

Research paper

Rationale and design of an international randomized placebo-controlled trial of a 36-ingredient micronutrient supplement for children with ADHD and irritable mood: The Micronutrients for ADHD in Youth (MADDY) study



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ABSTRACT

Background: Attention-Deficit/Hyperactivity Disorder (ADHD) is a chronic neurodevelopmental disorder affecting up to 9% of children and substantial numbers of adults. Existing pharmacologic treatments often improve symptoms, but concerns exist over side effects, stigma, potential long-term health effects, and residual irritability, often treated with adjunctive antipsychotics. To address public and clinician demand for non-pharmacologic evidence-based treatments, this study will examine efficacy of a 36-ingredient micronutrient (vitamin/mineral) supplement as treatment for children with ADHD and irritability.

Methods: An international team of experts in ADHD, mood dysregulation, nutrition, epidemiology, and clinical trials conferred to develop/refine a protocol powered to detect a medium effect. The study will employ a fully-blind randomized controlled trial (RCT) design, comparing the micronutrient supplement to matched placebo in 135 children aged 6–12 with ADHD symptoms and irritability, based on the parent-rated Child and Adolescent Symptom Inventory-5 (CASI-5). Irritability will be measured by at least one symptom of oppositional defiant disorder (ODD) or disruptive mood dysregulation disorder (DMDD). Based on research suggesting an irritable ADHD subtype, the primary outcome will be a composite score comprised of the CASI-5 subscales: ADHD, ODD, DMDD, and the Peer Conflict Scale, which assesses anger and aggression perpetrated towards peers. Participants will provide biological samples (blood, urine, saliva, hair and stool) to explore the micronutrients' mechanisms of action.

Discussion: This study is the first adequately powered RCT in North America to examine both behavioral responses to, and biological mechanisms of, micronutrients for ADHD and irritability in children. If found efficacious, broad-spectrum micronutrients, given at therapeutic doses, may provide an evidence-based alternative to prescription medications for ADHD and associated irritability.

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1. Introduction

Attention-Deficit/Hyperactivity Disorder (ADHD) is a chronic neurodevelopmental disorder affecting 6–9% of children [1,2]. Those affected, who also have irritability and aggression, have additional difficulties in emotional, cognitive, and social functioning, resulting in greater impairment and disability [3,4]. The comorbid aggression may result in adjunctive antipsychotic medication and its attendant risks [5].

While current treatments improve ADHD symptoms in some children [6], symptoms frequently persist into adulthood. ADHD in children commonly portends poor long-term outcomes, including lower educational and occupational achievement, unstable relationships, incarceration, and ongoing psychiatric problems [7–9]. Pharmacologic interventions are associated with side effects, and possible long-term health consequences [10]. Such concerns have led to investigations of other options [11], including nutrient-based treatments.

Nutritional inadequacy contributes to the etiology and persistence of ADHD symptoms [12]. Maternal nutrient deficiencies during fetal and infant development [13] and early childhood malnutrition are both risk factors for ADHD [14]. Dietary patterns also influence the disorder: low consumption of the Mediterranean Diet (fruit, vegetables and good fats) is linked to higher prevalence of ADHD [15], while consumption of the “Western diet” (processed food high in salt, fat, sugar, artificial colors, and sweeteners) is associated with a higher prevalence of ADHD [12,16,17]. Despite consumer and clinician interest in nutritional approaches as treatment for psychiatric disorders [18–22], research on nutrient supplementation is sparse. Literature reviews revealed less than 35 published studies, most using single-nutrients for ADHD [23,24]. These single nutrient studies have yielded modest findings [23,25], likely because the single-nutrient approach is at odds with human physiology and neurobiology. A variety of nutrients serve as cofactors for specific biochemical pathways in neurotransmitter production and function [26–28]. For example, iron is required for catecholamine synthesis, methylation, and lipid metabolism in energy production; vitamin B6 is required for neurotransmitter synthesis in the central nervous system; and vitamin D modulates glucose transport in the brain [29]. Optimal brain function necessitates availability of adequate amounts of a variety of nutrients, rather than a single nutrient in high doses [30]. Broad-spectrum micronutrient interventions have shown large effect sizes and improvement in ADHD and mood disorder symptoms, as well as improvement in acute stress after natural disasters [31–35,65,66]. Testing the efficacy of broad-spectrum micronutrient supplements for ADHD challenges conventional trial design where the deductive paradigm requires that one variable (e.g. a single nutrient) be manipulated at a time. Most studies have been open-label [31,32,36], retrospective database analyses [33], case reports [34], or patient preference studies [35]. Only one RCT in adults [37] and one RCT in children [41] have been conducted, both in New Zealand.

In the RCT of New Zealand children 7–12 years old ($n = 93$), broad spectrum micronutrient treatment reduced symptoms of anger, aggression, emotional dysregulation and inattention, with no serious safety concerns [41]. These preliminary results highlight the need for a controlled multi-site North American trial of micronutrients in children with ADHD and irritability. The proposed study will investigate changes in behavioral and biological measures in children with ADHD and irritability using a commercially available supplement containing 36 nutrients.

The study objectives are to:

1. Determine the acceptability and tolerability of a broad-spectrum micronutrient product for children with ADHD;
2. Assess effect size for symptom response in irritability and negative mood, as comorbid with ADHD
3. Collect samples of blood, saliva, urine, and stool to evaluate potential biological mechanisms including markers of methylation, metabolism, and microbiome biodiversity

4. Conduct qualitative interviews with a subgroup of parents, to learn about parental decision-making processes and treatment priorities for their child with ADHD, and to understand their experience participating in a RCT.

2. Rationale for nutrient supplementation

The fundamental premise of nutritional supplements' effectiveness is related to individual genetic and physiologic differences in absorption, metabolism, distribution locally within the gut, and systemically throughout the body. These differences result in significant individual variability in the need for various nutrients. The average needed daily intake, as reflected in the Recommended Daily Allowances/Recommended Dietary Intake (RDA/RDI) guidelines, now collectively referred to as the Dietary Reference Intakes (DRIs), are based on the average nutrient intakes required to prevent frank deficiency in an apparently healthy population [39] and do not consider optimal brain levels [40]. DRIs may be insufficient or excessive for some individuals based on polymorphism differences in enzymes, receptors, and other cell organelles [40]. Biological samples collected as part of the study will begin to examine some of these physiological differences between participants who do and do not respond to micronutrient treatment.

3. Design

In a randomized, fully-blind, placebo-controlled parallel-group trial, 135 children with ADHD and irritability will be randomized in a 3:2 ratio to the active treatment (60%) or a matching placebo (40%) for eight weeks, followed by an eight-week open-label extension. All participants will receive the active treatment during the open-label extension phase. The fully-blinded design means that none of the study staff or participants will know to which arm the participant was randomized during the RCT phase. The randomization ratio and the open-label extension were decided so as to maximize parents' willingness to enroll their child. The study will be conducted at three sites: two academic medical centers in the U.S.—Oregon Health & Science University, as the lead site (OHSU, Portland, OR), and The Ohio State University (OSU, Columbus, OH)—and one regional university in Canada, the University of Lethbridge (U of L, Lethbridge, Alberta). Participants will be screened for study eligibility by phone using a standardized list of inclusionary and exclusionary questions (see eligibility definitions below). Those who are eligible and provide consent/assent will be assessed at a baseline visit. Parents/guardians and children, using standardized rating measures, will report ADHD and irritability symptoms along with other psychological domains (see Table 1 for list of measures). At each subsequent visit, participants will be assessed for pill adherence and possible side effects using the Pediatric Adverse Events Rating Scale (PAERS), which was requested by the United States Food and Drug Administration (FDA) to standardize the assessment for possible adverse events. Blood and urine will be collected and analyzed at baseline and at week 8 (end of RCT) for medical eligibility and to assess supplement safety. Hair, stool, and saliva will be collected at baseline, weeks 8 and 16, and at 12-month follow-up (see Table 2). Other assessments, including depression, anxiety, obsessive compulsive disorder, and conduct disorder, will be obtained for sample characterization and secondary analyses. Data collection will occur in-person at baseline and at weeks 4, 8, 12, and 16. During follow-up phone calls (or optional in-person visits) at 1 and 2 months post-study completion and at 12 months post-study enrollment, measures will be repeated and information about ongoing use of micronutrients and/or use of other treatments (e.g. pharmacologic, behavioral) will be collected. See Fig. 1 for study flow diagram.

To better understand parents' motivation to use micronutrient supplements to treat their child with ADHD, qualitative data will be collected with a sub-sample of parents through semi-structured one-on-one interviews. Questions will be guided by a topic list, derived from

Table 1
Study measures.

Researcher-inputted Measures	
Biometrics	Height, weight, BMI, heart rate, blood pressure measured at baseline, week 8, and week 16.
Pill count compliance	Unused capsules will be collected, counted, and recorded, to check adherence at every visit after baseline.
Clinician-rated Measures	
Clinical Global Impressions Scale (CGI) [49]	Rates severity of illness at baseline; improvement via rating change over time. Clinician rating based on all available data: parent and child reports, observation by study staff with overview by PI, and cross-site discussion. Completed at baseline, end of RCT (week 8), end of open label (week 16), and at the 12 month follow-up.
Pediatric Adverse Event Rating Scale (PAERS)	Records adverse events reported by parent; scale requested by US Federal Drug Administration (FDA)
Parent-rated Measures	
Child & Adolescent Symptom Inventory (CASI-5)*	Based on DSM-5; rating scales for emotional and behavioral disorders in ages 5–18. ADHD, ODD and DMDD categories at all visits, rest of subscales at baseline, weeks 8 and 16, and 2 and 12 month follow-ups.
Patient-Reported Outcomes Measurement Information System (PROMIS)*	Self-report questionnaires developed by U. S. Dept. Health and Human Services for clinical trials. Parent will complete scales for anxiety, peer relationships, anger, depression, and sleep.
Strengths and Difficulties Questionnaire (SDQ)*	Measures positive and negative attributes based on five scales: emotional symptoms, conduct problems, hyperactivity/inattention, peer relationships, and prosocial behaviors. Administered at baseline, weeks 8 and 16, and at 12 month follow-up.
Temperament in Middle Childhood Questionnaire (TMCQ)*	Assesses temperament by asking about child's reaction to various situations. Divided into 16 categories. Completed once, at week 1.
Food Insecurity Questionnaire*	Measures food insecurity in the household. Administered once at baseline.
Food Frequency Questionnaire (FFQ)*	Records child's typical dietary intake at baseline and at week 8.
Parent Target Problem (PTP)	Nomination of one or two of child's biggest problems, and reports frequency, duration, impairment, and examples.
Blinding question	To measure the integrity of the blind, at week 8, study staff will ask the parent whether they think their child received active or placebo treatment during RCT. Study staff's response will be recorded also.
Treatment acceptability*	Rating the acceptability of the treatment after the completion of the study and at the 12 month follow-up.
Study Feedback*	Rating of study experience at the week 16 visit.
Reasons for Discontinuation*	Completed at 1, 2, and 12-month follow-up, if supplement is discontinued.
Child-rated Measures	
PROMIS Measures	Child report on anxiety, peer relationships, anger, and depression.
Columbia Suicide Severity Rating Scale (CSSRS)	Child report on presence of suicidal ideation or behavior (specific scale requested by the FDA).
Blinding question	Child's response regarding what they believe they were taking: the active compound or the placebo during RCT. Completed at week 8.
Other adult-rated Measures	
CASI-5 Teacher/Other Adult Report	Secondary reporter completes CASI-5 on child's symptoms of ADHD, ODD, DMDD and the Peer Conflict subscales at baseline and weeks 8 and 16.

*inputted by study participant

Table 2
Biological samples.

Blood	Function
Safety blood tests	Identify contraindications to participation; includes complete blood count, comprehensive metabolic panel, as well as thyroid and iron tests
Research blood tests	Measure changes in cytokines, metabolites, minerals, and fatty acids
Urine	
Safety urine	Identify contraindications to participation
Research urine	Measure changes in metabolites, hormones and neurotransmitters
Stool	
Omnigene GUT OMR-200	Explore micronutrient effects on the gut microbiome
Saliva	
Oragene DNA OG-500	Assess micronutrient effects on DNA methylation
Hair	
~100 strands from back of head	Measure changes in cortisol and mineral levels

consultation with parent advisors and the research team. Open-ended and probing questions will structure the conversation, allowing interviewees to communicate about their experiences and preferences in relation to micronutrient supplementation. Data collection will occur to saturation (when no new information is added), a standard end-point for qualitative interviews (Insert Fig. 1).

4. Methods

To develop the Micronutrients for ADHD in Youth (MADDY) Study protocol, an international consortium of experts in ADHD, nutrition, and clinical trials conferred by email and weekly teleconference for more than a year. The decision to focus on irritability and anger as a feature of ADHD was guided by previous research in children and adults indicating that irritability and mood dysregulation improved as much or more than core ADHD symptoms micronutrient treatment [38,41]. An Investigational New Drug (IND) application was submitted to the United States Food and Drug Administration (FDA), and was approved after review of micronutrient supplement analyses; approval of the study and the micronutrient use was also granted by Health Canada. Dr. Barbara Gracious at The Ohio State (OSU), holds IND#127832. The study protocol was approved by the respective Institutional Review Boards at OHSU, OSU, and the University of Calgary Conjoint Health Research Ethics Board, for the University of Lethbridge (U of L). Hardy Nutritionals (Raymond, Alberta, Canada) provided a letter of agreement for donated research product (Daily Essential Nutrients, "DEN") and matching placebo supply. No financial support was provided by the company, and they have relinquished rights to any data ownership, analyses, or publications.

5. Study population

5.1. Participant recruitment

Participants will be recruited through affiliated child psychiatry divisions and children's hospitals at the respective sites, referrals from local pediatricians and mental health providers, and social media websites (e.g. Facebook). At the two US sites, child and parent participants will be compensated for attending each visit. Additionally, child participants will receive compensation for each biological sample provided. In Alberta, Canada, providing incentives to study participants is not customary; parents will be reimbursed for cost of travel, including parking and mileage for each onsite visit.

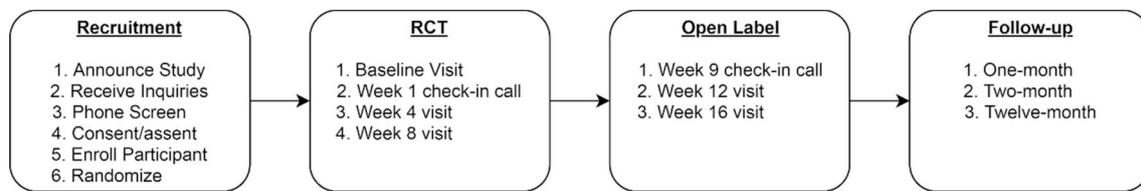


Fig. 1. Study flow diagram.

5.2. Eligibility determination

Each potential participant will undergo a phone screen describing the study purpose, procedures, and risks and benefits to the participants. It will also be used to assess willingness to participate in the study as well as to determine eligibility. If eligible based on parent responses, the parent will be emailed a link to complete the Child and Adolescent Symptom Inventory-5 (CASI-5) [42] eligibility questions, comprised of the ADHD, oppositional defiant disorder (ODD) or disruptive mood dysregulation disorder (DMDD) [43] symptom and impairment subscales, using the Research Electronic Data Capture (REDCap) system [44]. To be eligible, children must meet a clinical cut-off of six inattentive or hyperactive/impulsive symptoms from the ADHD subscale and at least one symptom of irritability or anger as assessed by the ODD or DMDD subscales; scored for severity as 1 point for each response of “often” or “very often” and 0.5 points each for responses of “sometimes”.

Phone screen data, including inclusion/exclusion criteria and the CASI-5 responses, will be reviewed and discussed weekly by the study PIs and the research assistants at each site, with access to a physician, if needed, regarding physical health-related eligibility questions. Difficult-to-decide cases will be discussed weekly at cross-site teleconferences, to reach consensus decisions on eligibility. Eligible participants will be scheduled for a baseline visit where parental consent and child assent for the study will be obtained. The child will be provided with visual and verbal information about the study using simple language appropriate for a child as young as six years old.

5.3. Inclusion and exclusion criteria

To be included in the study, participants will: 1) be 6–12 years old at the time of enrollment; 2) be willing/able to swallow 9–12 capsules per day with food and attend all study appointments to complete questionnaires; 3) meet criteria for ADHD as assessed by the CASI-5, with symptoms present in more than one setting and causing impairment in social or academic functioning; 4) have at least one symptom of irritability or anger from the ODD or DMDD subscale questions; 5) be medication-free, or washed out for at least 2 weeks prior to in-person baseline assessment (with medical supervision, requiring the parent to work with the child’s primary care provider); and; 6) be willing to give blood samples at two time points: baseline and week 8, with an optional week 16 blood draw.

Participants who report having trouble swallowing capsules will complete the pill swallowing program developed by Kaplan et al. [45] prior to the first study visit. The video can be viewed at: <http://research4kids.ucalgary.ca/capsuleswallowing>. Children who continue to be unable to swallow the capsules after training will be excluded.

Study exclusion criteria are based on parent report, consisting of: 1) having a neurological disorder involving brain or other central function (e.g., history of or suspected intellectual disability, autism spectrum disorder, epilepsy, narcolepsy) or other major psychiatric condition requiring hospitalization (e.g. significant mood disorder, active suicidal ideation, or psychosis); 2) having any serious medical condition, including inflammatory bowel disease, history of cancer, kidney or liver disease, hyperthyroidism, diabetes; 3) having a known allergy to any ingredient in the intervention; 4) having any known abnormality of mineral metabolism (e.g., Wilson’s disease, hemochromatosis); 5)

currently taking any other medication with primarily central nervous system activity, including stimulants, or having taken any within two weeks prior to enrollment; 6) having severe anxiety preventing separation from parent to answer study questionnaires; 7) having any disability that would interfere with participant answering questions verbally; 8) not able to communicate in English; 9) being pregnant or sexually active at baseline (specific to girls who started menstruating). Concomitant treatments, such as nutritional supplementation, will be permitted if the supplement does not contain ingredients already present in the treatment product. Other treatments, such as occupational therapy or counselling, will be permitted. Concomitant treatments will be asked about and recorded at each visit.

5.4. Target population size

For power = 0.8, with a two-tailed $\alpha = 0.05$ and a 3:2 randomization ratio, 123 participants are needed to detect an effect of $d > 0.6$ with a parent-rated primary outcome measure that is a composite of the CASI-5 [42] subscale scores. Given the three sites and two treatments in a 3:2 ratio, a number divisible by 15 is needed. Assuming two dropouts per arm per site, based on a previous study ($n = 93$ total) [41], 135 participants need to be randomized with 123 retained; thus, $n = 45$ participants per site. The recruitment target will be 135 participants between the three sites.

5.5. Randomization

A randomization scheme will be generated by the statistician prior to study initiation, with the randomization sequence arranged in permuted blocks of 5, in a ratio of 3:2 active to placebo, to randomize participants at each site. The scheme will extend beyond 45 participants to account for dropouts, ineligibility after enrollment, or other situations that may necessitate additional randomization allocation. There will be three randomization lists, separate for each site. Neither the study team, nor the participants, will have access to the randomization scheme until the end of the study, after the primary data are analyzed. Access will be available to the medical oversight team member at each site and to the Data Safety Monitoring Board (DSBM) members in the event of an 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 kit will include: 1) a seven-day pill caddy, divided into three daily time points, containing the capsules to be taken during the first week according to the titration schedule, 2) bottles of capsules sufficient to last for the rest of the four-week period, and 3) five days’ worth of extra capsules in the event of a delayed return visit. Upon receiving the pill kit, the parent will be instructed in how to refill the caddy after finishing the first week. The pill kits will be sequentially numbered and allocated to placebo or micronutrients based on the randomization scheme. Participating families will receive the name and contact information of the local study clinician (usually the PI or co-PI) in the case of a question or urgent notification.

5.6. Micronutrient formula, titration, and dosing

The micronutrient supplement to be used in the study is Daily

Essential Nutrients (DEN; www.hardynutritionals.com). It consists of a blend of 36 vitamins, minerals, amino acids, and antioxidants (see Table 3). Both active and matching placebo capsules (see Table 4) will be provided by Hardy Nutritionals (Raymond, Alberta, Canada). Participants will be instructed to take the capsules with sufficient amounts of water and food. They will titrate up to their therapeutic dose according to their age group, by taking one capsule three times per day for the first two days, then two capsules three times per day for the next two days, and finally three capsules three times per day for the rest of the first four weeks. For children 6–8 years old, nine pills per day will be the maximum dose; for children 9–12 years old, the dose can be increased to a maximum of twelve capsules per day as described below.

At week one, research staff will check in with the participant's parent via phone to inquire about the participant's tolerance, compliance, and symptom changes, and answer any follow-up questions. During the week 4 and week 12 study visits, a decision to increase the dose up to a maximum of 12 capsules for participants ages 9–12 can be made in consultation with the site PI, and will depend on: 1) parent/participant report of side effects, if any; 2) participant's ability to swallow the

Table 3
Daily essential nutrients (DEN) ingredients.

	Unit	One Pill	RDA* or AI [†]	LOAEL [⊖]
Vitamin A (as retinyl palmitate)	IU	480	2000	46667
Vitamin C (as ascorbic acid)	mg	50	45	3000
Vitamin D (as cholecalciferol)	IU	250	600	3800
Vitamin E (as d-alpha tocopheryl succinate)	IU	30	16.5	750
Vitamin K (as 75% phyloquinone, 25% menaquinone-7)	mcg	10	60	NE
Vitamin B1 (as thiamin mononitrate)	mg	5	0.9	NE
Vitamin B2 (riboflavin)	mg	1.5	0.9	NE
Niacin - Vitamin B3 (as niacinamide)	mg	7.5	12	50
Vitamin B6 (as pyridoxine hydrochloride)	mg	5.8	1.0	500
Folate - B9 (as calcium L-5 methyltetrahydrofolate)	mcg	66.6	300	5000
Vitamin B12 (as 75% adenosylcobalamin, 25%)	mcg	75	1.8	NE
Biotin - Vitamin H	mcg	90	20	NE
Pantothenic acid (as d-calcium pantothenate)	mg	2.5	4	NE
Calcium (as NutraTek™ chelation complex)	mg	110	1300	4000
Iron (as NutraTek™ chelation complex)	mg	1.15	8	70
Phosphorus (as NutraTek™ chelation complex)	mg	70	1250	10200
Iodine (as NutraTek™ chelation complex)	mcg	17	120	1700
Magnesium (as NutraTek™ chelation complex)	mg	50	240	360
Zinc (as NutraTek™ chelation complex)	mg	4	8	60
Selenium (as NutraTek™ chelation complex)	mcg	17	40	913
Copper (as NutraTek™ chelation complex)	mg	0.6	0.7	10
Manganese (as NutraTek™ chelation complex)	mg	0.8	1.9	15
Chromium (as NutraTek™ chelation complex)	mcg	52	25	NE
Molybdenum (as NutraTek™ chelation complex)	mcg	12	34	1500
Potassium (as NutraTek™ chelation complex)	mg	20	4500	NE

[⊖]Lowest Observed Adverse Effects Level.

*Recommended Daily Allowance.

[†]Adequate Intake.

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, vanadium, lithium orotate, nickel, gelatin capsule, microcrystalline cellulose, glycine, citric acid, magnesium stearate, silicon dioxide.

Table 4
Placebo ingredients.

	One Pill
Riboflavin Powder	0.10 mg
Magnesium stearate	5 mg
Silicon dioxide	2.5 mg
Microcrystalline cellulose	~650mg/capsule ^a

^a Sufficient amount to fill the capsule.

capsules; and 3) parent/participant report of symptom improvement. The dose can be lowered at any time, as clinically determined by the site PI or their designate. The participant can withdraw or be withdrawn at any point, without penalty. At every in-person visit, participants will receive additional study product, and will be asked to return their previously dispensed bottles with any remaining product (Insert Tables 3, 4).

5.7. Enrollment and assessment visits

After the consent and assent forms have been signed, the participant will complete a fasting blood draw. Both parent and child will then complete baseline questionnaires (Table 1) through the web-based data management platform REDCap, chosen to facilitate accurate and efficient data collection across sites. Some questionnaires are self-reported: participants enter their responses directly into REDCap, and other questionnaires are administered and entered by the study staff. The parent will be asked for the email address of a coach, teacher or other adult who knows the child and can serve as a secondary reporter. They will be asked to report on the child's ADHD, ODD, DMD, and peer conflict symptoms from the CASI-5 questionnaire at baseline, week 8 and week 16. To allow sufficient time for completion of repeated measures, teachers will be used as informants only if the participant's enrollment coincides with the first half of the school year. Details regarding the measures are noted in Table 1.

Blood, urine, saliva, stool, and hair samples will be collected from the child participant at baseline, week 8, and week 16 (Table 3). Aside from blood and urine analyzed for safety at baseline and week 8, all samples are optional. These will be stored, as noted below, for future analyses that will enable examination of DNA methylation, metabolomics, cortisol levels, and microbiome markers, as possible, pending sufficient funds. Safety blood tests include: complete blood count with differential, thyroid-stimulating hormone, and comprehensive metabolic panel. For girls who have started menstruation, a urine pregnancy test will be performed.

A notification-of-participation letter will be sent to the primary care physician of all enrolled participants. Upon completion of the study, if participants wish to continue taking the supplement, they will receive details on how to obtain, via purchase, more of the study product.

5.8. Post-study follow-up visits

Upon completion of the study, if participants are interested in continuing with the supplement, they are eligible to purchase the micronutrients at a 35% discount. The study provides the supplement to week 16. Families may keep any unused capsules from the open trial, after the capsules are counted for adherence. At the 1, 2, and 12 month follow-up, participants' parents will be contacted by phone and email to see whether the child is still taking the micronutrients. If the child is no longer taking the micronutrients, the parent will be asked to complete the Reasons for Treatment Discontinuation questionnaire. At 2 and 12-month follow-up, participants are invited to an in-person visit to complete questionnaires. If they are not able to attend in person, a phone call will be offered and the assessment questionnaires will be sent for completion via REDCap.

5.9. Adherence

At each visit, participants will be asked to return unused capsules so that adherence can be assessed by pill count. Participants, or their parent, will also be asked to download, on their mobile devices, a pill reminder app called Medisafe, to gather adherence information in real time. Parents will also be given the option to record, on paper, daily pill dosages consumed and any missed doses.

5.10. Assessment measures

The Child & Adolescent Symptom Inventory-5 (CASI-5) is a behavior rating scale for DSM-5 defined emotional and behavioral disorders in youth 5–18 years of age. The CASI-5 will be used as a baseline screening and diagnostic tool, as well as a measure of symptom change. During screening for ADHD, ODD, and DMDD categories, if a parent endorses a symptom, they will be asked for an example and detail about the setting (s) in which the behavior occurs, to capture qualitative detail supporting the validity of responses. Each symptom category also includes an impairment question (i.e., the degree to which symptoms interfere with the child's social or academic functioning). Subscales administered from the CASI-5 include ADHD, ODD, DMDD, conduct disorder (CD), anxiety, depression, post-traumatic stress disorder (PTSD), tics, mania, psychosis, separation anxiety, autism spectrum disorders [(ASD), which is an exclusion], enuresis/encopresis, and peer conflict. A weighted composite score comprised of CASI-5 ratings from the ADHD, ODD, DMDD and peer conflict subscales will be used as the primary outcome, based on findings from the New Zealand RCT in children [41], which reported symptom improvements in these areas as rated by parent, teacher and clinician, as well as in the RCT in adult, which noted improvements in mood and emotional dysregulation [46–48].

6. Demographics

Demographic information will include child's race and ethnicity, parents' marital status and level of education, and family income to estimate socioeconomic status (SES).

6.1. Study measures

Measures will be completed at all visits, unless otherwise noted in Table 1. (Insert Table 1).

6.2. Data and specimens

Biologic specimen collection: All samples will be collected in a clinical research services lab, with the exception of stool, which will be collected at home in most cases (Insert Table 2).

7. Data analysis plan

Our primary outcome measure, defined *a priori*, is the parent-rated CASI-5 subscales of ADHD, ODD, DMDD and the Peer Conflict Scale to test the hypothesis that the treatment targets symptoms of irritable mood and aggression, in addition to core ADHD symptoms. The change from baseline to the end of RCT at week 8, on a weighted composite score of these subscales plus the related impairment scores, will be compared between randomized groups by a mixed effects regression, using site as a covariate, which is standard practice for multisite RCTs. The differences between treatment groups will be summarized as the mean differences and 95% confidence intervals. The secondary outcome measure is the clinician-rated CGI-Improvement (CGI-I). A participant will be considered a treatment responder if the CGI-I is rated a 2 or a 1, "much" or "very much" improved. Quantitative data from these psychological questionnaires and the FFQ will be reviewed for completeness and scoring distributions examined for

normality to confirm that assumptions of statistical tests were met. Log-transformations will be applied where applicable on data not normally distributed, or the appropriate non-parametric test will be chosen.

Categorical outcomes will be compared between groups using Chi-square tests and will be described using odds ratios with 95% confidence intervals and relative risk. All analyses will be undertaken on an intention-to-treat (ITT) basis that includes all randomized participants who had at least one post-randomization assessment, analyzed according to the group to which they were randomized. For participants not completing the eight-week RCT, data from their final assessment (last observation carried forward) will be used to evaluate the change scores. A sensitivity analysis will be done excluding subjects with missing data at 8 weeks. Secondary analyses will be undertaken on all outcomes using the per-protocol analysis set (those that completed the study according to the protocol). All tests will be two-tailed and any *p* values less than 0.05 will be considered statistically significant.

Matched-pairs *t*-tests, change scores, Repeated Measures Analysis of Variance (or ANCOVA if warranted) may be employed to compare continuous outcomes across or within subgroups, adjusting for multiple comparisons when appropriate. Multinomial linear and logistic regression may also be performed to identify predictors of treatment response.

8. Safety considerations

Considering lowest-observed-adverse-effect level (LOAEL), only two nutrients, niacin and magnesium, are above the LOAEL at 9 capsules per day [49], the maximum dose for 6 to 8 year-olds. The amounts of these two nutrients for children of that age are unlikely to contribute to a serious adverse event, based on data of long-term consumption of these nutrients [50].

To standardize safety reporting across clinical trials, parents will be asked about 46 specific potential physical and mental health symptoms that may be considered side effects of exposure to a product, using the Pediatric Adverse Events Rating Scale (PAERS) [51]. This measure, requested by the US FDA, provides consistency of terminology to facilitate comparing across studies and disciplines. An adverse event, defined as "an untoward medical occurrence or...unfavorable...unintended sign, symptom or illness," [51](p.2) will be recorded if a parent responds affirmatively to, "The symptom is new or has increased in severity since starting the study," without need to establish causality between adverse event and micronutrient exposure. Serious adverse events deemed to be related to the study capsules will be reported to local IRB/Ethics boards and the FDA or Health Canada, as required.

Prior studies and database reviews have not revealed any serious safety concerns among participants, based on pre- and post-blood levels [33,52,53], including no differences between adverse events in participants taking the micronutrients compared to placebo [38,47]. In the proposed study, blood and urine samples will be analyzed at baseline for potential safety concerns that might preclude eligibility, and at week 8 after the RCT phase (see Table 2), to monitor safety after taking the micronutrients.

The Data Safety Monitoring Board (DSMB) will consist of a representative chosen from each of the three sites. The members will be free from any conflicts of interest with this study, and will consist of at least one person who is an expert on clinical trial safety monitoring. On a phone call every six months, or more frequently as needed, the DSMB will review and comment on study recruitment, subject demographics and characteristics, and any AEs and safety differences that emerge between the randomized arms, and by site.

9. Discussion

The MADDY study rationale, design, and implementation is unique in several ways. The rationale is based on the premise that intra-individual differences exist in the need for a variety of nutrients required for optimal brain functioning. Supplementation at therapeutic

doses may provide study participants with the nutrients needed to improve symptoms of irritability and ADHD. This study will be the first adequately-powered RCT in North America to investigate the effect of micronutrient supplementation to reduce ADHD among children ages 6–12 years old, recruited at three sites internationally. Further, this study will enroll participants who have an irritable mood component, as measured at baseline by ODD and/or DMDD symptoms, based on previous results in which children with high baseline levels of irritability, anger and aggression demonstrated benefit from micronutrient supplementation [41]. Irritability is often a driver of impairment in ADHD and is noted by parents and teachers as one of the most problematic symptoms [4,54], making it an important study design component. The focus enables confirmation-testing that an irritable ADHD subtype is valid [55, 56]. Use of a standardized measure for adverse events (the PAERS) will enable comparison of AEs to future treatment studies for ADHD or irritability. This study will also build on previous RCTs in children [41] and adults [47] through the collection of a range of biological samples to explore the micronutrients' hypothesized mechanisms of action, as well as qualitative interviews with participants' parents to capture their perspective on ADHD treatment options for their child.

The scientific rationale for using the broad-spectrum micronutrient intervention is based, in part, on animal research examining the prediction and treatment of tail biting in pigs [57]. This behavior is thought to be partially exacerbated by dietary insufficiencies; treatment with minerals reduces the behavior [57,58]. The intervention's synergistic blend of minerals and vitamins was designed to contain the nutrients needed to optimize brain functioning [30,59], while addressing other hypothesized mechanisms affecting mental health, including microbiome changes [60,61] and reduction in inflammatory markers [62]. Gut microbiota affects nutrient absorption and metabolism, and vice versa [63,64]. A role for the microbiome in relation to ADHD has also been suggested [60,61]. Through the collection and analyses of biological samples, these hypotheses will be testable.

Qualitative research enables researchers to gain insights into parents' perspectives beyond the constraints set by narrowly focused, pre-defined quantitative questionnaire data. This approach in the MADDY study is intended to capture parents' preferences in this vulnerable population of children who often receive stimulant or other medication as a primary treatment.

Despite its unique and well-timed design, this study has potential limitations. Using a broad-spectrum micronutrient formula with minerals requires participants to consume up to twelve capsules daily compared to pharmaceutical product studies that require the consumption of only 1 or 2 capsules. This amount may be difficult for some children and may result in poor adherence, although previous studies of micronutrients in children have demonstrated their ability and willingness to adhere to a relatively large number of capsules and other potentially difficult tasks in the protocols [38,41]. Study recruitment will happen year-round and changes in schedules, diet, and activities during the summer months may affect participants' behavior, compared to behaviors during the school year. The importance of a third-party rater for child behavior is acknowledged though teacher ratings may not be available for participants enrolled in late spring or summer months, so another adult who knows the child will be asked to complete questionnaires. Usual dietary intake will be assessed at two time-points to control for dietary variations over time and season. Differences in appearance or odor between active treatment and placebo capsules, despite identical encapsulation and efforts to conceal olfactory variances, may impact blinding, which will be measured and reported to document study blinding results.

To summarize, this study has the potential to test hypotheses related to several areas of ADHD research in a large, heterogeneous, international population. Specifically, to compare micronutrient-driven behavioral changes, the validity of an irritable ADHD subtype, and secondary, the micronutrients' mechanisms of action. In addition to behavioral measures, the study will collect stool, blood, saliva, hair, and

urine to investigate the stool microbiome and metabolome, blood metabolites and nutrient levels, salivary hormones, DNA, RNA, and hormone, metabolic and micronutrient residues in hair and urine. Future reports will describe specific tests, methodologies, and results of these exploratory measures, adding to an understanding of for whom and why micronutrient supplementation works. Qualitative data will complement the quantitative data by providing insights on parents' perspective of nutrient supplementation, which may help inform policy for coverage of this type of treatment. If significant, the study results have the potential to address currently unmet treatment needs in this child population, potentially improving outcomes throughout development and into adulthood.

Declaration of competing interest

Dr. Arnold has received research funding from Forest, Lilly, Noven, Shire, Supernus, Roche, and YoungLiving (as well as NIH and Autism Speaks); has consulted with Pfizer, Tris Pharma, and Waypoint; and been on advisory boards for Arbor, Ironshore, Otsuka, Pfizer, Roche, Seaside Therapeutics, Shire. Dr. Gracious has been or is a consultant to: AstraZeneca, Otsuka, and NovoNordisc. The other authors declare that they have no competing interests.

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Abbreviations

ADHD	Attention Deficit/Hyperactivity Disorder
AE	Adverse Event
BMI	Body Mass Index
CASI-5	Child & Adolescent Symptom Inventory, Fifth Edition
CGI	Clinical Global Impression
CSSRS	Columbia Suicide Severity Rating Scale
DEN	Daily Essential Nutrients
DMDD	Disruptive Mood Dysregulation Disorder
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
FFQ	Food Frequency Questionnaire
IND	Investigational New Drug
ITT	Intention to Treat
IRB	Institutional Review Board
LOAEL	Lowest-observed-adverse-effect Level
NUNM	National University of Natural Medicine
ODD	Oppositional Defiant Disorder
OHSU	Oregon Health & Science University
OSU	The Ohio State University
OL	Open Label
PAERS	Pediatric Adverse Event Rating Scale
PI	Principal Investigator
PROMIS	Patient-Reported Outcomes Measurement Information System
PTP	Parent Target Problem

RA Research Assistant
RCT Randomized Controlled Trial
SES Socioeconomic Status
U of L University of Lethbridge

Declarations

Ethics approval and consent to participate

The study has been approved by the United States Food and Drug Administration (IND #127832), Health Canada (Control #207742), Institutional Review Boards at OHSU IRB #16870, OSU IRB #2017H0188 and by the University of Calgary Conjoint Health Research Ethics Board (CHREB) REB #17-0325. All participants will provide written consent (parents) or assent (children) prior to participation. Prior to implementation of any protocol changes, amendments will be approved by the IRBs or Ethics Boards.

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Authors' contributions

Study inception and design: JJ, BG, BL, LEA; study implementation: JJ, BG, BL, LEA, GT, AS, LP; participant recruitment: JJ, BL, LP, GT, AS, AB, LEA; screening and enrollment: JJ, BL, LP, GT, AH, AS, AB, LEA; manuscript review, edits and final approval: all named authors. ICMJE authorship guidelines were followed in determining eligibility.

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