Evaluation of the Safety and Tolerability of a Nutritional Formulation in Patients with Angelman Syndrome (FANS): Study Protocol for a Randomized Controlled Trial

CURRENT STATUS: ACCEPTED

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DOI: 10.21203/rs.2.13020/v1

SUBJECT AREAS
Internal Medicine Integrative & Complementary Medicine

KEYWORDS
Ketogenic diet; ketosis; Angelman syndrome; placebo-controlled trial; children; exogenous ketone; ketone ester; ketone supplement
Abstract

Background Ketogenic and low glycemic index diets are effective in treating drug resistant seizures in children with Angelman syndrome. Cognition, mobility, sleep, and gastrointestinal health are intrinsically linked to seizure activity and overall quality of life. Ketogenic and low glycemic diets restrict carbohydrate consumption and stabilize blood glucose levels. The ketogenic diet induces ketosis, a metabolic state where ketone bodies are preferentially used for fuel. The use of exogenous ketones in promoting ketosis in Angelman syndrome has not been previously studied. The study formulation evaluated herein contains the exogenous ketone beta-hydroxybutyrate to rapidly shift the body towards ketosis, resulting in enhanced metabolic efficiency. Methods This is a 16 week, randomized, double blind, placebo-controlled crossover study to assess the safety and tolerability of a nutritional formula containing exogenous ketones. It also examines the potential for exogenous ketones to improve the patient’s nutritional status which can impact the physiologic, symptomatic, and health outcomes liabilities of living with Angelman syndrome. Discussion This manuscript outlines the rationale for a study designed to be the first to provide data on nutritional approaches for patients with Angelman syndrome using exogenous ketones. Trial Registration: ClinicalTrials.gov, identifier: NCT03644693; Registered on 23 August 2018. Last Updated on 23 August 2018.

Background

Angelman syndrome (AS) was first described in 1965 [1], and has a prevalence in the general population of 1:10,000-1:24,000 [2-4]. Loss or reduction of function of the maternal UBE3A gene (ubiquitin protein ligase E3A) was identified in the late 1990s as the causative mechanism in the majority of cases [5-6]. Patients with AS universally have global developmental delays including speech impairment and movement disorders [7]. Individuals are typically described as having a happy disposition with frequent laughter. Eighty percent of patients also experience seizures, and all individuals with AS have a disordered electroencephalogram (EEG) [8-9]. A large proportion of the population also has feeding problems (75%) and other gastrointestinal (GI) complaints [7,10]. Unfortunately, AS has no cure. Typical treatment protocols include pharmacotherapy for seizures, physical and behavioral therapies, and educational interventions [11]. However, approximately 77%
of the AS population experience treatment resistant seizures [8].

Refractory epilepsy has been treated successfully with specialized dietary approaches, such as the ketogenic (KD), medium chain triglyceride (MCT) based, and low glycemic index diets (LGIT); these diets have also been successful in AS [12-15]. The ketogenic diet allows the body to shift from carbohydrate-based metabolism to fat-based metabolism, thus stimulating hepatic ketogenesis.

Nutritional ketosis is a state where the blood levels of ketone bodies are significantly above baseline, typically >0.5 mmol/L. The ketone bodies (acetoacetate and beta-hydroxybutyrate (BHB)) are energy substrates used by mitochondria to produce adenosine triphosphate, and can be used as alternative fuels to glucose by the brain, heart and skeletal muscles.

The high rate of refractory seizures, feeding and GI problems, and severe communication impairments in AS lead to a high unmet medical need. Overwhelming challenges exist to ensuring appropriate diets that serve the complex needs of individuals with AS. Nutritional approaches that promote the body’s use of ketones as an alternative fuel may improve overall nutritional status and allow for a liberalized dietary therapeutic strategy in individuals with AS, therefore improve quality of life.

Dietary supplementation with ketogenic ingredients is hypothesized to have an important impact on meeting the nutritional ketosis needs of patients with AS. Ketone supplements can aid in achieving and maintaining ketosis, improve the patient’s overall nutritional health in AS symptom management, and may improve quality of life for patients and their families. This study is designed as the first-ever to evaluate the safety and tolerability of the ketogenic ingredient beta-hydroxybutyrate in AS. This manuscript describes the rationale, methods, and potential importance of ketone-based nutritional interventions in children with AS.

**Objectives**

The primary objective of the trial is to assess the tolerability of a nutritional formulation containing BHB in patients with AS ages 4-11 on a variety of dietary backgrounds. The secondary objectives include assessment of ketosis when consuming the nutritional formulation and safety of the nutritional formulation in patients with AS. These outcome measures were chosen as this is a
vulnerable, nonverbal population with limited ability to communicate difficulties with tolerability and safety concerns. Additionally, the degree of ketosis attained may inform future trials to assess the beneficial impact of patient nutritional status on health outcomes. All measures are assessed at the end of each four-week intervention period (placebo versus test formulation).

Methods And Trial Design

Population and Setting

This trial is sponsored by Vanderbilt University Medical Center in collaboration with Disruptive Nutrition, LLC, and the onsite Angelman Syndrome Foundation clinic at the Monroe Carell Jr. Children’s Hospital is the single study site. Patients are primarily recruited to the Nashville, Tennessee site. Subjects with AS are recruited through investigator clinical practices, physician referrals, outreach through social media, and parent support groups such as the Foundation for Angelman Syndrome Therapeutics and the Angelman Syndrome Foundation. Subjects are enrolled with the following inclusion criteria: genetically confirmed diagnosis of Angelman syndrome; age 4-11 years; currently on a LGIT, KD (conventional 4:1 or 3:1, MCT, modified Atkins), or standard diet (regular diet) consistently for at least three months; willingness to consume the investigational formulation; and willingness to undergo protocol examinations at home and clinic visits. Subjects are excluded if: they require parenteral nutrition; have major hepatic or renal dysfunction; history of diabetes or have diabetes; significantly underweight with body mass index <18.5; participation in other clinical intervention studies within 1 month prior to entry of the study, unwillingness to comply with protocol requirements; or have contraindications for the use of ketogenic or low carbohydrate diets which, in the opinion of the investigator, may influence patient’s ability to participate in the study. Subjects are permitted to continue using all previously prescribed medications, provided the prescriptions have not changed in the one month prior to randomization. Parents/guardians of all eligible subjects must sign informed consent prior to study enrollment, following adequate explanation of the aims, methods, objectives and potential hazards of the trial by the responsible investigator (see Additional File 3).

Study Design
This investigation is a sixteen week, randomized, crossover, double blinded, placebo controlled exploratory study of the safety and tolerability of a nutritional formulation containing d,l-beta-hydroxybutyrate mineral salts (sodium, calcium, magnesium) (trumacro™, Disruptive Nutrition, LLC, Durham, North Carolina) for use with a ketogenic diet, a LGIT diet, and standard diet in subjects with AS. It is being conducted in accordance with the Declaration of Helsinki and is approved by the Institutional Review Board (IRB) at Vanderbilt University (protocol number DUISJ10212017084407).

See Figure 1 (Standard Protocol Items: Recommendations for Intervventional Trials (SPIRIT) figure: schedule of enrollment, interventions and assessments), Additional File 1 for the SPIRIT 2013 Checklist (recommended items to address in a clinical trial protocol and related documents), and Additional File 2 for the Schematic of Trial Design.

Figure 1, Title: FANS Trial Design- Randomized, Placebo Controlled, Crossover Study. Legend:

Standard Protocol Items: Recommendations for Intervential Trials (SPIRIT) figure; schedule of enrollment, interventions and assessments. *May be conducted by phone or in person at the clinic site. ** An additional sample of blood will be collected during the baseline visit and added to the repository at the Division of Medical Genetics and Genomic Medicine at Vanderbilt University. Samples may be used for studies such as DNA and RNA, proteomics and metabolomics.
| Clinic Visit Number | 1* | 2 | 3 | -- | 4 |
|---------------------|----|----|----|-----|----|
| Timepoint           |    |    |    |     |    |
|                     | Scree ning | Baseline: weeks 1-2, Randomization day 14 | Intere ntion Period 1: weeks 3-6 | Washou t period: weeks 7-10 | Intere ntion Period 2: weeks 11-14 |
| Allowed Deviation Window | N/A | +/- 2 days | +/- 2 days | +/- 2 days | +/- 2 days |

**ENROLLMENT**
- Review patient eligibility criteria
- Review informed consent
- Report of natural and medical history
- Parent/caregiver instructional session

**INTERVENTIONS**
- Consume test or placebo formulation
- Cross over to opposite arm

**CLINICAL ASSESSMENTS**
- Physical exam, anthropometrics
- Blood collected (venipuncture)
- Clinical laboratory (hematology, biochemistry)
- Three-day diet and seizure recall
- Neuropsychological evaluations
- EEG
- ERP cognitive analysis
- Walkway gait analysis
- Rate convenience, taste, and acceptability
- Telephone contact (weekly)
- Review of home monitoring data (weekly)
- Adverse events recorded

**HOME MONITORING**
- Urine testing daily
- Record dietary intake daily
- Record seizures daily
- Nightly sleep monitoring
- GI health diary recorded daily
- Formula preparation, intake recorded daily
After obtaining informed consent from the parent/caregiver, subjects enter a 2-week run-in period. At the beginning of the run-in period the natural history and medical history is collected from the subject’s parent/caregiver. At baseline, a 3-day recall record of the subject’s diet, appetite, GI health, sleep, seizure, mood, and activity level are collected. While one goal of this study is to assess the effects of dietary background on the suitability of the nutritional formulation, there is no requirement for a specific number of patients per diet due to the rarity of the population. Additional study measures, including physiologic are also collected (Figure 1).

The study design for this nutritional intervention protocol is a randomized, double blind, placebo-controlled crossover study. There are two arms to the study, (1) investigational formulation period followed by placebo formulation period, or (2) placebo formulation period followed by investigational formulation period. Each patient will receive both investigational formulation and placebo formulation, with a washout period between each arm. The randomization schedule was created by the Investigational Drug Service Pharmacy using randomization software in a 1:1 ratio with a block size of 4. Participants are enrolled in the next open slot of on the randomization table. The nutritional formulations are coded by the manufacturer lot number and therefore the clinical staff is blind to the actual contents of the container (active verses placebo).

Once randomized, subjects start Intervention Period 1, a 4-week period in which they receive nutritional formulation containing BHB or placebo while maintaining the diet noted at entrance into the study. Home monitoring occurs as indicated in Figure 1. After 4 weeks of blinded intervention, subjects are evaluated in the clinic and then cease consumption of the ketone formulation or placebo for 4-week washout period. Following completion of the washout, subjects enter a second 4-week period in which they receive formulation versus placebo. They will continue the home monitoring throughout the duration of the study. Subsequently, at the completion of the second intervention, they undergo the final scheduled study visit and laboratory sampling. A washout period follows Intervention Period 2, and at approximately week 16 of the study, a follow up call is made to the family to review symptoms and ensure the patient is not experiencing adverse events during this
period. The trial schedule of enrollment, interventions, and assessments is outlined in Figure 1 (FANS Trial Schedule of Procedures).

Procedures And Interventions

Background information regarding the subject's natural history is collected using a REDCap© survey (Copyright 2006-2013 Vanderbilt University. All rights reserved) [16]. Body weight and height are measured at each study visit. Serum laboratory analysis is conducted to establish a metabolic baseline: comprehensive metabolic panel, complete blood count, ketones (beta-hydroxybutyrate and acetoacetate), and lipid panel. Electroencephalography (EEG), event-related potential recordings (ERP) and gait analysis using the ProtoKinetics Zeno™ Walkway are performed at each study visit (Figure 1). The utility of ERP and gait analysis to predict changes in patient function in an Angelman population has not been clearly established, but their appropriateness in similar populations has been detailed elsewhere [17-18]. Questionnaires completed during the study include the Vineland™-3, Developmental Behavioral Checklist (Pearson Education, Inc.), which has been used previously in studies of patients with AS [19]. Dietary intake, seizure frequency, sleep and GI health are evaluated throughout the protocol through at-home monitoring. Data collected during home monitoring is recorded on tablet devices provided to the parents/caregivers upon enrollment, preloaded with necessary tracking applications. Families are provided an EarlySense® monitor with a written guidance for home sleep monitoring. External sleep monitors are important in an AS population as wearable devices are typically removed by the patient. The monitors have not been studied with patients with AS, however their validity has been demonstrated in the general population [20]. Urinary ketones are measured by the patient’s caregivers throughout the protocol, using Ketostix which are a validated measure of urine acetoacetate [21-22].

Tolerability is determined at the end of each four-week intervention period. Protocol compliance is monitored during both investigational and placebo intervention periods by recording the amount of formulation consumed. Formulation acceptability is assessed at the end of each intervention period by a parent questionnaire evaluating convenience, taste and degree of acceptance of the nutritional formulation on a ten-point Likert scale. Similar ranking systems to assess formulation acceptability
have been used with other nutritional interventions in epilepsy populations [22-25].

Safety is assessed throughout the entire protocol period through (1) adverse event reporting and (2) assessment of clinical parameters collected throughout the protocol including anthropometrics, blood metabolism profile, dietary intake, seizure frequency, EEG, ERP, mobility, GI function, and sleep habits. In this population, the potential for negative effects of intervention must be closely monitored as patients are nonverbal. Additional File 4 contains details on collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events.

Investigational and placebo formulations are administered with the serving size based on subject body weight. The investigational formulation provides beta-hydroxybutyrate, 2g of carbohydrate, 1g of protein, and 9g of fat, plus minerals, per 100kcal. The placebo is matched for mineral content only. The formula is given orally as food or beverage three times per day.

Families will be encouraged to continue participation in the trial through weekly telephonic interviews. Data will be collected through three primary interfaces. Background information regarding the patient’s natural history will be collected by the clinical staff using the REDCap survey.

REDCap® is a secure web application that manages the trial database (21 CFR Part 11 and HIPAA compliant). Clinical visits will use either paper or electronic medical records. Data collected during home monitoring will be recorded using a tablet provided to the patient upon enrollment. The tablet is preloaded with the applications necessary to record all data during home monitoring. All data collected, regardless of patient completion status, will be shared with patients upon conclusion of the trial (last patient last visit) and assessment of the data. Additional File 4 contains details on data management and collection procedures.

**Statistical Considerations**

The primary outcome of the trial is to assess the tolerability of a nutritional formulation containing BHB in patients with AS. Tolerability is demonstrated through patient compliance with the protocol as determined by the amount of formulation consumed as compared to the amount prescribed and is assessed at the end of each intervention period. Premature discontinuation of the nutritional formulation by the family is also deemed as potential intolerability. Tolerability is also assessed at
the end of each intervention period through parental questionnaire. These outcome measures were chosen as this is a vulnerable, nonverbal population with limited ability to communicate and therefore determining if the formulation is suitable and well tolerated is an appropriate first step.

The secondary outcomes include assessment of ketosis when consuming the nutritional formulation and safety of the nutritional formulation in patients with AS. Ketosis is evaluated daily through urine testing as well as blood analysis at the end of each intervention period. The degree and timing of ketosis may be clinically relevant to demonstrating better patient nutrition as nutritional ketosis has been related to better patient outcomes as described in the background of this manuscript. Safety is evaluated by changes in motor function, cognitive function, GI tolerance, sleep and seizures, along with changes in height, weight and blood metabolic panels as assessed at the end of each intervention period. In addition, adverse event reporting is monitored in real time by the study coordinator.

No studies testing ketone supplementation in patients with AS exist. Therefore, sample size estimation is based on measures of ketosis. It is assumed from murine pre-clinical investigations, non-AS clinical studies using MCT and/or BHB, and low carbohydrate dietary interventions, that the minimal change of the secondary outcome, degree of ketosis, from the baseline to the end of the 4th week of the investigational formulation intervention period will be 100% change from baseline (baseline for standard diet is typically <0.5mMol/L of d-BHB in blood, or < 5mg/dL acetoacetate in urine). The acetoacetate test used is Ketostix (Bayer Corp. Diagnostics Division, Tarrytown, New York) which exhibits a sensitivity of 78% and a specificity of 96% when compared to a serum standard of 14.4 ml/dL. Considering a 25% drop out rate, an estimate of 25 subjects need to be screened to achieve 15 completers.

Descriptive statistics, such as mean and standard deviation, are used to describe continuous variables, and frequency for the categorical variables. Means are compared using a paired two-sample t-test. Statistical significance for all tests is p<0.05. Multiple comparisons can be made including: a) each subject to serve as his or her own control, comparing investigational formulation to placebo formulation, as well as to baseline, b) each dietary background group considered in
aggregate, comparing investigational formulation to placebo formulation, as well as to baseline, and
c) all subjects considered in aggregate, comparing investigational formulation to placebo formulation, as well as to baseline.

**Discontinuation/Withdrawal from Study Participation**

Participation in this study is voluntary. The parent or guardian of a participant may withdraw consent to participate in the study at any time for any reason they deem necessary. The administration of the study formulation may be discontinued abruptly and does not require any further medical intervention and no alternate therapy will be offered.

**Data Monitoring**

In order to ensure the safety of the study participants, members of a Data Safety Monitoring Board (DSMB) have been appointed by the principal investigator and Vanderbilt University. The board consist of a chairperson, biostatistician, and three independent reviewers with expertise in the research field. Each member of the board meets these minimum qualifications including: 1) expertise in the field, 2) experience in the conduct of clinical trials and statistical knowledge, 3) independence from the direct management of the clinical trial, and 4) no conflict of interest. The chairperson is responsible for overseeing the meetings, developing the agenda, summarizing the meeting, and is the contact person for the DSMB. Additional details on the charter of the DSMB can be had by contacting the Vanderbilt University Medical Center Human Research Protections Program at www.vumc.org/irb.

**Ethical Considerations**

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and institutional research policies and procedures. This protocol and any amendments will be submitted to the Vanderbilt University institutional review board (IRB) in agreement with local legal prescriptions, for formal approval of the study conduct. The DSMB will work in conjunction with the principal investigator and IRB to determine if a protocol amendment warrants additional patient consent or other communication to the enrolled patients and their families. The decision of the IRB concerning the conduct of the study will be made in writing to the
investigator before commencement of this study. Protection of participant confidentiality is described further in Additional File 4.

Discussion
Angelman syndrome is characterized by refractory epilepsy. In fact, the EEGs of individuals with AS are abnormal when compared to neurotypical controls even in the absence of seizures [26]. Fasting has been recognized as a potential treatment for seizures since ancient times, and dietary intervention that mimicked fasting was introduced in the early 1920’s [27-28]. These diets place the body in a state of nutritional metabolic ketosis. Using nutritional ketosis as a model, many dietary interventions have been studied in both animal models and in human clinical trials for a variety of neurological disorders.

For children with refractory epilepsy, dietary interventions leading to nutritional ketosis are efficacious and safe. A modified Atkin’s diet prospectively studied in children aged 3-18 years demonstrated moderate urinary ketosis developed within 4 days with significant improvement in seizure frequency at 6 months [29]. In AS, case reports also demonstrate efficacy [30]. For a 5-year-old girl with uncontrollable daily seizures resistant to 3-anticonvulsant therapy, a ketogenic diet proved to be effective in diminishing seizures from the first week of initiation. EEG with no epileptic activity confirmed the patient’s improvement along with improvement in sleep and hyperactivity. In a separate study in patients with AS, four months of LGIT resulted in decreased seizure frequency with correlative improvement post-trial EEG, with evidence of developmental progress. The diet was well tolerated and five of six subjects remaining on the LGIT after completion of the trial [14]. A retrospective medical record review of 23 subjects with AS who utilized LGIT suggested that 22% of subjects maintained complete seizure freedom, 43% maintained seizure freedom except in the setting of illness or non-convulsive status epilepticus, and 30% had a decrease in seizure frequency [13].

The literature suggests a safe transition from glucose to ketone bodies as fuel. A large study of the use of the LGIT diet in pediatric epilepsy revealed limited side effects and decreased seizure frequency [31]. Studies of the medium-chain triglyceride diet revealed gastrointestinal complaints as the greatest tolerability issue [32]. The risk of nutritional deficiency in children on restrictive dietary
treatments and a lack of ketogenic diet specific supplements raised concerns about micronutrient status. Vitamin A, E, zinc, selenium and magnesium levels have been measured in children with intractable epilepsy treated with the ketogenic diet [33]. The researchers suggested that changes in plasma vitamins A and E and the decline in magnesium status after 12 months may be suboptimal and require supplemental approaches to the ketogenic diet.

While clinical evidence suggests that nutritional ketosis through diet is effective, well tolerated and safe in seizure control; little clinical evidence is available regarding the consumption of exogenous ketones as part of nutritional support to improve a patient’s overall health, supporting dietary and pharmacologic management of seizures and other symptoms. Such an approach is supported by animal models. Transgenic mice, altered at E6-AP ubiquitin ligase gene known to be the causative factor in AS, resemble human AS with motor dysfunction, inducible seizures, and a context-dependent learning deficits [5-6, 34]. These mice were fed an exogenous ketone (R,S-1,3-butanediol acetoacetate diester) ad libitum for eight weeks, mixed into the standard rodent chow at 10% weight/weight. The animals were in nutritional ketosis and demonstrated improved motor coordination, learning and memory, and synaptic plasticity [35]. Exogenous ketone supplements (ketone ester 2.5 g/kg/day; BHB salt + medium chain triglyceride: 2.5 g/kg/day) administered orally for 7 days to genetically absence epileptic Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats modulated absence epileptic activity and significantly decreased the number of spike-wave discharges. These improvements returned to baseline levels on the first day without ketone supplementation [36].

The animal evidence supporting ketone supplementation and the clinical evidence for ketogenic diets in models of AS and seizure control, respectively, suggest that dietary interventions including exogenous ketones may be an important development in clinical management strategies. The current study is designed as an initial evaluation of ketogenic formulations used with a variety of dietary backgrounds. It represents a unique opportunity to study the potential benefits of nutritional intervention in a pediatric population (ages 4-11) with AS. The knowledge gained from the study may guide further evaluations in the use of exogenous ketones for nutritional strategies in the dietary management of patients AS.
It is known in the AS population that there is a large placebo effect. Our study also offers a unique design in which the use of objective measures is prioritized. We hope this will yield measures that benefit future trials in the Angelman community in addition to a clearer understanding of the overall effectiveness and mechanism of action of BHB for inducing ketosis. The ultimate goal will be to positively impact nutritional status, which should enhance the ability of the patient to overcome the symptoms of AS such as seizures, cognition and learning, gait/mobility, and/or sleep disturbances.

**Trial Status**

ClinicalTrials.gov, identifier: NCT03644693; Registered on 23 August 2018. Last Updated on 23 August 2018, recruitment start date November 2018, approximate completion date December 2019.

**List Of Abbreviations**

AS Angelman syndrome

UBE3A Ubiquitin Protein Ligase E3A

EEG electroencephalogram

GI Gastrointestinal

KD Ketogenic diet

MCT Medium chain triglycerides

LGIT Low glycemic index therapy

BHB beta-hydroxybutyrate

ERP Event related potential

IRB Institutional review board

**Declarations**

**Ethics Approval and Consent to Participate**

This clinical trial protocol was approved by the IRB at Vanderbilt University in November 2018 (protocol number DUISJ10212017084407). Potential parents/guardians of participants will be contacted initially by a member of the Angelman syndrome clinic at Vanderbilt University. Parents will be provided with the IRB approved version of the Informed Consent document (Additional File 3). Participants are children between the ages of 4 to 11 years and, therefore, consent obtained from the
person with parental rights after they have read the informed consent and had their questions answered.

**Consent for Publication/Publication Plan**

Not applicable.

**Availability of Data and Materials**

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study. The principal investigator and clinical team will have access to the final dataset. The research team at Disruptive Nutrition will only have access to de-identified data.

**Competing Interests**

DLH is employed by Disruptive Enterprises, LLC which has exclusively licensed the patents to the formula used in this study. EJW is an inventor on one of the patents covering the study formulation, has received research support from the Foundation for Angelman Syndrome Therapeutics, and is currently employed by PTC Therapeutics which engages in the development and commercialization of gene therapies for Angelman syndrome. DPD is an inventor on one of the patents covering the study formulation, and has received funding from Disruptive Enterprises, LLC and the Office of Naval Research. JD has received funding from the Foundation for Angelman Syndrome Therapeutics and the Angelman Syndrome Foundation.

**Funding**

This project is funded by the Foundation for Angelman Syndrome Therapeutics in collaboration with Disruptive Nutrition, LLC. The Foundation reviewed a grant application substantially equivalent to the protocol described herein, but did not have direct input into protocol design or writing the manuscript. DLH is employed by Disruptive Nutrition and was involved in the design of the study and writing the manuscript. Neither the Foundation nor Disruptive Enterprises has ultimate authority over study design, collection, management, analysis, and interpretation of data; writing of the report, or publication; this responsibility rests with the trial sponsor, Vanderbilt University, and principal investigator, Dr. Duis.

**Authors’ Contributions**
DLH participated in trial design drafting the manuscript. EJW and DPD participated in trial design. JD is the principal investigator for this investigator-driven clinical trial and the point of contact for all trial inquiries; she participated in trial design and drafting the manuscript. All authors read and approved the final manuscript.

**Acknowledgements**

A special thank you to Terry Jo Bichelle, Paula Evans, Allyson Berent, and Meagan Cross for their insight from the family’s perspective of living with a child with Angelman Syndrome. Their deep knowledge of this condition was invaluable. We are also grateful to Frank Cerasoli, PhD and Sophia Ononye, PhD for their professional writing services.

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DLH is the Chief Science Officer for Disruptive Enterprises, LLC, of which Disruptive Nutrition, LLC is a wholly owned subsidiary. EJW was formerly Professor, Molecular Pharmacology & Physiology, Chief Scientific Officer, USF Health Byrd Alzheimer’s Institute, Director, Murine Neurobehavioral Testing Facility, and Associate Professor, Molecular Pharmacology and Physiology at the University of South Florida. He is currently Vice President, Biology-Neuroscience at PTC Therapeutics. DPD is an Associate Professor, College of Medicine Molecular Pharmacology & Physiology at the University of South Florida and a Research Scientist at the Institution for Human and Machine Cognition. JD is an Assistant Professor, Division of Medical Genetics and Genomic Medicine, Director, Prader-Willi Syndrome Comprehensive Clinic at Vanderbilt and Director of the ASF supported Angelman Syndrome Comprehensive Clinic at Vanderbilt.

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Additional Files
Additional File 1: SPIRIT 2013 Checklist
Additional File 2: Schematic of Trial Design
Additional File 3: Patient Consent Document
Additional File 4: Additional Protocol Details

Supplementary Files
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