Choline Supplementation as a Neurodevelopmental Intervention in Fetal Alcohol Spectrum Disorders

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List of Abbreviations

CFR	Code of Federal Regulations
CNBD	Center for Neurobehavioral Development
CRF	Case Report Form
FAS	Fetal Alcohol Syndrome
FASD	Fetal Alcohol Spectrum Disorders
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GRAS	Generally Recognized as Safe
ICH	International Conference on Harmonization
IRB	Institutional Review Board
RDI	Recommended Dietary Intake

Study Summary

Title	Choline Supplementation as a Neurodevelopmental Intervention in Fetal Alcohol Spectrum Disorders
Short Title	Choline supplementation in FASD.
Protocol Number	1R01AA024123
Phase	Phase I and II
Methodology	Randomized, double-blind, placebo-controlled
Study Duration	Five years
Study Center(s)	Single-center
Objectives	To evaluate the feasibility and tolerability of post-natal choline supplementation in young children
Number of Subjects	60
Diagnosis and Main Inclusion Criteria	Fetal alcohol spectrum disorder; documented prenatal alcohol exposure
Study Product, Dose, Route, Regimen	Choline bitartrate,19 mg per kg body weight (typical range: 200-425 mg; max dose 500 mg), oral solution administered once daily
Duration of administration	Nine months
Reference therapy	Placebo
Statistical Methodology	Mixed models growth curve analyses of the outcome measures using and Intention to Treat (ITT) approach

1. Introduction

This document is a protocol for a human research study. This study will be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Fetal Alcohol Syndrome (FAS) is the largest single known cause of mental retardation and developmental disability (Abel, 1995). Estimates suggest that the incidence of FAS is nearly 1 per 1000 live births in the general population and 4.3% among heavy drinkers (Abel, 1995; May & Gossage, 2001). Perhaps more importantly, the incidence of the wider spectrum of disorders associated with prenatal alcohol exposure (FASD) is thought to be as high as .9% of the general population (Sampson et al., 1997). At present, a diagnosis of FAS is typically made with consideration of four criteria: 1. prenatal alcohol exposure (documented or not documented); 2. dysmorphic facial features; 3. growth deficiency; 4. central nervous system dysfunction (Hoyme et al., 2005; K. L. Jones & Smith, 1973). However, it is well-established that most prenatally exposed individuals do not display the physical characteristics of FAS, but many do have structural brain anomalies and neurocognitive impairment (Bookstein et al., 2001; Burden et al., 2005b; Mattson et al., 1998; Sowell et al., 2001a).

As it has become clear that alcohol exposure has serious and permanent adverse effects on cognitive, behavioral, emotional, and social development, even without producing the complete FAS syndrome, the disorder has been reframed numerous times in recognition of various “partial” FAS conditions including Fetal Alcohol Effects or FAE (Clarren & Smith, 1978) and Alcohol-Related Neurobehavioral Disorder or ARND (Institute of Medicine, 1996). Currently, the term Fetal Alcohol Spectrum Disorders (FASD) is now commonly used to describe the entire spectrum of disability resulting from prenatal alcohol exposure (Riley & McGee, 2005; Warren et al., 2004). This FASD continuum includes, by some estimates, 5 to 15 times as many children as those diagnosed with full-criteria FAS (Lupton et al., 2004; May & Gossage, 2001; Sokol et al., 1980). When one considers the estimates of the US economic impact, which are in the billions of dollars per year (Lupton et al., 2004; Rice et al., 1991), and the fact that FAS represents only the tip of the iceberg, the staggering nature of the public health problem posed by the full range of FASD becomes apparent.

At present, the literature on interventions for individuals with Fetal Alcohol Spectrum Disorders (FASD) is very limited, with only two published randomized controlled trials (Premji et al., 2007). However, there are promising new treatment options that have not been applied in humans. Specifically, recent pre-clinical studies have shown that dietary choline supplementation prenatally and even postnatally, as late as days 21-30 in the rodent (equivalent to human childhood), attenuates the memory and behavioral deficits associated with prenatal alcohol exposure (J. D. Thomas et al., 2007; J. D. Thomas et al., 2000). Perinatal choline levels directly influence brain development (Zeisel &

Niculescu, 2006) and, thus, a human clinical trial involving choline supplementation is warranted. The fact that postnatal choline supplementation is effective in rats as late as 21-30 days is particularly relevant because FASD is typically only diagnosed later in childhood. As no human choline supplementation studies have been done in children with prenatal alcohol exposure, it is not known if supplementation will be effective postnatally, prenatally, or both. Based on the time periods in which choline is effective in pre-clinical models of FASD, and on the fact that the first years of human life represent a period of intense brain development, choline supplementation in young children appears to have significant potential as an intervention for neurodevelopmental disorders including FASD.

1.2 Investigational Agent

Although the human body produces choline, the demand cannot be met entirely endogenously and thus, some choline must be consumed in food. Human cells require choline and, without it, die by apoptosis (Albright et al., 1998; Eagle, 1955; Zeisel et al., 1997). Choline has been classified as an essential nutrient, and guidelines for "adequate intake" of choline have been established for infants, children, and adults (Food and Nutrition Board - Institute of Medicine, 1998). The guidelines also identify adequate intake levels specifically for pregnant and lactating women because of the recognized importance of choline to fetal / neonatal development. A recent study suggests that daily consumption by adults in the United States (mean = 312 mg. per day for men and 314 mg. per day for women) is significantly below the recommended 'adequate daily intake' of 550 mg. per day for men and 425 mg. per day for women (Cho et al., 2006). Choline is found in many foods but it has only been within the last few years that the specific choline content of various foods and food products was published (Zeisel et al., 2003). Foods highest in choline include eggs, liver, nuts, fish, and soy. Choline is also considered part of the Vitamin B-complex.

Choline exists in a free form or esterfied in one of several phospholipid forms including phosphatidylcholine, glycerol-phosphocholine and sphingomyelin. It is also found in the neurotransmitter acetylcholine. As a precursor to acetylcholine, phospholipids, and betaine, choline is critical to the formation of cell membranes, transmembrane signaling, cholinergic neurotransmission, and the metabolism of lipids and cholesterol (Food and Nutrition Board - Institute of Medicine, 1998). In the context of brain development, choline is intricately linked to another essential nutrient, folate, and deficiencies in either nutrient lead to disruptions in neural tube closure (Shaw et al., 2004; Smithells et al., 1976; Zeisel, 2009b). Historical experiences with folate, iron, and iodine fortification have dramatically demonstrated the importance of essential nutrients during periods of development. For example, when cereal grains were first fortified with folic acid, the rates of neural tube birth defects dropped by 26% (Centers for Disease Control and Prevention, 2004). Although choline deficiency during pregnancy likely has less dramatic effects than folate deficiency, very little is actually known about the impact of deficiency or supplementation during critical developmental periods.

1.3 Preclinical Data

Pre-clinical studies have demonstrated that:

1. Perinatal choline deficiency results in abnormal brain development and cognitive impairment (Albright et al., 1999b; Holmes-McNary et al., 1997; Wong-Goodrich et al., 2008) and 2. Perinatal choline supplementation contributes to structural brain changes (Li et al., 2004) and enhanced performance on measures of cognition including memory (Cheng et al., 2008; Meck et al.,1988; Meck & Williams, 1997a). Choline deprivation in rats leads to a 4-fold increase in the rate of neural tube defects (Shaw et al., 2004). In addition, choline deficiency leads to permanent structural and biochemical brain abnormalities that may be related to underlying changes in neurogenesis and differentiation in fetal hippocampus and septum, areas of brain that are critical for normal spatial learning and memory (Cermak et al., 1999). Furthermore, prenatal choline supplementation leads to enhanced visual-spatial memory performance (Meck et al., 1988, 1989; Meck & Williams, 1997a, 1997b) and reductions in proactive interference during learning (Meck & Williams, 1999).

Perinatal choline levels have an impact at multiple levels of neurodevelopment. Choline contributes to increased dendritic arborization in hippocampal CA1 cells, larger somata, and critical functional changes to these cells (J. P. Jones et al., 1999; Li et al., 2004; Mellott et al., 2004; Montoya et al., 2000; Pyapali et al.,1998). Prenatal and postnatal choline supplementation also affects choline acetyltransferase levels in both hippocampus and frontal cortex in rats which, in turn, is associated with improved memory functioning, especially in visual-spatial memory (Meck et al., 1989; Williams et al., 1998). It has also been demonstrated that the ability of cholinergic neurons to produce acetylcholine is directly related to the availability of free choline, its precursor (Blusztajn & Wurtman, 1983). Furthermore, choline levels have an impact throughout the brain on apoptosis, the programmed cell death that is part of normal brain development (Holmes-McNary et al., 1997; Yen et al., 2001). In addition, choline is required for the production of a number of phopholipids (such as phosphatidylcholine, sphingomyelin, and plasmalogen) that are present in all cells including neurons. These membrane phospholipids are necessary for axonal growth and myelination, among other processes (Zeisel & Niculescu, 2006). Thus, the evidence suggests that the availability of free choline has important effects, depending on discrete time windows, on basic aspects of brain structure and function in the developing brain (J. P. Jones et al., 1999).

Importantly, pre-clinical choline studies provide strong evidence that choline levels during critical periods of brain development have a permanent impact on brain structure and function throughout the lifespan. For example, electrophysiological studies of hippocampal CA1 cells in brain slices of adult rats that were either deprived of choline or supplemented with choline during gestation show permanent changes in long term potentiation (LTP) (J. P. Jones et al., 1999). LTP reflects an increase in strength of the synaptic connection between neurons following high frequency stimulation (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973) and is thought to be an important substrate of learning and memory (Bliss et al., 2003). Meck and colleagues have

described the impact of perinatal choline availability on brain structure as “metabolic imprinting” because of the permanent changes that occur in the cholinergic system, affecting brain functioning throughout the lifetime (Meck & Williams, 2003).

Perinatal choline supplementation attenuates the negative effects of prenatal alcohol exposure. Thomas et al. (2000) initially demonstrated that administering choline to prenatally-exposed rat pups on postnatal days 2-21 resulted in improved performance on a discrimination task and a delayed discrimination task. Without postnatal choline supplementation, the alcohol-exposed rats were impaired on both tasks. Interestingly, choline improved the performance of a non-alcohol-exposed group as well, but the largest effects were seen in the alcohol-exposed rats, perhaps because they had the most room for improvement. In this study, as in others, the beneficial effects of choline on cognitive functioning continued (at least until postnatal day 45 when testing occurred) long after choline administration had been discontinued (post-natal day 21). A number of other studies have shown similar effects of postnatal choline improving cognitive functioning including visual-spatial learning, spatial reversal learning, and fear conditioning among rats prenatally exposed to alcohol (Wagner & Hunt, 2006). In addition, choline supplementation during postnatal days 4-30 has also been shown to reduce excess activity of rats in an open-field situation (J. D. Thomas et al., 2004). This finding is intriguing because prenatal alcohol exposure causes postnatal hyperactivity in rats, and is associated with hyperactivity and inattention in humans with FASD.

In normally developing rats, the “window” for the beneficial effects of choline appears to include gestation and the first 30 postnatal days with the most significant benefits obtained if choline supplementation is given during both prenatal and postnatal periods (Meck et al., 1989). However, the critical windows for choline’s effectiveness may in fact be different for animals whose brains have been damaged by prenatal alcohol exposure. Ryan et al. (2008) demonstrated that both “early” postnatal choline supplementation (postnatal days 11-20) and “late” choline supplementation postnatal days 21-30) significantly attenuated the cognitive deficits induced by prenatal alcohol exposure. There was a slight advantage for the early supplementation, but it was not a large difference. Thus, the authors concluded that “there is not a strict temporal window for choline to be effective, at least on some behavioral measures” and that “the developmental window for choline to be effective is either quite large, or spans between the PD (postnatal days) 11-20 and 21-30 time periods.”
Overall, these results suggest that, in humans, choline supplementation could attenuate the negative effects of prenatal alcohol exposure across a relatively wide period of postnatal development, perhaps even across the first 5 to 10 years of life.

Extrapolating from the pre-clinical trial findings of choline’s efficacy to potential applications in humans is challenging due in part to the problems of cross-species neurodevelopmental comparisons. Thomas et al.’s studies in FASD (J. D. Thomas et al., 2007; J. D. Thomas et al., 2004; J. D. Thomas et al., 2000) and much of the pre-clinical work in “normally developing” animals has been done with rats (Meck et al., 1988, 1989; Meck & Williams, 1997a). Fortunately, there have been significant advances in our understanding of cross-species comparisons (Clancy et al., 2001;

Clancy et al., 2007) and some have argued that the prenatal and early postnatal periods are the most reliable time period during the lifespan in which to make such comparisons because of the remarkable similarity in timing and order of critical neurodevelopmental events across species (Finlay & Darlington, 1995). This may be especially true with regard to specific neural systems or structures.

As an example, we know a great deal about the timing and sequencing of hippocampal development in rats and humans (Avishai-Eliner et al., 2002). In humans, there is a rapid increase in hippocampal development during the first two years of life, followed by a period of slow, steady growth until adolescence (Utsunomiya et al.,1999). Critical aspects of differentiation and synaptogenesis are occurring in the hippocampus between 3 and 5 years of age. This rapid development includes maturation of pyramidal cell dendrites in CA3. The equivalent developmental maturation in rats takes place by 21 postnatal days, within the timeframe of choline's efficacy in pre-clinical studies. Similarly, in humans, hippocampal-dependent learning and memory processes continue to mature until 4 or 5 years of age. The equivalent developmental maturation of memory processes in the rat occurs on postnatal days 15 and 16, again within the timeframe of choline's efficacy. MRI volumetric studies have now demonstrated that the human hippocampus undergoes a rapid growth spurt in the first two years of life and continues a slow, steady growth throughout childhood.

The principal of clinical equipoise, describing the state of genuine uncertainty in the expert medical community about the potential efficacy of an intervention (Freedman, 1987), is an important factor in decisions about the ethics and practicality of a randomized clinical trial. At the present time, despite impressive pre-clinical evidence, there is very little human data and no consensus about the potential neurodevelopmental benefits of postnatal choline supplementation in humans (Cockburn, 2003). This uncertainty supports the need for a comprehensive trial.

The ability of choline supplementation to modify certain aspects of brain development and mediate the effects of damage during development may prove useful in other neurodevelopmental disorders. For example, Holmes et al. (2002) have shown that prenatal choline supplementation of rats protects them from cognitive impairment typically seen in induced-seizures at a point much later in development. This work, and other similar work on choline supplementation suggests that nutritional factors during critical periods of brain development (prenatal and postnatal) has long-lasting effects on neuroplasticity (Williams, 2008). Intriguingly, the effects of early supplementation appear to last throughout the lifetime of the organism. For example Glenn et al. (2008) found that prenatal choline supplementation in rats on embryonic days 12-17 led to less age-related decline in cognitive functioning (open field exploratory behavior) at 25 months (considered old age in rats). This life-long protective effect of choline was associated with increased numbers of newly proliferated hippocampal cells as well as increased levels of vascular endothelial growth factor and neurotrophin-3 in the brains of the rats who received prenatal choline.

In addition, Nag et al. have recently demonstrated several neurodevelopmental benefits of postnatal choline supplementation in a rat model of Rett Syndrome (Mecp2(1lox) mice) including improved locomotion (Nag & Berger-Sweeney, 2007) and increased striatal nerve growth factor (Nag et al., 2008).

1.4 Clinical Data to Date

Dr. Steven Zeisel, Professor of Nutrition and Pediatrics at the University of North Carolina and director of the Nutrition Research Institute (nri.unc.edu) and a consultant on this project, is one of the world's leading nutrition experts. Dr. Zeisel has been studying choline for more than 30 years and has been responsible for many of the most important findings including the studies initially demonstrating that choline is an essential nutrient in humans (Sheard & Zeisel, 1986; Zeisel et al., 1991). Dr. Zeisel's work has identified choline levels in common foods (Howe et al., 2004), identified genetic polymorphisms associated with choline metabolism (daCosta et al., 2006; Zeisel, 2007, 2008), and investigated relationships between choline and numerous human diseases (Albright et al., 1997; Bidulescu et al., 2007; Cho et al., 2007; Xu et al., 2008; Zeisel, 1992). Most relevant to the current proposal, Dr. Zeisel has demonstrated the role of choline in normal brain development as well as the potential for choline supplementation during critical periods of brain development (Conlay & Zeisel, 1982; Magil et al., 1981; Sanders & Zeisel, 2007; Zeisel, 1997, 2000, 2004, 2006, 2009a; Zeisel & Niculescu, 2006).

Our research group has recently completed the first pilot studies to evaluate choline supplementation as a neurodevelopmental intervention in children with FASD. We first conducted a double- blind, randomized, placebo-controlled pilot study (R21AA019580) that focused on establishing the safety and tolerability of choline in children with FASD (n=20) (Wozniak, Fuglestad et al. 2013). That study evaluated the safety of choline (there were no serious adverse events) and demonstrated high tolerability (children received partial or full doses on 87% of 270 days in the study). We recently completed a larger double-blind, randomized, placebo controlled trial that included a total of 40 additional participants, ages 2.5 to 5 years (R33AA019580). Compared to placebo, choline supplementation improved 2-3 year old children's performance on an elicited imitation paradigm, a hippocampus-dependent memory task. These data are outlined in the preliminary studies section below. The results comprise the first available human evidence that the pre-clinical findings in rodents may translate to humans. The results from our initial two studies of choline in children with FASD directly inform the next phase of this work which will include 1. Testing a modified dosing scheme based on the efficacy and side effect data collected in the previous trials; 2. Determining the longevity of the cognitive changes attributable to choline; 3. Examining the specificity/generalizability of choline's effects by measuring additional cognitive domains; 4. Further evaluating a choline-specific gene as apotential

moderator of treatment response. The genomic hypothesis centers around a single nucleotide polymorphism (SMP) that is involved in endogenous choline synthesis (phosphatidylethanolamine N-methyltransferase (PEMT) rs12325817 SNP). The SNP is associated with significantly greater susceptibility to dietary choline insufficiency (Fischer, da Costa et al. 2010). The hypothesis is that children with one or two alleles of rs12325817 (CG or CC genotype) will show a significantly larger response to choline treatment on measures of cognitive functioning compared to those with the normal genotype (GG) because they have a lower capacity to synthesize choline endogenously.

A series of papers from a Cleveland Clinic research group have raised a potential link between trimethylamine-N-oxide or TMAO, a metabolite of choline and carnitine, and atherosclerosis in older adults with cardiovascular disease (Wang et al., 2011; Tang et al., 2013; Koeth et al., 2013). An initial metabolomic study of patients undergoing cardiac evaluation showed an association between plasma TMAO levels and acute cardiovascular events. A later study showed a similar relationship between plasma TMAO levels in patients undergoing coronary angiography and cardiovascular events over a 3-year follow-up period. Another study reported that atherosclerosis-prone (APOE--) mice that were fed choline or TMAO showed greater aortic root atherosclerotic plaque compared to control animals. In contrast, a study in hamsters demonstrated an inverse relationship between plasma TMAO and atherosclerosis (Martin et al., 2009). Two large studies of cardiovascular risk factors in humans, the Atherosclerosis Risk in Communities (14,430 men and women) study (Bidulescu et al., 2007) and the PROSPECT-European Prospective Investigation into Cancer and Nutrition (16,165 women) study (Dalmeijer et al., 2008) observed no significant increase in cardiovascular risk with increasing dietary intake of choline. An editorial accompanying the Tang et al. (2013) paper in the New England Journal of Medicine highlights the importance of further examining the new model but also cautions “These speculative comments and suggestive observations indicate that much remains to be done to determine the precise role of TMAO in atherothrombogenesis — whether it has a direct effect on pathogenesis, is an epiphenomenal biomarker, or is a precursor to a more direct effector.” (Loscalzo at al., 2013). In summary, these emerging data are important but do not imply a clear risk to healthy youhg individuals taking choline supplementation. We have informed the FDA of these developments in the literature with an Administrative Supplement and we will continue to monitor TMAO levels in the trial in order to provide additional data to the field.

1.5 Dose Rationale and Risk/Benefits

For children ages 1 to 3 years, the recommended dietary intake (RDI) for choline is 200 mg. and the maximum upper limit for tolerability is 1000 mg. (Food and Nutrition Board - Institute of Medicine, 1998). For children ages 4 to 8 years, the RDI for choline is 250 mg. and the maximum upper limit for tolerability is also 1000 mg. (eating two eggs would provide an equivalent of 545 mg. of choline). The goal of the current study is to provide choline supplementation as opposed to simply ensuring choline sufficiency. Our previous two studies used a fixed 500 mg. dose, regardless of the child’s weight

(Wozniak et al. 2013, and Wozniak et al., 2015). The dose was well-tolerated, with partial or full doses received on 88% of the 270 days in the study. There was no difference between 500 mg. choline and placebo in terms of doses received or participant drop-out. There were no serious adverse events and no adverse events that differed by group other than fishy body odor in the choline group (an expected adverse event). A substantial proportion of children in the choline group (52%) compared to the control group (4%) did experience fishy body odor at some point in the 9 month study. For the smallest children in the study by weight, nearly 100% experienced fishy body odor at some point.

For the current study, choline will be given at a 19 mg per kg dose, based on evidence from the prior two pilot studies showing increased rates of fishy body odor at higher doses and also on data indicating that memory performance was optimal at doses in the 19 mg per kg range. Choline will be administered in the form of a fruit-flavored drink mix containing choline bitartrate (compounded for the study by Fagron, St. Paul, MN; Investigational New Drug Application #107085). Balchem (New Hampton, NY) manufactures the choline bitartrate to USP standards. The parent will add the powder to plain water. The matching placebo drink mix (without choline bitartrate) will also be provided by Fagron. Material will be stored, labeled, allocated, and dispensed by the University of Minnesota's Investigational Drug Service (IDS) Pharmacy.

Participant families will be given a 65 day supply initially. Re-supply will be handled by packages mailed after the investigators have verified the participant's continued involvement in the study and freedom from serious adverse events during telephone visits. The preparation, coding, and tracking will be handled by the University of Minnesota's Investigational Drug Service pharmacy.

Choline is considered a GRAS substance (generally regarded as safe) by the FDA and the dose to be utilized in this study is lower than the maximum tolerable upper limit. Based on our previous study, tolerability for this lower dose of choline is expected to be high. Parents will be educated at the time of enrollment about potential side effects of choline which rarely occur and usually at much higher doses than those proposed (hypotension has been reported at 7.5 grams per day; gastrointestinal symptoms (loss of appetite, upset stomach), sweating, or body odor have occurred at 10-16 grams per day). As indicated, in the first 60 participants, we only observed fishy body odor and not other adverse events.

At one month, a full range of potential side effects will be screened. Although allergic reactions to choline are not expected (we observed none in the pilot studies), we will monitor for a range of allergic symptoms nonetheless. Adverse events and serious adverse events will be reported to the University of Minnesota IRB and to Quorum IRB. Serious adverse events will also be reported to the NIH program officer.

2. Study Objectives

Primary Objective
Evaluate the efficacy of choline in treating memory deficits and executive functioning deficits in children with FASD.

Secondary Objectives
Evaluate the long-term effects of choline supplementation by re-assessing children who took choline and placebo as part of the previous pilot study.

Evaluate the potential interaction between genotype ((for example PEMT, rs12325817) and response to choline intervention.

3. Study Design

3.1 General Design

This is a randomized, double-blind, placebo-controlled study. Sixty children, male and female, between the ages of 2 years and 5 years at enrollment will be included.

Eligible participants will be randomized to treatment or placebo in a 1:1 allocation ratio. A block randomization procedure with variable block sizes will be used to maximize unpredictability of assignment.

Participants will receive the test article for nine months and will have an in-person baseline visit followed by 11 additional visits (9 telephone visits; 2 additional in-person visits) for a total of 12 visits. A telephone visit for purposes of safety and compliance will occur at 2 weeks. A telephone visit will be scheduled for 1 month after administration of the test article begins. Formal complete follow-up assessments will occur at 6 months and 9 months. Telephone visits for safety and compliance will occur at months 2, 3, 4, 5, 7, 8 and 10. Parent-report measures of behavior will be collected. Behavioral paradigms will be used to assess memory and other cognitive functions. Formal psychometric testing to establish developmental levels will be completed. Blood will be collected to assess compliance with the treatment and effect on blood choline levels. DNA will also be extracted for analysis of single nucleotide polymorphisms related to endogenous choline production. Plasma will also be analyzed for immune function and micro-RNA.

As part of the project, participants from the previous study (1R21AA019580 / 4R33AA019580) will be brought back to the University for a set of cognitive evaluations to assess the long-term effects of having taken choline/placebo previously.

3.2 Primary Study Endpoints

The primary study endpoints will be cognitive assessments. These will include the Elicited Imitation memory task and the Stanford-Binet Intelligence Scales.

3.3 Secondary Study Endpoints

Secondary cognitive endpoints will include the Minnesota Executive Functioning Scale (Carlson, 2014), and the NIH Toolbox Flanker Inhibitory Control and Attention Task (MacDonald, 2014). We will also evaluate potential behavioral and/or emotional changes with a caregiver-report paper and pencil measure (Child Behavior Checklist).

We will also evaluate feasibility and tolerability by collecting empty and full choline packages to monitor compliance. Serum choline levels will be obtained.

As part of the project, we will bring up to 40 participants (20 choline and 20 placebo) from our previous study (1R21AA019580 / 4R33AA019580) back to the University for a brief visit focused on examining the potential long-term cognitive effects of having taken choline or placebo. All who participated in the previous study will be eligible for the return visit. No study drug will be used at this visit and no blood will be collected. The measures administered will be cognitive outcome measures. The Elicited Imitation paradigm, used in the initial study, will be repeated using materials/items that have not previously been seen by the child. The Stanford Binet Intelligence Scales will be administered. The NIH Toolbox Picture Sequence Memory Test is a measure of episodic memory appropriate for ages 3 and up (McDonald 2014). The NIH Dimensional Card Sort Task is a measure of executive functioning from the same NIH Toolbox. The NIH Flanker Task is a measure of attentional control from the same NIH toolbox. The Narrative Memory subtest from the NEPSY-II is a measure of verbal memory for ages 3 to 16 (Korkman, Kirk et al. 2007). The Memory for Names subtest is another verbal memory subtest from the NEPSY-II. The child's height and weight will also be measured.

We will also bring up to 20 participants (10 choline and 10 placebo) from our previous study (1R21AA019580 / 4R33AA019580) back to the University for a neuroimaging visit focused on examining the potential long-term brain development effects of having taken choline or placebo. Participants will undergo MRI scans and brief neurocognitive testing at this follow-up visit.

3.4 Primary Safety Endpoints

No safety endpoints have been defined. Choline is a normal component of the human diet, so there are relatively few risks associated with supplementation. The doses to be used in the proposed project are below the upper tolerable limit for choline (1000 mg per day) and at or below the doses previously used in our pilot studies. At much higher doses than we are proposing, choline supplementation has been associated with mild hypotension. We observed no evidence of hypotension in 60 participants in the previous pilot studies. In the literature, some individuals report gastrointestinal disturbance associated with very high levels of choline supplementation. We observed no significant increase in GI symptoms with choline in 60 participants during the previous pilot studies. A full range of potential side effects will be screened.

Although allergic reactions to choline are not expected, we will monitor for a range of allergic symptoms nonetheless. There are no reported manifestations of intoxication of any kind nor overdose from the choline preparation to be used in this study. No toxic

effects have been reported.

NOTE that a Data and Safety Monitoring Board (DSMB) is in place and will review the study progress and adverse events.

4. Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Ages 2.5 years to 5 years of age*
2. Available parent or legal guardian capable of giving informed consent for participation. Participation of children under guardianship (wards of the state) needs to be authorized by a court order.
3. Modified Institute of Medicine (IOM) criteria for Fetal Alcohol Syndrome (FAS), Partial Fetal Alcohol Syndrome (PFAS), or Alcohol-Related Neurodevelopmental Disorder (ARND) (Hoyme, May, et al., 2005).

4.2 Exclusion Criteria

1. Known history of a neurological condition (ex. epilepsy, traumatic brain injury)
2. Known history of a medical condition known to affect brain function.
3. Known history of other neurodevelopmental disorder (ex. autism, Down syndrome)
4. Known history of very low birthweight (<1500 grams)

*For the follow-up studies of returning participants from the previous study (1R21AA019580 / 4R33AA019580), the inclusion/exclusion criteria will be the same as above except that the age range will be extended to incorporate the fact that the children are now older. The inclusion/exclusion criteria for the follow-up study will be from 2.5 to 15 years of age.

4.3 Subject Recruitment and Screening

Participants will be recruited from two University of Minnesota Medical Center Clinics in which co-investigators see patients: The Fetal Alcohol Spectrum Disorders clinic (Dr. Christopher Boys, Ph.D., Dr. Amy Gross, Ph.D) and the International Adoption Clinic (directed by Dr. Judith Eckerle, M.D.). Families of potential participants will be contacted by research staff. Visitors to these clinics may also see flyers and brochures describing the study at the time of their clinic appointment although they will not be recruited directly by their clinician. Mailings will also be sent to families of children with FASD via lists provided by the Minnesota Organization on Fetal Alcohol Syndrome (MOFAS). MOFAS has close working relationships with Dr. Wozniak, Dr. Eckerle, Dr. Boys, Dr. Gross and both clinics. All recruitment materials will be submitted for IRB approval upon development, before use in the study. Telephone scripts will be utilized.

For the follow-up assessment, all participants from the previous study (1R21AA019580 / 4R33AA019580) will be invited to participate. Contact will be by letter and/or telephone call. Scripts will be utilized.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Participants may withdraw from the study at any time. The investigators may initiate the withdrawal of a study participant if significant side effects are apparent such as major gastrointestinal disturbance, diarrhea, hypotension, or allergic reaction.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

At the time of enrollment, contact information will be obtained for the participating family including address, home telephone number, cell phone number, and email address. We may also request contact information for a secondary caregiver of the child who would know their status in the future in case the study loses contact with the parent / primary caregiver. We will attempt to collect some follow-up information at 9 months for all withdrawn subjects (the normal point of study conclusion). If the withdrawn participant family is willing to be interviewed in person or by telephone about the child, we will collect side effect information, other health information such as the emergence of new medical problems, behavioral data (via the standard study behavioral rating instruments), and information about the reasons for study withdrawal.

5. Study Drug

5.1 Description

Choline, which is considered part of the Vitamin B-complex, is an essential nutrient normally consumed on a daily basis in many foods. Supplemental choline will be administered in the form of a fruit-flavored drink mix containing choline bitartrate (compounded for the study by Fagron, St. Paul, MN under IND#107085). Balchem (New Hampton, NY) manufactures the choline bitartrate to USP standards. The parent will add the powder to plain water. The matching placebo drink mix (without choline bitartrate) will also be provided by Fagron. Material will be stored, labeled, allocated, and dispensed by the University of Minnesota's Investigational Drug Service (IDS) Pharmacy.

5.2 Treatment Regimen

Choline will be administered at a dose of 19 mg per kg once daily, as an oral solution for nine months. Placebo will be administered as an oral solution once daily for nine months. Choline will be administered under FDA Investigational New Drug Application #107085. Parents will be informed that choline is being regulated as an investigational drug for purposes of this clinical trial.

5.3 Method for Assigning Subjects to Treatment Groups

Randomization will be managed by using a computer procedure for randomization and tracking. A block randomization with variable block sizes will be used to maximize unpredictability of assignment. The University of Minnesota Investigational Drug Service will have the randomization code and will assign subjects and distribute the test article according to the randomization schedule.

5.4 Preparation and Administration of Study Drug

Fagron will prepare the choline fortified fruit-flavored drink mix and placebo drink mix for the study (IND#107085). Participating families will be provided with a supply of the material in the form of individual packages. The parent / guardian will prepare the drink by adding the powdered drink mix to plain water and will then administer it to the child. The parent/guardian will be trained to prepare and administer the test article at the time of the baseline visit.

5.5 Subject Compliance Monitoring

The parent/guardian of each subject will be asked to keep all empty study packets in a provided Ziploc bag. Self-addressed, postage-paid large envelopes will be provided to participant families to facilitate the return of empty packets. At the time of Visit 8 and Visit 11, the parent/guardian will be asked to bring all used and unused packets of the test article. Study staff will count the used and unused packets. Information will be recorded on the compliance case report form for each follow-up visit.

Significant non-compliance will be reported to the investigator after each follow-up visit. Compliance will be discussed and encouraged at each follow-up visit including telephone visits.

5.6 Prior and Concomitant Therapy

At baseline, and at every follow-up visit, the parent/guardian will be asked to provide information about any over-the-counter or prescription medications the subject is currently taking or has been given since the last visit. The parent/guardian will be asked to provide the name of the substance, the dose, the starting date and the stopping date.

After enrollment, the new initiation of nutritional supplements containing choline or betaine will be disallowed. All other concomitant therapies, including potential behavioral interventions, will be allowed and recorded.

5.7 Packaging

The test article, a powdered drink mix, will be packaged in individual foil packets. The powdered drink mix in each foil pack will contain either choline bitartrate or placebo. The test article will be delivered by courier from the manufacturer (Fagron) to the

University of Minnesota Investigational Drug Service where the labels will be prepared and where the material will be kept prior to distribution to research participants. Each foil packet will be labeled with a study number, date of manufacture, a code by which the contents (choline bitartrate vs. placebo) can be tracked, cautionary note, and indication of “investigational drug” status.

5.8 Blinding of Study Drug

Each packet of test article will be coded with an arbitrary code number. The University of Minnesota Investigational Drug Service will keep the link between these codes and the contents (choline bitartrate vs. placebo). The investigators will remain blinded throughout the study. The study statistician, Ann Brearley, will have access to the link and, therefore, will be unblinded.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Balchem, the manufacturer of the active agent and the placebo, will ship the product to a compounding pharmacy (Fagron). The transportation and packaging will take place according to GMP (good manufacturing practices). Fagron will then ship the material in sealed foil packets to the University of Minnesota Investigational Drug Service (IDS). IDS will perform an inventory, complete a drug receipt log, and return any damaged material to the manufacturer. This will be documented and the study sponsor will be notified. There are no special storage requirements for this product.

5.9.2 Dispensing of Study Drug

The Investigational Drug Service will assign the test article (choline bitartrate or placebo) to each participant by consulting the randomization schedule. The investigators will pick up the coded test article from the IDS in several amounts. Initially, 30 packets will be distributed to participating family at the study baseline visit. The remaining material will be distributed in batches of 60 packets.

The investigator will keep a log onsite to ensure all medication that is dispensed is recorded the day of the baseline visit and the days of shipment.

5.9.3 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of test article distributed, consumed, and remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6. Study Procedures

6.1 Visits 1, 8, & 11 (Baseline and follow-up assessment visits)

Participants and their parent/guardians identified as described in Section 4.3 of this protocol will be asked to come for a baseline visit (Visit 1). This visit is expected to last for approximately 3 to 3.5 hours. Visits 8 and 11 will be similar, but slightly shorter (2.5 to 3 hours) because several tasks are not repeated. The following procedures will occur:

- Consenting process (Visit 1 only);
- Medical history (Visit 1 only) and physical exam
- Concomitant medications will be recorded
- Height
- Weight
- Head circumference
- Facial photograph to allow for detailed measurements (Visit 1 only)
- Blood pressure
- Blood collection for choline and metabolite (betaine, phosphatidylcholine, sphingomyelin, and trimethylamine-N-oxide (TMAO) level (3 ml tube).
- Plasma extracted from this whole blood will be analyzed for measures of immune function (cytokines) and micro-RNA..
- Extraction of DNA sample from any blood sample for single nucleotide polymorphisms related to endogenous choline synthesis (PEMT rs12325817)
- Astley & Clarren's FASD diagnostic evaluation including a photograph of the child's face for computer measurement and analysis (Visit 1 or Visit 3)
- Stanford Binet Intelligence Scales, Fifth Edition (Visit 1 and 11 only)
- Elicited Imitation Paradigm, including video-recording to facilitate scoring afterward
- Minnesota EF Scale (MEFS), Early Childhood Version
- NIH Toolbox Flanker Inhibitory Control and Attention Task
- Beery Developmental Test of Visual-Motor Integration (VMI) (any visit)
- Scales of Independent Behavior (SIB) (any visit)
- Child behavioral questionnaires (CBCL); a parent-report instrument
- 24-hour Diet interview (for the parent/guardian to complete about the subject)
- Child Eating Behavior Questionnaire (CEBQ); a parent-report instrument
- Randomization (Visit 1 only)
- Training on how to store, mix, and administer the study medication (Visit 1 only)
- Adverse events will be recorded (Visits 8 & 11 only)
- Dispense a supply of the test article (30 packages at Visit 1)
- Provide instructions for returning for the next follow-up visit (Visits 1 & 8 only)

6.2 Visits 2, 3, 4, 5, 6, 7, 9, 10 & 12 (compliance/safety phone contacts)

At each of these visits, study staff will place a phone call to the participant's parent/guardian. The following information will be collected:

- Adverse events will be recorded
- Concomitant medications will be recorded (Visits 2,3,4,5,6,7,9 & 10)
- Any challenges with compliance related to administration of the study medication or completing the medication diary (Visits 2,3,4,5,6,7,9 & 10)

Visit 2 will occur 14 days after the baseline visit; Visit 3 will occur at Month 1 after baseline; Visit 4 will occur at Month 2 after baseline; Visit 5 will occur at Month 3 after baseline; Visit 6 will be at Month 4 after baseline; Visit 7 will be at Month 5 after baseline; Visit 9 will be at Month 7 after baseline; Visit 10 will be at Month 8; Visit 12 will be at Month 10 after baseline (one month after discontinuation of the test article). The acceptable visit window for each of these visits is +/- 7 days for Visit 2 and +/-14 days for the remainder of the visits. Each phone call is expected to last approximately 15 minutes. The purpose of Visit 12 is to collect potential adverse event data after the participant is no longer on the test agent.

6.3 Return assessment visit (1R21AA019580 / 4R33AA019580) (2B)

- Consenting – using separate consent form; Assenting if age =>7 years
- Height
- Weight
- Elicited Imitation Paradigm, including video-recording to facilitate scoring afterward
- The NIH Toolbox Picture Sequence Memory Test
- The NIH Dimensional Card Sort Test
- The NIH Picture Sequence Memory Test
- The Narrative Memory subtest from the NEPSY-II
- Stanford Binet Intelligence Scales, Fifth Edition
- Child behavioral questionnaires (CBCL)
- 24-hour Diet interview

6.4 Second Return Visit (1R21AA019580 / 4R33AA019580) (2C)

- Consenting – using separate consent form; Assenting for those age =>7 years
- Delis-Kaplan Executive Function System (DKEFS) Trail Making and Color-Word Interference subtests
- NIH Toolbox List Sorting task
- Wechsler Intelligence Scale for Children – working memory subtests
- Child Behavior Checklist (CBCL)
- 1 hour MRI scan
- Subjects will be scanned at the Center for Magnetic Resonance Research on a Siemens Prisma scanner. High resolution structural scans will be collected
- MRI scans will not be interpreted clinically. Group MRI analysis will be done using already published approaches.
- Use of radiation: NA – MRI does not use ionizing radiation
- Use of Center for Magnetic Resonance Research: This study uses the University of Minnesota's CMRR and follows all CMRR protocols and procedures.

- De-identified neuroimaging data will be stored locally on servers supported by the Center for Magnetic Resonance (CMRR).
- De-identified cognitive and behavioral data will be stored in a secure, HIPAA-compliant RedCap database supported by the University of Minnesota.
- Data elements to be stored will include processed, de-identified neuroimaging results, de-identified neurocognitive test results, and de-identified behavioral questionnaire data.

NOTE: If a participant has recently participated in one of our other protocols, and there is recent appropriate MRI or neuropsychological data, signed permission will be obtained to utilize those data for the purposes of this protocol (in addition to the standard consent and assent forms for this study).

NOTE: Missing data for cognitive measures in studies of children with neurodevelopmental disorders are common (because of attention deficits, non-compliance, etc.). Behavioral checklists are always presented with the option to skip items if they are uncomfortable. Therefore, missing cognitive test data and behavioral checklist data are within protocol and not considered deviations.

7. Statistical Plan

7.1 Sample Size Determination

For purposes of sample size determination, the dependent measure was the percent of correct items on delayed recall of the EI task (averaged across two events per timepoint). The effect size from our completed trial was d=0.66. A power analysis was carried out using Optimal Design Software for Multi-level and Longitudinal Research (Version 3.01) to determine the sample size that would be necessary to detect a difference in linear change between treatment and placebo groups with this effect size (Feingold 2009). The analysis assumed that the data would be analyzed using a mixed models repeated measures design for a person-randomized trial, with an effect size d=.66, and a two-tailed type I error of 0.05. To achieve 80% power, a sample size of 58.5 participants (approximately 30 per treatment arm) is necessary. This is consistent with the significant group difference that was observed in the pilot trial. The sample size for the proposed study will therefore be fixed at 60 children. Combining participant data

for some outcome measures from the first set of pilot studies (N=60) and the proposed study (N=60) will provide additional power to further evaluate age, sex, and PEMT genotype as moderating variables.

7.2 Statistical Methods

Statistical analyses will utilize an Intention to Treat (ITT) approach. Mixed models analyses with growth curve estimates will form the basis of the core analyses.

7.3 Subject Population(s) for Analysis

Not applicable

8. Safety and Adverse Events

8.1 Definitions

8.1.1 Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

8.1.2 Serious Adverse Event

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

8.1.3 Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up (10 months in the case of this study).

8.1.4 Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

8.1.5 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

8.1.6 Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

8.1.7 Abnormal Laboratory Values

A clinical laboratory abnormality will be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

8.1.8 Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery will be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the participant family, the investigator will seek information about adverse events through specific questioning. At each in-person visit, the investigators will also directly evaluate for adverse events by examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). Clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document.

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events

8.3.1 Study Sponsor Notification by Investigator

A serious adverse event will be reported to the IRB and to the study sponsor (NIH) by telephone within 24 hours of the event. A Serious Adverse Event (SAE) form will be completed by the investigator and faxed to the IRB and to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events by phone and facsimile to:

Jeffrey R. Wozniak, Ph.D.
Voice: 612-273-9741
Pager: 612-899-8941
Cell: 612-598-0041
FAX: 612-273-9779

At the time of the initial report, the following information will be provided:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

Within the following 48 hours, the investigator will provide further information on the serious adverse event in the form of a written narrative. This will include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events will be provided promptly to the study sponsor

8.3.2 IRB Notification by Investigator

Reports of all serious adverse events (including follow-up information) will be submitted to the IRB within 10 working days including those that fall under the UPIRTSO guidelines. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

8.3.3 FDA Notification by Sponsor

The study sponsor will notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days from the sponsor's original receipt of the information.

If a previous adverse event that was not initially deemed reportable is later found to fit the criteria for reporting, the study sponsor will submit the adverse event in a written report to the FDA as soon as possible, but no later than 15 calendar days from the time the determination is made.

8.4 Unblinding Procedures

The principal investigators, Jeffrey R. Wozniak, Ph.D. and Michael K. Georgieff, M.D. will be blinded as will their co-investigators during the course of the study. However, in the event of a serious adverse event, the investigators will contact the Investigational Drug Service (IDS) within 24 hours to initiate the unblinding of the subject. This will be reported in the case report form. A written narrative of the events will be prepared within 48 hours.

8.5 Stopping Rules

The study will be monitored by a local DSMB. Based on the previous study experience, the investigational drug involved (choline), and the goals of the study, there will not be formal stopping rules based on efficacy or lack thereof. If a statistically significant group difference in serious side effects is identified by the DSMB, the study will be stopped.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.6.1 Internal Data and Safety Monitoring Board

The Data Safety and Monitoring Board will consist of three local clinical research experts / physicians who will review unblinded data. The group will comprise:

Rebecca Shlafer, Ph.D.
Assistant Professor of General Pediatrics and Adolescent Medicine

Brandon Nathan, M.D.
Associate Professor of Pediatrics

Claudia Fox, M.D.,MPH
Assistant Professor of Pediatrics

The DSMB will meet after 25%, 50% and 75% of the data have been collected and will be tasked with overseeing patient safety, study conduct, and study progress. All serious adverse events (SAEs) and adverse events (AEs) with a rating of 1 or higher on the NIH Common Terminology Criteria for Adverse Events (CTCAE) scale will be considered. Ann Brearley, the study statistician who will be unblinded, will prepare reports for the DSMB. The reports will contain information about patient accrual rate relative to targets, drop-outs, losses to follow-up, SAE counts, and AE counts by treatment condition.

The DSMB will record its summary in a formal report to be filed with the University of Minnesota IRB and Quorum IRB.

8.6.2 Independent Data and Safety Monitoring Board

None

9. Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use

PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

All participating parents / guardians will sign a HIPAA form for this study.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

Data generated by the methods described in the protocol will be recorded in the subjects' medical records and/or study progress notes. Data may be transcribed legibly on CRFs supplied for each subject or directly inputted into an electronic system or any combination thereof.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

Source documents will serve as case report forms for all test article and behavioral survey forms completed by the subjects' parent/guardian and for all of the standardized FASD and neurodevelopment tests implemented as a part of this research.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10. Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored according to FDA/GCP guidelines by the University's Clinical and Translational Science Institute monitoring service. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11.0 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12. Study Finances

12.1 Funding Source

The study is funded by grants from the National Institutes on Alcohol Abuse and Alcoholism / National Institutes of Health (1R01AA024123 and 3R01AA024123-05S1).

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must refer to the Regents Policies on Individual Conflict of Interest Policy or Institutional Conflict of Interest Policy. These policies require University Faculty and staff to report external professional activities and business and significant financial interests related to his or her University activities by submitting a REPA (Report of External Professional Activities) at least once per year. Faculty and staff should also file a REPA when substantial changes in business or financial interests occur, when an activity that presents a potential conflict of interest is anticipated, or when submitting an application for research support or technology transfer, submitting research protocols to the IRB, or receiving financial contributions. All University of Minnesota investigators will follow the University conflict of interest policy.

12.3 Subject Stipends or Payments

Participant families will receive payment for participation. Payment will be made using a pre-paid debit card called Greenphire ClinCard. It works like a bank debit card. We will give participants a debit card and the money will be added to the card after each completed visit. Because the participants are children, the payment will be provided to the parent on behalf of the child. Payments will be made following each visit. Payment will be based on procedures attempted and/or completed as follows:

Visits 1 & 11: $100 each
Visits 2, 3, 4, 5, 6, 7, 9, 10, 12: $10 each
Visit 8: $75

The maximum payment per participant will be $365 over the course of 10 months.

13. Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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15. Attachments

1. Study Schedule of Events

Visit Type	Clinic	Phone	Phone	Phone	Phone	Phone	Phone	Clinic	Phone	Phone	Clinic	Phone
Visit Title	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**	**Visit 10**	**Visit 11**	**Visit 12**
Time Point	Baseline	Day 14	Day 28	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Study Visit Window	n/a	+ or - 14 days	+ or - 14 days	+ or - 14 days	+ or - 14 days	+ or - 14 days	+ or - 14 days	+ or - 14 days	+ or - 14 days	+ or - 14 days	+ or - 14 days	+ or - 14 days
Consent	X											
Medical/developmental history	X											
Physical examination	X		X					X			X	
Height	X		X					X			X	
Weight	X		X					X			X	
Head circumference	X							X			X	
Blood pressure	X		X					X			X	
Blood collection for choline level	X							X			X	
DNA sample from same blood sample	X											
Astley and Clarren's FASD diagnosis with photograph of the child's face	X											
Stanford Binet	X										X	
Elicited Imitation with video	X							X			X	
MEFS Early Childhood Version	X							X			X	
NIH Toolbox Flanker Task	X							X			X	
Beery VMI Test								X				
Scales of Indep. Behavior								X				
Child Behavior Checklist	X							X			X	
24 hour diet Interview	X							X			X	
Child Eating Behavior Questionnaire	X											
Randomization	X											

Parent/guardian training in proper storage & administration of test article	X											
Dispense test agent (choline/placebo)	X from thereon as needed											
Record concomitant medications	X	X	X	X	X	X	X	X	X	X	X	
Record adverse events		X	X	X	X	X	X	X	X	X	X	X
Compliance check (packet count)								X			X	
Record parent-reported problems with administration of test article		X	X	X	X	X	X	X	X	X	X	