Chapter

Autism Spectrum Disorder (ASD): From Molecular Mechanism to Novel Therapeutic Approach

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Abstract

Autism spectrum disorder (ASD) is the joint name for neurodevelopmental impairments characterized by abnormal social interaction, communication difficulties, limited range of activities and areas of interest, and typical motor impairments. There is a remarkable increase in the prevalence of ASD over the past 30 years. Studies indicate that genetic, neurological, and environmental factors are involved in the emergence of ASD, and recent works describe the neuromolecular mechanism implicated in the basis of ASD. 3LT has now developed into a therapeutic procedure that is used for three main goals: to reduce inflammation, edema, and chronic orthopedic disorders; to promote healing of wounds, deeper tissues, and nerves; and to treat neurological injuries and pain. 3LT may treat neurological injuries by lowering levels of inflammation proteins and by stimulation of mitochondria to increase the production of adenosine triphosphate and neural growth factors. This review aims to discuss the current evidence for the effects and mechanisms of 3LT at the cellular level and the effects of 3LT-induced changes in brain development and function. Early and effective intervention, through the developmental time window of high ASD susceptibility, using tools that are directed to the mechanism of pathology, may minimize neurological and functional deficits.

Keywords: brain development, brain injury, ASD, autism, 3LT, low-level laser therapy, mitochondria

1. Introduction

Autism spectrum disorder (ASD) displays early in child development, during the time of human synapse formation and maturation [1], and usually results in long-term difficulties in social, communicational, emotional, adaptive, and cognitive functions [2]. The frequency of ASD occurrence continues to rise—from 1:110 in 2006 to 1:54 in 2016 [3], with at least one diagnosed coexisting neurodevelopmental disorder in most of the children [4]. Early diagnosis and treatment are very important as they may minimize neural injury and functional difficulty.

As ASD is still diagnosed only by behavioral criteria, it has been difficult to connect the numerous neurophysiologic findings to the clinical characteristics of ASD and to draw the mechanism and etiology of ASD [5]. This would allow an accurate treatment, directed to the mechanism of injury, with the best chance to make a change in the impaired developmental route.

The search for ASD brain mechanism may be reviewed from the neural circuit to the molecules and organelles involved.

In the late nineties of the twentieth century, a laboratory in Italy first documented neural activity from brain cycles, later named "Mirror Neurons" [6]. The innovation in its discovery was that it connected fields of neural control that were considered separate—motor and vision, that is, the same specific neuron cycles work both when a person does something and when he or she watches another person perform the same action, making an instant translation from visual to motor control [7]. This act of neural translation is considered the basis of the human ability to imitate, to anticipate others' goals, and to empathize others' pain or misery [8–11].

"Mirror Neurons" brain cycles showed altered activity in children with ASD, hinting that they are involved in the mechanism of ASD [12–14].

The scientific findings about mirror neurons and the possibility that their development may be related to the time window of temporary subcortical plate neurons (connecting thalamic and future cortical cycles) are indeed amazing [15]. But the mechanistic discussion in the level of neural cycle leaves many open questions—what may cause damaging alterations in these brain cycles? What cellular and molecular components are involved, and how can we target the therapeutic process to them?

Loss of synaptic stability and plasticity, or dysregulation of activity-dependent signaling networks that control synapse development, function, and plasticity, may cause injuries in neuronal circuits and contribute significantly to brain diseases, including ASD pathogenesis [16, 17].

Hence, alterations in synapse function, synaptic molecules, receptors, and neurotransmitters have been targets to research about the mechanism of ASD syndrome for the last 20 years. Studies showed that alterations in Glutamate receptors and enhanced GABA receptor–mediated inhibitory synaptic transmission are involved in ASD [18–20]. There may be various causes involved in psychiatric and neurologic diseases, including ASD—genetics, drug use, neurodegeneration, viral infections, and more. However, dysfunction of neuronal synaptic communication is almost always the underlying cellular mechanism. Epigenetic changes in synaptic genes encoding for synaptic adhesion molecules (neurexin, neuroligin, and N-cadherin) and for PSD proteins (i.e., Shank1, Shank3, and more) are involved in neuropsychiatric disorders including ASD, causing alterations in synaptic transmission [16, 21–25]. Studies have found that failure of the cellular machinery in pathways upstream of the synapse leads to synaptic dysfunction and neuropsychiatric characteristics. In addition, small non-coding microRNAs that repress the translation of target mRNAs seem to be important pathophysiologic mechanisms for neurologic and psychiatric diseases, and abnormal regulation of protein turnover, chromatin remodeling, and genomic imprinting may lead to synapse pathology. In some neuropsychiatric disorders, the basic neurobiological mechanisms underlying the symptoms are simple and easily solved, but the model of loss of function of a single gene or a limited number of genes is not suitable for most neuropsychiatric disorders, which are etiologically heterogeneous and complex and likely determined by the combination of variants/defects in multiple genes. For example, genome-wide association studies identified polymorphic variants in genes encoding synaptic proteins as important determinants of the risk of developing ASD [26–28].

2. Molecular mechanistic common denominator involved in ASD etiology

Multiple studies show that a mitochondrial disease or abnormality is involved in the etiology of ASD [29, 30] affecting about 80% of the children with ASD.

Mitochondria are the "cell powerplants," being responsible for most of cell energy production. Sufficient energy is required for everyday vitality and for brain survival and function. Brain cells need a lot of energy to function. Apart from energy production, mitochondria participate in the cellular metabolic processes of iron and the balance of calcium. The mitochondria are associated with normal and abnormal cell proliferation and participate in programed cell death. Each cell has hundreds to tens of thousands of mitochondria, depending on the role and energy consumption of that cell. Mitochondria are inherited only from the mother, through the ovum. They can develop mutations as they multiply and lack almost any repair mechanisms. Most of the proteins that make up mitochondria are encoded in the nucleus. Only 13 proteins are encoded by the circular mitochondrial genome.

As the mitochondria are inherited from the mother, hence, we do not have a "backup" from the father's genome when mutations or damage occurs. But since the ovum contains a lot of mitochondria to start with, some may be damaged without any clinical manifestations. Mitochondrial damage may be manifested over the generations; when the grandmother had a few damaged mitochondria, the mother happened to develop from an ovum with a greater concentration of damaged mitochondria, and her son already has very few normal mitochondria. A problem is revealed in such cases. A damage to mitochondria may be caused not only by maternal inheritance, when cells divide to form the fetus, but also by a coding error called "de novo mutation" (a new mutation in fetal cells or in mitochondria), due to environmental / epigenetic influence. Hence, when a diet contains fewer carbs, there is an increase in the number of mitochondria in liver and large muscle cells.

Mitochondrial abnormalities include either decreased [29, 31, 32] or increased [33–36] mitochondrial function; depending on the cause and developmental time window, they may lead to neurodevelopmental regression [30, 37–42] and the typical comorbidities of ASD (i.e., gastrointestinal problems, seizures, tiredness, and sensory dysregulation) [30, 43, 44]. The first findings, leading to this conclusion go back to the eighties of the twentieth century [45], reconfirmed about 20 years later [46] and continue with studies that examine the biomarkers of mitochondrial dysfunction [30, 47]. Neurodevelopmental regression, as typically described for many children with ASD, may be the hallmark of a mitochondrial disorder and abnormal mitochondrial physiology in ASD [38, 39].

As mitochondrial function is highly influenced by environmental factors, these findings connect mitochondrial dysfunction in ASD with environmental hazards [29, 30].

3. Therapeutic approaches

Since ASD was first defined, numerous treatments have been employed, with partial/sporadic mechanistic justification. Most of the treatment approaches target behavioral abnormalities of children with ASD and aim to improve the social and communicational function of the patient [48–50].

The website of the American Association of Communication Clinicians describes 30 common treatment programs for children with autism, divided into seven classes; however, parents cannot be given definite treatment recommendations, because of the heterogenous characteristics of children with ASD and because many therapies have not yet been investigated in a controlled and satisfactory manner.

In November 2020, the Australian governmental CRC top organization published a 502-page document written by 12 scientists. The paper is a meta-analysis based on 58 review articles analyzing more than a thousand research articles that examined the effectiveness of 111 different autism therapy programs [51].

The authors sorted the programs into nine categories (cognitive, behavioral, educational, developmental, animal assisted, sensory-based, naturalistic, technology-based, others). The review showed that intensive behavioral programs achieved good results, but the results were focused on specific goals in which the child has been practiced; only some of the developmental plans showed improvement, mainly programs that included parental involvement; only one sensory program has achieved clear results of reducing stimulation and improving learning habits and participation in the community; music therapy helped interpersonal communication and improved mental well-being in the family; various computer applications have improved cognitive ability but not mutual communication; alternative supportive communication programs have resulted in good results in communication, motor behavior, game levels, and learning ability. The authors note that in each category, only a very small number of studies were made in a controlled and satisfactory manner, meaning that the results should be treated with caution.

Altogether, children with autism spectrum disorders can be treated in a way that will lead to functional and communicational improvement, using various therapeutic approaches. These treatment plans are tailored to the unique behavioral profile of each child and each family at each point in time throughout their life journey with autism. However, as these treatments focus on external behavioral symptoms, and not on the internal mechanism, they aim at functional improvement and not actual repair of neurological damage. Hence, according to this approach, autism is not a "curable injury" but a developmental disorder whose treatment helps patients develop functional skills, improve communication skills, and rely on their strengths despite the disorder that will always remain a part of their lives.

Should we be satisfied with the important achievements of symptom-oriented therapeutic approach, or perhaps a persistent search into mechanistic questions may lead to a mechanism-oriented therapeutic approach?

Few therapeutic approaches for mitochondrial disorders were examined in clinical studies in children with ASD. These include cofactor supplementation and ketogenic diet. Nutritional supplements aimed to support the mitochondria, redox, and folate pathways, and contained L-carnitine, coenzyme Q10, and additional factors. They improved mitochondrial function and ASD symptoms [52–57], However, discontinuation of the supplement treatment caused worsening of the ASD behavior in children [31, 58].

Ketogenic diet has been studied for ASD, resulting in a mild-to-moderate improvement with 58% of the children who tolerated the 3-month diet [59–61]. In one out of three studies, worsening outcomes were observed. In the studies that used biomarkers to better understand the physiology of the ketogenic diet, an increase in chromium and creatine and a decrease in ornithine, acetoacetate, cesium, and N-acetylserotonin across the treatment period correlated with better outcomes [60, 61]. In addition, the ketogenic diet improved sociability and repetitive behaviors in two environmentally induced mice models of ASD [62–64]. With these results, the ketogenic diet needs more study for its use in children with ASD. In addition, the important limitation of the ketogenic diet is the child's ability to tolerate the diet, as dietary therapies are difficult to implement with children. For other dietary treatments, outcomes are related to the ability of the family to implement the diet adequately [65], and if it is impossible for the family to apply the diet properly, the expected outcome may not be achievable and other therapeutic options may be a better choice.

4. Low-level laser therapy (3LT)

Alternative medicine has become vastly used for managing health problems and developmental injuries in the modern western world, consisting of various

approaches stemming from traditional medicine combined with modern empirical techniques [66, 67].

Acupuncture and auricular therapy have been employed all over the world for the treatment of chronic and acute medical situations [68–71], for coping with pain in elderly [72, 73] and children [74–78]. For example, it was found that acupuncture increases the secretion of the natural neuromodulator adenosine, also known as anti-inflammatory and pain relief substance [79].

Lasers (light amplification by stimulated emission of radiation) are devices that generate electromagnetic radiation, which are uniform in wavelength, phase, and polarization. Low-level laser (3 L) is a special type of laser that affects biologic systems through nonthermal means [80, 81]. Low-level laser therapy (3LT) is the application of red and NIR (near infrared) light over injuries or lesions to improve wound and soft-tissue healing, reduce inflammation, and give relief for both acute and chronic pain (analgesia) [82–84].

3LT applies a therapeutic laser for the excitation of specific acupuncture points. This technique is considered nonintrusive, safe, and painless [85] and became an important tool for the treatment of patients at risk, such as premature neonates [86–92]. For example, excitation of specific pain acupuncture points using 3LT creates a local photochemical effect [93] that causes specific changes in neuronal brain activity [94, 95], apprehended by the patient as reduction in pain severity. These changes can be measured and quantified by imaging [96, 97].

3LT has a photochemical effect, meaning that when the correct parameters are employed (intensity and location), red or NIR light reduces tissue oxidative stress and increases ATP levels [98–101]. This improves cell metabolism and reduces inflammation. In addition, 3LT was proven to increase nociceptive threshold by altering the axonal flow [102] and elevate opioid-receptor binding [103] and endorphin production [104].

In the clinic, 3LT was found to cause an immediate decrease in acute and chronic pain and an increase in function [102, 105–107]. 3LT showed promising results for myocardial infraction [108], rejuvenating mesenchymal stem cells [109], skin injuries [110–113], brain trauma, TBI [114–116], diabetic retinopathy [117], oncology [118], and more.

3LT is a technique of noninvasive stimulation of which the irradiation of specific infrared wavelengths can penetrate the body [119]. These effects produce various biological responses, such as enhancing the formation of adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), and ribonucleic acid (RNA); releasing nitric oxide (NO) and cytochrome c oxidase (CCO); regulating reactive oxygen species (ROS); and altering intracellular organelle membrane activity, mainly in mitochondria, calcium flux, and stress proteins [66, 120–124]. 3LT produces a shift toward higher oxidation in the overall cell redox potential [125] and briefly increases the level of ROS [111, 126]. This change in the redox state of the mitochondria regulates several transcription factors [127]. These include redox factor-1 (Ref-1), cAMP response element (CREB), activator protein 1 (AP-1), p53, nuclear factor kappa B (NFjB), hypoxia-inducible factor (HIF-1), and HIF-like factor [127]. The activation and regulation of redox-sensitive genes and transcription factors are thought to be caused by ROS induced from 3LT [126]. In turn, both ATP levels and blood flow increase, improving oxygenation found in damaged areas of the brain [127].

5. Therapeutic potential

A wide range of seemingly unrelated disorders, such as schizophrenia, bipolar disease, dementia, Alzheimer's disease, epilepsy, migraine headaches, strokes, neuropathic pain, CP, TBI, diabetic retinopathy, Parkinson's disease, ataxia,

transient ischemic attack, cardiomyopathy, coronary artery disease, chronic fatigue syndrome, fibromyalgia, and SARS-CoV-2, have underlying pathophysiological mechanisms in common, namely reactive oxygen species (ROS) production and the accumulation of mitochondrial DNA (mtDNA) damage, resulting in mitochondrial dysfunction [114, 128–130].

3LT has been long recognized as an efficient therapeutic tool for brain injuries. Recent deciphering of the role of mitochondria in ASD etiology and in the 3LT therapeutic process gives us a great opportunity to improve mitochondria function and brain neural development, using suitable parameters of 3LT energy on specific ear and body locations.

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References

[1] Lord C, Cook EH, Leventhal BL, Amaral DG. Autism spectrum disorders. Neuron. 2000;**28**(2):355-363

[2] Thapar A, Cooper M, Rutter M. Neurodevelopmental disorders. Lancet Psychiatry. 2017;**4**(4):339-346

[3] Christensen DL, Braun KVN, Baio J, et al. Prevalence and characteristics of ASD among children aged 8 years—Autism and developmental disabilities monitoring network. MMWR Surveillance Summaries. 2018;**65**:1-23

[4] Saito M., Hirota T., Sakamoto Y., et al. (2020). Prevalence and cumulative incidence of autism spectrum disorders and the patterns of co-occurring neurodevelopmental disorders in a total population sample of 5-year-old children. Molecular Autism. 14;11(1):35

[5] Frye RE. Social skills deficits in autism spectrum disorder: Potential biological origins and progress in developing therapeutic agents. CNS Drugs. 2018;**32**:713-734

[6] Gallese V, Fadiga L, Fogassi L, Rizzolatti G. Action recognition in the premotor cortex. Brain. 1996;**119**: 593-609

[7] Rizzolatti G, Sinigaglia C. The functional role of the parieto-frontal mirror circuit: Interpretations and misinterpretations. Nature Reviews. Neuroscience. 2010a;**11**:264-274

[8] Mukamel R, Ekstrom AD, Kaplan J, Iacoboni M, Fried I. Single neuron responses in humans during execution and observation of actions. Current Biology. 2010;**20**:750-756

[9] Lamm C, Meltzoff AN, Decety J. How do we empathize with someone who is not like us? A functional magnetic resonance imaging study. Journal of Cognitive Neuroscience. 2010;**22**:362-376

[10] Perry A, Bentin S, Bartal IBA, Lamm C, Decety J. 'Feeling' the pain of those who are different from us: Modulation of EEG in the mu/alpha range. Cognitive, Affective, & Behavioral Neuroscience. 2010;**10**: 493-504

[11] Zaki J, Ochsner K. The neuroscience of empathy: Progress, pitfalls and promise. Nature Neuroscience. 2012;**15**:675-680

[12] Rizzolatti G, Fabbri-Destro M. Mirror neurons: From discovery to autism. Experimental Brain Research. 2010b;**200**:223-237

[13] Perkins T, Stokes T, McGillivray J, Bitter R. Mirror neuron dysfunction in autism spectrum disorders. Journal of Clinical Neuroscience. 2010;**17**: 1239-1243

[14] Dapretto M, Davies MS, Pfeifer JH, Scott AA, Sigman M, et al. Understanding emotions in others: Mirror neuron dysfunction in children with autism spectrum disorders. Nature Neuroscience. 2006;**9**:28-30

[15] Friedman H et al. INA early intervention for babies at risk. In: Neurodevelopment and Neurodevelopmental Disease. London, United Kingdom: IntechOpen; 2019

[16] Sudhof TC. Neuroligins and neurexins link synaptic function to cognitive disease. Nature. 2008;**455**(7215):903-911

[17] van Spronsen M, Hoogenraad CC. Synapse pathology in psychiatric and neurologic disease. Current Neurology and Neuroscience Reports. 2010;**10**:207-214

[18] Sacai H, Sakoori K, Konno K, et al. Autism spectrum disorder-like behavior caused by reduced excitatory synaptic

transmission in pyramidal neurons of mouse prefrontal cortex. Nature Communications. 2020;**11**:5140

[19] Tabuchi K, Blundell J, Etherton MR, et al. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science. 2007;**318**(5847):71-76

[20] Cetin FH, Tunca H, Guney E, Iseri E. Neurotransmitter systems in autism spectrum disorder. In: Fitzgerald M, editor. Autism Spectrum Disorder—Recent Advances. London, United Kingdom: IntechOpen; 2015

[21] Abrahams BS, Geschwind DH. Advances in autism genetics: On the threshold of a new neurobiology. Nature Reviews. Genetics. 2008;**9**(5):341-355

[22] Chen ACH, Arany PR, Huang YY, Tomkinson EM, Sharma SK, Kharkwal GB, et al. Low-level laser therapy activates NF-kB via generation of reactive oxygen species in mouse embryonic fibroblasts. PLoS One. 2011;**6**:e22453

[23] Brouwer JR, Willemsen R, Oostra BA. Microsatellite repeat instability and neurological disease. BioEssays. 2009;**31**(1):71-83

[24] Kaizuka T, Takumi T. Postsynaptic density proteins and their involvement in neurodevelopmental disorders. The Journal of Biochemistry. 2018;**163**(6): 447-455

[25] Ramocki MB, Zoghbi HY. Failure of neuronal homeostasis results in common neuropsychiatric phenotypes. Nature. 2008;**455**(7215):912-918

[26] Wang K, Zhang H, Ma D, et al. Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature. 2009;**459**(7246): 528-533

[27] Ebert DH, Greenberg ME. Activity-dependent neuronal signalling and autism spectrum disorder. Nature. 2013;**493**(7432):327-337

[28] Guang S, Pang N, Deng X, Yang L, et al. Synaptopathology involved in autism spectrum disorder. Frontiers in Cellular Neuroscience. 2018;**12**:470

[29] Rose S, Niyazov DM, Rossignol DA, et al. Clinical and molecular characteristics of mitochondrial dysfunction in autism spectrum disorder. Molecular Diagnosis & Therapy. 2018;**22**:571-593

[30] Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: A systematic review and meta-analysis. Molecular Psychiatry. 2012;**17**:290-314

[31] Delhey L, Kilinc EN, Yin L, et al. Bioenergetic variation is related to autism symptomatology. Metabolic Brain Disease. 2017;**32**:2021-2031

[32] Goldenthal MJ, Damle S, Sheth S, et al. Mitochondrial enzyme dysfunction in ASD: A novel biomarker revealed from buccal swab analysis. Biomarkers in Medicine. 2015;**9**:957-965

[33] Graf WD, Marin-Garcia J, Gao HG, et al. Autism associated with the mitochondrial DNA G8363A transfer RNA(Lys) mutation. Journal of Child Neurology. 2000;**15**:357-361

[34] Frye RE, Naviaux RK. Autistic disorder with complex IV overactivity: A new mitochondrial syndrome. Journal of Pediatric Neurology. 2011;**9**:427-434

[35] Palmieri L, Papaleo V, Porcelli V, et al. Altered calcium homeostasis in autism-spectrum disorders: Evidence from biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier AGC1. Molecular Psychiatry. 2010;**15**:38-52

[36] Hassan H, Gnaiger E, Zakaria F, et al. Alterations in mitochondrial

respiratory capacity and membrane potential: A link between mitochondrial dysregulation and autism. MitoFit Preprint Arch. 2020;**2020**:3

[37] Edmonds JL, Kirse DJ, Kearns D, et al. The otolaryngological manifestations of mitochondrial disease and the risk of neurodegeneration with infection. Archives of Otolaryngology – Head & Neck Surgery. 2002;**128**:355-362

[38] Shoffner J, Hyams L, Langley GN, et al. Fever plus mitochondrial disease could be risk factors for autistic regression. Journal of Child Neurology. 2010;**25**:429-434

[39] Singh K, Singh IN, Diggins E, et al. Developmental regression and mitochondrial function in children with autism. Annals of Clinical Translational Neurology. 2020;**7**:683-694

[40] Rose S, Frye RE, Slattery J, et al. Oxidative stress induces mitochondrial dysfunction in a subset of autism lymphoblastoid cell lines in a well-matched case control cohort. PLoS One. 2014;**9**:e85436

[41] Rose S, Frye RE, Slattery J, et al. Oxidative stress induces mitochondrial dysfunction in a subset of autistic lymphoblastoid cell lines. Translational Psychiatry. 2014;**4**:e377

[42] Rose S, Bennuri SC, Wynne R, et al. Mitochondrial and redox abnormalities in autism lymphoblastoid cells: A sibling control study. The FASEB Journal. 2017;**31**:904-909

[43] Frye RE, Rossignol DA. Mitochondrial dysfunction can connect the diverse medical symptoms associated with autism spectrum disorders. Pediatric Research. 2011;**69**:41R-47R

[44] Weissman JR, Kelley RI, Bauman ML, et al. Mitochondrial disease in autism spectrum disorder patients: A cohort analysis. PLoS One. 2008;**3**:e3815

[45] Coleman M, Blass JP. Autism and lactic acidosis. Journal of Autism and Developmental Disorders. 1985;**15**:1-8

[46] Oliveira G, Ataide A, Marques C, et al. Epidemiology of autism spectrum disorder in Portugal: Prevalence, clinical characterization, and medical conditions. Developmental Medicine and Child Neurology. 2007;**49**:726-733

[47] Frye RE. Biomarkers of abnormal energy metabolism in children with autism spectrum disorder. North American Journal of Medical Sciences. 2012;**5**:141-147

[48] Szabo TG. Acceptance and commitment training for reducing inflexible behaviors in children with autism. Journal of Contextual Behavioral Science. 2019;12:178-188

[49] Burnham RP, Khan M, Weiss JA. Measuring therapeutic alliance in children with autism during cognitive behavior therapy. Clinical Psychology & Psychotherapy. 2019;**26**(6):761-767

[50] Zwaigenbaum L, Bauman ML, Choueiri R, Kasari C, et al. Early intervention for children with autism spectrum disorder under 3 years of age: Recommendations for practice and research. Pediatrics. 2015;**136**:S60-S81

[51] Whitehouse A, Varcin K, Waddington H, Sulek R, et al. Interventions for Children on the Autism Spectrum: A Synthesis of Research Evidence. Brisbane: Autism CRC; 2020 Available from: Autismcrc.com.au

[52] Mousavinejad E, Ghaffari MA, Riahi F, et al. Coenzyme Q10 supplementation reduces oxidative stress and decreases antioxidant enzyme activity in children with autism

spectrum disorders. Psychiatry Research. 2018;**265**:62-69

[53] Adams J, Freedenfeld S, Audhya T, et al. Biochemical effects of ribose and NADH therapy in children with autism. Autism Insights. 2011;**3**:3

[54] Lonsdale D, Shamberger RJ, Audhya T. Treatment of autism spectrum children with thiamine tetrahydrofurfuryl disulfide: A pilot study. Neuro Endocrinology Letters. 2002;**23**:303-308

[55] Benke PJ, Duchowny M, McKnight D. Biotin and acetazolamide for treatment of an unusual child with autism plus lack of nail and hair growth. Pediatric Neurology. 2018;**79**:61-64

[56] Adams JB, Audhya T, Geis E, et al. Comprehensive nutritional and dietary intervention for autism spectrum disorder—A randomized, controlled 12-month trial. Nutrients. 2018;**10**:1-43

[57] Adams JB, Audhya T, McDonough-Means S, et al. Effect of a vitamin/mineral supplement on children and adults with autism. BMC Pediatrics. 2011;**1**(111):111-130

[58] Legido A, Goldenthal M, Garvin B, et al. Effect of a combination of carnitine, coenzyme Q10 and alpha-lipoic acid (MitoCocktail) on mitochondrial function and neurobehavioral performance in children with autism spectrum disorder (P3.313). Neurology **90**. 2018; n. pag

[59] Evangeliou A, Vlachonikolis I, Mihailidou H, et al. Application of a ketogenic diet in children with autistic behavior: Pilot study. Journal of Child Neurology. 2003;**18**:113-118

[60] Lee RWY, Corley MJ, Pang A, et al. A modified ketogenic gluten-free diet with MCT improves behavior in children with autism spectrum disorder. Physiology & Behavior. 2018;**188**: 205-211

[61] Mu C, Corley MJ, Lee RWY, et al. Metabolic framework for the improvement of autism spectrum disorders by a modified ketogenic diet: A pilot study. Journal of Proteome Research. 2020;**19**:382-390

[62] Ruskin DN, Svedova J, Cote JL, et al. Ketogenic diet improves core symptoms of autism in BTBR mice. PLoS One. 2013;**8**:e65021

[63] Ruskin DN, Murphy MI, Slade SL, et al. Ketogenic diet improves behaviors in a maternal immune activation model of autism spectrum disorder. PLoS One. 2017;**12**:e0171643

[64] Castro K, Baronio D, Perry IS, et al. The effect of ketogenic diet in an animal model of autism induced by prenatal exposure to valproic acid. Nutritional Neuroscience. 2017;**20**(343-350):2017

[65] Pennesi CM, Klein LC. Effectiveness of the gluten-free, casein-free diet for children diagnosed with autism spectrum disorder: Based on parental report. Nutritional Neuroscience. 2012;**15**(2):85-91

[66] Farivar S, Malekshahabi T, Shiari R. Biological effects of low level laser therapy. Journal of Lasers in Medical Sciences. 2014;**5**(2):58-62

[67] Nalamachu S. An overview of pain management: The clinical efficacy and value of treatment. The American Journal of Managed Care. 2013;**19**: s261-s266

[68] Hou PW, Hsu HC, Lin YW, Tang NY, Cheng CY, Hsieh CL. The history, mechanism, and clinical application of auricular therapy in traditional Chinese medicine. Evidence-based Complementary and Alternative Medicine: Ecam. 2015;**2015**:495684

[69] Raith W. Auricular medicine in neonatal care. Medical Acupuncture. 2018;**30**(3):138-140

[70] Binesh M, Daghighi M, Shirazi E, Oleson T, Fataneh Hashem-Dabaghian F. Comparison of auricular therapy with sham in children with attention deficit/hyperactivity disorder: A randomized controlled trial. The Journal of Alternative and Complementary Medicine. 2020;**26**(6):515-520

[71] Wong IV, Cheuk DKL, Chu V. Acupuncture for hypoxic ischemic encephalopathy in neonates (protocol). Cochrane Database of Systematic Reviews. 2009;**3**:CD007968

[72] Ruth M et al. Laser acupuncture for chronic back pain. A double-blind clinical study. Schmerz. 2010;**24**(5): 485-493

[73] Niemtzow RC. Battlefield acupuncture. Medical Acupuncture. 2007;**19**(4):225-228

[74] Reintal M, Andersson S, Gustafsson M, et al. Effects of minimal acupuncture in children with infantile colic—A prospective, quasi-randomised single blind controlled trial. Acupuncture in Medicine. 2008;**26**(3): 171-182

[75] Schlager A et al. Laser stimulation of acupuncture point P6 reduces postoperative vomiting in children undergoing strabismus surgery. British Journal of Anaesthesia. 1998;**81**:529-532

[76] Jindhal V et al. Safety and efficiency of acupuncture in children: A review of the evidence. Journal of Pediatric Hematology/Oncology. 2008;**30**(6): 431-442

[77] Wu S et al. Using acupuncture for acute pain in hospitalized children. Pediatric Critical Care Medicine. 2009;**10**(3):291-296

[78] Gottschling S et al. Laser acupuncture in children with headache: A double-blind, randomized, bicenter, placebo-controlled trial. Pain. 2008;**137**(2):405-412

[79] Freitas AC et al. Assessment of anti-inflammatory effect of 830nm laser light using c-reactive protein levels. Brazilian Dental Journal. 2001;**12**(3): 187-190

[80] Lin F, Josephs SF, Alexandrescu DT, Ramos F, Bogin V, Gammill V, et al. Lasers, stem cells, and COPD. Journal of Translational Medicine. 2010;**8**:16

[81] Zein R, Wayne S, Michael R, Hamblin MR. Review of light parameters and photobiomodulation efficacy: Dive into complexity. Journal of Biomedical Optics. 2018;**23**(12): 120901

[82] McKee MD, Kligler B, Fletcher J, Biryukov F, Casalaina W, Anderson B, et al. Outcomes of acupuncture for chronic pain in urban primary care. Journal of American Board of Family Medicine. 2013;**26**:692-700

[83] Fulop AM, Dhimmer S, Deluca JR, Johanson DD, Lenz RV, Patel KB, et al. A meta-analysis of the efficacy of laser phototherapy on pain relief. The Clinical Journal of Pain. 2010;**26**:729-736

[84] Chow RT, David MA, Armati PJ. 830 nm laser irradiation induces varicosity formation, reduces mitochondrial membrane potential and blocks fast axonal flow in small and medium diameter rat dorsal root ganglion neurons: Implications for the analgesic effects of 830 nm laser. Journal of the Peripheral Nervous System. 2007;**12**:28-39

[85] Weber M. Laser-needle therapy: An innovation in acupuncture. Natura. 2002;**17**(10):33-41

[86] Kurath-Koller S et al. Changes of locoregional skin temperature in neonates undergoing laser needle acupuncture at the acupuncture point large intestine. Evidence-based Complementary and Alternative Medicine. 2015;**2015**:571857

[87] Raith W et al. Near-infrared spectroscopy for objectifying cerebral effects of laser acupuncture in term and preterm neonates. Evidence-based Complementary and Alternative Medicine. 2013;**2013**:346852

[88] Raith W et al. Thermographic measuring of the skin temperature using laser needle acupuncture in preterm neonates. Evidence-based Complementary and Alternative Medicine. 2012;**2012**:614210

[89] Raith W et al. Active ear acupuncture points in neonates with neonatal abstinence syndrome (NAS). The American Journal of Chinese Medicine. 2011;**39**:29

[90] Raith W, Kutschera J, Müller W, et al. Ear acupuncture points in neonates with neonatal abstinence syndrome due to maternal substitution therapy. Zeitschrift für Geburtshilfe und Neonatologie. 2010;**214**(3):103-107

[91] Raith W, Pichler G, Zotter H, et al. Detection of psychic ear acupuncture points in a newborn infant with neonatal abstinence syndrome. Journal of Alternative and Complementary Medicine. 2010;**16**(4):345-346

[92] Raith W, Schmölzer GM, Resch B, et al. Laser acupuncture as a possible treatment for an agitated infant—A preterm after 28 weeks of gestation. Deutsche Zeitschrift für Akupunktur. 2008;**51**(3):33-36

[93] Smith KC. (1991) The photobiological basis of low level laser radiation therapy. Laser Therapy, 1991; 3: 19-24

[94] Walker J. Relief from chronic pain by low power laser irradiation. Neuroscience Letters. 1983;**43**:339-344

[95] Bischko JJ. Use of the laser beam in acupuncture. Acupuncture & Electro-Therapeutics Research. 1980;**5**:29-40

[96] Siedentopf CM et al. fMRI detects activation of the visual association cortex during laser acupuncture of the foot in humans. Neuroscience Letters. 2002;**327**:53-56

[97] Litcher G et al. Acupuncture using laser needles modulates brain function: First evidence from functional transcranial Doppler sonography and fMRI. Lasers in Medical Science. 2004;**19**:6-11

[98] Ferraresi C, de Sousa MV, Huang YY, Bagnato VS, Parizotto NA, Hamblin MR. Time response of increases in ATP and muscle resistance to fatigue after low-level laser (light) therapy (LLLT) in mice. Lasers in Medical Science. 2015 May;**30**(4): 1259-1267

[99] Enwemeka CS, Parker JC, Dowdy DS, Harkness EE, Sanford LE, Woodruff LD. The efficacy of low-power lasers in tissue repair and pain control: A meta-analysis study. Photomedicine and Laser Surgery. 2004;**22**:323-329

[100] de Freitas LF, Hamblin MR. Proposed mechanisms of photobiomodulation or low-level light therapy. IEEE Journal of Selected Topics in Quantum Electronics. 2016;**22**(3):7000417

[101] Mussttaf RA, Jenkins DFL, Jha AN. Assessing the impact of low level laser therapy (LLLT) on biological systems: A review. International Journal of Radiation Biology. 2019;**95**(2): 120-143

[102] Chow RT, Johnso MI, Lopes-Martins RA, Bjordal JM. Efficacy of low-level laser therapy in the management of neck pain: A systematic review and meta-analysis of randomised placebo or active-treatment controlled trials. Lancet. 2009;**374**:1897-1908

[103] Cidral-Filho FJ, Mazzardo-Martins L, Martins DF, Santos ARS.

Light-emitting diode therapy induces analgesia in a mouse model of postoperative pain through activation of peripheral opioid receptors and the L-arginine/nitric oxide pathway. Lasers in Medical Science. 2014;**29**:695-702

[104] Yamamoto H, Ozaki A, Iguchi N, Kinoshita S. Antinociceptive effect of laser irradiaion on Hoku points in rats. Pain Clinic. 1988;**8**:43-48

[105] Isolan C, Kinalski MD, Leão OA, Post LK, Isolan TM, Dos Santos MB. Photobiomodulation therapy reduces postoperative pain after third olar extractions: A randomized clinical trial. Medicina Oral, Patología Oral y Cirugía Bucal. 2021;**26**(3):e341-e348

[106] Djavid GE, Mehrdad R, Ghasemi M, Hasan-Zadeh H, Sotoodeh-Manesh A, Pouryaghoub G. In chronic low back pain, low level laser therapy combined with exercise is more beneficial than exercise alone in the long term: A randomised trial. The Australian Journal of Physiotherapy. 2007;**53**:155-160

[107] Leal EC, Johnson DS, Saltmache A, Demchak T. Adjunctive use of combination of super-pulsed laser and light-emitting diodes phototherapy on nonspecific knee pain: double-blinded randomized placebo-controlled trial. Lasers in Medical Science. 2014;**29**(6):1839-1847

[108] Gao X, Zhang W, Yang F, Ma W, Cai B. Photobiomodulation regulation as one promising therapeutic approach for myocardial infarction. Oxidative Medicine and Cellular Longevity. 2021;**2021**:9962922

[109] Eroglu B, Genova E, Zhang Q, Su Y, Shi X, Isales C, et al. Photo biomodulation has rejuvenating effects on aged bone marrow mesenchymal stem cells. Scientific Reports. 2021;**11**(1):13067

[110] Avci P, Gupta A, Sadasivam M, Vecchio D, Pam Z, Pam N, et al. Low-level laser (light) therapy (LLLT) in skin: Stimulating, healing, restoring. Seminars in Cutaneous Medicine and Surgery. 2013;**32**(1):41-52

[111] Amaroli A, Ravera S, Baldini F, Benedicenti S, Panfoli I, Vergani L. Photobiomodulation with 808-nm diode laser light promotes wound healing of human endothelial cells through increased reactive oxygen species production stimulating mitochondrial oxidative phosphorylation. Lasers in Medical Science. 2018;**34**(3):495-504

[112] Khan I, Rahman SU, Tang E, et al. Accelerated burn wound healing with photobiomodulation therapy involves activation of endogenous latent TGF-β1. Scientific Reports. 2021;**11**:13371

[113] Amini A, Soleimani H, Rezaei F, Ghoreishi SK, Chien S, Bayat M. The combined effect of photobiomodulation and curcumin on acute skin wound healing in rats. Journal of Lasers in Medical Sciences. 2021;**12**:e9

[114] Poiani G, Zaninotto AL, Carneiro A, Zangaro RA, Salgad A, Parreira RB, et al. Photobiomodulation using low-level laser therapy (LLLT) for patients with chronic traumatic brain injury: A randomized controlled trial study protocol. Trials. 2018;**19**(1):17

[115] Shah EJ, Hüttemann M, Sanderson TH, Gurdziel K, Ruden DM. Inhibiting mitochondrial cytochrome *c* oxidase downregulates gene transcription after traumatic brain injury in Drosophila. Frontiers in Physiology. 2021;**12**:628777

[116] Zhang Q , Ma HY, Nioka S, Chance B. Study of near infrared technology for intracranial hematoma detection. Journal of Biomedical Optics. 2000;**5**:206-213

[117] Ahmed SA, Ghoneim DF, Morsy ME, Hassan AA, Mahmoud A.

Low-level laser therapy with 670 nm alleviates diabetic retinopathy in an experimental model. Journal of Current Ophthalmology. 2021;**33**(2): 143-151

[118] Tam SY, Tam VCW, Ramkumar S, Khaw ML, Law HKW, Lee SWY. Review on the cellular mechanisms of low-level laser therapy use in oncology. Frontiers in Oncology. 2020;**10**:1255

[119] Zhang Q, Zhou C, Hamblin MR, Wu MX. Low-level laser therapy effectively prevents secondary brain injury induced by immediate early responsive gene X-1 deficiency. Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism. 2014;**34**(8):1391-1401

[120] Osipov AN, Machneva TV, Buravlev EA, Vladimirov YA. Effects of laser radiation on mitochondria and mitochondrial proteins subjected to nitric oxide. Frontiers in Medicine. 2018;**5**:112

[121] Ferraresi C, Kaippert B, Avci P, Huang YY, de Sousa MV, Bagnato VS, et al. Low-level laser (light) therapy increases mitochondrial membrane potential and ATP synthesis in C2C12 myotubes with a peak response at 3-6 h. Photochemistry and Photobiology. 2015;**91**(2):411-416

[122] Antunes F, Boveris A, Cadenas E. On the mechanism and biology of cytochrome oxidase inhibition by nitric oxide. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:16774-16779

[123] Khuman J, Zhang J, Park J, Carroll JD, Donahue C, Whalen MJ. Low level laser light therapy improves cognitive deficits and inhibits microglial activation after controlled cortical impact in mice. Journal of Neurotrauma. 2012;**29**:408-417

[124] Huang YY, Gupta A, Vecchio D, de Arce VJ, Huang SF, Xuan W, et al. Transcranial low level laser (light) therapy for traumatic brain injury. Journal of Biophotonics. 2012;**5**:827-837

[125] Karu TI, Pyatibrat LV, Kolyakov SF, Afanasyeva NI. Absorption measurements of a cell monolayer relevant to phototherapy: Reduction of cytochrome c oxidase under near IR radiation. Journal of Photochemistry and Photobiology B: Biology. 2005;**81**: 98-106

[126] Chen J, Yu S, Fu Y, Li X. Synaptic proteins and receptors defects in autism spectrum disorders. Frontiers in Cellular Neuroscience. 2014;**8**:276

[127] Thunshelle C, Hamblin MR. Transcranial low-level laser (light) therapy for brain injury. Photomedicine and Laser Surgery. 2016;**34**:587-598

[128] Vojdani A, Vojdani E, Kharrazian D. Reaction of human monoclonal antibodies to SARS-CoV-2 proteins with tissue antigens: Implications for autoimmune diseases. Frontiers in Immunology. 2021;**11**: 617089

[129] Brandes BL. Cerebral palsy research with low level laser therapy. 2010. Available from: http://rehab-med.blogspot.co.il/2010/09/cerebral-palsy-research-with-low-level.html

[130] Pieczenik SR, Neustadt J. Mitochondrial dysfunction and molecular pathways of disease. Experimental and Molecular Pathology. 2007;**83**(1):84-92