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Nutritional Formulation for Patients with Angelman Syndrome: A Randomized, Double-Blind, Placebo-Controlled Study of Exogenous Ketones

Robert P Carson,[1] Donna L Herber,[2] Zhaoxing Pan,[3] Fenna Phibbs,[4] Alexandra P Key,[5] Arnaud Gouelle,[6,7] Patience Ergish,[8] Eric A Armour,[1] Shital Patel,[9,10] and Jessica Duis[1]

[1]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, USA; [2]Disruptive Nutrition, LLC, Burlington, NC, USA; [3]Biostatistics Core, Children's Hospital Colorado Research Institute, University of Colorado School of Medicine Anschutz Medical Campus, Aurora, CO, USA; [4]Department of Neurology, Vanderbilt University Medical Center, Nashville, TN, USA; [5]Department of Hearing and Speech Sciences, Vanderbilt University Medical Center, Nashville, TN, USA; [6]Gait and Balance Academy, ProtoKinetics, Havertown, PA, USA; [7]Laboratory Performance, Health, Metrology, Society (PSMS) , Reims, France; [8]Clinical Nutrition, Vanderbilt University Medical Center, Nashville, TN, USA; [9]Department of Neurology, Baylor College of Medicine, Houston, TX, USA; and [10]Department of Pediatrics, Baylor College of Medicine, Houston, TX, USA

ABSTRACT

Background: Angelman syndrome (AS) patients often respond to low glycemic index therapy to manage refractory seizures. These diets significantly affect quality of life and are challenging to implement. These formulations may have benefits in AS even in the absence of biomarkers suggesting ketosis.

Objectives: We aimed to compare an exogenous medical food ketone formulation (KF) with placebo for the dietary management of AS.

Methods: This randomized, double-blind, placebo-controlled, crossover clinical trial was conducted in an academic center from 15 November, 2018 to 6 January, 2020. Thirteen participants with molecularly confirmed AS aged 4–11 y met the criteria and completed the 16-wk study. The study consisted of four 4-wk phases: a baseline phase, a blinded KF or placebo phase, a washout phase, and the crossover phase with alternate blinded KF or placebo. Primary outcomes were safety and tolerability rated by retention in the study and adherence to the formulation. Additional secondary outcomes of safety in this nonverbal population included blood chemistry, gastrointestinal health, seizure burden, cortical irritability, cognition, mobility, sleep, and developmental staging.

Results: Data were compared between the baseline, KF, and placebo epochs. One participant exited the trial owing to difficulty consuming the formulation. Adverse events included an increase in cholesterol in 1 subject when consuming KF and a decrease in albumin in 1 subject when consuming placebo. Stool consistency improved with KF consumption, from 6.04 ± 1.61 at baseline and 6.35 ± 1.55 during placebo to 4.54 ± 1.19 during KF ($P = 0.0027$). Electroencephalograph trends showed a decrease in Δ frequency power during the KF arm and event-related potentials suggested a change in the frontal memory response. Vineland-3 showed improved fine motor skills in the KF arm.

Conclusions: The exogenous KF appears safe. More data are needed to determine the utility of exogenous ketones as a nutritional approach in children with AS. This trial was registered at clinicaltrials.gov as NCT03644693. *J Nutr* 2021;151:3628–3636.

Keywords: Angelman syndrome, medical nutrition, pediatrics, seizure, ketogenic diet, ketosis

Introduction

Angelman syndrome (AS) was first described in 1965 (1) and has a prevalence in the population of ~1:10,000–1:24,000 (2–4). Loss of maternal ubiquitin protein ligase E3A (*UBE3A*) gene function was identified as the causative mechanism (5, 6). All patients with AS exhibit global developmental delays, including speech impairment and motor delays (7). Whereas 80% of patients have epilepsy, a disordered electroencephalogram (EEG) (8–10) is universally present. Many patients have feeding problems (75%) and other gastrointestinal (GI) complaints (7, 11). Individuals have a happy disposition with frequent laughter. Unfortunately, there is no cure for AS. Typical treatment protocols include pharmacotherapy for seizures and interventional therapies (12).

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Treatment-resistant seizures are prevalent in AS (8). Refractory epilepsy has been treated successfully with specialized dietary approaches, such as the ketogenic, medium-chain triglyceride (MCT)-based, and low glycemic index (LGIT) diets. These diets have also been successful in AS (13–16). The LGIT diet is successful for the treatment of seizures in AS, despite individuals on this diet lacking indications of nutritional ketosis when blood and urine ketones are monitored (13, 15). The ketogenic diet allows the body to shift from carbohydrate- to fat-based metabolism, thus entering nutritional ketosis. Nutritional ketosis is a state where the blood concentrations of ketone bodies are significantly above baseline, typically >0.5 mM. Ketone bodies [acetoacetate and β-hydroxybutyrate (β-OHB) measured from blood serum] are used as alternative fuels to glucose. In a previous study, researchers fed AS mice an exogenous ketone (R,S-1,3-butanediol acetoacetate diester) ad libitum for 8 wk and showed improved motor coordination, learning and memory, and synaptic plasticity (17).

The high rate of refractory seizures, feeding and GI problems, and severe communication impairments in AS all lead to challenges when developing diets to serve patients' complex needs. Data suggest dietary intervention to alter patient nutritional status could serve as an adjunct in the treatment of refractory seizures. Therefore, there is a significant unmet need for targeted nutritional support of patients with AS. Nutritional approaches that promote ketones as an alternative fuel may improve symptoms and allow for a liberalized diet in individuals with AS. Medical food formulations aim to improve a patient's nutritional status by supplementing deficiencies and enhancing the utilization of specific nutrients. This first-in-human trial assessed the use of an exogenous ketone formulation (KF) in individuals with AS, offering a potential approach for this population and others with neurodevelopmental disabilities and seizures.

Methods

Study population and setting

Healthy ambulatory individuals 4–11 y old with molecularly confirmed AS were recruited through the Multidisciplinary Angelman Clinic at the Monroe Carell Jr. Children's Hospital. Details of recruiting, setting, personnel, inclusion/exclusion criteria, sample size determination, and patient consent are found in an earlier publication (18). This study followed the CONSORT guidelines.

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Present address for JD: Section of Genetics & Inherited Metabolic Disease, Department of Pediatrics, Children's Hospital Colorado, University of Colorado Anschutz Medical Campus, 13123 E. 16th Ave., B065, Aurora, CO 80045.
Address correspondence to JD (e-mail: jessica.duis@childrenscolorado.org).
Abbreviations used: AS, Angelman syndrome; CAS, Callier-Azusa Scale; EEG, electroencephalogram; ERP, event-related potential; GI, gastrointestinal; IRB, Institutional Review Board; KF, ketone formulation; LGIT, low glycemic index therapy; MCT, medium-chain triglyceride; REM, rapid eye movement; TD, typically developing; β-OHB, β-hydroxybutyrate.

Study design

This study was a 16-wk, double-blind, placebo-controlled, crossover study to assess a ketogenic medical food formulation [hereafter, ketone formulation (KF)] in pediatric patients with AS. The study consisted of 4 × 4-wk epochs: baseline, followed by intervention period 1 consisting of consumption of placebo or KF, a 4-wk washout period, then intervention period 2, with KF subjects from intervention 1 crossed over to placebo and placebo subjects from intervention 1 crossed over to KF. After the baseline visit, patients were randomly assigned to consume flavored, powdered exogenous ketones (KF) or placebo 3 times daily with meals. The KF consisted of a total daily dose of 130 mg/kg of β-OHB (supplied as mineral salts) and 250 mg/kg of MCT. The placebo consisted of matched amounts of electrolytes. Randomization and blinding protocols were reported previously (18). Subjects were evaluated in the clinic 3 times, at baseline and after each of the 2 intervention periods. The clinical evaluations at each visit included neuropsychological questionnaires, dietary assessment, EEG, event-related potentials (ERPs), gait mat studies, and bloodwork. At home, families recorded daily nutritional intake, urine ketone concentrations, GI health, and seizure activity. Families used an at-home sleep monitor nightly throughout the study. Details and the full trial protocol were reported previously (18).

Outcome measures

The primary outcome measure was tolerability, demonstrated through patient compliance determined by the amount of nutritional formulation consumed compared with the amount prescribed. Secondary outcome measures assessed the formulation's suitability (convenience, taste, acceptability), nutrient intake, and degree of nutritional ketosis (urinary and serum). Primary safety endpoints were measured by monitoring the number of patients with adverse events and changes in laboratory parameters, anthropometrics, GI health, and seizure frequency. Additional safety-based measures included evaluation of mobility, cognition, cortical irritability, sleep, and adaptive function. These measures were assessed at each clinical visit with the exception of seizure counts, stool consistency, sleep, urinary ketones, and nutrient and formula intake, which were assessed daily.

Nutritional intake

Dietary intake was monitored throughout the protocol using the MyFitnessPal app (19). Families were encouraged to maintain their child's typical diet during the study. The data were exported to Microsoft Excel® for analysis using a deidentified username and password-protected Web application. Grams of protein, fat, and carbohydrate consumed each day were averaged to determine the macronutrient status during each epoch. In addition, a 24-h diet recall survey was conducted at each clinic visit. Compliance with consuming the study formulation 3 times/d was monitored through family interview.

Stool consistency

Stool consistency was scored daily for the duration of the study, when able, using an 11-point scale with anchors of 0 and 10, with 5 being considered normal.

Urine ketones

Urine ketones were recorded daily using Ketostix (Bayer) and documented via photos. For comparison of treatment epochs, the urine ketones from the last 7 d of the treatment epoch were averaged for each patient.

Laboratory values

At baseline and after both treatment epochs, metabolic parameters were obtained, including complete blood count, a complete metabolic panel, a lipid panel, and ketones. These were measured in-house using the Vanderbilt University Medical Center clinical laboratories.

EEG recordings

Awake EEGs were recorded for ~20 min in study patients at baseline and then at the end of each intervention period using a standard 10–20 montage. Data collection used a linked ear montage with filter settings as previously reported (20). Data were collected at 256 Hz and analyzed with a high-frequency filter of 70 Hz and low-frequency filter of 1.0 Hz. EEG spectra were generated using the spectral analysis tool integral to clinical NicVue 3.0.6 software. Spectra were acquired with a frequency resolution of 0.25 Hz and a spectral edge of 95% using a Hamming window. Frequency bands were defined as Δ 0.5–3.9 Hz, θ 4–7.9 Hz, α 8–12.9 Hz, and β 13–30 Hz. Three artifact-free 13-s EEG recording spans obtained during the awake state were identified manually by a board-certified epileptologist (RPC) blinded to treatment order. Spectra were generated from the O1 and O2 electrodes using both an average and a linked-ear montage, and power in the defined frequencies was determined. Relative Δ power was determined for each tracing and averaged to generate a single frequency value per patient per time point. Data from patients with AS were compared with a cohort of age-matched typically developing (TD) controls. Data from TD controls were collected under Vanderbilt Institutional Review Board (IRB) Protocol #200380.

ERPs

Study participants completed an auditory incidental memory ERP task at each of the 3 study visits (baseline and after the intervention periods) as an objective measure of cognition and to further assess the safety of the intervention (21–23). Data were acquired using 128-channel arrays (Electrical Geodesics, Inc.) at 250 Hz and analyzed using the procedures previously described (23). Statistical analyses focused on evaluating stimulus-related differences in ERPs within 250–500 ms and 500–800 ms after stimulus onset. The selected time windows correspond to the intervals where stimulus recall and familiarity effects have been reported for typical populations and for persons with AS.

Mobility assessment using Zeno Walkway

Gross motor capabilities as assessed by ambulation were measured using the Zeno Walkway system as previously reported (24). Subjects walked on a 20-ft pressure mat for ~20 passes at each clinical visit, including at baseline and after each of the intervention periods. Gait parameters were derived using a minimum of 4 complete passes and included step length, stride width, single and double support, the mean mediolateral cyclogram intersection point, stance center of pressure, velocity, and cadence.

Sleep assessment using EarlySense device

Sleep was monitored nightly throughout the protocol. Each patient was provided an EarlySense Monitor system, which has been validated to accurately measure sleep compared to the gold standard, polysomnography (25). The sensor is placed under the subject's mattress and captures data through sound waves while the subject is asleep, measuring heart rate, respiratory rate, and bed motion. The included EarlySense software uses these 3 data streams to stage sleep and calculate percentage time out of bed, time awake, rapid eye movement (REM) sleep, light sleep, deep sleep, and total time asleep (25, 26). Nocturnal awakenings were derived manually from hypnograms generated from the sleep staging data.

Cognition and motor skills

The Vineland-3^{TM} Parent/Caregiver Form (for ages 0 to $\geq$90 y) was utilized to assess adaptive and developmental skills. In parallel, the Callier-Azusa Scale (CAS) was used to assess motor function. Scaled scores were generated per guidelines for each test.

Adverse event monitoring

The study coordinator monitored all adverse events in real time and recorded the number of participants with adverse events and a description of events. Each family answered a closeout survey designed to elicit adverse event reporting.

Ethics

The procedures followed herein were conducted in accordance with the ethical standards of the Monroe Carell Jr. Children's Hospital at Vanderbilt University. The protocol was approved by the IRB at Vanderbilt University (IRB # 171969) and the University of Colorado (Protocol # 20-0366).

Statistics

This exploratory study was not powered on detecting a specific effect size for efficacy of the KF but to obtain preliminary data on feasibility and adaptability of the KF. We aimed to enroll 15 participants who completed the study. The study ended with 13 completers owing to the combination of early dropouts and the Covid-19 pandemic. Descriptive statistics, such as mean $\pm$ SD, are used to describe continuous variables, and frequency for the categorical variables. Data were graphed and statistics performed using GraphPad Prism. Normality of data was assessed visually using scatter plots. Means were compared using repeated-measures ANOVA followed by Tukey's multiple comparison test when $P < 0.05$. No data were imputed. The independent variables included 3 dummy variables for baseline, KF, and control treatment conditions, respectively. Statistical significance for all tests was defined by $P < 0.05$. Given the paucity of Vineland-3 data, a paired t test was used to compare either baseline or placebo with the KF epoch. When both baseline and placebo Vineland results were present, the higher of the 2 was used to generate the most conservative estimate of improvement.

Given the exploratory nature of the study, there is an increased risk of type 1 errors in our statistical analysis.

Results

Cohort characteristics

We recruited 26 patients with AS. Nineteen patients completed the baseline visit. Five patients withdrew after the baseline but before any intervention. One patient withdrew during the first intervention period because they were unable to tolerate the formula. A total of 13 patients completed the study. Demographics of the trial cohort are outlined in the CONSORT flow diagram in **Figure 1**. **Supplemental Tables 1** and **2** present clinical and developmental features of the cohort. A more detailed characterization of specific aspects of participants' development at baseline includes receptive language abilities (**Supplemental Table 3**), skills in use of augmentative communication (**Supplemental Table 4**), features of expressive language (**Supplemental Table 5**), gait and preferred activities (**Supplemental Table 6**), and behavioral features (**Supplemental Table 7**).

Tolerability

The nutritional formulation was well tolerated by 13 of 14 patients ($P < 0.1$). One patient refused to take the formulation and withdrew from the study. Thirteen completers consumed the formulation 3 times/d for 4 wk during the interventional period. Half of the families chose to continue using the formula after the conclusion of the study.

Dietary intake

There was variability in dietary background; most patients were consuming a standard American diet, 1 was classified as LGIT, and none were consuming a classic ketogenic diet (**Figure 2**A). Macronutrient values remained stable throughout the study with a slight, but significant, decrease in carbohydrate content between the baseline and KF intervention period. One patient demonstrated a marked increase in calories from protein during the KF phase, increasing from 14% at baseline to 30% during

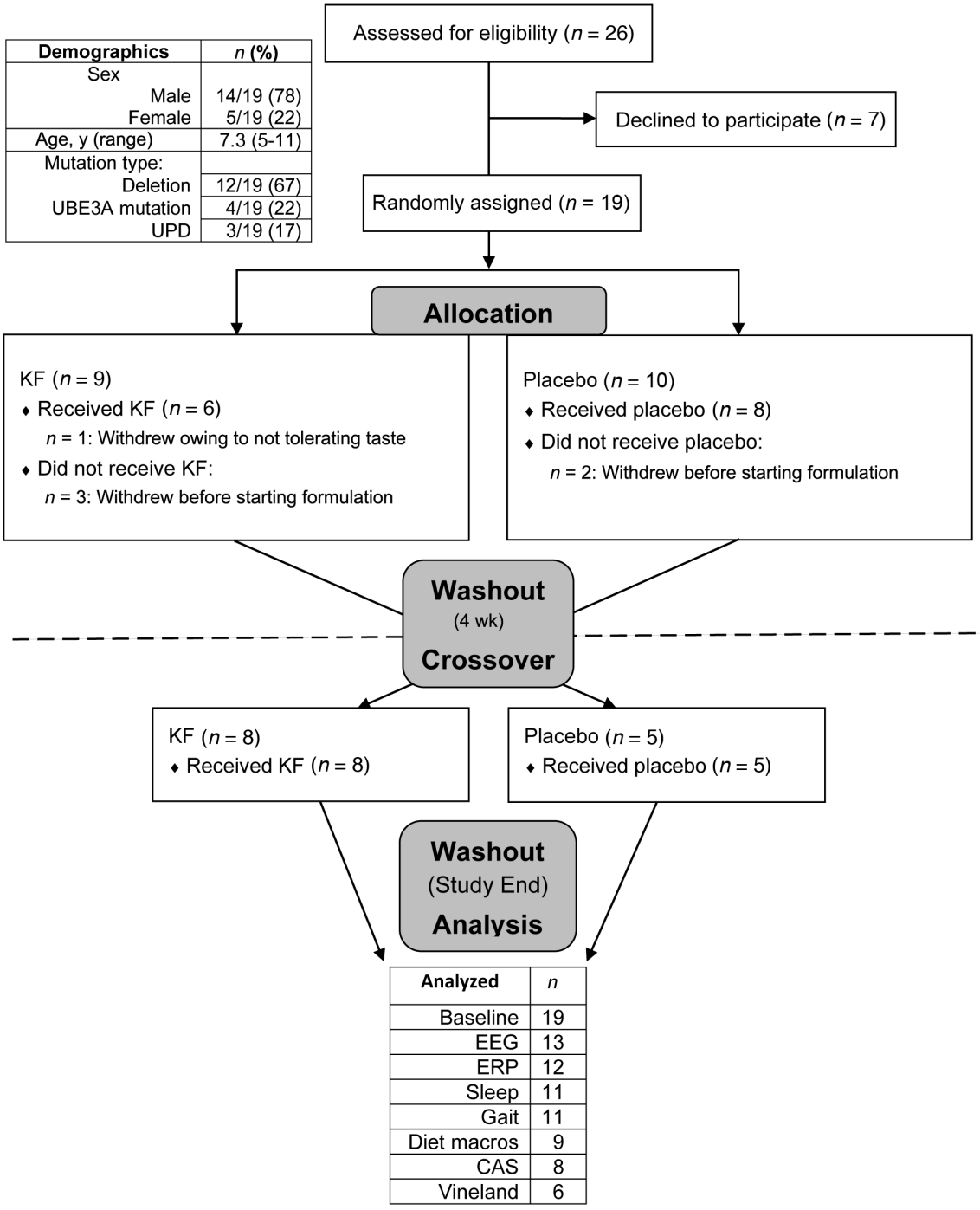

FIGURE 1 CONSORT diagram of patient flow from screening and random assignment to baseline assessment and intervention periods. Each patient received both KF and placebo, separated by a washout period. The 4 epochs of the study (baseline, intervention period 1, washout, intervention period 2) were 4 wk each. A final phone call occurred 2 wk after the last intervention period. Cohort demographics are shown in the top left, and the number of subjects with all 3 data points for each analysis is shown at the bottom. CAS, Callier-Azusa Scale; EEG, electroencephalogram; ERP, event-related potential; KF, ketone formulation; *UBE3A*, ubiquitin protein ligase E3A; UPD, uniparental disomy.

the KF phase. The values decreased to 22% during the placebo phase and 12% during the washout period.

GI health

Given concern for adverse GI effects of MCT oil (an ingredient in KF), the families monitored stools, appetite, and nausea. Twelve of 13 patients demonstrated decreased stool consistency (softening) during the KF period compared with baseline ($P < 0.05$) or placebo epochs ($P < 0.01$), with the 1 outlier showing decreased consistency during the baseline phase (Figure 2B). For most patients, appetite was stable during the study ($P = 0.0869$) (Figure 2C). We did not see a significant effect of KF on body weight (Figure 2D). Weight at baseline was significantly lower than during both the KF ($P < 0.01$) and placebo epochs ($P < 0.05$), a finding likely due to the natural trend to gain weight with time. Indeed, this result was driven by the group in which KF followed placebo. A trend toward decreased weight gain was seen in 7 of 12 patients during the

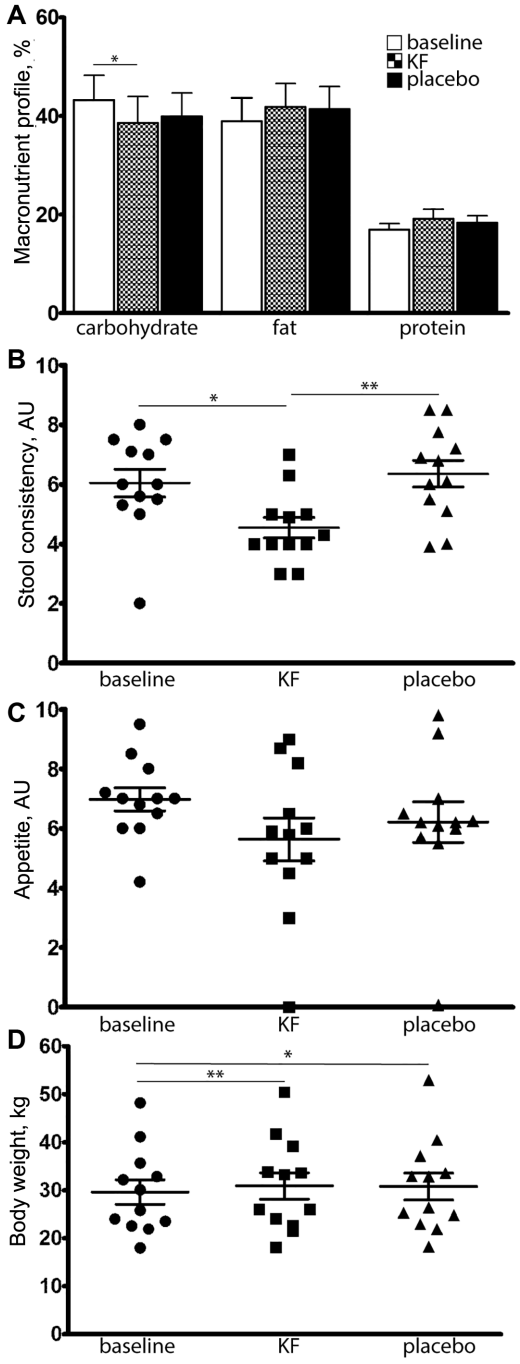

FIGURE 2 Changes in dietary macronutrient composition (A), stool consistency (B), appetite (C), and body weight (D) of Angelman syndrome patients treated with exogenous ketones. Results are presented with collective patient data for each epoch. (A) Dietary intake was recorded at home daily throughout the protocol, and macronutrient content was calculated as an average percentage of total daily calories for the given epoch. (B) Stool consistency and (C) appetite were reported daily in response to an email survey. For both surveys, a scale of 0–10, where 5 is normal, was used; 10 was the greatest appetite, and 10 was the hardest stool. (D) Body weight was recorded at each clinic visit. Where mean values are shown, error bars represent SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ by repeated-measures ANOVA. $n = 9$ for macronutrients, $n = 12$ for stool consistency and appetite. AU, arbitrary units; KF, ketone formulation.

KF epoch ($P > 0.1$), although only 1 patient demonstrated decreased weight during the study's entirety (−1.7% of baseline body weight).

Seizures

Seizures were uncommon, with only 4 patients reporting seizures during the baseline phase. Two patients had poorly controlled seizures (>60/mo). Of these, 1 subject had 89 seizures during the placebo phase and 69 during the KF phase. The second subject reported 1538 seizures during KF and 1767 during placebo.

Metabolic monitoring

No significant metabolic derangements were seen after consumption of KF or placebo. Bicarbonate concentrations measured from plasma remained >20 mmol/L for all patients, with no evidence of metabolic acidosis. Triglycerides and cholesterol, both measured from plasma, were also monitored. No significant treatment-related changes in total cholesterol were seen (Table 1). Triglycerides were not significantly increased across the group, but a single patient had a marked increase from 145 mg/dL to 348 mg/dL during the KF epoch. This returned to 130 mg/dL during the placebo epoch, which, for this patient, followed the KF epoch.

Evaluation of serum ketones demonstrated increased acetoacetate with KF in 2 patients, 1 of whom had detectable acetoacetate at baseline. In the remainder of the patients, acetoacetate was not detected. Similarly, no significant changes were seen in β-OHB concentrations, with the majority of serum concentrations <0.2 mmol/L. Two patients had slightly increased β-OHB concentrations with KF, although 1 demonstrated elevation at baseline. Paradoxically, 1 patient's β-OHB concentration was lower with KF than either at baseline or during placebo. When documented, urine ketones (acetoacetate) were mostly undetectable except for a single patient, who demonstrated urine ketones at baseline and throughout the study (data not shown).

EEGs

Data were collected at baseline and after each intervention period. Complete EEG data were obtained in 13 patients. Relative Δ frequency power from the occipital region during the waking state was significantly increased at baseline in AS patients compared with TD controls ($P < 0.0001$) (**Supplemental Figure 1**). The increased Δ power corresponded to a significant decrease in α frequency power in AS patients ($P < 0.0001$), consistent with a previous report (20). Likewise, the percentage of Δ power was lower in older patients than in younger patients ($P < 0.05$), whereas values were stable over time in TD children (20).

Comparison of Δ frequency power across intervention periods demonstrated a decrease of 5% during KF, although this was not statistically significant ($P = .1730$). To determine responsiveness on a patient-by-patient basis, we examined each patient separately. Seven of 13 patients demonstrated lower Δ frequency power during the KF period than during the baseline and placebo periods, with decreases ranging from 3% to 24% (Figure 3A). These data may suggest a trend towards normalization of Δ frequency power in a subset of patients consuming KF.

TABLE 1 Objective measures of metabolism, sleep, and mobility during baseline, treatment, and placebo epochs[1]

	Baseline	KF	Placebo	*P* value
Laboratory parameters				
Bicarbonate,[2] mmol/L	24.2 ± 2.57	23.8 ± 1.92	23.3 ± 2.25	0.52
Triglycerides,[2] mg/dL	84.5 ± 49.8	94.5 ± 81.9	75.6 ± 30.3	0.54
Total cholesterol,[2] mg/dL	156 ± 25.4	166 ± 22.7	162 ± 26.7	0.26
β-OHB,[3] mmol/L	0.19 ± 0.21	0.21 ± 0.20	0.16 ± 0.15	0.92
Albumin,[2] g/dL	4.33 ± 0.13	4.42 ± 0.16	4.29 ± 0.48	0.54
Sleep parameters				
REM sleep, %	7.45 ± 5.35	7.84 ± 7.38	6.44 ± 6.90	0.56
Light sleep, %	51.2 ± 9.37	47.5 ± 11.4	49.9 ± 7.84	0.47
Deep sleep, %	10.96 ± 6.52	11.7 ± 6.41	14.5 ± 12.9	0.52
Total sleep, min	346 ± 108	316 ± 91.9	324 ± 30.9	0.60
Sleep score, AU	58.6 ± 13.4	53.4 ± 16.7	56.0 ± 10.9	0.47
Sleep latency, min	40.5 ± 26.0	35.6 ± 32.1	33.2 ± 29.3	0.70
Time awake, min	138 ± 73.4	146 ± 83.0	128 ± 79.6	0.70
Time in bed, min	478 ± 155	448 ± 104	445 ± 93.5	0.70
Awakenings/h	2.78 ± 2.91	2.86 ± 2.05	2.42 ± 1.67	0.89
Gait parameters				
Step length, cm	41.2 ± 6.47	40.7 ± 10.2	40.3 ± 13.2	0.89
Stride width, cm	17.5 ± 4.81	18.2 ± 5.44	19.1 ± 6.27	0.47
Stride width SD, cm	3.80 ± 1.49	4.06 ± 1.85	3.65 ± 1.08	0.64
SS, %	37.3 ± 4.75	34.7 ± 4.40	33.95 ± 5.82	0.06
Double support, %	25.0 ± 9.43	31.1 ± 9.40	31.3 ± 9.86	0.04
SS COP distance, %	27.1 ± 10.2	20.5 ± 10.3	21.5 ± 12.1	0.08
Stance COP distance, cm	56.3 ± 13.3	55.7 ± 14.3	57.2 ± 17.2	0.87
Velocity, cm/s	105 ± 32.1	91.9 ± 31.4	92.9 ± 38.0	0.28
Cadence, steps/min	149 ± 29.2	133 ± 14.5	135 ± 19.3	0.05
Walk ratio	0.28 ± 0.04	0.30 ± 0.07	0.30 ± 0.09	0.50
eGVI	144 ± 6.79	147 ± 7.90	145 ± 12.2	0.64

[1] $n = 13$ for laboratory parameters, $n = 11$ for sleep and gait parameters. Values are mean ± SD unless indicated otherwise. Data within each group were compared with repeated-measures ANOVA at each time epoch. AU, arbitrary units; COP, center of pressure; eGVI, enhanced gait variability index; KF, ketone formulation; REM, rapid eye movement; SS, single support; β-OHB, β-hydroxybutyrate.
[2] Values were measured from serum.
[3] Values were measured from plasma.

ERPs

As an objective measure of cognition, ERP data were obtained using an auditory incidental memory task (23) during the baseline and intervention periods. The final ERP data set included 12 patients (1 patient was excluded owing to an insufficient amount of artifact-free data). Planned comparisons between baseline:placebo, baseline:KF, and KF:placebo visits consistently demonstrated that ERPs in response to the repeated stimuli were significantly different during the KF visit than those during baseline and placebo visits. There were no significant differences between baseline and placebo visits. The KF condition was associated with a change in the topographic distribution of the stimulus discrimination response, with loss of the parietal response and emergence of a frontal response driven by changes in the ERP amplitude for the repeated stimuli, although this was not statistically significant in the group analysis with the exception of the KF against placebo comparison of the 500- to 800-ms band (Figure 3B, C, **Supplemental Figure 2**).

Sleep

To determine the effect of the nutritional supplement on sleep, a remote sleep monitoring system was used. With KF, no significant changes in sleep latency, total sleep time, or sleep score were seen. During sleep, the percentage of time spent in light sleep, deep sleep, or REM sleep was not significantly changed with KF. The frequency of awakenings derived from hypnograms was not significantly changed by KF (Table 1).

Gait

Pressure mat recordings were collected at baseline and after the intervention periods. This system has previously been used to characterize gait in AS (24). Walkway data of adequate quality were obtained in 11 participants. No significant differences were seen in the KF group data when compared with baseline and placebo (Table 1).

Vineland Adaptive Behavior Scales-3

The Vineland-3™ Parent/Caregiver Form (for ages 0 to ≥90 y) was utilized to assess adaptive skills. Data were obtained during the KF period and either the baseline or placebo period in 6 individuals, but in only 2 patients for all 3 epochs. Given the paucity of data, we compared scores during the KF epoch with either baseline or placebo. In the 2 patients for whom both baseline and placebo data were obtained, we compared KF with the higher of the 2 scores to generate the most conservative estimate of change. Using this

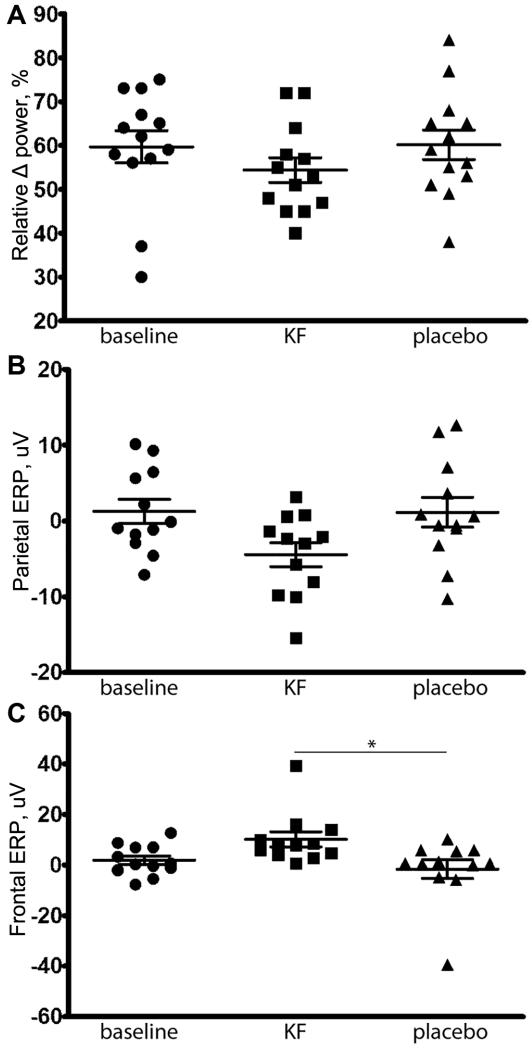

FIGURE 3 Characterization of brain cortical activity in Angelman syndrome patients treated with exogenous ketones. (A) Standard 20-lead awake EEGs were collected at each clinic visit, as were ERPs. Relative Δ power represents the power in the Δ frequency band as a percentage of total power. (B, C) ERP data were collected at each clinic visit using an auditory incidental memory task. Data are reported for both the (B) parietal and (C) frontal regions. The mean amplitude response during the indicated time frame poststimulus is shown for late segments. $^*P < 0.05$ by repeated-measures ANOVA. $n = 13$ for EEG, $n = 12$ for ERP. EEG, electroencephalogram; ERP, event-related potential; KF, ketone formulation.

approach, we were able to assess adaptive skills in 6 patients (Table 2). A significant group increase in fine motor skills was seen, with improvement in 5 of 6 patients with KF ($P = 0.04$). Although some patients demonstrated changes in scores in isolated domains, no significant group differences were seen elsewhere.

CAS

As an additional tool to assess motor function, the CAS was used to assess locomotion, visual motor skills, postural control, and development of fine motor skills. The form was completed by the caregiver. No significant changes were seen in the recorded domains during KF compared with baseline or placebo ($P > 0.1$) (Table 2).

Adverse events

Adverse events perhaps connected to the KF were changes in serum laboratory values. One patient had elevated triglycerides during the KF intervention. Separately, a different patient had depressed serum albumin concentrations during the placebo phase, which was the second intervention period for this patient. This patient continued to have hypoalbuminemia after the study's cessation despite termination of the formulation at study completion.

Discussion

We report a randomized, double-blind, placebo-controlled, crossover trial to assess the utility of exogenous ketones in patients with Angelman syndrome. Patients consumed a mixture of ketogenic ingredients including MCT and mineral salts of β-OHB. We hypothesized that shifting a patient's nutritional status toward ketone utilization would improve neurological features of AS even in the absence of metabolic signs of ketosis, consistent with the improvement in symptoms when individuals are consuming the LGIT diet. This study was a follow-up to preclinical work suggesting an exogenous ketone improved motor development, seizure activity, and learning in a mouse model of AS (17).

Our primary outcome was to assess the tolerability of the formula. It was not known if *1*) it was feasible to feed children with AS a flavored formulation 3 times/d for several weeks, *2*) a formulation high in minerals and fats would be well tolerated, or *3*) such a feeding protocol would be safe for patients with AS. Our primary endpoint was met. Individuals with AS accepted and tolerated the powder formulation. One patient withdrew from the study owing to refusal to consume the formulation. Adverse events that cannot be ruled out as related to the study formulation included a transient increase in triglycerides during the KF arm ($n = 1$) and a decrease in albumin during the placebo arm ($n = 1$). Multiple safety measures were characterized in this study. No negative effects were seen concerning cognition, seizure count, EEG, gait, mobility, sleep, GI health, or developmental stage.

A total of 13 participants completed the study; therefore, the study is underpowered to draw significant conclusions beyond the primary endpoint of safety and tolerability. Recruitment was ceased in early 2020 because of funding limitations and the implementation of Covid-19 restrictions. Each participant consumed both KF and placebo during separate intervention periods; therefore, future trajectory analysis combining natural history data and study data for these patients would help to understand the impact of nutritional ketosis.

Individuals consuming the KF had a consistent, gentle, and statistically significant improvement in constipation. Clinically, constipation can negatively affect seizures (27), behavior, and sleep. Families felt this was a considerable improvement despite remaining blind to the interventional and placebo periods. Approximately half of the completed patients remained on the formulation after trial completion. Decreased food-seeking behaviors were anecdotally reported as highly motivating for families who have had difficulty controlling their child's food intake to the detriment of their overall health.

We saw very little evidence of ketosis (elevated serum ketones) with KF, even though the children consumed ketones. Dietary background could affect metabolic response to exogenous ketones, with a more significant benefit of the study formulation in individuals on a LGIT background. Although

TABLE 2 Survey measures of cognitive and motor development during the baseline, treatment, and placebo epochs[1]

	Baseline/placebo[2]	KF	Placebo	*P* value
Vineland-3				
Receptive	4.71 ± 4.49	5.57 ± 4.43	—	0.17
Expressive	3.00 ± 3.46	3.14 ± 3.19	—	0.69
Written	3.43 ± 1.72	4.71 ± 2.75	—	0.23
Personal	5.14 ± 3.34	6.57 ± 5.09	—	0.28
Domestic	7.28 ± 2.06	8.28 ± 2.49	—	0.27
Community	4.57 ± 1.62	6.14 ± 2.48	—	0.13
Interpersonal	7.29 ± 1.38	8.57 ± 2.22	—	0.14
Play	7.71 ± 2.22	8.57 ± 2.94	—	0.31
Coping	7.43 ± 1.81	8.71 ± 3.35	—	0.23
Gross motor	7.33 ± 3.02	9.00 ± 3.03	—	0.12
Fine motor	4.17 ± 3.31*	6.33 ± 3.33*	—	0.04
CAS				
Postural control	13.4 ± 2.07	12.4 ± 3.11	14.4 ± 2.44	0.13
Locomotion	13.1 ± 2.36	12.6 ± 2.07	13.1 ± 1.25	0.67
Fine motor	13.9 ± 2.42	13.8 ± 1.98	15.1 ± 2.17	0.11
Visual motor	9.63 ± 1.60	10.5 ± 2.51	11.0 ± 2.39	0.12

[1] $n = 6$ for Vineland-3 data, $n = 8$ for CAS data. Values are mean ± SD unless indicated otherwise. Vineland-3 data were compared using a paired *t* test. CAS data were compared with repeated-measures ANOVA. *$P < 0.05$ using paired *t* test for Vineland-3 data. CAS, Callier-Azusa Scale; KF, ketone formulation.

[2] Values are baseline/placebo for Vineland-3 data, baseline for CAS data.

we enrolled patients of all dietary backgrounds, many of our patients, including those reporting a standard American diet, did have diets with relatively high amounts of fat. The diet's high fat content affected both fasting blood concentrations of β-OHB and urine ketone concentrations in the baseline period. The dietary background's overall contribution was difficult to assess owing to the small number of participants in this study classified as consuming LGIT or ketogenic diets. The absence of ketones in serum or in the urine may suggest that the dose of exogenous ketones in this initial trial was too low. However, this may also be complicated by the short half-life of β-OHB, the times when samples were taken, and the individual's fed compared with fasted state when consuming the ketones (28). Individuals consuming an LGIT diet have demonstrated improved seizure control despite the absence of ketones in serum and urine in individuals adherent to the diet (13–15), suggesting that KF may not have precise biomarkers to suggest a metabolic transition. Additional study is indicated in this area.

Owing to the combination of a nonverbal patient population and poor insight into appropriate objective measures for clinical trials in AS, we collected many measurements beyond the standard safety laboratory measurements. There was no indication that the intervention was harmful to the patients and it may have provided benefit in some cases. Seven of 13 patients demonstrated a decrease in Δ frequency power on the EEG. To our knowledge, this trial is the first to see a change in Δ power during an intervention. Although there was a statistically significant topographical change in ERP responses during KF, the overall clinical significance is difficult to assess in the absence of clear objective improvements in neuropsychological measures. To fully interpret the significance of the EEG and ERP changes, a more extensive and prolonged study focused on correlation to objective neuropsychological measures and clinically relevant improvements will be required (29).

Regarding motor ability, more knowledge is needed concerning the natural history of gait, because our experience suggests such knowledge is essential to treating AS (J Duis, A Skinner, A Tagawa, R Carson, F Phibbs, A Gouelle, D Eggenspieler, M Annoussamy, L Moore, J Silverman, S Petkova, S Apkon, L Servais, J Carollo, unpublished results, 2021; manuscript in review) (30). Although the number of participants in this study with a full data set was small, an improvement in fine motor skills was noted in the KF phase. This suggests that consuming exogenous ketones as a dietary approach may affect neural and adaptive functioning, but further study is needed to draw specific conclusions.

Limitations to our study include the overall duration of the study. Ketogenic diet therapy for epilepsy is often trialed for ≤6 mo before final decisions on efficacy are made. Cognitive benefits may require neuronal network alterations which may evolve slowly and which may have not been evident over 4 wk. Similarly, we cannot exclude potential adverse effects which may not have been evident within the 4-wk treatment window. Participant attrition and missing data are limitations in this study. The study's time commitments (e.g., daily reports), challenges of travel with AS children, and technical difficulties with the study equipment for in-home monitoring increased the burden on families, resulting in a significant withdrawal rate postconsent and before baseline, contributing to an incomplete data set. Such challenges with AS children, some of which had refractory seizures, were likely overwhelming. Although it may appear counterintuitive, the use of electronic tablets for monitoring was, in many ways, more cumbersome than requesting a paper-based diary. This experience reinforces that we must consider the needs of the AS community when designing future trials with the intent to limit burden and challenges to compliance. Identification of focused primary outcomes for future studies will aid in achieving this goal.

In conclusion, this study of exogenous ketones in AS demonstrates that the formula is well tolerated in children with AS. The ketogenic formulation positively affected constipation, a common ailment in AS that, when present, may correlate with the severity of other features of AS, including seizure burden, behavior, and sleep quality. Although no significant adverse

effects were seen in this study, a larger and longer randomized placebo-controlled trial of exogenous ketones is needed, both to confirm safety and to determine efficacy, perhaps with a specific focus on AS patients with drug-resistant epilepsy. This study has informed the dosing approach and reinforces the need for clear and focused clinical endpoints in future studies.

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Data availability

Data described in the article will be made available upon request pending approval through the Critical Path Institute (https://c-path.org/).

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