Neurobiology of Disease 96 (2016) 38–46

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Ketone ester supplementation attenuates seizure activity, and improves behavior and hippocampal synaptic plasticity in an Angelman syndrome mouse model

Stephanie L. Ciarlone [a,b], Joseph C. Grieco [a,b], Dominic P. D'Agostino [b], Edwin J. Weeber [a,b,*]

[a] USF Health Byrd Alzheimer's Institute, Tampa, FL 33613, United States
[b] Department of Molecular Pharmacology and Physiology, University of South Florida, Tampa, FL 33620, United States

ARTICLE INFO

Article history:
Received 3 May 2016
Revised 28 July 2016
Accepted 16 August 2016
Available online 18 August 2016

Keywords:
Angelman syndrome
Seizure
Ketone ester
Motor coordination
Metabolism
Synaptic plasticity

ABSTRACT

Angelman syndrome (AS) is a rare genetic and neurological disorder presenting with seizures, developmental delay, ataxia, and lack of speech. Previous studies have indicated that oxidative stress-dependent metabolic dysfunction may underlie the phenotypic deficits reported in the AS mouse model. While the ketogenic diet (KD) has been used to protect against oxidative stress and has successfully treated refractory epilepsy in AS case studies, issues arise due to its strict adherence requirements, in addition to selective eating habits and weight issues reported in patients with AS. We hypothesized that ketone ester supplementation would mimic the KD as an anticonvulsant and improve the behavioral and synaptic plasticity deficits in vivo. AS mice were supplemented *R*, *S*-1,3-butanediol acetoacetate diester (KE) ad libitum for eight weeks. KE administration improved motor coordination, learning and memory, and synaptic plasticity in AS mice. The KE was also anticonvulsant and altered brain amino acid metabolism in AS treated animals. Our findings suggest that KE supplementation produces sustained ketosis and ameliorates many phenotypes in the AS mouse model, and should be investigated further for future clinical use.

1. Introduction

Angelman syndrome (AS) is a devastating neurological disorder with a prevalence of 1 in 15,000 that currently has no treatment (Williams et al., 2010). AS often presents with ataxia, frequent smiling and laughter, lack of speech, and severe, debilitating seizures (Valente et al., 2006; Pelc et al., 2008; Williams et al., 2010). It is estimated that approximately 80% of individuals with AS have epilepsy, with difficulty in controlling seizures being a primary reason for patient hospitalization (Valente et al., 2006; Pelc et al., 2008; Thibert et al., 2009). Epilepsy in AS is often refractory to many prescribed medications, and frequently involves many seizure types (Valente et al., 2006; Pelc et al., 2008; Thibert et al., 2009). Approximately 70% of AS cases involve deletion within 15q11.2-q13.1 and generally exhibit increased frequency and severity of seizure. The larger deletions include the gene encoding the $GABA_A$ receptor β3 subunit, leading to cortical hyperexcitability and seizure activity. Importantly, chronic, intractable epilepsy has been shown to cause hippocampal damage and is associated with cognitive decline (Helmstaedter et al., 2003). Effective anti-epileptic drugs (AEDs) are limited in AS, and those medications are generally known to have side effects that can alter cognition (Drane and Meador, 1996; Campos-Castello, 2006; Mula and Trimble, 2009). Therefore, it is crucial to find or develop novel therapeutics to treat this aspect of the disorder.

Recent findings demonstrate an overall decrease in cortical and cerebellar inhibition in AS mice, and dietary therapies may help overcome this imbalance and affect neuronal excitability (Egawa et al., 2012; Thibert et al., 2012; Wallace et al., 2012). Both the ketogenic diet (KD), a high fat, low carbohydrate, moderate protein diet, and the low-glycemic index treatment (LGIT), a high fat diet with limited carbohydrates, are described as well-tolerated and successful in case reports involving patients with AS (Valente et al., 2006; Evangeliou et al., 2010; Stein et al., 2010; Thibert et al., 2012). Additionally, the KD has been shown to stimulate mitochondrial biogenesis, which can improve some of the hippocampal deficits in AS mice (Bough et al., 2006; Su et al., 2011), and enhances motor performance in neurological and neurodegenerative disorders (Friedman et al., 2006; Mantis et al., 2009; Beckett et al., 2013; Brownlow et al., 2013). However, non-pharmacological management is rarely considered and little data has been published on dietary therapies in AS (Pelc et al., 2008; Thibert et al., 2009), limiting additional information regarding efficacy of the KD in the overall AS population. Additionally, investigation of the KD in AS

* Corresponding author at: Byrd Alzheimer's Institute, 4001 East Fletcher Avenue, Tampa, FL 33613, United States.
E-mail address: eweeber@health.usf.edu (E.J. Weeber).
Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2016.08.002

specifically is hampered by individuals having selective eating habits, weight loss, and difficulties gaining weight (Clayton-Smith, 1993; Clarke and Marston, 2000).

Oral administration of ketone esters, which mimic the KD and are precursors to ketone bodies, may help circumvent Angelman syndrome-specific issues, as they have been shown to significantly elevate blood ketones in rats (D'Agostino et al., 2013) independent of carbohydrate restriction (Kesl et al., 2016). Preliminary work also suggests ketone esters elevate blood ketones and are generally safe and well-tolerated in healthy human patients (Clarke et al., 2012; Kemper et al., 2015). A number of hypotheses have sought to isolate the neuroprotective and anticonvulsant mechanism(s) underlying ketosis, including a metabolic shift of [GABA/glutamate], resulting in increased tonic inhibition (Bough and Rho, 2007; Yudkoff et al., 2007). Therefore, the use of therapeutics that increase the GABA/glutamate ratio may serve to dampen overall neuronal excitability in various areas of the brain, resulting in decreased seizure activity.

In this study, we evaluated the potential of a ketone ester (KE), *R,S*-1,3-butanediol acetoacetate diester (BD-$AcAc_2$), to induce therapeutic ketosis in an AS mouse model and act as an anticonvulsant. Additionally, we examined the effects of the ketone ester on behavioral and metabolic outcomes in our mouse model. We hypothesized that supplementation of BD-$AcAc_2$ with a standard diet would mimic results of the KD as an anticonvulsant and a treatment for the cognitive and motor dysfunction reported in AS.

2. Materials and methods

2.1. Animals

UBE3A$^{tm1Alb/J}$ null mutation (AS) mice, described previously (Jiang et al., 1998) were purchased from the Jackson Laboratory. Wild-type (WT) and AS mice were obtained through breeding of heterozygous female mice with WT males to produce maternally-deficient AS offspring and age-matched, wild-type littermate controls. Animals were housed with a standard 12 h light/dark cycle and supplied with food and water ad libitum at the University of South Florida, and were housed in groups of three to four per cage. Experiments were performed on 12–14 week-old male and female mice. All animal testing procedures and care followed the NIH guidelines and were approved by the University of South Florida's Institutional Animal Care and Use Committee (Approval ID number A4100-01).

2.2. Ketone ester administration

4–6 week-old experimental and control mice were fasted for 8 h prior to initial ketone ester administration and study initiation to ensure rapid feeding compliance and to establish a similar metabolic starting point as previously described (Poff et al., 2014). Control mice were fed standard rodent chow (Teklad 2018) ad libitum. Mice receiving the ketone supplement were administered BD-$AcAc_2$ with their standard rodent chow ad libitum. These mice received standard rodent chow mixed at 10% BD-$AcAc_2$ by volume and 1% saccharin for palatability (Sigma-Aldrich) as previously described (Poff et al., 2014), which prevented food aversion observed in initial pilot studies. The KE was synthesized in collaboration with Savind, Seymour IL, as previously described (D'Agostino et al., 2013). Diets were continuously monitored and replaced twice weekly or as needed to maintain freshness for 8 weeks.

2.3. Blood and weight measurements

Blood was collected once weekly from the tail using approved methods. Behavioral testing was not performed on these days, and food availability was limited for 4 h and returned for 2 h prior to blood collection. Blood glucose and β-hydroxybutyrate (BHB) were measured with the Precision Xtra™ Blood Glucose & Ketone Monitoring System (Abbott Laboratories). Mice were weighed twice weekly for the duration of the study and were removed from experimentation if >20% of their initial body weight was lost. For measurement of plasma ketones, blood samples (200 μl) were collected into heparinized Eppendorf tubes. Samples were processed for the detection and quantification of BHB and acetoacetate (AcAc) at Case Western Reserve University, Mouse Metabolic Phenotyping Center. Blood samples were chilled on ice for 30 s and centrifuged in a microcentrifuge (13,000*g*) for 3–5 min. Plasma was removed and immediately frozen on dry ice before being stored at −80 °C. Samples were stored at −80 °C until analyzed for ketones. Internal standards of [$^2H_6$]BHB or [$^2H_8$]isopropanol were added to the treated plasma samples (50 μl) and the BHB and AcAc (as M + 1 of BHB) were analyzed by gas chromatography-mass spectrometry (GC-MS) using an Agilent 5973 mass spectrometer, linked to a 6890 gas chromatograph equipped with an autosampler. Briefly, GC-MS conditions were either electron ionization (EI) mode or chemical ionization (CI) mode; the samples were detected by selected ion monitoring as the BHB- and AcAc-trimethylsilyl derivatives (EI).

2.4. Behavioral testing

Open field behavior was assessed to determine general locomotor activity and anxiety. Mice were placed in an acrylic chamber (40 cm × 40 cm × 27 cm) and were allowed to explore for 15 min. ANY-Maze animal activity system (Stoelting Co.) was used to monitor movement and distance traveled.

Elevated plus maze was used to assess anxiety levels in the mice. The EPM consisted of four arms: two (30 cm × 5 cm) open, well-lit arms and two (30 cm × 5 cm × 15 cm) enclosed arms facing each other. Each arm attached to a common open square center platform (4.5 cm). Mice were placed in the center platform and allowed to explore for 5 min. A digital camera (XV-BP330, Panasonic) was used to monitor activity, and ANY-Maze animal activity system (Stoelting Co.) was used to record and analyze behavior. Total time spent in open arms versus closed arms was measured, and anxiety levels were assessed by comparing percent time spent in the open arms.

Rotarod was used to assess motor coordination, motor learning and stamina. Mice were placed on a 3 cm diameter rod with an initial rotation of 4 rpm and accelerated to 40 rpm over a maximum of 5 min (Ugo Basile, Italy). Mice were tested for latency to fall off the rod for four trials per day for two consecutive days.

Wire hang test was used to measure subacute muscle function and fatigue. A horizontal wire (2 mm in diameter, 40 cm in length) was suspended above a padded table. The animal was allowed to cling to the middle of the wire with its forepaws for one 60 s trial, and latency to fall was recorded.

Hindlimb clasping is used as a marker for neurological dysfunction, including certain ataxias. The clasping test evaluated the animal's hindlimb response during tail suspension 10 cm above their home cage. If the hindlimbs were consistently splayed outward, away from the abdomen, the mouse was assigned a score of 0. If one hindlimb was retracted toward the abdomen, the animal received a score of 1. If both hindlimbs were partially retracted toward the abdomen, it received a score of 2. If the animal's hindlimbs were entirely retracted and touching the abdomen it received a score of 3.

Fear conditioning was used to assess hippocampal function and memory formation. Mice were placed in a 25 cm × 25 cm sound attenuation chamber with a wire grid flooring. Mice were allowed to explore the context for 3 min before they received the conditioned stimulus (CS, 90 db tone) for 30 s. At the end of the 30 s, mice received a mild foot shock (0.5 mA, unconditioned stimulus, US). After 1.5 min, the mice received a second CS/US pairing and monitoring continued for 1.5 min after food shock administration. 24 h following CS/US presentation, mice were placed back into the chamber and allowed to explore for

3 min. Learning was assessed by measuring freezing behavior consisting of lack of motion for at least 2 consecutive *sec*.

Novel object recognition was used to evaluate recognition memory. Each mouse was habituated in the test arena (40 cm × 40 cm) for 10 min. 24 h following habituation, two identical objects similar in size to the mouse were placed along the center line of the arena approximately 3–5 cm from the outside wall, and mice were allowed to explore for 10 min. 24 h following training, a novel object replaced one of the familiar objects presented during the training session, and mice were allowed to explore for 5 min. Animals were monitored and behavior was quantified by video tracking (ANY-Maze, Stoelting, IL).

2.5. Audiogenic seizures

For audiogenic seizure testing, a separate cohort of mice were habituated to a sound attenuation chamber for 60 s and exposed to sound stimulation (115 dB) for 60 s or until tonic or clonic episodes occurred. An occurrence of sound-induced seizure was defined as tonic, clonic, or tonic-clonic seizures during sound stimulation. Animals were tested only once. Seizure testing was carried out between 1:00 PM and 6:00 PM to limit effects of diurnal variation on results.

2.6. Kainic acid injections

Seizures were induced in a separate cohort of mice by intraperitoneal injection of kainic acid (KA) at 20 mg/kg. Following injection, animals were returned to cages where seizure severity was assessed at 5-min intervals for up to 50 min according to a modified Racine's scale (Dunleavy et al., 2013).

2.7. Extracellular recordings

Following behavioral testing, a cohort of mice was euthanized and the hippocampi dissected out to be used in hippocampal LTP experimentation as previously described (Trotter et al., 2013). The brain was rapidly dissected and placed in ice-cold, oxygenated cutting solution containing (in mM): 110 sucrose, 60 NaCl, 3 KCl, 28 $NaHCO_3$, 1.25 NaH_2PO_4, 5 glucose, 0.6 ascorbate, 7 $MgCl_2$, and 0.5 $CaCl_2$. Hippocampal slices (400 μm) were prepared on a vibratome and allowed to equilibrate in a 50% cutting saline and 50% artificial cerebrospinal fluid solution containing (in mM): 125 NaCl, 2.5 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 25 glucose, 1 $MgCl_2$, and 2 $CaCl_2$. Slices were maintained in this solution with constant 95% O_2/5% CO_2 perfusion for 10 min before being transferred to the brain slice recording chamber supported by nylon mesh or maintained in a holding container. Slices were recovered for a minimum of 1 h before recording. The recording chamber was held at 30° ± 0.5 °C with a ACSF flow rate of 1 ml/min. Field EPSPs (fEPSPs) were recorded from stratum radiatum in hippocampal area CA1 via glass microelectrodes filled with artificial cerebrospinal fluid (resistance 1–4 mΩ). Responses were generated by stimulation of Schaffer collaterals arising from the CA3 region. Stimulating electrodes consisted of formvar-coated nichrome wire, which was used to deliver biphasic stimulus pulses (1–15 V, 100 μs duration, 0.05 Hz). Delivery of stimulation, controlled by pClamp 9.0 software (Molecular Devices), was via the Digidata 1322 A interface (Molecular Devices) and a stimulus isolator (model 2200; A-M Systems). Signals were amplified using a differential amplifier (model 1800; A-M Systems), filtered at 1 kHz, and digitized at 10 kHz. For all experiments, baseline stimulus intensity was set at the level that elicited ~50% of the maximum fEPSP response as determined from the input–output curve. The input–output relationship was determined by stimulating slices from 0 to 15 mV at 0.5 mV increments. Short-term plasticity was measured via paired-pulse facilitation (PPF), which was induced by stimulating slices at half-max intensity with sequential pulses spaced at 20 ms intervals from 20 to 300 ms. LTP was induced by a theta-burst protocol, which consisted of five trains of four pulse bursts at 200 Hz separated by 200 ms, repeated six times with an intertrain interval of 10 s. For analysis, the last 10 min of recording was averaged and compared.

2.8. Western blot analysis

Whole hippocampal brain tissue from male mice was lysed on ice in lysis buffer (radio-immunoprecipitation assay buffer supplemented with protease/phosphatase inhibitor cocktail, Thermo Scientific). Protein concentrations were determined using the BCA Protein Assay Kit (Thermo Scientific). Equal amounts of protein from each sample were loaded for SDS-PAGE, and transferred to a PVDF transfer membrane. The membranes were blocked in 0.1 M TBS with 0.1% Tween 20 and 5% nonfat milk, then incubated overnight at 4 °C with primary antibodies anti-E6AP (#A300-352A, Bethyl Laboratories, Inc.), anti-GAD65/67 (#AB1511, Millipore), and anti-β-actin (#4967L, Cell Signaling Technology) diluted in blocking solution. Membranes were washed and incubated with anti-rabbit IgG-HRP secondary antibody (Southern Biotech) diluted at 1:2000 in blocking solution. Proteins were detected using Pierce ECL Western Blotting Substrate (Thermo Scientific) and audioradiography. The films were digitized and optical densities were measured using a high-powered scanner and the software program ImageJ (v1.46r, National Institutes of Health).

2.9. Metabolic analysis of brain amino acids

GABA and glutamate (Glu) were measured in brain homogenate at Case Western Reserve University, Mouse Metabolic Phenotyping Center. This approach enabled metabolites to be measured with a high degree of sensitivity (Yang et al., 2008; Kombu et al., 2011; Zhang et al., 2015).

2.9.1. Analytical methods

Brains were dissected immediately, frozen in liquid nitrogen within 30 s of removal and stored at −80 °C. Hippocampal sections (25–30 mg tissue) were then dissected under frozen conditions in dry ice (−80 °C). For the isolation of metabolic intermediates the frozen tissue samples were then homogenized using an organic solvent mixture containing 5% acetic acid and methanol (1:1; methanol to water) (Zhang et al., 2015). Briefly, frozen samples were spiked with internal standards (0.1 μmol each): [$^2H_6$]GABA and [$^{13}C_5$]glutamate, and then homogenized with 3 ml of cold methanol-H_20 solvent mixture (1:1, v/v) using a Polytron homogenizer.

2.9.2. GC-MS assays

Following homogenization, the homogenates were extracted using a mixture of acetonitrile and 2-propanol (3:1), vortexed and centrifuged for 30 min at 2500 RCF at 4 °C. Extracts were then dried by nitrogen gas for 0.5 h or until completely dry and then chemically derivatized using MTBSTFA + 1% TBDMCS reagent (*N*-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide + 1% tert-butyldimetheylchlorosilane, Regis Technologies, Inc. Morton Grove, IL, USA) and reacted at 70 °C for 30 min. The derivatized products were measured under Agilent 6890 Gas-Chromatography and Agilent 5973 Mass Spectrometry (GC-MS). A DB-17 MS capillary column (30 m × 0.25 mm × 0.25 μm) was used in all analysis. The starting oven temperature was set to 80 °C, the pressure was 14.82 psi, and the flow velocity was 45 cm/s. Temperature was then increased linearly to 220 °C and held for 1 min. The mass spectrometer was in electron-impact (EI), sim mode. Ions for glutamate (m/z = 432) and GABA (m/z = 274) were monitored and data acquisition collected and stored for further analysis.

2.10. Statistical analysis

All data is represented as the mean ± SEM. To compute *p* values, data was analyzed using Student's *t*-test and two-way ANOVA with

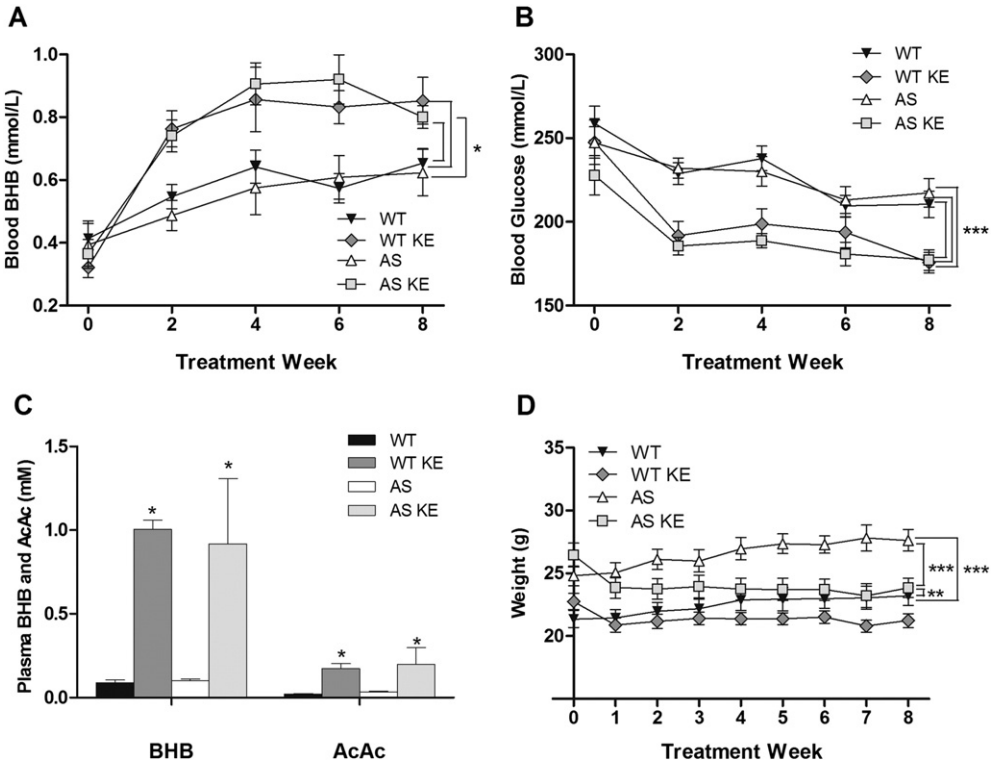

Fig. 1. *R,S*-1,3-butanediol acetoacetate diester (BD-AcAc$_2$) induces ketosis, lowers glucose, and normalizes body weight in WT and AS mice. (A) BD-AcAc$_2$ elevated whole blood β-hydroxybutyrate (BHB) in treated WT and AS animals compared to controls. (B) WT and AS treated mice demonstrated decreased whole blood glucose compared to controls following 8 weeks of ketone ester supplementation. (C) Both plasma BHB and acetoacetate (AcAc) levels were significantly elevated in treated compared to non-treated WT and AS animals (n = 4/group). (D) AS mice demonstrated a significant increase in body weight while AS KE animals had a significant normalization in body weight that was sustained throughout the duration of the study (WT and AS controls: n = 15; WT KE: n = 16; AS KE: n = 20; $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$).

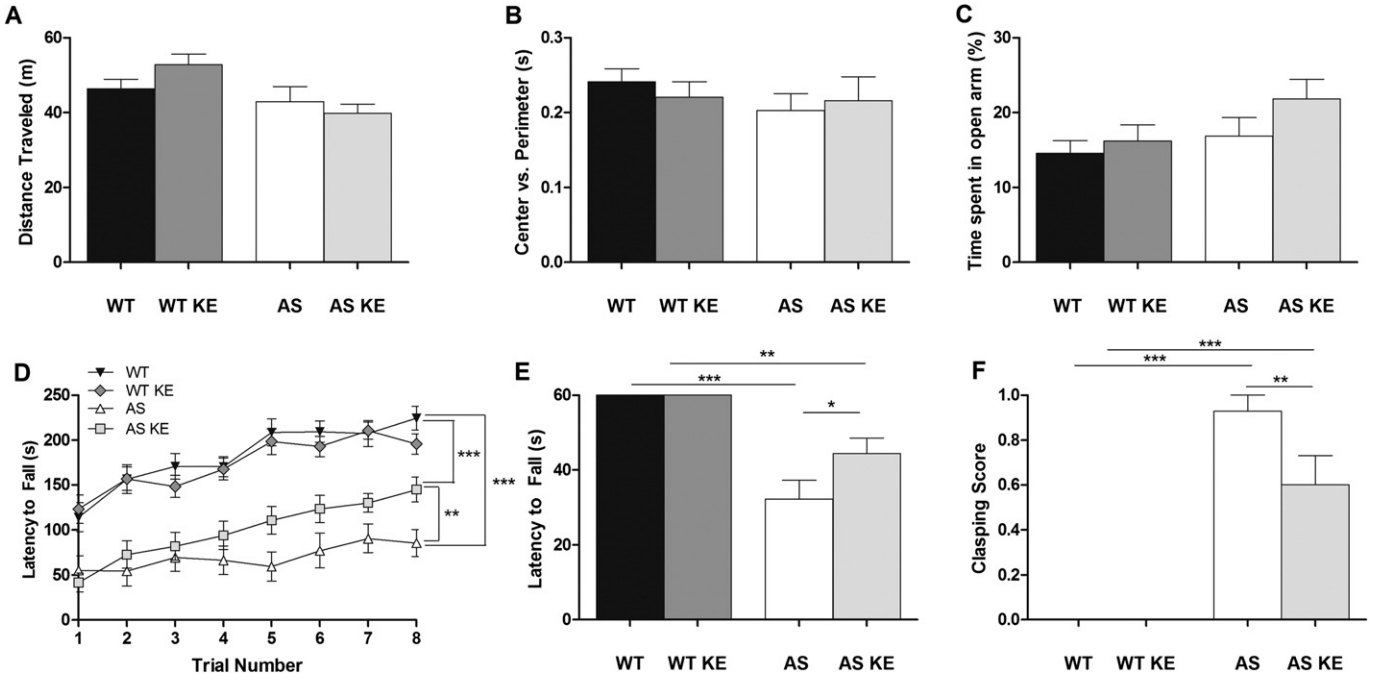

Fig. 2. Ketone supplementation improves motor coordination but does not affect general locomotor activity or anxiety levels in AS mice. (A) Open field: distance traveled. Following 8 weeks of ketone ester supplementation, mice underwent open-field testing as a locomotor and general anxiety control for behavioral testing. Data represent the overall distance traveled in the open field. There were no significant differences between experimental groups. (B) Open field: time spent in center vs. perimeter. Data represents the ratio of time spent (*sec*) in the open field vs. the perimeter of the field, with no significant differences between groups. (C) Elevated plus maze: anxiety levels in WT and AS mice are unaffected with ketone ester supplementation. Data represent percentage of total time spent in open arms of the elevated maze. There were no significant differences between experimental groups. (D) Average latency to fall on the accelerating rotarod was significantly reduced in AS control animals, while ketone ester supplementation significantly enhanced motor performance in AS treated mice. (E) AS mice performed poorly on the wire hang task compared to WT controls, while AS KE animals demonstrate a significant increase in the latency to fall (WT and AS controls: n = 18; WT KE: n = 19; AS KE: n = 20). (F) Severity of the hindlimb clasping score was significantly decreased in AS KE-treated mice (WT and AS controls: n = 14; WT KE and AS KE: n = 15; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

genotype and treatment as main factors followed by Bonferroni post hoc tests, set at a significance of $p < 0.05$ (GraphPad Prism software). Statistical outliers that fell outside two standard deviations of the mean were excluded from analysis. Data is also reported from both male and female mice (Supplementary Table 1).

3. Results

3.1. Ketone supplementation lowered blood glucose, elevated blood ketones, and normalized body weight in AS mice

Whole blood ketone (BHB) and glucose measurements were similar at baseline, and chronic ketone supplementation resulted in elevated ketones and lowered blood glucose in WT KE and AS KE treated mice (Fig. 1A and B, repeated measures ANOVA, $p < 0.01$ and $p < 0.0001$; WT vs. WT KE, WT vs. AS KE, and AS vs. AS KE $p < 0.05$ and $p < 0.001$, respectively). BD-AcAc_2 also significantly increased plasma BHB (Fig. 1C, ANOVA $p < 0.05$, $F_{(3,13)} = 9.156$; WT vs. WT KE and AS vs. AS KE $p < 0.05$) and AcAc (Fig. 1C, ANOVA $p < 0.01$, $F_{(3,13)} = 9.038$; WT vs. WT KE and AS vs. AS KE $p < 0.05$) in WT KE and AS KE compared to control animals. Standard high-carbohydrate rodent chow with ketone supplementation also significantly decreased body weight in AS mice during the two-month KE treatment, which did not differ significantly from WT control body weights by the end of the study (Fig. 1D, repeated measures ANOVA, $p < 0.0001$; WT vs. AS and AS vs. AS KE $p < 0.001$, WT vs. AS KE $p < 0.01$).

3.2. Ketone supplementation had no effect on general locomotor activity or anxiety

General locomotion and anxiety were examined in BD-AcAc_2 fed mice, in addition to WT and standard diet (SD) control animals. There were no significant alterations in general locomotor activity and anxiety behavior in animals fed the ketone ester diet, as measured by the open field test (Fig. 2A and B) and elevated plus maze (Fig. 2C).

3.3. Administration of a ketone ester improved motor coordination, learning, and overall neurologic function

AS mice display abnormalities in gait, motor learning, and motor coordination, as evidenced by increased hind stride length and base width and deficits in rotarod, wire hang, and paw abduction tests (Jiang et al., 1998; Van Woerden et al., 2007; Heck et al., 2008; Egawa et al., 2012; Meng et al., 2013). Following two months of BD-AcAc_2 administration, AS KE mice demonstrated significant improvements in rotarod performance compared to AS controls (Fig. 2D, repeated measures ANOVA $p < 0.0001$; AS vs. AS KE $p < 0.01$; WT vs. AS and AS KE $p < 0.001$). AS mice displayed significant deficits in the wire hang task, while AS KE diet-fed mice showed improvement in wire hang endurance, although not to WT levels (Fig. 2E). A two-way ANOVA revealed a significant effect of genotype ($F_{(1,65)} = 43.43$, $p < 0.0001$; interaction of group and treatment: $p = 0.0667$; Bonferroni post-hoc tests: WT vs. AS $p < 0.001$; AS vs. AS KE $p < 0.05$; WT and WT KE vs. AS KE = $p < 0.01$). All WT and WT KE mice were able to hang for the maximum time of 60 s, while 17.6% of AS and 38.8% of AS KE mice reached the maximum trial duration. As previously reported, AS animals also demonstrated a significant hindlimb clasping phenotype compared to WT controls (Egawa et al., 2012), while AS KE-fed mice showed significant improvement (Fig. 2F). A two-way ANOVA revealed a significant effect of genotype ($F_{(1,51)} = 90.13$, $p < 0.0001$) and treatment ($F_{(1,51)} = 4.16$, $p < 0.05$) with a significant interaction of group and treatment ($p < 0.05$) (Bonferroni post-hoc tests: WT vs. AS $p < 0.001$; AS vs. AS KE $p < 0.01$; WT and WT KE vs. AS KE $p < 0.001$).

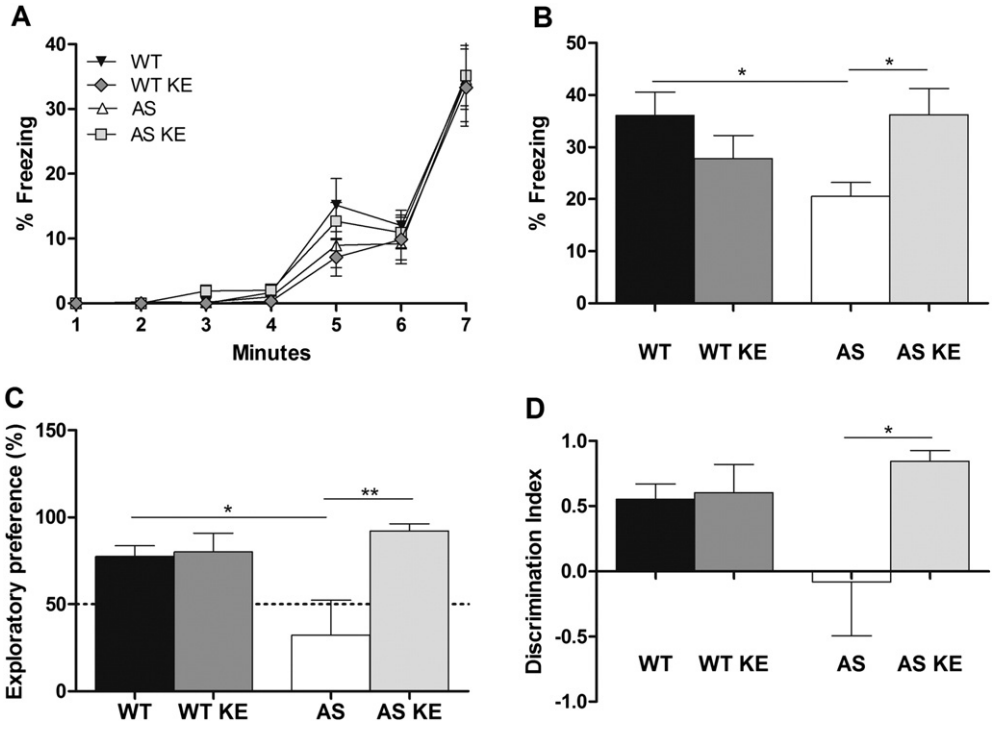

Fig. 3. BD-AcAc_2 recovers associative learning and recognition memory deficits observed in AS mice. AS mice were trained with a standard 2-shock contextual fear conditioning protocol following 8 weeks of treatment. (A) There were no significant differences in freezing between experimental groups during training. (B) Contextual fear conditioning was assessed 24 h post-training. BD-AcAc_2 administration increased the average context-dependent freezing in AS KE animals compared to nontreated AS mice (WT controls: n = 16; AS controls and AS KE: n = 18; WT KE: n = 10; $^*p < 0.05$). (C) Effect of BD-AcAc_2 on recognition memory in AS mice in the novel object recognition test. Exploratory preference 24 h following training; the dotted line at 50% indicates equal preference for both familiar and novel object, indicative of visual memory impairment. (D) Effect of BD-AcAc_2 on the discrimination index post-treatment. AS KE-treated mice demonstrated a significant increase in the discrimination index compared to AS controls (WT controls: n = 17; WT KE: n = 11; AS controls: n = 5; AS KE: n = 9; $^*p < 0.05$, $^{**}p < 0.01$).

3.4. Ketone supplementation improves associative learning and recognition memory in AS mice

During training, all animals showed similar levels of freezing after presentation of the US (Fig. 3A). Changes in fear memory to the cue were not altered in WT KE, AS KE, or control animals (data not shown). AS KE mice had a significant enhancement of associative memory similar to WT controls as demonstrated by increased freezing behavior. A two-way ANOVA revealed a significant interaction of group and treatment (Fig. 3B, $F_{(1,51)} = 6.98$, $p < 0.05$; Bonferroni post-hoc tests: WT vs. AS $p < 0.05$, AS vs. AS KE $p < 0.05$).

AS mice demonstrated impaired exploratory preference for the novel object and KE treatment in AS mice reversed the exploratory preference for the novel object to virtually the same level as WT mice (Fig. 3C). A two-way ANOVA revealed a significant effect of treatment ($F_{(1,34)} = 9.67$, $p < 0.01$) and significant interaction of genotype and treatment ($F_{(1,34)} = 8.11$, $p < 0.01$). (Bonferroni post-hoc tests: WT vs. AS $p < 0.05$, AS vs. AS KE $p < 0.01$). The ability to discriminate between the familiar and novel object was calculated as the discrimination ratio [(time spent exploring novel object – time spent exploring familiar object)/(total time spent exploring both objects)] (Fig. 3D). A two-way ANOVA revealed a significant effect of treatment ($F_{(1,35)} = 5.90$, $p < 0.05$) and significant interaction of genotype and treatment ($F_{(1,35)} = 4.70$, $p < 0.05$). Post hoc tests revealed that while there was no significant difference between WT and AS controls, KE treatment significantly increased the discrimination ratio in AS mice (ANOVA, $p < 0.05$ $F_{(3,38)} = 2.697$; AS vs. AS KE $p < 0.05$).

3.5. BD-AcAc$_2$ decreases audiogenic and chemically-induced seizure activity in AS mice

Following audiogenic stimulation, we observed seizures in 84% of the AS mice, whereas no seizures were observed in WT animals (data not shown). AS KE animals demonstrated a 48% reduction in seizure activity as compared to AS controls (Fig. 4A, $p < 0.05$ Fisher's exact test), as well as a significant increase in latency to seize (Fig. 4B, $p < 0.05$). A significant decrease in behavioral seizure score was recorded 10 and 45 min post-kainic acid injection in AS KE vs AS control animals (Fig. 4C, Two-way repeated measures ANOVA, Bonferroni post-hoc tests, $p < 0.01$). There was also a significant increase in the latency to a seizure score of 2 post-injection (Fig. 4D, $p < 0.05$), suggesting an overall initial delay in seizure severity.

3.6. Ketone ester supplementation in AS mice results in improvements in early phase LTP

Using a TBS-LTP protocol, the extent of LTP, calculated by averaging the slope values of fEPSPs recorded between 50 and 60 min after TBS, was significantly lower in slices from AS mice (113.2 ± 0.7) than those from WT animals (165.7 ± 0.73) (Fig. 5C and D, ANOVA, Bonferroni post-hoc tests, $p < 0.001$). Ketone ester supplementation partially rescued impairment of LTP in area CA1 of AS KE mice (Fig. 5C and D, 128.5 ± 0.88, $p < 0.001$) compared to AS mice on the standard diet, although not to the extent of WT animals. There were no recorded changes in basal synaptic transmission (input-output relationship,

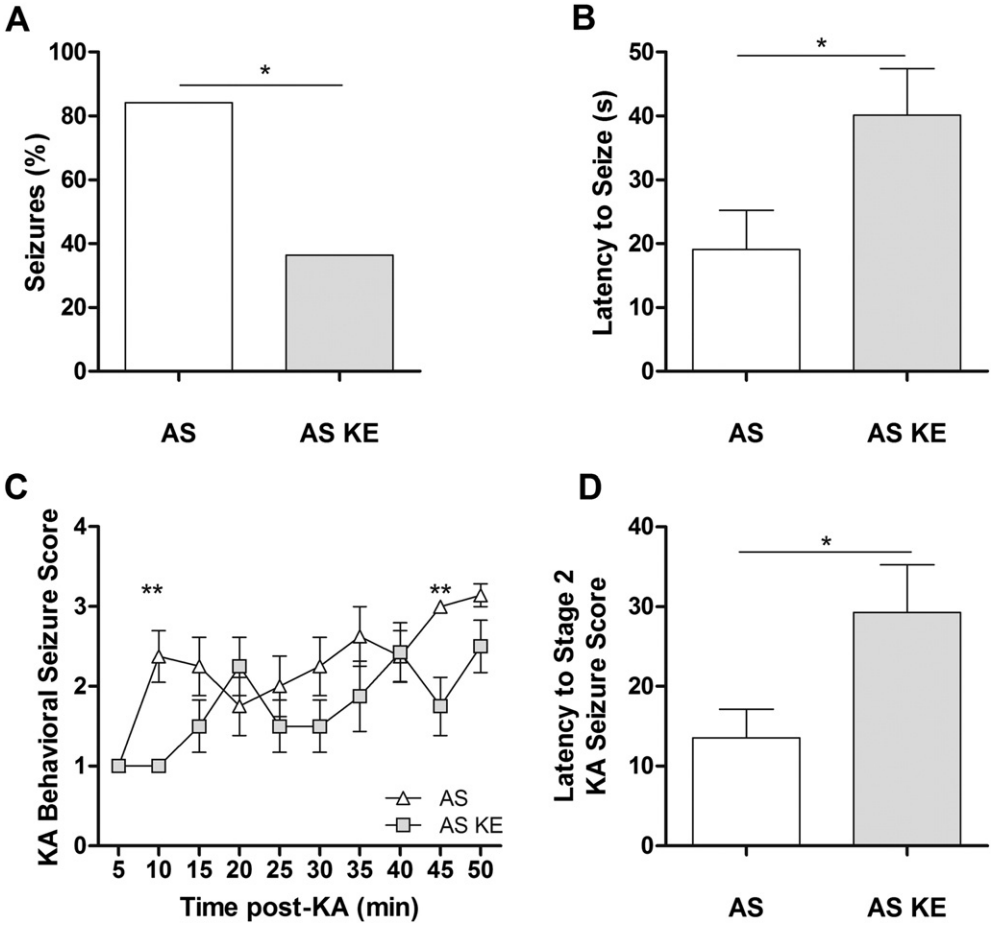

Fig. 4. Ketone ester supplementation attenuated audiogenic and kainic acid-induced seizure activity. (A) Percentage of AS KE and control animals that demonstrated behavioral seizure activity following a 115 dB sound stimulation. (B) Latency to seizure following audiogenic stimulation was significantly increased in AS KE mice (AS controls: n = 11; AS KE: n = 13; $^*p < 0.05$). (C) Kainic acid behavioral seizure scores in AS treated and nontreated animals. Scores were tabulated every 5 min (AS controls: n = 8; AS KE: n = 9; $^*p < 0.01$). (D) Latency to behavioral seizure score of 2 was significantly increased in AS KE mice (AS controls: n = 8; AS KE: n = 9; $^*p < 0.05$).

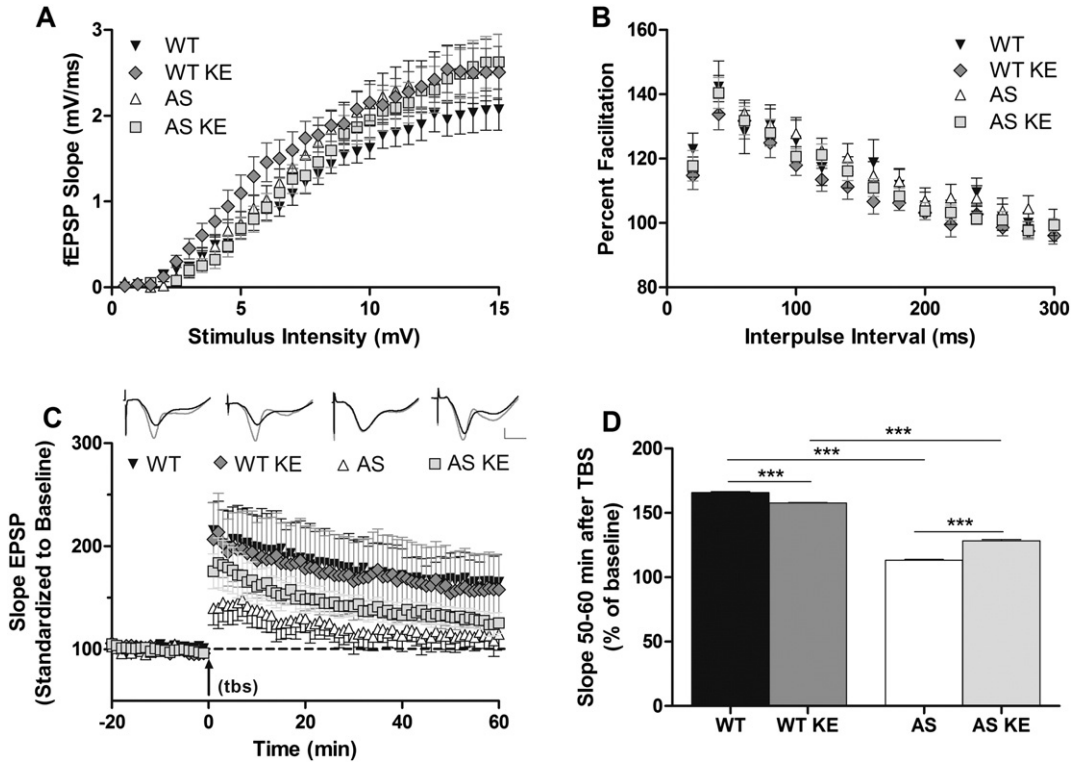

Fig. 5. AS animals given oral ketone ester supplementation show increased LTP induction without changes in synaptic transmission. (A) Normal input-output curve at hippocampal SC-CA1 synapses in WT, WT KE, AS, and AS KE-treated mice. (B) Short-term synaptic plasticity was evaluated by the amount of PPF with IPIs ranging from 20 to 300 ms. There were no significant differences between experimental groups. (C) Long-term potentiation induced by 5 trains of theta-burst stimulation (arrow). Representative traces are shown for all groups at baseline (black trace) and 50 min after tetanic stimulation (grey trace). Scale bar = 1 mV and 5 ms. (D) LTP induction calculated between 50 and 60 min after TBS. Data expressed as mean ± SEM. (WT = 15 slices, n = 4 mice; WT KE = 14 slices, n = 4 mice; AS = 20 slices, n = 5 mice; AS KE = 19 slices, n = 5 mice; $p < 0.001$).

Fig. 5A) or short-term synaptic plasticity (paired-pulse facilitation, Fig. 5B).

3.7. Alterations in hippocampal GAD65 and GAD67 expression and GABA/Glutamate ratio following ketone ester administration

Hippocampal protein expression of GAD65 and GAD67 was significantly decreased in AS mice compared to WT controls by 32.8% and 49.1%, respectively (Fig. 6A and B). There were no significant changes in GAD65/67 in WT KE mice compared to WT controls. AS KE-fed mice displayed significant increases in both hippocampal GAD65 (two-way ANOVA, $p < 0.01$ $F_{(1,26)} = 11.89$; WT vs. AS $p < 0.01$, AS vs. AS KE $p < 0.001$) and GAD67 (two-way ANOVA, $p < 0.01$ $F_{(1,26)} = 10.59$; WT vs. AS $p < 0.05$, AS vs. AS KE $p < 0.01$), comparable to WT levels. Feeding a ketone ester diet to AS mice also resulted in a significant increase in the GABA/glutamate ratio when compared to standard diet-fed AS animals, similar to WT levels (Fig. 6C, two-way ANOVA, $p < 0.05$ $F_{(1,13)} = 11.55$; WT vs. AS $p < 0.05$, AS vs. AS KE $p < 0.01$).

4. Discussion

Approximately 80% of children with AS have epilepsy, with ~77% of those individuals remaining refractory to AEDs (Thibert et al., 2009). These patients are at a high risk of early death due to seizures, and many suffer considerable side effects from AEDs. Accordingly, examination of alternative therapies should be prioritized. In the present study, we examined the use of a KE to induce therapeutic ketosis and improve behavioral phenotypes in the AS mouse model. The major findings of this study demonstrate ketosis induced by dietary KE administration, rather than strict adherence to a ketogenic diet, is anticonvulsant and improves motor function. Surprisingly, we find the KE also improves

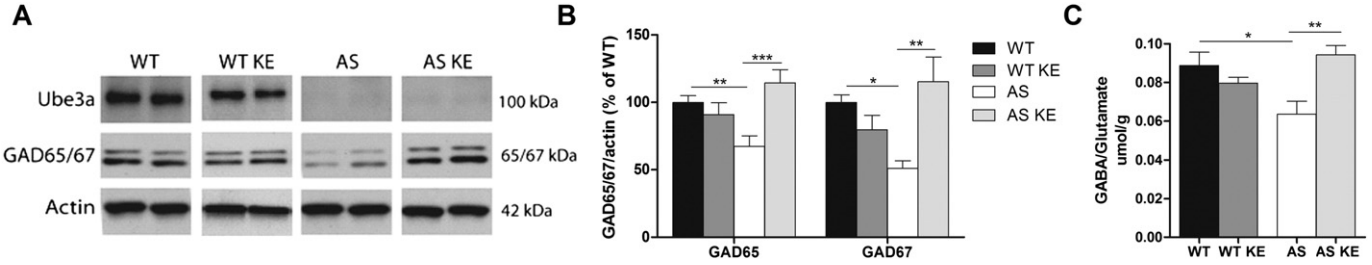

Fig. 6. Increased GAD65/67 and GABA/glutamate ratio in the AS mouse hippocampus following ketone ester administration. (A) Expression of GAD65 and GAD67 in the hippocampus were evaluated in WT, WT KE, AS, and AS KE mice by Western blotting (representative blot shown). (B) Densitometric quantification of Western blots in (A) was performed as described in Experimental Procedures (n = 9 WT and AS KE, n = 8 AS, n = 4 WT KE). Expression of GAD65 and GAD67 was significantly reduced in AS mice compared to WT controls and AS KE animals. (C) Brain amino acids GABA and glutamate were measured. The GABA/glutamate ratio is significantly decreased in AS mice compared to WT controls and AS treated mice ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

recognition memory, associative learning, and enhances hippocampal synaptic plasticity in AS mice.

Chronic ketone supplementation decreased blood glucose and body weight and sustained ketosis in our AS treated mice, as evidenced by significantly increased plasma and whole blood ketones. In addition, eight weeks of KE administration was sufficient to normalize body weight in AS mice via chronic ketosis. The ability of the KE to decrease blood glucose has been reported previously (Kashiwaya et al., 2010; Poff et al., 2014). It has also been established that ketogenic diets can cause weight loss in overweight humans (Astrup et al., 2000; Volek et al., 2004), and these adult AS mice are significantly overweight compared to WT controls. KEs can have an appetite suppressing effect via an increase in the anorexigenic metabolite malonyl-CoA, which may decrease food intake and, in turn, decrease blood glucose and body weight (Kashiwaya et al., 2010). It is also important to note that the mice in this study were fed ad libitum, therefore glucose and ketone measurements could be affected by variable feeding behavior prior to weekly glucose monitoring. Any of these factors could have an effect on the decreased blood glucose and body weight seen in the AS KE treated animals.

In our study, KE-fed AS mice presented an overall improved behavioral phenotype that correlates to an equally significant improvement in hippocampal synaptic function. In support of our findings, several studies have reported similar effects of ketosis on motor and cognitive performance in various rodent models including models of aging, Alzheimer's disease (AD), traumatic brain injury, and amyotrophic lateral sclerosis (Appelberg et al., 2009; Xu et al., 2010; Beckett et al., 2013; Brownlow et al., 2013; Kashiwaya et al., 2013; Ari et al., 2014). Furthermore, mitochondrial dysfunction has also been reported in the AS mouse model, demonstrated by impaired mitochondrial structure and a partial oxidative phosphorylation defect, resulting in increased oxidative stress (Su et al., 2011; Llewellyn et al., 2015). Several reports of oxidative stress have also been linked to memory deficits in rodents (Fukui et al., 2001; Silva et al., 2004), and ketones can induce synaptic protection and prevent oxidative impairment of hippocampal LTP (Maalouf and Rho, 2008; Abdelwahab et al., 2015). The data suggest that incorporating ketones as alternative fuel substrates into the diet may bypass potential mitochondrial deficiencies and protect against oxidative stress, ameliorating some of the behavioral and altered synaptic phenotypes in the AS mouse model.

KE supplementation produced anticonvulsant effects in AS mice in both audiogenic- and chemically-induced paradigms, affecting inhibition. However, future studies should extend seizure monitoring following kainic acid injections in order to record potential differences in later seizure stages. Both synaptic GAD65 and cytosolic GAD67 are responsible for GABA synthesis, and AS mice hippocampi displayed significant decreases in GAD65/67 compared to WT, suggesting altered brain amino acid metabolism. AS KE mice demonstrated increased protein expression of both enzymes in the hippocampus, indicating a significant alteration in GAD activity. In order to explore the potential metabolic alterations further, we measured GABA and glutamate concentrations in the hippocampi of AS KE and control mice, and examined the GABA/Glu ratio as an indicator of neurotransmitter turnover. The GABA/Glu ratio was significantly increased in AS KE mice, suggesting the KE has a significant impact on brain amino acid metabolism, and is likely affecting neuronal inhibition.

Alterations in GAD, GABA, and neuronal inhibition do not solely have an effect on seizure activity. Excitatory and inhibitory imbalances have been reported in the AS mouse brain, which could affect synaptic function, sensory detection and integration, and result in impaired learning and memory (Wallace et al., 2012), although recent findings suggest GABAergic *Ube3a* loss specifically underlies the circuit hyperexcitability in AS (Judson et al., 2016). Decreased hippocampal GABA resulting from increases in GAT1, a GABA transporter, impairs learning and memory in mice (Hu et al., 2004), and increased GAT1 has been measured in the AS mouse cerebellum (Egawa et al., 2012). Learning triggers a rapid increase in GABA content (Jasinska et al., 2010), leading to increased GABA released from hippocampal GABAergic interneurons (Nitz and McNaughton, 2004; Cui et al., 2008). Increased inhibitory synaptic plasticity and GABA release may therefore be essential for learning and memory tasks (Andrews-Zwilling et al., 2012). It has been suggested that treatments that alter GABA, GABAergic interneuron function, or GABA/glutamate ratio may be beneficial for improving cognition and synaptic plasticity in disorders such as AD (Andrews-Zwilling et al., 2010; Andrews-Zwilling et al., 2012) and AS (Egawa et al., 2012; Ciarlone and Weeber, 2015).

The present findings strongly suggest that KE supplementation in addition to a standard diet induces therapeutic ketosis in AS and may be a promising mitigation strategy for many of the devastating phenotypes of the disorder, including seizures, motor difficulties, and severe developmental delay. With limited treatment options available for human AS, it is important to examine this therapeutic option for seizure control and for increased cognitive acuity. Future studies will seek to determine its safety and efficacy for potential future clinical trials.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nbd.2016.08.002.

Funding Sources

This work was supported by Foundation for Angelman Syndrome Therapeutics, Grant Number: 6143108900.

Disclosure

SLC, DPD and EJW are named inventors on issued and pending U.S. Patent Applications directed toward similar subject matter that has been assigned to the University of South Florida and is subject to the rules and regulations of the University.

Acknowledgements

The authors thank Dr. Michelle Puchowicz at the Case Western Reserve University Mouse Metabolic Phenotyping Center for assisting with the whole blood ketone and brain amino acid measurements.

References

Abdelwahab, M.G., Lee, S.H., O'Neill, D., Thompson, R.J., Duff, H.J., Sullivan, P.G., Rho, J.M., 2015. Ketones prevent oxidative impairment of hippocampal synaptic integrity through K ATP channels. PLoS One 10, e0119316.

Andrews-Zwilling, Y., Bien-Ly, N., Xu, Q., Li, G., Bernardo, A., Yoon, S.Y., Zwilling, D., Yan, T.X., Chen, L., Huang, Y., 2010. Apolipoprotein E4 causes age-and tau-dependent impairment of GABAergic interneurons, leading to learning and memory deficits in mice. J. Neurosci. 30, 13707–13717.

Andrews-Zwilling, Y., Gillespie, A.K., Kravitz, A.V., Nelson, A.B., Devidze, N., Lo, I., Yoon, S.Y., Bien-Ly, N., Ring, K., Zwilling, D., 2012. Hilar GABAergic interneuron activity controls spatial learning and memory retrieval. PLoS One 7, e40555.

Appelberg, K.S., Hovda, D.A., Prins, M.L., 2009. The effects of a ketogenic diet on behavioral outcome after controlled cortical impact injury in the juvenile and adult rat. J. Neurotrauma 26, 497–506.

Ari, C., Poff, A.M., Held, H.E., Landon, C.S., Goldhagen, C.R., Mavromates, N., D'Agostino, D.P., 2014. Metabolic therapy with Deanna protocol supplementation delays disease progression and extends survival in amyotrophic lateral sclerosis (ALS) mouse model. PLoS One 9, e103526.

Astrup, A., Ryan, L., Grunwald, G.K., Storgaard, M., Saris, W., Melanson, E., Hill, J.O., 2000. The role of dietary fat in body fatness: evidence from a preliminary meta-analysis of ad libitum low-fat dietary intervention studies. Br. J. Nutr. 83, S25–S32.

Beckett, T.L., Studzinski, C.M., Keller, J.N., Murphy, M.P., Niedowicz, D.M., 2013. A ketogenic diet improves motor performance but does not affect β-amyloid levels in a mouse model of Alzheimer's disease. Brain Res. 1505, 61–67.

Bough, K.J., Rho, J.M., 2007. Anticonvulsant mechanisms of the ketogenic diet. Epilepsia 48, 43–58.

Bough, K.J., Wetherington, J., Hassel, B., Pare, J.F., Gawryluk, J.W., Greene, J.G., Shaw, R., Smith, Y., Geiger, J.D., Dingledine, R.J., 2006. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. Ann. Neurol. 60, 223–235.

Brownlow, M.L., Benner, L., D'Agostino, D., Gordon, M.N., Morgan, D., 2013. Ketogenic diet improves motor performance but not cognition in two mouse models of Alzheimer's pathology. PLoS One 8, e75713.

Campos-Castello, J., 2006. The neuropsychology of epilepsy: what factors are involved? Rev. Neurol. 43, S59–S70.

Ciarlone, S.L., Weeber, E.J., 2015. Towards Targeted Therapy for Angelman Syndrome. Expert Opinion on Orphan Drugs.
Clarke, D.J., Marston, G., 2000. Problem behaviors associated with 15q-Angelman syndrome. Am. J. Ment. Retard. 105, 25–31.
Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., King, M.T., Musa-Veloso, K., Ho, M., Roberts, A., Robertson, J., VanItallie, T.B., 2012. Kinetics, safety and tolerability of (*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate in healthy adult subjects. Regul. Toxicol. Pharmacol. 63, 401–408.
Clayton-Smith, J., 1993. Clinical research on Angelman syndrome in the United Kingdom: observations on 82 affected individuals. Am. J. Med. Genet. 46, 12–15.
Cui, Y., Costa, R.M., Murphy, G.G., Elgersma, Y., Zhu, Y., Gutmann, D.H., Parada, L.F., Mody, I., Silva, A.J., 2008. Neurofibromin regulation of ERK signaling modulates GABA release and learning. Cell 135, 549–560.
D'Agostino, D.P., Pilla, R., Held, H.E., Landon, C.S., Puchowicz, M., Brunengraber, H., Ari, C., Arnold, P., Dean, J.B., 2013. Therapeutic ketosis with ketone ester delays central nervous system oxygen toxicity seizures in rats. Am. J. Phys. Regul. Integr. Comp. Phys. 304, R829–R836.
Drane, D., Meador, K., 1996. Epilepsy, anticonvulsant drugs and cognition. Baillieres Clin. Neurol. 5, 877–885.
Dunleavy, M., Provenzano, G., Henshall, D.C., Bozzi, Y., 2013. Kainic acid-induced seizures modulate Akt (SER473) phosphorylation in the hippocampus of dopamine D2 receptor knockout mice. J. Mol. Neurosci. 49, 202–210.
Egawa, K., Kitagawa, K., Inoue, K., Takayama, M., Takayama, C., Saitoh, S., Kishino, T., Kitagawa, M., Fukuda, A., 2012. Decreased tonic inhibition in cerebellar granule cells causes motor dysfunction in a mouse model of Angelman syndrome. Sci. Transl. Med. 4, 163ra157.
Evangeliou, A., Doulioglou, V., Haidopoulou, K., Aptouramani, M., Spilioti, M., Varlamis, G., 2010. Ketogenic diet in a patient with Angelman syndrome. Pediatr. Int. 52, 831–834.
Friedman, J.R., Thiele, E.A., Wang, D., Levine, K.B., Cloherty, E.K., Pfeifer, H.H., De Vivo, D.C., Carruthers, A., Natowicz, M.R., 2006. Atypical GLUT1 deficiency with prominent movement disorder responsive to ketogenic diet. Mov. Disord. 21, 241–244.
Fukui, K., Onodera, K., Shinkai, T., Suzuki, S., Urano, S., 2001. Impairment of learning and memory in rats caused by oxidative stress and aging, and changes in antioxidative defense systems. Ann. N. Y. Acad. Sci. 928, 168–175.
Heck, D.H., Zhao, Y., Roy, S., LeDoux, M.S., Reiter, L.T., 2008. Analysis of cerebellar function in Ube3a-deficient mice reveals novel genotype-specific behaviors. Hum. Mol. Genet. 17, 2181–2189.
Helmstaedter, C., Kurthen, M., Lux, S., Reuber, M., Elger, C.E., 2003. Chronic epilepsy and cognition: a longitudinal study in temporal lobe epilepsy. Ann. Neurol. 54, 425–432.
Hu, J.-H., Ma, Y.-H., Jiang, J., Yang, N., S-h, D., Jiang, Z.-H., Mei, Z.-T., Fei, J., Guo, L.-H., 2004. Cognitive impairment in mice over-expressing γ-aminobutyric acid transporter I (GAT1). Neuroreport 15, 9–12.
Jasinska, M., Siucinska, E., Cybulska-Klosowicz, A., Pyza, E., Furness, D.N., Kossut, M., Glazewski, S., 2010. Rapid, learning-induced inhibitory synaptogenesis in murine barrel field. J. Neurosci. 30, 1176–1184.
Jiang, Y.H., Armstrong, D., Albrecht, U., Atkins, C.M., Noebels, J.L., Eichele, G., Sweatt, J.D., Beaudet, A.L., 1998. Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation. Neuron 21, 799–811.
Judson, M.C., Wallace, M.L., Sidorov, M.S., Burette, A.C., Gu, B., van Woerden, G.M., King, I.F., Han, J.E., Zylka, M.J., Elgersma, Y., 2016. GABAergic neuron-specific loss of Ube3a causes Angelman syndrome-like EEG abnormalities and enhances seizure susceptibility. Neuron 90, 56–69.
Kashiwaya, Y., Pawlosky, R., Markis, W., King, M.T., Bergman, C., Srivastava, S., Murray, A., Clarke, K., Veech, R.L., 2010. A ketone ester diet increases brain malonyl-CoA and uncoupling proteins 4 and 5 while decreasing food intake in the normal Wistar rat. J. Biol. Chem. 285, 25950–25956.
Kashiwaya, Y., Bergman, C., Lee, J.-H., Wan, R., King, M.T., Mughal, M.R., Okun, E., Clarke, K., Mattson, M.P., Veech, R.L., 2013. A ketone ester diet exhibits anxiolytic and cognition-sparing properties, and lessens amyloid and tau pathologies in a mouse model of Alzheimer's disease. Neurobiol. Aging 34, 1530–1539.
Kemper, M.F., Srivastava, S., King, M.T., Clarke, K., Veech, R.L., Pawlosky, R.J., 2015. An Ester of β-hydroxybutyrate regulates cholesterol biosynthesis in rats and a cholesterol biomarker in humans. Lipids 50, 1185–1193.
Kesl, S.L., Poff, A.M., Ward, N.P., Fiorelli, T.N., Ari, C., Van Putten, A.J., Sherwood, J.W., Arnold, P., D'Agostino, D.P., 2016. Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in Sprague–Dawley rats. Nutrition & metabolism 13, 1.
Kombu, R.S., Brunengraber, H., Puchowicz, M.A., 2011. Analysis of the citric acid cycle intermediates using gas chromatography-mass spectrometry. Metabolic Profiling: Methods and Protocols 147–157.
Llewellyn, K.J., Nalbandian, A., Gomez, A., Wei, D., Walker, N., Kimonis, V.E., 2015. Administration of CoQ 10 analogue ameliorates dysfunction of the mitochondrial respiratory chain in a mouse model of Angelman syndrome. Neurobiol. Dis. 76, 77–86.
Maalouf, M., Rho, J.M., 2008. Oxidative impairment of hippocampal long-term potentiation involves activation of protein phosphatase 2A and is prevented by ketone bodies. J. Neurosci. Res. 86, 3322–3330.
Mantis, J.G., Fritz, C.L., Marsh, J., Heinrichs, S.C., Seyfried, T.N., 2009. Improvement in motor and exploratory behavior in Rett syndrome mice with restricted ketogenic and standard diets. Epilepsy Behav. 15, 133–141.
Meng, L., Person, R.E., Huang, W., Zhu, P.J., Costa-Mattioli, M., Beaudet, A.L., 2013. Truncation of Ube3a-ATS unsilences paternal Ube3a and ameliorates behavioral defects in the Angelman syndrome mouse model. PLoS Genet. 9, e1004039.
Mula, M., Trimble, M.R., 2009. Antiepileptic drug-induced cognitive adverse effects. CNS drugs 23, 121–137.
Nitz, D., McNaughton, B., 2004. Differential modulation of CA1 and dentate gyrus interneurons during exploration of novel environments. J. Neurophysiol. 91, 863–872.
Pelc, K., Boyd, S.G., Cheron, G., Dan, B., 2008. Epilepsy in Angelman syndrome. Seizure 17, 211–217.
Poff, A., Ari, C., Arnold, P., Seyfried, T., D'Agostino, D., 2014. Ketone supplementation decreases tumor cell viability and prolongs survival of mice with metastatic cancer. Int. J. Cancer 135, 1711–1720.
Silva, R., Abilio, V., Takatsu, A., Kameda, S., Grassl, C., Chehin, A., Medrano, W., Calzavara, M., Registro, S., Andersen, M., 2004. Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. Neuropharmacology 46, 895–903.
Stein, D., Chetty, M., Rho, J.M., 2010. A "happy" toddler presenting with sudden, life-threatening seizures. Seminars in Pediatric Neurology. Elsevier, pp. 35–38.
Su, H., Fan, W., Coskun, P.E., Vesa, J., Gold, J.-A., Jiang, Y.-H., Potluri, P., Procaccio, V., Acab, A., Weiss, J.H., 2011. Mitochondrial dysfunction in CA1 hippocampal neurons of the UBE3A deficient mouse model for Angelman syndrome. Neurosci. Lett. 487, 129–133.
Thibert, R.L., Conant, K.D., Braun, E.K., Bruno, P., Said, R.R., Nespeca, M.P., Thiele, E.A., 2009. Epilepsy in Angelman syndrome: a questionnaire-based assessment of the natural history and current treatment options. Epilepsia 50, 2369–2376.
Thibert, R.L., Pfeifer, H.H., Larson, A.M., Raby, A.R., Reynolds, A.A., Morgan, A.K., Thiele, E.A., 2012. Low glycemic index treatment for seizures in Angelman syndrome. Epilepsia 53, 1498–1502.
Trotter, J., Lee, G.H., Kazdoba, T.M., Crowell, B., Domogauer, J., Mahoney, H.M., Franco, S.J., Müller, U., Weeber, E.J., D'Arcangelo, G., 2013. Dab1 is required for synaptic plasticity and associative learning. J. Neurosci. 33, 15652–15668.
Valente, K.D., Koiffmann, C.P., Fridman, C., Varella, M., Kok, F., Andrade, J.Q., Grossmann, R.M., Marques-Dias, M.J., 2006. Epilepsy in patients with Angelman syndrome caused by deletion of the chromosome 15q11-13. Arch. Neurol. 63, 122.
Van Woerden, G.M., Harris, K.D., Hojjati, M.R., Gustin, R.M., Qiu, S., de Avila, F.R., Y-h, J., Elgersma, Y., Weeber, E.J., 2007. Rescue of neurological deficits in a mouse model for Angelman syndrome by reduction of αCaMKII inhibitory phosphorylation. Nat. Neurosci. 10, 280–282.
Volek, J., Sharman, M., Gomez, A., Judelson, D.A., Rubin, M.R., Watson, G., Sokmen, B., Silvestre, R., French, D.N., Kraemer, W.J., 2004. Comparison of energy-restricted very low-carbohydrate and low-fat diets on weight loss and body composition in overweight men and women. Nutr. Metab. (Lond.) 1, 13.
Wallace, M.L., Burette, A.C., Weinberg, R.J., Philpot, B.D., 2012. Maternal loss of Ube3a Produces an excitatory/inhibitory imbalance through neuron type-specific synaptic defects. Neuron 74, 793–800.
Williams, C.A., Driscoll, D.J., Dagli, A.I., 2010. Clinical and genetic aspects of Angelman syndrome. Genitourin. Med. 12, 385–395.
Xu, K., Sun, X., Eroku, B.O., Tsipis, C.P., Puchowicz, M.A., LaManna, J.C., 2010. Diet-induced ketosis improves cognitive performance in aged rats. Oxygen Transport to Tissue XXXI. Springer, pp. 71–75.
Yang, L., Kombu, R.S., Kasumov, T., Zhu, S.-H., Cendrowski, A.V., David, F., Anderson, V.E., Kelleher, J.K., Brunengraber, H., 2008. Metabolomic and Mass Isotopomer Analysis of Liver Gluconeogenesis and Citric Acid Cycle I. Interrelation Between Gluconeogenesis And Cataplerosis; Formation Of Methoxamates From Aminooxyacetate And Ketoacids. J. Biol. Chem. 283, pp. 21978–21987.
Yudkoff, M., Daikhin, Y., Melø, T.M., Nissim, I., Sonnewald, U., Nissim, I., 2007. The ketogenic diet and brain metabolism of amino acids: relationship to the anticonvulsant effect. Annu. Rev. Nutr. 27, 415–430.
Zhang, Y., Zhang, S., Marin-Valencia, I., Puchowicz, M.A., 2015. Decreased carbon shunting from glucose toward oxidative metabolism in diet-induced ketotic rat brain. J. Neurochem. 132, 301–312.