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Research Article

Clinical Improvements in Autism Spectrum Disorder with Novel Pulsatile Insulin Therapy

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Abstract

Background: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition often associated with underlying mitochondrial dysfunction, leading to impaired cellular energy metabolism. Emerging evidence suggests that insulin plays a key role in mitochondrial regulation and BIONICA-MDI (pulsatile insulin therapy) may offer a novel therapeutic pathway.

Methods: Twenty patients (n= 20) with clinically diagnosed ASD were enrolled. Baseline assessments included clinical features and developmental milestone scales. Patients received BIONICA-MDI therapy, a modality designed to mimic physiologic pulsatile insulin release. Post- therapy evaluations measured changes in clinical symptoms and behavioural domains. Statistical analyses were performed to assess treatment response.

Findings: The improvement was linear in nature and was not strongly dependent on the sessions attended by the patients (p=0.413). This indicates that even a limited number of sessions may confer therapeutic benefit. BIONICA-MDI therapy was associated with improvements in behavioural parameters, attention span, social interaction and reduction in hyperactivity. No major adverse effects were reported, highlighting the safety and tolerability of the intervention.

Conclusion: This preliminary study suggests that BIONICA-MDI improves cellular activities and clinical outcomes in patients with ASD. By targeting a fundamental cellular pathway, this approach holds promise as a novel adjunctive therapy for autism. Larger, controlled studies would be accepted to confirm these findings.

Keywords: Autism Spectrum Disorder; Mitochondrial Dysfunction; Pulsatile Insulin Therapy; BIONICA-MDI; Regenerative Medicine; Carbohydrate and Lipid Metabolism

Abbreviations

ASD: Autism Spectrum Disorder; ATP: Adenosine Triphosphate; DDM: Delayed Development Milestone; DNA: Deoxyribose Nucleic Acid; ETC: Electron Transport Chain; FADH: Flavin Adenine Dinucleotide + Hydrogen; FFA: Free Fatty Acid; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; LHRH: Gonadotrophin- Releasing Hormone; LPO: Lipid Peroxidation; NADH: Nicotinamide Adenine Dinucleotide + Hydrogen; ROS: Reactive Oxygen Species; SD: Standard Deviation; SPSS: Statistical Package for the Social Sciences; T2DM: Type 2 Diabetes Mellitus; TCA: Tri Carboxylic Acid

Introduction

Autism Spectrum Disorder (ASD) is a concept coined in 1911 by the German psychiatrist Eugen Bleuler [1]. ASD is a biologically based neurodevelopmental disorder characterized by persistent deficits in social communication and social interaction, restricted by repetitive patterns of behavior, interests and activities [2,3]. Between 9 months and 3 years of age parents may notice defects in social skills, language skills and behavior. Caregivers may also observe frequent prolonged tantrums often with unclear triggers and intolerance to changes in routines or transition. Parents may also notice loss of skills, for example; not speaking words they once were using, delayed language skills and also developmental regression. Child may be wanting to be alone or lack of joint attention and orientation to name and lack of shared interests. Child does not engage in interactive games or reduced use of gestures or muteness. Spoken language if present may be atypical, for example; unusual presage of speech (rate, rhythm, tone, volume), echolalia and non- specific like vocalization. Reduced, absent or atypical nonverbal communications (for example; eye contact, gestures, facial expression, body orientation) may be present [3]. Poor understanding or following of social conventions (for example; greetings, farewell behaviors). Repititve or stereotypical motor and vocal mannerism, for example; hand tapping, rocking behavior, echolalia. Extremes of extended reactivity that are excessive for circumstances. Aberrant responses to sensory stimuli (over and under sensitive), for example; excessive touching people or objects, preferred to be in the dark, deliberately smelling objects [4].

Autism is a collection of neurodevelopmental disorders characterized by repetitive and stereotyped behaviors, as well as differing degrees of difficulty in social interaction, communication, attention, cognition, learning, speech and sensory processing issues [5,6]. Over time, the prevalence of ASD has risen steadily [7,8]. The incidence of autism is seen to be one in 127 people worldwide in 2021 and that the prevalence and health burden persisted throughout life [9]. About 1 in 31 (3.2%) children aged 8 years has been identified with ASD according to estimates from CDC's ADDM Network [10]. There is a 4:1 prevalence ratio of ASD in males compared to females [11].

The human brain, weighs 2% body weight approximately and consumes 20% of the whole body's oxygen. Most of the mitochondrial oxygen is utilized for Adenosine Triphosphate (ATP) production by the mitochondrial Electron Transport Chain (ETC) through Oxidative Phosphorylation (OXPHOS) [12,13]. Importantly, 93% of the ATP necessary for normal brain functioning is supplied by mitochondria (14). This energy supports synaptic transmission, which is a very energy- demanding process. ATP provides energy for ion pumps, supporting ion gradients, to ensure vesicle recycling and mitochondrial motility [13]. The ATP production is a chain process where it begins with glycolysis followed by the conversion of pyruvate to oxaloacetate in Tricarboxylic Acid (TCA) cycle from which the proton gradient Nicotinamide Adenine Dinucleotide + Hydrogen (NADH) and Flavin Adenine Dinucleotide + Hydrogen (FADH2) is maintained in the mitochondrial complexes (IeV) by two-electron carriers; ubiquinone and cytochrome c [15,16]. This addresses the brain's high energy needs, as neuronal cells consume 4.7 billion ATP molecules every second [16,17].

The primary cause of oxidative cell damage and a contributing factor to autism may be the brain's and neuron's high energy requirements [16].

Mitochondria are multifaceted organelles involved in many cellular functions and play a crucial role in ASD pathophysiology [18]. Recent studies have demonstrated the fundamental involvement of mitochondria in the maturation of brain neurons [19,20]. The state-of-the-art current research regards ASD as a systemic disorder due to intertwined aberrations in immune-inflammatory pathways, mitochondrial and gastrointestinal functions and the impact of environmental and epigenetic factors [21-25]. The metabolic profiles of individuals with autism indicate a potential involvement of the pathways of mitochondria [26].

Mitochondria are the only cellular organelle possessing own genome; however, their functions also depend on the nuclear genes; thus, mitochondrial disorders may be caused by defects in both nuclear and mitochondrial Deoxyribose Nucleic Acid (DNA) [27,28].

Several reports have indicated accrued levels of different Lipid Peroxidation (LPO) markers in autism, confirming a rise in oxidative stress in autism [16,29]. Oxidative stress results from dysfunction in mitochondria, specifically deficits in the respiratory chain complexes, which could increase Reactive Oxygen Species (ROS) levels and cause an imbalance of ROS production or antioxidant defenses [30]. Additionally, the same study reported significantly decreased activity of antioxidant enzyme superoxide dismutase, that has a crucial role in the defense mechanisms against ROS, in the set of patients with autism [30,31]. The outcome from this study have supported others researches that have found the link between dysfunction in

mitochondria, abnormal levels of pyruvate and lactate with ASD signifying that the abnormal activity of pyruvate dehydrogenase may be the reason for the elevated level of metabolites in autism [30,32,33].

Mitochondria Dysfunction Due To Impaired Insulin and B-Cell Failure

Several studies suggest that neurodevelopmental regression is associated with mitochondrial dysfunction, specifically in patients who are autistic [34-36]. In neural cells, mitochondrial dysfunction is associated with heightened production of ROS [20,37]. Decreased glucose metabolism can be a marker causing a gap in energy that affects mitochondrion while gestation and post birth [38,39]. The detection of persistent neuroinflammation in post-mortem brain tissues from individuals with ASD ranging in age from 4 to 45 years has been an important finding in ASD research [40-43].

Current researches have suggested that the failure of β-cell might correspond with the increase in insulin resistance and failure of β-cell might be the reason of insulin resistance [44,45]. For around 5-min cycles, insulin is infused into the portal vein in a pulsatile pattern [46,47]. Up to 70% of the total amount of insulin secretion in the basal state, may be due to insulin pulses [46]. Intracellular Ca^{2+} oscillations have an intrinsic rhythm controlling pulsatile β-cell secretion pattern [48]. Insulin and somatostatin production are anti-synchronous with pulsatile glucagon secretion [47]. It results into 20-fold variations of insulin-to-glucagon ratio in portal vein that can be crucial for a short duration for controlling the hepatic glucose metabolism. With the advancing insulin resistance, insulin is insufficient to meet the requirement and β-cell dysfunction will emerge. C-peptide is a better measure of portal insulin secretion than insulin itself [49]. The reduced C-peptide level at 30 minutes indicated that there might be pancreatic β-cell dysfunction in patients with autism [50]. The surviving β-cells generate higher levels of insulin, to make it up for the lost β-cell mass, nonetheless the pulsatile secretion pattern has altered so that the insulin pulses are noted as decreased [46]. Change in insulin secretion has various consequences, like the impacts on the autocrine regulation of secretion of insulin, elevated plasma levels of uncleaved proinsulin and progress of hepatic insulin resistance in both animals and humans [51,52]. Furthermore, it spears to be that more insulin eludes the hepatic retention, causing higher levels of peripheral insulin [53]. It has long been known that plasma levels of insulin vary rhythmically and that this periodicity reflects the release of the hormone in a pulsatile pattern through the pancreas. Results in a periodic depolarization with influx of Ca^{2+} in the Langerhans islets' β-cells. Within the islets, cells have an intrinsic rhythmicity which is coordinated by both cells contact and through extracellular messengers, primarily ATP. The adaptation of the different islets of Langerhans in pancreas to the same oscillatory phase occurs via nerve impulses from autonomic ganglia [54]. These results offer a firm truth that the loss of pulsatile insulin secretion develops as a result of intrahepatic molecular alterations and changes in gene expression constantly with the developing hepatic insulin resistance impaired insulin secretion and inflammatory processes causing ASD [53].

Pulsatile Insulin Therapy for Autism Spectrum Disorder

BIONICA-MDI (pulsatile insulin therapy) is a newer mode of delivery and works differently. It aims to more closely stimulate the function of a healthy pancreas, which delivers insulin from the pancreatic Beta cell to the circulation in a pulsatile manner that varies with levels of glucose into bloodstream. The objective of BIONICA-MDI is to synchronize the timing and pulsed levels/quantities of insulin secretion, imitating the way healthy pancreas work. These pulses are in approximate 5-6 min intervals and adjust insulin dosage to the glucose levels in the patient's blood [51,52]. It was found that pulsatile insulin therapy could change hepatic glucose metabolism preferring carbohydrate metabolism over that of lipid, which lowers Free Fatty Acids (FFA) that promotes inflammatory responses in other areas [52,53].

Using BIONICA-MDI, we aim to study its efficacy in treating mitochondrial dysfunction in autism patients and health status reported by patients using Delayed Development Milestone (DDM) scale. Our goal is to investigate the association between patients' number of weeks in BIONICA- MDI treatment (differences between baseline and previous or most recent treatment) and DDM scale score results.

Our study for 3 weeks to 3 months focuses on clinical measures (DDM scale scores). This investigation is a rare study that uses surveys by patients' reporting health and better quality of life. Similar to the findings of Dong, et al., and Elliott, et al., patients have testified significant improvement in health post the 12 week course of BIONICA-MDI treatment (Fig. 1) [51-53].

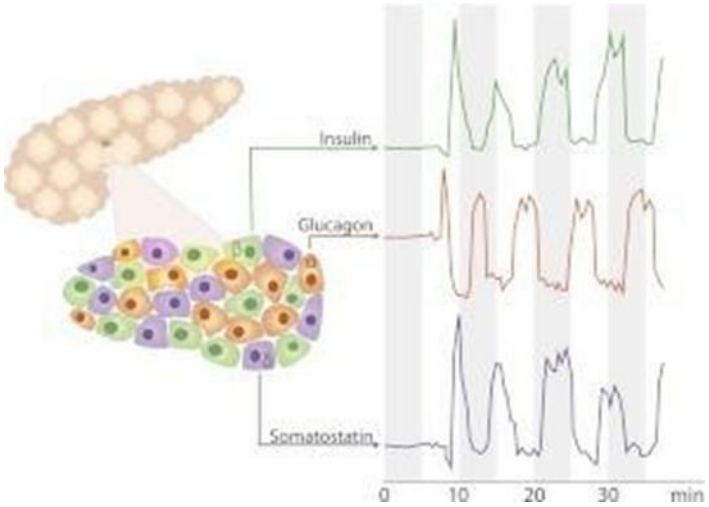

Figure 1: Schematic depiction of the pulsatile character and temporal associations of insulin, glucagon and somatostatin secreted by glucose stimulated human β-cells [53].

Furthermore, pulsatile insulin delivery was associated with a decent reduction in levels of plasma glucose, while both the continuous infusion and imitation of pulsatile infusion results in elevated levels of plasma glucose. Loss of insulin pulsatility and β-cell dysfunction during fasting, could not only be opening events, triggering development of hepatic insulin resistance and autism [55]. The insulin secretion pattern seems to impact blood glucose regulation as well as tissue insulin resistance [53].

Hepatic insulin signaling and glycemic control depend on the natural pulsatile pattern of insulin delivery. A complementary implication is that pulsatile insulin administration may determine the hepatic actions of insulin, e.g., in suppressing hepatic glucose release [56]. Hepatocytes are subjected to an oscillating insulin concentration with an increase in amplitude of 0.5-1.0 nmol/L during fasting to 5.0 nmol/L post meal ingestion [57,58]. The vascular anatomy of hepatic sinusoids allows direct contact of hepatocytes to oscillations of insulin disrupted when insulin is administered in a nonpulsatile manner or when Type 2 Diabetes Mellitus (T2DM) results in defects in insulin secretion [59].

This is analogous to Gonadotrophin- Releasing Hormone (LHRH) having a greater effect with pulsatile release than with steady levels [60,61]. Receptors become less sensitive to hormonal stimuli when exposed to high steady concentrations; thus, oscillatory hormonal stimuli could help to maintain receptor integrity [62-64].

Post 3 months, 47.5% of patients conveyed resolution of symptoms completely, 45.5% stated that their symptoms were improved and 7% stated no change in symptoms [65]. Thus, this advancement is a viable treatment option for neurodevelopmental disorders.

Insulin receptor expression was demonstrated to increase significantly in conjunction with oscillatory insulin release [66]. These patterns may lead to down-regulation of insulin receptors, that is influential because of the presence of insulin receptors on beta cell [67].

Our study aims in evaluating effects of BIONICA-MDI therapy and its clinical outcomes in individuals with ASD.

Methodology

Study Design

This was an interventional study evaluating the impact of BIONICA-MDI sessions on patients diagnosed with ASD.

Participants/Subjects

A total of twenty patients (n = 20) with a confirmed diagnosis of ASD were involved in the study.

Inclusion and Exclusion Criteria

The study included patients with Autism Spectrum Disorder. Patients other than ASD like Alzheimer's, Parkinsons, ADHD etc were excluded from this study.

Data Collection

The Delayed Developmental Milestone Scale was used to assess developmental progress in participants throughout the intervention.

Data Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) [68]. The Shapiro-Wilk test was applied to confirm that the data met the assumption of normal distribution. A repeated measures ANOVA was conducted to assess the effect of time on autism scores. Additionally, the relationship between the number of BIONICA-MDI sessions and changes in autism scores was analysed. Two main comparisons were carried out:

1. Changes in autism severity scores over time
2. Frequency of BIONICA-MDI sessions in relation to improvements in autism scores

Results

Autism Severity Score

Descriptive statistics indicated a progressive reduction in autism severity scores following BIONICA-MDI therapy. The mean score at baseline (Pre-treatment) was 123.90 (Standard Deviation, SD = 29.71), which decreased to 110.35 (SD = 24.68) immediately post-intervention and further declined to 97.80 (SD = 29.78) at the 3-month follow-up.

A Shapiro-Wilk test confirmed that the data met the assumption of normality ($p > 0.05$ for all time points).

A repeated measures ANOVA revealed a statistically significant effect of time on autism scores, $F(1,19) = 48.35$, $p < 0.001$, with a large effect size ($\eta^2 = 0.718$). This indicates that BIONICA- MDI therapy was associated with a steady and clinically meaningful reduction in autism severity across the study period (Fig. 2).

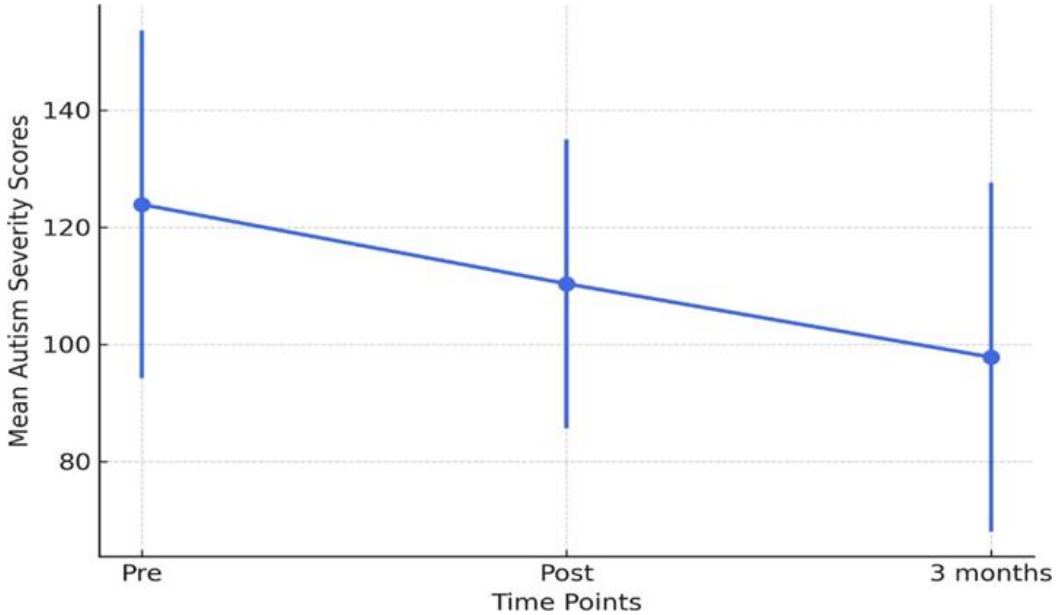

Figure 2: Change in Autism Severity Scores Over time (BIONICA-MDI Therapy): The line graph shows mean autism scores at baseline (Pre-treatment), immediately post- intervention and at 3 months follow-up. A clear linear decline in scores was observed, indicating sustained and meaningful clinical development in autism severity following BIONICA-MDI therapy.

The improvement was linear in nature, with no evidence of plateau or reversal (quadratic p = 0.792). Correlation with Frequency of BIONICA-MDI Session Pearson correlation analysis between the frequency of BIONICA-MDI sessions and change in autism severity (Pre-Post 3-months difference) revealed a weak positive but non-significant relationship, r (20) = 0.194, p = 0.413 (Fig. 3).

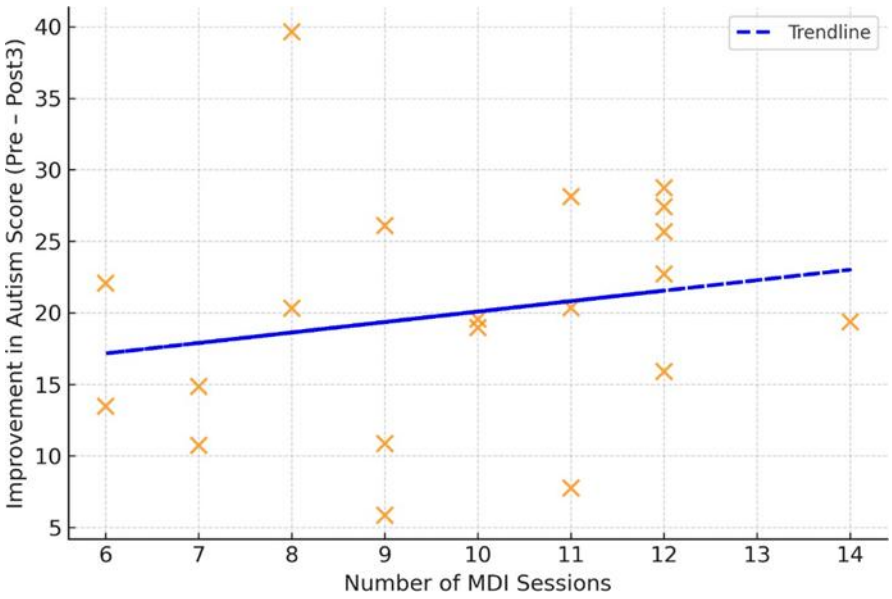

Figure 3: Frequency Of BIONICA-MDI Sessions vs. Improvement in Autism Score. Each point represents an individual patient, with the dashed line indicating the trend. Although a weak positive trend was observed, the correlation was not significant (r=0.194, p=0.413), suggesting the improvement in autism scores was not strongly dependent on the number of sessions attended.

This suggests that the degree of improvement was not strongly dependent on the number of sessions attended. The repeated measures ANOVA revealed a significant effect of time on autism scores, F (1,19) = 48.35, $p < 0.001$, with a large effect size (η^2 = 0.718). This indicates that BIONICA-MDI therapy led to a steady and meaningful clinical development in autism-related outcomes. The improvement followed a linear trend without evidence of plateau or reversal, suggesting that the benefits of BIONICA-MDI were sustained across the study period. These results align with prior evidence that metabolic modulation, particularly targeting mitochondrial and oxidative stress pathways, can contribute to symptomatic improvement in ASD.

Importantly, the descriptive data showed a progressive decline in mean scores from 123.9 at baseline to 110.3 post-intervention and 97.8 at three months, highlighting not only statistical but also clinical significance. Such reductions are particularly relevant given the high variability in therapeutic responsiveness typically observed in ASD populations.

Interestingly, correlation analysis revealed no significant relationship between the frequency of BIONICA-MDI sessions and the magnitude of improvement (r = 0.194, p = 0.413). This suggests that even a limited number of sessions may confer therapeutic benefit. The mechanism may therefore rely more on the biological modulation of cellular energy metabolism and insulin signalling rather than the cumulative number of sessions. This observation is clinically valuable, as it indicates that meaningful improvement may be achievable without requiring intensive or frequent interventions, thus enhancing the feasibility of BIONICA-MDI as a supportive therapy in ASD.

The longer the patients were in treatment, they experienced improvements in lab values and self-reported measures equally. We observed changes in patients in 3 weeks and 3 months. Given the encouraging primary outcome of this study, further research is necessary. With the development in pulsatile insulin therapy brought by BIONICA-MDI and initial signs of its effectiveness, with the right timing and in-dept research the patients can achieve better outcomes. BIONICA-MDI's comparative effectiveness vis-à-vis traditional treatments, are cost effective.

The present study evaluated the effects of BIONICA MDI on ASD severity, with a focus on changes in autism scale scores across baseline, post-intervention and three-month follow-up. Our findings demonstrate a significant and consistent reduction in autism severity scores over time, supporting the potential of BIONICA-MDI in ASD.

Discussion

In autistic patients, HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) may show decrease of glucose metabolism in certain areas of the brain in comparison to healthy individuals [69,70]. Uptake of glucose in neurons is determined by the energy uptake and not based on the levels of plasma glucose, indicating that glucose uptake in neurons does not increase due to hyperglycemia [38]. Decrease in glucose uptake may correspondingly disrupt the connections found in ASD patients. Mostly patients with autism have low levels of connectivity (or hypo-connectivity) within distant regions of the brain (like the frontal and parietal lobes) and elevated levels of connectivity (or hyper-connectivity) within the local regions of the brain (like in the frontal lobe) in comparison to the normal individuals that are developing [39,71].

Insulin delivered in pulsatile pattern markedly reduces hepatic glucose production in comparison to continuous insulin delivery, possibly due to elevated insulin sensitivity by limiting the downregulation of hepatic insulin receptors [72]. Reduced variation in the amplitude of peripheral insulin pulses was led by an elevated level of hepatic insulin extraction found along with higher amplitude of insulin pulses than decreased insulin pulses or constant infusion of insulin [56]. The findings indicated that this kind of pulsed insulin infusion has a significant result on carbohydrate metabolism, surpassing the decrease in the resting metabolic rate observed along with traditional subcuticular insulin treatment. Such significant results are due to the failure to metabolize carbohydrates properly that characterizes an essential dysfunction in autism patients. Individuals with autism escape from the effects of increased levels of free fatty acids, that trigger a pathway of inflammatory processes in the brain, by the transition of energy production to carbohydrate metabolism, comparative to lipids.

In this investigative study, we evaluated that BIONICA-MDI (pulsatile insulin therapy) is effective in treating ASD. Pulsatile insulin therapy, also called as BIONICA-MDI, comprises of bursts of pulsatile intravenous infusion of insulin at recurring durations of 6 mins for 3 hours [73]. It helps to bring back insulin levels to the normal physiological level. This can occur through stimulation of the pulsatile component in hormone release from existing β-cells and rhythmic delivery of insulin towards the portal vein [54].

The normal pancreatic stimulation of the liver (with insulin) is mimicked by BIONICA-MDI; which is important to release the enzymes that facilitate ATP production in every organ of the body. It results in normalized carbohydrate and lipid metabolism. It stops and reverses complications by reducing the damage from high lipid metabolism and restoring missing cellular energy. Suppressing the free fatty acid reduces the byproducts of improperly high lipid metabolism [73]. Inhibiting the proinflammatory cytokines improves the retention of nitric oxide, suppresses inflammatory repair (growth) factors, upregulates metabolic enzymes of glycolysis and the Krebs cycle, provides additional ATP [73]. Pulsatile Bursts of Insulin maintains peripheral insulin receptor activity and peripheral glucose uptake [54].

The integration of BIONICA-MDI (pulsatile insulin therapy) therapy in treating autism patients provided a significant turning point in the overall recovery of the patients. BIONICA-MDI therapy, combined with conventional physiotherapy, intonation therapies and diet facilitated in reducing inflammation and regression, increase in cognition, understanding and speech. This study highlights the potential of pulsatile insulin administration as a transformative adjunct in treating autism as a spectrum. The results are constant with emerging research that positions ASD as a systemic disorder with intertwined metabolic and neuroinflammatory dysregulation. By mimicking physiological pulsatile insulin secretion, BIONICA-MDI (pulsatile insulin therapy) may improve mitochondrial function, reduce oxidative stress and promote neuronal energy balance, thereby addressing fundamental pathophysiological mechanisms in ASD. The crucial part of this treatment was the use of BIONICA-MDI therapy (pulsatile insulin therapy) that significantly improved cellular repair and regeneration of tissues. BIONICA-MDI therapy (pulsatile insulin therapy) enhanced mitochondrial function, improved carbohydrate and lipid metabolism, decreasing brain inflammation and increasing cognition and speech. Its ease of administration also supports greater patient compliance and more effective healthcare delivery.

Limitations

Nevertheless, this study has certain limitations, including a relatively small sample size and the absence of a randomized control group, which restricts the portability of the findings. Additionally, although normality assumptions were met, future studies with larger and more diverse populations are warranted to validate these results. Future studies should also examine the long-term sustainability of improvements and explore potential moderators such as age, baseline severity and genetic background.

Conclusion

BIONICA- MDI (pulsatile insulin therapy) therapy demonstrated a robust and clinically meaningful reduction in autism severity scores, with a large effect size and sustained benefits over time. These findings support BIONICA-MDI (pulsatile insulin therapy) as a promising adjunctive therapeutic strategy in ASD, warranting further investigation through larger controlled trials.

Conflict of Interest

The authors declare no conflicts of interest.

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Informed Consent Statement

Informed Consent was obtained from the participants involved in this study.

Authors Contribution details

Dr. Pradeep Mahajan- Conceptualization, supervision, final manuscript review and approval. Dr. Harsadbhai Patel - Conceptualization, supervision, final manuscript review and approval. Dr. Anilkumar Daxini - Clinical inputs, literature review, critical revision of the manuscript.
Dr. Masira Khan - Data collection, manuscript drafting, contribution to the discussion.
Marilyn Dsouza - Manuscript drafting, literature review, clinical data collection and formatting.
Jyoti Gupta- Statistical data analysis, contribution to discussion.

Financial Disclosure

StemRx Bioscience Solutions, Pvt Ltd; with established expertise in regenerative medicine and translational research, is well-positioned to advance innovative approaches in autism care. Securing funding for this project to support the development of scientifically validated interventions with the potential to significantly improve neurodevelopmental outcomes.

Consent for Publication

Informed consent for publication was obtained from the patients involved in this study, as documented in the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

References

1. Evans B. How autism became autism: The radical transformation of a central concept of child development in Britain. Hist Hum Sci. 2013;26(3):3-31.
2. American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-5. 5th ed. Washington (DC): American Psychiatric Publishing; 2013.
3. Hyman SL, Levy SE, Myers SM. Identification, evaluation and management of children with autism spectrum disorder. Pediatrics. 2020;145(1):e20193447.
4. Centers for Disease Control and Prevention (CDC). Signs and symptoms of autism spectrum disorder. Autism Spectrum Disorder (ASD). 2024. [Last accessed on: October 20, 2025]. https://www.cdc.gov/autism/signs-symptoms/index.html
5. Zhang B, Angelidou A, Alysandratos KD, Vasiadi M, Francis K, Asadi S, et al. Mitochondrial DNA and anti-mitochondrial antibodies in serum of autistic children. J Neuroinflammation. 2010;7:80.
6. Volkmar FR, State M, Klin A. Autism and autism spectrum disorders: diagnostic issues for the coming decade. J Child Psychol Psychiatry. 2009;50(1-2):108-15.
7. Maenner MJ, Warren Z, Williams AR, Amoakohene E, Bakian AV, Bilder DA, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 sites, United States, 2020. MMWR Surveill Summ. 2023;72(2):1-14.
8. Teke H, Balci S, Neselioglu S, Teke S, Erel O, Tamer L, et al. Oxidative stress and dynamic thiol/disulfide homeostasis in autism: A focus

on early childhood. J Mol Neurosci. 2025;75(2).
9. Santomauro DF, Erskine HE, Mantilla AM, Miller PA, Shadid J, Hagins H, et al. The global epidemiology and health burden of the autism spectrum: findings from the Global Burden of Disease Study 2021. Lancet Psychiatry. 2024;12(2).
10. Centers for Disease Control and Prevention (CDC). Data and statistics on autism spectrum disorder. Autism Spectrum Disorder (ASD). 2025. [Last accessed on: October 20, 2025]. https://www.cdc.gov/autism/data-research/index.html
11. Usui N, Kobayashi H, Shimada S. Neuroinflammation and oxidative stress in the pathogenesis of autism spectrum disorder. Int J Mol Sci. 2023;24(6):5487.
12. Hall CN, Klein-Flugge MC, Howarth C, Attwell D. Oxidative phosphorylation, not glycolysis, powers presynaptic and postsynaptic mechanisms underlying brain information processing. J Neurosci. 2012;32(26):8940-51.
13. Khaliulin I, Hamoudi W, Amal H. The multifaceted role of mitochondria in autism spectrum disorder. Mol Psychiatry. 2024.
14. Harris JJ, Jolivet R, Attwell D. Synaptic energy use and supply. Neuron. 2012;75(5):762-77.
15. Rossignol DA, Frye RE. A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. Mol Psychiatry. 2011;17(4):389-401.
16. Balachandar V, Rajagopalan K, Jayaramayya K, Jeevanandam M, Iyer M. Mitochondrial dysfunction: a hidden trigger of autism? Genes Dis. 2020;7(4):511-20.
17. Zhu XH, Qiao H, Du F, Xiong Q, Liu X, Zhang X, et al. Quantitative imaging of energy expenditure in human brain. Neuroimage. 2012;60(4):2107-17.
18. Grabrucker AM, editor. Autism spectrum disorders. Brisbane (AU): Exon Publications. 2021.
19. Khacho M, Harris R, Slack RS. Mitochondria as central regulators of neural stem cell fate and cognitive function. Nat Rev Neurosci. 2018;20(1):34-48.
20. Qiao L, Yang G, Wang P, Xu C. The potential role of mitochondria in the microbiota-gut-brain axis: Implications for brain health. Pharmacol Res. 2024;209:107434.
21. Gevezova M, Sarafian V, Anderson G, Maes M. Inflammation and mitochondrial dysfunction in autism spectrum disorder. CNS Neurol Disord Drug Targets. 2020;19(6):458-65.
22. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: A systematic review and meta-analysis. Mol Psychiatry. 2011;17(3):290-314.
23. Ormstad H, Bryn V, Saugstad OD, Skjeldal O, Maes M. Role of the immune system in Autism Spectrum Disorders (ASD). CNS Neurol Disord Drug Targets. 2018;17(7):489-95.
24. Havdahl A, Niarchou M, Starnawska A, Uddin M, van der Merwe C, Warrier V. Genetic contributions to autism spectrum disorder. Psychol Med. 2021;51(13):1-14.
25. Gevezova M, Sbirkov Y, Sarafian V, Plaimas K, Suratanee A, Maes M. Autistic Spectrum Disorder (ASD)-gene, molecular and pathway signatures linking systemic inflammation, mitochondrial dysfunction, transsynaptic signalling and neurodevelopment. Gene Rep. 2023;30:100646.
26. Citrigno L, Muglia M, Qualtieri A, Spadafora P, Cavalcanti F, Pioggia G, et al. The mitochondrial dysfunction hypothesis in autism spectrum disorders: current status and future perspectives. Int J Mol Sci. 2020;21(16):5785.
27. Legido A, Jethva R, Goldenthal MJ. Mitochondrial dysfunction in autism. Semin Pediatr Neurol. 2013;20(3):163-75.
28. Babinská K, Repiská G, Gvozdjáková A, Kucharská J, Vokálová L, Vidošovičová M, et al. What is the evidence of mitochondrial dysfunction in autism spectrum disorders? Act Nerv Super Rediviva. 2017;59(2):217-8.
29. Ji L, Chauhan A, Wegiel J, Essa MM, Chauhan V. Gelsolin is proteolytically cleaved in the brains of individuals with Alzheimer's disease. J Alzheimers Dis. 2009;18(1):105-11.
30. Siddiqui MF, Elwell C, Johnson MH. Mitochondrial dysfunction in autism spectrum disorders. Autism Open Access. 2016;6(4):1-8.
31. Tang G, Gutierrez Rios P, Kuo SH, Akman HO, Rosoklija G, Tanji K, et al. Mitochondrial abnormalities in temporal lobe of autistic brain. Neurobiol Dis. 2013;54:349-61.
32. Weissman JR, Kelley RI, Bauman ML, Cohen BH, Murray KF, Mitchell RL, et al. Mitochondrial disease in autism spectrum disorder patients: A cohort analysis. PLoS One. 2008;3(11):e3815.
33. Oliveira G, Diogo L, Grazina M, Garcia P, Ataíde A, Marques C, et al. Mitochondrial dysfunction in autism spectrum disorders: A population-based study. Dev Med Child Neurol. 2005;47(3):185-9.
34. Edmonds JL, Kirse DJ, Kearns DB, Deutsch R, Spruijt L, Naviaux RK. The otolaryngological manifestations of mitochondrial disease and the risk of neurodegeneration with infection. Arch Otolaryngol Head Neck Surg. 2002;128(4):355-5.
35. Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: A systematic review and meta-analysis. Mol Psychiatry. 2011;17(3):290-314.
36. Shoffner J, Hyams L, Langley GN, Cossette S, Mylacraine L, Dale J, et al. Fever plus mitochondrial disease could be risk factors for autistic regression. J Child Neurol. 2009;25(4):429-34.
37. Zhao W, Xu Z, Cao J, Fu Q, Wu Y, Zhang X, et al. Elamipretide (SS-31) improves mitochondrial dysfunction, synaptic and memory impairment induced by lipopolysaccharide in mice. J Neuroinflammation. 2019;16(1):239.

38. Cunnane SC, Trushina E, Morland C, Prigione A, Casadesus G, Andrews ZB, et al. Brain energy rescue: an emerging therapeutic concept for neurodegenerative disorders of ageing. Nat Rev Drug Discov. 2020;19(9):609-33.
39. Manco M, Guerrera S, Ravà L, Ciofi Degli Atti M, Di Vara S, Valeri G, et al. Cross-sectional investigation of insulin resistance in youths with autism spectrum disorder: any role for reduced brain glucose metabolism? J Diabetes Clin Res. 2021;1(2).
40. Goldenthal MJ, Damle S, Sheth S, Shah N, Melvin J, Jethva R, et al. Mitochondrial enzyme dysfunction in autism spectrum disorders: A novel biomarker revealed from buccal swab analysis. Biomark Med. 2015;9(10):957-65.
41. Showkat UN, Rehman MU, Arafah A, Taifa S, Khan IS, Khan A, et al. Treatment of autism spectrum disorders by mitochondrial-targeted drug: Future of neurological diseases therapeutics. Curr Neuropharmacol. 2023;21(5):1042-64.
42. Abbas AK, Lichtman AH, Pillai S. Cellular and molecular immunology. 9th ed. Philadelphia: Elsevier Health Sciences; 2017.
43. Bantug GR, Galluzzi L, Kroemer G, Hess C. The spectrum of T-cell metabolism in health and disease. Nat Rev Immunol. 2017;18(1):19-34.
44. Leahy JL, Hirsch IB, Peterson KA, Schneider D. Targeting beta-cell function early in the course of therapy for type 2 diabetes mellitus. J Clin Endocrinol Metab. 2010;95(9):4206-16.
45. Schofield CJ, Sutherland C. Disordered insulin secretion in the development of insulin resistance and type 2 diabetes. Diabet Med. 2012;29(8):972-9.
46. Porksen N, Munn S, Steers J, Vore S, Veldhuis J, Butler P. Pulsatile insulin secretion accounts for 70% of total insulin secretion during fasting. Am J Physiol Endocrinol Metab. 1995;269(3):E478-88.
47. Hellman B, Salehi A, Gylfe E, Dansk H, Grapengiesser E. Glucose generates coincident insulin and somatostatin pulses and antisynchronous glucagon pulses from human pancreatic islets. Endocrinology. 2009;150(12):5334-40.
48. Gylfe E, Grapengiesser E, Hellman B. Propagation of cytoplasmic Ca^{2+} oscillations in clusters of pancreatic β-cells exposed to glucose. Cell Calcium. 1991;12(2-3):229-40.
49. Shapiro E, Hartmut T, Rubenstein A, Polonsky K. Peripheral insulin parallels changes in insulin secretion more closely than C-peptide after bolus intravenous glucose administration. J Clin Endocrinol Metab. 1988;67(5):1094-9.
50. Zhang M, Ding R, Wang Z, Zhang J, Liu J, Wang J. Altered peak C-peptide and fasting blood glucose in children with autism spectrum disorder. J Diabetes Clin Res. 2019;1(2).
51. Stapelfeldt W, Bender H, Schusdziarra V, Pfeiffer EF. Effect of continuous and oscillatory portal vein insulin infusion upon glucose-induced insulin release in rats. Res Exp Med. 1984;184(1):67-71.
52. Temple R, Luzio S, Schneider A, Carrington C, Owens D, Sobey W, et al. Insulin deficiency in non-insulin-dependent diabetes. Lancet. 1989;333(8633):293-5.
53. Wahren J, Kallas Å. Loss of pulsatile insulin secretion: A factor in the pathogenesis of type 2 diabetes? Diabetes. 2012;61(9):2228-9.
54. Hellman B, Gylfe E, Grapengiesser E, Dansk H, Salehi A. Insulin oscillations-clinically important rhythm. Lakartidningen. 2007;104(32-33):2236-9.
55. O'Rahilly S, Turner RC, Matthews DR. Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes. N Engl J Med. 1988;318(19):1225-30.
56. Meier JJ, Veldhuis JD, Butler PC. Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans. Diabetes. 2005;54(6):1649-56.
57. Song SH, McIntyre SS, Shah H, Veldhuis JD, Hayes PC, Butler PC. Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. J Clin Endocrinol Metab. 2000;85(12):4491-9.
58. Pørksen N, Munn S, Steers J, Veldhuis JD, Butler PC. Effects of glucose ingestion versus infusion on pulsatile insulin secretion: The incretin effect is achieved by amplification of insulin secretory burst mass. Diabetes. 1996;45(10):1317-23.
59. Matveyenko AV, Liuwantara D, Gurlo T, Kirakossian D, Dalla Man C, Cobelli C, et al. Pulsatile portal vein insulin delivery enhances hepatic insulin action and signaling. Diabetes. 2012;61(9):2269-79.
60. Belchetz P, Plant T, Nakai Y, Keogh E, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. Science. 1978;202(4368):631-3.
61. Davies TF, Gomez-Pan A, Watson MJ, Mountjoy CQ, Hanker JP, Besser GM, et al. Reduced gonadotroph response to releasing hormone after chronic administration to impotent men. Clin Endocrinol. 1977;6(3):213-8.
62. Pezzino V, Vigneri R, Pliam NB, Goldfine ID. Rapid regulation of plasma membrane insulin receptors. Diabetologia. 1980;19(3):211-5.
63. Insel JR, Kolterman OG, Saeko M, Olefsky JM. Short-term regulation of insulin receptor affinity in man. Diabetes. 1980;29(2):132-9.
64. Matthews DR, Naylor BA, Jones RG, Ward GM, Turner RC. Pulsatile insulin has greater hypoglycemic effect than continuous delivery. Diabetes. 1983;32(7):617-21.
65. Goodner CJ, Sweet IR, Harrison HC. Rapid reduction and return of surface insulin receptors after exposure to brief pulses of insulin in perifused rat hepatocytes. Diabetes. 1988;37(10):1316-23.
66. Xu GG, Rothenberg PL. Insulin receptor signaling in the β-cell influences insulin gene expression and insulin content: Evidence for autocrine β-cell regulation. Diabetes. 1998;47(8):1243-52.
67. Nayak A, Khuntia R. Socio-demographic correlates of autism spectrum disorder: A study in Odisha. Ind Psychiatry J. 2024;33(Suppl 1):S222-5.
68. Alavi A, Reivich M. Guest editorial: the conception of FDG-PET imaging. Semin Nucl Med. 2002;32(1):2-5.

69. Baker LD, Cross DJ, Minoshima S, Belongia D, Watson GS, Craft S. Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. Arch Neurol. 2011;68(1):51-7.
70. Mohammad-Rezazadeh I, Frohlich J, Loo SK, Jeste SS. Brain connectivity in autism spectrum disorder. Curr Opin Neurol. 2016;29(2):137-47.
71. Bratusch-Marrain PR, Komjati M, Waldhausl WK. Efficacy of pulsatile versus continuous insulin administration on hepatic glucose production and glucose utilization in type I diabetic humans. Diabetes. 1986;35(8):922-6.
72. Mahajan PV, Patel HD, Daxini A, Thorat S, Khan M, Dsouza M. Novel pulsatile insulin therapy in foot crush syndrome using BIONICA-MDI: A case study. Int J Case Rep Orthop. 2025;7(2):73-7.
73. Aoki TT, Grecu EO, Arcangeli MA, Benbarka MM, Prescott P, Ahn JH. Chronic intermittent intravenous insulin therapy: A new frontier in diabetes therapy. Diabetes Technol Ther. 2001;3(1):111-23.