	Synopsis
Protocol Title	Effect of a Wide Spectrum Nutritional Supplement on Mitochondrial Function in Children with Autism Spectrum Disorder (ASD)
IRB	WCG Institutional Review Board
Protocol Ver	Wednesday, August 3, 2022
Sponsor	Richard E Frye, Rossignol Medical Center, Phoenix AZ
IND # / FDA	IND 142751; FDA Division of Psychiatry; Protocol Version 1.4
NCT ID	NCT03835117
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Clinical Sites	1. SARRC, Phoenix, AZ
Laboratory Sites	1. Laboratory of Dr. Edward Quadros, State University of New York Downstate—450 Clarkson Ave, Brooklyn, NY 2. Religen Inc, Plymouth Meeting, PA 3. Laboratory of Dr Arora, Icahn School of Medicine at Mount Sinai, NY NY 4. Laboratory of Dr. Madeleine Cunningham at the University of Oklahoma Health Sciences Center in Oklahoma City, OK 5. 3billion Laboratory in Seoul, South Korea 6. Iliad Inc, Plymouth Meeting, PA
Study Design	Prospective Randomized 24-week Double-Blind Placebo-Controlled Cross-over
Objectives	To evaluate the metabolic effects of a comprehensive wide-spectrum supplement for children with ASD to determine whether it physiologically targets mitochondrial pathways known to be abnormal in children with ASD.
Participants	Up to 50 children, aged 2 years 6 months to 17 years 3 months with confirmed ASD and mitochondrial dysfunction. Participants will have moderate disease severity (clinic global impression of 4 or above) and stable behavioral, education and medical treatment. Those with severe intellectual impairment, disruptive behavior or medical conditions such as epilepsy will be excluded.
Investigational Product	Weight adjusted wide-spectrum oral nutritional supplement powder and gel capsules that targets mitochondrial support produced by NeuroNeeds (Old Lyme, CT) taken in two divided doses vs oral placebos.
Safety	Physical examination, safety labs, Adverse Event Reporting, safety monitoring
Statistical Methods	The primary analysis will be intent-to-treat, defined as all randomized subjects. Mixed-model regression will determine whether blinded treatment produces a greater normalization in mitochondrial function over the 12-week period as compared to placebo treatment over the same time period. Similar analysis will test secondary outcome measures. Assuming a medium effect size of 0.5, the study provides an 80% power assuming a 0.01 alpha (allowing correction for multiple primary end-points). Secondary outcome measures will include the Improvement item of the Clinical Global Impression Scale and behavioral scales such as the Aberrant Behavior Checklist. Multivariable logistic regression will be used to select biomarkers that can significantly predict treatment response.

Funding	Turnabout for Autism (Private Foundation), Pleasanton CA

Contents

1. ABSTRACT

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder often with life-long consequences that affects young children during critical developmental periods. The Centers for Disease Control estimates that ASD affects as many as 17 per 1000 children (1 in 59) in the United States suggesting that the prevalence is higher than previous estimates.[1] Despite the dramatic rise in the detected prevalence of ASD over the past two decades, there is no effective medical treatment for core ASD symptoms (social communication and repetitive behavior), the closely associated problem of language impairment, or the underlying pathophysiology of ASD. Currently, the only accepted treatment for **core** ASD symptoms is behavior therapy, which may entail intensive one-on-one treatment over several years. Such therapy is inconsistently covered by medical insurance and may not be available in special education settings, thereby limiting its application. The *de facto* standard-of-care for treating ASD is a combination of educational and behavioral interventions.

Although interventions such as speech and occupational therapy may help modify neural pathways, they may not address the pathophysiology that underlies ASD. Despite these interventions, most children with ASD have incomplete recovery and need for long-term supportive care. Thus, a safe, effective and well-tolerated medical treatment that improves core and closely associated ASD symptoms, addresses the underlying physiology associated with ASD, and augments ongoing behavioral and educational interventions would have disease-modifying potential. To better understand if treatments commonly used in children with ASD are indeed normalizing physiological disturbances associated with ASD we aim to determine if a commonly used wide-spectrum nutritional supplement theoretically designed to normalize mitochondrial function does have the hypothesized effect of physiology in individuals with ASD.

The *primary aims* of this study are to evaluate the effect of a wide-spectrum nutritional supplement on mitochondrial function in individuals with ASD. Participants entered into the trial will have abnormalities in mitochondrial function that are associated with ASD (such abnormalities are found in approximately 50% of children with ASD) but are not diagnostic of mitochondrial disease. Specifically, children with mitochondrial disease (approximately 5% of the ASD population), which is a very severe disorder, will be excluded from the study so they can pursue more intensive appropriate treatment. As we believe 50% of children with ASD have some form of atypical variations in mitochondrial function, we will target these children to improve the function of their mitochondria. We hypothesizes that a nutritional supplement designed for children with ASD will have a physiological action of normalizing mitochondrial function and cellular physiology throughout the body. To test whether the targeted nutritional supplement is superior to placebo, we will study 50 children, between the ages of 2 years 6 months and 17 years 3 months, with confirmed ASD and known abnormal variations in mitochondrial at baseline. Participants will be randomly assigned to receive active treatment or placebo for 12-weeks under double-blind conditions and at the end of the 12 weeks switch to the opposite treatment arm. Mitochondrial function will be measured at baseline and after each treatment arm in order to determine if the supplement positively influences cellular biochemistry.

2. BACKGROUND

ASD is a heterogeneous neurodevelopmental disorder often with life-long consequences that affects young children during critical times in their development.[2] The latest estimates from the Center for Disease Control estimates an ASD affects as many as 17 per 1000 children (1 in 59) in the United States with recently estimation demonstrating that the provenience continues to rise.[1] ASD is defined by impairments in social communication as well as the presence of restrictive interests and repetitive behaviors[2]. ASD is frequently associated with co-occurring problems such as gastrointestinal abnormalities[3], seizures[4], attention deficits[5], anxiety[6] and allergies[7] - to name a few.

Despite the fact that the prevalence of ASD has dramatically increased over the past two decades, there currently is no effective medical treatment for children with ASD. A recent multicenter study showed that optimal outcomes can be achieved in a subset of ASD children.[8] Moreover, a study that analyzed the annual change in ASD symptoms in 6,975 children showed that some children with ASD can substantially improve.[9] However, these studies did not address whether particular therapies were associated with better outcomes. In addition, the findings suggest that only a minority of children with ASD show substantial improvement over time, underscoring the need for more effective treatments.

Currently the only well-accepted treatment for **core** ASD symptoms is behavior therapy such as Applied Behavioral Analysis (ABA) and Early Intensive Behavioral Intervention. However, such therapy requires near full-time engagement with a one-on-one therapist over several years. The evidence for early intervention rests primarily on single-subject design studies with few randomized controlled studies and these interventions rarely result in complete recovery.[12] Moreover, access to such intensive treatments is often limited and may only be available in highly specialized centers.[13] Behavior therapy is inconsistently covered by medical insurance and not uniformly available in the education system.[10,11] Thus, the *de facto* standard-of-care for treating ASD is a combination of educational and allied health therapies (speech and occupational therapies) in various settings. Although these interventions may promote development in children with ASD, they may not address the underlying pathophysiological abnormalities in ASD. Most individuals with ASD require life-long supportive care and do not achieve independent living in adulthood.[12]

Evidence-based medical treatments for ASD are limited. There is no United States (US) Food and Drug Administration (FDA) approved medical therapy that addresses **core** ASD symptoms or the pathophysiological processes that underlie ASD.[13] The two FDA approved drugs, both atypical antipsychotics, are indicated for serious behavioral problems in children with ASD. Although these drugs, risperidone and aripiprazole, may improve social interactions as a secondary benefit,[18] the detrimental effects on lipid and glucose metabolism and body weight are well established and may develop quickly (within 12 weeks).[14-16] These metabolic changes increase the risk of cardiovascular disease and type-2 diabetes. [17 17 17] Although the incidence is low, tardive dyskinesia, a potentially permanent movement disorder, is an additional risk.[18] Thus, there is a pressing need for safe medications that affect target pathophysiological processes and treat core ASD symptoms.

Several medications are used off-label to treat associated, but not core, ASD symptoms. These medications do not address underlying pathophysiological abnormalities. A systematic review of attention-deficit hyperactivity disorder (ADHD) treatments for ASD highlights the complicated nature of medication treatment due to the diverse nature of children with ASD and associated adverse effects (AEs).[19] A recent Cochrane review found no evidence for efficacy of selective serotonin reuptake inhibitors in ASD.[23] Although studies show positive effects of methylphenidate and extended-release guanfacine on hyperactivity, these medications did not improve core features of ASD and the AE burden was higher than found in children with ADHD.[24,25] Pharmaceutical companies have developed medications targeting excitatory-inhibitory neurotransmission imbalances identified in animal models of ASD[20], but the first of these compounds failed to demonstrate efficacy in a double-blind placebo-controlled (DBPC) trial, leading to termination of the research program.[21]

3. An Integrated Approach to Understanding Autism Spectrum Disorder

Our novel approach to treating ASD is to address the underlying physiological abnormalities associated with ASD as we hypothesize that these physiological abnormalities are driving abnormal behavior and cognition. Of note, ASD is associated with physiological disturbances including abnormal redox and mitochondrial metabolism. In fact, between 5%-80% of children with ASD demonstrate evidence of mitochondrial dysfunction and 5% of children with ASD appear to have

mitochondrial disease.[22-24] This is in comparison to the general population where mitochondrial disease is believed to affect less than 0.1% of the population.[25]

Repeated studies have suggested that genetic defects are found only in a minority of children with ASD, including a minority of children with ASD with mitochondrial disease[22] and many case reports and series have described only moderate, rather than severe, deficiencies in the electron transport chain (ETC) activity in those with ASD and mitochondrial disease.[24,26] Perhaps more striking is the fact that ETC activity in muscle,[26,27] skin,[24] buccal epithelium[28-30] and brain [31] has been documented to be significantly increased, rather than decreased, in some individuals with ASD. This is consistent with our *in vitro* data showing elevated mitochondrial activity in lymphoblastoid cell lines (LCLs) derived from children with ASD.[32-38] We recently demonstrated that this alteration in mitochondrial function is associated with more severe repetitive behaviors.[35] Thus, the current data points to possible non-genetic defects or changes causing abnormal mitochondrial function as well as unique types of mitochondrial dysfunction in individuals with ASD.[24,27]

Mitochondrial dysfunction has profound significance for health. Mitochondria are essential for a wide range of functions in almost every cell in our body. Best known for their role in the production of adenosine triphosphate (ATP), mitochondria are also intimately involved in other essential cellular functions such as calcium buffering, redox regulation, apoptosis and inflammation and have a role in non-energy producing metabolic pathways, such as the urea cycle and porphyrin pathway. Additionally, ATP produced by the mitochondria is essential for many cellular systems. Thus, abnormal mitochondrial function can adversely affect a wide range of cellular systems. Mitochondrial dysfunction is recognized to contribute to the pathophysiology of many common diseases, especially those thought to have a substantial environmental component, including psychiatric diseases,[39-42] neurodegenerative disorders,[43] persistent systemic inflammation,[44] cardiac disease,[45] cancer[46] and diabetes.[47] Abnormalities in mitochondrial function are closely associated with neurodevelopmental disorders, including genetic syndromes closely associated with ASD including phosphatase and tensin homolog (PTEN) and WDR45 mutations,[48,49] septo-optic dysplasia[50] and Rett[51-53], Phelan-McDermid,[54] 15q11-q13 duplication,[55,56] Angelman[57] and Down[58,59] syndrome.

Most significantly, there is evidence that nutritional supplement used in children with ASD target the mitochondrial. Several studies have demonstrated that nutritional supplements, such as carnitine, nicotinamide adenine dinucleotide (NAD), ubiquinol and ubiquinone designed to treat individuals with mitochondrial disease may be helpful in children with ASD.[21] Two DBPC studies demonstrated improvement in ASD symptoms with carnitine supplementation[60] with some improvements directly related to the change in blood carnitine levels.[61] In another study, NAD and ribose appeared to improve metabolic biomarkers in children with ASD and symptoms of mitochondrial dysfunction.[62] Two studies have reported behavioral improvements in children with ASD using ubiquinol[63] and ubiquinone.[64] While these aforementioned studies did not specifically target individuals with mitochondrial dysfunction, one recent study examined the behavioral effects of a customized mitochondrial supplement in ASD children with mitochondrial dysfunction. Legido et al[65] in an open-label study treated 11 children with ASD and abnormal Complex 1 and/or Complex 4 activity, as measured by the buccal swab method, with a mitochondrial cocktail containing carnitine, coenzyme Q10 and alpha-lipoic acid. Three months of treatment improved the index of mitochondrial function and improved several behavior scales including lethargy and inappropriate speech subscales from the Aberrant Behavior Checklist (ABC). Three months after withdrawal of the treatment the lethargy and inappropriate speech subscales significantly worsened.

Thus, we aim to build on this previous evidence in order to demonstrate that a mitochondrial targeted nutritional supplement commonly given to children with ASD indeed positively affect mitochondrial

function. Previous studies have been low quality because they were either open-label or did not measure physiological response of the treatment. We wish to perform a rigorous study that targets the subset of children with ASD with abnormal variations in mitochondrial function associated with ASD in order to increase the scientific evidence to understand how the physiological mechanisms which nutritional supplements may affect children with ASD.

4. SPECIFIC AIMS

Primary Aim: To determine if a wide-spectrum nutritional supplement can influence mitochondrial activity in 50 individuals with ASD and signs and symptoms of abnormal mitochondrial function. The trial design will be a 24-week randomized, DBPC cross-over study. We hypothesize that the supplement will result in a greater ability to normalize mitochondrial activity as compared to placebo. To test this hypothesis we will:

a. Test mitochondrial activity at baseline and after the placebo and supplement arms of the study and determine if the nutritional supplement normalizes mitochondrial activity significantly more than placebo.
b. Test redox metabolism at baseline and after the placebo and supplement arms of the study and determine if the nutritional supplement improves redox metabolism significantly more than placebo.
c. To determine if specific biomarkers (Folate Receptor Autoantibody, Single Nucleotide Polymorphism) can predict response to treatment.

Secondary Aim: To evaluate the effectiveness of a wide-spectrum metabolic supplement on core and associated ASD symptoms. We will determine is symptoms associated with ASD will be effected by a nutritional supplement which targets normalizing mitochondrial function. We will evaluate:

a. Core ASD symptoms as measured by the Childhood Autism Rating Scale (CARS).
b. Clinical Global Impression Severity and Improvement
c. Behavior, including repetitive behavior Children Yale Brown Obsessive Compulsive Scales Modified for ASD (CYBOCS-ASD), Irritability, Social Withdrawal, and Hyperactivity subscales of the Aberrant Behavior Checklist (ABC), Parent-rated Anxiety Scale-ASD (PRAS-ASD), Caregiver Strain Questionnaire (CGSQ), Parent Targeted Problems and adaptive behavior (Vineland III).
d. Somatic manifestations of pain, fatigue and gastrointestinal symptoms

Tertiary Aim: To collect samples which will allow the evaluation of the effect of a wide-spectrum metabolic supplement on changes in cellular regulation and to determine if specific gene abnormalities or cellular regulatory factors or autoantibodies results in abnormal mitochondrial function. In the future we wish to determine the changes in cellular regulation as a result of the treatment by examining several aspects of cellular regulatory elements. We also wish to determine if certain genetic or epigenetic changes are associated with mitochondrial dysfunction in ASD. The current funding does not allow these advanced analysis to be performed at this time but we will collect samples so these studies can be performed in the future. In the future we will examine:

a. Gene expression by measuring RNA expression
b. Cellular regulation by measuring microRNA expression
c. Concentrations of key regulatory proteins
d. Methylation of targeted genes
e. Abnormal gene sequences (mutations, rare polymorphisms)
f. Autoantibodies to Brain tissue and the folate receptor alpha

5. RESEARCH PLAN

Trial Design

50 children with ASD and signs and symptoms of abnormal mitochondrial activity will be randomized in 1:1 ratio to receive a wide-spectrum nutritional supplement that targets mitochondrial function or placebo for 12 weeks under DBPC conditions. Each participant will serve as their own control in the cross-over design. Using a phone screen, we will identify children (age ≥ 2:6 and < 17:3) with a diagnosis of ASD and evidence of mitochondrial function which is significantly higher or lower than average (here forth referred to as mitochondrial dysfunction). Children with suspicion of mitochondrial dysfunction will be invited to participate in a screening visit to make sure they meet including and exclusion criteria. If the screening visit is longer than 6 weeks from the prescreening, we will repeat the baseline metabolic testing to ensure an accurate baseline mitochondrial measurement. If they meet criteria, the baseline visit will be completed within 2 week but we will attempt to complete it on the same day (Table 1). Upon successful completion of the baseline visit, the participant will enter Phase 1 of the trial and will be randomized to receive active treatment or placebo for 12 weeks under DBPC conditions. At the end of the 12 weeks (end of Phase 1), the participant will return for evaluations similar to the ones conducted at baseline. Upon successful completion of the post-Phase 1 visit, the participant will start Phase 2 where they will receive active treatment or placebo, depending on what they received during Phase 1 – they will be crossed over to the opposite treatment regimen. Potential AEs, compliance and concomitant treatments will be monitored every four weeks.

Inclusion criteria

- Boys and girls ≥ 2 years 6 months of age to < 17 years 3 months of age;
- Weight ≥ 15 kg and ≤ 100kg;
- DSM-5 diagnosis of *Autism Spectrum Disorder* as established by formal clinical assessment which includes a gold-standard tool such as the Autism Diagnostic Observational Schedule.
- Current Childhood Autism Rating Scale, 2nd Edition (CARS-2) score meeting criteria for Autism Spectrum Disorder (Standard Form, cutoff ≥ 30 for children < 13 years of age; ≥ 28 for children ≥ 13 years of age; High Functioning Form, cutoff ≥ 28)
- IQ at least 30 as measured by the Differential Abilities Scale (DAS)
- Stable educational and therapy plan (one month) with no **planned** changes in the intensity of treatment for 12 weeks.
- English spoken in the home and at least one parent is able to read, write and speak English.
- Stable medication (no changes in past 6 weeks) and no **planned** changes for the study duration.
- Signs and symptoms of mitochondrial dysfunction (See Figure Below)

Clinical Abnormalities Found in Mitochondrial Disease

Not Typically Clinically Abnormal in ASD		Clinically Abnormal in Some ASD Individuals	
Ophthalmoplegia	Stroke-like episodes	Development Delay	Abnormalities in Endocrine/Growth,
Facies myopathica	Migraine	Loss of Skills	Gastrointestinal
Hematology, Vision	Cortical blindness	Seizures	System, Lactate,
Hearing, Brainstem abnormalities		Muscle Weakness	Pyruvate, Alanine
		Peripheral Neuropathy	

Exclusion criteria

- IQ below 30 as measured by the Differential Abilities Scale (DAS)
- Presence of serious behavioral problems (tantrums, aggression, self-injury) for which another treatment is warranted.
- Significant medical condition by history or by physical examination or lab tests that would be incompatible with the treatment.
- Children taking anticonvulsant medication for seizures or active epilepsy.
- Diagnosis of Mitochondrial Disease
- Current use of SpectrumNeeds or QNeeds

Procedures

Randomization: Randomization will be carried out within site using permuted blocks of 4 subjects without additional stratification to nutritional supplement or placebo in a 1:1 ratio. Statisticians will prepare the permuted blocks for each site and will be sent to the Investigational Pharmacist at each site. Investigators and study staff will be blind to the allocation sequence.

Compensation Participants will be compensated $50 for each of the four physical visits.

Measures:

Parent Ratings

1. Vineland Adaptive Behavior Social Subscale (VABS) III: The Vineland Adaptive Behavior Scale (VABS) III is a widely used standardized, well-validated assessment tool for children with developmental delays that measures functional abilities within several domains [66]. It is particularly useful for children with intellectual disability which commonly co-occurs with ASD [67] and has valid measures of social impairments in children with ASD [68]. The VABS relies on an informant (caretaker) to complete the survey about the child's adaptive behavior – i.e., what the child actually does in the course of daily living. We will use the parent checklist version and examine the social domain.
2. Aberrant Behavior Checklist (ABC): The ABC is a validated questionnaire that rates symptoms of hyperactivity, irritability, social withdrawal, and stereotypic behavior in individuals with developmental disabilities.[69] It has convergent and divergent validity [70] and is used in multiple ASD clinical trials.
3. Parent Rated Anxiety Scale-ASD (PRAS-ASD): Twenty five-item scale designed to measure anxiety in children with ASD. It has demonstrated reliability and validity (L. Scahill unpublished data).
4. Caregiver Strain Questionnaire (CGSQ)-Short Form: This version of the CGSQ is a 7-item, parent self-report designed to measure the burdens associated with raising a child with special needs.
5. Sensory Profile 2: This assessment provides information about sensory sensitivity and interpretation in children with developmental disorders.

6. Parent Rated Autism Symptomatic Change Scale (PRASC): The PRASC is a 12-item questionnaire that asks parent to rate changes in symptoms from “much better” to “much worse” on a 7 point Likert scale from the beginning of the intervention. This instrument was developed by Frye and Rossignol (2010) and produces a reliable measure of changes The parent will be asked to complete this scale at each remote and onsite visit following randomization to monitor changes in ASD symptoms in the study.

Table 1. Schedule of Measures				Double blind Phase							
Measure	Pre-Screening	Screening	Baseline	*1 wk	*2 wk	*4 wk	*8 wk	12 wk	*16 wk	*20 wk	24 wk
Phase 1											
Phase 2											
Parent Ratings											
Demographics		X									
Vineland III Caregiver Form [1]			X					X			X
ABC [2]		X						X			X
PRAS-ASD [3]			X					X			X
CGSQ-Short form [4]			X					X			X
Sensory Profile 2 [5]			X								
PRASC [6]						X	X	X	X	X	X
Laboratory Assessments											
Safety Laboratory [7]	X							X			X
Biomarker Laboratories [8]	X							X			X
Pregnancy (B-HCG)	X										X
Vital Signs	X										
Height and Weight	X		X					X			X
Head Circumference	X										
Medical Assessments											
Medical/Psych History [9]		X									
Physical Exam		X									
Adverse Effects Review			X	X	X	X	X	X	X	X	X
Concomitant Treatments	X	X	X	X	X	X	X	X	X	X	X
Suicidality Assessment [10]		X	X			X	X	X	X	X	X
Compliance				X	X	X	X	X	X	X	X
Developmental Assessments											
CARS [11]		X						X			X
DAS [12]		X						X			X
OACIS [13]			X					X			X
CGI-Severity [14]			X					X			X
CGI-S-ASD			X					X			X
CGI-Improvement [15]								X			X
CGI-I_ASD								X			X
Parent Target Problems [16]			X					X			X
CYBOCS-ASD [17]			X					X			X
Other Assessments											
Eye tracking task [18]			X					X			X

Laboratory Assessments

7. Safety laboratories: Safety labs will be measured at the screening visit. These will include a complete blood count and a comprehensive metabolic panel.

8. Biomarker: Most biomarkers will be collected at baseline and after each treatment arm to determine if the treatment results in a change in these biomarkers. Baseline biomarkers will help understand whether treatment response can be predicted from biomarker tests.

a. Buccal swabs will be collected to assess mitochondrial function.
b. Plasma, Peripheral Blood Mononuclear Cells (PBMCs), DNA, and RNA will be collected and stored locally for future analysis, such as oxidative and methylation metabolism, bioenergetics, immune biomarkers, gene expression, epigenetics, and gene sequencing. 3billion Laboratory in Seoul, South Korea will perform genetic sequencing to look for causative mutations if good quality DNA is obtained and can be isolated. Samples will be stored until they are used up or destroyed, when possible. The PI, Dr. Richard Frye, will retain control over the blood sample unless it is requested that the sample be destroyed and then it will not be used for any future studies. If the participant wishes to have the sample removed from storage and destroyed at any time, they may contact a study coordinator or PI. IRB permission will be obtained prior to sharing samples for future studies. These samples will be de-identified and no protected health information will be shared with collaborators. If samples are shared, a log of those who withdraw samples will be documented thoroughly.
c. Deciduous teeth and hair samples will also be collected and sent to Dr Arora's laboratory. Recent research suggests prenatal nutritional metal concentrations measured in deciduous teeth correlate with long-term language development and may predict treatment response and other recent studies support the measurement of unique metabolic markers using hair samples.
d. Folate receptor α autoantibody (FRAA) titers will be sent to the laboratory of Dr. Edward Quadros, Ph.D. at the State University of New York, Downstate (Brooklyn, NY) or Iliad Neurosciences. We have demonstrated in one study that FRAA are related to methyl-B12 metabolism.[71]
e. Saliva will be collected from the participant and biological parents to investigate whether single nucleotide polymorphism (SNPs). Parental SNPs are important to understanding whether the SNPs in the children are significant or not – for example, if parents have the same SNP profile as the child, the SNP is unlikely to have a significant effect of pathophysiology.
f. Autoantibodies to neuronal tissue may be assessed by the Laboratory of Dr. Madeleine Cunningham at the University of Oklahoma Health Sciences Center in Oklahoma City, OK if enough sample is available for the assay.

Medical Assessments

9. Medical and Psych History: This structured questionnaire form assists with the elicitation and assessment of health complaints, appetite, sleep, activity level and general health at **baseline**. The companion measure, the Adverse Effects Review, is used in follow up visits to monitor and document new events during the trial.

10. Suicidality Assessment: Although we do not anticipate any symptoms consistent with suicidality, an assessment of suicidality is required in FDA trials involving psychiatric disorders including ASD and is a standard assessment in FDA regulated ASD trials. An assessment similar to other studies examining ASD children will be used. The Columbia-Suicide Severity Rating Scale (C-SSRS) fulfills this requirement.

Developmental Assessments

11. Childhood Autism Rating Scale (CARS): This 15-item scale can be used to both diagnose autism and ASD, and to assess the overall severity of symptoms.

12. Differential Abilities Scale (DAS): This scale is a scale that assesses cognitive abilities and early learning and has been validated against other cognitive ability scales (Wechsler, Bayley, etc.). This scale is designed to identify clinical risk and disability.
13. Ohio Autism Clinical Impression Scale (OACIS): A clinical evaluation by the study physician of initial severity of 10 ASD-related symptoms at baseline on a 7-point scale (Part 1), and a clinical evaluation of change of those symptoms using a different 7-point scale (very much improved to very much worse). The 10 items include social interaction, aberrant/abnormal behavior, repetitive/ritualistic behavior, verbal communication, non-verbal communication, hyperactivity/inattention, anxiety/fears, sensory sensitivities, restricted/narrow interests, and autism.
14. Clinical Global Impression - Severity (CGI-S): This is a 7-item scale ranging from a score of 1 for "Normal" to 7 for "Extreme" [72]. All aspects of the child's condition need to be integrated to assign the CGI-S score. Only participants of CGI-S of 4 ("moderate") or greater severity will be included in the trial. By definition, a child with ASD and language impairment (which is established at screening) has moderate severity ASD. Thus, the CGI-S will be completed at baseline since it requires all available information about the child. CGI-S will be rated by an assessor who is blind to treatment assignment. Both the standard CGI-S and the newly developed CGI-S-ASD will be completed, with the CGI-S completed before the CGI-S-ASD.
15. Clinical Global Impression for Improvement (CGI-I): The CGI-I is a 7-point measure of overall symptomatic change compared to baseline [72] that will be used as a **key primary** outcome measure. Scores range from 1 (Very Much Improved) through 4 (Unchanged) to 7 (Very Much Worse). All aspects of the child's condition need to be integrated to assign the CGI-I score. The CGI-I will be rated by an assessor who is blind to treatment assignment, AEs, and medication dose. Both the standard CGI-I and the newly developed CGI-I-ASD will be completed, with the CGI-I completed before the CGI-I-ASD. Ratings of Much Improved or Very Much Improved on the CGI-I will be used to define positive response.
16. Parent Target Problems (PTP): At baseline, parents will be asked to nominate the two most important ASD problems for the child. Through brief discussion, the frequency (for episodic behaviors such as insists on daily routines) or constancy (for problems such as language delay or rigidity reflecting more enduring patterns), intensity and impact of the behavior on the family are established. Responses from this systematic inquiry are documented in a brief narrative. The narrative will be reviewed monthly and a new narrative documented. This review will assist with the scoring of the CGI-I in real time. [73] At the end of the trial, the narratives will be reviewed by a panel blind to treatment assignment. This method has been shown to be reliable and valid in placebo-controlled studies.[73,74]
17. Children's Yale-Brown Obsessive-Compulsive Scales-ASD (CYBOCS-ASD): The CYBOCS-ASD is a modified version of the CYBOCS developed for children with Obsessive-Compulsive Disorder.[75] The modified version is a semi-structured clinician-rated scale designed to rate the current severity of repetitive behavior in children with ASD (i.e., Time Spent, Interference, Distress, Resistance, and Control). Each item is scored from 0 (least symptomatic) to 4 (most symptomatic), yielding a Total score from 0 to 20. It has established reliability and validity [76] and is sensitive to change.[77]

Other Assessments
18. Eye Tracking Task: This 15-item scale can be used to both diagnose autism and ASD, and to assess the overall severity of symptoms. The foremost criterion for the diagnosis of autism spectrum disorder (ASD) is a pervasive deficit in social communication and interaction, including abnormal speech, impaired reciprocity in relationships and conversation, and poor use and integration of nonverbal communication behaviors. A key behavior indicative of impaired social function in ASD is inappropriate or absent eye contact. For example, when gazing at faces, individuals with autism tend to direct gaze toward the mouth rather than the eyes. Changes in social communication are typically measured using multiple clinical assessments and caregiver-reported questionnaires normed for the

ASD population. We wish strengthen measures of abnormal attention to social stimuli. We believe that objective changes in attentional gaze will be reflected in clinical presentation and overall clinician and caregiver reports of symptom severity. We may use the Tobii eye tracking software and Noldus Face Reader software to collect data about the location and duration of eye gaze while participants watch an age-appropriate video. The video will include both social and nonsocial stimuli. In each age-appropriate video, we will present a variety of simulated social interactions as well as nonsocial behaviors. We will also present speech sounds and non-speech sounds, as well as background distractors. Each of these elements will be presented across the four quadrants of the screen. We will measure the percentage of time the participant spends gazing at social stimuli and nonsocial stimuli, as well as time spent away from the screen. We will also analyze the duration of fixation for each shift in gaze. This will be performed if equipment is available and operational.

Investigational Product

Table 2. Daily Serving Size		**6.6g**	**8.8g**	**11g**	**13.2 g**	**Tolerable Upper Intake Levels**
Patient Body Weight	**Units**	**15kg-20kg**	**21kg-40kg**	**41kg - 60kg**	**61kg - 100kg**	**Age 4-8 years**
NeuroNeeds						
Vitamin C (ascorbic acid)	Mg	450	600	750	900	ND
Vitamin D (cholecalciferol)	Mcg	11.25	15	18.75	22.5	75
Vitamin E (d-α-tocopheryl acetate and mixed tocopherols)	Mg	37.5	50	62.5	75	300
Thiamin (as thiamin HCl)	Mg	18.75	25	31.25	37.5	ND
Riboflavin	Mg	18.75	25	31.25	37.5	ND
Niacinamide	Mg	18.75	25	31.25	37.5	15[1]
Vitamin B_6 (50% as pyridoxine HCl and 50% as pyridoxal-5-phosphate)	Mg	15	20	25	30	40
(6S)-5-methyltetrahydrofolic acid	mcg	1250	1667	2084	2500	ND
Calcium folinate	mcg	1250	1667	2084	2500	400[2]
Vitamin B_{12} (as methylcobalamin)	Mcg	375	500	625	750	ND
Biotin	Mcg	750	1000	1250	1500	ND
Pantothenic acid (as D-Ca pantothenate)	Mg	225	300	375	450	ND
Calcium	Mg	45	60	75	90	2,500
Iodine (as K iodide)	Mcg	75	100	125	150	300
Magnesium (Mg citrate and Mg malate)	Mg	150	200	250	300	110
Zinc (Zn gluconate)	Mg	9	12	15	18	12
Selenium (as selenomethionine)	Mcg	37.5	50	62.5	75	150
Manganese (as manganese amino acid chelate)	Mg	0.75	1	1.25	1.5	3
Chromium (Cr picolinate)	mcg	37.5	50	62.5	75	ND
Molybdenum (Na molybdate)	mcg	75	100	125	150	600
Potassium (K citrate)	mg	37.5	50	62.5	75	
Creatine monohydrate	mg	937.5	1250	1562.5	1875	
L-Arginine	mg	750	1000	1250	1500	
Carnitine Blend (acetyl L-carnitine HCl and L-carnitine)	mg	375	500	625	750	
Alpha ketoglutaric acid	mg	225	300	375	450	
Ubiquinone	mg	187.5	250	312.5	375	
Choline bitartrate	mg	112.5	150	187.5	225	1000
Inositol	mg	75	100	125	150	
Alpha lipoic acid	mg	75	100	125	150	
D-Beta, D-Gamma, D-Delta tocopherols	mg	3.3	4.4	5.5	6.6	300
QNeeds						
Ubiquinol (QNeeds)[4]	mg	200	300	400	400	

The nutritional supplement used will be a combination of SpectrumNeeds and QNeeds, both produced by NeuroNeeds (Old Lyme, CT; See Table 1). Weight based dosing will be use. The daily serving size will divided into two daily doses in the form of a powder which can be mixed into liquid of food. Together, there are 34 different dietary supplements in the products. Thirty-two of the 34 supplements do not exceed the Tolerable Upper Intake Limits set by the United States Institute of Medicine. The exceptions are:

1. The niacin dosing is about the upper limit. Niacin in the form of nicotinic acid is well known for causing benign flushing. Niacinamide (also known as nicotinamide) is the form of niacin in SpectrumNeeds, and this form does not cause flushing.
2. Folate exceeds the upper limit. It should be noted that half of the total folate provided is in the form of Quatrefolic™ (6S)-5-Methyltetrahydrofolic acid Glucosamine salt (55% FOLATE), which is microbial sourced, not synthetic. There is no known upper tolerable limit for non-synthetic folate, only for synthetic folate. The other half is in the form of calcium folinate. Dosage of folate used in this supplement are about one-half that commonly recommended during pregnancy to women with a prior child with a neural tube defect who may get pregnant. Dosages of folinate are one half to one-twenty-fifth the dosing commonly recommended in children with ASD. These dosages are known to be generally well tolerated in ASD children.

Except for ubiquinol, all of these nutrients are provided in a powder form in SpectrumNeeds. The physical stability and bioavailability of ubiquinol is insufficient in powder form, and thus ubiquinol is provided separately in QNeeds gel capsules. These capsules can be swallowed whole, or cut with scissors and the contents squeezed out and added to SpectrumNeeds just before ingestion.

Placebos will have the same look, feel and taste as the active treatments. For example, the SpectrumNeeds is a silica powder flavored by monk fruit extract, so the inert silica powder with the same favoring will be used as a control. Similarly, in QNeeds, the ubiquinol is carried by purified water and glycerin colored by caramel liquid, so for the placebo the same carrier and color will be used in the gel capsule without ubiquinol.

Ongoing Examiner Reliability and Fidelity Assessments: A psychologist will train assessors at each site on the administration of the confrontational assessment. The psychologist will review the first three administrations of the language test to confirm reliable administration and conduct quarterly conference calls to review cases and to promote a common approach to conducting assessments across sites.

Data Management: Study data will be collected and managed using the REDCap software specifically developed for research and clinical trial data collection and management. REDCap is a secure web application and fully compliant with privacy guidelines and communication security as defined in the HIPAA, Federal Information Security Management Act (FISMA), and the U.S. Food and Drug CFR for electronic records.

Food and Drug Administration (FDA) Approval

The FDA has approved our Investigational New Drug (IND) application.

6. DATA MANGEMENT AND STATISTICS

Storage and Security

Study data will be managed using a 21 CFR Part 11 compliant web-based data entry system. Thus, data collection templates will be modified accordingly for data entry.

Statistical Analysis
All data analysis for the primary study aims will be conducted by the principal investigator.
The primary analysis will be intent-to-treat, defined as all randomized subjects. Exploratory "per protocol" analysis will be conducted on participants who adhered to protocol. Analyses will be performed with R 3.02 (http://www.r-project.org) or SAS 9.3 (SAS Institute, Cary, NC).

Sample Size Justification and Power Analysis: Assuming a medium effect size of 0.5, the study provides an 80% power assuming a 0.01 alpha (allowing correction for multiple primary end-points)..

Primary Hypothesis: After 12 weeks of taking the nutritional supplement will improve mitochondrial function more than taking the placebo for 12 weeks. To test this hypothesis, we will use a mixed model using baseline and all post-randomization measures of mitochondrial activity. To deal with missing post-treatment data, the model will be conditioned on all baseline values.[72] This conditional joint response model is more tolerant of missing data than ANCOVA and is less biased than carrying baseline data forward to endpoint.

Safety analyses: Adverse events (AE) will be systematically monitored using a 34-item form. It includes specific queries about major body systems, activity level, sleep, appetite, and general health. New events, whether presumed related to treatment or not, are classified as AEs and rated as Mild, Moderate or Severe (see details below). The occurrence of an AE will be counted once at the highest level of severity (e.g., a report of mild nausea followed by a report of moderate nausea in the same child would counted as an occurrence of moderate nausea). The frequency of AEs by severity will be evaluated using a Fisher's exact test. We will pursue exploratory analyses such as survival analyses using time to AE as the response variable.

Validation of the CGI-ASD: In addition to the standard CGI-S and CGI-I we will use new a structured CGI rating scale called the CGI-S-ASD and CGI-I-ASD respectively. We will compare the performance of the standard and new CGI rating scales. Since the CGI-ASD provide guidance for completing the ratings we will have the rater complete the standard scale before the new scale. The correspondence between each scale and other outcome measures will be studied as well as the performance of the scales as outcome measures.

Extra Sample Collection: As an optional portion to the research, deciduous teeth and hair samples can also be collected and sent to Dr Arora's laboratory. Recent research suggests prenatal nutritional metal concentrations measured in deciduous teeth correlate with long-term language development and may predict response to various treatments and other recent studies support the measurement of unique metabolic markers using hair samples.

7. HUMAN SUBJECTS CONCERNS

Sources of Materials
The sources of the research material will be data collected in the course of the clinical trial to determine the efficacy and also to ensure the safety of study participants. The principal types of data will be clinical rating scales and standardized tests (e.g., IQ test and the CELF-4). Routine laboratory measures (blood chemistry, blood cell counts) and biomarkers will be obtained.

Future Studies
Although the long-term use of the SpectrumNeeds and QNeeds multivitamin is believed to be safe, long-term tolerability and therapeutic effect has not been evaluated. Furthermore, the optimal length of treatment is not known. Further studies will identify the optimal treatment for this therapy and long-term safety.

The biomarkers to be collected for future research include DNA, and RNA. These samples will be collected and sent to Dr Frye's laboratory for storage for future analysis such as gene expression, epigenetics, gene sequencing, proteomics or metabolomics.

Potential Risks
The risks associated with these procedures include exposure to the study supplement or placebo, and the risk associated with venipuncture. The blood draws poses a possible risk of bruising at the site of the draw, light-headedness and fainting along with a remote chance of infection.

Although highly unlikely, there are potential privacy risks associated with participating in such a study. There is minimal risk associated with the completion of rating scales and assessment measures. Blood draws are common procedures and will be performed by a certified phlebotomist. We will offer a numbing cream prior to the blood drawing procedure. We will systematically review adverse at follow up phone calls and clinic visits (see below). Parents will be given phone numbers of the principal investigator and study physician – who will be available after hours. The PI and study physician have access to the pharmacy after hours – if it is necessary to break the blind.

The nutritional supplement and supplements similar to it have been on the market and used widely in the autism community. There have been few reported side effects of this supplement. They include nausea and hyperactivity, both of which resolve on their own. One similar supplement produced by Dr. Adams at Arizona State University has been studied in three clinical trials without reported significant adverse effects. In addition, the majority of the ingredients in the nutritional supplement are well within the proposed upper tolerable limits recommended by the institute of medicine and the two vitamins that exceed these limits are known to be well tolerated at doses well above the recommend upper limits. Increased consumption of B vitamins may also temporarily change urine color.

Confidentiality will be maintained throughout the project period. With the exception of tests that require date of birth, research records will not include names or contact information. Subjects will be identified only by their code numbers. A file with personal health information (e.g., names and addresses) will be kept separately in a locked file cabinet.

Recruitment
The research team will use targeted recruitment, such as posting to the local website, contacting clinical populations, using social media and contacting local organizations, physicians and schools. All recruitment materials such as flyers, letters and social media posts will be IRB approved. There must be a suspicion of mitochondrial dysfunction for the participants to qualify for the study.

Compensation
Subjects will be compensated $50 for the following visits: screening, baseline, end of Phase 1 and end of Phase 2.

Consent Process

Interested families will be screened on the telephone by the study coordinator. Presumably eligible and willing families will be invited to the site for a formal screening visit. Parental consent will be obtained at the screening visit prior to the collection of any study data. The consent procedure will include the following: (a) provide the consent forms to parents and guardians together with a verbal summary of the contents of all paragraphs, and (b) a description of alternatives to participating in the study (not to take part, use of other treatments, and obtaining similar treatment through other clinical services). Parents (guardians) will also be told that declining participation will not prejudice their right to pursue other treatments at the local site or any of its affiliates.

Parental consent will be obtained by trained investigators as documented on each sites delegation of authority log. Children whose parents indicate notable barriers to meeting the demands of the study or whose parents lack capacity to understand the purpose and expectations of the study will not be randomized in the study. These judgments will be made by the investigator conducting the consent discussion in consultation with the research team as needed. Parents will be given the opportunity to ask questions. Once all questions have been answered, parent(s) will be asked to sign and date the consent form. Parents will be provided with a copy of the singed consent form.

Child Assent: The children in this study will be diagnosed with ASD. Most will also be cognitively delayed. The site investigator or designated Co-investigator conducting the consent procedure will evaluate the child's capacity to provide written or verbal assent. The investigator's judgment will be documented on the assent form.

Consent for Video Recording Consent for video recording for research purposes will be obtained from parents. If parents are willing, video recordings will be used for verifying behavioral scoring but additional observers, monitoring of the testing for ensuring high quality evaluations and training evaluators. Parents can indicate agreement or disagreement with this additional use of the videos directly on the video consent form. Agreement to use the videos for these additional training purposes is not required for study participation.

Parental Consent for Saliva Biological parents may choose to provide saliva samples. This is not required for child participation in the study. Parents will be asked to accept or decline by initialing under the "Making Your Choice" option in the future and genetic research sections. There will also be a separate consent page for each biological parent at the end of the consent form. If one of the parents is unable to consent to providing saliva at the time of initial consent, they may return to the clinic and be consented by the PI at a later date. This will be documented in the subject record, and the family will be provided a copy of the updated consent form.

Study Withdrawal Parents are free to withdraw from the study at any time. This will be clearly stated during the consent process. Subjects may need to discontinue participation in the study before completion for various reasons (e.g., family move, withdrawal of consent, adverse events, need for a different treatment). Parents who indicate intention to withdraw the child from the study will be offered to have a case conference with the study team to discuss the matter. If the discussion indicates that the child needs another treatment, we will assist the family to locate that treatment. Parents who drop out of treatment will be invited to return for scheduled assessments or an early termination visit. Data collected from children who drop out of treatment, but return for assessments, will be analyzed in their originally randomized group.

CONFIDENTIALITY

Case report forms (CRFs) contain the subject's unique ID number – but no identifying information. The RedCap Clinical Trial Management System is a secure, password protected data base. The folders containing CRFs data will be kept in locked files and access to these files is only granted to members of the research team. Standardized tests (e.g. IQ and language tests) record will not record the child's date of birth but will use age calculate normative data. No other personal health information will be documented on research forms. Contact information for the family is kept in a separate file that is in a locked cabinet.

Certain measures will be recorded on video. The digital files are stored on a secure, password-protected site. Recordings may also be stored electronically on an encrypted external hard drive that is kept in a locked closet or cabinet. The digital files will be marked with a Subject ID instead of names of children or their parents. It is possible that names may be mentioned on the recordings. Video recordings from this research study will be retained for up to 5 years after the study is over. Recordings may be used for other purposes (e.g. presentations at professional meetings) if separate consent is obtained from the parent (or guardian).

Access to study records (case report forms) will be restricted to study staff. Others that may review research records include study monitors, representatives from Food and Drug Administration (FDA). We may submit de-identified study data to the National Database for Autism Research (NDAR).

Potential Benefits of the Proposed Research to the Subjects and Others
Participants may directly benefit by the receipt of the supplement. Overall, the risks to the subjects in this research are similar to those that exist in standard clinical practice and the proposed research project offers considerable promise in improving mitochondrial function in children with ASD. Indeed, this research may lead to a new treatment option for children with ASD. Thus, the potential benefits of the research outweigh the risks involved.

Importance of the Knowledge To Be Gained Children with ASD are impaired and, untreated, their long-term prognosis is guarded. Although current treatments are helpful in some cases, significant gaps in our treatment options remain. There are no large, controlled clinical trials of nutritional supplements in children with ASD and no medication treatments for core features of ASD or language delay. This study may provide new information about nutritional supplements in children with ASD and may identify subgroups of affected children who could benefit from a nutritional supplement.

Data Safety and Monitoring Plan The medical monitor will review adverse effects every three months. If potential safety concerns are identified, the medical monitor will review the risk-benefit ratio with the unblended statistician. If treatment-emergent safety concerns alter the risk-benefit ratio, the medical monitor will contact the PI for discussion of potential AEs.

Monitoring for Adverse Events (AEs):
At each visit, including baseline and interim contacts, the treating clinician will systematically review for AEs and concomitant medications using an AE Review form developed for this study. We have used versions of this form, in several multi-site clinical trials in children with ASD which contains a general inquiry including several questions about daily activities (e.g., sleep, appetite, energy level, bowel and bladder functions). The general inquiry includes an open-ended question about any problems or complaints, as well as questions regarding the need for other medications and doctor or health care encounters since the last study visit. The last section includes specific questions about daily activities. All **new** AEs will be documented on the AE log. The status of previously-reported AEs

will be monitored as well.

When the treating clinician elicits an AE, it will be documented regardless of suspected relationship to the supplement. The AE log requires the treating clinician to label the AE and to document the severity, onset, course, outcome, and attribution (study drug related or not).

Attribution is classified as follows:
- Definite: AE is clearly related to the study drug.
- Probable: AE is likely to be related to the study drug.
- Possible: AE may be related to the study drug.
- Unlikely: AE is doubtfully related to the study drug.
- Unrelated: AE is clearly not related to the study drug.

Severity of AEs is classified as follows:
- mild= present, but no intervention required;
- moderate = present, may be bothersome or may require intervention;
- severe = present, bothersome, requires intervention and may produce sequelae.

Decisions on the appropriate care of the subject will be made by the treating clinician (who will remain blind to treatment assignment), in collaboration with the family's primary care provider whenever possible. Abnormal findings on laboratory tests that exceed established alarm values will be investigated to determine the nature of the adverse events.

Routine reporting of AEs Adverse events will be documented on the Adverse Event Log as described above. All AE data will be captured in the electronic database. Investigators will provide this information to local IRBs at the time of annual renewal. The investigators will decide if modifications to the protocol or consent form are required based on ongoing AEs.

AEs resulting in treatment discontinuation: Adverse events that are the primary reason for study discontinuation will be tracked separately. High rates of such events in the active supplement group compared to the placebo group will prompt evaluation of potential significant safety concerns in the trial. Rates of AEs will also be monitored during the open-label extension trial.

Serious adverse events: A serious adverse event (SAE) is defined as an event that poses a threat to the participant's life or functioning. We note that "severe" is not necessarily equivalent to "serious." Thus, a severe rash is not a serious adverse event, whereas a heart attack of any severity is likely to be a serious adverse event. A serious adverse event is defined as any event that entails one of the following:
- Death;
- Threat to the individual's life;
- Inpatient hospitalization or prolongation of existing hospitalization;
- Persistent or significant disability or incapacity;
- Intentional drug overdose;
- Any other significant event that jeopardizes the participant.

Assessment of Serious Adverse Events: The SAE will be classified as: a) serious AND anticipated; b) serious AND anticipated, BUT occurring with a greater frequency than expected; c) serious AND unanticipated. **SAEs that are unanticipated or anticipated SAEs that occur at a greater frequency than expected and deemed at least possibly related to the study treatment will be reported with seven days.** The report will be sent to study IRBs. The report will be sent to the FDA within 14 days. **SAEs** that do not meet either of these criteria will be included in annual reports to the IRBs.

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