Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Effect of fatty acids on mitochondrial biogenesis during mouse embryonic stem cell differentiation

A.H. Taha

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Article information

Article history:
Received 23 March 2025
Accepted 20 July 2025
Published 01 November 2025

Keywords:
Fatty acids
Mitochondrial biogenesis
Mouse embryonic stem cells
ImageJ

Correspondence:
A.H. Taha
amer.taha@uomosul.edu.iq

Abstract

Fatty acids (FAs) and their metabolites may affect and improve mitochondrial biogenesis in mouse embryonic stem cells (mESCs). Mitochondria are dynamic organelles that are responsible for adenosine triphosphate (ATP) production and are implicated in cellular functions such as proliferation, differentiation, cell cycle, reprogramming, aging, and apoptosis, with normal morphology required for cellular functions. Thus, mitochondrial morphology may change to meet the energy requirement. This study examines the impact of FAs, such as eicosapentaenoic acid (EPA) and linoleic acid (LA), on mitochondrial biogenesis through a quantitative analysis of multiple parameters. Three-day-old mESCs under differentiation conditions were treated with 50 µM FAs for 48 hours, and the results were compared with untreated and vehicle (dimethyl sulfoxide, DMSO). Mitochondrial configurations in the two-dimensional (2D) projection were determined and quantified using ImageJ. Treatment with 50 µM of FAs significantly increased the quantity of mitochondria, including mitochondrial count, area, perimeter, form factor, the number of branches, the number of branch junctions, and the length of branches, when compared to the control and vehicle groups, hence signifying improved mitochondrial interconnectivity, suggesting the enhancement of mitochondrial biogenesis. These findings explain the role of FAs in promoting mitochondrial biogenesis and suggest potential therapeutic applications for controlling metabolic diseases associated with impaired mitochondrial function.

DOI: 10.33899/ijvs.2025.158114.4176,

Introduction

Dietary fat is a contributing factor to chronic diseases such as diabetes, obesity, and arteriosclerosis (1). Research indicates that FAs, such as omega-3 and omega-6, are advantageous for preventing and treating conditions such as Alzheimer's, cancer, and cardiovascular disease (2,3) by activating specific protein-targeting biogenesis pathways in the cytosol that mitigate numerous human diseases (4). These FAs are allocated to cells and incorporated into cellular membranes, impacting metabolism and viability (5). They participate in multiple mitochondrial functions, encompassing calcium homeostasis, gene expression, respiratory function, reactive oxygen species (ROS) generation, and apoptosis (3). The protective benefits of FAs depend on their structural characteristics, cellular uptake, metabolic processes, competition with intracellular reserves, and the intrinsic properties of their metabolites (6,7). In addition, FAs increased ROS and nitric oxide production and elevated intracellular calcium levels. They promoted endothelial NO synthase (eNOS) in mESCs (8). Mitochondria are cell powerhouses responsible for ATP synthesis and regulation of cellular metabolism, and they are engaged in cellular functions such as proliferation, differentiation, the cell cycle, reprogramming, aging, and apoptosis (9). They undergo constant fission and fusion events, which regulate mitochondrial size, number, and function (10,11). Mitochondrial biogenesis indicates the maturation and division of pre-existing mitochondria to generate new ones. It is influenced by cellular stimuli and

increased energy needs, implicating diverse biological processes, such as the synthesis of both inner and outer mitochondrial membranes, mitochondrial proteins, oxidative phosphorylation, mtDNA replication, and mitochondrial fusion and fission (12-15). Mitochondrial dynamics, which encompass mitochondrial fission, fusion, biogenesis, and autophagy (mitophagy), are a dynamic, actively controlled trait that affects mitochondrial morphology (16). Alterations in MD have been linked to the control of calcium homeostasis, oxidative metabolism, and necrotic or apoptotic cell death (17). Differentiated cells and mESCs have variable mitochondrial dynamics. Mitochondria in stem cells are often described as spherical, punctate, fragmented, perinuclear, and having rarer cristae (18-20). It is commonly acknowledged that stem cells have immature mitochondria with low quantities of ATP, ROS, and OXPHOS (21). This mitochondrial condition is consistent with the general role of stem cells, which is to maintain the nuclear, epigenomic, and mitochondrial genomes of differentiated cells. Thus, mESCs are protected from ROS-induced genotoxicity by their immature mitochondria, which may have more severe and pervasive effects on mESCs than differentiated cells. Changes in mitochondrial morphology, notably the appearance of larger, elongated, and tubular forms, coincide with an increase in mitochondrial content during cell differentiation into final cell types. Differentiated cells have tightly packed mitochondria, some of which are widely dispersed throughout the cytoplasm and have many branches. ROS, OXPHOS, and mitochondrial ATP levels in differentiated cells also increase with maturity. It has been shown that when many mESC populations differentiate, their cellular metabolism shifts from glycolysis to oxidative metabolism (9,20,22-24).

In light of the established roles of FAs in membrane remodeling and the signaling pathways that influence mitochondrial function, the present study investigates the regulation of mitochondrial biogenesis in mESCs exposed to two different FAs: EPA, an omega-3, and LA, an omega-6, both of which are unsaturated FAs.

Materials and methods

Ethical approval

The College of Veterinary Medicine at the University of Mosul provided ethical approval for the research project UM.VET.2024.107. on August 18, 2024.

Cell culture

In summary, Iscove's basal media was used to grow mESCs on feeder layers derived from mouse embryonic fibroblasts. In humidified conditions with 5% CO_2 at a temperature of 37°C, the growth medium was supported with 15% heat-inactivated foetal calf serum, 1mM Pyruvic acid sodium salt, 2 glutamic acid γ-amide, 1% nonessential amino acids, 100 µM β-mercaptoethanol, 0.4% penicillin/ streptomycin, and 1,000 U/ml Leukemia inhibitory factor (LIF). To create three-day-old, three-dimensional (3D) spherical structures of embryoid body tissue, the attached cells were separated using 0.05% trypsin-EDTA. Following dissociation, 250 ml siliconized spinner flasks containing 125 ml of Iscove's medium supplemented as previously mentioned, but with LIF excluded, were used to seed single cells at a concentration of 3×10^6 cells/ml. Finally, 250 ml was achieved by adding 125 ml of media after 48 hours. An Integra Biosciences stirrer device was used to agitate the medium in the spinner flask at 20 rpm. The rotation direction was reversed every 1440° to improve uniform aggregation. Every day, 125 millilitres of cell culture media were swapped out (8,25).

Single-cell preparation and FAs exposure

On the third day of cell culture, embryoid bodies were formed and subsequently enzymatically digested for 30 minutes at 37°C in 1X phosphate-buffered saline (PBS) containing 2 mg/ml collagenase B to produce single-cell preparations. Dissociated single cells were grown in Iscove's media and supplemented with additional nutrients after being seeded onto gelatin-coated coverslips on 24-well cell culture plates. Cells were treated with physiological concentrations of EPA or LA (50 µM) for 48 hours. EPA and LA were dissolved in DMSO (final DMSO concentration ≤ 0.1% in the culture). This concentration of EPA and LA has previously been shown to have a physiological impact on mESCs' differentiation (8). Alternatively, they were exposed to DMSO at suitable concentrations as a vehicle control. The cell culture medium enriched with EPA and LA was refreshed every 48 hours (Figure 1) (8,26).

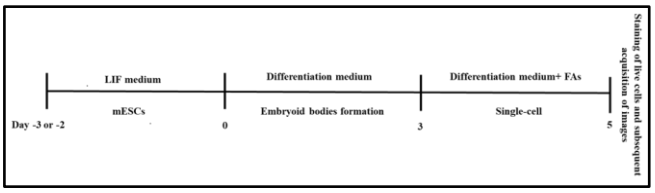

Figure 1: A schematic overview of the experimental setup.

Live cell staining and image acquisition by confocal microscopy

MitoTracker Green FM (MTG) (Ca. no.: M7514; Thermo Fisher Scientific) was used to stain the mitochondria in live cells. It was done according to the manufacturer's protocol. After 48 hours, individual cells were adhered and subsequently incubated under standard conditions in serum-free Iscove's basal medium with 50 nM MTG for 30 minutes. Thereafter, the coverslips were transferred to an incubation slide chamber containing freshly prepared Iscove's basal medium free of serum before placement on the stage of the confocal laser scanning microscope (Wetzlar, Germany) SP8 FALCON, which is outfitted with an HCX PL APO 63x/1.2

water immersion objective. The images were captured at 512 x 512 pixels, with a magnification of 63x and a digital zoom of 4X. Mitochondria were quantified as previously described, and fluorescence recordings were obtained using a confocal laser-scanning microscope mounted on an inverted microscope (8,27-29).

Utilising the image analysis software ImageJ (ImageJ 1.54 f USA), the mitochondrial configurations within the 2D projection were quantified by the mitochondrial image analysis plugin. This procedure assessed mitochondrial elements, which were clearly delineated. The 2D analysis commenced with processing and thresholding, followed by employing the resultant binary images as input for the analyze particles command. This command facilitates the quantification of various parameters, including the number of mitochondria, area, perimeter, form factor, aspect ratio, branch length, branches per mitochondrion, and branch junctions per mitochondrion. Considered as the "length-to-width ratio," the aspect ratio is computed as the quotient of the major and minor axes.

Furthermore, reflecting the complexity and branching properties of mitochondria, the form factor is represented as the square of the circumference divided by 4π multiplied by the surface area (21). The skeletonize 2D function was used on the threshold image to generate a structure map, which was subsequently analyzed for quantity within the skeletonized network (30-32). Our method for analyzing mitochondrial morphologies enables large-scale measurement of cells within a single experiment by combining automated imaging, computational high-content analysis, and machine-learning-derived classifiers.

Statistical analysis

The PRISM statistics program (GraphPad version 8.0) was used for statistical analysis. The statistics are presented as mean ± SD, representing the average of at least 3 separate cell cultures. One-way ANOVA was utilized for statistical evaluation when applicable. The significance level of *$P \leq 0.05$* was established.

Results

The study investigates confocal imaging of mitochondria in live mESCs under either standard or treatment conditions. Thus, 8 -10 images were taken, particularly with 50 µM of LA or EPA of FAs over a duration of 48 h during the differentiation of mESCs. This study meticulously uses fluorescent dyes to tag mitochondria. It optimizes photographic capture to achieve high resolution and superior image quality. Following a comprehensive image thresholding procedure, the next phase involves quantifying the morphological characteristics of the identified mitochondrial structures (Figure 2a). A 2D analysis was performed, and mitochondrial counts were assessed. The results indicate that the mitochondrial count exhibited a relative increase across all experimental groups (control, vehicle, 50 µM LA, 50 µM EPA), demonstrating significant variation as illustrated in Figure 2b.

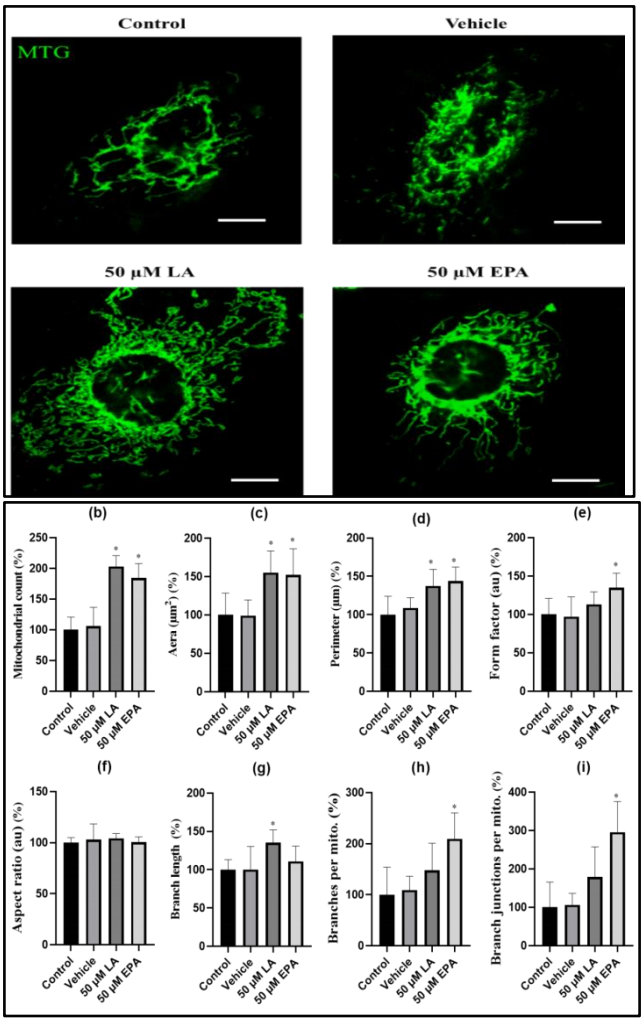

Figure 2: The effect of LA and EPA on mitochondrial morphology in mESCs. Embryoid bodies were enzymatically dissociated, followed by the cultivation of single cells on gelatin-coated coverslips under 50 µM LA or EPA conditions, and were either left untreated or exposed to a vehicle (DMSO). Modifications in mitochondrial morphology were monitored using MTG dye. a) A representative image of MTG labeled mitochondrial. The scale bars represent 10 µm. The data were analyzed for parameters such as b) mitochondrial count, c) area, d) perimeter, e) form factor, f) aspect ratio, g) branch length, h) branches per mitochondrion, and i) branch junctions per mitochondrion. The data presented are representative of the 8 to 10 analyzed images obtained from distinct and separate plates across 3 different studies. *P<0.05 denotes statistically significant variations relative to the untreated control.

The group treated with 50 µM LA and EPA shows a noticeable increase compared with the control and vehicle groups, suggesting a potential stimulatory effect on mitochondrial count. Furthermore, the vehicle group displayed no significant change.

However, mitochondrial dimensions are defined by the evaluation of area and perimeter. The mean area is modestly increased across all groups, with significant disparities observed between the control, vehicle, 50 µM LA, and 50 µM EPA groups. Additionally, perimeter values increased significantly in the EPA and LA groups compared with the control, with EPA showing the largest increase (Figures 2c and 2d). Furthermore, mitochondrial shape is characterized using form factor and aspect ratio. The form factor shows an improvement in results with only 50 µM EPA treatment, highlighting noticeable differences among the groups. An increased form factor indicates enhanced elongation and branching typical of active mitochondria. The aspect ratio appears to remain stable, with no significant variation in the treatment group (Figure 2, e and f). The skeletonized structure evaluates the connectivity and morphological complexity of the mitochondrial network, quantifying these attributes by counting branches, branch junctions, and the length of branches within the skeleton. The results reveal significant increases in these parameters in treated groups with FAs compared with control groups, and no significant changes were observed in the vehicle group (Figure 2, g, h, and i).

Discussion

The polyunsaturated fatty acids, EPA (Omega-3) and LA (Omega-6), are distinguished by a single carbon-carbon double bond. EPA's carbon-carbon double bond is located at the ω-3 site, which is the third bond from the fatty acid's methyl end. The ω -6 situated, which is the sixth bond from the methyl end of FA, is where LA, on the other hand, displays the double bond (33). This study elucidates the effects of FAs, specifically LA and EPA, on mitochondrial biogenesis in mESCs. Mitochondria are capable of ATP production within cells via oxidative phosphorylation (34). This intricate mechanism involves the movement of electrons between complexes in the respiratory chain, which facilitates the translocation of protons across the mitochondrial membrane, ultimately driving ATP synthesis (35). In stem cells, during cardiomyocyte development, mitochondrial oxidative enzymes play a greater role, and the energy source for cardiomyocytes gradually shifts from glycolysis to β-oxidation of FAs (16,36).

Cardiomyocytes derived from human-induced pluripotent stem cells using FAs as the essential metabolic substrate have been demonstrated as follows (37), and another study found that FAs are necessary for the differentiation of blood cell progenitors (38). Moreover, ROS generated by electron leakage within mitochondria during cellular respiration primarily manifests as superoxide, which then undergoes dismutation to form hydrogen peroxide (H_2O_2) (39,40). Thus, ROS is involved in several physiological functions, such as cell proliferation, differentiation, and apoptosis, by acting as signaling molecules. Given that FAs induce ROS and mitochondrial remodeling, our findings suggest that EPA and LA might promote early differentiation in mESCs via mitochondrial reorganization (40). However, Mitochondria play an essential role in energy metabolism, signaling, and cellular differentiation. The observed variations in mitochondrial characteristics (27,41), particularly branching and junction formation, underscore the importance of FAs as modulators of mitochondrial behavior. Omega-3 supplementation has been found to enhance mitochondrial respiration sensitivity and increase mitochondrial ROS release capacity without altering the levels of oxidative products. This indicates that omega-3 FAs contribute to the reorganization of mitochondrial membrane composition (42). In previous studies, FAs have been shown to enhance mESC differentiation by producing ROS and nitric oxide (NO). FAs promote mESC differentiation through mechanisms involving biochemical components (ROS and NO) and alterations in intracellular calcium levels, which are associated with the energy detectors AMP-activated protein kinase (AMPK-α) and peroxisome proliferator-activated receptors (PPAR-α) (8). However, Mitochondrial morphology is partially influenced by dynamics. The modulation of mitochondrial dynamics is evident in circumstances that require enhanced mitochondrial proliferation (43), is modified by hypoxia signaling mechanisms (44), as previously reported, and is closely linked to ROS production. Actually, extreme oxidative stress can lead to network fragmentation (45), and network modification may be regulated by elevated ROS level generation (46).

The findings of the present investigation demonstrate that mitochondrial counts differ notably between the treatment group and the untreated or vehicle groups. Some studies have also shown that high glucose concentrations enhance cell-specific morphological rearrangements of mitochondria and the mitochondrial network (47). In addition, this study's findings show significant alterations in mitochondrial branching and junctions, suggesting that LA and EPA may influence mitochondrial network dynamics. The study also explored form factor and aspect ratio, which are critical metrics for assessing mitochondrial shape (21). Significant changes were observed in these modifications following EPA treatment, indicating that while FAs may affect branching, they may also alter mitochondrial shape. The study also explored form factor and aspect ratio, which are critical metrics for assessing mitochondrial shape. Significant changes were observed in the form factor upon EPA treatment, while the aspect ratio remained unchanged. The observed aspect ratio suggests that mitochondria are not

undergoing elongation, but rather branching, increasing in number through division, or undergoing morphological changes without altering their overall shape. This proposes a proportion between fusion and fission processes, or changes in mitochondrial shape without altering the length-to-width ratio (17).

The modulation of mitochondrial dynamics plays a pivotal role in stem cell differentiation (48). Implications of FAs on cellular processes. FAs are known to be integral to cellular signaling pathways that govern stem cell fate (49). A different study mentions that these long-chain FAs, such as palmitic acid and stearic acid, enhanced gastrointestinal permeability and energy deprivation, causing elevated proton leaks, mitochondrial transformation, and improved ROS generation in intestinal cells, in contrast to dodecanoic acid and tetradecanoic acid, which induced significant lipid storage and enhanced mitochondrial network fusion (6). Furthermore, research shows that a prolonged culture of cardiovascular myocytes in glucose media improves mitochondrial function and FA metabolism. Supplementation with palmitate and oleate aids mitochondrial remodeling, oxygen consumption, and ATP production. However, glucose-maintained cardiomyocytes show underdeveloped ultrastructural architecture and suboptimal development (50).

Mitochondrial biogenesis induction activates transcription factors and local protein translation in response to natural products such as 6-gingerol (ginger extracts) and ursolic acid. At the same time, a few synthetic drugs are recognized as such inducers (51-53). Natural extracts such as Kaempferia parviflora, tangeretin, salidroside, spice saffron, and polydatin have been shown to promote mitochondrial biogenesis by activating different pathways, including SIRT1/AMPK/PGC-1α/PPARδ, miR22/SIRT1, and resveratrol (54-56). Cells respond to energy demands by up-regulating transcription factors, stimulating or inhibiting mitochondrial biogenesis. Pathology-associated disturbances involve either impaired or abnormally elevated mitochondrial biogenesis (57). There is growing evidence that the regulation of mESCs' behaviours is actively mediated by mitochondria. Specific processes (biogenesis, fission, fusion, and mitophagy) occur in mitochondria during the self-renewal, proliferation, and differentiation of mESCs. Significant effects on mESCs' behavior result from changes in mitochondrial dynamics, which are regulated by stress signaling and mESCs' niche variables (20).

Finally, the analysis of the mitochondrial network indicated that all FA treatments, with either LA or EPA, altered it. This remodelling could manifest as either mitochondrial fusion, as evidenced by an increased average network branch length, or fission and fragmentation, as indicated by a decreased average network branch length (20).

Conclusion

The study shows that treatment with either 50 µM LA or EPA significantly alters mitochondrial metrics, including number, area, perimeter, and branching, while maintaining some morphological characteristics. This suggests potential effects on energy metabolism and mESC differentiation. These findings may have implications for the design of stem cell-based therapies or for the treatment of mitochondrial disorders.

Acknowledgment

I sincerely thank Professor Dr. Heinrich Sauer, affiliated with Justus Liebig University Giessen, Germany, for his collaboration and the invaluable assistance of the College of Veterinary Medicine at the University of Mosul.

Conflict of interest

The author declares that there are no conflicts of interest.

References

1. Rohrbach S. Effects of dietary polyunsaturated fatty acids on mitochondria. Curr Pharm Des. 2009;15(36):4103-4116. DOI: 10.2174/138161209789909692
2. Jerab D, Blangero F, da Costa C, de Brito Alves L, Kefi R, Jamoussi H, Morio B, Eljaafari A. Beneficial Effects of Omega-3 Fatty Acids on Obesity and Related Metabolic and Chronic Inflammatory Diseases. Nutrients. 2025;4:17(7):1253-1281. DOI: 10.3390/nu17071253
3. Fat UK. Dietary fat and chronic diseases. Bahrain Med Bull. 1998;9:20(3):77-80. [available at]
4. Kar A, Ghosh P, Patra P, Chini DS, Nath AK, Saha JK, Patra BC. Omega-3 fatty acids mediated Cellular signaling and its regulation in Human Health. Clin Nutr Open Sci. 2023;12:52:72-86. DOI: 10.1016/j.nutos.2023.10.004
5. Gu C, Philipsen M, Ewing A. Omega-3 and-6 Fatty Acids Alter the Membrane Lipid Composition and Vesicle Size to Regulate Exocytosis and Storage of Catecholamines. ACS Chem Neurosci. 2024;2:15(4):816-826. DOI: 10.1021/acsche mneuro.3c00741
6. Guerbette T, Rioux V, Bostoën M, Ciesielski V, Coppens-Exandier H, Buraud M, Lan A, Boudry G. Saturated fatty acids differently affect mitochondrial function and the intestinal epithelial barrier depending on their chain length in the in vitro model of IPEC-J2 enterocytes. Front Cell Dev Biol. 2024;12:1266842. DOI: 10.3389/fcell .2024.1266842
7. Dikalov S, Panov A, Dikalova A. Critical role of mitochondrial fatty acid metabolism in normal cell function and pathological conditions. Int J Mol Sci. 2024;25(12):6498. DOI: 10.3390/ijms25126498
8. Taha A, Sharifpanah F, Wartenberg M, Sauer H. Omega-3 and Omega-6 polyunsaturated fatty acids stimulate vascular differentiation of mouse embryonic stem cells. J Cell Physiol. 2020;235(10):7094-7106. DOI: 10.1002/jcp.29606
9. Wai T, Langer T. Mitochondrial dynamics and metabolic regulation. Trends Endocrinol Metab. 2016;2:27(2):105-117. DOI: 10.1016/j.tem.2015.12.001
10. Liesa M, Palacín M, Zorzano A. Mitochondrial dynamics in mammalian health and disease. Physiol Rev. 2009;89(3):799-845. DOI: 10.1152/physrev.00030.2008
11. Putti R, Sica R, Migliaccio V, Lionetti L. Diet impact on mitochondrial bioenergetics and dynamics. Front Physiol. 2015;6:109. DOI: 10.3389/fphys.2015.00109

12. Jornayvaz FR, Shulman GI. Regulation of mitochondrial biogenesis. Essays Biochem. 2010;47:69-84. DOI: 10.1042/bse0470069
13. Li PA, Hou X, Hao S. Mitochondrial biogenesis in neurodegeneration. J Neurosci Res. 2017;95(10):2025-2029. DOI: 10.1002/jnr.24042
14. Golpich M, Amini E, Mohamed Z, Azman Ali R, Mohamed Ibrahim N, Ahmadiani A. Mitochondrial dysfunction and biogenesis in neurodegenerative diseases: Pathogenesis and treatment. CNS Neurosci Ther. 2017;23(1):5-22. DOI: 10.1111/cns.12655
15. Liu L, Li Y, Chen G, Chen Q. Crosstalk between mitochondrial biogenesis and mitophagy to maintain mitochondrial homeostasis. J Biomed Sci. 2023;30(1):86. DOI: 10.1186/s12929-023-00975-7
16. Persad KL, Lopaschuk GD. Energy metabolism on mitochondrial maturation and its effects on cardiomyocyte cell fate. Front Cell Dev Biol. 2022;7:10:886393. DOI: 10.3389/fcell.2022.886393
17. Luz AL, Rooney JP, Kubik LL, Gonzalez CP, Song DH, Meyer JN. Correction: Mitochondrial Morphology and Fundamental Parameters of the Mitochondrial Respiratory Chain Are Altered in Caenorhabditis elegans Strains Deficient in Mitochondrial Dynamics and Homeostasis Processes. PloS One. 2016;12:11(12):e0168738. DOI: 10.1371/journal.pone.0168738
18. Folmes CD, Nelson TJ, Martinez-Fernandez A, Arrell DK, Lindor JZ, Dzeja PP, Ikeda Y, Perez-Terzic C, Terzic A. Somatic oxidative bioenergetics transitions into pluripotency-dependent glycolysis to facilitate nuclear reprogramming. Cell Metab. 2011;8:14(2):264-271. DOI: 10.1016/j.cmet.2011.06.011
19. Zhou W, Choi M, Margineantu D, Margaretha L, Hesson J, Cavanaugh C, Blau CA, Horwitz MS, Hockenbery D, Ware C, Ruohola-Baker H. HIF1α induced switch from bivalent to exclusively glycolytic metabolism during ESC-to-EpiSC/hESC transition. EMBO J. 2012;5:31(9):2103-21016. DOI: 10.1038/emboj.2012.71
20. Fu W, Liu Y, Yin H. Mitochondrial dynamics: Biogenesis, fission, fusion, and mitophagy in the regulation of stem cell behaviours. Stem Cells Int. 2019;2019(1):9757201. DOI: 10.1155/2019/9757201
21. Picard M, White K, Turnbull DM. Mitochondrial morphology, topology, and membrane interactions in skeletal muscle: A quantitative three-dimensional electron microscopy study. J Appl Physiol. 2013;1:114(2):161-171. DOI: 10.1152/japplphysiol.01096.2012
22. Noguchi M, Kasahara A. Mitochondrial dynamics coordinate cell differentiation. Biochem Biophys Res Commun. 2018;500(1):59-64. DOI: 10.1016/j.bbrc.2017.06.094
23. Chen H, Chan DC. Mitochondrial dynamics in regulating the unique phenotypes of cancer and stem cells. Cell Metab. 2017;26(1):39-48. DOI: 10.1016/j.cmet.2017.05.016
24. Xu X, Duan S, Yi F, Ocampo A, Liu GH, Belmonte JC. Mitochondrial regulation in pluripotent stem cells. Cell Metab. 2013;9:18(3):325-332. DOI: 10.1016/j.cmet.2013.06.005
25. Ali E, Sharifpanah F, Taha A, Tsang S, Wartenberg M, Sauer H. The milk thistle (*Silybum marianum*) compound silibinin inhibits cardiomyogenesis of embryonic stem cells by interfering with angiotensin ii signaling. Stem Cells Int. 2018;2018(1):9215792. DOI: 10.1155/2018/9215792
26. Rai Y, Pathak R, Kumari N, Sah DK, Pandey S, Kalra N, Soni R, Dwarakanath BS, Bhatt AN. Mitochondrial biogenesis and metabolic hyperactivation limits the application of MTT assay in the estimation of radiation induced growth inhibition. Sci Rep. 2018;8(1):1531. DOI: 10.1038/s41598-018-19930-w
27. Leonard AP, Cameron RB, Speiser JL, Wolf BJ, Peterson YK, Schnellmann RG, Beeson CC, Rohrer B. Quantitative analysis of mitochondrial morphology and membrane potential in living cells using high-content imaging, machine learning, and morphological binning. Biochim Biophys Acta Mol Cell Res. 2015;1853(2):348-360. DOI: 10.1016/j.bbamcr.2014.11.002
28. Valente AJ, Maddalena LA, Robb EL, Moradi F, Stuart JA. A simple ImageJ macro tool for analyzing mitochondrial network morphology in mammalian cell culture. Acta Histochem. 2017;119(3):315-326. DOI: 10.1016/j.acthis.2017.03.001
29. Gooz M, Maldonado EN. Fluorescence microscopy imaging of mitochondrial metabolism in cancer cells. Front Oncol. 2023;13:1152553. DOI: 10.3389/fonc. 2023.1152553
30. Merrill RA, Flippo KH, Strack S. Measuring mitochondrial shape with ImageJ. In: Strack S, Usachev YM, editors. Techniques to investigate mitochondrial function in neurons. USA: Humana; 2017. 31-48 p. DOI: 10.1007/978-1-4939-6890-9_2
31. Chaudhry A, Shi R, Luciani DS. A pipeline for multidimensional confocal analysis of mitochondrial morphology, function, and dynamics in pancreatic β-cells. Am J Physiol Endocrinol Metab. 2020;318(2):E87-101. DOI: 10.1152/ajpendo.00457.2019
32. Hemel IM, Engelen BP, Luber N, Gerards M. A hitchhiker's guide to mitochondrial quantification. Mitochondrion. 2021;59:216-224. DOI: 10.1016/j.mito.2021.06.005
33. Kaur N, Chugh V, Gupta AK. Essential fatty acids as functional components of foods-a review. J. Food Sci Technol. 2014;10(51):2289-2303. DOI: 10.1007/s13197-012-0677-0
34. Gilkerson RW, Selker JM, Capaldi RA. The cristal membrane of mitochondria is the principal site of oxidative phosphorylation. FEBS Lett. 2003:546(2-3):355-358. DOI: 10.1016/S0014-5793(03)00633-1
35. Nolfi-Donegan D, Braganza A, Shiva S. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. Redox Biol. 2020;10:37:101674. DOI: 10.1016/j.redox.2020.101674
36. Yang X, Rodriguez ML, Leonard A, Sun L, Fischer KA, Wang Y, Ritterhoff J, Zhao L, Kolwicz SC, Pabon L, Reinecke H. Fatty acids enhance the maturation of cardiomyocytes derived from human pluripotent stem cells. Stem Cell Rep. 2019;10:13(4):657-668. DOI: 10.1016/j.stemcr.2019.08.013
37. Mummery CL, Zhang J, Ng ES, Elliott DA, Elefanty AG, Kamp TJ. Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: A methods overview. Circ Res. 2012;111(3):344-358. DOI: 10.1161/CIRCRESAHA. 110.227512
38. Tiwari SK, Toshniwal AG, Mandal S, Mandal L. Fatty acid β-oxidation is required for the differentiation of larval hematopoietic progenitors in Drosophila. Elife. 2020;9:e53247. DOI: 10.7554/eLife.53247
39. Liu Y, Fiskum G, Schubert D. Generation of reactive oxygen species by the mitochondrial electron transport chain. J Neurochem. 2002;80(5):780-787. DOI: 10.1046/j.0022-3042.2002.00744.x
40. Palma FR, Gantner BN, Sakiyama MJ, Kayzuka C, Shukla S, Lacchini R, Cunniff B, Bonini MG. ROS production by mitochondria: function or dysfunction?. Oncogene. 2024;43(5):295-303. DOI: 10.1038/s41388-023-02907-z
41. Chen W, Zhao H, Li Y. Mitochondrial dynamics in health and disease: mechanisms and potential targets. Signal Transduct Target Ther. 2023;8(1):333. DOI: 10.1038/s41392-023-01547-9
42. Herbst EA, Paglialunga S, Gerling C, Whitfield J, Mukai K, Chabowski A, Heigenhauser GJ, Spriet LL, Holloway GP. Omega-3 supplementation alters mitochondrial membrane composition and respiration kinetics in human skeletal muscle. J Physiol. 2014;592(6):1341-1352. DOI: 10.1113/jphysiol.2013.267336
43. Garnier A, Fortin D, Zoll J, N'Guessan B, Mettauer B, Lampert E, Veksler V, Ventura-Clapier R. Coordinated changes in mitochondrial function and biogenesis in healthy and diseased human skeletal muscle. FASEB J. 2005;19(1):43-52. DOI: 10.1096/fj.04-2173com
44. Trigo D, Goncalves MB, Corcoran JP. The regulation of mitochondrial dynamics in neurite outgrowth by retinoic acid receptor β signaling. FASEB J. 2019;33(6):7225. DOI: 10.1096/fj.201802097R
45. Wu S, Zhou F, Zhang Z, Xing D. Mitochondrial oxidative stress causes mitochondrial fragmentation via differential modulation of mitochondrial fission–fusion proteins. FASEB J. 2011;278(6):941-954. DOI: 10.1111/j.1742-4658.2011. 08010.x
46. Yu T, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. Proc Natl Acad Sci. 2006;103(8):2653-2658. DOI: 10.1073/pnas.0511154103
47. Belosludtseva NV, Serov DA, Starinets VS, Penkov NV, Belosludtsev KN. Alterations in Mitochondrial Morphology and Quality Control in Primary Mouse Lung Microvascular Endothelial Cells and Human Dermal Fibroblasts under Hyperglycemic Conditions. Int J Mol Sci. 2023;24(15):12485. DOI: 10.3390/ijms241512485

48. Liu Y, Yang Z, Na J, Chen X, Wang Z, Zheng L, Fan Y. In vitro stretch modulates mitochondrial dynamics and energy metabolism to induce smooth muscle differentiation in mesenchymal stem cells. FASEB J. 2025;1:39(2):e70354. DOI: 10.1096/fj.202402944R
49. Kang JX, Wan JB, He C. Concise review: Regulation of stem cell proliferation and differentiation by essential fatty acids and their metabolites. Stem Cells. 2014;5:32(5):1092-1098. DOI: 10.1002/stem.1620
50. Ramachandra CJ, Mehta A, Wong P, Ja KM, Fritsche-Danielson R, Bhat RV, Hausenloy DJ, Kovalik JP, Shim W. Fatty acid metabolism driven mitochondrial bioenergetics promotes advanced developmental phenotypes in human induced pluripotent stem cell derived cardiomyocytes. Int J Cardiol. 2018;272:288-297. DOI: 10.1016/j.ijcard.2018.08.069
51. Deng X, Zhang S, Wu J, Sun X, Shen Z, Dong J, Huang J. Promotion of mitochondrial biogenesis via activation of AMPK-PGC1α signaling pathway by Ginger (*Zingiber officinale* Roscoe) extract, and its major active component 6-Gingerol. J Food Sci. 2019;84(8):2101-2111. DOI: 10.1111/1750-3841.14723
52. Chen J, Wong HS, Leong PK, Leung HY, Chan WM, Ko KM. Ursolic acid induces mitochondrial biogenesis through the activation of AMPK and PGC-1 in C2C12 myotubes: A possible mechanism underlying its beneficial effect on exercise endurance. Food Funct. 2017;8(7):2425-2436. DOI: 10.1039/C7FO00127D
53. Cameron RB, Beeson CC, Schnellmann RG. Development of therapeutics that induce mitochondrial biogenesis for the treatment of acute and chronic degenerative diseases. J Med Chem. 2016;59(23):10411-10434. DOI: 10.1021/acs.jmedchem.6b00669
54. Kim MB, Kim T, Kim C, Hwang JK. Standardized Kaempferia parviflora extract enhances exercise performance through activation of mitochondrial biogenesis. J Med Food. 2018;21(1):30-38. DOI: 10.1089/jmf.2017.3989
55. Kou G, Li Z, Wu C, Liu Y, Hu Y, Guo L, Xu X, Zhou Z. *Citrus tangeretin* improves skeletal muscle mitochondrial biogenesis via activating the AMPK-PGC1-α pathway in vitro and in vivo: a possible mechanism for its beneficial effect on physical performance. J Agric Food Chem. 2018;66(45):11917-11925. DOI: 10.1021/acs.jafc.8b04124
56. Mao GX, Xu XG, Wang SY, Li HF, Zhang J, Zhang ZS, Su HL, Chen SS, Xing WM, Wang YZ, Dai JH. Salidroside delays cellular senescence by stimulating mitochondrial biogenesis partly through a miR-22/SIRT-1 pathway. Oxid Med Cell Longev. 2019;2019(1):5276096. DOI: 10.1155/2019/5276096
57. Popov LD. Mitochondrial biogenesis: An update. J Cell Mol Med. 2020;5:24(9):4892-4899. DOI: 10.1111/jcmm.15194

تأثير الأحماض الدهنية على التخليق الحيوي للميتوكوندريا أثناء تمايز الخلايا الجذعية الجنينية للفأر

عامر حسين طه

فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

قد تؤثر الأحماض الدهنية ومستقلباتها على التخليق الحيوي للميتوكوندريا في الخلايا الجذعية الجنينية للفأر. الميتوكوندريا هي عضيات ديناميكية مسؤولة عن إنتاج الأدينوسين ثلاثي فوسفات، وتشارك في العمليات الخلوية مثل التكاثر والتمايز ودورة الخلية وإعادة البرمجة والشيخوخة والموت الخلوي المبرمج، مع الشكل الطبيعي المطلوب للوظائف الخلوية. وبالتالي، قد يتغير شكل الميتوكوندريا ليتوافق مع متطلبات الطاقة. تبحث هذه الدراسة في تأثير الأحماض الدهنية مثل حمض الإيكوسابنتاينويك وحمض اللينوليك على التخليق الحيوي للميتوكوندريا من خلال تحليل كمي لتقييم المعايير المختلفة. تم معاملة الخلايا الجذعية الجنينية للفأر التي يبلغ عمرها ثلاثة أيام في ظل ظروف التمايز بـ ٥٠ ميكرومولر من الأحماض الدهنية لمدة ٤٨ ساعة، وتمت مقارنة النتائج بكل من المجاميع الغير المعاملة والمذيب ثنائي ميثيل سلفوكسيد. تم تحديد التركيب الهيكلي للميتوكوندريا من خلال تحليل ثنائي الأبعاد وقياسها كميًا باستخدام برنامج تحليل الصور. أدى العلاج بـ ٥٠ ميكرومولار من الأحماض الدهنية إلى زيادة ملحوظة في كمية الميتوكوندريا، بما في ذلك عدد الميتوكوندريا، ومساحتها، ومحيطها، وعامل شكلها، وعدد فروعها، وعدد تقاطعاتها، وطولها، مقارنةً بمجموعتي الغير معاملة والمذيب، مما يدل على تحسن الترابط بين الميتوكوندريا، مما يشير إلى تعزيز التكوين الحيوي للميتوكوندريا. تشرح هذه النتائج دور الأحماض الدهنية في تحسين التخليق الحيوي للميتوكوندريا وتشير إلى تطبيقات علاجية محتملة في السيطرة على الأمراض الأيضية المرتبطة بضعف نشاط وظيفة الميتوكوندريا.