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Obesity and Mitochondrial Function in Children: A case–control study

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Abstract

OBJECTIVES: Childhood obesity has increased over the past years worldwide. Therefore, changes in mitochondrial function as the risk factors of obesity in children need to consider.

AIM: The study aimed to evaluate the connection between obesity and mitochondrial function in obese children.

PATIENTS AND METHODS: This study was a case–control study conducted in the primary school children in Mosul city. The study included 100 children, with an age ranged from 6 to 12 years. Fifty child with obesity (BMI ≥ 95th percentile) for children enrolled in this work and compared to 50 control with BMI <95th percentile. Mitochondrial function assessed by measurement of serum lactic acid, lactate/pyruvate ratio, and L-carnitine and mt-DNA copy number.

RESULTS: Serum lactate and the lactate/pyruvate ratio were significantly higher in obese children than in the control group, while serum pyruvate levels in children with obesity are not significantly different from those in the control group. Serum levels of L-carnitine and mt-DNA copy number significantly reduced in obese children comparison to the control group.

CONCLUSION: Changes of mitochondrial function may be involved in obesity of children.

Introduction

Obesity in children is a rapidly growing community health issue that has reached epidemic proportions around the world. From 1975 (4%) to 2016, the global prevalence of obesity in children and adolescents tripled (over 18%) [1], [2].

Nourishment and daily life before gustation and during pregnancy, breast feeding, infancy, and early childhood have been presented to induce long-term effects on the risk of o obesity.

Body mass index (BMI) can be used to define obesity. “BMI = weight in kg/(height in meters) [2].” The criterion for obesity is BMI ≥95th percentile [3].

Both nutritional state and the degree of obesity can regulate lactate production [4].

As a result, the lactate threshold used to develop an additional evidence-based exercise program for obesity in children [1].

Lactic and pyruvic acids have been stated as additional products of glucose metabolism in adipocyte. However, experimental studies that carried out in rats and humans showed that the glucose conversion to lactic acid is quantitatively significant [4].

Mitochondrial dysfunction is one of the very important and basic factors in the development of many metabolic syndromes. Animal studies have recently revealed that maternal obesity has an effect on the critical regulators of mitochondrial fusion and fission. Obesity in mothers alters mitochondrial targeting, resulting in mitochondrial malfunction and an increased risk of obesity in children [2].

Obesity is associated with high calorie consumption; this causes elevation in free radical formation, accumulation of partially oxidized substrates, and finally mitochondrial malfunction. [5], [6].

This mitochondrial dysfunction induces oxidative stress (OS) pathways, that reflecting as lowering cellular insulin sensitivity and reducing nutrition influx. Chronically, this leads to lower mitochondrial metabolic capacity, which induce hyperinsulinemia with insulin resistance and food diversion to fat tissue storage [7].

Insulin resistance is a frequent problem of obesity, and it has thought to be cause by disruptions in mitochondrial function [8].

Reduced skeletal muscle mitochondrial oxidative phosphorylation was closely link to insulin resistance and metabolic phenotype in children, according to research. Although obesity in children has

not been directly related to mitochondrial malfunction, its function has been discovered to be a major factor to altered metabolism [9]. Furthermore, increased fatty acid oxidation results in an increase in reactive oxygen species (ROS), which is supposed to perform a vital role in the development of insulin resistance and a subsequent drop in electron transport chain activity [2].

BMI is highly related with genetic variations in the cytochrome c subunits 1, 3, and the NADH dehydrogenase subunits 1, 2, and 4L of the mt-DNA. Increased mt-DNA mutations result in lower mitochondrial protein production, reduced enzyme function, and altered respiration. The membrane potential lowered by changes in respiratory activity and capacity, which diminish ATP concentration in the cells, and signal cellular apoptotic processes, because mitochondria's major duty is to create ATP to preserve cell's energy status. Altogether these elements are expected to participate in a rise in BMI, and growing data recommends that the majority of these alterations can be avoided or mitigated by increasing physical exercises. Some speculate that these changes are produced by the accumulation of oxidative damage induced by long-term ROS generation. The cytochrome c and NADH dehydrogenase complexes are both engaged in mitochondrial ROS control. Actually, preventing ROS generation by expressing antioxidant enzymes at specific locations in animal body's can reduce dysfunction and recovery. The mitochondrial NADH dehydrogenase subunit (MT-ND1) is one of the seven respiratory complex I subunits engaged in the first step of the oxidative phosphorylation electron transport chain. Mutations in MT-ND1 may affect electron transport components and disrupt normal electron flow, resulting in an increase in superoxidase radical production and OS in a variety of cell types [10]. Multiple researchers have reported connections between central obesity, OS, excess fat accumulation, and metabolic disease [11]. NOX activation is one of the most important mechanisms for ROS production. One of the biological targets for the NOX signaling pathway, which produces ROS is the mitochondria. This organelle is largely considered as being responsible for the creation of cell energy through oxidative phosphorylation, during which, a modest excess of electrons leads an oxygen molecule to be reduced, resulting in the formation of a potentially toxic free radical [12], [13].

Children's mitochondrial activity is poorly understood in comparison to adult mitochondrial function. The association between mitochondrial function and obesity was investigated in this study.

Patients and Methods

This is a case–control study was conducted on the primary school children in Mosul city, Iraq, between September 1, 2021 and December 31, 2021 on 100 children, with an age ranged from 6 to 12 years. All of them were healthy. All children undergo height and weight measurement, and BMI is calculated (BMI = weight in kg/height in meters). The study was approved by the Medical Ethical Research Committee of the College of Medicine-University of Mosul (Ref. No. UOM/COM/MREC/21-22(7). Fifty children with obesity (BMI 95th percentile) were enrolled in this work and compared to fifty controls with BMI less than the 95th percentile. After receiving a written concept from one of their parents, 5 mL of blood were aspirated from each of them. Indirect mitochondrial function was assessed by measurement of serum lactate using Cayman L-lactate kit No. 700510, serum pyruvate, Cayman pyruvate kit No. 700470, and serum L-carnitine, which was detected using L-carnitine assay kit No. MAK063. The molecular study involves DNA extraction using AddPrep Genomic DNA extraction kit No. 10023. The quantity and quality of products were assayed using an Implen® Nanophotometer. Primers were designed using the NCBI website. Homosipens VDR forward primer (CATCTTCTTGGATCCTCGCC), reverse primer (TATGAGGGCTCCGAAGGCAC), and ND1 gene forward and reverse sequences (ATTATCGCCCACCCTCTC) and (GCTCGTAGGG CTCCGAATAG). The GAPDH gene is used as a housekeeping gene with a forward sequence (CGGGTCTTTGCAGTCGTATG) and a reverse sequence (CTGTTTCTGGGGACTA GGG G). The data were analyzed by Eco Study software. The data in this study represent data as the mean and standard deviations. For all statistical studies, Excel 2010 was utilized to analyze the data. The Student's t-test was used to examine the differences in parameters between the two groups. $p < 0.05$ is considered statistically significant.

Results

Serum lactate and the lactate/pyruvate molar ratio significantly increased in obese children comparison to those in control group. While serum pyruvate levels show no significant differences between children with obesity and those in the control group. Both serum levels of L-carnitine and mt-DNA copy number significantly reduced in obese children in comparison to those in control group, as showed in Table 1 and Figure 1.

Table 1: Mitochondrial function tests comparing children with obesity (BMI ≥ 95th percentile) to children with control (BMI <95th percentile)

Parameters	Obesity	Control	p-value
Lactate (μM)	1698 ± 171	1131 ± 285	0.0001*
Pyruvate (μM)	69.34 ± 7.8	70.28 ± 27.5	0.82
L:P molar ratio	24.68 ± 5.65	16.5 ± 2.69	0.0001*
L-carnitine (μM)	24.38 ± 3.74	43.08 ± 17	0.0001*

*Significant at $p \leq 0.01$.

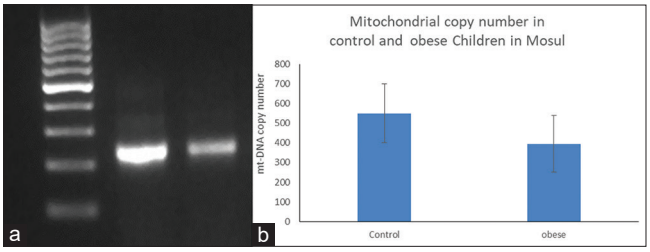

Figure 1: Mitochondrial copy number in control and obese child in Mosul city. (a) Gel image of MT-DNA using ND1 gene. (b) Histogram showed the changes in MT-DNA in control and obese child

Discussion

Overweight and obesity are connected with health risks during the lifetime [2]. Mitochondria function as threat sensors, stress signaling regulators, and cytotoxicity effectors in addition to being the cell's metabolic powerhouse; therefore, mitochondrial dysfunction is a common thread of obesity [14].

The association between elevated level of serum lactate and obesity can explained by fact that fat cells from obese humans can metabolize about 50–70% of the glucose to lactate. It was recently proposed that the organ with adiposity supplies lactic acid for hepatic gluconeogenesis during fasting and, for hepatic glycogen synthesis after food consumption [4]. As a result of the enlarged fat mass of obesity, greater lactic acid construction by the larger fat cell of patients with obesity, blood lactate levels may be expected to rise. A previous study discovered a link between circulating lactate levels and adipocyte size in humans [15].

A number of studies are in agreement with our results. When obese participants were compared to lean controls, they found that those who were at the overnight fasted condition had greater circulating lactate levels [16], [17], [18], [19].

Doar *et al*. found a substantial alteration in fasting lactic acid levels between lean and obese subjects in their studies [16].

In healthy lean and obese subjects, there was a direct and substantial linear association between BMI (kg/m^2) and lactic acid levels. ($r = 0.59$, $p = 0.001$) [18].

These findings corroborate those of Andersen *et al*. in severely obese people. Furthermore, in obese patients, weight loss by hypocaloric diets leads circulation lactate levels to recover to normal, lean levels [18], [19].

As a result, there is compelling evidence that fat people have higher lactate levels in the overnight fasted state than lean people. It appears doubtful that obesity's elevated lactate levels are due to aberrant lactate clearance rates, suggesting an increase in lactate generation [4].

The discovery that glucose conversion to lactic acid is mainly takes place in fat tissue, especially as adipocytes increase and adipose mass rises in obesity, which has elevated the possibility that lactate overproduction is linked to the numerous metabolic abnormalities that go together with obesity and carbohydrate intolerance [4].

The process of fusion and fission, which repairs damaged mitochondria and removes those that are irreparable, which is largely responsible for mitochondrial maintenance in cells. Obesity has been associated to an imbalance in the fusion and fission processes, which has been shown to impact mitochondrial morphology, size, number, and integrity [2].

In disagreement with results of our study, Fleischman *et al*. (2009) found that obesity, *per se*, is not associated with mitochondrial dysfunction in children [20].

According to the findings of the current study, serum pyruvate levels in children with obesity are non-significant ($p = 0.82$) when matched to those in the control group, implying that pyruvate is not required for adipogenesis and obesity.

Mitochondrial pyruvate carriers (MPCs) are transmembrane proteins that transport pyruvate from the cytosol into the mitochondrial matrix, at which it is oxidized to acetyl – CoA and carbon dioxide by the pyruvate dehydrogenase complex. The importance of MPC during adipocyte formation was investigated utilizing two different techniques in a new study. The manifestation and/or activity of MPCs were not necessary for adipogenesis, as established by pharmacological inhibition of MPCs and siRNA-mediated suppression of MPC1 [21].

Furthermore, this study found that serum levels of L-carnitine have significantly reduced in children with obesity. The recovery of L-carnitine in OS might be important for health [22].

Total L-carnitine levels in the blood were established to be considerably and inversely linked with pre-pregnancy body weight, pre-pregnancy BMI, pregnancy body weight, and pregnancy BMI, respectively, according to Tipi-Akbas *et al*. [23].

L-carnitine supplementation prevented obesity in female and male mice nourished a high-fat diet by increasing lipolysis and decreasing lipogenesis in white adipose tissues [24].

Mitochondrial DNA (mtDNA) damage results in mitochondrial dysfunction, which is a feature of many diseases [25].

Copy number variation in MtDNA, that indicates oxidant induced cell injury, has been found in a variety of human disorders [26]. However, whether it correlates with obesity in children, which has not been explained before.

Alteration of mitochondrial metabolism is a defining feature, which is frequently accompanied by a

rise in the formation of ROS. ROS may have harmed nDNA, mtDNA, lipoproteins, and cell membranes [10].

Intracellular oxidants were shown to be considerably higher in white blood cells in a previous study. Mitochondria in circulating blood cells can detect OS and get harmed, resulting in mitochondrial malfunction, which could exacerbate peripheral ROS production [26]. Obesity significantly increases mitochondrial activity and thus ROS production [11].

The link between childhood obesity and a metabolically unhealthy state could be explained by OS [27].

Increased levels of OS have been linked to the progress of obesity in children. There has also been evidence of antioxidant defense dysregulation, as well as opposite relationships between antioxidant capacity and body fat percentage [11]. This could explain the reduced L-carnitine levels in obesity group in the present study, which may play a role in the loss of mitochondrial function since that L-carnitine have a role in the recovery from OS [22].

The majority of reports, particularly in human studies, have concentrated on measuring oxidatively damaged nuclear DNA. However, due to its proximity to ROS production sources and the protection provided by higher order levels of structure accompanying with nuclear DNA, mtDNA is mainly prone to damage from both endogenous and external causes [25].

Characterizing mitochondrial dysfunction in obese children could lead to the development of regulated antioxidant therapy that could be useful in the treatment of obesity in the future [27].

Conclusion

This study discovered that specific mitochondrial function parameters vary with obesity. This implies that mitochondrial function has a direct impact on BMI, raising the possibility that mitochondria-targeted interventions could play a role in the prevention of developmental regression and the treatment of abnormal body weight. Furthermore, this significant clinical finding must be considered when enrolling children with obesity to study mitochondrial function, as participant characteristics will have a direct impact on the mitochondrial function of the sample.

References

1. Tan S, Yang C, Wang J. Physical training of 9-to 10-year-old children with obesity to lactate threshold intensity. Pediatr Exerc Sci. 2010;22(3):477-85. https://doi.org/10.1123/pes.22.3.477
   PMid:20814042
2. Pop TL, Maniu D, Rajka D, Lazea C, Cismaru G, Stef A, *et al.* Prevalence of underweight, overweight and obesity in school-aged children in the urban area of the northwestern part of Romania. Int J Environ Res Public Health. 2021;18(10):5176. https://doi.org/10.3390/ijerph18105176
   PMid:34068152
3. Gahagan S. Overweight and obesity. In: Kliegman RM, Stanton BF, St. Geme JW, Schor NF, Behrman RE, editors. Nelson Textbook of Pediatrics. 21th ed. Philadelphia, PA: Elsevier Inc.; 2016. p. 1876-77.
4. DiGirolamo M, Newby FD, lovejoy J. Lactate production in tissue: A regulated function with extra-adipose implications. FASEB J. 1992;6(7):2405-11. https://doi.org/10.1096/fasebj.6.7.1563593
   PMid:1563593
5. Wang Y, Wang JQ. A comparison of international references for the assessment of child and adolescent overweight and obesity in different populations. Eur J Clin Nutr. 2002;56(10):973-82. https://doi.org/10.1038/sj.ejcn.1601415
   PMid:12373618
6. Ajejas Bazán MJ, Jiménez Trujillo MI, Wärnberg J, Fernández SD, De Andrés AL, Farinós NP. Differences in the prevalence of diagnosis of overweight-obesity in Spanish children according to the diagnostic criteria set used. Gac Sanit. 2018;32(5):477-80. https://doi.org/10.1016/j.gaceta.2017.07.014
   PMid:28965658
7. Kurth BM, Schaffrath Rosario A. Overweight and obesity in children and adolescents in Germany. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2010;53(7):643-52. https://doi.org/10.1007/s00103-010-1083-2
   PMid:20631974
8. World Health Organization. Growth Reference 5-19 Years. 2007. Available from: http:/www.who.int/growthref/who2007_bmi_for_age/en/[Last accessed on 2020 Jul 10].
9. Deren K, Nyankovskyy S, Nyankovska O, Łuszczki E, Wyszynska J, Sobolewski M, *et al.* The prevalence of underweight, overweight and obesity in children and adolescents from Ukraine. Sci Rep. 2018;8(1):3625. https://doi.org/10.1038/s41598-018-21773-4
   PMid:29483604
10. Xu Y, Zhou J, Yuan Q, Su J, Li Q, Lu X, *et al.* Quantitative detection of circulating MT-ND1 as a potential biomarker for colorectal cancer. Bosn J Basic Med Sci. 2021;21(5):577-86. https://doi.org/10.17305/bjbms.2021.5576
    PMid:33823124
11. Nono Nankam PA, Nguelefack TB, Goedecke JH, Blüher M. Contribution of adipose tissue oxidative stress to obesity-associated diabetes risk and ethnic differences: Focus on Women of African Ancestry. Antioxidants (Basel). 2021;10(4):622. https://doi.org/10.3390/antiox10040622
    PMid:33921645
12. Sakurai T, Ogasawara J, Shirato K, Izawa T, Oh-Ishi S, Ishibashi, Y *et al.* Exercise training attenuates the dysregulated expression of adipokines and oxidative stress in white adipose tissue. Oxid Med Cell Longev. 2017;2017:9410954. https://doi.org/10.1155/2017/9410954
    PMid:28168013
13. Fernandez-Sanchez A, Madrigal-Santillan E, Bautista M, Esquivel-Soto J, Morales-Gonzalez A, Esquivel-Chirino C, *et al.* Inflammation, oxidative stress, and obesity. Int J Mol Sci. 2011;12(5):3117-32. https://doi.org/10.3390/ijms12053117
    PMid:21686173
14. Bhatraju NK, Agrawal A. Mitochondrial dysfunction linking obesity and asthma. Ann Am Thorac Soc. 2017;14:368-73.

https://doi.org/10.1513/annalsats.201701-042aw2017
PMid:29161084

15. Vendsborg PB, Bach-Mortensen N. Fat cell size and blood lactate in humans. Scand J Clin Lab Invest. 1977;37(4):317-20. https://doi.org/10.3109/00365517709092635
PMid:616057
16. Doar JW, Wynn V, Cramp DG. Blood pyruvate and plasma glucose levels during oral and intravenous glucose tolerance tests in obese and non-obese women. Metabolism. 1968;17(8):690-701. https://doi.org/10.1016/0026-0495(68)90053-x
PMid:5676215
17. Molnar D, Varga P, Rubecz I, Hamar A, Mestyan J. Food-induced thermogenesis in obese children. Eur J Pediatr. 1985;144(1):27-31. https://doi.org/10.1007/BF00491920
PMid:3894027
18. DiGirolamo M, Newby FD, Hill JO. Blood lactate levels in human obesity. Int J Obes. 1989;13:394-50.
19. Andersen T, McNair P, Fogh-Andersen N, Nielsen TT, Hyldstrup L, Transbol I. Increased parathyroid hormone as a consequence of changed complex binding of plasma calcium in morbid obesity. Metabolism. 1986;35(2):147-51. https://doi.org/10.1016/0026-0495(86)90116-2
PMid:3080652
20. Fleischman A, Kron M, Systrom DM, Hrovat M, Grinspoon SK. Mitochondrial function and insulin resistance in overweight and normal-weight children. J Clin Endocrinol Metab. 2009;94(12):4923-30. https://doi.org/10.1210/jc.2009-1590
PMid:19846731
21. Burrell JA, Richard AJ, King WT, Stephens JM. Mitochondrial pyruvate carriers are not required for adipogenesis but are regulated by high-fat feeding in brown adipose tissue. Obesity (Silver Spring). 2020;28(2):293-302. https://doi.org/10.1002/oby.22678
PMid:31970913
22. Liu D, Zeng X, Li L, Ou Z. Carnitine promotes recovery from oxidative stress and extends lifespan in *Caenorhabditis elegans*. Aging (Albany NY). 2021;13(1):813-30. https://doi.org/10.18632/aging.202187
PMid:33290254
23. Tipi-Akbas P, Arioz DT, Kanat-Pektas M, Koken T, Koken G, Yilmazer M. Lowered serum total L-carnitine levels are associated with obesity at term pregnancy. J Matern Fetal Neonatal Med. 2013;26(15):1479-83. https://doi.org/10.3109/14767058.2013.789847
PMid:23560471
24. Gao X, Sun G, Randell E, Tian Y, Zhou H. Systematic investigation of the relationships of trimethylamine N-oxide and L-carnitine with obesity in both humans and rodents. Food Funct. 2020;11(9):7707-16. https://doi.org/10.1039/d0fo01743d
25. Chao MR, Evans MD, Hu CW, Ji Y, Møller P, Rossnerg P, *et al.* Biomarkers of nucleic acid oxidation-a summary state-of-the-art. Redox Biol. 2021;42:101872. https://doi.org/10.1016/j.redox.2021.101872
PMid:33579665
26. Huang J, Tan L, Shen R, Zhang L, Zuo H, Wang DW. Decreased peripheral mitochondrial DNA copy number is associated with the risk of heart failure and long-term outcomes. Medicine (Baltimore). 2016;95(15):3323. https://doi.org/10.1097/md.0000000000003323
PMid:27082579
27. Rupérez AI, Mesa MD, Anguita-Ruiz A, González-Gil EM, Vázquez-Cobela R, Moreno LA, *et al.* Antioxidants and oxidative stress in children: Influence of puberty and metabolically unhealthy status. Antioxidants (Basel). 2020;9(7):618. https://doi.org/10.3390/antiox9070618
PMid:32679739