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OFFICIAL STUDY TITLE:

Micronutrients for ADHD in Youth:
The “MADDY” study

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Protocol for
Micronutrients for ADHD in Youth:
The "MADDY" study
at
Oregon Health & Science University
The Ohio State University/Nationwide Children's Hospital
University of Lethbridge

Aims and Objectives

Aim 1: Replicate acceptability/tolerability of a multi-ingredient micronutrient product for children with ADHD [1].

Hypothesis 1: Consumption of 9-12 capsules, in 3 divided doses daily, of a product containing a blend of vitamins, minerals, amino acids and antioxidants (see Appendix A for ingredients), will be achieved at >80% success, as measured by self-report adherence and pill counts and with no or transient side effects (e.g. nausea, loose stool); and no directly attributable serious adverse events (AEs) (see definition of AE in the section on page 21) recorded via the Pediatric Adverse Events Rating Scale (PAERS).

Aim 2: Determine the prevalence, severity, and effect size for symptoms of irritability and negative mood, which are often associated with ADHD.

Hypothesis 2: Treatment will improve mood, irritability and aggression by a medium effect (d>=.50) [2] as measured by the parent-rated Child & Adolescent Symptom Inventory – 5 (CASI-5), Categories B, C, CD, K, L, Rz, Q, and the child-rated Patient Reported Outcomes Measurement Information System (PROMIS) measures: Depression, Anger, Anxiety, and Peer Relations.

Aim 3: Estimate true effect sizes on ADHD symptoms in this age group, to inform a larger trial.

Hypothesis 3: The micronutrients will reduce participants' ADHD symptoms of inattention and/or hyperactivity/impulsivity, as measured by the parent-rated CASI-5, Question Category A, and the Clinician-rated Clinical Global Impression (CGI) by a medium effect (d>=.50) compared to the placebo [1].

Aim 4: Collect and store biological samples of blood, hair, saliva, urine, and feces at three time points. The blood samples are being collected per a request from the FDA for safety screening. We will collect additional blood to look at nutrient level changes and metabolomics, both targeted and untargeted, blood. Collection of other biological samples will enable future examination of nutrient mechanisms including markers of methylation, inflammation, metabolism, and microbiome biodiversity, as funds are available.

Hypothesis 4: Ingestion of micronutrients will alter one, two or three of the areas under examination. The rationale for the collection of these samples is to begin to identify nutrient biomarkers that indicate mechanisms of the treatment within participants and between participants that may account for clinical symptom change, an important step forward in ADHD treatment [3]. Blood levels from a complete blood count, as well as thyroid and iron tests, will show no safety concerns following ingestion of micronutrient treatment. Levels of certain supplemented nutrients (vit D, B12, folate) will increase in those who take the active product [4]. Hair is being collected to examine mineral distribution and changes over time as well as to record baseline levels of lead and mercury. Salivary DNA may show peripheral epigenetic marks that are relevant to ADHD symptom expression [5]. For urine, metabolomics may detect amino acid metabolism , such as tryptophan, which has been found to be related to autism in children [6], which may contribute to increased oxidative stress, and altered gut microbiomes byproducts, which have been found in children with Autism Spectrum Disorders (ASD)[7], but not yet studied in ADHD. In the case of feces, as gut bacteria synthesize water-soluble vitamins [39], it is possible to examine the impact of micronutrient supplementation on gut bacteria and biodiversity, which has not been described yet in this population. Lower bifidobacterium species have been found to be associated with rates of ADHD and ASD [40] and indigenous gut microbiota bacteria have been found to regulate serotonin [8] which may impact mood symptoms related to ADHD.

Background

Overview of ADHD

Attention-Deficit/Hyperactivity Disorder (ADHD) is a chronic developmental disorder affecting about 5% of children[9]. Existing treatments, both behavioral and pharmacological, suppress symptoms in many cases[10]. However, behavioral interventions are unavailable for many families and pharmacological interventions continue to raise significant public concerns over side effects, stigma, and the long-term health effects[11]. These concerns lead scientists to call for further development of alternative interventions [12]. As well, there is a strong public desire for a scientific investigation of the efficacy of non-pharmacological treatments for ADHD; for example, up to 70% of US families use some form of complementary alternative medicine[13]. Since the 1960s, there has been ongoing documentation of the long-term outcome of having ADHD symptoms as a child, including increased risk for incarceration, substance abuse, disability, poor educational achievements and ongoing psychiatric problems [14-16], *despite* receiving conventional treatments. To address this public health need, the present study examines a novel nutritional intervention.

Recent research highlights the importance of considering nutritional factors in the expression of ADHD, based on investigations into the impact that processed foods play in symptom severity[17], the roles of food dyes[18] and essential fatty acids[19], and long-term studies showing that early malnutrition is an important risk factor for ADHD[20]. One treatment that logically stems from these studies is providing individuals with ADHD with the nutrients required for optimal brain functioning. One method to achieve this would be through supplementation with micronutrients (vitamins and minerals), based on the assumption that micronutrients serve as cofactors for synthesizing essential neurotransmitters. Such an approach may seem to challenge conventional understanding that only one variable at a time should be manipulated. However, a single ingredient strategy is already known to be of limited utility and also is at odds with human physiology as optimal functioning requires presence of all nutrients in balance rather than one nutrient provided in high doses[21]. Indeed, single nutrient studies have yielded only modest findings[22] whereas research with multi-ingredient nutrient interventions are proving more promising[23-27]. Pilot research has demonstrated on-off-on-off control of symptoms in ADHD children using micronutrients and a just-published RCT in children in New Zealand has shown clinical symptom improvement, particularly in children with comorbid irritability, aggression and anger [4]. These results all point to the need for a controlled trial with children, run in the US, as well as trials that investigate mechanisms of action of the micronutrients to document the transformational potential of nutritional therapy for ADHD.

Rationale and approach of RCT

The randomized clinical trial (RCT) is viewed as the gold standard for testing treatment efficacy and is the appropriate next step to test the claim that micronutrients provided in combination, yield clinically meaningful effect on these debilitating psychiatric symptoms in children. This proposed research will use RCT methodology and compare micronutrients with placebo in 135 children with ADHD in 3:2 ratio. The study will extend beyond efficacy in terms of clinical outcome, by gathering data that can be used in follow up investigations of how micronutrients may work via changes in physiology. Many theories have been proposed as to how micronutrients exert an effect on psychiatric symptoms, including: 1) affecting dopamine function in the frontal-striatal pathway[28], 2) correcting

inborn errors of metabolism that slow metabolic reactions[29], increasing methylation, 3) correcting deficiencies present in western diets[30, 31], and/or 4) increasing the production of adenosine triphosphate (ATP), the energy source produced by mitochondria[32]. Some of these ideas are testable. Although not without controversy, changes in methylation marks can be investigated through DNA sampling from saliva as well as via sampling of the microbiome (e.g. stool samples).

The evidence to date

Studies assessing micronutrients for the treatment of ADHD have reported positive benefits and large effect sizes; however, they have been either open-label[23, 24, 33], retrospective database analyses[25], case reports[26], or patient preference studies[27]. The field is generally plagued with poorly designed and uncontrolled studies, often leading to erroneous conclusions about the utility of micronutrients in treating serious psychiatric conditions. Despite the popularity with consumers of using nutritional methods for the treatment of psychiatric illnesses [13, 34-37] the research is surprisingly sparse. A recent review showed that there were only 27 published studies using nutritional approaches (most of them single nutrient studies) in the treatment of ADHD [22] compared with hundreds using conventional medications. An RCT of micronutrients in youth with ADHD found positive results with a high safety profile [4]. The role of micronutrients in the pathophysiology and management of other psychiatric disorders including bipolar disorder, anxiety states, and autism is receiving considerable international attention because of large and beneficial effects [24, 38-42], suggesting that this approach could prove meaningful.

Concerns about long-term safety of supplementary nutrients have been raised; however, these studies typically use one nutrient at a time [43, 44]. It is not definitively known whether nutrients given in combination hold the same risk as when given alone, however, our data and that of prior studies have provided support for the safety and tolerability of the planned intervention at the dosages proposed here [40, 45, 46]. With the exception of niacin and magnesium, no one ingredient is given in a dose higher than the specified Lowest Observed Adverse Effect Level (LOAEL) for that nutrient based on the Dietary Reference Intake recommendations [47]. Details on risk management, data safety monitoring, and product safety are covered starting in the Risk Management section which begins on page 14.

Study Design

Design Overview

The design is an 8-week randomized, double-blind, placebo-controlled trial, with an 8-week open-label extension, of Daily Essential Nutrients (DEN; Hardy Nutritionals), and a 30-, 60-, and 365-day follow-up phone call or in person-visit. The follow-up calls will also assess for safety following study completion. Parent/guardian and child ratings, using standardized measures, will evaluate adherence, side effects and AEs (Aim 1); ADHD-associated mood and irritability (Aim 2); and ADHD symptoms (Aim 3), thus allowing evaluation for clinical significance. Other domains of psychopathology will be evaluated for purposes of sample characterization and secondary analyses. The design consists of three study periods, which are individualized for each participant as they enroll:

1. Screening and baseline. Participants will be screened by phone through a conversation with the parent/guardian for study eligibility. Those who are eligible and consent/assent to the study will receive a baseline assessment (see below for definition of eligibility and assessment).
2. An 8-week period of fully randomized, placebo-controlled, treatment.
3. An 8-week open-label extension period (OL).

Study data collection will occur in-person at baseline and at weeks 4, 8, 12, and 16 and via phone 30-60- days after end-of-open label and 365- days after enrollment. Details of data collection are outlined in Tables 1 & 2 located on the last two pages of this proposal.

Study Population

Number of subjects

Sample size power calculations are based on using power = 0.8, with a two-tailed α=0.05, and the a priori decision to detect a medium effect size (d>=.5) with a primary parent-rated measure that is a combination of CASI-5 subscale scores. Given the three sites and two treatments; a 3:2 randomization ratio, active-to-placebo; and assuming two dropouts per arm, per site, based on a previous study (Rucklidge, 2017), (n = 12 total), sample needed to recruit = at least 135, with at least 123 retained. To account for the 3 sites and the 3:2 randomization, a number divisible by 15 is needed, so, n = 45 participants per site.

In terms of feasibility, we estimate being able to recruit the required number of participants over a period of 12-18 months, given proven success in recruitment for a vitamin D supplementation trial (Dr. Gracious) and established success recruiting participants in a longitudinal study on ADHD (Dr. Johnstone).

Inclusion Criteria

This study will include a vulnerable population: children.

1) Age inclusive of and between 6 and 12 years at the time of enrollment; 2) Verbally willing to agree to swallowing a maximum of 9-12 capsules/day with food, attend all study appointments and complete questionnaires; and 3) Meet criteria for ADHD as assessed by the clinical cut-off (6+ questions scored as 2s or 3s, "often," or "very often," or sufficient "sometimes" responses to meet a score of 6) on the Category A: ADHD questions from on the Child & Adolescent Symptom Inventory-5 (CASI-5) with at least several symptoms present in more than 1 setting, based on the DSM-5 symptom criteria, including significant impairment in functioning socially and/or academically[41]; 4) Demonstrate at least one symptom of irritability or anger as assessed by a score of 2 or 3 from an "often," or "very often" response on one question from Category B or Rz from the CASI-5; or two "sometimes" responses; 5) Be medication-free, or washout with medical supervision to be provided by the child's pediatrician or primary care physician, reliant on the parent/guardian to work with that physician, for *at least 2 weeks* prior to in-person study assessment. 5) Willing to give blood sample at two time points: baseline, week 8; week 16 is optional.

Participants identified as having trouble swallowing pills during the phone will be asked to complete the pill swallowing program developed by Kaplan et al[48] and successfully complete this prior to being invited to come in for a lab visit. The video can be viewed at:

http://research4kids.ucalgary.ca/pillswallowing. Children who continue to be unable to swallow the pills after completing the pill swallowing program will not be included in the study.

Exclusion criteria

1) Neurological disorder involving brain or other central function (e.g., history of or suspected intellectual disability, autism spectrum disorder, epilepsy, multiple sclerosis, narcolepsy) or other major psychiatric condition requiring hospitalization (e.g. significant mood disorder, active suicidal ideation, or psychosis), based on parent/guardian self-report of child's condition and response to category M on the CASI-5 subscale; 2) Any serious medical condition, including inflammatory bowel disease, history of cancer, kidney or liver disease, hyperthyroidism, diabetes Type I or II; 3) Known allergy to any ingredients of the intervention; 4) Any known abnormality of mineral metabolism (e.g., Wilson's disease, hemochromatosis); 5) Taking any other medication with primarily central nervous system activity, including stimulants, within the last 2 weeks prior to in-person assessment; participants must be off these medications for a minimum of two weeks prior to the screening 6) Severe separation anxiety that would preclude separating from parent/guardian to answer study questionnaires; 7) Any disability that would interfere with participant answering questions verbally; 8) Non-English speaking; 9) Pregnancy or sexually active at baseline. Exclusion criteria 1-6 and 9, will be based on parent/guardian self-report of child's condition. If the parent/guardian reports medical exclusion criteria, or concerns about eligibility, data provided by parent/guardian will be confirmed by review of medical records with release of information signed by parent/guardian. Potential participant may be reviewed in-person by study physician in the case of any concerns about participation.

The data collected from potential participants who are not included in the study due to screening failure will be securely stored (as outlined below) until the end of the study for the purpose of including in the recruitment section of the CONSORT diagram.

Setting and Recruitment methods

Participants will be recruited, enrolled and will participate in study procedures at three locations: two in the US and one in Canada. In Oregon, participants will be recruited at Oregon Health & Science University (OHSU) through Child & Adolescent Psychiatry, the Child Development and Rehabilitation Center, the ADHD Research Lab Newsletter, and the National University of Natural Medicine (NUNM), OHSU social media, a study-specific Facebook profile that would initiate contact with local parenting groups on Facebook and request to post a study flier through that page's administrator, and referrals from community pediatricians and mental health providers. Approved flyers may be placed on the windshield, under the windshield wiper on cars at local parks where sporting events are occurring. Peachjar, https://www.peachjar.com/, will be used to email flyers to parents at participating local elementary schools. Study procedures themselves, including enrollment, will only occur at OHSU, not at NUNM. In Ohio, participants will be recruited at Nationwide Children's Hospital and The Ohio State University through the intake services where parent/guardians and youth are typically on wait lists for outpatient ADHD assessment for up to 6 months. In Canada, participants will be recruited from schools, community agencies, and clinics (e.g. pediatricians, psychologist, and counsellors) within the surrounding communities of the University of Lethbridge in Southern Alberta. Bearing in mind that the population base in Canada is smaller than in Oregon or Ohio, a detailed plan for recruitment has been created to address the process locally. Recruitment materials will include a flyer which will be displayed in clinics and onsite at each of the three locations, as well as posting of the YouTube video: https://youtu.be/Gslb9cLkt-c, of a family whose son has experienced

success with using the intervention. He was non-randomized (given the active intervention) due to low behavioral functioning at baseline.

At the two US sites, the child participant will be paid $10 per in-person assessment (n=5), in the form of a gift card, (see Table 1 for assessment schedule) for a total of $50. Gift card payment will be made for each instance the child completes the questionnaires and measures associated with that assessment time point. If the child declines to answer any questions, questionnaires and measures despite reassurance or assistance including by parent and the study coordinator, or actively declines to participate at any visit, s/he will be discontinued from the study. Children will also be paid $10 for each blood sample provided, and $5 for each of the other biological samples they provide, for up to $105 for each of the 3 time points. Except for blood, children will not be withdrawn from the study for not providing a biological sample (hair, saliva, stool, urine), but the samples collection will be prioritized as part of the study. Parent/guardians will be paid $20 for traveling to the site for in-person assessments, for a total of 5 visits during the main, 16 weeks of the study ($100), and additionally, $10 for questionnaires completed online and over the phone at 2-month follow up, or $20 for parents and $10 for the child, if 2-month follow up occurs in person. One year after enrollment in the study, participant families will be contacted regarding their willingness to provide follow-up data including questionnaires and, potentially, biological samples. Parents and children will be paid for that in-person visit, or phone visit, according to the previously described schedule: $10 for phone completion; $20 to parent for in-person completion; $10 to child for in-person completion; $5 for each biological sample provided, except blood, $10.

At the Canadian site, payment to participants is not expected or customary. As such, parents will be given $25 gift vouchers for their participation in the qualitative part of the study (i.e. in-person interviews) and will be reimbursed for cost of travel, including parking and mileage per onsite visit. In Canada, blood and urine will not be collected due to storage limitations. Health Canada, the Canadian equivalent of the FDA, has not required collection of blood.

Each site will submit an identical protocol to their internal IRB or ethics committee. All study protocols and materials (identical for each site, except where differentiation or specification is required by an individual IRB) will be approved by the IRB/ethics committee at that site. Details concerning coordination of activities are located in the Multi-Site Coordination section.

Phone screen

At initial phone contact, which will be initiated following contact from the potential participants, a study coordinator/research associate will conduct a phone screen with interested families by outlining the purpose of the study, confirming willingness to participate by asking eligibility questions from the inclusion and exclusion criteria below. Parent/guardians of females will be asked if they have begun menstruating. If yes, parent/guardian will be asked whether child is sexually active. If yes, or unsure, potential participant will need to take a urine test at baseline as a pregnancy screen. Any questions of the parent/guardian and potential participant will be answered as well. Parent will be asked for the email address of a teacher, coach, or other adult who knows the child, and CASI-5 questions will be sent to that person to answer on behalf of the child. The questionnaire will be sent to the teacher or named adult using the email provided, via the secure REDCap system.

Only children who are ADHD-medication-free for at least 2 weeks prior to in-person assessment will be scheduled for an in-person screening assessment and considered for participation. A phone screen process has been used with success in multiple previous studies by one of the co-investigators [49] If the child is eligible based on the screening, the parent will be send the CASI-5 screening questions to their email via REDCap. The screening questions come from the ADHD and DMDD/ODD sections of the CASI-5 (see details of this process in the Baseline Assessment section below).
Phone screen data, including inclusion/exclusion criteria, and the CASI-5 responses will be reviewed and discussed by the study PIs and the research assistant. Any questions or concerns regarding enrollment, based on information provided by the parent/guardian or participant, may be handled with phone or in-person consultation with an onsite study physician. If necessary, the consultation or meeting enables a brief review of medical history and the opportunity to identify any potential concerns prior to enrollment.

Consent and Assent

After eligibility is established based on phone screen and CASI-5 responses, and review of medical records and/or site physician assessment, if necessary, the parent/guardian and child will be invited to meet with a research staff team member for a baseline visit, in designated office space at one of the three sites to discuss the study and to complete consent and assent prior to any study procedures. Prior to the first visit, the consent and assent forms will be sent to the parent/guardian for review in before the first meeting. This will facilitate the parent's ability to formulate and ask questions in advance of the first visit. The consent will be signed onsite, using REDCap.

As this study represents minimal risk to the child and offers potential benefit, the consent of one parent/guardian will be sufficient for participation. The child will be provided with information about the study using simple language that is appropriate for a child as young as 6 years of age. The child will be clearly told that s/he does not have to participate, s/he can change his/her mind at a later time. Provided that both the parent/guardian and child want to participate, both will provide electronic consent/assent via the secure, HIPAA-compliant REDCap web portal. Documentation of assent/consent, via an electronic signature, will be captured for each participant and parent/guardian. If the parent/guardian or child does not consent/assent or is excluded for any other reason, data already collected will be retained for the purpose of characterizing those who made contact. The reason for not enrolling will be noted in REDCap. Parents will be asked whether they are interested in communicating regarding the MADDY research study via text. A separate consent form will be provided and the text communication explained.

Procedures

Baseline Assessment

Once consent and assent are given, the parent/guardian and child will complete the other baseline questionnaires electronically, also via the REDCap web portal. Details regarding the measures are noted in the section below (starting on page 13). Participant will also have weight, height and blood pressure measures taken and recorded electronically in the REDCap portal. Any paper-collected ratings data will be entered weekly.

Blood, hair, saliva, and urine samples will also be collected from the child participant at the baseline assessment, along with the stool sample, if possible. If the child cannot give a stool sample at the baseline visit, the parent will be given the collection kit and instructed to collect the sample later that day or the next morning, before the child begins taking the capsules. The samples will be stored as below, in order to examine nutrient levels, systemic inflammation, DNA methylation, metabolomics and microbiome markers. Except for the blood sample at baseline and week 8, the child will not be excluded from the study without providing any one, or all, of the biological samples (hair, saliva, urine or feces). Child-appropriate information sheets will be provided to the participants to explain the biological sample collection and receipt of parent/guardian consent and child assent will be documented. Aside from blood, which will be collected and stored per laboratory requirements, samples that are collected will be entered on a paper study sample log, by site (A1 A=Oregon, sample 1, saliva; etc), along with the participants ID number. For stool and hair, a record of the serial number of the collection container will be added to participant's REDCap record. Hair and urine collection will be noted in participants' REDCap record.

Blood pressure, heart rate, height and weight will also be assessed at in-person visits by a trained research assistant using instruments calibrated according to hospital maintenance schedule. Participants will be asked to complete the VioScreen version of the Food Frequency Questionnaire (FFQ) at baseline and at the end of eight weeks with input from one parent/guardian.

Enrollment

Once parent/guardian has signed consent and child participant has signed assent, and following the completion of baseline questionnaires and recording of vitals, including blood pressure, pulse, height and weight, the participant will be considered to be enrolled in the study. Enrollment may be delayed until any questions that may have arisen during the baseline assessment are discussed with and answered by the study physician. Once enrolled, the participant will be randomized to a treatment, either active or placebo, and a notification of participation letter will be sent to the child's primary care physician with the parent/guardian signature on the release of information form. Parent/guardian will be given study capsules for the initial 4 weeks as noted below.

Randomization

The randomization scheme will be generated for all three sites, as a whole, by the study statistician at OHSU using REDCap, with the randomization sequence arranged in permuted blocks of size 5, in a 3 active to 2 placebo ratio. The randomization scheme will be emailed to the other two sites. Neither the participants nor the clinicians involved in the study will have access to the randomization until end of study, but access will be available to the medical oversight person at each site and Data Safety Monitoring Board members in event of emergency. A pharmacist or designated unblinded research team member at each site will prepare individual participant pill kits in advance, per the randomization scheme. The pill kits include 1-week pill caddy containing the capsules to be taken during the first week (titrated up to the full dose by Day 5), from pre-labeled and packaged bottles sent from the manufacturer. The pill caddy, together with the bottle of pills for the rest of the month will be individually labeled and put inside a white paper bag to be given to each participant. These kits will be sequentially numbered and allocated to placebo or micronutrients based on the randomization list. The pharmacy research staff at OHSU, who is unblinded, will be available 24/7

as per on-call schedule, to break the blind for an individual subject if medically necessary. Each participating family will be given the appropriate local clinician name and contact information in the case of a side effect or question or urgent notification for any medical emergencies.

Micronutrient formula, titration and dosing

The study will be using a product called Daily Essential Nutrients (DEN) from Hardy Nutritionals, which consists of a blend of vitamins, minerals, amino acids and antioxidants; see Appendix A for ingredients of both DEN and placebo, beginning on page 27. Participants will be given one pill caddies with 7-day sections divided into 3x/day dosing that contain the pills for the first week of the study and will receive instructions on how to store the caddie at home. The remaining pills will be in a labeled bottle with the participant's name. Participants will be asked to take one capsule, 3 times each day, with plenty of food and water, and asked to increase the dose by three capsules every two days, up to 9 capsules per day, in 3 divided doses, for the first four weeks. For 6-8 year olds, this will represent the maximum dose; for 9-12 years old, up to 12 capsules per day is the maximum. At week one, research staff will check in with each participant's parent/guardian via phone to see how the participant is responding and answer any questions. At this call and during any subsequent contact (other phone calls, week-4 study visit), any decision to increase dose up to a maximum of 12 capsules for participants ages 9-12 will be made in consultation with the site PI, and will depend on parent/guardian/participant report of side effects, if any; participant's ability to swallow the capsules; and parent/guardian/participant report of symptom profile, noting whether any improvement or worsening has occurred. Symptom questions from the CASI-5 eligibility questions will be used to ascertain any symptom change. These data will inform the site PI- or designate-rated CGI-Improvement scale to determine if dose needs to be increased to 12 capsules/day, based on a score of 3 or more on the CGI, for participants who are 9-12 years old. Dose can be lowered from 9 or 12 at any time for concerns regarding any AEs, as clinically determined by the site PI or their designate. Designates will be physicians or PhD-level licensed psychologists with clinical experience using micronutrients. At the return visit, participants will return the pill caddie and any remaining pills to receive the bottle of pills for the next month. The participant's parent will be responsible for filling the pill caddie with a reminder from study staff.

Adherence

At the four- and eight-week visits in the RCT and the twelve- and sixteen-week visits in the open label phases, participants will return the pill caddies to the site coordinator with any remaining capsules, to assess adherence. The returned pills will be returned to the research pharmacy, and the pill caddies will be refilled using pills dispensed by the pharmacy or research staff (site dependent) at each in-person assessment. Parents will also be asked to download Medisafe, a pill reminder app and will receive detailed instructions on how to download it and use it.

End-of-RCT, Open Label Extension, and End-of-Trial

At the completion of the 8-week RCT, participants will come in to the research site for an in-person visit at each site to be re-assessed, and assessments repeated (see schedule below in Table 1). Participants will then enter an 8-week open-label extension in which they have the opportunity to knowingly take the active product. The capsules, both active and placebo, will be donated by Hardy Nutritionals, Raymond, Alberta, Canada. Study participants will be eligible to purchase the product,

DEN, at a price of $85 per bottle (65% of current retail price) for a person of one year after the completion of the study. At the end of the open label trial (end of month 4, week 16), participants and their parent/guardian, will return to the research lab for a final in person end-of-trial assessment. Questionnaires to be completed are noted below.

Post-study follow up: 30- and 60- and 365-days

Thirty and sixty days following the completion of the open label portion of the study, participants' parents will be contacted by phone to see whether the child participant is still taking the micronutrients. If not, parent/guardian will be asked the questions on the Treatment Cessation questionnaire. In either case, parent/guardian and child will be sent, via REDCap, the assessment questions to complete: e.g. CASI-5, PROMIS measures and others as noted below. At 365-days after study enrollment, participants will be given the opportunity to come in for a visit, as described above, and to provide optional biological samples.

Assessments

ADHD, Emotional and Behavioral Disorder Assessment

The Child & Adolescent Symptom Inventory-5 (CASI-5) will be used as a baseline screening and diagnostic tool. The CASI-5 is a behavior rating scale for DSM-5-defined emotional and behavioral disorders in youths between 5 and 18 years old. Subscales of the parent-version will be used. When the parent/guardian endorses a symptom question, s/he will be asked for an example, when a symptom question is answered "often" or "very often" and also asked for additional detail about the setting or settings in which the behavior occurs. Each symptom category includes an Impairment question (i.e., the degree to which symptoms interfere with the youth's social or academic functioning). Subscales to be used at baseline, end-of-RCT and end-of-Open Label are questions from: Categories A, B, C, Cz, D, E, G, I, Rz, K, L, M, Q. These include questions about ADHD, Disruptive Mood Dysregulation Disorder (DMDD), anxiety, depression, Obsessive Compulsive Disorder (OCD)/Post Traumatic Stress Disorder (PTSD), tics, mania, psychosis, separation anxiety, Autism Spectrum Disorders (ASD) (which is an exclusion), enuresis/encopresis, and Oppositional Defiant Disorder (ODD).

Demographics

Demographic information will also be collected including ethnicity, parent/guardians' occupation, parent/guardians' level of education, and family income so that Socioeconomic Status (SES) can be estimated.

Study personnel-rated measures

Completed at baseline and at assessments every 4 weeks – time: 30 minutes:

1. ***Vital Signs and Body mass index-BMI*** **-** height and weight plus pulse and blood pressure, adverse events, will be assessed at every in-person visit. BMI will be calculated using the standard formula from the participant's height and weight, as measured with a scale and stadiometer. Blood pressure and pulse will be measured using standardized hospital equipment.
2. ***Adverse events*** will be measured using the Pediatric Adverse Event Rating Scale (PAERS), and the Columbia Suicide Severity Rating Scale (CSSRS) with questions asked of the

parent/guardian and participant. Both of these measures were requested by the Federal Drug Administration upon review of an IND application by Dr. Gracious for a similar proposed study, specifically to standardize reports.

3. ***Pill count compliance*** will be monitored by the assessor at in-person visits by counting the remaining pills.
   Completed at end of week 8:
4. ***Clinician/RA and rater blinding*** will be recorded to verify integrity of the blind. Clinicians and raters will be asked whether they believe the child is assigned to the placebo, active nutrient supplement, or are unsure.

Clinician- reviewed measures

Completed at baseline, at week 8 and 16:

1. ***Clinical Global Impressions Scale (CGI)*** The CGI is a single-item rating of the clinician's assessment of the severity of symptoms[50]. Its goal is to allow the clinician to rate the efficacy of treatment, change over time and the severity of illness. CGI-rating will be based on 1) participant self-report, 2) parent ratings, 3) observation by RA and, 4) overview by PI.

Parent/guardian-rated measures

Completed at appropriate times during trial – see Table 1 – total time: 40 minutes or less:

1. ***CASI – 5:*** Subscales as noted above will be used from this behavior rating scale for DSM-5-defined emotional and behavioral disorders in youths between 5 and 18 years old
2. ***PROMIS measures:*** The Patient-Reported Outcomes Measurement Information System (PROMIS) measures are simple self-reports developed by the U.S. Dept. Health and Human Services to be pragmatic outcome measures in clinical trials. (http://www.healthmeasures.net/explore-measurement-systems/promis/obtain-administer-measures). The child's parent/guardian will complete PROMIS scales for sleep, anxiety, peer relationships, anger, and depression. There are 4 to 13 Likert-style questions in each form. The parent/guardian answers will be used to compare to the child's responses.
3. ***Strengths and Difficulties Questionnaire*** (SDQ): The Strengths and Difficulties Questionnaire (SDQ) is a brief behavioral screening questionnaire designed to measure positive and negative attributes, divided between 5 scales: emotional symptoms, conduct problems, hyperactivity/inattention, peer relationships, and prosocial behaviors.
4. ***Temperament in Middle Childhood Questionnaire*** (TMCQ): The Temperament in Middle Childhood Questionnaire (TMCQ) will be used to assess children's reaction to a number of scenarios. It is divided in 17 categories: activation control, activity level, affiliation, anger/frustration, assertiveness/dominance, attention focusing, discomfort, fantasy/openness, fear, high intensity pleasure, impulsivity, inhibitory control, low intensity pleasure, perceptual sensitivity, sadness, shyness, and soothability/falling reactivity.
5. ***Food Frequency Questionnaire*** (FFQ): The children's usual dietary intake captured over 90 days, will be assessed using a standardized, validated VioScreen FFQ, which includes 1,200 food images that users may slect, based on branching logic that tracks their choices in food families.
6. ***Food Insecurity Measure:*** A brief measure asking to what degree the family has experienced food insecurity in the past 12 months, asked at baseline and week 8.

7. ***Parent Target Problems:*** (PTP): At baseline, parents will be asked to nominate the child's biggest problem. If the parent cannot select just one, two may be named. The research assistant and parent discuss the named problem and the RA asks questions about the frequency, duration and interference level of the problem. The example field allows the parent to construct a brief narrative of the behavior and its impact on family life. The target symptoms are reviewed and the narratives revised at subsequent visits. A panel of four judges, blind to treatment condition, will independently review the narratives to rate change from baseline on a 9-point scale: 1, normal; 2, markedly improved; 3, definitely improved; 4, equivocally improved; 5, no change; 6, possibly worse; 7, definitely worse; 8, markedly worse; 9, disastrously worse. The mean of the four raters will be compared with primary and key secondary ratings from the original study.
8. **Concomitant treatment measure** will ask parents about any current treatments received (behavioral or physical), for what condition, when started and stopped, and details on dosing or frequency. This measure will be repeated at each in-person visit.
9. ***Parent/guardian assessment of the blind*** will be reviewed by recording a direct query of whether the parent/guardian believes the child is taking the placebo or the active product, to monitor integrity of the blind.
10. ***Treatment acceptability rating*** – upon study completion, the parent/guardian will be asked how participating in the study went, any suggestions they have to improve the study, and whether they would recommend the trial to others with symptoms.
11. ***Treatment Review*** – at 30-, 60- and 365-days post-study, parent/guardian will be asked whether his or her child is still taking the micronutrients and if yes, dosage information. Also parent/guardian will be asked about any other treatments used or obtained in the time period since last interviewed.
12. ***Treatment cessation*** – at 30-, 60- and 365-days post-study, parent/guardian will be asked whether his or her child is still taking the micronutrients. If not, the parent/guardian will complete this measure to report on the reasons for stopping the treatment.

13. ***(Optional) Qualitative assessment –*** a smaller sample of participants will be asked whether they want to provide optional qualitative input regarding the study. The scripted, phone-based interview, will be completed a member of the study staff who will conduct all the interviews. See details of the qualitative assessment in separate document, included as part of IRB submission.

    For this qualitative component, interviews with parents of children enrolled in the clinical trial will be asked about their perspectives and experience of using medications and the micronutrients. Questions will address the following concept domains and questions:

    a. Current and past treatments/therapies used – what are the perceived benefits and limitations?

    b. Reasons for micronutrients – what are the perceived benefits? What are the facilitators and barriers to micronutrients as a treatment option; e.g. adherence and tolerance (# capsules/day), swallowing difficulties, side effects, cost, practitioner knowledge

    c. Accessibility –how would micronutrients incorporated into part of healthcare services? E.g. after nothing else has worked, or be the first step in treatment?

d. Patient oriented outcomes – what is important for parents/patients to find out from the research? What would be impactful for them?

Methodology

For this qualitative component of the study, we will use *qualitative descriptive analysis* (Sandelowski, 2000; Magilvy & Thomas, 2009) to explore the perspectives and experiences of parents whose child is enrolled in the clinical trial. Qualitative description is a pragmatic approach to qualitative data analysis that enable health research to gain preliminary insight into a specific topic and help to focus on the experiences of the participants (e.g. patients), gaining their views and insights on their interaction within the health care system (Neergaard, et al, 2009). With qualitative data, we will gain a deeper understanding of the issues and give patients/parents a voice. Furthermore, it allows us to understand the issues from a range of patients' narratives, without constraints set by the researcher or by the use of narrowly focused questionnaires and pre-defined items (Festen et al., 2014). The findings from patients' experiences will help to formulate new theories or hypotheses for further testing.

We will interview parents of children in the current clinical trial. We chose to conduct one-on-one interviews in place of focus groups for two important reasons. Personal interviews allow for the tailoring of inquiries specific to different families and their individual experiences. Thus, interviews allow for in-depth questioning to solicit personal experiences, and enable for more direct, customized, probing questions. As well, interviews will allow parents to speak openly or freely about potentially intimate issues, and not be constrained as it would in a group setting (Powell & Single, 1996).

Data collection: Semi-structured interviews will be guided by a topic list with open-ended questions and probing questions (see sample below). The questions will be used to roughly structure the conversation, but allow for flexibility to enable interviewees to express their experiences (Sandelowski, 2000).

Interview questions will be piloted to assess for language (understandability), relevance (i.e. makes sense to patients), comprehension (i.e. content validity), sensitivity, and the length of time to complete the interview. Interviews are anticipated to take about 60 minutes. The interviews may be conducted face-to-face at the University of Lethbridge, at the participant's home, or by phone for participants outside of the Lethbridge area. A Research Associate with experience in qualitative interviewing techniques will conduct the interviews. [Participants will be offered a gift certificate for their time].

The interview process will involve building rapport, empathic listening, probing, and asking open-ended questions. The interviews will be conducted in a way that the interviewee will feel at ease, decrease social desirability, and deepen the conversation. In addition to constructing the topic list, the research team will discuss the expectations of participant responses, as well as acknowledge assumptions and biases of team members. As well, the interviewer will record thoughts and insights in field notes during each interview. These discussion points and field notes will help guide or adjust the interview process as needed. A professional transcriptionist will transcribe the interview data.

Child-rated measures

In person at baseline, end of RCT, end of open label;
Table 1 at the end of this protocol – time: 15 minutes

1. ***PROMIS measures***: The child will complete the PROMIS scales for sleep, anxiety, peer relationships, anger, and depression. There are 4 to 13 Likert-style questions in each form.
2. ***Columbia Suicide Severity Rating Scale (CSSRS):*** The child will complete the CSSRS baseline or follow up version, together with a research coordinator viewing the responses to ensure safety; a minimum of 3 questions will be asked. Numerous studies support the psychometric properties of the C-SSRS. These studies attest to the scale's divergent, convergent, predictive, and incremental validity, as well as to its sensitivity to change, internal consistency, inter-rater reliability, cross-cultural and multi-lingual application, and more. Evidence also supports the effectiveness of the C-SSRS as an intervention tool for preventing suicides, as well as a measurement tool for treatment response (http://cssrs.columbia.edu/the-columbia-scale-c-ssrs/cssrs-for-research/).
3. ***Child Target Problem-*** At baseline, child will be asked to nominate his or her biggest problem or symptom. If the child cannot select just one, two may be named. The research assistant and child discuss the named problem and the RA asks questions about the frequency, duration and interference level of the problem. The example field allows for the construction of a brief narrative of the behavior and its impact on family life. The target symptoms are reviewed and the narratives revised at subsequent visits. A panel of four judges, blind to treatment condition, will independently review the narratives to rate change from baseline on a 9-point scale: 1, normal; 2, markedly improved; 3, definitely improved; 4, equivocally improved; 5, no change; 6, possibly worse; 7, definitely worse; 8, markedly worse; 9, disastrously worse. The mean of the four raters will be compared with primary and key secondary ratings from the original study.
4. ***Treatment Acceptability***– upon study completion, the child participant will be asked for their opinion on participation and any suggestions they have to improve the study, and whether they would recommend the trial to others with symptoms.

In person at the end of the RCT

5. ***Child assessment of the blind*** will be captured by asking the youth whether they believe they are taking the placebo or real product.

Teacher or Other Adult-rated measures

Completed at baseline and 8 week RCT, 16-week end-of-study – time: 10 minutes:

1. ***CASI-5*** selected subscales (3 eligibility subscales and peer relationships) will used at baseline and to monitor the effectiveness of the child's response to intervention at two other time points.

Data and Specimens

Blood, hair, saliva, urine, and feces collection

Collected at baseline, 8 weeks and week 16 (end-of-study) – time: 5-10 minutes

Blood collection

Per FDA requirements, participants will be **required** to provide blood samples at each in-person visit. These samples will be used for safety measures and for research purposes. Analysis will include CBC, TSH, iron, a number of supplemented nutrients including B vitamins and Vit D, and

blood for future analysis of targeted and untargeted metabolomics and IL-6 and TNF-a. Total blood collection will be approximately 18ml, or 3 teaspoons. See "Blood Sample Collection Info for OHSU" chart included in IRB submission, for details.

Hair Collection

At least 20 strands of hair, approximately the diameter of a pencil lead will be cut close to the root from the posterior aspect of the head. The hair will be tied at the cut end to identify the most recent portion of hair growth. Samples will be wrapped in static free paper, zip-locked, placed in a light occlusive envelope, labeled with participant's study identification only and stored at the individual lab sites until all hair is collected. Then it will be shipped to OHSU for analyzes.

Saliva collection

Using the Oragene – DNA, OG-600 collection kit, participants will be asked to provide saliva samples during their visits at: baseline, 8 weeks, 16 weeks, in order for us to begin to understand the effect that micronutrient treatment might have on DNA methylation. They will be provided with instructions on how to spit into the collection tube and seal up the kit, which will be labelled with the participant's ID number and recorded on a log, then stored in the lab, in a specially labelled and locked file drawer and/or the Clinical Research Center, until all samples are collected from participants. The specimens collected at the other two sites will be stored at their onsite location. Once the study finishes, and all samples have been collected, the saliva samples will be sent to the lab determined most appropriate for analyses, once funding is available.

Stool sample collection

Participants will be asked to provide three stool samples, collected in the home setting, within 48 hours of an in-person study visit, using the OMNIgene Gut OMR-200 collection kit: at baseline, end of RCT (8 weeks) and end of open label (16 weeks); in order for us to begin to understand the effect that micronutrient treatment might have on the microbiome. These data are exploratory components of the study and seen as a pilot investigation because this is a very new area and there is little scientific literature to direct these analyses. However, a series of studies have now documented the impact of the gut microbiome on behavioral disorders such as autism, anxiety, and depression directly and indirectly. See [51] for an example. The purpose of collecting the samples for storage is to identify the relationship between the gut microbiome and clinical symptoms of ADHD at a later date, once funds are available to generate and analyze the samples.

Procedures for stool sample collection

Participants will be asked if they would be willing to provide a stool specimen. If they agree, they will be mailed a stool specimen collection kit and instructions, to be performed ideally within 24-48 hours of their next visit. This kit includes a swab system and sterile tube for collection of stool material from tissue wipe after defecation, as well as a biohazard bag and brown paper bag for transportation. After defecation at home, the subject or parent will wipe the provided swab on the toilet paper to collect the stool specimen to be put in the tube. The swab will then be thrown away, and the tube sealed and brought to the research assistant at the participant's next visit. If the participant is not returning for another visit, the participant will be given a mailer and instructions on

how to mail the sample back. After being delivered to the lab, the specimen will be frozen at -80 C until further processing, once funding is available. In Oregon, freezer space in the Core Lab run by Clive Woffendin, PhD, will be used to store the samples. Stool sample collection and storage will be identical at the other two sites per best practice recommendations [52]. University of Lethbridge will ship their samples to OHSU for storage; Ohio State will store their samples locally until sample generation and analyses funds are available.

Urine sample collection

Participants will be asked to provide three urine samples, collected in the home setting or at the lab, first thing in the morning: at baseline, end of RCT (8 weeks) and end of open label (16 weeks) in order for us to begin to understand how metabolism of the micronutrients might impact response to treatment. Participants will be given a sterile urine specimen collection container and instructed to fill it at least half full (a minimum of 4ml). If done at home, participant will store urine in the refrigerator until lab appointment. The urine samples will be frozen at -80 C, in the Core Lab run by Clive Woffendin, PhD, until further processing, pending available funds.

Sharing of Results

Due to the exploratory nature of this research and the time delay from collection to generation and analyzation of the samples, results from the biological samples will not necessarily be shared with participants' families.

However, once samples are analyzed, participants may be re-contacted, even if they have completed the study, if any safety concerns arise from the analyses. Participants will be asked whether they want to know the results. As the collected samples may or may not be analyzed by a CLIA-approved lab the reliability of the results may not be unknown. Participants will be advised to run further analyses, at their own expense. The risks associated with receiving this information include, but are not limited to: costs of additional medical care and testing, impact on insurability, and psychological risks, if you receive information that is upsetting.

Specimen Banking and Repository

As noted in each section pertaining to the biological samples, specimens will be stored (at each of the three sites, unless specified) for future generation and analyses, which will occur once sufficient funding is available. Specimens may be accessed by any of the named PIs or their representative. Permission via an email granting access will be sufficient. Study-generated identification (coded) information will be provided with the specimens so that the data may be matched up by treatment arm. Data and samples from this study may be shared with other study investigators at Ohio State or University of Lethbridge for future research studies. A code number is assigned to each child, their cells and genetic information, as well as to information about them. Only the investigators and people involved in the conduct of the study will be authorized to link the code number to the participant. Other investigators or outside labs, including ZRT Laboratory, who receive samples, saliva/genetic information/other data for research, will be given only the code number, but not identifying information.

ZRT Laboratory in Beaverton, Oregon, will be analyzing approximately .5-1ml of blood and up to 2 mls of urine for neurotransmitters, diurnal analytes, sex steroids, hormones and mineral. All samples will be de-identified. _

Data Analysis

Our primary outcome measures defined *a priori,* reflecting the often co-morbid ADHD symptoms of irritable mood and aggression are the CASI-5 subscales and the clinician CGI. The changes from baseline to the end of treatment will be compared between randomized groups using repeated-measures ANCOVA, with the baseline level as the covariate. The differences between treatment groups in these measures will be summarized as the mean differences and 95% confidence intervals generated from the ANCOVA/ANOVA models. Categorical outcomes will be compared between groups using Chi-square tests and will be described using odds ratios and 95% confidence intervals. All analyses will be undertaken on an intention-to-treat (ITT) basis that includes all randomized participants analyzed according to the group to which they were randomized. For those participants not completing the 8 weeks, data from their final assessment (last observation carried forward) will be used to evaluate the change scores. Secondary analyses will be undertaken on all outcomes using the per-protocol (completers) analysis set. All tests will be two tailed and any *p* values less than 0.05 will be considered statistically significant.

Privacy, Confidentiality, and Data Security

All staff involved in the conduct and/or monitoring of this study will have completed the requisite Human Subject Protection Training for each IRB. Documentation of training will be held in locked cabinets or secure password protected computers at each site. Prior to implementation of any protocol changes, amendments will be submitted to the Institutional Review Boards for approval. The site PIs will be responsible for continuous data and safety monitoring of all participants enrolled in this study at their respective sites; discussion of any data or safety monitoring concerns will be routinely brought up as part of weekly study coordination meetings across sites. Overt identifying information will be separated from collected data and specimens.

All information collected in this study will remain confidential. Only designated study staff will have access to the participant information. Electronic data and documentation of consent will be maintained in REDCap. Electronic data is password protected with permissions for log-ins given only to the PIs and approved research assistants. Standard security measures ensure confidentiality including double encryption of electronic files and double locking of paper files and removal of identifying information from data files. The identification key will be locked in a secure office cabinet apart from the data. Computerized versions of the list of participants and the recruitment log will be encrypted and stored on the secure, HIPAA compliant REDCap system at OHSU.

However, after study completion, the ID-key will be destroyed to anonymize the data. In the case of birthdates, age at time of study participation will be retained, but actual birth dates will not. No information that could personally identify any participant will be used in any manuscripts or reports on this study. In cases where there is a concern about the safety of a participant, or of others, or any mandatory audit, confidentiality may be breached in accordance with applicable regulations.

Data Safety Monitoring Plan (DSMP)

All three sites place the highest priority on ensuring the safety and protection of participants in a

clinical trial, and the integrity of the trial data. Study participation is voluntary and participants may withdraw at any time without penalty; we would request permission to retain questionnaire follow-ups in this instance to preserve the ITT analysis. Participant data collected to-date would remain part of the database, per FDA regulations.

Data Safety and Monitoring Board

A Data Safety and Monitoring Board (DSMB) will be created with a representative at each site. The members will consist of at least one person who is an expert on clinical trial safety monitoring and free of any conflicts of interest with this study. DSMB participants who have agreed to be on the board include: Dr. Sarah Feldstein Ewing, at OHSU, Dr. Bob Kowatch, at Nationwide Children's Hospital/The Ohio State University, and Dr. Darren Christensen from the University of Lethbridge. Every six months, the DSMB will review study recruitment, subject demographics and characteristics, and any adverse events (AEs) and safety differences that emerge between the randomized arms, including looking for differences by site. We anticipate the DSMB will meet via Skype or teleconference. To facilitate this review, the PIs will provide the DSMB with the data related to safety and efficacy. If the DSMB determines that there are significant and/or serious emergent risks within the study for any reason, the DSMB could make a recommendation to the PIs and IRBs that the study be closed for accrual of participants until a more detailed review can be conducted.

Risk and Benefits

As Co-PIs, the overseeing the risk management of the project will be handled by Dr. Johnstone, Dr. Gracious, and Dr. Leung, in consultation with site-specific study physicians including Dr. Meg Cary at OHSU, Dr. Gene Arnold at Ohio State, and Dr. Megan Rodway for the University of Calgary. The Co-PIs have been and will be involved on all aspects of the project, and will, therefore, monitor for any foreseeable risks.

Risk/benefit analysis

One risk for participants, if they were taking stimulant medication that conferred a benefit in terms of ADHD symptoms, is that by coming off the medication, their symptoms may return. Risks to participants in terms of taking the micronutrients are low, but as with any intervention there are potential side effects. These may include transient gastrointestinal upset (loose stool, nausea), and headache, particularly if product is taken on an empty stomach. If these side effects are experienced and believe to be related to taking the micronutrients, these symptoms should go away if pills are taken with food and water as directed, and tend to lessen after a day or two of taking the pills, or when participant ceases taking the pills. Not all participants will receive the active treatment in the first 8-weeks, but everyone will have the opportunity to knowingly take the active treatment in the second 8-week, open-label phase. In terms of specific personal benefit, some participants may find that taking the micronutrients help with the symptoms of ADHD and irritability, as well as aggressive behavior. However, participants may or may not personally benefit from being in this study.

The product to be studied has the largest amount of published research in the mental health field for a complex micronutrient product. Data were examined from all known published and unpublished

studies of the micronutrient product to be used in the study [1, 25, 46, 53]. Biological safety data from 237 children and adults from six datasets revealed no occurrences of negative outcomes or effects [38]. Further, no abnormal blood tests indicated toxicity. In an RCT of 93 children, aged 7-12 years of age, which was completed in New Zealand in December 2016, there were no differences between adverse events in the micronutrient versus the placebo groups [4] Comparing tolerability data between a micronutrient group and a group taking psychotropic medication, those in the micronutrient group reported 1/6th the number of AEs, such as appetite suppression or metabolic side effects, than participants treated with medication [31]. Further, no abnormal blood tests could be attributed to toxicity. In previous studies [53, 54], the only side effect noted was transitory gastrointestinal difficulties (loose stool, nausea), when the product was taken on an empty stomach, contrary to recommendation. These difficulties reduced or disappeared when taking the capsules on a full stomach. As such, participants are asked to *always take their capsules with food*. In the extension phase of the child open label study [1], as well as in a child RCT just completed in NZ, a reformulated version of the product is being used with no gastrointestinal difficulties noted (J.J. Rucklidge, personal communication, December 19, 2016). This is the version of the product to be used in this study. Another way to prevent potential side effects is increase the dose slowly over several days, so participants will begin with three (3) capsules per day (one capsule 3x/day) and increase gradually to the full dose. The safety concerns of nutrient supplementation generally pertain to giving one nutrient at a time whereas this product includes 38 ingredients. Considering lowest-observed-adverse-effect level (LOAEL), only two nutrients: niacin and magnesium, are above the LOAEL at 9 capsules per day (maximum dose) for 6 to 8-year-olds. See Appendix A on page 27 for ingredients. Given the significant issues facing children with ADHD, the possibility of reducing their symptomology and improving their quality of life, and the rigorous nature of our safety procedures, the benefits significantly outweigh the risks of this study.

Risk assessment and right to withdraw

Participant safety is the most important consideration. Participants may be discontinued from the study if they show adverse symptoms of either a physical or psychological nature in response to the treatment. Evidence of significant or poorly tolerated side-effects will stop a participant's participation in the study, in the judgement of the PI and in consultation with the study physicians. Mandatory stop concerns will include: possible or probable allergic reaction, new onset of severe and persistent suicidal ideation deemed related to study product, or emergence of exclusion criteria. If a participant's psychological state deteriorates to a clinically significant degree during the trial, the investigator will discuss with the participant and parent/guardians the possibility of withdrawing from the study, or may decide that the participant should be withdrawn. Participants or their parent/guardian may request to be withdrawn from the study or withdraw consent at any time without penalty. If a participant, for any reason, requires treatment with certain therapeutic agents (i.e. antibiotics), we will note what they are taking and for how long. If a protocol exclusion violation has occurred (i.e. participant requires psychiatric medications), his or her involvement will be discontinued, but they will be followed for the duration initially anticipated for descriptive purposes. If any patient is discontinued from the trial, we will carry out follow-ups and appropriate referrals for treatment linkage, to ensure participant well-being.

Human subjects' protection

All participants will be required to provide informed assent should they wish to participate in the trial and their parents/guardians will be asked to provide written informed consent. Any side effects or AEs will be asked about and recorded during in-person visits using the Pediatric Adverse Events Rating Scale (PAERS). This measure was selected to standardize outcomes for studies using the proposed micronutrient treatment in a previously submitted Investigational New Drug application for the product. Any AEs reported during participant visits will be discussed with onsite PI, and on weekly calls with the study physicians at one of the three sites, for assignment as to whether they are related to the study product and to determine if symptoms represent unanticipated problems involving risk to subjects. If AE severity is mild or moderate; was not unexpected (is listed as a possible AE); unrelated, unlikely related, or possibly-related to study product, PI may allow research staff to sign off on the AE, by proxy, after discussion. Medical oversight will be provided to participants as needed throughout the trial by one of the medical physicians at each site. While any reported side effects or AEs, will be discussed with one of the study doctors weekly, any medical concerns or questions may be directed to the appropriate on-site physician. Consultation between PI and doctor will determine the appropriate action to be taken, which may include unblinding and trial cessation or dose change depending on the nature and severity of the symptoms. If a protocol exclusion violation has occurred, involvement will be discontinued, but follow-up will be carried out to ensure participant well-being.

Definition of an Adverse Event or Serious Adverse Event

An adverse event (AE) is a negative experience encountered by an individual during the course of a clinical trial that may be associated with the drug or treatment provided. An AE can include previously undetected symptoms, or the exacerbation of a pre-existing condition. A serious adverse event (SAE) is: 1) any medical occurrence that results in death; 2) is life-threatening, requires or prolongs hospitalization; 3) causes persistent or significant disability/incapacity; 4) or, in the opinion of the investigators represents other significant hazards or potentially serious harm to research subjects or others. An AE/SAE is considered unexpected if it is not described in the Package Insert or in the Investigator's Brochure (for FDA investigational agents), in the protocol, or in the informed consent document.

Investigational New Drug

The product to be used has an approved Investigational New Drug application through the FDA. Results will be reported to the FDA, per requirements. Researchers, working closely with the FDA, will follow applicable Research Pharmacy policies and procedures. Plan for drug storage, handling, and accountability have been outlined in the appropriate sections above. The study has been registered as a clinical trial through clinicaltrials.gov #NCT03252522 and results will be submitted per requirements.

Multi-Site Coordination

Research Sites

Oregon Health & Science University, Portland, OR
Nationwide Children's Hospital/The Ohio State University, Columbus, OH
University of Lethbridge, Alberta, Canada

IRB

Prior to participant recruitment or data collection, all study materials including consent/assent forms will be approved by the IRBs at all three sites. The most current version of all study materials, including the protocol, consent documents and HIPAA authorization will be stored on the Box, the OHSU-policy compliant password-secure cloud storage. The Box system is already in place and being used by the three named PIs. However, no participant data will be stored here. Any modifications will be communicated to each of the sites and approved by each sites IRB before the modification is implemented. Any non-compliance with study protocol or applicable requirements will be reported in accordance with local policy. All staff involved in the conduct and/or monitoring of this study will have completed the requisite Human Subject Protection Training for each IRB. Documentation of training will be held in locked cabinets or secure password protected computers at each site. Prior to implementation of any protocol changes, amendments will be submitted to the Institutional Review Boards for approval. All staff and volunteers working on the study will be registered with the appropriate institutional IRB office and must complete annual HIPAA and IRB compliances as required. The compliances ensure that all have passed knowledge tests regarding protection of data, confidentiality, and research participants.

Cross Site Coordination

To ensure continuity across sites, there will be weekly phone meetings amongst the three site PIs and investigators; common training of Research Assistants (RAs) prior to the start of the study via Skype meetings to ensure identical screening, assessment, and scoring procedures are used at each site; adherence/problem-solving meetings every 3 months or sooner if needed/requested by one of the PIs;a protocol which follows the same procedures and processes at each of the sites and will be clearly outlined using a Standard Operating Procedure applicable across sites, and operationalized by a Manual of Procedures, which will be tailored for each site; simultaneous access to most recent version via the centralized online repository, The Box; use of central data repository, REDCap, housed at OHSU, but accessible to other site PIs and appropriate research team members at any time online; recruitment and enrollment managed via REDCap, with de-identified information; use of identical forms/questionnaires across sites; and similar recruitment materials using same text and pictures, at each site.

Investigators

Jeanette M. Johnstone, PhD – Dr. Johnstone will serve in the lead investigator role, and will oversee the OHSU site and the IRB approval process. OHSU's REDCap data collection will serve as the data repository for all three sites, and the study randomization for the three sites will be handled through OHSU's pharmacy. Dr. Johnstone will provide oversight, support and supervision to research assistants and trained student volunteers on the project, being available at all times should any issues or difficulties arise. Joseph Thoits, MD, a child and adolescent psychiatrist, will provide medical oversight..

Barbara L. Gracious MD – Dr. Gracious will oversee and take responsibility for the OSU site and the FDA IND approval process. She will provide oversight, support and supervision to research assistants and any other team members, including other study doctors on the project, being available at all times should any difficulties arise. She will oversee any safety issues including clinical concerns.

Brenda Leung, ND, PhD – Dr. Leung will oversee the University of Lethbridge site and the IRB approval process. She will provide oversight, support and supervision to research assistants and trained student volunteers on the project, being available at all times should any issues or difficulties arise. An affiliated physician will oversee any patient related safety issues at this site.

Data ownership

All data associated with the study, and all manuscripts resulting from said data, will be owned by the three named investigators. In the case of a disagreement, consensus will decide the outcome. Input from Professor Emeritus Bonnie Kaplan or Dr. Gene Arnold may be sought as needed.

Resources

A generous anonymous donor has given $125,000 to the Foundation for Excellence in Mental Health Care (FEMHC) to be used for the aforementioned study, with monies earmarked for Oregon Health & Science University. Dr. Johnstone and collaborators also won a competitive grant, issued through the FEMHC, of $100,000. In addition, a number of other generous donors have contributed additional funds to provide $100,000 to Ohio State University; and, another generous donor has given $95,000 (Canadian) to be used by University of Lethbridge.

Anticipated costs

Costs will vary by site. See associated budget for cost breakdown.

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Appendix A: **Daily Essential Nutrients (DEN) Ingredients**	**Unit**	**1 Cap**[^]	**9 Caps**	**12 Caps**	**RDA**[*] **or AI**[+]	**LOAEL**[Θ]
Vitamin A (as retinyl palmitate)	IU	480	4320	5760	2000	46667
Vitamin C (as ascorbic acid)	mg	50	450	600	45	3000
Vitamin D (as cholecalciferol)	IU	250	2250	3000	600	3800
Vitamin E (as d-alpha tocopheryl succinate)	IU	30	270	360	16.5	750
Vitamin K (as 75% phylloquinone, 25% menaquinone-7)	mcg	10	90	120	60	NE
Vitamin B1 (as thiamin mononitrate)	mg	5	45	60	0.9	NE
Vitamin B2 (riboflavin)	mg	1.5	13.5	18	0.9	NE
Niacin - Vitamin B3 (as niacinamide)	mg	7.5	67.5	90	12	50
Vitamin B6 (as pyridoxine hydrochloride)	mg	5.8	52.5	70	1.0	500
Folate - B9 (as calcium L-5 methyltetrahydrofolate)	mcg	66.6	600	799.9	300	5000
Vitamin B12 (as 75% adenosylcobalamin, 25%	mcg	75	675	900	1.8	NE
Biotin – Vitamin H	mcg	90	810	1080	20	NE
Pantothenic acid (as d-calcium pantothenate)	mg	2.5	22.5	30	4	NE
Calcium (as NutraTek™ chelation complex)	mg	110	990	1320	1300	4000
Iron (as NutraTek™ chelation complex)	mg	1.15	10.35	13.8	8	70
Phosphorus (as NutraTek™ chelation complex)	mg	70	630	840	1250	10200
Iodine (as NutraTek™ chelation complex)	mcg	17	153	204	120	1700
Magnesium (as NutraTek™ chelation complex)	mg	50	450	600	240	360

[Θ]**Lowest Observed Adverse Effects Level;** [*]**Recommended Daily Allowance;** [+]**Adequate Intake**

Unit measurements: mg – milligram (1,000 milligrams = 1 gram); mcg – microgram (1,000 micrograms = 1 milligram)

Appendix A: **DEN Ingredient List (continued)**	**Unit**	**1 Cap^**	**9 Caps**	**12 Caps**	**RDA* or AI+**	**LOAEL$^{\Theta}$**
Zinc (as NutraTek™ chelation complex)	mg	4	36	48	8	60
Selenium (as NutraTek™ chelation complex)	mcg	17	153	204	40	913
Copper (as NutraTek™ chelation complex)	mg	0.6	5.4	7.2	0.7	10
Manganese (as NutraTek™ chelation complex)	mg	0.8	7.2	9.6	1.9	15
Chromium (as NutraTek™ chelation complex)	mcg	52	468	624	25	NE
Molybdenum (as NutraTek™ chelation complex)	mcg	12	108	144	34	1500
Potassium (as NutraTek™ chelation complex)	mg	20	180	240	4500	NE
$^{\Theta}$Lowest Observed Adverse Effects Level; *Recommended Daily Allowance; +Adequate Intake						
Unit measurements: mg – milligram (1,000 milligrams = 1 gram); mcg – microgram (1,000 micrograms = 1 milligram)						

DEN Proprietary blend ingredients:
Choline bitartrate
Alpha-lipoic acid
Mineral wax (shilajit)
Inositol
Acetyl-L-carnitine
Grape seed extract
Ginkgo biloba leaf extract
L-methionine
N-acetyl-L-cysteine
Boron (as NutraTek™ chelation complex)
Vanadium (as chelate)
Lithium orotate (as chelate)
Nickel (as chelate)
DEN Proprietary blend ingredients (continued):
Other ingredients:
Gelatin capsule (Bovine derived, 100% BSE-free)

Microcrystalline cellulose
Glycine
Citric acid
Magnesium stearate
Silicon dioxide

Appendix B: Placebo Formula

INGREDIENTS	**One capsule**	**9 capsules**	**12 capsules**
Riboflavin Powder	0.10 mg	0.90 mg	1.2 mg
Magnesium stearate	5 mg	45 mg	60 mg
Silicon dioxide	2.5 mg	22.5 mg	30 mg
Microcrystalline cellulose	~ 650mg/capsule^{+}	~ 650mg/capsule^{+}	~ 650mg/capsule^{+}
$^{+}$ Sufficient amount to fill the capsule			

Table 1: Schedule of Events								
ITEM or TASK	**Phone Screen**	**Base-Line**	RCT Wk 4	RCT Wk 8	Open Wk 12	Open Wk 16	30-, and 60-Day F/U Phone Call	365-Day F/U Phone Call or in person visit*
Phone Screen with CASI-5 eligibility Qs	X							
PARENT/GUARDIAN								
Consent		X						
CASI-5 Subscales		X	X	X	X	X	X	X
PROMIS Scales		X	X	X	X	X	X	X
SDQ		X		X		X		X
FFQ		X		X				
PTP		X		X		X		X
Food Insecurity		X		X				
Temperament Q.			X					
Concomitant Treatment		X	X	X	X	X	X	X
Adverse Events – also at wk 1		X	X	X	X	X	X	X
Placebo Question				X				
Treatment Acceptability						X		
Treatment Review							X	X
Treatment Discontinuation							X	X
CHILD								
Assent		X						
PROMIS Scales		X	X	X	X	X	X	X
PAERS - also at week 1		X	X	X	X	X	X	X
CSSRS – also at week 1		X	X	X	X	X		
CTP		X	X	X	X	X		X*
Placebo Question				X				
Trial feedback						X		
RESEACH STAFF								
Pill caddy – receive /return		X	X	X				
How to take your pills		X						
Return unused pills			X	X	X	X		
Pedi study info.		X						
Height/weight/BP		X	X	X	X	X		X*
Placebo/active Question				X				
TEACHER/Other Adult								
CASI-5 Subscales		X		X		X		
CLINICIAN								
CGI		X		X		X		
BIOLOGICAL SAMPLES								
Collection Instructs.		X						
Blood		X		X		X		X
Hair		X		X		X		X
Urine		X		X		X		X

Saliva		X		X		X		X
Stool Sample		X		X		X		X

Table 2: Assessment Measures and Time Points

Measures	Construct	Time to complete[1]	Who	How	When
Ped. Adverse Events*	AEs	20	P	Q, I	M
CSSRS	Suicidality	1 min	C	I	B, E x2
Concomitant Txts	Other txts	5 mins	P	I	B, E x2
MediSafe App	Adherence	2	P	Q, I	M
CASI-5; wide range of subscales	Gen. Clinical	25	P	Q	B, E x2,
CASI-5; targeted symptoms subscales	Mood, Irritability	10	P	Q	B, M, Post×
SDQ – strengths & difficulties	Difficulties prosocial	10	P	Q	B, E x2
PTP – parent target problem	Target problem	10	P	Q	B, E x2
CTP- child target prob	Target problem	10	C	Q	B, M
Food Frequency	Diet	20	P,	Q	B, E
Food Insecurity	Food Insecurity	5	P	Q	B, E
CASI-5 subscales	ADHD, anger	10	T	Q	B, E x2
PROMIS Anger	Anger	1	P, C+	Q	M
PROMIS Anxiety	Anxiety	2	P, C+	Q	M
PROMIS Depression	Mood	2	P, C+	Q	M
PROMIS Peer R/Ship	Social	2	P, C+	Q	M
PROMIS Sleep	Sleep	2	P, C+	Q	M
Temperament	Temperament	15	P	Q	B
Treatment Acceptability	Acceptability	2	P	Q	Wk 16
Treatment Review€	Follow up	5	P	I	30, 60, 365×
Reasons for Treatment Discontinuation	Discontin-uation	1	P	Q	30,60, 365×

[1]In minutes P=parent, C=child, T=teacher, Q=questionnaire, I=interview, E=week 8, B=baseline, x2=end of RCT and Open Label, M=monthly, [+] completed separately, [€] Only given if parent reports that child has stopped taking the DEN, [×]30, 60 after finishing the study and 365 days after enrollment