REVIEW

High fat diet and associated changes in the expression of micro-RNAs in tissue: Lessons learned from animal studies

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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, noncoding RNAs that are important regulators of gene expression at posttranscriptional 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 summarize 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.

Keywords:
Disease / Gene regulation / High fat diet / miRNA / Obesity

1 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 labor 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 nonpeptide 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 nonalcoholic fatty liver disease (NAFLD), which is associated with hepatic insulin resistance, altered glucose, fatty acid, and

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Abbreviations: Akt, also known as protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; aP2, adipocyte fatty acid-binding protein; ApoE, apolipoprotein E; Cab-39, calcium-binding protein 39; CAT1, cationic amino acid transporter 1; CVD, cardiovascular diseases; ERK, extracellular signal regulated kinase; FABP, fatty acid-binding protein; FASN, fatty acid synthase; Fgf10, fibroblast growth factor 10; LDLR, low density lipoprotein receptor; 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; NALFD, nonalcoholic fatty liver disease; PPAR\textsubscript{\alpha}, peroxisome proliferator-activated receptor-alpha; PPAR\textsubscript{\gamma}, peroxisome proliferator-activated receptor-gamma; Pref-1, preadipocyte factor 1; RNA, ribonucleic acids; TGF-\textbeta, transforming growth factor beta; TNF-\textalpha, tumor necrosis factor alpha

Colour Online: See the article online to view Fig. 1 in colour.
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 signaling, 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 remodeling, and most recently discovered, miRNA expression regulation [12–14].

MiRNAs (micro ribonucleic acids or miRNAs or miR) are a group of small, noncoding RNAs that regulate gene expression at the posttranscriptional 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 nonobese 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 summarize 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 has also been noted to demonstrate their clinical relevance and potential as a therapeutic target.

2 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 Fig. 1. Livers from maternal high fat fed offspring were also included to demonstrate the impact that diet and obesity can have on the intratuerine environment during pregnancy. In order to minimize 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).

2.1 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 overfeeding, 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, five 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].

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

<table>
<thead>
<tr>
<th>Animal model used</th>
<th>miRNA identified</th>
<th>Change in tissue expression under high fat conditions—animal model</th>
<th>Change in serum/plasma expression under high fat conditions—animal model</th>
<th>Clinical implications—therapeutic target for pathological condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>miR-21</td>
<td>↓ Adipose, ↓ Liver</td>
<td>↓</td>
<td>Obesity [16,147], Cancer [27], Heart failure [148]</td>
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<tr>
<td>C57BL/6</td>
<td>miR-103</td>
<td>↑ Adipose</td>
<td>↓</td>
<td>Obesity [149], Obesity [150]</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>miR-221</td>
<td>↑/↓ Adipose, ↑/↓ Liver</td>
<td>↓</td>
<td>Type 2 diabetes [16], Insulin resistance [4]</td>
</tr>
<tr>
<td>Baboon</td>
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<tr>
<td>C57BL/6</td>
<td>miR-16</td>
<td>↓ Liver</td>
<td>↓</td>
<td>Nonalcoholic fatty liver disease [151], Cancer [152]</td>
</tr>
</tbody>
</table>

MiR-143 was originally thought to play a key role in cardiac morphogenesis and tumor 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 that 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.
Table 2. List of papers and summary findings included in the review

<table>
<thead>
<tr>
<th>Title of the study</th>
<th>Animal model and sex</th>
<th>Diet</th>
<th>Duration of feeding (weeks)</th>
<th>miRNA upregulated</th>
<th>miRNA downregulated</th>
<th>Target gene/mRNA (predicted)</th>
<th>Predicted function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adipose tissue</strong></td>
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</tr>
<tr>
<td>1 Upregulated expression of microRNA-143 in association with obesity in adipose tissue of mice fed high-fat diet [30]</td>
<td>Mice (C57BL/6)</td>
<td>High-fat diet (60%)</td>
<td>8–9</td>
<td>miR-143</td>
<td></td>
<td>PPARγ, aP2, ERK5</td>
<td>Adipocyte differentiation</td>
</tr>
<tr>
<td>2 MicroRNAs induced during adipogenesis that accelerate fat cell development are downregulated in obesity [29]</td>
<td>Mice (C57BL/6)</td>
<td>High-fat Diet (55%)</td>
<td>12</td>
<td>miR-221, 222,</td>
<td>miR-21</td>
<td></td>
<td>Adipogenesis</td>
</tr>
<tr>
<td>3 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]</td>
<td>Mice (C57BL/6)</td>
<td>High-fat Diet (60%)</td>
<td>10</td>
<td>miR-21</td>
<td></td>
<td></td>
<td>Adipocyte proliferation and differentiation</td>
</tr>
<tr>
<td>4 Differential expression of microRNAs in adipose tissue after long-term high-fat diet-induced obesity in mice [31]</td>
<td>Mice (C57BL/6)</td>
<td>High-fat diet (60%)</td>
<td>20</td>
<td>miR-221, 222,</td>
<td>miR-21</td>
<td>BMP-4, Fgf10, Akt1, interferon γ, Hif-1α, TNFα, zeb1, zeb2, UCP-1, Runx2</td>
<td>Adipogenesis, inflammation response, adipocyte differentiation, epithelial to mesenchymal transition, osteogenesis</td>
</tr>
<tr>
<td>5 Altered microRNA expression in bovine subcutaneous and visceral adipose tissues from cattle under different diet [32]</td>
<td>Bovine</td>
<td>High-fat Diet (5.85%)</td>
<td>14</td>
<td>miR-19a, 19b,</td>
<td>miR-200, 204, 30a*, 30e*, 378, 379, 122, 133p, 1, 30a, 192, 203</td>
<td>SOCS3, SGK1, ADRB1, ABHD5, TEF, SLCL12A2, PKRCE, PPAR GC1B, KITLG, GSK3B, APP, BDNF, CLEFT, PTGS2, ACSL6, CAV2, REST, ABHD4</td>
<td>Lipid metabolism, connective tissue differentiation, and function</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
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<tr>
<td>1 Expression profiles of miRNA-122 and its target CAT1 in minipigs (Sus scrofa) fed a high-cholesterol diet [65]</td>
<td>Male Göttingen Minipigs, 2% Cholesterol + 22.7% Fat</td>
<td>High-fat Diet (60%)</td>
<td>11</td>
<td>miR-122</td>
<td></td>
<td>Essra, Ihh, Chr1, FASN, and MYC</td>
<td>Cholesterol levels, obesity</td>
</tr>
<tr>
<td>2 Murine hepatic miRNAs expression and regulation of gene expression in diet-induced obese mice [67]</td>
<td>Mice (C57BL/6)</td>
<td>High-fat diet (60%)</td>
<td>6</td>
<td>miR-351, 669</td>
<td>miR-16, 103, 107, 451, 29a</td>
<td></td>
<td>Fatty acid synthesis, obesity, osteoarthritis</td>
</tr>
<tr>
<td>3 High-fat diet-induced downregulation of microRNA-467b increased lipoprotein lipase in hepatic steatosis [61]</td>
<td>Mice (C57BL/6)</td>
<td>Lard 20%</td>
<td>8</td>
<td>miR-467b</td>
<td></td>
<td>LPL</td>
<td>Hepatic steatosis</td>
</tr>
<tr>
<td>4 Lycopene inhibits hepatic steatosis via microRNA-21-induced downregulation of fatty acid-binding protein 7 in mice fed a high-fat diet [63]</td>
<td>Mice (C57BL/6)</td>
<td>High-fat diet (49.29)</td>
<td>8</td>
<td>miR-21</td>
<td></td>
<td>FABP7</td>
<td>Hepatic steatosis</td>
</tr>
<tr>
<td>Title of the study</td>
<td>Animal model and sex</td>
<td>Diet</td>
<td>Duration of feeding (weeks)</td>
<td>miRNA upregulated</td>
<td>miRNA downregulated</td>
<td>Target gene/mRNA (predicted)</td>
<td>Predicted function</td>
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<tr>
<td>5 Differential microRNA response to a high-cholesterol, high-fat diet in livers of low, and high LDL-C baboons [66]</td>
<td>Baboons High-fat Diet (40%) + cholesterol (1.7 mg/kcal)</td>
<td>7</td>
<td>miR-122, 27a</td>
<td>miR-221, 222, 155, 146a, 26b</td>
<td>LDLR, VLDLR, LIPA, PTEN, TENC1, ACVR1B</td>
<td>Atherosclerosis, cardiovascular diseases</td>
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<td><strong>Skeletal muscle</strong></td>
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<tr>
<td>1 Altered microRNA expression in skeletal muscle results from high-fat diet-induced insulin resistance in mice [77]</td>
<td>Mice (C57BL/6) Males High-fat diet (58%)</td>
<td>12</td>
<td>miR-106, 143, 125a-3p</td>
<td>miR-1, 133a, 206, 23b</td>
<td>MAPK, Wnt, neurotrophic, hedgehog, VEGF, and TGF-β</td>
<td>Cell proliferation, metabolism, protein degradation, oncogenic pathways, and insulin resistance</td>
<td>Cell proliferation, metabolism, protein degradation, oncogenic pathways, and insulin resistance</td>
</tr>
<tr>
<td>2 MyomiRs as markers of insulin resistance and decreased myogenesis in skeletal muscle of diet-induced obese mice [87]</td>
<td>Mice (C57BL/6) Males High-fat diet (59%)</td>
<td>12</td>
<td>miR-133a</td>
<td>miR-1</td>
<td>IGF-1R, P13K Myf-5, HDAC4</td>
<td>Myogenesis, insulin resistance</td>
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<td><strong>Intestine</strong></td>
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<tr>
<td>1 Effects of calorie restriction and diet-induced obesity on murine colon carcinogenesis, growth, and inflammatory factors, and microRNA expression [7]</td>
<td>FVB Male High-fat Diet (45%)</td>
<td>20</td>
<td>miR-425p, 100a, 194–1-a, 378–3p, 422b-a, 718p, 196-a, 2a, 669-a, 155a</td>
<td>miR-150a, 351-a, 694-p, 34c-p, 133a*-5-p-a, 138a, 133a-1–3-p-a</td>
<td>IGF-1/Akt/mTOR</td>
<td>Cell proliferation and apoptosis</td>
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<td><strong>Nervous tissue</strong></td>
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<td>1 MicroRNA 375 mediates palmitate-induced enteric neuronal damage and high-fat diet-induced delayed intestinal transit in mice [8]</td>
<td>Mice (C57BL/6) and CF1, Males High-fat diet (60%)</td>
<td>11</td>
<td>miR-375</td>
<td></td>
<td>14-3-3sj, Pdk1, embryonic lethal abnormal vision, drosophila-like 4, and myotrophin</td>
<td>Apoptosis</td>
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<td><strong>Endothelium</strong></td>
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<tr>
<td>1 MicroRNA-1 prevents high-fat diet-induced endothelial permeability in apoE knock-out mice [106]</td>
<td>apoE knock-out mice 2% Cholesterol + 5% lard oil</td>
<td>16</td>
<td>miR-1</td>
<td></td>
<td>MLCK, ERK</td>
<td>Endothelial permeability</td>
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<tr>
<td>2 MicroRNA-29b promotes high-fat diet-stimulated endothelial permeability and apoptosis in apoE knock-out mice by downregulating MT1 expression [108]</td>
<td>apoE knock-out mice, Male 2% Cholesterol + 5% lard oil</td>
<td>16</td>
<td>miR-29b</td>
<td></td>
<td>MT1</td>
<td>Apoptosis</td>
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<tr>
<td>Title of the study</td>
<td>Animal model and sex</td>
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<td>miRNA upregulated</td>
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<td>Predicted function</td>
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<td><strong>Cardiac tissue</strong></td>
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<tr>
<td>1 Analysis of microRNA-22 on cardiac hypertrophy induced by high-fat diet [10]</td>
<td>Mice (C57BL/6)</td>
<td>High-fat diet (60%)</td>
<td>10</td>
<td>miR-22</td>
<td></td>
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<td>Cardiac hypertrophy</td>
</tr>
<tr>
<td>2 MicroRNA-451 exacerbates lipotoxicity in cardiac myocytes and high-fat diet-induced cardiac hypertrophy in mice through suppression of the LKB1/AMPK pathway [114]</td>
<td>Mice (C57BL/6)</td>
<td>High-fat diet (45%)</td>
<td>20</td>
<td>miR-451</td>
<td></td>
<td>GATA4, Calc39</td>
<td>Cardiac hypertrophy</td>
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<tr>
<td><strong>Maternal on offspring</strong></td>
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<tr>
<td>1 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]</td>
<td>Mice (C57BL/6)</td>
<td>High-fat diet (22.6% fat (W/W))</td>
<td>4 weeks before conception, during pregnancy and lactation</td>
<td>miR-503*, 770–3p, 369–3p, 197, 667</td>
<td>miR-122, 709, Let-7s, 194, 26a</td>
<td>Methyl-CpG-binding protein 2, Histone 4</td>
<td>Hepatic fatty acid metabolism and foetal growth</td>
</tr>
<tr>
<td>2 Maternal high-fat diet consumption modulates hepatic lipid metabolism and microRNA-122 (miR-122) and microRNA-370 (miR-370) expression in offspring [119]</td>
<td>Mice (C57BL/6)</td>
<td>High-fat diet (24% fat (W/W))</td>
<td>2 weeks before conception, during pregnancy and lactation</td>
<td>miR-370</td>
<td>miR-122</td>
<td>CPT1α and ACADVL</td>
<td>Fatty acid β-oxidation</td>
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<td><strong>Corporal tissue</strong></td>
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ACADVL, acyl-CoA dehydrogenase, very long chain; BMP-4, bone morphogenetic protein 4; Crhr1, corticotropin releasing hormone receptor 1; GATA4, transcription factor that belongs to GATA family; Hif-1α, hypoxia-inducible factor 1-alpha; IGF-1, insulin-like growth factor-1; Ihh, Indian hedgehog; LIPA, lipase A precursor; mTOR, mechanistic target of rapamycin; MYC, V-Myc avian myelocytomatosis viral oncogene homolog; Pdk1, pyruvate dehydrogenase lipoamide kinase isozyme 1; PTEN, phosphatase and tensin homolog; TENC1, tensin-like C1 domain containing phosphatase; UCP-1, uncoupling protein 1; VEGF, vascular endothelial growth factor.
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 transforming growth factor beta (TGF-β) signaling pathway [38]. In 2012, Kim et al. [27] investigated the expression levels of miR-21 in epididymal adipose tissue of high fat diet-induced obese mice. Interestingly, a biphasic 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 preadipocyte factor 1 (Pref-1), a preadipocyte 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 downregulation) 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 preadipocytes 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 two miRNAs (miR-221 and miR-222) decreasing their expression [29]. The study also used a leptin-deficient B6.V-Lepob/f(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 upregulation in preadipocytes (cell culture model) and downregulation in mature adipocytes (animal model). The authors suggested that the contrasting changes in miRNAs, specifically the downregulation in later stages, could be due to chronic local inflammation environment and enhanced tumor necrosis factor alpha (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 upregulated 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 proadipogenic effect by regulating high-mobility group AT-hook 2, and miR-27b demonstrating an anti-adipogenic effect by regulating PPARγ [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 tumor 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 PPARγ, 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 postdietary intervention. Researchers reported an upregulation 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

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downregulation 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, 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 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-alpha and 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, which have been implicated in adipogenesis and obesity [54]. MiR-1, a skeletal muscle-specific miRNA, is reported to target uncoupling protein 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 downregulation of miR-204 and 133b may result in noninhibition 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 for high fat feeding studies. MiR-16 has proapoptotic 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 upregulated and are suggested to manifest their effects by targeting PPARγ/ERK5 and TGFβ, 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.

2.2 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 that can lead to a reversible pathological condition called 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, five 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. Downregulation 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 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 downregulation 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 downregulation of miR-21 and upregulation 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 mouse model. Some of the changes were already known to be involved in
obesity and other diseases. For example, miR-451 was reported to be down-regulated, a miRNA already known to be associated with non-alcoholic steatohepatitis [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 (Essra), Indian hedgehog, corticotropin releasing hormone receptor 1 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, 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 downregulation [65], while another study reported an upregulation following high fat diet [66]. The differences could be due to the animal models utilized 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 (1.7 mg/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 downregulation 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, phosphatase and tensin homolog, tensin-like C1 domain containing phosphatase, and activin A receptor, type IB isoform C precursor [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 tumor 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 that were upregulated and downregulated, respectively in liver following a high fat diet target low density lipoprotein receptor adapter protein 1 that interacts with LDL cholesterol receptors.

### 2.3 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 downregulated [79–85], whereas miR-144 and miR-106b were upregulated. Gene annotation and signaling 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 signaling 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, and TGF-β], metabolism
lysine degradation, heparin sulphate biosynthesis, and glycogen 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 p1 UK 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 fiber type analyzed with the Chen et al. [77] study using the gastrocnemius that is predominantly type IIB and IIDb fibers, whereas the soleus muscle used by Frias et al. [87] study is predominantly IIB fibers [88]. Notwithstanding, the downregulation 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.

2.4 Intestine tissue

Obesity is considered a risk factor for various types of cancers and high fat diets are associated with colorectum 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-425p, 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 that are downstream pathways of insulin-like growth factor-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 ten miRNAs that were downregulated 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 tumor growth by increasing apoptosis and decreasing proliferation [95]. Other members of tumor suppressing miRNAs, namely miR-34c, let-7, and miR-16 have also been downregulated 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 tumor progression in the small intestine of genetically susceptible, Kras(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 tumors 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 tumor 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.

2.5 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), pyruvate dehydrogenase lipoamide kinase isozyme 1, 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 miR-375 inhibitor preventing high fat diet-induced delays in intestinal transit and morphologic changes [8]. Although these preclinical findings are acute, it does suggest a potential target for clinical gastrointestinal dysmotility.

### 2.6 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 summarizing 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 that 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 knockout 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 downregulation 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 upregulation 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 upregulated in both arteries and mononuclear cells in apoE−/− mice [110]. MiR-155 has been shown to be upregulated 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.

### 2.7 Cardiac tissue

Long-term high fat diet feeding induces cardiac remodeling and dysfunction that can lead to obesity-induced heart failure [10, 113]; whether miRNAs play a role is 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, 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 knockout mouse studies [114]. Cab-39 is a scaffold protein of liver kinase B1 (LKB1), and an upstream kinase of S’ AMP-activated protein kinase (AMPK). Indeed, enhanced AMPK phosphorylation has been demonstrated in miR-451 knockout models [114]. It has been suggested that suppression of Cab-39 through miR-451 upregulation 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 upregulated 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 signaling pathways that we affected by the increased miR-22 expression, it is difficult to comment on the significance of such a finding.

### 2.8 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.

2.9 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 that regulates developmental timing and miR-122 that regulates fat metabolism. The change in let-7c could be due to the upregulation of PPARα, as PPARα 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 coordinated changes in key metabolic genes and miRNAs that regulate early fetal growth and fat metabolism [120]. Similarly, Benatti et al. [120] demonstrated reduced expression of miR-122 as well as carnitine palmitoyl-transferase 1alpha (CPT1α) in the offspring livers of high fat fed dams. In addition, miR-370 was increased whereas acyl-CoA dehydrogenase, very long chain 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 downregulation of five miRNAs (miR-709, let-7, miR-122, miR-194, miR-269) that have methyl-CpG-binding protein 2 as their predicted target gene and the upregulation of five 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 that 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.

3 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 has 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 coincidently 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 et al. [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].

4 Clinical implications and therapeutic applications

Identification of therapeutic miRNAs requires well-planned experimental designs, development of antagonim (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.

Table 3. List of common miRNAs included in the review

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adipose, muscle, intestine</td>
</tr>
<tr>
<td>16</td>
<td>Liver, intestine</td>
</tr>
<tr>
<td>21</td>
<td>Adipose, liver</td>
</tr>
<tr>
<td>26</td>
<td>Liver, corporal, offspring liver</td>
</tr>
<tr>
<td>29</td>
<td>Liver, endothelium</td>
</tr>
<tr>
<td>103</td>
<td>Adipose, liver</td>
</tr>
<tr>
<td>106</td>
<td>Adipose, muscle</td>
</tr>
<tr>
<td>107</td>
<td>Adipose, liver</td>
</tr>
<tr>
<td>122</td>
<td>Adipose, liver, offspring liver</td>
</tr>
<tr>
<td>133</td>
<td>Adipose, muscle, intestine</td>
</tr>
<tr>
<td>143</td>
<td>Adipose, muscle</td>
</tr>
<tr>
<td>146</td>
<td>Adipose, liver</td>
</tr>
<tr>
<td>155</td>
<td>Liver, intestine</td>
</tr>
<tr>
<td>194</td>
<td>Intestine, offspring liver</td>
</tr>
<tr>
<td>221</td>
<td>Adipose, liver</td>
</tr>
<tr>
<td>222</td>
<td>Adipose, liver</td>
</tr>
<tr>
<td>351</td>
<td>Liver, intestine</td>
</tr>
<tr>
<td>378</td>
<td>Adipose, intestine</td>
</tr>
<tr>
<td>425</td>
<td>Intestine, corporal</td>
</tr>
<tr>
<td>451</td>
<td>Liver, heart</td>
</tr>
<tr>
<td>669</td>
<td>Liver, intestine</td>
</tr>
<tr>
<td>Let7</td>
<td>Intestine, offspring liver</td>
</tr>
</tbody>
</table>

5 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 ribonucleoprotein miRNA induced silencing complex 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 C57BL6J 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
Finally, from a clinical viewpoint, plasma miRNA profiles typically help facilitate the generation of therapeutic targets. Currently, miRNAs may be used to distinguish healthy, obese, and diseased states, but more importantly, help facilitate the generation of therapeutic targets.

Characterization of miRNAs in circulation and tissues will be impacted by dietary-induced stress, further identification and increased calorie/fat intake. Given the numerous tissues that seem to be affected and possibly dysregulated by the increased calorie/fat intake. The influence of 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 characterization 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.

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6 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 posttranscriptional regulation by miRNAs in humans is unknown. Further, prediction models suggest 45,000 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.

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