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Research Article
Influence of L-carnitine on the Expression Level of Adipose Tissue miRNAs Related to Weight Changes in Obese Rats

[1]Maryam Nazari, [6]Alihossein Saberi, [1]Majid Karandish, [2]Niloofar Neisi, [3]Mohammad Taha Jalali and [4,5]Manoochehr Makvandi

[1]Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2]Research Center for Infectious Diseases of Digestive System and Department of Virology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3]Hyperlipidemia Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[4]Health Research Institute, Infectious and Tropical Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[5]Department of Virology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[6]Department of Medical Genetics, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Golestan Highway, Ahvaz, Iran

Abstract

Background and Objective: Molecular mechanisms of most anti-obesity drugs are remained to be clear. MicroRNAs that are noncoding RNA molecules supposed to regulate biological processes concomitant to obesity and have attracted a lot of attention to themselves. The *miR-27a* and *miR-143* expression levels in obese and non-obese rats during weight changes and L-carnitine (LC) effects on them was investigated in this study. **Materials and Methods:** In the present study 12 male Wistar rats were randomly divided into normal fat diet and high fat diet groups to develop obesity. After 8 weeks rats were weighted and half of diet induced obese rats were randomly selected to receive 200 mg LC kg^{-1} b.wt. for 4 weeks. At the end epididymal fat was isolated to investigate expression level of microRNAs by real-time PCR. **Results:** After 12 weeks, high fat diet in comparison with normal fat diet mediated significant decrease and increase in expression levels of *miR-27a* and *miR-143*, respectively. These changes were modified in groups, which had received LC in a 4 weeks period. Furthermore, rats in this group gained less weight. **Conclusion:** Findings of this study suggest that the changes of microRNAs expression probably play a role in pathogenesis of obesity, might be modulated by means of dietary agents and supplements and modify weight gain trend.

Key words: High fat diet, normal fat diet, L-carnitine, *miR-27a*, *miR-143*, obesity, weight gain, adipogenesis

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Corresponding Author: Alihossein Saberi, Department of Medical Genetics, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Golestan Highway, Ahvaz, Iran Tel: +989161133488 Fax: +98613332036

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity has emerged as an epidemic globally and has detrimental effects on the quality of life, increases rates of mortality and morbidity via increasing the risk of different complications like cardiovascular diseases, some types of cancers and metabolic syndrome. According to the Center for Disease Control (CDC) and prevention the costs for medical payments related to overweight and obesity in the USA were about $75 billion in 2003 and it reached an annual rate of $147 billion[1,2] in 2008. As a result, increasing demand for safe and effective anti-obesity drugs arises from this phenomenon, which is recognized as a disease by the American Medical Association in 2013.

Unfortunately different strategies, such as diet therapy, physical activity, surgery and medication had limited success. In addition, many anti-obesity drugs are withdrawn from the market due to their serious adverse effects[3]. Hence, pursue an appropriate action to control the obesity is a priority. In this regard, the growing interest in study of epigenetic regulatory mechanisms during the development of obesity has specified some investigations to microRNAs (miRNAs).

The miRNAs are 21-23 nucleotide fragments of noncoding RNAs that by coupling with the 3'-untranslated region (UTR) of their target mRNAs regulate gene expression. The miRNAs play important roles in highly regulated processes, such as proliferation, differentiation, apoptosis, growth, development and some metabolic procedures. It seems that several miRNAs are out of regulation in obese animals and humans, however little is known about the exact role of these tiny molecules in metabolism particularly in adipose tissue[4,5].

Researches on miRNAs have been demonstrated their roles in different complications like cancers, neurological, autoimmune, metabolic and cardiovascular diseases[6]. Understanding miRNA genetic targets, which regulated adipogenesis via their pro or anti-adipogenic roles may potentially detect new pathways in metabolic diseases, such as obesity and influences future approaches to its treatment[7].

Several miRNAs exist in adipose tissue but just a few are differentially expressed in obesity or show regional difference in their expression[8]. Numerous experiments have verified an altered miRNA expression profile in this situation. In contrast, limited studies have assessed the effect of different kinds of diet like cholesterol, glycemic load or other dietary agents on the miRNA alterations[9,10].

Few recent studies have reported that expression of miRNAs in adipose tissue is affected by changing diet composition, which underlie pathogenesis of chronic diseases like obesity[11,12]. So, this idea comes to mind that dietary agents used for weight loss like fat burners, might modify these changes and explain probable mechanisms for their anti-obesity effects[13]. For instance, increasing evidence has confirmed lipolysis boosting role for L-carnitine and hence, its supplementation is widely used for losing weight although the exact molecular mechanism of action of L-carnitine has not been clarified[14-16].

Several studies have shown that *miR-27* family (a and b) as a potential anti-adipogenic factor are down regulated and *miR-143* as a pro-adipogenic agent are up regulated during adipocyte differentiation[17-19]. In fact, *miR-27a* could accelerate adipolysis by releasing more glycerol and free fatty acids from adipose tissue[20] and *miR-143* could promote adipogenesis by accumulating more triglycerides in the adipocytes[21].

So, it seems rational to find *miR-27a* inducers or *miR-143* inhibitors for deceleration of adipocyte differentiation. Therefore, this study aimed to determine the changes in *miR-143* and *mi-R27a* expression in adipose depots of epididymal tissue of rats fed diets containing high levels of dietary fat before and after the use of dietary L-carnitine.

MATERIALS AND METHODS

Animal design: A total of 12 male albino rats of Wistar strain from Laboratory Animal Unit of Jundishapur University of Medical Sciences (Ahvaz, Iran) were acclimatized for 1 week period. They aged about 8 weeks and weighing 150-200 g were housed in groups of two per cages and maintained on a 12 h light: Dark cycle at 22°C and the relative humidity at 50±5%. Then they were randomly allocated into 2 different diet group: Normal Fat Diet (NFD) all over the study (semi-purified normal fat diet) (n = 4) as control group and High Fat Diet (HFD) all over the study (semi-purified high fat diet to induced obesity) (n = 8).

The animals fed a semi-purified form of American Institute of Nutrition (AIN)-93M *ad libitum*. Proportional composition of the diets have been presented[22] in Table 1. Mentioned diets were prepared freshly twice a week and were stored at 4°C. After 8 weeks HFD groups was randomly subdivided into 2 categories (n = 4 in each) and maintained on their own diet to receive the main intervention by oral gavage as follows for more 4 weeks:

- Control group, which received HFD+1 mL water
- L-carnitine group, which received HFD+200 mg kg^{-1} b.wt., L-carnitine in 1 mL water

The L-carnitine was levocarnitin (So.Se PHARM, Italy), which contains 1 g L-carnitine per 10 mL solution. Throughout the experiment body weight gain were recorded weekly to confirm development of obesity in the HFD-fed rats. At 12th week animals were fasted for 10 h before sacrifice and then White Adipose Tissue (WAT) from epididymal fat were isolated and were immediately submerged in RNA stabilization reagent (Qiagen, Hilden, Germany) to stabilize and protect cellular RNA *in situ* and kept at -80°C until analyzed.

WAT miRNA extraction: To isolate miRNA from WAT NFD or HFD-fed rats, approximately 100 mg of tissue was homogenized using a digital homogenizer (WiseTis HG-15D, Germany) in 1-2 mL of Qiazol (Qiagen). Subsequently, miRNA extraction was performed using the RNeasy Mini Kit (Qiagen) according to the company's instructions. The concentration of isolated microRNA was measured using the NanoDrop Spectrophotometer 2000c (USA) and its integrity confirmed by 2% agarose gel electrophoresis. The RNA samples were directly frozen and stored at -80°C.

For miRNA quantification, RNA samples were first reverse-transcribed and then amplified by power SYBR green PCR master mix (Applied Biosystems, Foster city, CA) and specific primers. Reverse transcription and quantitative PCR were done using miScript reverse transcription kit (Qiagen), miScript SYBR green PCR kit (Qiagen) and miScript primer assay (Qiagen) according to the manufacturer's instructions. All primers were synthesized by Qiagen (Hs_RNU6-2_11 cat# MS00033740, RN_*miR-143*_1 cat# MS00000420 and RN_*miR-27a*_1 cat# MS00000147) and their sequences were not revealed by the manufacturers.

Table 1: Modified* American institute of nutrition diet composition

Ingredients in 1 kg diet	Normal fat diet (g)	High fat diet (g)
Corn starch	465.6	237
Casein (>85% protein)	140	140
Dextrinized corn starch	155	78
Sucrose	100	50
Soybean oil	40	185
Margarine	0	185
Fiber	50	50
Mineral mix (AIN-93M-MX)	35	35
Vitamin mix (AIN-93-VX)	10	10
L-cysteine	1.8	3
Choline bitartrate	2.5	2.5
Tert-butylhydroquinone	0.008	0.01
Total energy (kcal)	3600	5350

*AIN-93M purified diets for laboratory Rodents[22] was modified, fiber was replaced by wheat bran in formulation

The *miR-143* and *miR-27a* levels were normalized to RNA U6. Real-time RT-PCR was performed on step one real time PCR (Applied Biosystems). The qRT-PCR thermal cycling included of 15 min incubation at 95°C followed by 40 cycles of a 3-stage temperature profile of 94°C for 15 sec, 55°C for 30 sec and final 70°C for 30 sec. All samples were run in triplicates and the fold changes in the miRNA level was calculated by comparative cycle threshold (Ct) method $2^{-\Delta\Delta Ct}$, where, ΔCt = Ct miRNA-Ct U6 and ΔΔCt = ΔCt treated samples -ΔCt untreated controls.

Ethics statement: All procedures performed in this study involving animals were approved by ethical committee of experimental animal care at Jundishapur University of Medical Sciences (NRC9204).

Statistical analysis: Results are expressed as Means±Standard Error of the Mean (SEM). Student t-test and one-way analysis of variance (ANOVA) followed by LSD test were used to compare mean differences between groups (Statistical Package for Social Sciences version 17.0, SPSS Inc., Chicago, 2008) and a $p<0.05$ was considered as statistically significant.

RESULTS

Body weight changes: At the baseline animal's body weights were not different between NFD and HFD groups ($p<0.05$). At the end of 8 weeks, HFD-fed rats appeared obese phenotype and significantly gained more weight in comparison with controls (Table 2).

Four weeks treatment with L-carnitine, significantly reduced weight gain during the treatment period (Table 3).

Table 2: Effect of NFD and HFD on rat's body weight during 8th weeks

Groups	n	Baseline weight (g)	p-value	Eight-week weight (g)	p-value
NFD	4	170.66±8.91	0.67[a]	246.38±6.48	0.009[b]
HFD	8	174.00±4.02		297.43±5.36	

Values are expressed as Means±Standard Error, [a]Insignificant weight difference between NFD and HFD at baseline, [b]Significant weight difference between NFD and HFD at 8th week, NFD: Normal fat diet and HFD: High fat diet

Table 3: Effect of L-carnitine on body weight and weight gain rate during 4 weeks in obese rats

Groups	n	Eight-week weight (g)	Twelve-week weight (g)	Weight gain (g)	p-value
NFD	4	246.38±6.480	264.62±8.030	18.0 (7.3%)	
HFD	4	300.50±13.16	336.43±15.10	35.9 (11.9%)	
LC	4	294.38±7.600	316.68±11.80	22.5 (7.6%)	0.044[a]

Values are expressed as Means±Standard Error, [a]Significant difference between L-carnitine and control HFD in weight gain during 4 weeks ($p = 0.044$), LC: L-carnitine, NFD: Normal fat diet and HFD: High fat diet

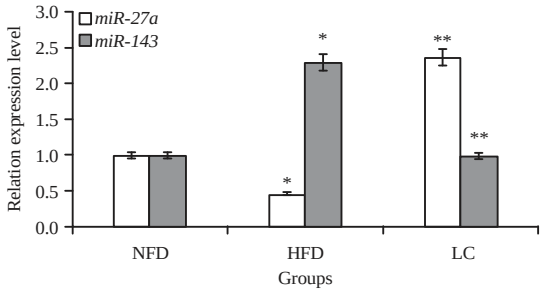

Fig. 1: Relative expression levels of *miR-143* and *miR-27a* in the adipose tissue of rats fed a normal fat diet or high fat diet in comparison with L-carnitine group. The miRNA levels were normalized to U6 small nuclear RNA. The Means±SEM of 4 rats is shown. Values with significant changes (*p>0.05) relative to NFD group. Values with significant changes (**p>0.05) relative to HFD group, NFD: Normal fat diet, HFD: High fat diet and LC: L-carnitine

***miR-143* and *miR-27a* expression levels:** According to the weight gain observed in HFD-fed rats, the expression of the *miR-27a* and *miR-143* was changed in the adipose tissue of obese rats compared to the rats fed NFD. When compared to the NFD-fed rats, HFD after 8 weeks influenced *miR-27a* expression by reducing it to 0.46 and *miR-143* expression by rising it up to 2.3 (Fig. 1).

Four weeks of L-carnitine administration counteracted the overexpression of *miR-143* and down regulation of *miR-27a* in the adipose tissue, which was induced by the HFD in rats. Surprisingly, compared to the obese rats fed only the HFD, *miR-27a* expression was increased by almost 5 folds and *miR-143* expression was reduced to half after treatment with daily 200 mg of L-carnitine kg^{-1} of b.wt.

DISCUSSION

Animal models of obesity are used extensively for the investigations of therapeutic strategies to establish effective remedies for obesity and its related complications. However, understanding of the mechanisms is vital for anti-obesity drug development, too. To the best of our knowledge this is the first study on relationship between L-carnitine supplementation and miRNA level changes in adipose tissue of diet induced obese rats, which could conclusively approve our hypothesis.

In the present study, a synthetic NFD was used to adjust confounding effects of control diet, which is chow diet in most of the studies although its basic composition is totally different from experimental HFD and this is a noteworthy point[23]. So, AIN-93M based two kinds of diets were formulated, which in fat content differed.

The main finding of this study was that L-carnitine supplementation in obese rats caused a significant alteration in the *miR-27a* and *miR-143* expression in adipose tissue suggesting at least probable pathways, which L-carnitine through them mediates its biological effects.

The relationship between abnormal miRNA expression and anomalies in adipogenesis and obesity may explain a reason for targeting these molecules in obesity treatment. Statistical analysis revealed a positive but insignificant correlation between weight changes and *miR-143* levels. Furthermore, *miR-27a* levels in adipose tissue showed an insignificant negative association with weight gain.

A possible explanation for non-significant association between miRNAs expression levels and weight changes is that a gene is usually subject to be regulated by a group of miRNAs. Additional reason might be that the L-carnitine mediated changes in the level of certain miRNAs was not strong enough to induce a significant changes in as short duration as 4 weeks in the expression of the targeted miRNAs. Furthermore, post-transcriptional variations by L-carnitine might also be responsible for undetectable miRNA-mRNA interactions *in vivo*[10].

Esau *et al.*[24] examined miRNA expression patterns in pre-adipocyte and revealed for the first time that *miR-143* normally stimulates adipocyte differentiation. However, it has already shown anti-differentiation properties[8]. Takanabe *et al.*[18] reported a 3.3-fold amplified expression of *miR-143* in adipose tissue of obese mice, which was correlated with highly expressions of adipocyte differentiation markers like *PPARγ* and *aP2*. Moreover, adipocytes differentiation is controlled by key transcriptional genes like C/EBP (α, β, δ), fatty acid synthase and fatty acid binding proteins genes[25], which is suggested to be modulated by adipose tissue miRNAs like *miR-143* that acts mainly via *ERK5* (Extracellular signal-regulated kinase 5)[8,24,26] and *MAPK7*. Furthermore, it has been shown that use of conjugated linoleic acid as a fat lowering agent was accompanied by decreased *miR-143* levels that is similar to results of this study[27].

In contrast, the expression level of *miR-27a* in mature adipocytes from obese mice was less than its level in lean mice and it confirmed down regulation of *miR-27* in adipocyte hypertrophy[19]. Consistently, diet with inducing non-alcoholic fatty liver disease (NAFLD) properties (high fat or high carbohydrate content) could down regulate the expression of *miR-27*, *-122* and *-451*, while up-regulate *miR-429*, *-200a* and *-200b* expression in rat liver[28], which resembles results of the present study.

It would be interesting to investigate the protein and mRNA levels of key molecules, considering expression level of some genes like *CEBPs*, *PPARγ*, *SREBP*, *FAS* or genes related to adipogenesis or lipolysis in a longer intervention period. Since, every single miRNA can control hundreds of target mRNAs, i.e., one single gene could be regulated by many miRNAs, it is preferred to investigate miRNA profile alteration, by which researchers in the present study have not been able to perform.

CONCLUSION

In conclusion, these results confirmed that adipocyte *miR-143* and *miR-27a* show different levels in diet induced obese rats, which could be modulated by L-carnitine while, weight gain decelerated. As dietary agents or supplements have been revealed to modify miRNAs expression, miRNA profiling could be a beneficial tool in mechanistically studies. Complementary and focused studies about the expression of other related genes are needed not only to confirm the potential of miRNAs as novel prognostic metabolic biomarkers but also to target more precise and novel goals for treatment in obesity and other metabolic disorders.

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REFERENCES

1. Padula, W.V., R.R. Allen and K.V. Nair, 2014. Determining the cost of obesity and its common comorbidities from a commercial claims database. Clin. Obes., 4: 53-58.
2. Alexander, R., H. Lodish and L. Sun, 2011. MicroRNAs in adipogenesis and as therapeutic targets for obesity. Expert Opin. Ther. Targets, 15: 623-636.
3. Kumar, P. and U. Bhandari, 2015. Letter to the editor: Obesity pharmacotherapy: Current status. EXCLI J., 14: 290-293.
4. Ortega, F.J., J.M. Moreno-Navarrete, G. Pardo, M. Sabater and M. Hummel *et al*., 2010. MiRNA expression profile of human subcutaneous adipose and during adipocyte differentiation. PloS ONE, Vol. 5. 10.1371/journal.pone.0009022
5. Xie, H., L. Sun and H.F. Lodish, 2009. Targeting microRNAs in obesity. Expert Opin. Ther. Targets, 13: 1227-1238.
6. Lundstrom, K., 2012. MicroRNA and diet in disease prevention and treatment. J. Med. Res. Sci., 2: 11-18.
7. Williams, M.D. and G.M. Mitchell, 2012. MicroRNAs in insulin resistance and obesity. Exp. Diabetes Res., Vol. 2012. 10.1155/2012/484696
8. Arner, P. and A. Kulyte, 2015. MicroRNA regulatory networks in human adipose tissue and obesity. Nat. Rev. Endocrinol., 11: 276-288.
9. McCann, S.E., S. Liu, D. Wang, J. Shen and Q. Hu *et al*., 2013. Reduction of dietary glycaemic load modifies the expression of microRNA potentially associated with energy balance and cancer pathways in pre-menopausal women. Br. J. Nutr., 109: 585-592.
10. Keller, J., R. Ringseis and K. Eder, 2014. Supplemental carnitine affects the microRNA expression profile in skeletal muscle of obese Zucker rats. BMC Genomics, Vol. 15. 10.1186/1471-2164-15-512
11. Chartoumpekis, D.V., A. Zaravinos, P.G. Ziros, R.P. Iskrenova, A.I. Psyrogiannis, V.E. Kyriazopoulou and I.G. Habeos, 2012. Differential expression of microRNAs in adipose tissue after long-term high-fat diet-induced obesity in mice. PloS ONE, Vol. 7. 10.1371/journal.pone.0034872
12. Romao, J.M., W. Jin, M. He and T. McAllister, 2012. Altered microRNA expression in bovine subcutaneous and visceral adipose tissues from cattle under different diet. PLoS ONE, Vol. 7. 10.1371/journal.pone.0040605
13. Jeukendrup, A.E. and R. Randell, 2011. Fat burners: Nutrition supplements that increase fat metabolism. Obes. Rev., 12: 841-851.
14. Osorio, J.H., 2011. Supplementation with carnitine for weight loss: A biochemical approach. Colombia Medica, 42: 529-535.
15. Wu, T., A. Guo, Q. Shu, Y. Qi and Y. Kong *et al*., 2015. L-Carnitine intake prevents irregular feeding-induced obesity and lipid metabolism disorder. Gene, 554: 148-154.
16. Mingorance, C., M.G. del Pozo, M.D. Herrera and M.A. de Sotomayor, 2009. Oral supplementation of propionyl-l-carnitine reduces body weight and hyperinsulinaemia in obese Zucker rats. Br. J. Nutr., 102: 1145-1153.
17. Sacco, J. and K. Adeli, 2012. MicroRNAs: Emerging roles in lipid and lipoprotein metabolism. Curr. Opin. Lipidol., 23: 220-225.
18. Takanabe, R., K. Ono, Y. Abe, T. Takaya and T. Horie *et al*., 2008. Up-regulated expression of microRNA-143 in association with obesity in adipose tissue of mice fed high-fat diet. Biochem. Biophys. Res. Commun., 376: 728-732.
19. Kim, S.Y., AY. Kim, H.W. Lee, Y.H. Son and G.Y. Lee *et al*., 2010. miR-27a is a negative regulator of adipocyte differentiation via suppressing PPARγ expression. Biochem. Biophys. Res. Commun., 392: 323-328.
20. Yang, Z., T. Cappello and L. Wang, 2015. Emerging role of microRNAs in lipid metabolism. Acta Pharm. Sin. B, 5: 145-150.
21. Wang, T., M. Li, J. Guan, P. Li and H. Wang *et al*., 2011. MicroRNAs miR-27a and miR-143 regulate porcine adipocyte lipid metabolism. Int. J. Mol. Sci., 12: 7950-7959.

22. Reeves, P.G., F.H. Nielsen and G.C. Fahey Jr., 1993. AIN-93 purified diets for laboratory rodents: Final report of the American institute of nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.
23. Warden, C.H. and J.S. Fisler, 2008. Comparisons of diets used in animal models of high-fat feeding. Cell Metab., 7: 277-277.
24. Esau, C., X. Kang, E. Peralta, E. Hanson and E.G. Marcusson *et al*., 2004. MicroRNA-143 regulates adipocyte differentiation. J. Biol. Chem., 279: 52361-52365.
25. Lin, Q., Z. Gao, R.M. Alarcon, J. Ye and Z. Yun, 2009. A role of miR-27 in the regulation of adipogenesis. FEBS J., 276: 2348-2358.
26. McGregor, R.A. and M.S. Choi, 2011. microRNAs in the regulation of adipogenesis and obesity. Curr. Mol. Med., 11: 304-316.
27. Parra, P., F. Serra and A. Palou, 2010. Expression of adipose MicroRNAs is sensitive to dietary conjugated linoleic acid treatment in mice. PLoS ONE, Vol. 5. 10.1371/journal.pone. 0013005
28. Garcia-Segura, L., M. Perez-Andrade and J. Miranda-Rios, 2013. The emerging role of MicroRNAs in the regulation of gene expression by nutrients. J. Nutrigenet. Nutrigenomics, 6: 16-31.