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Aberrant expression of miRNA profiles in high-fat and high-sucrose fed rats

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SUMMARY

High-fat and high-sucrose intakes were shown to contribute to syndromes such as hyperlipidemia, glucose intolerance, hypertension, and atherosclerosis. The pathogenesis of such diseases caused by high-fat and/or high-sucrose diet is unclear. The aim of the present study was to investigate plasma microRNA expression profiling in high-fat and high-sucrose fed rats. In total, 28 adult Wistar albino postnatal (8–12 weeks) male rats were involved in this experiment. The experimental animals were randomly divided four groups and fed with either standard rat chow, high-fat diet, high-sucrose diet and high-fat & high-sucrose diet for a period of 4 weeks. miRNAs were extracted from plasma and detected to miRNA expression profiling (Eighty four miRNAs) by quantitative real-time PCR (qRT-PCR) with the Fluidigm integrated microfluidic circuit technolog.

Among miRNAs, expression profiles of twenty miRNAs were found to be significantly different ($P < 0,05$) between groups. Of these twenty miRNAs, 1 were upregulated and 19 were downregulated with high-fat and high-sucrose feeding. The following miRNAs were downregulated: miR-130a-3p, miR-320-3p, miR-17-5p, miR-16-5p, miR-144-3p, miR-93-5p, miR-192-5p, miR-532-5p, miR-106b-5p, miR-26b-5p, miR-208b-3p, miR-23a-3p, miR-25-3p, miR-15b-5p, miR-195-5p, miR-103-3p, miR-122-5p, miR-29b-3p and miR-30a-3p and the following miRNAs were upregulated: miR-375-5p.

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These results indicate that aberrant expression of miRNA profiles may be associated with the development of hyperlipidemia, insulin resistance and cancer in the high-fat & high-sucrose-fed rat. © 2019 Published by Elsevier Ltd on behalf of European Society for Clinical Nutrition and Metabolism. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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1. Introduction

Glucose, fructose and sucrose are the most frequently consumed sugars which is commonly used to describe mono- and disaccharides [1–3]. Approximately 10% of daily caloric intake can be attributed to fructose, and fructose consumption has dramatically increased [2,3]. High-fat and high-sucrose intakes were detected to contribute to promote the development of type 2 diabetes mellitus, which is associated with obesity and insulin resistance [4–6]. It is well known that a high-fat and high-sucrose leads to hyperlipidemia and hypercholesterolemia, and increases the risk of cardiovascular disease. In some articles have indicated that the consumption of high-fat and high-sucrose diet induced a significant rise in the arterial blood pressure of normotensive rats [7–9]. Addition, epidemiological observations showed that the risk factors for the development of various cancers are intimately associated with metabolic symptoms such as obesity, hyperlipidemia, and insulin resistance caused by the excess consumption of high-fat and high-sucrose diet [10].

Many scientific studies might provide insight into the diet, lifestyle, and genetic factors that influence miRNA expression [11]. MicroRNAs [miRNAs] are one of a growing class of noncoding RNAs that are involved in the regulation of a wide range of metabolic processes including cellular differentiation, cell proliferation, inflammation and apoptosis [12]. Ross SA et al. have reported that dietary factors may influence cancer, cardiovascular disease, type 2 diabetes mellitus, obesity, and nonalcoholic fatty liver disease through modulation of miRNA expression [11]. Additionally, circulating miRNAs are emerging as putative biomarkers of disease, susceptibility, and perhaps dietary exposure. Research needs to move beyond associations in cells and animals to understanding the direct effects of diet on miRNA expression and function in human health and disease. Therefore, in this study, we have investigated plasma miRNA expression profiling [obesity, hyperlipidemia, cardiovascular disease, cancer and diabetes-related] in high-fat and high-sucrose fed rats.

2. Materials and methods

2.1. Experimental groups and diets

Twenty eight male Wistar rats, weighing $259,36 \pm 28,3$ g and aged 8–12 weeks, were obtained from Necmettin Erbakan University Experimental Medicine Research and Application Center (Konya, Turkey). The rats were housed in a climate controlled room (22 ± 2 °C temperature and $50 \pm 5\%$ humidity) on a 12/12 h light/dark cycle (lights on between 07:00 and 19:00), with ad libitum food and fresh water. The experimental animals were randomly divided four groups (n:7 for each group) and fed with either standard rat chow, high-fat diet, high-sucrose diet and high-fat & high-sucrose diet for a period of 4 weeks. It was prepared to be high fat diet in which the total energy (kcal35%) was supplied by suet [13], a high-sucrose diet in which 69% of carbohydrate-derived energy content was provided by sucrose [14], and the high-fat & high-sucrose diet, which was the combination of the high-fat and high-sucrose diets. All animals were sacrificed after an overnight fasting by cervical dislocations under ether anesthesia. Cardiac puncture was used to collect all blood samples in empty vacuum tubes and in tubes containing EDTA at the indicated sacrifice times. Blood samples were obtained after suitable centrifugation and samples were stored frozen at -80 °C until the day of analysis. The protocols of the animal experiments were approved by the internal ethical committee of the University.

2.2. Selection of candidate miRNAs

The criteria for further investigation of the most promising candidates were as follows: (1) higher fold change of differentially expressed miRNAs in obesity, hyperlipidemia, cardiovascular disease, diabetes and cancer, (2) higher expression levels and signal intensity of differential miRNAs in the mentioned diseases, and (3) well-known miRNAs that have been reported by literatures. We used these criteria to generate a list of 84 miRNAs: miR-27a-5p, miR-130a-3p, miR-106b-3p, miR-24-3p, miR-96-5p, miR-379-3p, miR-27b-3p, miR-758-3p, miR-217-3p, miR-208b-5p, miR-15b-3p, miR-378a-3p, miR-184, miR-103-3p, miR-23b-3p, miR-26a-5p, miR-217-5p, miR-29a-3p, miR-760-5p, miR-410-5p, miR-216a-3p, miR-500-3p, miR-369-5p, miR-33-5p, miR-107-3p, miR-532-3p, miR-142-5p, miR-122-5p, miR-29b-3p, miR-337-5p, miR-16-3p, miR-23b-5p, miR-500-5p, miR-216a-5p, miR-760-3p, miR-320-3p, miR-17-5p, miR-143-3p, miR-16-5p, miR-379-5p, miR-195-3p, miR-93-3p, miR-377-5p, miR-222-3p, miR-144-3p, miR-210-3p, miR-378a-5p, miR-93-5p, miR-30c-5p, miR-192-5p, miR-532-5p, miR-223-5p, miR-143-5p, miR-27b-5p, miR-30a-3p, miR-23a-5p, miR-200a-3p, miR-106b-5p, miR-30d-5p, miR-223-3p, miR-26b-5p, miR-133a-5p, miR-370-5p, miR-208b-3p, miR-30d-3p, miR-375-3p, miR-21-3p, miR-25-5p, miR-26b-3p, miR-23a-3p, miR-369-3p, miR-107-5p, miR-133b-5p, miR-210-5p, miR-21-5p, miR-25-3p, miR-15b-5p, miR-377-3p, miR-124-5p, miR-27a-3p, miR-195-5p, miR-26a-3p, miR-200a-5p and miR-375-5p.

2.3. miRNA expression profiling

RNAs isolated from plasma samples by using High Pure miRNA Isolation Kit (Roche Life Science, Mannheim, Germany). RNA samples converted to cDNA by using miScript II RT Kit (Qiagen, Hilden, Germany). cDNA samples are PreAmplified by using miScript Microfluidics PreAMP Kit (Qiagen, Hilden, Germany). qRT-PCR analysis performed by using miScript miRNA Assays (Qiagen, Hilden, Germany) with Dynamic Array 96.96 (Fluidigm, South San Francisco, CA, USA) on BioMark System (Fluidigm, South San Francisco, CA, USA).

2.4. Biochemical analyses

Serum total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol and blood glucose was measured by commercially available kits based on routine methods by the Abbott Architect C16000 auto-analyzer (Architect C16000 auto-analyzer; Abbott Laboratory, Abbott Park, IL, USA).

2.5. Statistical analysis

Statistical analysis of qRT-PCR data analyzed by using $2^{-\Delta\Delta Ct}$ method [15,16]. Basic student t test used for statistical analysis. Statistical analyses of biochemical data were done using SPSS v. 16.0 (SPSS Inc.,IL, USA). Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. All results are presented as mean \pm standart deviations (SD). Differences were considered significant at a probability level of $p < 0.05$.

3. Results

At the end of 4 weeks, there was no significant difference in the body weight of groups [Table 1]. In addition, regardless of the dietary treatment, none of the rats exhibited signs of toxicity, discomfort or behavioural anomalies, sickness and decreased activity or mortality during the study period.

Serum total cholesterol, triglycerides, HDL cholesterol and blood glucose levels of rats are shown in Table 1. The ANOVA test showed the serum glucose and triglycerides levels to be significantly higher ($p < 0.05$ for glucose, and $p < 0.01$ for triglycerides) in group 1 compared to control group. Similarly, serum total cholesterol levels to be significantly higher ($p < 0.01$) in group 2 compared to control group. We also observed a significant decrease in serum triglycerides levels in group 2 (high-fat & high-sucrose-fed rat) and group 3 (high-fat-fed rat) compared to group 1 ($p < 0.01$). On the other hands, serum total cholesterol and HDL cholesterol levels significantly increased ($p < 0.05$) in group 2 compared to group 1 (high-sucrose-fed rat).

To investigate the impact of high-fat diet, high-sucrose diet and high-fat & high-sucrose diet on plasma miRNA expression, miRNAs were extracted from plasma and detected to miRNA expression profiling (Eighty four miRNAs) by quantitative real-time PCR (qRT-PCR) with the Fluidigm integrated microfluidic circuit technolog. Among them, expression profiles of twenty miRNAs were found to be significantly different ($P < 0.05$) between groups. Of these twenty miRNAs, 1 were upregulated and 19 were downregulated with high-fat and high-sucrose feeding. The following miRNAs were down-regulated: miR-130a-3p, miR-320-3p, miR-17-5p, miR-16-5p, miR-144-3p, miR-93-5p, miR-192-5p, miR-532-5p, miR-106b-5p, miR-26b-5p, miR-208b-3p, miR-23a-3p, miR-25-3p, miR-15b-5p, miR-195-5p, miR-103-3p, miR-122-5p, miR-29b-3p and miR-30a-3p and the following miRNAs were upregulated: miR-375-5p (Table 2). Relative expression of these miRNAs in the plasma of high-fat, high-sucrose and high-fat & high-sucrose-fed rat is presented in Table 2. These results indicate that aberrant expression of miRNA profiles may be associated with the development of hyperlipidemia, insulin resistance and cancer in the high-fat & high-sucrose-fed rat.

4. Discussion

Numerous studies showed that a high-fat and/or high-sucrose diet induces insulin resistance in rodents [17–21]. The pathogenesis of insulin resistance caused by high-fat and/or high-sucrose diet is unclear. miRNAs, modulate gene expression, are significant regulators of beta cell function. miR-375 is

Table 1
Baseline characteristics and biochemical parameters of groups.

Parameters	Control	Group 1	Group 2	Group 3	<i>p</i>
Glucose (mg/dL)	222.86 \pm 26.0	382.57 \pm 153.3 ^b	308.71 \pm 50.1	259.29 \pm 20.1	0.002
Triglycerides (mg/dL)	60.71 \pm 5.8	182.71 \pm 65.6 ^c	103.43 \pm 31.3 ^d	93.57 \pm 9.6 ^d	$P < 0.001$
Cholesterol (mg/dL)	53.85 \pm 2.6	55.71 \pm 6.1	67.42 \pm 8.6 ^{a,e}	60.71 \pm 1.5	0.003
HDL (mg/dL)	32.67 \pm 2.1	29.62 \pm 4.9	37.45 \pm 4.4 ^e	33.72 \pm 1.2	0.032
First weight (g)	267.43 \pm 11.2	255.0 \pm 18.7	250.0 \pm 37.9	265.0 \pm 9.9	0.644
Last Weight (g)	339.14 \pm 16.9	342.7 \pm 61.7	355.8 \pm 34.5	343.86 \pm 11.7	0.906

^a Compared to control group $P < 0.01$.

^b Compared to control group $P < 0.05$.

^c Compared to control group $P < 0.001$.

^d Compared to sucrose group $P < 0.01$.

^e Compared to sucrose group $P < 0.05$; Group 1 (high-sucrose-fed rat); group 2 (high-fat & high-sucrose-fed rat) and group 3 (high-fat-fed rat).

Table 2
Plasma microRNAs expression profile of groups.

microRNAs	Up-Down Regulation (comparing to control group)					
	Group 1		Group 2		Group 3	
	Fold Regulation	p value	Fold Regulation	p value	Fold Regulation	p value
rno-miR-27a-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-130a-3p	1.0697	0.955246	-2.0269	0.046887	-2.0292	0.110149
rno-miR-106b-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-24-3p	-1.5502	0.571954	-1.8012	0.080903	-2.6312	0.081976
rno-miR-96-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-379-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-27b-3p	-1.3993	0.627018	-1.8155	0.091452	-2.8647	0.053682
rno-miR-758-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-217-3p	1.341	0.499492	-1.7449	0.174217	1.2092	0.517113
rno-miR-208b-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-15b-3p	-1.1371	0.464718	-1.864	0.096305	-1.8089	0.137787
rno-miR-378a-3p	1.2559	0.488626	-1.8293	0.068046	-2.2937	0.091703
rno-miR-184	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-103-3p	1.048	0.982409	-2.0132	0.096589	-1.8156	0.027968
rno-miR-23b-3p	-1.127	0.290709	-1.7497	0.090072	-3.1783	0.052978
rno-miR-26a-5p	-1.4087	0.801266	-1.5432	0.114874	-2.8812	0.062007
rno-miR-217-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-29a-3p	-1.3301	0.762735	-1.7441	0.098383	-2.7579	0.107653
rno-miR-760-5p	1.6434	0.201685	-1.5323	0.143954	1.0954	0.506938
rno-miR-410-5p	1.6434	0.201685	-1.5323	0.143954	1.0561	0.631547
rno-miR-216a-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-500-3p	1.9061	0.155185	-1.0766	0.575394	-1.0595	0.489269
rno-miR-369-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-33-5p	1.6434	0.201685	-1.2962	0.288244	-1.0678	0.76393
rno-miR-107-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-532-3p	1	0	1	0	1	0
rno-miR-142-5p	1.7369	0.262817	-1.1391	0.337123	-1.0964	0.565516
rno-miR-122-5p	-1.3351	0.469784	-1.4787	0.948927	-3.9469	0.019819
rno-miR-29b-3p	1.127	0.504795	-1.4388	0.198305	-2.1996	0.019611
rno-miR-337-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-16-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-23b-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-500-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-216a-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-760-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-320-3p	-1.2889	0.763113	-1.9647	0.023402	-2.2083	0.091909
rno-miR-17-5p	-1.4055	0.918266	-2.2084	0.028795	-2.5445	0.087
rno-miR-143-3p	-1.3124	0.575751	-1.4935	0.60915	-2.9394	0.074074
rno-miR-16-5p	-1.6752	0.898091	-2.1089	0.028465	-2.1047	0.148134
rno-miR-379-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-195-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-93-3p	1.7501	0.210351	-1.8668	0.114223	-1.0983	0.622263
rno-miR-377-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-222-3p	1.4559	0.816394	-1.1437	0.297778	-1.6826	0.134572
rno-miR-144-3p	1.4029	0.301274	-2.5324	0.020835	-2.5616	0.03013
rno-miR-210-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-378a-5p	1.4446	0.231556	-1.4127	0.348065	-1.2147	0.959731
rno-miR-93-5p	-1.4575	0.802125	-2.7646	0.016273	-2.5631	0.051444
rno-miR-30c-5p	-1.0807	0.601875	-1.4698	0.13813	-1.7248	0.267954
rno-miR-192-5p	-1.1423	0.815603	-1.805	0.025758	-1.905	0.075779
rno-miR-532-5p	-1.1534	0.610267	-2.1956	0.038373	-2.4699	0.144797
rno-miR-223-5p	1.911	0.115054	-1.2783	0.360983	1.2462	0.358974
rno-miR-143-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-27b-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-30a-3p	1.1059	0.877508	-1.6932	0.232584	-2.7326	0.013924
rno-miR-23a-5p	1.9083	0.115936	-1.5323	0.143954	-1.0678	0.76393
rno-miR-200a-3p	1.6018	0.400698	1.0142	0.825888	-1.1336	0.394047

(continued on next page)

Table 2 (continued)

Up-Down Regulation (comparing to control group)						
microRNAs	Group 1		Group 2		Group 3	
	Fold Regulation	p value	Fold Regulation	p value	Fold Regulation	p value
rno-miR-106b-5p	-1.1985	0.796421	-2.0318	0.043876	-2.4999	0.090531
rno-miR-30d-5p	-1.1596	0.975619	-1.6045	0.080375	-2.2635	0.062225
rno-miR-223-3p	-1.9508	0.795228	-1.5693	0.0927	-2.2659	0.190934
rno-miR-26b-5p	-1.6021	0.621515	-1.7444	0.044856	-2.8494	0.068011
rno-miR-133a-5p	1.4604	0.227394	-1.7243	0.052619	-1.2016	0.933276
rno-miR-370-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-208b-3p	1.4141	0.241246	-1.7808	0.034535	-1.1169	0.907308
rno-miR-30d-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-375-3p	1.1235	0.942837	-1.0864	0.616708	-1.5845	0.052018
rno-miR-21-3p	1.4108	0.355336	-1.6259	0.956045	-1.6276	0.201237
rno-miR-25-5p	1.8503	0.135504	-1.5323	0.143954	1.1647	0.48905
rno-miR-26b-3p	2.2163	0.053116	-1.3376	0.844037	1.0451	0.648159
rno-miR-23a-3p	-2.0226	0.494662	-1.8182	0.043459	-3.0453	0.041946
rno-miR-369-3p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-107-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-133b-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-210-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-21-5p	-1.5453	0.653863	-1.3824	0.158811	-2.0691	0.134963
rno-miR-25-3p	-1.8243	0.558067	-2.5087	0.005392	-2.8649	0.018447
rno-miR-15b-5p	-1.8615	0.610252	-2.6133	0.011735	-2.8578	0.055593
rno-miR-377-3p	1.6434	0.201685	-1.2388	0.508719	-1.0678	0.76393
rno-miR-124-5p	1.6434	0.201685	-1.5323	0.143954	-1.0678	0.76393
rno-miR-27a-3p	-1.3173	0.82141	-1.7317	0.099514	-2.8926	0.070883
rno-miR-195-5p	-1.633	0.979098	-2.026	0.036817	-2.1524	0.093739
rno-miR-26a-3p	2.2956	0.080374	-1.205	0.610107	-1.0678	0.76393
rno-miR-200a-5p	1.9043	0.117237	-1.5323	0.143954	1.0575	0.629388
rno-miR-375-5p	2.0914	0.049481	-1.0853	0.923048	1.1898	0.611777

Group 1, (high-sucrose-fed rat); group 2, (high-fat & high-sucrose-fed rat) and group 3, (high-fat-fed rat).

one of the most abundant miRNAs in the pancreatic islets of Langerhans [22]. Studies have shown that miR-375 has a role in beta cell function, including effects in development and differentiation, proliferation and regulation of insulin secretion [22]. Also, miR-375 is reported to be a potential circulating biomarker in type 1 [23–26] and type 2 diabetes [27–30]. We found that circulating miR-375-3p was increased in high-sucrose-fed rat. According to our hypothesis, the level of 375-3p increased due to high sucrose consumption. Our result is supported by a hypothesis is that obese diabetes model the ob/ob mouse compensate for an increased metabolic demand, due to insulin resistance, by increasing the beta cell mass and thereby elevated release of insulin. Additionally, concomitant with the increase in beta cell mass, miR-375 levels are also increased in ob/ob islets as compared to control islets [31]. The results of these latter studies also support our results [23–30]. However, to our knowledge, our study is the first one performed on the high-fat & high-sucrose-fed rat. Octoń E et al. shown that the expression level of hsa-miR-144-3p was lower in epicardial adipose tissue [porcine] in response to hyperglycemia [32]. The results of this study support our results. On the other hand, in the literature, there are conflicting findings about miR-144 levels and diabetes. An opposing view expresses that miR-144 expression is highly up-regulated in type 2 diabetes and it also seemed to exhibit a linear relationship with increasing glycaemic status [33]. The different ethnic group and size of the plasma sample may be one of the sources in the variance. In addition, the effect of different data normalization methods on the final results can't be ignored.

Dysregulation of miR-192 has been associated with a number of pathologic conditions including type 2 diabetes and liver diseases [34]. Roy S et al. have reported that miR-192-5p is involved in the regulation of liver cell death during acute liver injury and might represent a potent marker of hepatic injury [35]. In our study, miR-192-5p were downregulated in the high-fat & high-sucrose-fed rat. Also, triglyceride levels significantly increased in high-fat & high-sucrose-fed rat, but no significant difference was observed in HDL levels. Consistent with our data, Mysore R et al. have reported that the

quantity of miR-192-3p in the visceral adipose tissue correlated in morbidly obese subjects negatively with serum triglyceride content and positively with HDL-cholesterol [34]. The miR-29 family [miR-29a, miR-29b-3p, and miR-29c] has been previously implicated in multiple pathological changes in cardiovascular diseases. Furthermore, in accordance with our results, all members of the miR-29 family were downregulated in myocardial tissue adjacent to the infarct and during cardiac fibrosis after acute myocardial infarction in mice and humans [36]. In addition, accumulating evidence suggests that miR-29 downregulation inhibits dilation of aneurysms and is helpful in an early fibrotic response at the aortic wall by increasing target gene expression in murine models of experimental aneurysms and human aneurysm tissues [37].

Serum hsa-miR-532-5p level was significantly decreased during relapse in relapsing-remitting multiple sclerosis. This miRNA was also decreased in patients with a gadolinium enhancement on brain magnetic resonance imaging. In vitro secretion of this miRNA by peripheral blood mononuclear cells was also significantly impaired in relapsing-remitting multiple sclerosis [38]. Rats that displayed vulnerability to subsequent chronic stress exhibited reductions in circulating miR-532-5p levels [39]. The expression of miR-532-5p significantly decreased in the serum of ischemic stroke patients. Serum miR-532-5p may serve as potential diagnostic biomarkers for ischemic stroke [40]. In our study, this miRNA was found to be down-regulated in accordance with the literature.

Westernized diets are to a great extent high in fat and/or sugar, making these varieties of food highly obesogenic. As stated in the literature, combined high-carbohydrate/high-fat diet caused an increase in body weight, energy intake, and abdominal fat deposition. This occurred due to impaired glucose tolerance, dyslipidaemia, and hyperleptinaemia and hyperinsulinaemia in the rats or humans. These were accompanied by damage to the heart, liver, and pancreas. Also, chronic stress has been shown to have a strong link with these pathological conditions [41]. Epidemiological studies have provided evidence suggesting an important role for diet in the development of cancer. Specifically, lipid and sucrose nutrients of the diet have been identified as important regulators of tumor development and progression [42,43]. In this case, we believe that our findings on microRNAs discussed above will provide a significant contribution to diagnosis and treatment of feeding a high-fat & high-sucrose diet-related diseases and our finding will contribute to further understanding of the etiopathogenesis of feeding a high-fat & high-sucrose diet-related diseases.

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Conflicts of interest

None declared.

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