pathway was reduced thus supporting the altered expression in key genes regulating fat metabolism observed by the Zhang et al. [120] study. Liver inflammation was evident as well in the Benatti study [119], which could be attributed to the negative changes in uterine environment and hepatic metabolic enzymes from the high fat fed dams. Other miRNA changes of significance in the Benatti et al. [119] study, were the down-regulation of 5 miRNAs (miR-709, let-7, miR-122, miR-194, miR-269) which have methyl-CpG binding protein 2 as their predicted target gene and the up-regulation of 5 miRNAs (miR-503*, miR-773-3p, miR-369-3p, miR-197 and miR-667) that have

histone 4 as their predicted target gene target [119]. It has been suggested that 23 miRNAs expressed during early stages of life play an active role regulating metabolism and foetal growth [119]. Although no significant phenotypic changes were observed in the offspring of high-fat diet fed dams, alteration in important genes and miRNA expression indicate that cellular changes are still occurring which may have significant implications later in life. This is an important area of preventative medical research and requires investigation to understand the short- and long-term impact on the offspring.

NUTRITIONAL CONSIDERATIONS – IMPACT OF DIFFERENT DIETARY LIPIDS

The impact of different dietary lipids at the miRNA level is poorly defined. While it is clear that dietary lipids play a key role in human health and disease, the type and the amount of ingested fat can significantly affect the outcome. For example, n-3 long chain PUFAs have been suggested to promote or maintain cardiovascular health [123, 124], mainly due to their anti-lipidemic [125, 126], anti-inflammatory [127, 128], anti-platelet [129, 130], and anti-arrhythmic effects [130-132]. Other dietary lipids are more controversial. The impact of different dietary bioactive compounds on the expression of miRNAs have primarily focused on the chemopreventive effects of some dietary compounds through the modulation of cancer-related miRNAs [133, 134]. However, recent studies have shown that manipulation of dietary fat content such as varying levels of conjugated linoleic acid in the diet can impact the expression of miRNAs, but also phenotypic changes such as adipose tissue levels [135, 136]. In addition, different forms of PUFAs can also differentially impact the expression of miRNA's, with a recent study demonstrating a diet rich in omega-3 rather than omega-6 reduced inflammation *in vivo* by regulating the transcription of three miRNAs (rno-miR-19b-3p, -146b-5p and -183-5p), and thus suppression of a set of inflammation-related genes [137]. Of therapeutic importance is the ability of nutrient supplements to reduce the adverse consequences of a high-fat diet and also coincidentally modulating miRNA expression in tissues [136, 138]. Although the nutritional impact on the expression of metabolically relevant miRNAs needs to be further addressed, these examples suggest that dietary manipulation may play an important role in the regulation of metabolically-relevant miRNAs that have been linked with obesity and its associated comorbidities [139]. Predicting an individual's nutritional status by assessing their miRNA profile may help decide which diet(s) will elicit the most beneficial effects. Lastly, complicating the impact of diet on miRNA expression is the identification of food-derived miRNAs (XenomiRs), which may potentially affect host gene expression [140]. Witwer and colleagues [141] proposed the existence of miRNAs that could be absorbed with the diet and would contribute directly or indirectly to the apparent expression of circulating miRNAs, although there is still no convincing evidence on the putative effect of food-derived miRNAs [142].

CLINICAL IMPLICATIONS AND THERAPEUTIC APPLICATIONS

Identification of therapeutic miRNAs requires well planned experimental designs, development of antagomir (oligonucleotides used to silence miRNAs), knock out animal models and the use of cutting edge bioinformatics tools. More than 100 miRNAs have been shown to be differentially expressed in a variety of tissues following high fat feeding. Approximately 22 of those miRNAs were reported in more than one tissue type (See Table 3 for a list of common miRNAs reported) indicating the potential of these miRNAs as diagnostic and prognostic tools. However, only a handful of miRNAs modulated by high fat diet in tissue have also been observed in circulation. Regardless, a number of tissue specific miRNAs discussed in this review have been identified as plausible therapeutic targets to date. Specifically, miR-375 and miR-451 have been suggested as potential targets for treating gastrointestinal dysmotility and cardiac hypertrophy, respectively. Additionally, miR-1, miR-29b, miR-155 and miR-146 were identified as therapeutic targets for treatment and prevention of atherosclerosis. Although not discussed in this review given the impact of high fat diet on its expression has not been directly studied, pharmacological inhibition of miR-208, a heart specific miRNA, resulted in less weight gain, improved systemic insulin sensitivity and glucose tolerance in mice consuming a high-fat diet compared to control, thus demonstrating therapeutic potential for metabolic disorders [143]. Finally, in addition to pharmacological interventions, it is also evident that dietary and/or lifestyle modifications can combat the negative effects high fat diet-induced obesity. In fact, a study published in 2015 demonstrated a reversal of obesity-related changes in circulating miRNA's expression following metabolic alterations associated with weight reduction following low fat dieting [139]. These results highlight the idea that dietary modification could represent a realistic therapeutic strategy to treat obesity and other obesity-associated comorbidities.

SUMMARY

MiRNAs have been identified to play important roles in both healthy and diseased conditions. With the increased consumption of calorie dense foods (high fat and sugar), many individuals are becoming predisposed to various health issues, specifically obesity. During adipogenesis, miRNAs can accelerate or inhibit adipocyte differentiation and hence regulate fat cell development. In addition, miRNAs may regulate adipogenic lineage commitment in multipotent stem cells and hence govern fat cell numbers. Few miRNA targets have been experimentally validated in adipocytes, but miR-143, miR-21 and miR-103 seem to be identified as important regulators of fat cell development during high fat-induced obesity. Although adipose tissue has been the major focus of high fat diet and miRNA research, especially in context of obesity development; it is clear that many other tissues are also impacted.

A finding of interest, though not discussed in detail in the review, was the differential expression of Passenger miRNA strands (miRNA*). MiRNA synthesis involves synthesis of the RNA duplex, separation of duplex strands, incorporation of one strand (called guide strand- miRNA) into RISC

assembly and degradation of the other strand (passenger strand denoted by star (miRNA*)). The guide strand is the mature form of miRNA and is mainly the prime focus of miRNA related research. However, it has been reported in number of studies that these passenger miRNAs* exhibit differential expression irrespective of the mature miRNA [144]. For example, miR-30a*, miR-30e* and miR-2368* reported in adipose tissue, miR-222* in liver, miR-133a* in intestine and miR-503* in livers of offspring from high-fat fed mothers. These findings indicate the passenger miRNAs are also involved in various cellular functions, and thus represent a future area of research focus.

Finally, the majority of existing studies examining the impact of high fat feeding on miRNA expression have used the C57BL/6 mouse models, usually feeding for a period of about 3 months to induce obesity. Animal models, especially rodents, have marked similarity to human physiology. When subjected to high fat diet, these animal models have been reported to gain weight and exhibit other metabolic consequences in a pattern which closely, if not exactly, mimics humans [145]. While obesity is the primary outcome, other comorbidities can often arise such as insulin resistance, hepatic steatosis etc. Under high fat conditions, it is expected that a number of core miRNAs that are fundamental to the regulation of genes involved in adipose metabolism, but also obesity development, will be expressed. However, it is clear that differences in miRNA number and regulation can exist between animal species or disease models, but also the same animal species consuming different diets. For example, two separate studies reported contrasting expression of miR-133a (an important miRNA for skeletal muscle development) in the same animal model (C57BL/6) consuming slightly different high fat diets (59% kcal fat, 15% kcal protein, and 26% kcal carbohydrate [87] versus 58% kcal fat, 25% kcal protein, and 17% kcal carbohydrate [77]). Moreover, using different animal models of disease (diet-induced diabetic mice (C57BL/6) [77] versus streptozotocin-induced type 2 diabetic rats [83]) also produced contrasting observations in miRNA expression. These observations indicate that each species responds to the environment uniquely and/or that the interaction between genetic regulation and dietary influence is complex. Moreover, since the disease state can be induced by various methods which may require different mechanisms, this could suggest that different therapeutic strategies are required.

Conclusion and future directions

Despite advances in understanding the molecular basis of obesity, a number of gaps in the literature still exist. Firstly, it is evident that the majority of studies that have demonstrated regulation of protein levels by miRNAs have been performed in cellular and animal models, and thus, the extent of post-transcriptional regulation by miRNAs in humans is unknown. Further, prediction models suggest 45000 putative miRNA binding sites may exist in human protein coding genes [146]. However, many of the putative miRNA binding sites remain to be experimentally validated. Secondly, to gain a better understanding of the role high fat diet plays in the development of obesity and associated disorders, more time course studies are needed that monitor the acute changes over the dietary intervention period. The majority of studies report changes in miRNA expression at the end of the feeding period when the animal has developed obese phenotype. Thus it is unclear as to

whether the changes in miRNA expression are due to the dietary intake alone or obese phenotype. Furthermore, determining if and how miRNA changes are causal in the development of obesity as a result of high fat diet is the main challenge of diet -induced obesity-related miRNA research. Thus, greater time-course studies that allow the observations of acute miRNA changes as a result of diet are clearly needed. Lastly, studies to investigate the diet effects on the same animal models are necessary to clarify if the expression of miRNAs is directly regulated by the diet.

In conclusion, following high fat feeding, researchers have identified key miRNAs that are involved in the early and later stages of obesity development and other obesity associated comorbidities. Moreover, tissues other than adipose seem to be affected and possibly dysregulated by the increased calorie/fat intake. Given the numerous tissues impacted by dietary-induced stress, further identification and characterisation of miRNAs in circulation and tissues will be essential for establishing plasma miRNA profiles that distinguish healthy, obese and diseased states, but more importantly, help facilitate the generation of therapeutic targets. Finally, from a clinical viewpoint, plasma miRNA profiles could be used to track the efficacy of miRNA based systemic therapeutics for obesity and disease management as a result of poor diet.

AUTHOR CONTRIBUTIONS

RW proposed the concept of this review and wrote the majority of the paper. MC wrote several sections, reviewed and edited the manuscript. WD and AH reviewed and edited the manuscript.

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CONFLICT OF INTEREST STATEMENT

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References

- [1] Guilherme, A., Virbasius, J. V., Puri, V., Czech, M. P., Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol.* 2008, 9, 367-377.
- [2] Greenberg, A. S., Obin, M. S., Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr.* 2006, 83, 461S-465S.
- [3] Hilton, C., Neville, M. J., Karpe, F., MicroRNAs in adipose tissue: their role in adipogenesis and obesity. *Int J Obes.* 2013, 37, 325-332.
- [4] Deiluiis, J. A., MicroRNAs as regulators of metabolic disease: pathophysiologic significance and emerging role as biomarkers and therapeutics. *Int J Obes.* 2016, 40, 88-101.
- [5] Olefsky, J. M., Glass, C. K., Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol* 2010, 72, 219-246.
- [6] Corcoran, M. P., Lamon-Fava, S., Fielding, R. A., Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise. *Am J Clin Nutr.* 2007, 85, 662-677.
- [7] Olivo-Marston, S. E., Hursting, S. D., Perkins, S. N., Schetter, A., *et al.*, Effects of calorie restriction and diet-induced obesity on murine colon carcinogenesis, growth and inflammatory factors, and microRNA expression. *Plos One.* 2014, 9, e94765.
- [8] Nezami, B. G., Mwangi, S. M., Lee, J. E., Jeppsson, S., *et al.*, MicroRNA 375 mediates palmitate-induced enteric neuronal damage and high-fat diet-induced delayed intestinal transit in mice. *Gastroenterology.* 2014, 146, 473-483.

- [9] Collins, N. T., Cummins, P. M., Colgan, O. C., Ferguson, G., *et al.*, Cyclic strain-mediated regulation of vascular endothelial occludin and ZO-1: influence on intercellular tight junction assembly and function. *Arterioscler Thromb Vasc Biol.* 2006, 26, 62-68.
- [10] Guedes, E., Diniz, G., Analysis of microRNA-22 on cardiac hypertrophy induced by high fat diet. *FASEB J.* 2015, 29.
- [11] Barberry, C. E., Celigoj, F. A., Turner, S. D., Smith, R. P., *et al.*, Alterations in microRNA Expression in a Murine Model of Diet-Induced Vasculogenic Erectile Dysfunction. *J Sex Med.* 2015, 12, 621-630.
- [12] Choi, S. W., Friso, S., Epigenetics: A new bridge between nutrition and health. *Adv Nutr.* 2010, 1, 8-16.
- [13] Ehlert, T., Simon, P., Moser, D. A., Epigenetics in sports. *Sports Med* 2013, 43, 93-110.
- [14] Milagro, F. I., Miranda, J., Portillo, M. P., Fernandez-Quintela, A., *et al.*, High-throughput sequencing of micrnas in peripheral blood mononuclear cells: identification of potential weight loss biomarkers. *Plos One.* 2013, 8, e54319.
- [15] McGregor, R. A., Choi, M. S., MicroRNAs in the regulation of adipogenesis and obesity. *Curr Mol Med.* 2011, 11, 304-316.
- [16] Ortega, F. J., Mercader, J. M., Catalan, V., Moreno-Navarrete, J. M., *et al.*, Targeting the circulating microRNA signature of obesity. *Clin Chem.* 2013, 59, 781-792.
- [17] McGregor, R. A., Poppitt, S. D., Cameron-Smith, D., Role of microRNAs in the age-related changes in skeletal muscle and diet or exercise interventions to promote healthy aging in humans. *Ageing Res Rev.* 2014, 17, 25-33.

- [18] Peng, Y., Yu, S., Li, H., Xiang, H., *et al.*, MicroRNAs: Emerging roles in adipogenesis and obesity. *Cell Signal*. 2014, 26, 1888-1896.
- [19] Lynn, F. C., Meta-regulation: microRNA regulation of glucose and lipid metabolism. *Trends Endocrinol Metab*. 2009, 20, 452-459.
- [20] Bartel, D. P., MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*. 2004, 116, 281-297.
- [21] MacFarlane, L. A., Murphy, P. R., MicroRNA: Biogenesis, function and role in cancer. *Current Genomics*. 2010, 11, 537-561.
- [22] Palmer, J. D., Soule, B. P., Simone, B. A., Zaorsky, N. G., *et al.*, Review: MicroRNA expression altered by diet: Can food be medicinal? *Ageing Res Rev*. 2014, 17, 16-24.
- [23] Xie, H., Sun, L., Lodish, H. F., Targeting microRNAs in obesity. *Expert Opin Ther Targets*. 2009, 13, 1227-1238.
- [24] Guay, C., Regazzi, R., Circulating microRNAs as novel biomarkers for diabetes mellitus. *Nat Rev Endocrinol*. 2013, 9, 513-521.
- [25] 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*. 2011, 96, E846-850.
- [26] Apostolopoulos, V., de Courten, M. P., Stojanovska, L., Blatch, G. L., *et al.*, The complex immunological and inflammatory network of adipose tissue in obesity. *Mol Nutr Food Res*. 2016, 60, 43-57.

- [27] Kim, Y. J., Hwang, S. H., Cho, H. H., Shin, K. K., *et al.*, MicroRNA 21 regulates the proliferation of human adipose tissue-derived mesenchymal stem cells and high-fat diet-induced obesity alters microRNA 21 expression in white adipose tissues. *J Cell Physiol.* 2012, 227, 183-193.
- [28] Halberg, N., Khan, T., Trujillo, M. E., Wernstedt-Asterholm, I., *et al.*, Hypoxia-inducible factor-1alpha induces fibrosis and insulin resistance in white adipose tissue. *Mol Cell Biol.* 2009, 29, 4467-4483.
- [29] Xie, H., Lim, B., Lodish, H. F., MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity. *Diabetes.* 2009, 58, 1050-1057.
- [30] Takanabe, R., Abe, Y., Takaya, T., Wada, H., *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.* 2008, 376, 728-732.
- [31] Chartoumpekis, D. V., Ziros, P. G., Iskrenova, R. P., Psyrogiannis, A. I., *et al.*, Differential expression of microRNAs in adipose tissue after long-term high-fat diet-induced obesity in mice. *Plos One.* 2012, 7, e34872.
- [32] Romao, J. M., Jin, W., He, M., McAllister, T., Guan, L. L., Altered microRNA expression in bovine subcutaneous and visceral adipose tissues from cattle under different diet. *Plos One.* 2012, 7, e40605.
- [33] Das, A. V., Pillai, R. M., Implications of miR cluster 143/145 as universal anti-oncomiRs and their dysregulation during tumorigenesis. *Cancer Cell Int.* 2015, 15, 92.

- [34] Maeda, K., Cao, H., Kono, K., Gorgun, C. Z., *et al.*, Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metab.* 2005, *1*, 107-119.
- [35] MacDougald, O. A., Lane, M. D., Transcriptional regulation of gene expression during adipocyte differentiation. *Annu Rev Biochem.* 1995, *64*, 345-373.
- [36] Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., Spiegelman, B. M., mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev.* 1994, *8*, 1224-1234.
- [37] Esau, C., Davis, S., Murray, S. F., Yu, X. X., *et al.*, Article: miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metabolism.* 2006, *3*, 87-98.
- [38] Kim, Y. J., Hwang, S. J., Bae, Y. C., Jung, J. S., MiR-21 regulates adipogenic differentiation through the modulation of TGF-beta signaling in mesenchymal stem cells derived from human adipose tissue. *Stem Cells.* 2009, *27*, 3093-3102.
- [39] Morin, C. L., Eckel, R. H., Marcel, T., Pagliassotti, M. J., High fat diets elevate adipose tissue-derived tumor necrosis factor-alpha activity. *Endocrinology.* 1997, *138*, 4665-4671.
- [40] Karbiener, M., Fischer, C., Nowitsch, S., Opriessnig, P., *et al.*, microRNA miR-27b impairs human adipocyte differentiation and targets PPARgamma. *Biochem Biophys Res Commun.* 2009, *390*, 247-251.
- [41] Sun, T., Fu, M., Bookout, A. L., Kliewer, S. A., Mangelsdorf, D. J., MicroRNA let-7 regulates 3T3-L1 adipogenesis. *Mol Endocrinol.* 2009, *23*, 925-931.

- [42] Wang, Q., Li, Y. C., Wang, J., Kong, J., *et al.*, miR-17-92 cluster accelerates adipocyte differentiation by negatively regulating tumor-suppressor Rb2/p130. *Proc Natl Acad Sci U S A*. 2008, *105*, 2889-2894.
- [43] Gao, Y., Schug, J., McKenna, L. B., Le Lay, J., *et al.*, Tissue-specific regulation of mouse microRNA genes in endoderm-derived tissues. *Nucleic Acids Res*. 2011, *39*, 454-463.
- [44] Lagos-Quintana, M., Rauhut, R., Yalcin, A., Meyer, J., *et al.*, Identification of tissue-specific microRNAs from mouse. *Curr Biol*. 2002, *12*, 735-739.
- [45] Goto, T., Lee, J. Y., Teraminami, A., Kim, Y. I., *et al.*, Activation of peroxisome proliferator-activated receptor-alpha stimulates both differentiation and fatty acid oxidation in adipocytes. *J Lipid Res*. 2011, *52*, 873-884.
- [46] Ortega, F. J., Moreno-Navarrete, J. M., Pardo, G., Sabater, M., *et al.*, MiRNA expression profile of human subcutaneous adipose and during adipocyte differentiation. *Plos One*. 2010, *5*, e9022.
- [47] Gimble, J. M., Morgan, C., Kelly, K., Wu, X., *et al.*, Bone morphogenetic proteins inhibit adipocyte differentiation by bone marrow stromal cells. *J Cell Biochem*. 1995, *58*, 393-402.
- [48] Yun, S. J., Kim, E. K., Tucker, D. F., Kim, C. D., *et al.*, Isoform-specific regulation of adipocyte differentiation by Akt/protein kinase Balpha. *Biochem Biophys Res Commun*. 2008, *371*, 138-143.
- [49] Huang, Z. H., Gu, D., Mazzone, T., Role of adipocyte-derived apoE in modulating adipocyte size, lipid metabolism, and gene expression in vivo. *Am J Physiol Endocrinol Metab*. 2009, *296*, E1110-1119.
- [50] Tanaka, T., Yoshida, N., Kishimoto, T., Akira, S., Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene. *EMBO J*. 1997, *16*, 7432-7443.

- [51] Taganov, K. D., Boldin, M. P., Chang, K. J., Baltimore, D., NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A*. 2006, *103*, 12481-12486.
- [52] Xu, H., Sethi, J. K., Hotamisligil, G. S., Transmembrane tumor necrosis factor (TNF)-alpha inhibits adipocyte differentiation by selectively activating TNF receptor 1. *J Biol Chem*. 1999, *274*, 26287-26295.
- [53] Yun, Z., Maecker, H. L., Johnson, R. S., Giaccia, A. J., Inhibition of PPAR gamma 2 gene expression by the HIF-1-regulated gene DEC1/Stra13: a mechanism for regulation of adipogenesis by hypoxia. *Dev Cell*. 2002, *2*, 331-341.
- [54] Saykally, J. N., Dogan, S., Cleary, M. P., Sanders, M. M., The ZEB1 transcription factor is a novel repressor of adiposity in female mice. *Plos One*. 2009, *4*, e8460.
- [55] Yang, B., Lin, H., Xiao, J., Lu, Y., *et al.*, The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat Med*. 2007, *13*, 486-491.
- [56] Feldmann, H. M., Golozoubova, V., Cannon, B., Nedergaard, J., UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab*. 2009, *9*, 203-209.
- [57] Enomoto, H., Furuichi, T., Zanma, A., Yamana, K., *et al.*, Runx2 deficiency in chondrocytes causes adipogenic changes in vitro. *J Cell Sci*. 2004, *117*, 417-425.
- [58] Alkhouri, N., Gornicka, A., Berk, M. P., Thapaliya, S., *et al.*, Adipocyte apoptosis, a link between obesity, insulin resistance, and hepatic steatosis. *J Biol Chem*. 2010, *285*, 3428-3438.

- [59] Felli, N., Fontana, L., Pelosi, E., Botta, R., *et al.*, MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down-modulation. *Proc Natl Acad Sci U S A.* 2005, *102*, 18081-18086.
- [60] Fabbrini, E., Sullivan, S., Klein, S., Obesity and nonalcoholic fatty liver disease: Biochemical, metabolic, and clinical implications. *Hepatology.* 2010, *51*, 679-689.
- [61] Ahn, J., Lee, H., Chung, C. H., Ha, T., High fat diet induced downregulation of microRNA-467b increased lipoprotein lipase in hepatic steatosis. *Biochem Biophys Res Commun.* 2011, *414*, 664-669.
- [62] Cho, J., Kim, S., Lee, S., Kang, H., Effect of training intensity on nonalcoholic fatty liver disease. *Med Sci Sports Exerc.* 2015, *47*, 1624-1634.
- [63] Ahn, J., Lee, H., Jung, C. H., Ha, T., Lycopene inhibits hepatic steatosis via microRNA-21-induced downregulation of fatty acid-binding protein 7 in mice fed a high-fat diet. *Mol Nutr Food Res.* 2012, *56*, 1665-1674.
- [64] Szabo, G., Csak, T., Role of MicroRNAs in NAFLD/NASH. *Dig Dis Sci.* 2016, *61*, 1314-1324.
- [65] Cirera, S., Birck, M., Busk, P. K., Fredholm, M., Expression profiles of miRNA-122 and its target CAT1 in minipigs (*Sus scrofa*) fed a high-cholesterol diet. *Comp Med.* 2010, *60*, 136-141.
- [66] Karere, G. M., Glenn, J. P., VandeBerg, J. L., Cox, L. A., Differential microRNA response to a high-cholesterol, high-fat diet in livers of low and high LDL-C baboons. *BMC Genomics.* 2012, *13*, 320.
- [67] Park, J. H., Ahn, J., Kim, S., Kwon, D. Y., Ha, T. Y., Murine hepatic miRNAs expression and regulation of gene expression in diet-induced obese mice. *Mol Cells.* 2011, *31*, 33-38.

- [68] Marchesini, G., Brizi, M., Morselli-Labate, A. M., Bianchi, G., *et al.*, Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med.* 1999, *107*, 450-455.
- [69] Liu, G., Friggeri, A., Yang, Y., Milosevic, J., *et al.*, MiR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *J Exp Med.* 2010, *207*, 1589-1597.
- [70] Marquez, R. T., Wendlandt, E., Galle, C. S., Keck, K., McCaffrey, A. P., MicroRNA-21 is upregulated during the proliferative phase of liver regeneration, targets Pellino-1, and inhibits NF-kappaB signaling. *Am J Physiol Gastrointest Liver Physiol.* 2010, *298*, G535-541.
- [71] Berndt, J., Kovacs, P., Ruschke, K., Kloting, N., *et al.*, Fatty acid synthase gene expression in human adipose tissue: association with obesity and type 2 diabetes. *Diabetologia.* 2007, *50*, 1472-1480.
- [72] Iliopoulos, D., Malizos, K. N., Oikonomou, P., Tsezou, A., Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *Plos One.* 2008, *3*, e3740.
- [73] Lanford, R. E., Hildebrandt-Eriksen, E. S., Petri, A., Persson, R., *et al.*, Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science.* 2010, *327*, 198-201.
- [74] Chang, J., Nicolas, E., Marks, D., Sander, C., *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.* 2004, *1*, 106-113.
- [75] Huang, R. S., Hu, G. Q., Lin, B., Lin, Z. Y., Sun, C. C., MicroRNA-155 silencing enhances inflammatory response and lipid uptake in oxidized low-density lipoprotein-stimulated human THP-1 macrophages. *J Investig Med.* 2010, *58*, 961-967.

- [76] Yang, K., He, Y. S., Wang, X. Q., Lu, L., *et al.*, MiR-146a inhibits oxidized low-density lipoprotein-induced lipid accumulation and inflammatory response via targeting toll-like receptor 4. *FEBS Lett.* 2011, 585, 854-860.
- [77] Chen, G. Q., Lian, W. J., Wang, G. M., Wang, S., *et al.*, Altered microRNA expression in skeletal muscle results from high-fat diet-induced insulin resistance in mice. *Mol Med Rep.* 2012, 5, 1362-1368.
- [78] McClelland, A. D., Kantharidis, P., MicroRNA in the development of diabetic complications. *Clin Sci.* 2014, 126, 95-110.
- [79] Anderson, C., Catoe, H., Werner, R., MIR-206 regulates connexin43 expression during skeletal muscle development. *Nucleic Acids Res.* 2006, 34, 5863-5871.
- [80] Gallagher, I. J., Scheele, C., Keller, P., Nielsen, A. R., *et al.*, Integration of microRNA changes in vivo identifies novel molecular features of muscle insulin resistance in type 2 diabetes. *Genome Med.* 2010, 2, 9.
- [81] Herrera, B. M., Lockstone, H. E., Taylor, J. M., Ria, M., *et al.*, Global microRNA expression profiles in insulin target tissues in a spontaneous rat model of type 2 diabetes. *Diabetologia.* 2010, 53, 1099-1109.
- [82] Huang, B., Qin, W., Zhao, B., Shi, Y., *et al.*, MicroRNA expression profiling in diabetic GK rat model. *Acta Biochim Biophys Sin.* 2009, 41, 472-477.
- [83] Karolina, D. S., Armugam, A., Tavintharan, S., Wong, M. T., *et al.*, MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus. *Plos One.* 2011, 6, e22839.

- [84] Ling, H. Y., Ou, H. S., Feng, S. D., Zhang, X. Y., *et al.*, Changes in microRNA (miR) profile and effects of miR-320 in insulin-resistant 3T3-L1 adipocytes. *Clin Exp Pharmacol Physiol.* 2009, *36*, e32-39.
- [85] McCarthy, J. J., Esser, K. A., MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. *J Appl Physiol.* 2007, *102*, 306-313.
- [86] Gehart, H., Kumpf, S., Ittner, A., Ricci, R., MAPK signalling in cellular metabolism: stress or wellness? *EMBO Reports.* 2010, *11*, 834-840.
- [87] Frias Fde, T., de Mendonca, M., Martins, A. R., Gindro, A. F., *et al.*, MyomiRs as markers of insulin resistance and decreased myogenesis in skeletal muscle of diet-induced obese mice. *Front Endocrinol.* 2016, *7*, 76.
- [88] Augusto, V., Padovani, C. R., Campos, G. E. R., Skeletal muscle fiber types in C57BL6J mice. *Braz J Morphol Sci.* 2004, *21*, 89-94.
- [89] La Vecchia, C., Cancers associated with high-fat diets. *J Natl Cancer Inst Monogr.* 1992, 79-85.
- [90] Takagi, T., Naito, Y., Mizushima, K., Hirata, I., *et al.*, Increased expression of microRNA in the inflamed colonic mucosa of patients with active ulcerative colitis. *J Gastroenterol Hepatol.* 2010, *25 Suppl 1*, S129-133.
- [91] Schimanski, C. C., Frerichs, K., Rahman, F., Berger, M., *et al.*, High miR-196a levels promote the oncogenic phenotype of colorectal cancer cells. *World J Gastroenterol.* 2009, *15*, 2089-2096.
- [92] Ribeiro, D. L., Goes, R. M., Pinto-Fochi, M. E., Taboga, S. R., *et al.*, AKT and AMPK activation after high-fat and high-glucose in vitro treatment of prostate epithelial cells. *Horm Metab Res.* 2014, *46*, 471-476.

- [93] Lashinger, L. M., Malone, L. M., Brown, G. W., Daniels, E. A., *et al.*, Rapamycin partially mimics the anticancer effects of calorie restriction in a murine model of pancreatic cancer. *Cancer Prev Res.* 2011, *4*, 1041-1051.
- [94] Moore, T., Beltran, L., Carbajal, S., Strom, S., *et al.*, Dietary energy balance modulates signaling through the Akt/mammalian target of rapamycin pathways in multiple epithelial tissues. *Cancer Prev Res.* 2008, *1*, 65-76.
- [95] Watanabe, A., Tagawa, H., Yamashita, J., Teshima, K., *et al.*, The role of microRNA-150 as a tumor suppressor in malignant lymphoma. *Leukemia.* 2011, *25*, 1324-1334.
- [96] Toyota, M., Suzuki, H., Sasaki, Y., Maruyama, R., *et al.*, Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. *Cancer Res.* 2008, *68*, 4123-4132.
- [97] Schulz, M. D., Atay, C., Heringer, J., Romrig, F. K., *et al.*, High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. *Nature.* 2014, *514*, 508-512.
- [98] Murphy, E. A., Velazquez, K. T., Herbert, K. M., Influence of high-fat diet on gut microbiota: a driving force for chronic disease risk. *Curr Opin Clin Nutr Metab Care.* 2015, *18*, 515-520.
- [99] Liu, S., da Cunha, Andre P., Rezende, Rafael M., Cialic, R., *et al.*, The host shapes the gut microbiota via fecal microRNA. *Cell Host Microbe.* 19, 32-43.
- [100] Putignani, L., Human gut microbiota: onset and shaping through life stages and perturbations. *Front Cell Infect Microbiol.* 2012, *2*, 144.
- [101] Al Mushref, M., Srinivasan, S., Effect of high fat-diet and obesity on gastrointestinal motility. *Ann Transl Med.* 2013, *1*, 14.

- [102] Moraes, J. C., Coope, A., Morari, J., Cintra, D. E., *et al.*, High-fat diet induces apoptosis of hypothalamic neurons. *Plos One*. 2009, 4, e5045.
- [103] Packer, A. N., Xing, Y., Harper, S. Q., Jones, L., Davidson, B. L., The bifunctional microRNA miR-9/miR-9* regulates REST and CoREST and is downregulated in Huntington's disease. *J Neurosci*. 2008, 28, 14341-14346.
- [104] Thum, T., MicroRNA therapeutics in cardiovascular medicine. *EMBO Mol Med*. 2012, 4, 3-14.
- [105] Vickers, K. C., Rye, K.-A., Tabet, F., microRNAs in the onset and development of cardiovascular disease. *Clin Sci*. 2014, 126, 183-194.
- [106] Wang, H., Zhu, H. Q., Wang, F., Zhou, Q., *et al.*, MicroRNA-1 prevents high-fat diet-induced endothelial permeability in apoE knock-out mice. *Mol Cell Biochem*. 2013, 378, 153-159.
- [107] Zhu, H. Q., Cheng, X. W., Xiao, L. L., Jiang, Z. K., *et al.*, Melatonin prevents oxidized low-density lipoprotein-induced increase of myosin light chain kinase activation and expression in HUVEC through ERK/MAPK signal transduction. *J Pineal Res*. 2008, 45, 328-334.
- [108] Zhu, H. Q., Li, Q., Dong, L. Y., Zhou, Q., *et al.*, MicroRNA-29b promotes high-fat diet-stimulated endothelial permeability and apoptosis in apoE knock-out mice by down-regulating MT1 expression. *Int J Cardiol*. 2014, 176, 764-770.
- [109] Hu, Z. P., Fang, X. L., Fang, N., Wang, X. B., *et al.*, Melatonin ameliorates vascular endothelial dysfunction, inflammation, and atherosclerosis by suppressing the TLR4/NF-kappaB system in high-fat-fed rabbits. *J Pineal Res*. 2013, 55, 388-398.
- [110] Zhu, J., Chen, T., Yang, L., Li, Z., *et al.*, Regulation of microRNA-155 in atherosclerotic inflammatory responses by targeting MAP3K10. *Plos One*. 2012, 7, e46551.

- [111] Nazari-Jahantigh, M., Wei, Y., Noels, H., Akhtar, S., *et al.*, MicroRNA-155 promotes atherosclerosis by repressing Bcl6 in macrophages. *J Clin Invest.* 2012, *122*, 4190-4202.
- [112] O'Connell, R. M., Taganov, K. D., Boldin, M. P., Cheng, G., Baltimore, D., MicroRNA-155 is induced during the macrophage inflammatory response. *Proc Natl Acad Sci U S A.* 2007, *104*, 1604-1609.
- [113] Wang, Z., Li, L., Zhao, H., Peng, S., Zuo, Z., Chronic high fat diet induces cardiac hypertrophy and fibrosis in mice. *Metabolism.* 2015, *64*, 917-925.
- [114] Kuwabara, Y., Horie, T., Baba, O., Watanabe, S., *et al.*, MicroRNA-451 exacerbates lipotoxicity in cardiac myocytes and high-fat diet-induced cardiac hypertrophy in mice through suppression of the LKB1/AMPK pathway. *Circ Res.* 2015, *116*, 279-288.
- [115] Liang, Q., De Windt, L. J., Witt, S. A., Kimball, T. R., *et al.*, The transcription factors GATA4 and GATA6 regulate cardiomyocyte hypertrophy in vitro and in vivo. *J Biol Chem.* 2001, *276*, 30245-30253.
- [116] Toque, H. A., da Silva, F. H., Calixto, M. C., Lintomen, L., *et al.*, High-fat diet associated with obesity induces impairment of mouse corpus cavernosum responses. *BJU Int.* 2011, *107*, 1628-1634.
- [117] Kulshreshtha, R., Ferracin, M., Wojcik, S. E., Garzon, R., *et al.*, A microRNA signature of hypoxia. *Mol Cell Biol.* 2007, *27*, 1859-1867.
- [118] Mahizir, D., Briffa, J. F., Hryciw, D. H., Wadley, G. D., *et al.*, Maternal obesity in females born small: Pregnancy complications and offspring disease risk. *Mol Nutr Food Res.* 2015, *60*, 8-17.

- [119] Benatti, R. O., Melo, A. M., Borges, F. O., Ignacio-Souza, L. M., *et al.*, Maternal high-fat diet consumption modulates hepatic lipid metabolism and microRNA-122 (miR-122) and microRNA-370 (miR-370) expression in offspring. *Br J Nutr.* 2014, *111*, 2112-2122.
- [120] Zhang, J., Zhang, F., Didelot, X., Bruce, K. D., *et al.*, Maternal high fat diet during pregnancy and lactation alters hepatic expression of insulin like growth factor-2 and key microRNAs in the adult offspring. *BMC Genomics.* 2009, *10*, 478.
- [121] Gonzalez, F. J., Shah, Y. M., PPARalpha: mechanism of species differences and hepatocarcinogenesis of peroxisome proliferators. *Toxicology.* 2008, *246*, 2-8.
- [122] Shah, Y. M., Morimura, K., Yang, Q., Tanabe, T., *et al.*, Peroxisome proliferator-activated receptor alpha regulates a microRNA-mediated signaling cascade responsible for hepatocellular proliferation. *Mol Cell Biol.* 2007, *27*, 4238-4247.
- [123] Delgado-Lista, J., Perez-Martinez, P., Lopez-Miranda, J., Perez-Jimenez, F., Long chain omega-3 fatty acids and cardiovascular disease: a systematic review. *Br J Nutr.* 2012, *107 Suppl 2*, S201-213.
- [124] Mozaffarian, D., Wu, J. H., Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol.* 2011, *58*, 2047-2067.
- [125] Jump, D. B., Fatty acid regulation of hepatic lipid metabolism. *Curr Opin Clin Nutr Metab Care.* 2011, *14*, 115-120.
- [126] Harris, W. S., Bulchandani, D., Why do omega-3 fatty acids lower serum triglycerides? *Curr Opin Lipidol.* 2006, *17*, 387-393.
- [127] Serhan, C. N., Novel lipid mediators and resolution mechanisms in acute inflammation: to resolve or not? *Am J Pathol.* 2010, *177*, 1576-1591.

- [128] Micallef, M. A., Garg, M. L., Anti-inflammatory and cardioprotective effects of n-3 polyunsaturated fatty acids and plant sterols in hyperlipidemic individuals. *Atherosclerosis*. 2009, 204, 476-482.
- [129] Dangardt, F., Osika, W., Chen, Y., Nilsson, U., *et al.*, Omega-3 fatty acid supplementation improves vascular function and reduces inflammation in obese adolescents. *Atherosclerosis*. 2010, 212, 580-585.
- [130] Daimiel-Ruiz, L., Klett, M., Konstantinidou, V., Micó, V., *et al.*, Dietary lipids modulate the expression of miR-107, a miRNA that regulates the circadian system. *Mol Nutr Food Res*. 2015, 59, 552-565.
- [131] Kumar, S., Sutherland, F., Rosso, R., Teh, A. W., *et al.*, Effects of chronic omega-3 polyunsaturated fatty acid supplementation on human atrial electrophysiology. *Heart Rhythm*. 2011, 8, 562-568.
- [132] Brouwer, I. A., Raitt, M. H., Dullemeijer, C., Kraemer, D. F., *et al.*, Effect of fish oil on ventricular tachyarrhythmia in three studies in patients with implantable cardioverter defibrillators. *Eur Heart J*. 2009, 30, 820-826.
- [133] Davidson, L. A., Wang, N., Shah, M. S., Lupton, J. R., *et al.*, n-3 Polyunsaturated fatty acids modulate carcinogen-directed non-coding microRNA signatures in rat colon. *Carcinogenesis*. 2009, 30, 2077-2084.
- [134] Davis, C. D., Ross, S. A., Evidence for dietary regulation of microRNA expression in cancer cells. *Nutr Rev*. 2008, 66, 477-482.

- [135] Qi, R. L., Chen, Y., Huang, J. X., Yang, F. Y., Effects of conjugated linoleic acid on the expression levels of miR-27 and miR-143 in pig adipose tissue. *Genet Mol Res.* 2015, *14*, 6985-6992.
- [136] Parra, P., Serra, F., Palou, A., Expression of adipose microRNAs is sensitive to dietary conjugated linoleic acid treatment in mice. *Plos One.* 2010, *5*, e13005.
- [137] Zheng, Z., Ge, Y., Zhang, J., Xue, M., *et al.*, PUFA diets alter the microRNA expression profiles in an inflammation rat model. *Mol Med Rep.* 2015, *11*, 4149-4157.
- [138] Murase, T., Misawa, K., Minegishi, Y., Aoki, M., *et al.*, Coffee polyphenols suppress diet-induced body fat accumulation by downregulating SREBP-1c and related molecules in C57BL/6J mice. *Am J Physiol Endocrinol Metab.* 2011, *300*, E122-133.
- [139] Hsieh, C.-H., Rau, C.-S., Wu, S.-C., Yang, J. C.-S., *et al.*, Weight-reduction through a low-fat diet causes differential expression of circulating microRNAs in obese C57BL/6 mice. *BMC Genomics.* 2015, *16*, 699.
- [140] Zhang, L., Hou, D., Chen, X., Li, D., *et al.*, Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. *Cell. Res* 2012, *22*, 107-126.
- [141] Witwer, K. W., XenomiRs and miRNA homeostasis in health and disease: evidence that diet and dietary miRNAs directly and indirectly influence circulating miRNA profiles. *RNA Biol.* 2012, *9*, 1147-1154.
- [142] Tarallo, S., Pardini, B., Mancuso, G., Rosa, F., *et al.*, MicroRNA expression in relation to different dietary habits: a comparison in stool and plasma samples. *Mutagenesis.* 2014, *29*, 385-391.

- [143] Grueter, C. E., van Rooij, E., Johnson, B. A., DeLeon, S. M., *et al.*, A cardiac microRNA governs systemic energy homeostasis by regulation of MED13. *Cell*. 2012, 149, 671-683.
- [144] Vaz, C., Ahmad, H. M., Sharma, P., Gupta, R., *et al.*, Analysis of microRNA transcriptome by deep sequencing of small RNA libraries of peripheral blood. *BMC Genomics*. 2010, 11, 288.
- [145] Barrett, P., Mercer, J. G., Morgan, P. J., Preclinical models for obesity research. *Dis Model Mech*. 2016, 9, 1245-1255.
- [146] Friedman, R. C., Farh, K. K., Burge, C. B., Bartel, D. P., Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*. 2009, 19, 92-105.
- [147] Murri, M., Insenser, M., Fernandez-Duran, E., San-Millan, J. L., Escobar-Morreale, H. F., Effects of polycystic ovary syndrome (PCOS), sex hormones, and obesity on circulating miRNA-21, miRNA-27b, miRNA-103, and miRNA-155 expression. *J Clin Endocrinol Metab*. 2013, 98, E1835-1844.
- [148] Dong, S., Ma, W., Hao, B., Hu, F., *et al.*, microRNA-21 promotes cardiac fibrosis and development of heart failure with preserved left ventricular ejection fraction by up-regulating Bcl-2. *Int J Clin Exp Pathol*. 2014, 7, 565-574.
- [149] Karolina, D. S., Tavintharan, S., Armugam, A., Sepramaniam, S., *et al.*, Circulating miRNA profiles in patients with metabolic syndrome. *J Clin Endocrinol Metab*. 2012, 97, E2271-2276.
- [150] Prats-Puig, A., Ortega, F. J., Mercader, J. M., Moreno-Navarrete, J. M., *et al.*, Changes in circulating microRNAs are associated with childhood obesity. *J Clin Endocrinol Metab*. 2013, 98, 2013-1496.
- [151] 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*. 2011, 6, e23937.

[152] Yan, X., Liang, H., Deng, T., Zhu, K., *et al.*, The identification of novel targets of miR-16 and characterization of their biological functions in cancer cells. *Mol Cancer*. 2013, 12, 92.

Figure 1: Effect of high fat diet on microRNA expression in different tissues.

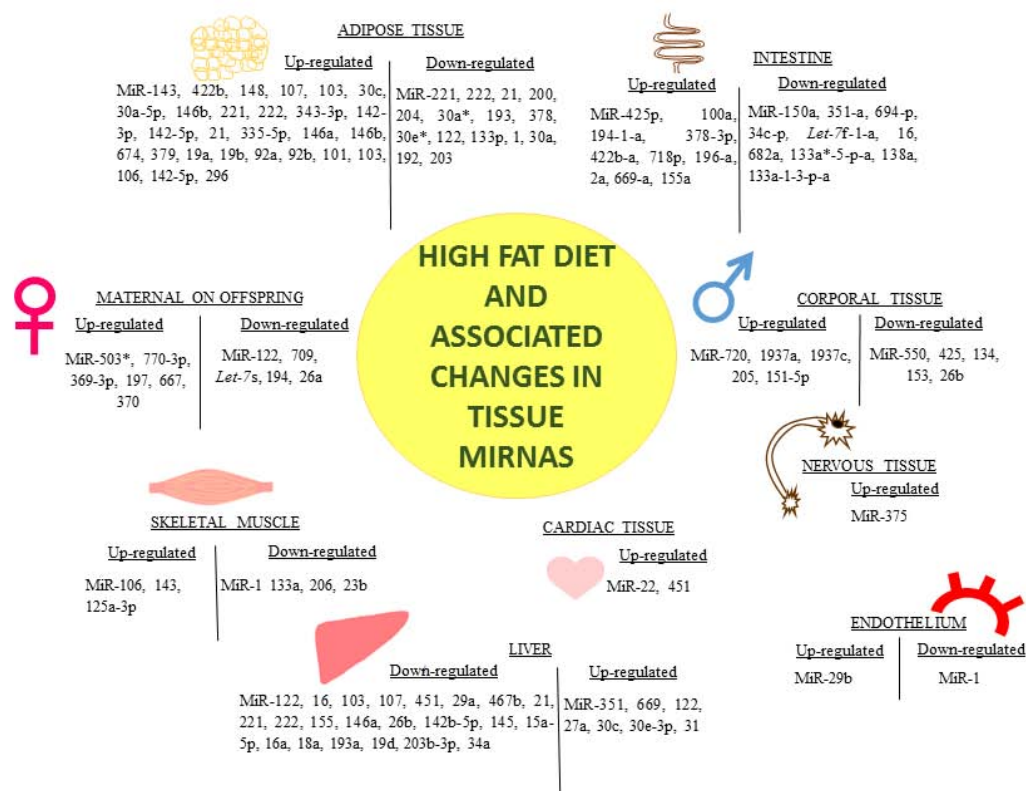


Table 1. List of tissue and circulatory specific miRNA changes following high fat diet.

Animal model used	miRNA identified	Change in tissue expression under high fat conditions –Animal model	Change in serum/plasma expression under high fat conditions –Animal model	Clinical implications – therapeutic target for pathological condition
C57BL/6	miR-21	↓ Adipose ↓ Liver	↓	Obesity [16, 147] Cancer [27] Heart Failure [148]
C57BL/6	miR-103	↑ Adipose	↓	Obesity [149]
C57BL/6 Baboon	miR-221	↑/↓ Adipose ↑/↓ Liver	↓	Obesity [150] Type 2 Diabetes [16] Insulin resistance [4]
C57BL/6	miR-16	↓ Liver	↓	Non-alcoholic fatty liver disease[151] Cancer [152]

Table 2. List of papers and summary findings included in the review

Title of the study	Animal model & sex	Diet	Duration of feeding (weeks)	miRNA Up-regulated	miRNA Down-regulated	Target gene/ mRNA (Predicted)	Predicted function
ADIPOSE TISSUE							
Up-regulated expression of microRNA-143 in association with obesity in adipose tissue of mice fed high-fat diet [30]	Mice (C57BL/6) Males	High-fat Diet (60%)	8-9	MiR-143		PPAR γ , aP2, ERK5	Adipocyte differentiation
MicroRNAs induced during adipogenesis that accelerate fat cell development are down-regulated in obesity [29]	Mice (C57BL/6) Males	High-fat Diet (55%)	12	MiR-422b, 148, 107, 103, 30c, 30a-5p, 146b and 143	221, 222		Adipogenesis
MicroRNA 21 regulates the proliferation of human adipose tissue-derived mesenchymal stem cells and high-fat diet-induced obesity alters microRNA 21 expression in white adipose tissues [27]	Mice (C57BL/6) Males	High-fat Diet (60%)	10		MiR-21		Adipocyte proliferation and differentiation
Differential expression of microRNAs in adipose tissue after long-term high-fat diet-induced obesity in mice [31]	Mice (C57BL/6) Males	High-fat Diet (60%)	20	221, 222, 343-3p, 142-3p, 142-5p, 21, 335-5p, 146a, 146b, 674, 379,	MiR-200, 204, 30a*, 193, 378, 30e*, 122, 133p, 1, 30a, 192, 203	BMP-4, Fgf10, Akt1, interferon γ , Hif-1 α , TNF α , zeb1, zeb2, UCP-1, Runxx2	Adipogenesis, inflammation response, adipocyte differentiation, epithelial to mesenchymal transition, osteogenesis

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Altered microRNA expression in bovine subcutaneous and visceral adipose tissues from cattle under different diet [32]	Bovine	High-fat Diet (5.85%)	14	19a, 19b, 92a, 92b, 101, 103, 106, 142-5p, 296		SOCS3, SGK1, ADRB1, ABHD5, TEF, SLC12A2, PKRCE, PPARGC1B, KITLG, GSK3B, APP, BDNF, CLOCK, PTGS2, ACSL6, CAV2, REST, ABHD4	Lipid metabolism, connective tissue differentiation and function
LIVER							
Expression profiles of miRNA-122 and its target CAT1 in minipigs (<i>Sus scrofa</i>) fed a high-cholesterol diet [65]	Male Göttingen Minipigs,	2% Cholesterol + 22.7% Fat	11		MiR-122		Cholesterol level, obesity
Murine hepatic miRNAs expression and regulation of gene expression in diet-induced obese mice [67]	Mice (C57BL/6) Males	High-fat Diet	6	MiR-351, 669	MiR-16, 103, 107, 451, 29a	Essra, lhh, Crhr1, FASN and MYC	Fatty acid synthesis, obesity, osteoarthritis
High-fat diet-induced down-regulation of microRNA-146b increased lipoprotein lipase in hepatic steatosis [61]	Mice (C57BL/6) Males	Lard 20%	8		MiR-467b	LPL	Hepatic steatosis
Lycopene inhibits hepatic steatosis via microRNA-21-induced down-regulation of fatty acid-binding protein 7 in mice fed a high-fat diet [63]	Mice (C57BL/6) Males	High-fat Diet (49.29)	8		MiR-21	FABP7	Hepatic steatosis
Differential microRNA response to a high-cholesterol, high-fat diet in livers of low and high LDL-C baboons [66]	baboons	High-fat Diet (40%) + cholesterol (1.7 mg/kcal)	7	MiR-122, 27a	MiR-221, 222, 155, 146a, 26b	LDLR, VLDLR, LIPA, PTEN, TENC1, ACVRIB	Atherosclerosis, cardiovascular diseases
SKELTAL MUSCLE							
Altered microRNA expression in skeletal muscle results from high-fat diet-induced insulin resistance in mice [77]	Mice (C57BL/6) Males	High-fat Diet (58%)	12	MiR-106, 143, 125a-3p	MiR-1, 133a, 206, 23b	MAPK, Wnt, neurotrophic, hedgehog, VEGF, and TGF- β	Cell proliferation, metabolism, protein degradation, oncogenic pathways and insulin resistance

MyomiRs as Markers of Insulin Resistance and Decreased Myogenesis in Skeletal Muscle of Diet-Induced Obese Mice [87]	Mice (C57BL/6) Males	High-fat Diet (59%)	12	MiR-133a	MiR-1	IGF-1R, P13K Myf-5, HDAC4	Myogenesis, insulin resistance
INTESTINE							
Effects of calorie restriction and diet-induced obesity on murine colon carcinogenesis, growth and inflammatory factors, and microRNA expression [7]	FVB Male	High-fat Diet (45%)	20	MiR-425p, 100a, 194-1-a, 378-3p, 422b-a, 718p, 196-a, 2a, 669-a, 155a	MiR-150a, 351-a, 694-p, 34c-p, Let-7f-1-a, 16, 682a, 133a*-5-p-a, 138a, 133a-1-3-p-a	IGF-1/Akt/mTOR	Cell proliferation, apoptosis
NERVOUS TISSUE							
MicroRNA 375 mediates palmitate-induced enteric neuronal damage and high-fat diet-induced delayed intestinal transit in mice [8]	Mice (C57BL/6) and CF1, Males	High-fat Diet (60%)	11	MiR-375		14-3-3 ζ , Pdk1, embryonic lethal abnormal vision, drosophila-like 4 and myotrophin	Apoptosis
ENDOTHELIUM							
MicroRNA-1 prevents high-fat diet-induced endothelial permeability in apoE knock-out mice [106]	apoE knock-out mice	2% Cholesterol + 5% lard oil	16		MiR-1	MLCK, ERK,	Endothelial permeability
MicroRNA-29b promotes high-fat diet-stimulated endothelial permeability and apoptosis in apoE knock-out mice by down-regulating MT1 expression [108]	apoE knock-out mice, Male	2% Cholesterol + 5% lard oil	16	MiR-29b		MT1	Apoptosis
CARDIAC TISSUE							
Analysis of microRNA-22 on cardiac hypertrophy induced by high-fat diet [10]	Mice (C57BL/6) Males	High-fat Diet (60%)	10	MiR-22			Cardiac hypertrophy
MicroRNA-451 exacerbates lipotoxicity in cardiac myocytes and high-fat diet-induced cardiac hypertrophy in mice through suppression of the LKB1/AMPK pathway	Mice (C57BL/6)	High-fat Diet (45%)	20	MiR-451		GATA4, Cab39	Cardiac hypertrophy

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MATERNAL ON OFFSPRING							
Maternal high-fat diet during pregnancy and lactation alters hepatic expression of insulin like growth factor-2 and key microRNAs in the adult offspring [120]	Mice (C57BL/6)	High-fat Diet (22.6% fat (W/W))	4 weeks before conception , during pregnancy & lactation	MiR-503*, 770-3p, 369-3p, 197, 667	MiR-122, 709, Let-7s, 194, 26a	Methyl-CpG binding protein 2, Histone 4	Hepatic fatty acid metabolism and foetal growth
Maternal high-fat diet consumption modulates hepatic lipid metabolism and microRNA-122 (miR-122) and microRNA-370 (miR-370) expression in offspring [119]	Mice (C57BL/6)	High-fat Diet (24% fat (W/W))	2 weeks before conception , during pregnancy & lactation	MiR-370	MiR-122	CPT1 α and ACADVL	Fatty acid β -oxidation
CORPORAL TISSUE							
Alterations in microRNA Expression in a Murine Model of Diet-Induced Vascular Dysregulation [11]	Mice (C57BL/6) Males	High-fat Diet (60%)	22	MiR-720, 1937a, 1937c, 205, 151-5p	MiR-550, 425, 134, 153, 26b		Vasodilation, hypoxia-induced apoptosis

Table 3. List of common miRNAs included in the review.

MiRNA	Tissues
1	Adipose, Muscle, Intestine
16	Liver, Intestine
21	Adipose, Liver
26	Liver, Corporal, offspring liver
29	Liver, endothelium
103	Adipose, liver

106	Adipose, Muscle
107	Adipose, liver
122	Adipose, liver, offspring liver
133	Adipose, Muscle, Intestine
143	Adipose, muscle
146	Adipose, liver
155	Liver, Intestine
194	Intestine, offspring liver
221	Adipose, liver
222	Adipose, liver
351	Liver, Intestine
378	Adipose, Intestine
425	Intestine, Corporal
451	Liver, Heart
669	Liver, Intestine
Let7	Intestine, offspring liver