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Dysregulation of Some Skeletal Muscle miRNAs in High-Fat Diet-Induced Obesity: Implications for Metabolic Disorders

Elham M. Youssef[a], Asmaa M. Elfiky[b], Lamiaa M. Ramadan[a]*

[a]*Biochemistry Department, National Research Centre, Cairo, Egypt, 12622*

[b]*Environmental and Occupational Medicine Department, Environmental and climate change Research Institute, National Research Centre, Cairo, Egypt, 12622*

Abstract

Skeletal muscle-adipose tissue crosstalk is crucial for developing therapeutic strategies for various metabolic disorders. While obesity is known to disrupt microRNA (miRNA) expression profiles in skeletal muscle, a comprehensive understanding of this phenomenon remains elusive. Therefore, our study aims to investigate the impact of high-fat diet (HFD)-induced obesity on miRNA dysregulation within skeletal muscle tissue at distinct time points. In HFD model, rats received HFD for 2, 4, 6, 8, or 10 weeks. Body weight was determined at 2, 4, 6, 8, and 10 weeks. At the end of each interval, group of animals (n = 8) were sacrificed and skeletal muscle tissues were harvested to assess miRNAs expression levels. HFD administration for 8 and 10 weeks resulted in marked weight changes in comparison to control group. There were not much significant changes in body weight seen in low durations of HFD feeding. Alterations in miR130a, miR30a-5p, miR133a-5p, miR193a-5p, and miR125a-5p expression levels were observed at different time point relative to control rats. While miR let-7 and miR107-5p were upregulated at all-time points compared to control animals. Thus, skeletal muscle miRNA dysregulation likely plays a role in HFD-induced obesity.

***Keywords*:** High-fat diet, Obesity, microRNAs, Skeletal muscles, Expression profiling, Body weight.

1. Introduction

Obesity is considered a major risk factor for chronic diseases such as coronary heart disease, hypertension, insulin resistance, type 2 diabetes, and certain types of cancer [1], with over 4 million people dying each year as a result of being overweight or obese according to the global burden of disease in 2017 [2]. Dietary fat intake is often cited as a cause of increased obesity [3, 4].

Increased adiposity is associated with reduced muscle energetic efficiency with more reliance on glycolysis and can promote catabolic events in skeletal muscle [5]. One possible mechanism by which obesity promotes protein turnover pathways and muscle loss involves the effect of inflammatory cytokines that abundantly expressed by adipose tissue [6].

MicroRNAs (miRNAs) are short noncoding RNAs that regulate the expression of complementary messenger RNAs. They have been shown to play important roles in mammalian organogenesis and also appear in the pathophysiology of various diseases [7]. This element acts on cellular processes related to cell development and differentiation, immunity, reproduction, and metabolism [8, 9]. Dysregulation of miRNAs expression affects the function of various organs and may lead to obesity-related metabolic diseases [10, 11]. They are considered a class of epigenetic regulators of metabolism and energy homeostasis and have emerged as key regulators of skeletal muscle development [12].

Beyond their individual roles within muscle and adipose tissue, microRNAs (miRNAs) also play an important role in communication between these two tissues. This crosstalk can be influenced by

* *Corresponding author E-mail: l_mmr1@yahoo.com ;(Lamiaa M. Ramadan).*
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various factors like diet or exercise. Modifications in miRNA expression within one tissue can trigger a cascade of effects in the other through altered signaling pathways or metabolite production. This coordinated exchange serves to maintain a delicate equilibrium between muscle and adipose tissue function, ultimately impacting whole-body metabolic homeostasis [13].

Some miRNAs, termed the myogenic miRNAs (myomiR) family, are preferentially detected in muscle tissue and act as regulators of myogenesis, proliferation, and metabolism, as well as skeletal muscle hypertrophy. MyomiRs include miR133a and miR133b [14-16]. One such myomiR, miR133a, demonstrates a broader presence beyond skeletal muscle tissue. Although classified as a muscle-specific miRNA, miR133a expression is also detectable in both brown adipose tissue (BAT) and subcutaneous white adipose tissue (SAT). Interestingly, miR133a plays a critical role in regulating adipocyte browning, a process with significant implications for metabolic health [17, 18].

As well as, inflammatory condition induces miR130 that leads to adipocyte dysfunction [19]. Furthermore, it has been reported that transgenic mice with inducible muscle-specific overexpression of let-7 exhibit insulin resistance and impaired glucose tolerance [20, 21]. The miR30 family of miRNAs promotes myogenic cell differentiation [22]. A lipid-modulated miRNA, miR107 has a role in inflammation and diet-induced obesity [23]. Moreover, in obese and pre-diabetic adults, blood level of miR193 was found to be increased [24]. miR125a trigger cellular mechanisms in adipose tissue associated with inflammation. miR125a also regulates energy metabolism processes as adipocyte differentiation and metabolism of glucose and amino acid [25].

Therefore, the purpose of this study was to investigate miRNA dysregulation in skeletal muscle during obesity development and progression and to identify potential biomarkers and determine the role of skeletal muscle miRNA expression, and so new therapeutic targets for obesity and obesity-related muscle disorders. Overall, this study provides valuable insights into the identification of miRNAs biomarkers for obesity and associated muscular changes in rats.

2. Materials and Methods

2.1 Study design

Eighty male Wister rats (6-week old) were maintained in the Modern Veterinary House Egypt (five rats/cage). The animals randomly divided into two groups: Control group received normal diet for 10 weeks and high-fat diet (HFD) group received a high-fat diet for 10 weeks. Diet content for each group was presented in Table 1, the normal diet utilized in our study contained enough nutrient and energy for rats' growth and development. As well as, a high-fat diet was performed by adjusting the proportion of fat of normal diet according to previous studies [26-28]. Body weight was determined at 2, 4, 6, 8, and 10 weeks for each group. A comprehensive metabolic analysis, including fasting blood glucose, insulin level, leptin, adiponectin hormones and lipid parameters were carried out at 2 and 10 weeks.

At the end of each interval, group of animals (n = 8) were sacrificed and gastrocnemius (GC) and soleus muscles tissue were harvested, then frozen in liquid nitrogen and stored at −80°C. All experiment procedures were revised and approved by Research Ethical Approval Committee, National Research, Egypt.

Table (1): Diet content for ND group and HFD group

Components of the diet (g/kg diet)	Control	HFD
Carbohydrates in the form of corn starch	65%	50%
Proteins in the form of casein	23%	23%
Fats	6% (in the form of corn oil)	20% (in the form of sheep tallow)
Fibers in the form of cellulose	3%	3%
Vitamins and minerals	1–4%	1–4%
Total	100 %	100%

2.2 Metabolic Analysis

Fasting blood glucose levels were measured by a hexokinase colorimetric technique. Hormone levels of leptin, adiponectin, and insulin were quantified by enzyme-linked immunosorbent assay (ELISA). Serum lipids, including total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol, were estimated by enzymatic methods using a Spectrum kit from the Egyptian Company for Biotechnology (S.A.E.), Obour City, Cairo, Egypt. Low-density lipoprotein (LDL) cholesterol was calculated using the formula: LDL cholesterol = Total cholesterol - HDL cholesterol - (Triglycerides / 5).

2.3 RNA extraction and quantitative RT-PCR

Differential expression of miR130a, miR let7, miR30a-5p, miR107-5p, miR133a-5p, miR193a-5p, and miR125a-5p were assessed in GC and soleus

muscles tissue of both groups using quantitative RT-PCR (qRT-PCR). Muscular tissues (100 mg) were used for total RNA isolation using Mini Kit (QIAGEN, GmbH, Hilden) based on instructions of the manufacturer. Then RNA quantitation was performed using Nanodrop Lite (ThermoScientific, Wilmington, DE).

After this, the total purified RNA (1 µg) was reverse transcribed into cDNA by mi Script II RT Kit (QIAGEN, GmbH, Hilden) as manufacturer guided. Then miRNA expression was evaluated by real-time PCR. The reaction mixture contains 100 ng/µLcDNA (2.0 µL), 2× SYBR Green PCR Master Mix (10 µl), 10× miScript Universal Primer (2.0 µL), 10× miScript Primer Assay (2.0 µL) specific for each mature miRNA, then nuclease-free water is added to complete the total volume 20 µL. Then PCR was performed as follows:95 °C for 10 min; 40 cycles in three steps: 94 °C for 15 se, 55 °C for 30 s and 70 °C for 30 s, followed by 4 °C forever (Real-Time System, BIO-RAD, Hercules, CA)).

2.4 Statistics and data presentation

All data are expressed as mean ± SEM. The two-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was applied to compare between week 0, week2, week 4, week6, week8, and week 10 using GraphPad Prism software (V.8, California, USA). $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Metabolic Changes in High-Fat Diet Group

Rats fed a high-fat diet exhibited elevated fasting blood glucose and insulin levels at week 10 compared to those on a normal diet (**Table 2**).

Table (2): Fasting blood glucose an fasting insulin levels at weeks 2 and 10 compared to the normal diet group

	Blood glucose level (mg/dl)	Insulin level (ulU/ml)
Control		
2-week	67.64± 3.7	3.68±0.1
10-week	99.28± 3.4*	6.5±0.44
HFD		
2-week	81±1.6	5.96±0.3
10-week	150± 2.8*	10.76±0.4*

Results are expressed as means ± SEM (n = 8) and two-way ANOVA followed by Tukey's –multiple comparisons post hoc test was used to perform comparisons.

Leptin concentration showed a significant ($p < 0.05$) increase after the high-fat feeding for 10 weeks. In contrast, adiponectin level decreased significantly ($p < 0.05$) compared to the control group (**Table 3**).

Table (3): Effect of High-Fat Diet on Leptin and Adiponectin Levels at weeks 2 and 10

	Leptin µg/l	Adiponectin µg/l
Control		
2-week	2± 0.19	10.92±0.4
10-week	39.14±2.3*	263±3.3
HFD		
2-week	4.28± 0.178	12.88±0.2
10-week	68.7± 2.02*	199±2.4*

Results are expressed as means ± SEM (n = 8) and two-way ANOVA followed by Tukey's –multiple comparisons post hoc test was used to perform comparisons.

Moreover, the blood levels of lipids in the group fed a high fat diet significantly increased by the end the experiment at 10 weeks ($p < 0.05$). These changes consisted of an increase in triglycerides, total and low-density lipoprotein cholesterol and a reduction in high-density lipoprotein cholesterol **(Table 4).**

Table (4): Effect of High-Fat Diet on Lipid parameters at weeks 2 and 10

	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	High density lipoprotein (mg/dl)	Low density lipoprotein (mg/dl)
Control				
2-week	32± 0.6	52.74±1.00	13.7±0.4	7.9±0.4
10-week	85.84±2.5*	82.14±0.9	53.4 ±0.9	16±0.6
HFD				
2-week	53.54± 0.7	60.34±1.1	15.98±0.53	26±1.6
10-week	114.54±2.9*	122.2± 4.4*	30.18±0.6*	60±7.9*

Results are expressed as means ± SEM (n = 8) and two-way ANOVA followed by Tukey's –multiple comparisons post hoc test was used to perform comparisons

In current experiment, we assessed the changes in rats' body weight after feeding of high-fat diet and normal diet. After 2-, 6-, 8-, and 10-week HFD consumption, there were significant elevation in total body weight relative to the 2-, 6-, 8-, and 10-week control groups; respectively. Furthermore, body weight of 4-, 6-, 8-, and 10-week HFD groups markedly raised in comparison with 2-week HFD group while, 6-, 8-, and 10-week HFD groups showed significant increase when compared to 4-week HFD group. Both 8- and 10-week HFD rats revealed substantial increase in body weight relative to 6-week HFD animals. Moreover, there is statistical increase in body weight of 10-week HFD group in compared with 8-week HFD group (**Table 5**).

Table (5): Effect of HFD administration on total body weight after different time intervals

	Total body weight(g)
Control	
2-week	122 ± 10.8
4-week	158.5 ± 12.2
6-week	162 ± 9.6
8-week	182 ± 11.7
10-week	240.5 ± 15.4
HFD	
2-week	155.4 ± 8.2*
4-week	173 ± 13.1$^{\#}$
6-week	180.5 ± 14.3$^{*\#@}$
8-week	222.5 ± 6.9$^{*\#@\$}$
10-week	267.5 ± 20.2$^{*\#@\$\%}$

Results are expressed as means ± SEM (n = 8) and two-way ANOVA followed by Tukey's –multiple comparisons post hoc test was used to perform comparisons. *, #, @, $, % $p<0.05$ versus each interval control, 2-week, 4-week, 6-week, and 8-week HFD groups; respectively

In addition, we examined the alterations in the expression of some miRNAs in skeletal muscle tissue during feeding of high-fat diet and normal diet. The present data showed that 2-week HFD group revealed significant increase on miR let7, miR107, and miR133a expression and 4-week HFD animals have marked changes on miR130a, miR let7, miR30a, miR107, miR133a, miR193a, and miR125a expression compared to control group. In addition, 6-week HFD rats demonstrated marked raised expression of miR let7, miR30a, miR107, miR193a, and miR125a expression while 8-week HFD group showed significant changes on miR130a, miR let7, miR30a, miR107, miR133a, and miR125a expression relative to control group. The pattern expression of miR let7, miR107, and miR133a in 10-week HFD group represented marked alterations compared to control animals (**Figure 1**).

Current results showed significant variation in 4-week HFD miR130a, miR30a, miR107, miR133a, miR193a, and miR125a expression compared to 2-week HFD group. Additionally, at week 6during HFD there are statistically changes in miR let7, miR30a, miR107, miR133a, miR193a, and miR125a expression whereas, after 8 weeks from HFD feeding there are significant alterations on miR130a, miR let7, miR30a, miR107, miR133a, miR193a, and miR125a expression compared with 2-week HFD group. However, 10-week HFD rats showed a marked change on miR let7, miR107, miR133a, and miR125a expression compared to 2-week HFD rats (**Figure 1**).

miR130a, miR30a, miR107, miR133a, and miR193a expression of 6-week HFD animals revealed statistical alterations whilst miR130a, miR let7, miR30a, miR107, and miR125a expression at week 8 during HFD and 10-week HFD animals have significant changes in miR130a, miR let7, miR30a, miR107, miR133a, and miR125a expression compared with 4-week HFD rats (**Figure 1**).

The expression level of miR let7, miR30a, miR107, miR133a, miR193a, and miR125a of 8-week HFD and 10-week HFD groups were significantly changed compared with 6-week HFD animals. Moreover, the expression of miR30a, miR107, miR133a, and miR125a in 10-week HFD group were markedly changed relative to 8-week HFD group (**Figure 1**).

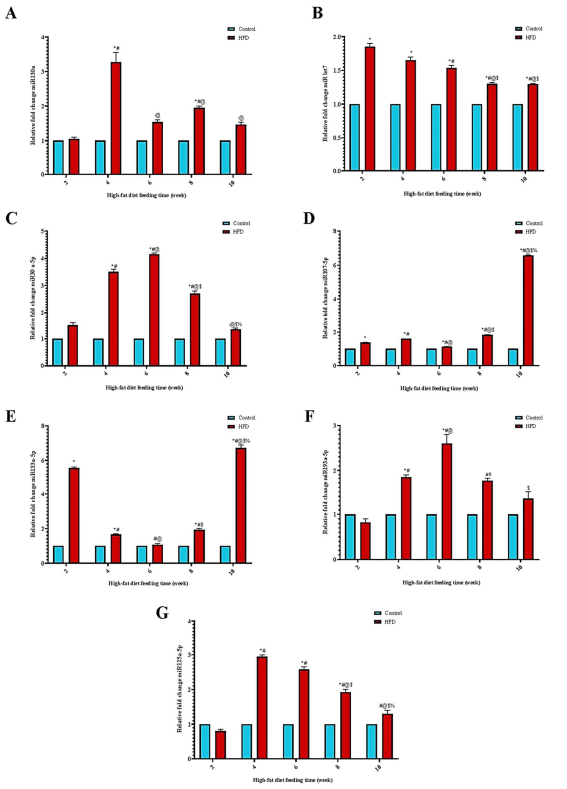


Figure (1): Effect of HFD feeding on relative fold change of miRNA expressions in gastrocnemius and soleus muscles tissue. Where (A) relative fold change of miR130a, (B) relative fold change of miR let7, (C) relative fold change of miR30a, (D) relative fold change of miR107, (E) relative fold change of miR133a, (F) relative fold change of miR193a, and (G) relative fold change of miR125a expressions.
Results are expressed as means ± SEM (n = 8) and two-way ANOVA followed by Tukey's –multiple comparisons post hoc test was used to perform comparisons. *, #, @, $, % $p<0.05$ versus control, 2-week, 4-week, 6-week, and 8-week HFD groups; respectively

4. Discussion

High caloric intake together with inactive habits are the main causes of obesity and increased fat accumulation that occurs not only in adipose tissue but also in other sites including skeletal muscle [29]. This deposition results in compositional and functional alterations within skeletal muscle [30, 31]. Therefore, this study investigated whether dysregulation of muscle miRNAs contributes to metabolic problems like fat gain and dyslipidemia in a high-fat diet-induced obesity model.

Obesity results from excessive animal's energy intake relative to energy expenditure. Dietary fat intake is considered as a cause of increased obesity [3]. This comes in line with the results of our study that revealed the duration-dependent weight gain that attributed to the significant elevation in rats' body weight after two, six, eight, and ten weeks of HFD feeding compared to each interval control groups. As well as, the marked difference in animals' body weight 4-, 6-, 8-, and 10-week HFD groups relative to 2-week HFD group. The substantial raise in body weight of 6-, 8-, and 10-week HFD animals compared to 4-week HFD group. Additionally, there was a significant raise in 8-week and 10-week HFD groups' body weight relative to 6-week HFD rats, and a significant difference between 8-week and 10-week HFD groups' body weight. Previous studies by Carlsen et al., 2009 [32] and Youssef et al., 2020 [28], supported our results.

Growing evidence documented that miRNAs are the main regulators of various physiological and pathological processes [33]. Moreover, miRNAs in skeletal muscle are considered as multifaceted regulators of skeletal muscle development and homeostasis [14, 15]. Therefore, miRNAs that can be detected in skeletal muscle tissue could reflect the health status, and so, they could serve as potential biomarkers for detection and monitoring the progression of diseases. Thus, the expression profiles of miR130a, miR let7a-5p, miR30a-5p, miR107-5p, miR-133a-5p, miR193a-5p, and miR125a-5p were assessed in current study. Some of these miRNAs have been linked with skeletal muscle in obesity, while others are first identified.

Elevation in miR130 is induced by inflammatory condition leading to adipocyte dysfunction. Whereas, a mouse mature adipocyte cell line treated with tumor necrosis factor (TNF) showed an elevated miR130 levels, an adipogenesis regulator, via inducing binding of transcription factor p65 to miR130 promoter region [19]. In parallel, our results demonstrated marked increase in miR130a in skeletal muscle homogenates after four and eight weeks of HFD feeding rats relative to control and 2-week HFD rats, with the highest level in 4-week HFD group compared to other groups.

Let7 has a role in skeletal muscle, where Let7a was high in the skeletal muscle tissue of type2 diabetes mellitus patients. As well as, let7 alters the inflammation in muscle through interleukin-13 (IL-13) repression [20]. Additionally, let7 showed strong regulation of glucose metabolism and peripheral insulin resistance in skeletal muscle tissues in transgenic mouse experiments [34]. Furthermore, let-7 anti-miR administration partially alleviated HFD effect on insulin resistance in mice through anti-let-7-mediated insulin receptor derepression [35]. In our model, along with previous studies, miR let7

expression in skeletal muscle tissues revealed marked increase in 2-, 4-, 6-,8-, and 10-week groups compared with control group, and the highest level in 2–week group compared to 6-,8-, and 10-week HFD rats.

miR30a showed a critical role in activating peroxisome proliferator-activated receptor-γ (PPARγ) to drive white-to-beige fat conversion and subcutaneous white adipose tissue expansion [36]. As well as, miR-30a amends insulin resistance in obesity [37, 38]. Furthermore, miR-30 family of miRNAs promotes myogenic cell differentiation [22]. Of note, on the contrary, our findings showed unexplained raise in miR30 a-5p in 4-, 6-, and 8-week HFD groups relative to control group, with highest level after 6 weeks from HFD feeding that returned to control level at 10-week HFD group, which needed further investigations.

Moreover, miR107 has a role in inflammation, diet-induced obesity [23], as well as lipid and energy metabolism in metabolic tissues as white adipose tissue [39]. Our results, in the line with these, demonstrated significant elevations of miR107 in skeletal muscle homogenates of 2-, 4-, 6-, 8-, and 10-week HFD groups compared with control group, and revealed the highest increase at 10-week HFD group.

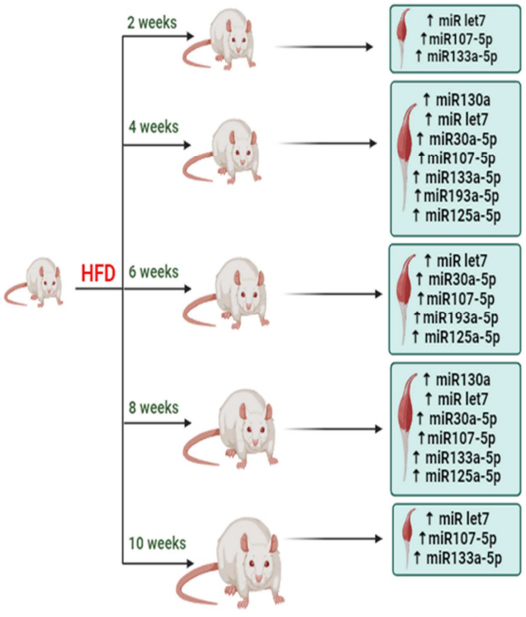
miR133a, myomiRs, is recognized as regulator of skeletal muscle development [40], and it has a role in myogenesis, and insulin resistance in soleus muscle [41]. Frias Fde et al., 2016 examined the effect of HFD feeding mice on insulin sensitivity and metabolism in skeletal muscle and reported a substantial raise in miR133a expression in soleus muscle relative to control mice [41]. In accordance with that, our findings revealed significant increase in skeletal muscle miR133a-5p expression after 2-, 4-, 6-, 8-, and 10-week HFD feeding compared to control rats, and showed the highest level at 10-week HFD group.

Moreover, miR193 blood level was reported to be elevated in obese and pre-diabetic adults [24] and showed to be associated with type 2 diabetes [42]. Contrariwise, HFD-induced obesity revealed miR193 downregulation [43, 44], and this resulted in decrease in lipid deposition, and so, adipogenesis biomarkers [45]. Our study showed significant raise in miR193a-5p in skeletal muscle after four and six weeks of HFD feeding relative to control animals whereas there were no difference between 2-, 8-, 10-week HFD groups and control rats, which indicates that relations needs further investigations.

The impact of miR125a in inflammation is critical pathophysiological feature in obesity [46]. Moreover, miR125a expression is increased during multi-potent stromal cells adipogenic differentiation [47]. Contrariwise, Bengestrate et al., 2011 reported downregulation of miR125a expression in obese ob/ob mice [48]. Current results demonstrated substantial elevation in miR125a-5p in skeletal muscle in 4-, 6-, and 8-week HFD rats compared to control group, but there were no difference after two and ten weeks from HFD feeding in skeletal muscle expression of miR125a-5p relative to control rats.

5. Conclusion

It is concluded that the high fat diet administration induced obesity and has resulted in defects in different miRNAs expressions in skeletal muscle tissues during obesity development **(Schematic diagram 1)**. In this experiment, we manifest adipose tissue/muscle crosstalk function. In addition, it examined the potential use of miRNAs as biomarkers to screen various medicinal plants and drugs as well as potential diagnostic biomarkers that target obesity and secondary consequences of obesity in skeletal muscle. However, further research is necessary to elucidate the molecular mechanisms by which these specific miRNAs contribute to the disease process and explore their potential as therapeutic targets.



Schematic diagram (1): Simplified chart showing HFD- induced changes in rats' body weight and miRNAs expressions in skeletal muscle tissues.

Conflict of interest

The authors have no conflict of interests related to this publication.

Acknowledgments

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