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Brain glutamate and sleep efficiency associations following a ketogenic diet intervention in individuals with Alcohol Use Disorder

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ABSTRACT

Background: We previously showed that ketogenic diet (KD) was effective in curbing alcohol withdrawal and craving in individuals with alcohol use disorder (AUD). We hypothesized that the clinical benefits were due to improvements in sleep. To test this, we performed a secondary analysis on the KD trial data to (1) examine the effects of KD on total sleep time (TST) and sleep quality and (2) investigate the association between KD-induced alterations in cingulate glutamate concentration and changes in TST and sleep quality.

Methods: AUD individuals undergoing alcohol detoxification were randomized to receive KD (n = 19) or standard American diet (SA; n = 14) for three weeks. TST was measured weekly by self-report, GENEActive sleep accelerometer, and X4 Sleep Profiler ambulatory device. Sleep quality was assessed using subjectively ratings of sleep depth and restedness and Sleep Profiler (Sleep Efficiency [%]). Weekly ^{1}H magnetic resonance spectroscopy scans measured cingulate glutamate levels.

Results: TST was lower in KD than SA and increased with time. Sleep depth, restedness, and Sleep Efficiency improved with time, but exhibited no effect of diet. In KD and SA combined, week 1 cingulate glutamate levels correlated positively with Sleep Efficiency, but not with TST.

Conclusions: Although cingulate glutamate levels correlated positively with Sleep Efficiency in week 1, KD-induced glutamate elevation did not produce significant sleep improvements. Rather, KD was associated with lower TST than SA. Given the well-established associations between sleep and alcohol relapse, longer follow up assessment of KD's impact on sleep in AUD is warranted.

1. Introduction

Alcohol use disorder (AUD) is a chronic relapsing brain disorder that accounts for 5% of deaths globally (Rehm and Imtiaz, 2016). It stems from neurobiological adaptations following chronic alcohol consumption that eventuates compulsive behavior and loss of control in limiting alcohol consumption (Koob and Colrain, 2020). Among its numerous detrimental consequences, alcohol has been linked to impaired sleep quality and altered sleep architecture (Koob and Colrain, 2020). Sleep consists of three stages of progressively deeper N1, N2, and N3 non-REM sleep and REM sleep that are differentiated in electroencephalogram waveforms and cycle in 60–90 min intervals (Carley and Farabi, 2016). In healthy volunteers, administration of alcohol to obtain a peak breath alcohol concentration (BrAC) of 0.10 g% impaired sleep continuity (Arnedt et al., 2011). Moreover, individuals with AUD demonstrated significant reduction in total sleep time (TST) and in percentage of time spent in N2, N3, or REM sleep in comparison to non-dependent healthy subjects (Zhang et al., 2021).

In AUD individuals attempting to abstain, disturbances in sleep increase the risk of relapse and pose an additional obstacle in recovery (Brower et al., 1998; Feige et al., 2007). One study recruited individuals with AUD from treatment programs and categorized abstained vs. relapsed participants following an average 5-month follow-up period. Participants reporting incidences of relapse at follow-up exhibited more subjective and objective measures of sleep disturbances at baseline than those who successfully abstained from alcohol (Brower et al., 1998).

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Abbreviations

ACC	anterior cingulate cortex;
AUD	alcohol use disorder;
BHB	β-hydroxybutyrate;
KD	ketogenic diet;
SA	standard American diet;
TST	total sleep time

An electroencephalographic (EEG) study evidenced differences in brain activity during REM sleep between abstained vs. relapsed participants, such that relapsed individuals exhibited greater β2 REM sleep spectral power at baseline than those who abstained (Feige et al., 2007). Several pharmacological interventions have attempted to treat AUD and decrease incidences of relapse by mitigating alcohol-induced sleep disturbances. However, the studies have demonstrated mixed results. As such, Gabapentin demonstrated effectiveness in improving sleep (Mason et al., 2014) and increased rates of abstinence from alcohol (Anton et al., 2020; Mason et al., 2014). Conversely, Trazodone, despite eliciting beneficial outcomes on sleep parameters in AUD participants (Friedmann et al., 2008; Le Bon et al., 2003), hindered abstinence (Friedmann et al., 2008). Here, we proposed to delineate the effects of ketogenic diet (KD) intervention on sleep parameters in AUD participants with the goal of identifying safe and efficacious strategies for treating AUD.

The KD (low carbohydrates, high fat, adequate protein) promotes the production of ketone bodies from the breakdown of fats. A state of ketosis ensues, and the body shifts its primary fuel source from glucose to ketone bodies (Hartman et al., 2007). Preclinical and clinical studies have supported the effective use of KD to reduce alcohol craving and mitigate withdrawal (Blanco-Gandía et al., 2021; Castro et al., 2018; Wiers et al., 2021), but its effects on alcohol-induced sleep disturbances have largely been unexplored. Here, we proposed to monitor sleep in AUD individuals following a KD intervention and explore the role of glutamate in mediating changes in sleep. KD alters glutamate availability by shifting the flux of carbon in the metabolic pathway, tricarboxylic acid (TCA) cycle, and increasing the utilization of glutamic acid decarboxylase for glutamate to gamma-aminobutyric acid (GABA) conversion (Mahajan et al., 2021). We previously reported elevated dorsal anterior cingulate cortex (ACC) glutamate levels in AUD individuals receiving KD compared to those on a standard American diet (SA) (Wiers et al., 2021). A previous ^{1}H-MRS study associated elevated ACC glutamate with better sleep quality in both healthy volunteers and in individuals with Schizophrenia (Korenic et al., 2020). Under normal conditions, different wake and sleeping states correspond to varying concentrations of glutamate. In rodents, extracellular glutamate concentration in the cerebral cortex and activity of glutaminergic neurons in the ventral tegmental area increase during wake and REM sleep and decrease during non-REM sleep (Dash et al., 2009; Yu et al., 2019). Sleep deprivation induces changes in glutamate concentration, such that forced extended wakefulness progressively decreases glutamate concentration as the pressure of sleep increase (Dash et al., 2009). Subsequent recovery sleep is characterized by a drastic drop in glutamate concentrations during non-REM sleep and the rate of glutamate decrease covaried with sleep intensity (Dash et al., 2009). Despite the well-studied interrelationships between KD, glutamate, and sleep, extant clinical studies have inconsistently demonstrated both positive and null effects of KD on sleep (Castro et al., 2018; Hallböök et al., 2007; Iacovides et al., 2019; Klement et al., 2021; Siegmann et al., 2019; Willi et al., 1998).

Our previous report described the beneficial effects of KD in mitigating acute alcohol withdrawal and curbing craving in individuals with AUD undergoing detoxification (Wiers et al., 2021). We hypothesized that the beneficial effects of KD may be mediated in part by improvements in sleep and brain glutamate levels. The objectives of this study were to: (1) examine the effects of KD on subjective and objective measures of sleep in AUD participants undergoing detoxification, (2) assess the association between glutamate levels in the dorsal ACC and sleep measures, and (3) correlate sleep measures with alcohol craving/wanting. The findings of this study will help elucidate factors implicated in varying AUD outcomes and further explore the role of KD as a treatment option.

2. Methods

2.1. Participants

Detailed information on study intervention and subject characteristics was previously published (Wiers et al., 2021). In short, participants who met the Substance Abuse and Mental Health Services Administration criteria for heavy drinking for > 5 years were admitted to the National Institute on Alcohol Abuse and Alcoholism inpatient unit for detoxification and randomized to receive KD or standard American diet while receiving treatment (Wiers et al., 2021). A total of 33 participants completed the three-weeks dietary intervention (19 KD/ 14 SA). The groups did not exhibit any baseline differences in demographics or clinical characteristics of alcohol use (**Table 1**). Alcohol withdrawal symptoms were monitored using the Clinical Institute Withdrawal Assessment–Alcohol revised (CIWA-Ar) (Sullivan et al., 1989) and, if became too severe, patients were provided with benzodiazepine by medical staff blinded to the study. Participants were excluded if they had any contraindications for MRI, psychiatric or substance use disorder (other than AUD or nicotine), major medical problems, head trauma or neurological problems, or chronic use of psychoactive medications. Also excluded were women who were pregnant or breast feeding. All participants had to be free of any psychoactive medication within 24 h of study initialization and produced a negative urine drug screen on the study day (with the exception of benzodiazepine for treatment of withdrawal symptoms or Δ^{9}-tetrahydrocannabinol [THC]). If 11-Nor-9-carboxy-Δ^{9}-tetrahydrocannabinol (THC–COOH) was present in the urine, confirmatory saliva drug screen (Dräger DrugTest 5000; Draeger Safety Diagnostics, Lübeck, Germany) was used to test for THC (cut-off 5 ng/mL) to rule out recent use on Sleep Profiler and MRI days (Newmeyer et al., 2017). THC saliva tests were performed for 5 individuals, of whom 2 were assigned to the KD group and 3 to the SA group. An overview of study procedures is provided in **Fig. 1**.

2.2. Dietary intervention

Participants were randomized to receive eucaloric KD or SA within 2 days of admissions and maintained on their respective diets for 3 weeks. KD contained the classic 4:1 ratio by weight of fats to carbohydrate and protein, whereas SA contained 50% kcal carbohydrates, 35% kcal fat, and 15% kcal protein. The meals were provided as a shake at breakfast, lunch, and dinner and contained 75% of the total calories. The remaining 25% calories were provided in the form of a solid ketogenic snack (i.e., scrambled eggs, yogurt with nuts, and broth). To quantify the effects of KD on ketone bodies levels and assess study compliance, daily urinary ketones (acetoacetate) were measured before breakfast, and weekly blood β-hydroxybutyrate (BHB) levels were collected using finger prick tests.

2.3. Questionnaires

The participants completed the Timeline Follow-back (TLFB) to estimate alcohol consumption during the 90-days period prior to study (Sobell et al., 1996), Lifetime Drinking History (LDH) to assess lifetime alcohol use behavior (Skinner and Sheu, 1982), and Alcohol Dependence Scale (ADS) to evaluate alcohol dependence severity (Skinner and Allen, 1982). Alcohol craving was assessed weekly with Desires for Alcohol Questionnaire (DAQ) (Love et al., 1998), for which week 3 DAQ measurements were missing for 2 KD participants.

Table 1
Participant Characteristics (mean ± SD).

	Ketogenic Diet (n = 19)	Standard Diet (n = 14)	P value
Demographics			
Age	39.3 ± 11.2	44.2 ± 16.4	0.311
Sex	7 females/ 12 males	3 females/ 11 males	0.341
BMI (kg/m^2)	24.5 ± 3.4	27.6 ± 5.5	0.051
Race	6 African American/ 11 White/ 2 Multiracial	6 African American/ 6 White/ 2 Multiracial	0.694
Years of Education	12.7 ± 2.8	14.2 ± 2.5	0.110
Household Income	3.4 ± 2.2	3.6 ± 1.9	0.839
Alcohol			
Lifetime Drinks	70,353 ± 67,537	101,031 ± 104,468	0.320
Age at First Drink	16.3 ± 8.6	14.2 ± 3.4	0.213
Heavy Drinking Yrs.	12.5 ± 8.1	16.0 ± 8.1	0.122
ADS	21.0 ± 9.8	23.6 ± 7.2	0.399
TLFB Drinks/ Day	15.2 ± 8.4	17.0 ± 9.8	0.584
CIWA- Ar at admission	6.4 ± 4.9	5.4 ± 4.2	0.540
Oxazepam since admission (mg)[A]	122.1 ± 167.5	240.0 ± 268.1	0.127
Oxazepam after KD/SA diet initiation (mg)	34.2 ± 79.4	156.4 ± 206.2	0.052

ADS, alcohol dependence score.
CIWA, clinical institute withdrawal assessment (revised version), collected initially at admission.
TLFB, timeline follow back.
[A] Cumulative oxazepam dose (2:1 conversion for diazepam) administered to study participants during the first week of detoxification.

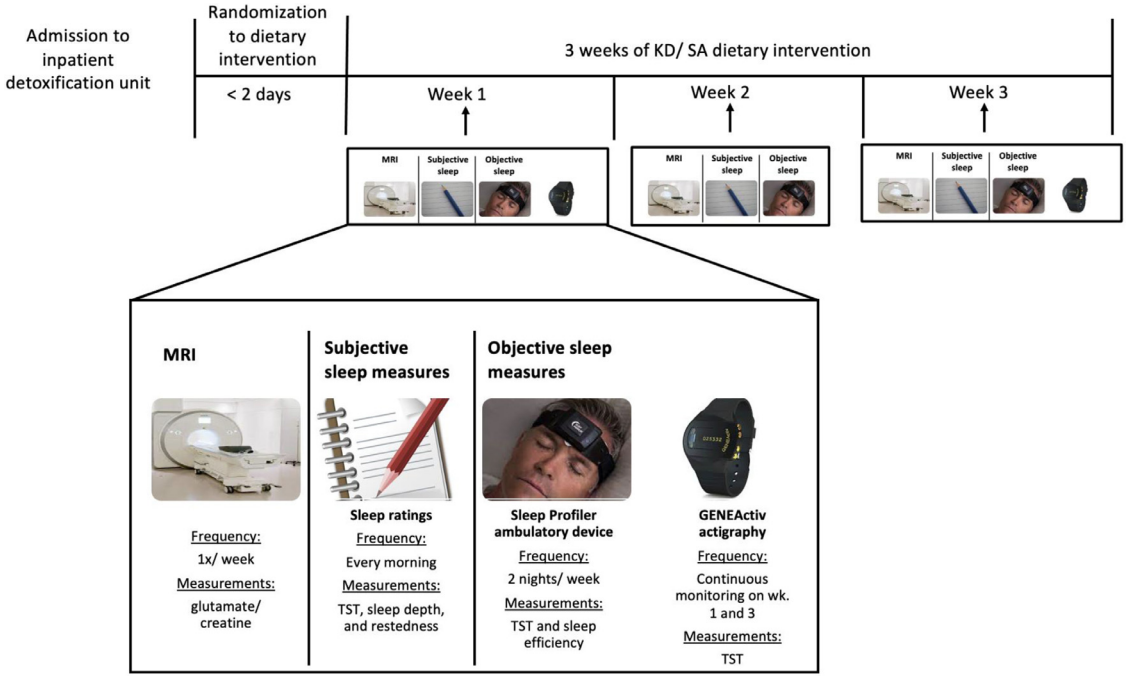

Fig. 1. Overview of study procedures. Abbreviations: TST, total sleep time.

2.4. MRI acquisition and ^{1}H magnetic resonance spectroscopy (MRS)

All participants underwent weekly magnetic resonance imaging (MRI) on a 3.0T Magentom Prisma scanner (Siemens Medical Solutions USA Inc., Malvern, PA) with a 32- channel head coil. Brain images were acquired by T1-weighted three-dimensional magnetization-prepared rapid gradient- echo [MP-RAGE; repetition time/ echo time (TR/TE)= 2200/4.25 ms, flip angle (Stubbs et al.)= 9°, 1- mm isotropic resolution]. Localized proton ^{1}H- MRS was performed in the dACC (2 cm x 2 cm x 2 cm) using a PRESS pulse sequence (TE/ TR= 30/3000 s, 64 averages). Spectral fitting of the datasets with LCModel program determined glutamate concentration relative to total creatine. Nonrandom residuals and baseline fitting were inspected and spectra with signal-to- noise ration of ⟨ 15 or line width of ⟩ 0.1 ppm were excluded from analysis. Cramér- Rao lower bound of 20% served as an additional quality criterion (Wiers et al., 2021).

2.5. Sleep monitoring

2.5.1. Sleep ratings

Sleep was monitored subjectively using daily morning questionnaire regarding total sleep time (TST [hrs.]), sleep depth, and feelings of restedness (Kaplan et al., 2017; Laffan et al., 2010). Weekly responses were reported as the average across 7 days. One outlier for TST measures was removed in the SA group at week 2.

2.5.2. Sleep profiler ambulatory device

Study participants also received an X4 Sleep Profiler ambulatory device (Advanced Brain Monitoring Inc., Carlsbad, CA) across two non-consecutive nights every week (6 times in total). The Sleep Profiler provides 3 channel frontopolar EEG signals, photoplethysmographic signal for calculating pulse rate, and head position/movement measurements via an accelerometer. Sleep was staged and characterized by an automated Sleep Profiler software using filtered and unfiltered EEG power spectral data as described in detail by Levendowski et al. and sleep biomarkers were averaged across both nights (Levendowski et al., 2017). We focused on TST and used sleep efficiency (percentage of TST to time in bed) as a proxy for sleep quality. Sleep Profiler data were unavailable for 1 SA participant at week 2.

2.5.3. GENEActiv actigraphy device

Participants wore a tri-axial GENEActiv actigraphy device (Activinsights Ltd., Kimbolton, England) during weeks 1 and 3. The devices were placed on the non- dominant wrist and ankle of the participants and detect sleep as sustained periods of inactivity. The data were summarized into 60- seconds epoch and analyzed using the GENEActiv Software. TST was averaged across 7 days of 24 h continuous monitoring (Ramirez et al., 2018; van Hees et al., 2015). At least one time-point of GENEActiv sleep measures were missing from 5 KD and 4 SA participants.

2.6. Statistical analysis

Measurements pertaining to sleep and glutamate concentrations were analyzed with repeated-measures ANOVAs with time as the within-subject factor and diet (KD/ SA) as the between-subject factor. Greenhouse- Geisser correction was applied when Mauchly's test of sphericity was significant. Outliers > 3 standard deviations from the mean were removed. Effect sizes are reported in partial η^2. Post-hoc pairwise comparisons were subjected to Bonferroni correction. The association between glutamate and sleep measures were tested with linear regression. The role of diet (KD/SA) in moderating significant correlations between glutamate and sleep measures were assessed by including a centered glutamate levels-by-diet interaction term in regression model. Additionally, associations between sleep measures and alcohol wanting (DAQ) were assessed with linear regression. Exploratory associations between sleep monitoring outcomes are provided in Supplementary Figure 1. All analyses were performed with SPSS (IBM, Armonk, NY).

3. Results

Table 1 provides demographics and clinical characteristics of alcohol use in KD and SA participants and shows no significant between-group differences (Wiers et al., 2021).

In AUD individuals, main diet effect of KD intervention lowered TST obtained by self-report ($F_{1,30}$= 5.6, p = 0.024, partial η^2 = 0.16) and GENEActiv actigraphy device ($F_{1,22}$= 5.5, p = 0.028, partial η^2 = 0.20), but not with Sleep Profiler ambulatory device ($F_{1,30}$= 0.3, p = 0.57, partial η^2 = 0.011) **(Fig. 2)**. GENEActiv, but not self-report or Sleep Profiler ambulatory device, measurements additionally showed a significant time effect ($F_{1,22}$= 7.1, p = 0.014, partial η^2 = 0.24), such that total sleep hours increased throughout the 3-weeks study period. No significant diet x time interaction was observed for subjective or objective measures of TST. Correlations between measures of TST collected using subjective report, GENEActiv actigraphy device, and Sleep Profiler ambulatory device are available in **Supplementary Figure 1**. Specifically, subjective reports of TST correlated with that obtained using the GENEActiv (r = 0.351, p< 0.05) and the Sleep Profiler (r = 0.484, p< 0.05) in week 1, but no other associations were evidenced (p> 0.05).

Sleep quality was additionally assessed with self-report questionnaires using a 1–10 scale regarding sleep depth and feelings of restedness. Ratings of sleep depth ($F_{1.7,\ 51.6}$ = 16.8, p< 0.001, partial η^2 = 0.35) and restedness ($F_{1.6,\ 48.0}$ = 10.03, p< 0.001, partial η^2 = 0.24) significantly increased over time **(Fig. 3A)**, but there were no significant effects of diet (depth: $F_{1,31}$= 0.23, p = 0.63, partial η^2 = 0.007; restedness: $F_{1,31}$= 0.95, p = 0.34, partial η^2 = 0.03). The Sleep Profiler-measures of sleep efficiency increased with time during the 3-weeks detoxification period ($F_{2,60}$= 7.6, p = 0.001, partial η^2 = 0.20) **(Fig. 3B)**, but did not show a main effect of diet ($F_{1,30}$= 2.6, p = 0.12, partial η^2 = 0.08). X4 Sleep Profiler sleep measures in KD and SA for all time points are listed in **Supplementary Table 1**.

We previously reported that ACC glutamate concentrations was greater in KD compared to SA ($F_{1,29}$= 4.7, p = 0.039, partial η^2 = 0.25) (Wiers et al., 2021). Here, we aimed to identify the relationship between glutamate levels with sleep measures in AUD individuals receiving SA or KD. Glutamate levels in ACC at week 1 correlated positively with Sleep Profiler-measures of sleep efficiency (r = 0.536, p = 0.001) **(Fig. 4A)**, but did not correlate with TST (r = 0.341, p = 0.052) **(Fig. 4A).** Moderation analysis did not indicate significant interactive effects of diet x glutamate on sleep parameters ($TST_{\ week\ 1}$: p = 0.41; sleep efficiency $_{week1}$: p = 0.65; $TST_{\ week\ 1\Delta3}$: p = 0.98; Sleep efficiency $_{\ week\ 1\Delta3}$ = 0.54). Therefore, no follow-up correlations between glutamate and sleep parameters were performed separately in SA and KD groups. Glutamate changes across the three-week study period did not significantly associate with any sleep measures changes (r = 0.067–0.174, p = 0.35–0.72) **(Fig. 4B)**. Additional exploratory correlation analysis between glutamate and sleep parameters is available in **Supplemental Figure 2**.

Exploratory correlation analysis did not reveal significant associations between any Sleep Profiler measures and DAQ scores at week 1, 2, or 3 (r = 0.038–0.197, p = 0.29–0.94) (**Supplemental Table 2**).

4. Discussion

This study is a secondary analysis of a KD intervention trial that previously demonstrated clinical efficacy in ameliorating withdrawal symptoms and elevating ACC glutamate levels in individuals undergoing inpatient detoxification treatment for AUD. We hypothesized that improvements in sleep contributed to the clinical benefits of KD during the first 3 week of detoxification in AUD individuals. Although our findings demonstrated a strong positive correlation between ACC glutamate concentration and sleep efficiency at week 1, the KD-associated glutamate elevation was not associated with significant sleep improvements. Contrarily, individuals receiving KD administration demonstrated reduced sleep duration in comparison to those on SA. Sleep duration and quality increased throughout the three-week detoxication period irrespective of diet assignment, but exploratory correlation analysis did not evidence significant relationships between sleep parameters and alcohol craving.

The sleep-deprived state of our participants at week 1 (~5.8 hrs.) coincides with the high prevalence of sleep disturbances reported in individuals with AUD, especially in those undergoing withdrawal (Kolla et al., 2020, 2014; Laniepce et al., 2020). In our AUD participants, sleep duration (GENEActiv measures only) and quality improved throughout the 3-weeks study period. Due to diet manipulations and possible confounding factors, such as acclimation to the inpatient treatment center, we can only speculate and cannot conclusively attribute the observed sleep improvements to alcohol detoxification. However, previous studies demonstrated significant improvements in Pittsburgh Sleep Quality Index from treatment onset to discharge (Kolla et al., 2014; Wallen et al., 2014), possibly as a function of restoration of alcohol-induced aberrations in neuronal structure and neurotransmitter signaling (i.e., glutamate and GABA) (Colrain et al., 2014).

Extant literature exploring the role of KD on sleep has reported mixed findings. Previous studies in obese participants have demonstrated the effectiveness of KD in improving sleepiness score (Castro et al., 2018) and normalizing sleep architecture (Willi et al., 1998). Another study in children with therapy-resistant epilepsy similarly demonstrated efficacy of KD in prolonging REM sleep in addition to confirming its well-

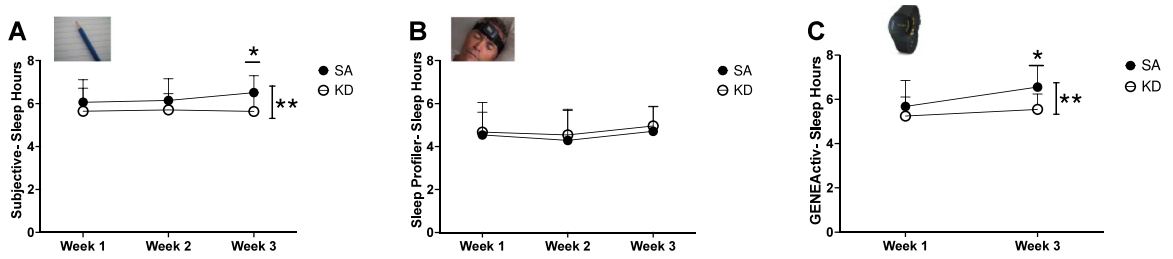

Fig. 2. Total sleep time in SA and KD participants. Measures of TST were obtained by **(A)** self- report (n = 19 KD/ 14SA), **(B)** X4 Sleep Profiler ambulatory device (n = 19KD/ 13–14 SA), and **(C)** GENEActiv wrist- worn actinography device (n = 14 KD/14SA). Data are presented as mean ± SEM. ** Indicates significant main effect of diet between KD and SA*Indicates significant differences between KD and SA at a particular time point, p< 0.05, p< 0.05. Abbreviations: SA, standard American diet; KD, ketogenic diet.

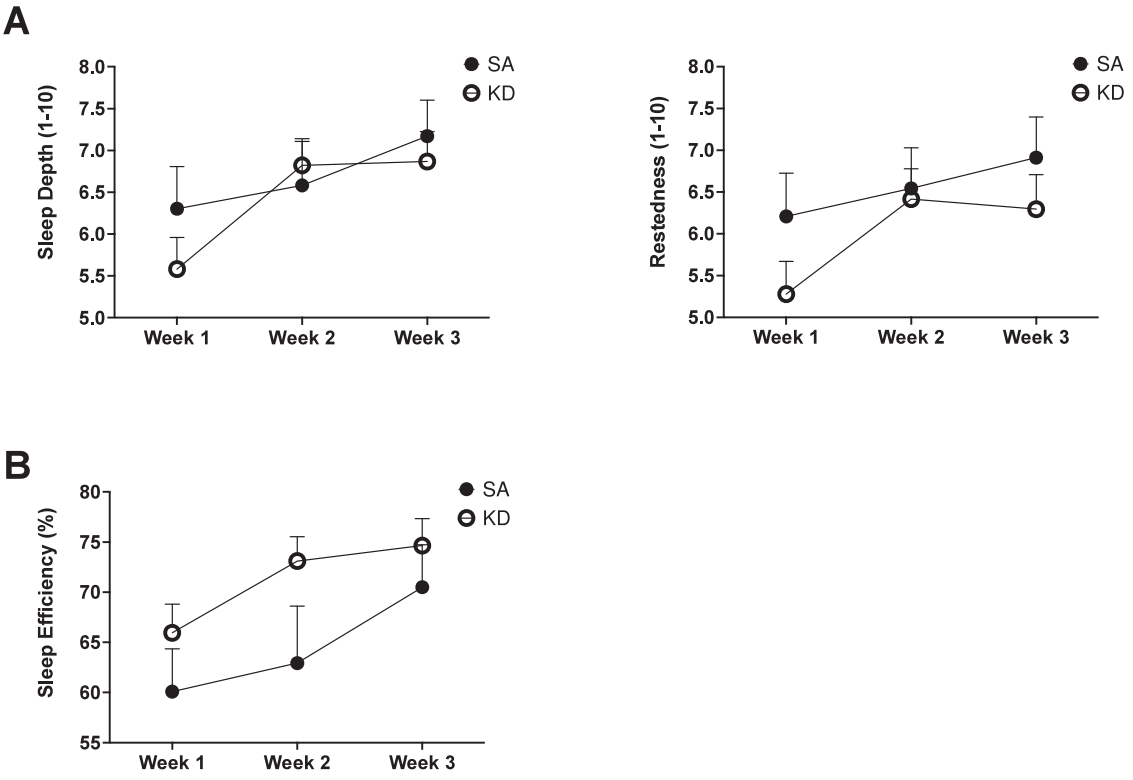

Fig. 3. Sleep quality in SA and KD participants. (A) Subjective ratings of sleep depth and restedness (n = 19 KD/ 14 SA) and **(B)** Sleep Profiler-measured sleep efficiency (n = 19KD/ 13–14 SA) significantly increased with time. Data are presented as mean ± SEM. Abbreviations: SA, standard American diet; KD, ketogenic diet.

evidenced anti-seizure effects (Hallböök et al., 2007). Contrarily, our study in AUD individuals and another in healthy participants did not evidence beneficial effects of KD on sleep (Iacovides et al., 2019). Given impaired sleep is a symptom of obesity and epilepsy (Malow, 2007; Valencia-Flores et al., 2004), it is plausible that improvement in sleep parameters reported in these studies were not directly attributable to KD but are rather indications of alleviation from disease pathologies. Future studies are needed to better distinguish the dietary effects of KD on sleep and that associated with disease recuperation. Additionally, our study was limited in sample size so it is possible that it was inadequately powered to demonstrate significant dietary differences in sleep quality and future studies with larger samples sizes are warranted.

The reduced sleep duration observed in our study mirrored that previously reported in children with therapy-resistant epilepsy following 3-months KD intervention (Hallböök et al., 2007). The KD is a high-fat, low-carbohydrate diet and several cross-sectional studies have noted significant effects of macronutrition composition on sleep duration, albeit mixed results. One study stratified individuals based on fat intake at dinner and found those in the highest quartile for dinner fat intake displayed increased risk for persistent short sleep (Cao et al., 2016). In contrast, another study utilized linear regressions and demonstrated that long sleep duration was significantly associated with lower carbohydrate%kcal and higher fat%kcal intake (Martinez et al., 2017). Alternatively, the reduction in sleep duration in our study may be brought upon by the colloquial “keto flu,” characterized by flu-like symptoms, reported in some individuals during the initial transition in fuel source (Bostock et al., 2020). It is possible that the effects are only transitory and normal sleep may be restored following physiological acclimation to the diet.

We postulated elevation in glutamate concentration following KD as one possible mechanistic pathway mediating sleep alterations. One rodent study demonstrated that microinjection of 15- and 30-ng L-glutamate in the pedunculopontine tegmentum increased REM sleep at the expense of decreased slow-wave sleep. Injection of higher 60- and 90-ng dose L-glutamate increased wakefulness (Datta et al., 2001). In a clinical study, a positive correlation between glutamate and sleep

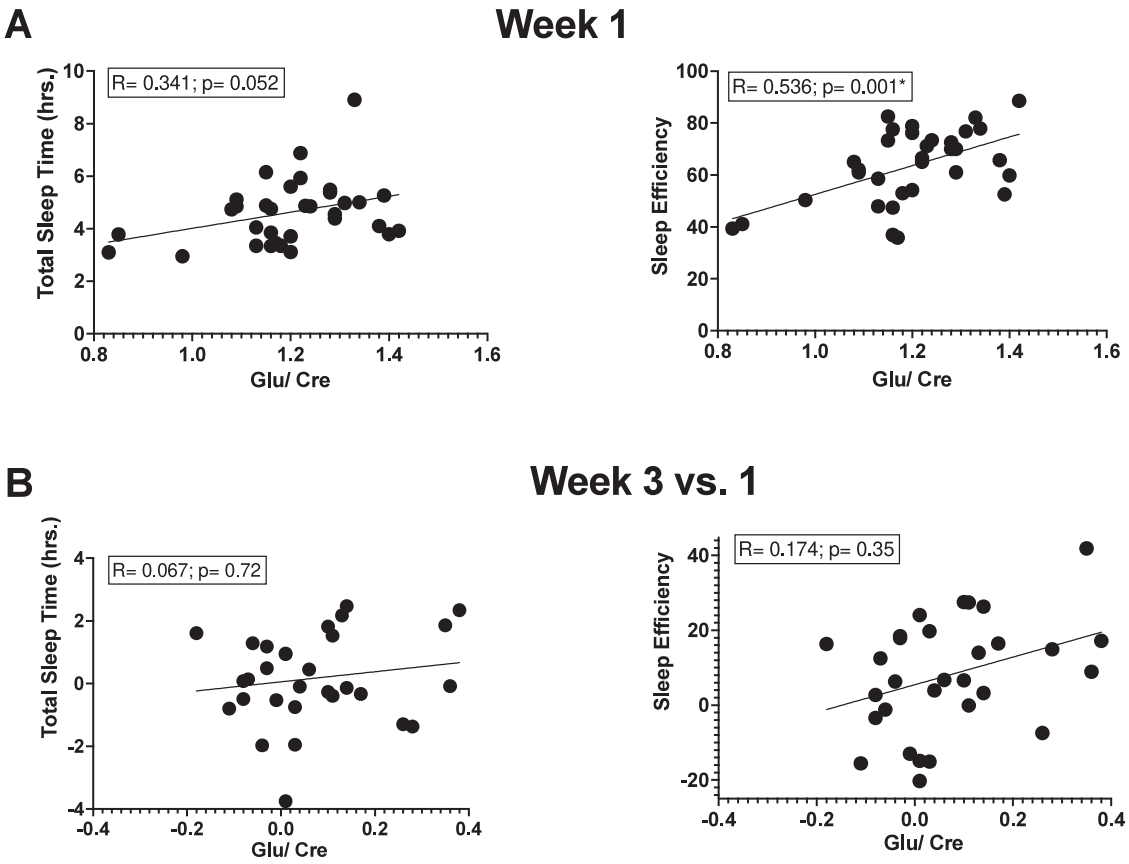

Fig. 4. Associations between sleep measures and ACC glutamate levels. (A) Correlations between Sleep Profiler sleep measures and ACC glutamate levels during week 1 (n = 19 KD/ 14 SA) and **(B)** changes over three weeks (n = 18 KD/ 13 SA). *Indicates significant correlations, p< 0.05. Abbreviations: Glu/Cre, glutamate/creatine.

quality was indicated in Schizophrenic individuals and healthy volunteers (Korenic et al., 2020) and we anticipated similar findings in our study population. Although ACC glutamate concentration significantly correlated with sleep efficiency at week 1 of study intervention, no significance persisted into subsequent weeks. Furthermore, KD-associated glutamate elevation did not produce clinical improvements in sleep outcomes. We utilized ^{1}H-MRS here because it provided a non-invasive method to quantify glutamate and other biochemical metabolites. However, limitation of utilizing ^{1}H-MRS is the inability to differentiate intracellular versus extracellular glutamate and assess the actions of glutamate on ionotropic or metabotropic receptors. Moreover, we were unable to assess GABA levels with the standard ^{1}H-MRS PRESS sequence performed at 3Tesla. Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter that acts in opposition to excitatory glutamate to maintain equilibrium and has also been implicated in sleep regulation. One study categorized individuals based on high vs. low sleep duration and found reduced ACC GABA levels in individuals with shorter sleep duration (Park et al., 2020). Therefore, possible interceding effects of GABA on glutamate-induced sleep alterations are unknown and need further elucidation.

Another limitation of the study is the significantly lower dosage of benzodiazepine needed by the KD group than the SA group during the first week of detoxification (Wiers et al., 2021). Chronic alcohol consumption produces imbalance between the GABAergic and glutaminergic systems that, upon alcohol cessation, results in hyperexcitability of the central nervous system (Ntais et al., 2005). Benzodiazepine alleviates withdrawal symptoms by acting on $GABA_A$ receptors to enhance the inhibitory functions of GABA and mitigate central nervous system hyperexcitability (Gottesmann, 2002). One study utilized transcranial magnetic stimulation (TMS) in healthy individuals and demonstrated increased cortical inhibition following benzodiazepine administration (Ferland et al., 2021). In AUD individuals undergoing detoxification, an ^{1}H-MRS study negatively associated benzodiazepine dosage with ACC glutamate and GABA concentration (Wang et al., 2021). Given the effects of benzodiazepine on neuroexcitation and previous studies implicating its involvement in sleep (Nowell et al., 1997; Winsky-Sommerer, 2009), differences in benzodiazepine between KD and SA groups may have confounded our study results. However, exploratory correlation analyses did not evidence benzodiazepine dosage to significantly correlate with glutamate and sleep measures in our study **(supplementary Table 3 and 4)**.

Our study utilized three methods for assessing sleep that included both subjective responses to questionnaires and objective measurements with commercially available devices. The GENEActive actinography device indirectly infers sleep using triaxial movement detection (Ramirez et al., 2018), whereas the Sleep Profiler ambulatory device characterizes sleep using electroencephalography (Levendowski et al., 2017). TST served as a proxy to evaluate congruity amongst the sleep measurements and the data suggest inter-variabilities between the measurement methods. The discordance amongst the three sleep measuring methods is also evidenced in our study results, in which KD decreased TST measured by subjective responses and GENEActiv, but not with the Sleep Profiler. High inter-individual variabilities in sleep were also noted in our study population and evidenced by high standard deviations. Our study recruited both male and female AUD individuals and inter-sex differences may have contributed to the high standard deviations observed. Pre-clinical ethanol withdrawal studies have evidenced differential GABAergic and glutaminergic neurotransmission in male and females mice (Alele and Devaud, 2005), which has implications for sex differences in neurotoxicity/ excitotoxic brain damages (Hashimoto

and Wiren, 2008) and behavioral responses (Jury et al., 2017). Sex differences in sleep following alcohol intoxication or withdrawal have been previously reported in some (Arnedt et al., 2011), but absent in others (Kolla et al., 2020). Analysis of sex differences in KD-associated sleep alterations was limited in our study due to the small sample size. Despite such limitations, our study underscored the role of alcohol in disrupting sleep and highlighted associations between bioenergetics and sleep measures, which may have potential implication for treatment interventions.

Declaration of interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dadr.2022.100092.

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