Protocol Title: Ketogenic Diet (KD) in Alcoholism
Abbreviated Title: KD in Alcoholism
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Principal Investigator

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Total requested accrual: 100 subjects with the aim of completing 50 subjects total:
(50) Patients with Alcohol Use Disorder (AUD)

Project Uses Ionizing Radiation:	☒ No	☐ Yes
IND/IDE	☒ No	☐ Yes
Durable Power of Attorney	☒ No	☐ Yes
Multi-institutional Project	☒ No	☐ Yes
Data and Safety Monitoring Board	☒ No	☐ Yes
Technology Transfer Agreement	☐ No	☒ Yes
Samples are being stored	☐ No	☒ Yes

Flesch-Kincaid reading level of consent form: AUD = 8.0

Précis:

Alcohol intoxication leads to marked reductions in brain glucose metabolism that reflect in part the use of ketones (including acetate) as alternative energy sources by the brain during intoxication [1]. With repeated alcohol exposure both clinical and preclinical studies have shown a shift of brain substrate preference towards ketones [1, 2, 3, 4]. This has led us to question the potential value of a ketogenic diet in alcohol detoxification in order to prevent the ketone deprivation that would follow alcohol detoxification in alcoholics.

- ***Objectives****:* Here we propose a blinded randomized design to assess the effects of a ketogenic diet on symptoms of alcohol withdrawal and on brain function in alcoholics undergoing inpatient treatment of alcohol detoxification. We hypothesize that a ketogenic diet will increase acetate levels in brain resulting in improved brain function in alcoholics as well as a reduction of alcohol withdrawal symptoms during detoxification.
- ***Study population****:* Participants diagnosed with alcohol use disorder (AUD) as per DSM IV or DSM 5. Males and females ages 18 years and older will be included.
- ***Design****: This will include an inpatient component and outpatient follow-up.* Patients are admitted to the Clinical Center (CC) for detoxification, where they undergo treatment as usual (TAU) and will be randomized into a regular versus a ketogenic diet. Patients will be given benzodiazepines only if withdrawal symptoms emerge while receiving either the ketogenic or the regular diet. Within 2-6 days after admission, all patients will undergo an MRI (brain structure and function, functional connectivity and spectroscopy, i.e. MRS) and a battery of neuropsychological tests (NP). MRI scans will also be obtained in week 2. After 3 weeks of inpatient care the MRI scans and NP studies will be repeated. We will complete all study procedures in n=25 patients with AUD with the ketogenic diet and n=25 with the regular diet.
- ***Outcome parameters:*** Main outcome: To assess the effects of a ketogenic diet in patients hospitalized for the treatment of alcohol detoxification, on: (1) withdrawal symptoms including the need of medications to control them (benzodiazepines); (2) brain function as assessed by fMRI (at rest and during task conditions), (3) MRS, and (4) structural MRI. Secondary Outcomes: To assess the effects of a ketogenic diet on performance of cognitive tests, sleep, mood and craving.

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List of Abbreviations

AUD	alcohol use disorder
ADS	alcohol dependence scale
ASI	Addiction Severity Index
ATP	adenosine triphosphate
BOLD	blood-oxygenation level-dependent
CANTAB	Cambridge Neuropsychological Test Automated Battery
CBF	cerebral blood flow
CIWA	Clinical Institute Withdrawal Assessment
CMRGlc	cerebral metabolic rate of glucose
DDT	Delayed discounting task
EPI	echo-planar imaging
FTND	Fagerström Test for Nicotine Dependence
KD	Ketogenic Diet

MPQ	Multidimensional personality questionnaire
MPRAGE	magnetization-prepared rapid gradient-echo
MRI	magnetic resonance imaging
mI	myo-inositol
NADPH	nicotinamide adenine dinucleotide phosphate
NEM	negative emotionality
NP	neuropsychological tests
PEM	positive emotionality
RFC	resting functional connectivity
RS	resting state
SA	Standard American diet
TLFB	Alcohol Timeline Follow-back
TS	task state
VAT	visual attention task
VBM	voxel-based morphometry

1. Introduction and Background

Effects of Alcohol on Brain Glucose and Acetate Metabolism

Alcohol is widely consumed; approximately 56% of Americans 18 years or older drink alcohol at least once a month [5]. Although the majority of individuals use alcohol in moderation, approximately 7.1% are heavy drinkers (5 or more drinks/day on at least 5 different days in the past 30 days) [6]. Thus, understanding the effects of heavy alcohol drinking on the human brain is crucial for developing interventions to minimize potential adverse effects.

Brain imaging studies have shown that acute alcohol administration decreases glucose metabolism in the human brain, which initially was thought to reflect decreased brain activity [7, 8, 9, 10]. However, subsequent studies showed that even low doses of alcohol and minimal behavioral effects also decreased baseline brain glucose metabolism [11], and that alcohol-induced reductions in brain glucose metabolism were significantly larger than those induced by benzodiazepines at doses that induced sedation [12]. Moreover in alcoholics the glucose metabolic decrements triggered by acute alcohol were significantly larger than those in controls even when the symptoms of intoxication where much lower than in controls [12]. This prompted us to hypothesize that the reduction in brain glucose metabolism during alcohol intoxication reflected the increased utilization by the brain of the alcohol metabolite acetate as an energy substrate. Specifically, acetate, which serves as an energy substrate for astrocytes [13], is readily taken up into the brain, and though its concentration in blood is normally low, it rises significantly during intoxication and this is enhanced in heavy alcohol users (see below).

Indeed using PET and [^{11}C]acetate, we recently showed that during alcohol intoxication the brain increased metabolism of acetate while decreasing metabolism

of glucose an effect that was more pronounced in heavy drinkers (HD) than in controls [1]. Similarly, a recent magnetic resonance spectroscopy (MRS) study also reported greater acetate metabolism in the occipital cortex for heavy drinkers studied during sobriety than for controls [4], corroborating a shift to acetate metabolism that appears to persist beyond the acute intoxication state. Preclinical studies have corroborated that repeated exposure to alcohol increases the metabolism of acetate by the brain [2, 14].

Plasma Acetate levels during Alcohol Intoxication

During alcohol intoxication plasma acetate levels increase significantly as alcohol is oxidized to acetaldehyde by alcohol dehydrogenases and subsequently oxidized to acetate by aldehyde dehydrogenase [15]. Acetate is readily taken up by the brain and is metabolized by glia [13]. Under normal physiological conditions plasma acetate levels are constitutively low (about 0.2 to 0.3 mM) and glucose levels typically high (about 5 mM) so acetate brain metabolism is one order of magnitude lower than glucose metabolism [16, 17]. However, during alcohol intoxication the concentration of acetate in blood increases (around 1 mM) [18] to levels that could support 10-20% of the total brain metabolic rate [19]. The increases in blood acetate concentrations after alcohol intoxication are higher for alcoholics than for controls [20], which could facilitate their reliance on ketones as an energy substrate.

Ketogenic Diet for Alcohol Detoxification

The above findings led us and others to question the potential therapeutic benefits of a ketogenic diet in managing alcohol withdrawal and detoxification. The justification being that during alcohol detoxification as the acetate levels in plasma decrease, the brain may encounter an energy deficit state that might contribute to neurotoxicity and the enhanced excitability observed during the acute phase of abstinence [21].

To our knowledge no clinical studies have been reported, yet preclinical studies in rodents exposed chronically to alcohol have provided evidence of a beneficial effects of a ketogenic diet during alcohol discontinuation. Rodents chronically exposed to alcohol have higher levels of ketones (butyrate, lactate and the beta-hydroxybutyrate) in blood that can be used as substrates for energy generation though the Krebs cycle [22]. Thus, studies in rodents evaluated the effects of ketone on the suppression of alcohol withdrawal syndrome in rats exposed chronically to ethanol through intragastric administration (doses of 9-15 g/kg per day, over a period of 4 days). These studies showed that ketones suppressed the signs of ethanol withdrawal in the rats [22].

Evidence of Disrupted Brain Function in Alcoholics

Numerous NP studies have documented cognitive impairment in multiple domains in alcoholics including perceptuomotor, visuospatial and executive functions (working memory, attention control and response inhibition) [23] with some evidence of recovery of cognitive function with abstinence from alcohol [23, 24, 25]. Brain imaging technologies have revealed structural and functional changes in the brain of alcoholics including cortical atrophy, ventricular enlargement and white matter degradation [25, 26, 27, 28] with evidence that some of these changes recover with

abstinence [29, 30].

Functional imaging studies using fMRI have revealed disrupted brain activation during task processing, cues exposure or exposure to emotional stimuli [31]. These include: deficits in executive function associated with abnormal prefrontal activity [23, 32], deficits in decoding negative emotional facial expressions associated with abnormal rostral anterior cingulate activity [33], enhanced reactivity to cues associated with increased activation of reward (NAc, midbrain and ventral PFC) [34], and limbic regions (amygdala, hippocampus and insula) [35] and the cue reactivity has been associated with craving and clinical severity [36, 37, 38]. Some of these changes and has shown improve after detoxification and treatment [37, 39, 40, 41]. The delay discounting tasks (DDT) is also disrupted in alcoholics (as well as other substance use disorders), and involves a greater discount of delayed reinforcers [42][43] that reflect impairments in self-control [44] and associated with deficits in prefrontal and cortical limbic regions (insula/orbitofrontal cortex)[45, 46].

More recently studies using resting functional connectivity (RFC), showed disrupted connectivity in the default mode network (DMN) [47], and in the saliency network in alcoholics [48]. Magnetic Resonance Spectroscopy (MRS) can quantify a variety of neurochemicals, including various neurotransmitters and their byproducts (glutamate, glutamine and GABA) constituents of metabolic pathways (choline and creatine) as well as the glial marker myo-inositol (mI) [49]. In recently detoxified alcoholics, higher concentrations of mI in thalamus, anterior cingulum and prefrontal white matter [50] have been reported when compared to controls. However, to our knowledge there are no studies that have assessed if these changes recover with detoxification.

These studies providence evidence that chronic alcohol impairs brain structure and function and cognitive performance but they also indicate that some of these changes recover with detoxification. We hypothesize that some of the cognitive structural and functional changes reported in alcoholics reflect the effects of energy substrate deprivation that follows withdrawal from alcohol. Here we will test if a ketogenic diet can protect the brain from the negative effects of energy deprivation that we hypothesize occurs during alcohol detoxification. We hypothesize that this will result in a faster recovery of brain function.

Ketogenic Diet

Ketogenic diets are based on foods high in fat but very low in carbohydrates. Ketogenic diets are approved non-pharmacological interventions for the treatment of child epilepsy [51, 52], [Metanalyses:53, 54]. In addition to the clinical efficacy of a ketogenic diet in epilepsy in decreasing seizures [55, 56], the diet has also been shown to improve sleep in children with epilepsy [57], and improved executive functioning in elderly healthy volunteers [58]. There is also increased interest on the value of ketogenic diets for other conditions, including diseases of substrate insufficiency or of insulin resistance, diseases of free radical damage and disorders of hypoxia [59], and preliminary evidence suggests a potential therapeutic role of ketogenic diets in brain and other cancers, hypoxia or ischemic encephalopathy, stroke, heart failure,

Alzheimer's, Parkinsonism, amyotrophic lateral sclerosis, diabetes, autism, inflammatory disorders, migraine, severe hyperactivity [60, 61, 62].

Despite its clinical importance, studies on the effects of a ketogenic diet on the brain are scarce. Using MRS, the detection of cerebral β-hydroxybutyrate, acetone and acetoacetate have been reported in children with diabetic ketoacidosis during an episode [63], in a child with Ohtahara syndrome treated with a ketogenic diet [64], and in children with epilepsy on a ketogenic diet [65]. Further, an MRS study in patients with diabetic ketoacidosis showed increased myo-inositol and choline metabolism in gray and white matter; yet reduced N-acetyl metabolites in the parietal cortex, as compared to controls [66]. These studies suggest MRS to be sensitive for measuring ketone bodies in the human brain.

Ketogenic diets are generally very well tolerated and safe. However, they are not recommended in patients that have a history of metabolic disorders and in the presence of clinical complications such as kidney stones, dyslipidemia, liver disorders, gastroesophageal reflux, constipation, cardiomyopathy and chronic metabolic acidosis [67]. Most complications of the ketogenic diet include short-lived gastrointestinal disturbances, acidosis, and dyslipidemia [54].

Genetic Testing (genotyping)
We will collect blood samples to test for specific biomarkers associated with responses to the ketogenic diet. We will determine if polymorphisms in the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) genes predict the response to the ketogenic diet in alcoholics.

Innovation
This is the first study to evaluate the potential beneficial effects of a ketogenic diet for alcohol detoxification in alcoholics.

Summary and Significance
If beneficial effects of a ketogenic diet can be documented in the brain of patients with AUD, then this could be targeted as a therapeutic intervention to improve brain function and enhance success in recovery.

2. Study Objectives

a. Primary objectives

To assess the effects of a ketogenic diet in alcoholic patients hospitalized for the treatment of alcohol detoxification, on: (1) withdrawal symptoms, which will be measured using the Clinical Institute Withdrawal Assessment for Alcohol-Revised (CIWA-Ar), as well as with the quantification of medications required to control withdrawal symptoms (benzodiazepines); (2) brain functions as assessed by fMRI (rest and activation), (3) MRS and (4) structural MRI. We will complete studies in 50 alcoholics (25 given a ketogenic diet and 25 a regular diet).

b. Secondary objectives

To assess the effects of a ketogenic diet on cognitive performance, alcohol craving, sleep and mood.

3. Subjects

a. Description of study populations

Subjects include individuals diagnosed with an alcohol use disorder (AUD) that is moderate or severe and who are interested in treatment for their AUD. The accrual number for the total study will be 100, of which the estimated number to complete studies are 50 AUD; half of whom are randomized to receive KD food and shakes for up to 4 weeks while inpatient. We expect that 50% of subjects that undergo screening will not complete the study or don't fulfill criteria.

b. Inclusion criteria

Patients with AUD

1. Age 18 years and older.
2. Ability to provide written informed consent as determined by clinical examination and verbal communication. Capacity to consent will be determined by those giving the informed consent.
3. DSM-IV diagnosis of alcohol dependence or alcohol abuse or DSM 5 diagnosis of moderate or severe AUD (established through history and clinical exam).
4. Participants seeking treatment for their AUD (self-report)
5. Minimum 5-year history of heavy drinking (SAMSHA's criteria for heavy drinking: for men 5 or more drinks/day on at least 5 different days per month; and for women 4 or more drinks/day on at least 5 different days per month. [self-report]).
6. Alcohol specified as the preferred drug (self-report).
7. NIH employees with an AUD may participate in this study.

c. Exclusion criteria

1. Unwilling or unable to refrain from use, within 24 hours of MRI and NPT procedures, psychoactive medications or medication that may affect study results (e.g., analgesics containing narcotics, antibiotics [must finish course at least 24 hours prior to a scheduled procedure], antidiarrheal preparations, anti-inflammatory drugs [systemic corticosteroids are exclusionary], antinauseants, cough/cold preparations) (self-report, medical history). The following medications are allowable for entry on this study: analgesics (non-narcotic); antacids; antiasthma agents that are not systemic corticosteroids; antifungal agents for topical use; antihistamines (non-sedating); H2-Blockers/PPI (proton pump inhibitors); laxatives. The use of antihyperlipidemics and/or diuretics are permitted as long as they have been taken for at least 1 month before procedure visits and dose has been stabilized.

2. Current DSM-IV or DSM 5 diagnosis of a major psychiatric disorder (other than alcohol and nicotine use disorders, or substance use disorders that are mild/moderate) that required hospitalization, or that required daily medications for over 4 weeks in the past year (i.e., antidepressants; anticholinergics; antipsychotics; anxiolytics; lithium; psychotropic drugs not otherwise specified (nos) including herbal products (no drugs with psychomotor effects or with anxiolytics, stimulant, antipsychotic, or sedative properties); sedatives/hypnotics). Chronic benzodiazepine use prior to alcohol detox will also be excluded. Note that nicotine and/or caffeine use will not exclude participation.
3. Chronic use of the following medications: analgesics containing narcotics; anorexics (sibuteramine); antianginal agents; antiarrhythmics; antiasthma agents that are systemic corticosteroids; antibiotics; anticoagulants; anticonvulsants; antidiarrheal preparations; antifungal agents (systemic); antihistamines (sedating); antihypertensives (except angiotensin-converting enzyme (ACE) inhibitors such as Lisinopril, or Angiotensin receptor blockers (ARB) such as Losartan); anti-inflammatory drugs (systemic); antineoplastics; antiobesity; antivirals (except for treatment of HSV with agents without CNS activity, e.g. acyclovir, ganciclovir, famciclovir, valacyclovir); cough/cold preparations (dextromethorphan preparations, pseudoephedrine); hormones (exceptions: thyroid hormone replacement, oral contraceptives, and estrogen replacement therapy); insulin; and muscle relaxants.
4. Major medical problems that can impact brain function or the use of a ketogenic diet (e.g., epilepsy, diabetes, liver disease, kidney disease, kidney stones (current and/or in the past), chronic metabolic acidosis or a cardiomyopathy) as determined by EKG, history and clinical exam.
5. Clinically significant laboratory findings that could affect brain function (e.g. HIV+).
6. Head trauma with loss of consciousness for more than 30 minutes (self-report, medical history).
7. Pregnant or breast-feeding: Females of childbearing potential, or with tubal ligation, or are post-menopausal and are age 60 or less will undergo a urine pregnancy test and it must be negative to continue participation. Urine pregnancy tests will be repeated on subsequent days of study (i.e., within 24 hours before study procedures). Females must not be currently breastfeeding.
8. Presence of ferromagnetic objects in the body that are contraindicated for MRI of the head, fear of enclosed spaces, or other standard contraindication to MRI (self-report checklist).
9. Cannot lie comfortably flat on his/her back for up to 2 hours in the MRI scanner (self-report).
10. Body weight > 550 lbs. The MR scanner bed is tested to a weight limit of 550 lbs.
11. Milk or soy allergy (self-report).

Note that subjects will not be excluded on initial screening from enrollment onto this study if their breath alcohol test is positive; or if their urine test is positive for drugs. The following guideline will be followed for positive alcohol/drug screens on study procedure days:

- If an AUD subject's breath alcohol and/or urine drug screen test is/are positive on study days (i.e., within 24 hours before study procedures except for benzodiazepines during detox, including oxazepam [™Serax]), the procedures will be postponed and rescheduled to another day. If the urine drug screen is positive for THC-COOH, a saliva drug screen will be performed and subject may proceed with MRI/NPT procedures if saliva results for THC are negative. We will not place a limit on rescheduling study days.

4. Study Design and Methods

a. Study overview

This study includes an inpatient component and outpatient follow-up.

Patients are admitted to the Clinical Center (CC) for detoxification and treatment as usual (TAU) under protocol 14-AA-0181. Under the current protocol, patients will be randomized into a meal and shake plan consisting of either a standard American (SA) versus a ketogenic diet (KD). Patients will be given benzodiazepines only if withdrawal symptoms emerge while receiving either the KD or the SA diet. Subjects may be administered benzodiazepines only if withdrawal symptoms emerge. On day 2-6 after admission, all patients will undergo an MRI (brain structure, functional reactivity, functional connectivity and MRS) and will have a battery of neuropsychological tests (NP). Personality tests will be obtained during week 1 at the time of NP testing. The MRI scans will be repeated at 1 week intervals (Week 2 and 3). The Sleep Profiler device will be obtained 2X p/week in weeks 1-3. The ™GENEActiv (actigraphy) will be done for an entire week in weeks 1 and 3. The NP studies will be repeated coincident with the last MRI session. We will complete studies on at least 25 alcoholics with the ketogenic diet and 25 with the regular diet.

Procedures	**Week 1**	**Week 2**	**Weeks 3-4**
KD/SA Randomization (solid food and shake) 3 snacks and 3 shakes p/day until stopped – up to 4 weeks while inpatient	x	x	x
Neuropsychological test battery (NPT)	x		x
Personality tests	x		
MRI Scan session (structure, functional reactivity, functional connectivity, MRS) ~ 3 hrs	x	x	x
Sleep Profiler	2x	2x	2x

™GENEActive (actigraphy) – full week	x		X
Urine tests for ketones	Daily	Daily	Daily
Finger pricks for ketones	up to 5x	up to 5x	up to 5x
Blood tests for ketones and ghrelin	2x	1x	1x
Blood tests for fatty acids, liver function, platelets and coagulation	1x	1x	1x

Time points are targets, and the order can be adjusted for practical considerations during the inpatient stay.

Upon discharge from the NIH CC, participants in the study will be followed up for 3 months. For this purpose, we will call the participants by phone at weeks 1, 2, 4, 8 and 12 to ask questions about relapse and sobriety. This will take about 5 minutes or less. The following questions which are from the Alcohol Timeline Follow Back (ATFB) will be asked during the follow up:

i. In the last 7 days, did you drink any alcohol?; **Y___N___**
ii. If yes we then ask "On what days did you drink"?; and
iii. How much did you drink?

b. Recruitment

Participants will be recruited through referrals from the NIH Volunteer Office, the Patient Recruitment and Public Liaison (PRPL) Office, the NIAAA Intramural Research Program screening protocol 14-AA-0181 and through ResearchMatch.org and www.craigslist.com.

Participants (including NIH employees with an AUD) will also be recruited by word of mouth and through IRB-approved local advertisements. There will be no direct solicitation of employees by supervisors or coworkers. These will include: (1) Flyers posted on NIH campus, campuses of universities and colleges, public places and public transportation services in the greater Washington DC area; (2) Advertisements in electronic and printed local media (newsletters, websites including www.niaaa.nih.gov/join-study#, researchstudies.drugabuse.gov, newspapers including the Baltimore Beat, Baltimore Sun, the Beacon, Washington City Paper, Streetsense, the Express, craigslist.org, NIH and other local email distribution lists), following IRB approval, (3) through substance abuse treatment programs of clinics (e.g. Caron Treatment Facilities).

ClinicalTrials.gov may also represent a source of recruitment.

Participants will also be recruited via the NIAAA clinical program.

c. Telephone Pre-Screening

Initial contact with potential subjects will be done through use of a telephone pre-screening questionnaire. Subjects will be briefly interviewed over the telephone to determine that they report meeting inclusion and not meeting exclusion criteria

as described above. Subjects who appear to meet criteria will be scheduled for a screening visit. Subjects who do not meet criteria based on this telephone interview will not be scheduled for a screening visit and their telephone screening form will be shredded in a cross-cut shredder.

d. Admission and Initial Screening: Participant will be admitted to the NIAAA inpatient unit (under NIAAA protocol #14-AA-0181) for detoxification where they will remain for about 4 weeks. Participants will be screened under the protocol 14-AA-0181 titled "*Unit and Clinic Evaluation, Screening, Assessment and Management*" and the results will be shared with this protocol. All inclusion/exclusion tests will be done under 14-AA-0181. Tests may be repeated to assure eligibility. In addition, any test required to insure ongoing eligibility (pregnancy testing prior to MRIs for women of childbearing potential) will be done as necessary in subsequent visits under the ketogenic diet protocol.

The first step for our screening is to obtain informed consent for 14-AA-0181 prior to any procedures being implemented. Subjects will undergo a comprehensive medical history and physical examination by a clinically credentialed licensed independent practitioner. The purpose of the medical history and examination is to rule out serious medical conditions that may be contraindications to protocol participation. A copy of the medical evaluation will be placed in the subjects NIH chart. Screening procedures will include: Demographics; vital signs including height, weight and temperature; standard laboratory tests (Chem Screen, CBC, and Urinalysis); STAT urine tests to identify drug abuse and NP testing; a urine pregnancy test for females who are able to become pregnant; and an EKG. We will also interview the subject to obtain a lifetime drug history (including but not limited to everything from caffeine, tobacco, alcohol, and common illicit drugs) and we will administer self-report questionnaires, which provide descriptive data necessary to our brain imaging program. Some of the self-report assessments will be administered to evaluate the inclusion of participants as well as to provide descriptive data in all of our subjects. It is possible that subjects could be excluded from the ketogenic diet study based on the information they provide to us. If on the 14-AA-0181 admission and screening day the subject agrees to participate, they are given the informed consent for the ketogenic diet study.

e. Study procedures

Study Timeline: After admission and initial 14-AA-0181 screening, AUD patients who agree to participate will first sign the consent for this study and then begin to undergo detoxification in the 1SE unit under 14-AA-0181. The current protocol will involve being randomized into one of two diets. One diet consists of a ketogenic diet (KD) snacks and shakes 3x p/day (high in fat). The other liquid diet consists of a standard American (SA) diet shakes 3x p/day in the proportions of carbohydrates, protein and fat of traditional western diet. SA will receive the same KD solid snacks, in order to keep the diets blind to participants. Both diets will be eucaloric such that a standard equation based on weight, height, sex and

age will be used to determine how many calories are provided to each participant. While on the KD/SA diet, patients are limited to drink non-caloric drinks (water, diet soda, coffee and tea without milk or sugar). In addition to the diets, MRI/fMRI/MRS scans, genetic tests, self-report ratings, motor activity, sleeping behavior, NP testing and personality measures will be obtained in all participants. The first MRI session and the first NP testing will be performed within the first 2-6 days of admission. The MRI sessions will be repeated on a weekly basis (second and third week of hospitalization; the later corresponding to the last week of their inpatient stay) and the NP testing will be repeated during the third week of hospitalization. MRI scanning session and NP tests are described below and are for research purpose only.

During the inpatient period, participants will be periodically monitored for signs of withdrawal and evaluated for mood, sleep and alcohol cravings. Compliance with diet adherence (KD or SA) will be closely monitored during the hospital stay using daily urine tests.

After the final MRI sessions, the study diets are discontinued and the individual blind will be broken on a per subject basis. If KD is discontinued this will be done by slowly increasing the levels of carbohydrates to the diets of the SA group [68]. The levels of carbohydrates in the control group remains constant throughout the study, but patients are still blind to treatment group.

Participants will then be released at about 3-4 weeks from the Clinical Center and will have a 3 month follow up period under this protocol which is discussed below in section 4.d.12 *Follow-Up*.

1. **Ketogenic Diet (KD) / Standard American (SA) Snacks and Shakes***:* Participants will be randomized to a meal plan of either a ketogenic diet (KD) or standard American (SA) diet at the time they sign the consent (day 1 or 2 of admission). For each meal at breakfast, lunch and dinner, the diets will consist of SA meal (carbohydrate rich) or KD meal depending on treatment randomization. Compliance tests are done daily with urine tests measuring ketone levels by an independent person (BSN RN) [69], results of which are retained. To ensure that the diet is double blind in both the outpatient and inpatient phases, solid snacks are always ketogenic and shakes are either KD or SA.

   To measure the effectiveness of the ketogenic diet in increasing ketone bodies, we will measure ketones in urine daily as recommended for monitoring ketogenic diets in the management of epilepsy [70]. Specifically, we will measure acetoacetate (AA) in urine, in the early morning starting the day after initiation of the KD diet or the control diet and every day thereafter. We will use over-the-counter reagent strips (Ketostix, Bayer Vital GmbH, Leverkusen, Germany), which determine the presence of AA. Moreover,

beta-hydroxybutyrate (BHB) will be measured up to 5 times per week with a finger prick (Precision xtra, Abbott).

We will also measure beta-hydroxybutyrate and acetoacetate in blood at baseline and once each study week and free fatty acids once per week (no more than 8mL per week). These measures will be associated with MRS measures (e.g., correlate beta-hydroxybutyrate and acetoacetate in blood with β-hydroxybutyrate and acetone concentrations in the anterior cingulate and frontal white matter), and will provide information on potentially unwanted consequences of KD (e.g., potential disturbing effect of KD on free fatty acid concentrations). If the Laboratory of Medicine at the Clinical Center cannot provide quantitative measures for beta-hydroxybutyrate and acetoacetate, assays will be used from Wako Chemicals GmbH, Neuss, Germany). We will also measure plasma ghrelin (up to 6cc) at baseline and on a weekly basis.

Weekly blood draws are scheduled to monitor liver function (2.5 cc), platelet counts (2cc) and coagulation (INR, 3cc).

2. **Randomization and blinding:** The study has a blinded and randomized design. A randomization code will be generated prior to recruiting and patient randomization will be assigned accordingly. Since we expect a larger dropout in the KD group, we pre-set the ratio of KD:SA participants as 3:2 in the randomization generation.* Nurses and research staff who do CIWA, craving ratings and other study procedures with patients will not have access to the randomization key. An independent person (BSN RN) will perform and analyze daily ketone testing and blood pricks and will have access to the key. The nutrition department also has access to the randomization key to prepare the diets, but patients' diets will be sent out without information on its contents.* This ratio will be set for the first 20 participants. After the enrollment of 20 participants, the ratio will be evaluated by an dindependent person (BSN RN) based on diet compliance during their 3 weeks inpatient stay as per questionnaire and urine screen. Results are shared with the PI who will modify the ratio if needed to aim for a 50% KD versus 50% SA study completion.

3. **Diet Compliance:** In addition to daily urine tests (described above in section 4.e.1) and blood pricks, a daily questionnaire on whether additional food was consumed and whether the participant was compliant will be assessed by an independent person (BSN RN). On each day, participants will be asked if they were compliant to the diet (i.e. the shakes and snacks provided), or if additional food was consumed. If yes, which food items and at what time. At that time, they will also be encouraged to be compliant with the diet. All participants will be motivated on a daily basis to be compliant to their diet by nutrition department staff. If after the first 3 days of diet initiation a participant (in either KD or SA) self-reports non-compliance to the diet for 4

days, the PI will be informed and participant will be withdrawn and excluded from further study participation.

Additionally, if after the first 5 days of diet initiation, a participant in the KD group has 3 urine tests that are negative for ketone bodies, this participant will also be removed from the study. In this case, the independent person who monitors daily urine testing, will inform study staff about the compliance breach and the patient will be removed from the study.

In this way, all participants will remain in the study for at least the first 7 days and will be included in the analysis on the effects of KD on withdrawal symptoms, benzodiazepine use, MRI and NPT within the first 7 days. After 7 days participants may be withdrawn from the study based on non-complicance to the diet.

4. **MRI scans**: MRI evaluations of the brain are being performed at 3Tesla (3T) (Siemens Prisma). These MRI scans are performed on FDA-approved scanners with approved radiofrequency coils, and their use conforms to the corresponding FDA labels. The MR scanner is being used in normal mode (not research mode) using the following pulse sequences: magnetization-prepared rapid gradient-echo (MPRAGE), Prospective Acquisition Correction (3D PACE), diffusion tensor imaging (DTI), echo-planar imaging (EPI), arterial spin-labeling (3D ASL), and Magnetic Resonance Spectroscopy (MRS).

   MR acquisition: A high resolution T1-weighted magnetization-prepared rapid gradient-echo pulse sequence (MP2RAGE) will be used to map brain structure with high spatial resolution and excellent gray-white contrast and to map T1 relaxation in the brain. DTI, an MRI technique that can quantify isotropic and anisotropic water diffusion, will be used to map fractional anisotropy and the apparent diffusion coefficient, and to assess the structural connectivity of the brain. ASL, an MRI-based method that utilizes blood as an endogenous tracer and provides noninvasive quantification of tissue perfusion, will be used to map CBF in the resting state. BOLD-weighted functional images will be collected to measure brain activation and to map functional connectivity. *Brain activation paradigms:* We will use passive viewing of neutral, food and drug related pictures [40 pictures per category; the neutral, alcohol and food pictures will be selected from freely available online and in-house collections) [71, 72]. Pictures will also be rated for characteristics such as valence, liking and wanting. The stimuli are presented to the subjects on an MRI-compatible 32-inches LCD screen synchronized with the MR acquisition using an MRI trigger pulse.

   We will use the delayed discounting task (DDT) *to* evaluate effects on self-regulation. The DDT task has been used extensively [for review see [73]. It

allows measurement of BOLD response to immediate reward responses versus responses to delayed rewards.

Additionally, we will measure the visual attention task (VAT), which is obtained to assess the attentional network, which we and other have shown to result in reproducible and robust activation patterns and to be sensitive to cognitive impairment in substance use disorders [74, 75].

fMRI for Functional Connectivity: For functional connectivity we will collect three 8 min long RFC scans while subjects keep their eyes open (for resting scans this allows us to ensure patients don't fall asleep) while the subjects watch a fixation cross on a black screen. During the first minute of the RFC scans the subject will watch a 1 minute long alcohol or nature movie scenes or a black screen. An eye tracker will be used to measure subjects' gaze and pupil size during the RFC scan.

Eye blinking: as part of the fMRI procedures subjects blinking will also be monitored using an eye tracker [76]. Specifically, we will use a semi-automatic model to detect the eyelid contour from high-speed video data from an MRI compatible eye tracker. This method provides eye-blink measurements with a detection rate better than 90%.

MRS acquisition: Large voxels (8 cm^3) in anterior cingulate cortex and prefrontal white matter will be used for maximal SNR and minimal spectral linewidth. MRS data will be acquired using a point-resolved spectroscopy sequence (PRESS) with short echo time (TE/TR = 30/2000 ms). First and second order shimming will be used to maximize the magnetic field homogeneity in the voxel. The chemical shift selective CHESS technique will be used for water suppression. Water suppressed spectra will be analyzed using the commercial LC model program using a metabolite spectra database. An absolute quantification approach with the "brain water" amplitude as a concentration reference will be used during spectral fitting in LC model [77].

Repeating MRI/MRS scans - Because fMRI and functional connectivity scans are sensitive to excessive head movement, participants who generate unusable data (or if there is an equipment failure) during their scanning session may be asked to repeat the session. The likelihood of repeating MRI/MRS is small. However, if it becomes necessary we will only repeat one session.

5. **Ratings for Mood, Sleep, and Alcohol Craving:** Participants rate the quality of their sleep on a daily basis (estimated sleep length in hrs, depth: 1-10, rested 1-10). Patients are also asked questions about their mood on a scale of 1-10 (e.g., Alert, Tired, Happy, Energetic).

Prior to diet initiation, and then on each MRI day (up to 5 times in total), participants fill out the Desire for Alcohol Questionnaire [78] – a 14 item alcohol craving scale.

Mood will be assessed since dysphoria is frequently present during alcohol withdrawal and contributes to relapse [79]. We will obtain these measures since we want to assess if improving brain energetics would decrease withdrawal symptoms and improve brain function along with improved mood, and sleep and a reduction in alcohol craving.

6. **Motor Activity and Sleeping Behavior measured with ™GENEActiv:** To assess spontaneous motor activity we will ask participants to wear the GENEActiv accelerometer during their inpatient stay during two separate weeks. The recording from the GENEActiv will also allow us to determine the average number of hours slept per day and the subject's levels of spontaneous motor activity. The GENEActiv measures can be obtained prior to or after completion of the PET scans. The actigraphy will give us a measure of hours slept per day, which is of interest since insomnia is a key symptom of alcohol withdrawal [80].

   ™Sleep Profiler: Participants will be hooked up to the Sleep Profiler twice per week during their inpatient stay. Sleep Profiler is an ambulatory device providing a flexible and relatively easy way to detect sleep/wake stages compared to traditional polysomnographic devices. It is worn on the head, where the main part holds onto the forehead with plastic straps with 3 electroencephalogram (EEG) electrodes placed underneath. It also has two extension cables connected to two electrodes placed on the collar bones which measures Electrocardiography (ECG). With the inclusion of head position sensors and snore detection via microphone, this FDA approved device provides full scale detection of sleep status. The sleep measure is of interest since insomnia is a key symptom of alcohol withdrawal [80]. Preclinical studies have demonstrated a role of ketone bodies in sleep regulation [81], and a ketogenic diet has been found to improve sleep quality in children with epilepsy [57]. If KD also improves sleep in alcoholism, this may mediate improvement in alcohol withdrawal and brain function.

7. **Neuropsychological Tests:** We will obtain a NP battery of tests at baseline once it is determined that the subject meets eligibility and after 3-4 weeks of detoxification and ketogenic/control diet. This battery takes about 90 minutes. We will use the Cambridge Neuropsychological Test Automated Battery (CANTAB), which is a computerized battery of tests [82]. The CANTAB has normative data and has good reliability [83] and is sensitive to cognitive deficits in alcoholism [23, 84]. We will obtain the following CANTAB tests:
   - Reaction Time –Multiple choices reaction time test.
   - Stop Signal- it's a measure of response inhibition

- Intra/Extra-Dimensional Set Shift–Attention test, similar to the Wisconsin Card Sorting Test.
- Pattern Recognition Memory-Visual pattern recognition memory test.
- Stockings of Cambridge–Visuospatial planning test that is on the 'Tower of London' test.
- Spatial Span–Spatial working memory span test.
- Spatial Working Memory–This is a test of a subject's ability to retain and to manipulate spatial information in working memory.
- *Cambridge Gamble Task, test of decision making
- Affective Go/No-go – test for emotional bias
- Emotion Recognition Task–test to assess social cognition

* We selected the gambling task since it is disrupted in alcoholism and will serve as measure of risk taking and impulse control.

http://www.cambridgecognition.com/?gclid=CNH9qq-96roCFWrNOgodvHsA8Q

Personality Measures and Questionnaires: Within the first week of admission we will obtain the:

- *Multidimensional personality questionnaire (MPQ)*, which measures three main personality dimensions: Positive Emotionality or PEM (or extraversion), Negative emotionality or NEM (or neuroticism) and constraint [85]. These measures tap into characteristics associated with vulnerability to substance use disorders and correlate with specific patterns of brain activity [86]. The MPQ takes about 35-45 minutes to complete.
- *Morningness-Eveningness Questionnaire (MEQ)*, which provides a self-report circadian typology that is based on the preferred timing for activity, sleep/wake preferences, and optimal timing for mental alertness [87]. Responses to these items are considered to represent underlying circadian function.
- *The Beck Depression Inventory Second Edition (BDI-II)* is a 21-question multiple choice self-report inventory for measuring the severity of symptoms of depression as listed in the DSM-IV [88].

Personality measures with the MPQ will be collected to determine if they are associated with the biochemical measures. Our group has shown that MPQ's Positive Emotionality covaried with baseline glucose metabolism in the brain (i.e, orbitofrontal cortex and default mode network) [86] and that in alcoholics Negative Emotionality was associated with decreased expression of D2R in striatum in alcoholics [89].

8. **Genetic Testing (genotyping):** All participants will be asked to give a total of 10 ml of blood split into two vials containing 5 ml each (1 teaspoon) for

genetic research purposes. Providing blood for genotyping is optional. The genetic material, DNA, will be extracted from one of the samples and analyzed in order to identify polymorphisms in the genes related to alcohol metabolism. Specifically, DNA is collected in order to assess if polymorphisms in the alcohol dehydrogenase (ADH) and alcohol aldehyde dehydrogenase (ALDH) genes predict the response to the ketogenic diet in alcoholics.. Genetic testing will not influence diagnoses or treatment in AUD participant. Leftover DNA and the other 5 ml sample will be stored for future use.

9. **Plasma Ghrelin:** It was recently found that a KD intervention decreases plasma ghrelin levels and subjective appetite ratings in healthy volunteers [90]. This finding is relevant to AUD, since ghrelin levels have been positively associated with alcohol craving [91, 92, 93] and with alcohol consumption [94] in AUD. Therefore, we will also measure plasma ghrelin (up to 6cc) at baseline and on a weekly basis, and associate these scores with alcohol craving.

10. **End of diet questionnaire**
    At the end of study participation, patients will be asked to fill out an end of diet questionnaire:
    - Which diet did you think you were on? Ketogenic diet / Control diet
    - How certain are you about this answer? (0-10; not at all certain – extremely certain)
    - What makes you believe you were on the ketogenic or control diet? [..]
    - How pleasurable was it to be on the diet? (0; not at all – extremely)
    - Do you think your diet improved your health? (0; not at all – extremely)
    - Would you continue the diet if you could? Y___N___ Maybe if [..]

11. **Saliva THC screen**
    On days of MRI/NPT, if a urine drug screen is positive for THC-COOH, a saliva drug screen will be performed using the Draeger DrugTest 5000 (Draeger Safety Diagnostics, Lübeck, Germany). The Draeger DrugTest 5000 has a 5 µg/L THC screening cutoff, and has shown high diagnostic sensitivity (90.7%), specificity (75.0%), and efficiency (87.9%) [95, 96]. DrugTest 5000 test results were positive for a median of 12h after last cannabis (range: 4-24h) for occasional smokers, and 21h (range: 1-≥30h) for frequent smokers [97].

    The DrugTest 5000 test cassette is equipped with a polymeric non-compressible pad for oral fluid collection. Oral fluid will be collected by swiping the test cassette on the tongue and side of the cheeks. The test cassette collects 270µL±15% oral fluid, as indicated by the volume adequacy indicator. Oral intake (eating, drinking, cigarette smoking) will be prohibited 10 min before oral fluid collection. The analysis procedure takes about 5 minutes.

The saliva drug screens are for qualitative research purposes only and will not be stored.

12. **Follow-Up:** We assess participants post discharge at weeks 1, 2, 4, 8 and 12 to ask questions about relapse and sobriety. This will take about 5 minutes or less. The following questions which are from the Alcohol Timeline Follow Back (ATFB) will be used:
    In the last 7 days:
    - Did you drink any alcohol?; Y___N___
    - If yes we then ask "On what days did you drink"?; and
    - How much did you drink?

f. End of participation

While all steps are always taken to ensure quality data from all participants, we may ask participants who generate unusable data (or if there is an equipment failure) on one of their experimental sessions to repeat that session. This will have the effect of maximizing the risk/benefit ratio as it will prevent having to discard usable data from one session and will minimize the number of participants recruited overall to obtain sufficient data to meet power requirements. We may ask the participant to return at a later date or to repeat study procedures for genetic testing (genotyping) and/or MRI scans. Subjects will receive additional compensation if any MRI/MRS session scans are repeated. Compensation for repeated imaging scans will be the same as described in Section 23 *Research and Travel Compensation* table.

5. Management of Data and Samples

a. Storage

Data will be stored on an NIH data server, under the management of the ORIT, NIAAA. The existence and types of information contained in the data management system have been publicly reported as required by the FOIA.

Subject data will be stored in one of two locations. Fully identifiable data will be stored in the NIH Clinical Center CRIS system, where clinical patient data are routinely stored and adequate privacy protections are implemented by design. Additional data will be stored in the B2L124 NIAAA/LNI space in Building 10 on an access controlled NIAAA server located in secured space to prevent physical theft of storage media. We will feed some of the data residing in CRIS and on the NIAAA servers to the NIH Biomedical Translational Research Information System (BTRIS). Data on the NIAAA server will be kept in a coded form. The code key will be kept separately by the trial leader or designee.

Biological specimens obtained under this protocol will be stored in coded form (protocol identifier plus randomization number), in freezers located in Bldg 10 CRC Room 1528 area of NIAAA. We will collect 10 ml of venous blood from

each participant, which will be divided into 2 parts; one of which will be frozen and stored at -80ºC and the other to be used for DNA isolation and subsequent genotyping tests.

We will collect DNA so that in the future when new findings emerge with respect to genes implicated in brain diseases so we can assess across integrated data from various brain imaging studies if they are associated with changes in brain connectivity. However, DNA collection will be optional and will not exclude subjects from participating in this study if they do not want to give blood for genotyping.

b. Data including genomic data and sample sharing plan

Samples and data, including genomic data may be shared with collaborators.

The following questionnaires/assessments/interviews collected under the 14-AA-0181 will be shared with this protocol: Smoking History Questionnaire [98]; Fagerström Test for Nicotine Dependence (FTND) [99]; The Timeline Follow-Back (TLFB) [100]; Alcohol Use Disorders Identification Test (AUDIT) [101]; Barratt's Impulsivity Scale (BIS-11) [102]; Structured Clinical Interview (SCID) for DSM-IV or DSM 5 [103]; Alcohol Dependence Scale (ADS) [104]; and Clinical Institute Withdrawal Assessment – Alcohol revised (CIWA-Ar) [105].

Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Repositories receiving data and/or samples from this protocol may be open-access or restricted access.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

6. Additional Considerations

a. Research with investigational drugs or devices

N/A

7. Risks and Discomforts

Potential risks from this study include the following:

a. *Medical examination and Laboratory testing*: Medical examinations are associated with minimal risks. We will first explain and familiarize the subjects with the laboratory testing to minimize discomfort, if any, during testing. Tests may disclose that the potential subject's health is at risk. If there are abnormal tests uncovered as part of the physical examination, this information will be given to the subject and explained by a physician who will encourage them to seek medical evaluation with their primary care physician.

b. *Ketogenic diet (KD)*: Ketogenic diets are not recommended in patients with urolithiasis, liver disorders, cardiomyopathy and chronic metabolic acidosis [67]. Despite been a controversial treatment, the ketogenic diet has been studied as a treatment aid for dyslipidemia, obesity diabetes, epilepsy, brain tumors, autism, and neuroprotection with positive results. We will exclude participants with a history of urolithiasis and liver disorders other that mild alcoholic hepatic changes to decrease the risk to the participants. Liver function will be monitored during the study on a weekly basis as it is standard during the detox and treatment of alcoholism. The diet will be discontinued if there is a progression of liver damage after the detox is completed (3-5 x the upper-bound limit). KD may also have potential side effects, including GI discomfort and constipation, dyslipidemia, and low platelet counts and coagulation problems. Platelet counts and coagulation will be monitored on a weekly basis and the diet will be discontinued if platelet count < 100.000 or INR > 1.5. Free fatty acid levels in blood will also be monitored on a weekly basis. To facilitate compliance and decrease the burden on the participants the diet will consist of a primarily liquid diet (house-made by Nutrition Department), in combination with ketogenic solid snacks (e.g., eggs, meat, fish, vegetables). The meals will be provided after an assessment with the nutritionist. Most of our treatment seeking alcohol dependent subjects have chronic problems with malnutrition and have a very poor intake of solids while they drink. Therefore, the liquid diet was considered a better option to improve compliance. An additional benefit is that it provides a way to make patients unware of whether they are in the ketogenic- or control diet group. We will measure ketones on a weekly basis for diet compliance. If a subject is withdrawn from the study for non-compliance to the diet, he/she will be paid for procedures completed up to the point of withdrawal.

c. *Venous line placements*: Subjects may have some discomfort and bruising from the needle insertion. Some people feel light-headed or faint. The risks of an

intravenous catheter also include bleeding, infection, or inflammation of the skin and vein with pain and swelling. These will be treated if they occur.

d. *Blood sampling*: Blood draw volumes will not exceed the limits allowed by the NIH Clinical Center (Medical Administrative Policy 95-9: Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://internal.cc.nih.gov/policies/list_policies.asp?index=med_chrono). Blood sampling may lead to the formation of a small subcutaneous hematoma caused by blood leaking from a punctured blood vessel. This hematoma causes only minor discomfort. It is not dangerous and requires no treatment other than reassuring the patient. There is also a small risk of infection at the site of the needle puncture, which can be readily treated with antibiotic therapy. Subjects will be asked not to donate blood for a period of eight weeks after the participation is completed. The approximate amount of blood drawn in this protocol is as follows: Screening labs: 30 cc (obtained under 14-AA-0181); and Genotyping: 10 cc; plus up to 48 cc for research purpose to measure free fatty acid and ketone concentrations, 24 cc for ghrelin, and up to 23 cc for weekly liver function, platelet count and coagulation. Total amount over course of study not to exceed 140 cc.

e. *MRI Risks*: People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. Subjects will be screened for these conditions before having any scan, and if he/she has any, he/she will not receive an MRI scan. If a subject has a question about any metal objects being present in his/her body, he/she should inform the staff. In addition, all magnetic objects (for example, watches, coins, jewelry, and credit cards) must be removed before entering the MRI scan room.

It is not known if MRI is completely safe for a developing fetus. Therefore, all women of childbearing potential will have a pregnancy test performed no more than 24 hours before each MRI scan. The scan will not be done if the pregnancy test is positive.

People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. If the hearing protection comes loose during the scan, the subject should let a member of the staff know right away. The subject will be asked to notify the investigators if he/she has hearing or ear problems. The subject will be asked to complete an MRI screening form

for each MRI scan he/she has. There are no known long-term risks of MRI scans.

f. *Genotyping (genetic tests)*: The genetic testing that will be done as part of this study is for research purposes only and results will not be placed in the medical record. It will not provide any information about the participant's health or ancestry. It is our policy to not provide the results of such genetic testing to study participants. Problems, such as with insurance or employment discrimination, may occur if a participant discloses information about this genetic testing or if he/she agrees to have his/her medical records released. We will not release any information about a participant to any physician, insurance company or employer unless he/she signs a document allowing release of the information.

g. *Blood Banking For Future Genetic Studies*: This will allow us to repeat our genetic tests, thus to ensure and validate the genotyping results. In addition, we will be able to conduct analyses of new variations in the brain related genes that are yet to be discovered. Blood samples will be stored in secured freezers on the NIH campus. The participants name and identifying information will not be on the samples; we will assign them a code. The key to the code will be kept in a separate, secure area. The samples may be used for other research projects. If participants do not want his/her sample used for other projects, he/she should not participate in this study. If a participant withdraws from this research project before it is complete, any remaining samples he/she have contributed will be discarded. Results obtained before he/she withdraws will be kept and the participant's privacy will be protected.

h. *NP and personality measures*: These tests are not harmful, but may be frustrating or stressful. Subjects may refuse to answer any question that makes them uncomfortable and may stop a test at any time and for any reason.

i. ™*GENEActiv (actigraphy)*: Actigraphy is a non-invasive method of monitoring rest/activity cycles and is used to study sleep/wake patterns. It can be worn on a participant's wrist to continually record movement, therefore measuring gross motor activity. The data can later be transferred to a computer for analysis. There are no known risks of wearing the ™GENEActiv watch.

j. *Sleep Profiler Risks*: Sleep Profiler is a non-invasive method of monitoring sleep/wake cycles. It is a device that is worn on the head, where the main part holds onto the forehead with plastic straps with 3 electroencephalogram (EEG) electrodes placed underneath. It also has two extension cables connected to two electrodes placed on the collar bones which measures Electrocardiography (ECG). This device is FDA approved for the use indicated in the protocol. There is minimal risk associated with this device. The conductive gel and/or adhesive used to attach the electrodes to skin may cause mild irritation.

8. Subject Safety Monitoring

Subjects' safety will be monitored by the medical advisory investigator (MAI).

If any participant reports an adverse reaction to the experiment or demonstrates adverse responses during the post-experimental assessment of adverse events (AE), or appears to be in distress at any point during the experiment, the procedures will stop and the patient will meet with the medical advisory investigator to assess suitability for continuation in the study. Additionally, procedures will be stopped for any participant who asks to stop them at any point. The reasons for participants' discontinuation from the study will be logged and changes to procedures necessary to prevent future adverse reactions will be made.

Any individual may withdraw from the study at any time and with no penalty. This option is clearly stated in the consent form and will be emphasized to participants.

NCI Common Terminology Criteria for Adverse Events v. 3.0 will be used for grading and reporting adverse events (CTCAE, 2007).

Criteria for individual subject withdrawal

- Significant AE, including clinically significant changes in mood or behavior such as aggression, agitation, depression or suicidal ideation or suicidal behavior.
- Pregnancy
- At the discretion of the Principal Investigator (PI) and Medical Investigator, based on adverse event (AE) severity
- Non-compliance with protocol procedures or investigator request(s)
- Patient request

9. Outcome Measures

a. Primary outcome measures:

To assess ketogenic diet on:

1. Withdrawal ratings (CIWA) and Benzodiazepine use during withdrawal in the first week of detoxification.
2. Brain function (during rest and activation assessed with fMRI), neurochemistry (assessed with MRS) and structure (assessed with MRI).
3. NP performance.

b. Secondary outcome measures

To determine if ketogenic diet improves:

1. sleep.
2. mood.
3. alcohol craving.

10. Statistical Analysis

a. **Analysis of data/ study outcomes**

Withdrawal symptoms: We will use the CIWA-Ar to measure alcohol withdrawal, which will be measured daily [106, 107]. Because there are no studies on the KD on withdrawal symptoms we cannot predict the effect size to be expected. We will compare the scores on the CIWA-Ar between participants on the KD and participants on the control diet. Based on effects of medications used for treatment of withdrawal symptoms we expect a 30 % reduction in CIWA-Ar scores with KD [108].

fMRI data will be analyzed using standard software such as statistical parametric mapping (SPM) software, AFNI, FSL, and/or in-house routines. For a standard fMRI analysis, all image volumes will be pre-processed to correct for inter-scan movement, and realigned within and between scans. Each subject's EPI images will be co-registered with the subject's own T1 anatomical images for individual analysis or co-registered to a template, generated by the population under study or a standard template. These co-registered images will also be normalized to a standard EPI template such as the Montreal Neurological Institute (MNI) reference brain for potential group analysis. Statistical analyses will be performed using fixed effects (for individual data), conjunctions analysis (between and/or within subjects), and random effects model (for group analysis such as gender differences). The fMRI signal change produced by a task will be estimated relative to baseline activation, or in comparison with other task. Resting state or steady state scans will also be analyzed using standard or in-house software and network analysis might be performed. Statistical contrasts of activation maps may be analyzed with within-subjects t-statistics. The analysis may include single individual cases, a group of participants with similar characteristics, or a comparison between males and females. Region of interest or whole brain analysis will be based on functional or anatomical landmarks. Data may be combined with other modalities and behavioral results.

MRI data will be processed using adequate software such as SPM8, Freesurfer, Tortoise or DTI studio, to obtain, for each subject, images of gray and white matter, diffusion or perfusion maps. Images will then be compared using standard statistical parametric mapping software such as SPM, FSL, or AFNI, or even other more advanced programs as available. The statistical program will generate a group-specific adjusted mean value and an associated adjusted error variance for each voxel or region of interest that will be derived. Statistical significance may be established based on multiple-comparison corrections, permutations or simulations, in accordance with the analysis method selected. Data may be combined with other modalities and behavioral results. Accounting for multiple non-independent comparisons inherent in the analyses, a voxel level corrected P value < 0.05 will be used as the final threshold for significance. However, in line with functional imaging literature, results will also be reported as significant at a statistical threshold of $P < 0.001$, uncorrected, when a priori hypotheses require.

MRS data will be processed using specialized software packages such as LCModel, jMRUI or in house routines. These will be used to calculate ratios, such as GABA/Cr, NAA/Cr and, NAA/Cho. Anatomical images may be used to identify the ROI. Data may be combined with other modalities and behavioral results.

In case of group differences in severity of addiction or age due to randomization, we will add these variables as covariates to the analyses.

Plasma ghrelin

Weekly plasma ghrelin (total and acyl-ghrelin) will be analyzed using standard lab procedures [94]. Ghrelin levels will be compared between KD and SA group, and associated with weekly alcohol craving scores in both groups. We expect ghrelin to decrease in the KD versus SA group, and to be associated with the expected decreases in alcohol craving in KD.

b. Power analysis

Empirical knowledge regarding the necessary sample size for significant effects of a ketogenic diet on withdrawal, NP or brain functioning in AUD during withdrawal does not exist. Previous behavioral and pharmacological treatment studies in AUD with behavioral, fMRI and MRS as outcome measures have studied on average 20-25 patients per group [treatment versus control: reviewed in 109]. Clinical efficacy of a ketogenic diet in epilepsy was established in randomized-controlled trials in 20 [55] or 94 epilepsy patients [56]. In 20 elderly participants, an acute dose of ketogenic meal improved executive functioning 1.5-3 hours after, as compared to a control meal on a different day [58]. Further, beneficial effects of KD on sleep was established in a study in 18 children with epilepsy [57]. Thus, for within-subject fMRI studies in individuals with AUD sample sizes of 25 patients per group have been effective for obtaining interpretable data regarding behavioral and neurocognitive effects of treatment, suggesting that the sample size of the current protocol is adequate for the identification of effects of ketogenic diet in AUD.

Independent sample t-test will be used to test **Hypothesis 1**, "withdrawal symptom severity within the first week of detoxification will be lower for patients with KD compared to control diet".

Paired t-test on BOLD signals will be used to test **Hypothesis 2**, "limbic activation to alcohol cues would be reduced after 3 weeks of treatment"; **Hypothesis 3**, "the functional connectivity of the saliency network will be stronger after 3 weeks of treatment"; **Hypothesis 4**, 'prefrontal cortex atrophy and ventricular enlargement will stronger decrease after 3 weeks of KD treatment compared to control diet'. Assuming a threshold probability for rejecting the null hypothesis (Type I error rate), $\alpha = 0.05$, and a probability of failing to reject the null hypothesis under the alternative hypothesis (Type II error rate), $\beta = 0.2$, the

proposed sample sizes (25 KD versus 25 ND) will allow us to detect an effect size of 0.66.

To test KD treatment effects, mixed ANOVAs will be used to assess whether KD group shows reduced brain activation (Hypothesis 2), higher functional connectivity (Hypothesis 3), and prefrontal cortex atrophy and ventricular enlargement (Hypothesis 4) compared to the control diet group. Assuming $\alpha = 0.05$ and $\beta = 0.2$, the proposed sample sizes will allow us to detect an effect size of 0.9.

Pearson correlation will be used to test **Hypothesis 5**, "acetone and β-hydroxybutyrate concentration in the anterior cingulate and frontal white matter will increase linearly as a function of time week #1, #2 and #3". Assuming $\alpha = 0.05$ and $\beta = 0.2$, the proposed sample sizes will allow us to detect statistically significant correlation coefficients, $r > 0.6$.

From our past experience with neuroimaging of patients with AUD, it is expected that, even with careful screening and preparation, approximately 50% of participants will not be able to complete protocols with neuroimaging like the current one being proposed. Thus, we are proposing to recruit 100 participants in order to complete studies in 50 of them (25 on KD and 25 on SA diet).

*** We expect that more than 25 patients per group will finish the first week of KD and its assessment of withdrawal symptoms, and will therefore include all patients who finished these assessments to test Hypothesis 1.**

11. Human Subjects Protection

a. Subject selection

Adults who fulfill the eligibility criteria will be included, regardless of race, ethnicity, sex, or religious affiliation. We predict the racial composition is approximately 60% Caucasian, 35% African American, and remaining 5% proportion of Asian or other. We predict the Hispanic ethnicity to be approximately 25%. On the same basis, the expected breakdown is 70% male and 30% female. The age range of 18 years and older will provide a sample size that hits our target inclusion criteria of a minimum of 5 year of heavy drinking history.

b. Justification for exclusion of children

Children are excluded since it is unlikely that they will meet the ten-year criteria of heavy drinking required for the study.

c. Justification for exclusion of other vulnerable subjects

Vulnerable populations such as those who lack capacity to consent, are also excluded due to the likely inability to follow instructions for tasks to be performed during diet (KD or SA meal/shake protocol) randomization, MR scans and NP

testing. We also exclude individuals with major psychiatric disorders since this may affect brain function and structure, and may influence study results.

d. Justification for sensitive procedures
N/A

e. Safeguards for vulnerable populations and sensitive procedures
Vulnerable populations such as pregnant and/or breastfeeding women will not be studied due to the administration of a ketogenic diet, and MRI tests that have potential unknown risks to these populations. Urine pregnancy tests will ensure that MRIs are not done to women who may be pregnant and we will rely on self-report for women who are breast feeding and they too will be excluded.

Protections for employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "FAQs- Research Involving NIH Staff" which is in compliance with Policy 404 Research Involving NIH Staff prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff. Co-workers will not consent each other as a 3rd party monitor must be present when informed consent is obtained. Waivers are not permitted. This study collects sensitive information on drug/alcohol use, medical, neurologic and psychiatric histories. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

12. Anticipated Benefit

There is no direct benefit to the subjects participating in the study. The results of the study may improve our understanding of the mechanism underlying neurotoxicity in alcoholism.

13. Consent Documents and Process

a. Designation of those obtaining consent
All study investigators obtaining informed consent have completed the NIMH HSPU 'Elements of Successful Informed Consent' training.

b. Consent procedures
Participants will be consented on this protocol after they sign the 14-AA-0181 (UnCLESam protocol). They will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing. Either the PI or an AI will conduct the

consent process in accordance with NIH policy. Only subjects that can give informed consent will be included in this study. The PI and/or AI's will determine that the subjects are able to give informed consent based on their clinical assessment of the subject's ability to understand the nature of the study and the risks. If patient's BAL is more than 0 and/or if their CIWA is more than 8 at the time of consent, or if the subject's capacity to consent remains otherwise questionable, the Ability to Consent Assessment team (ACAT) will be consulted. HSPU advocates will monitor the informed consent discussion conducted between investigator and subject assessing subject capacity to enroll in the study and providing ongoing monitoring over the course of research participation. Co-workers will not consent each other if possible, but if a coworker does consent another, HSPU will monitor the consent process.

c. Consent documents

The consent form contains all required elements. The original consent is filed with their NIH medical record and a digital copy is uploaded into the CRIS system. A copy of the signed consent is also made for the subject's research binder for regulatory purposes.

14. Data and Safety Monitoring

a. Data and safety monitor

Data and safety monitoring will be monitored by the PI, who will review all safety data for each participant. The PI will consult with the Medically Accountable Investigator (MAI) should any concerns about safety arise.

b. Data and safety monitoring plan

This study will be monitored on a yearly basis at the time of continuing review. We will look at possible trends in AE's and UP's to determine if a change to the protocol/consent/study procedures becomes necessary via study amendment. In addition, individual subject case report forms will be monitored on an ongoing basis upon completion of study visits. Frequency of monitoring may increase if the PI determines it is necessary.

c. Criteria for stopping the study or suspending enrollment or procedures

This study will be stopped and enrollment suspended if any serious adverse events occur that is determined to potentially be related to one of the study procedures.

15. Quality Assurance (QA)

a. Quality assurance monitoring

The Principal Investigator, Lead Investigator and the MAI will be monitoring data collection and the study yearly or more often. Quality assurance (QA) will be performed by NIDA QA and NIAAA QA staff as indicated in the NIDA and NIAAA Quality Assurance Monitoring Plan on a schedule determined by the

Clinical Director. In addition to yearly monitoring of data collection, the Laboratory of Neuroimaging (LNI) utilizes a multi-layer study monitoring program. As a general practice, LNI lab staff records study procedures using a case report form (CRF) checklist on the day of each study. The study procedure checklist is completed on the day of study as well as within a few business days after the procedures were performed. Once a subject has completed all procedures per the protocol, a final study CRF monitoring log is completed for each participant. The CRF monitoring log captures that procedures were verified, data has been pushed to appropriate databases if necessary, questionnaire data entry verified if necessary, and the participant CRF folder monitoring log is signed by the LNI lab staff who verified the data. Following that process, each folder is monitored independently by other lab staff (i.e., post bac IRTA, clinical protocol coordinator, CRNP) as a final independent check that procedures were performed per protocol followed by signoff by the Principal Investigator.

b. Quality assurance plan

Quality assurance (QA) will be performed by the PI, as well as by an independent QA monitor, which according to established practice is considered sufficient for small, single-site trials, like the present project. The QA monitor for this study will have considerable expertise in clinical trials in drug dependence, but s/he will not work in the same section of the PI. Therefore, his/her role as QA monitoring will assure expertise and independence in the same time.

16. Reportable Events

Reportable events will be tracked and submitted to the IRB as outlined in Policy 801.

17. Alternatives to Participation

Participants neither receive nor forego treatment in order to participate in this study. The alternative, therefore, is not to participate.

18. Privacy

All research activities will be conducted in as private a setting as possible.

19. Confidentiality

a. For research data and investigator medical records

Medical records will be handled according to standard NIH Clinical Center policies, designed to prevent breach of privacy at a level considered sufficient for sensitive health care data. Investigators will be trained to respect the privacy and confidentiality of all participants including NIH employees and staff. Senior staff members (i.e., PI, MD, CRNP) will assure that all staff has completed the "Just in time" human subject's protection training course on "Biomedical- Vulnerable

Subjects-Workers/Employees" prior to engaging in recruitment of NIH employees. Privacy and confidentiality for NIH Employees will be adhered to the same as for other subjects. The certificate of confidentiality (CoC) that will be applied for this study will be used to resist demands for subject information as allowed by law. Staff will be reminded when an NIH employee has been recruited to use extra precautions so that identifying information will be protected. Confidentiality and information technology standards are in place at the NIAAA and NIDA intramural programs to protect electronic repositories of patient data as well as other clinical patient related material. It is reasonably expected that these safeguards will protect participants' medical and personal health information, ensuring their privacy.

Information obtained in the course of being screened for or participating in this protocol will become part of the patient's NIH medical record. This includes potentially sensitive information, such as results of urine tests that are positive for illicit drugs. Access to this information may be requested by third parties. Such access will not be granted without the explicit, written consent of the subjects. However, failure on the part of the subject to provide access to the information may in itself be to the disadvantage of the subject, e.g. in the case of a potential employer or insurer requesting the information. This situation will be made clear in the consent.

b. For stored samples

Samples and data will be stored using codes that we assign. Data will be kept in password protected computers. Samples will be kept in locked storage. Only study investigators will have access to the samples and data.

c. Special precautions

The Investigators are applying for a Certificate of Confidentiality (CoC) issued by NIH to further protect subject confidentiality.

20. Conflict of Interest

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

There are no conflicts of interest to report.

c. Role of a commercial company or sponsor

There is no drug company involved.

21. Technology Transfer

This study now has two separate data transfer agreements (DTA's) in place under the following docket numbers:

1. AA DTA 22-11002: This DTA is between UPENN and NIAAA/LNI. We will send deidentified (coded) MRI imaging data for further analysis which includes: MRS and fMRI time series and associated data as well as craving data in response to alcohol cues from the fMRI scan sessions to our collaborator at UPENN for analyzation. UPENN will send back the results and we will match it back up to the subjects on our end.
2. AA DTA 22-11006: This DTA is between Zhejiang University, China, and NIAAA/LNI. We will send coded MRI imaging data for further analysis which includes: fMRI resting state time series and associated data as well as craving data in response to alcohol cues from the fMRI scan sessions to Zhejiang University China, for analysis. Zhejiang University will send back the results and we will match it back up to the subjects on our end.

22. Research and Travel Compensation

Volunteers will be compensated for time and research-related inconveniences, to the extent to which they complete them, as follows:

Procedures	Inconvenience Units	$ Payment	Frequency	Total
Week 1 Study Procedures				
KD/SA Randomization Week	10	100	1	100
Genetics blood draw	1	10	1	10
Urine Drug Screen prior to MRI/NPT	1	10	1	10
Saliva Drug Screen prior to MRI/NPT	1	10	1	10
Urine pregnancy if female who can become pregnant prior to MRI/NPT	1	10	1	10
Ketone blood test #1 – obtained prior to start of KD/SA start	1	10	1	10
Ghrelin blood test #1 – obtained prior to start of KD/SA start	1	10	1	10
MRI session #1 (structure, functional reactivity, functional connectivity, MRS)	10	100	1	100
Neuropsychological Test Battery and Personality tests (NPT) including questionnaires Session #1	13	130	1	130

Sleep Profiler #1	10	100	1	100
Sleep Profiler #2	10	100	1	100
™GENEActiv #1 (entire week of wear)	10	100	1	100
Ratings for Mood, Sleep, and Alcohol Craving	7	70	daily	total of 70
Daily urine ketone test	1	10	daily	total of 70
Ketone finger prick	1	10	Up to 5x/week	maxl of 50
Ketone blood test #2	1	10	1	10
Ghrelin blood test #2	1	10	1	10
Liver function, FFA platelets/coagulation blood test #1	1	10	1	10
Week #1 – Total Compensation up to				**$910**
Week 2 Study Procedures				
KD/SA Randomization Week 2	10	100	1	100
Urine Drug Screen prior to MRI	1	10	1	10
Saliva Drug Screen prior to MRI	1	10	1	10
Urine pregnancy if female who can become pregnant prior to MRI	1	10	1	10
MRI session #2 (structure, functional reactivity, functional connectivity, MRS)	10	100	1	100
Sleep Profiler #3	10	100	1	100
Sleep Profiler #4	10	100	1	100
Ratings for Mood, Sleep, and Alcohol Craving	7	70	daily	total of 70
Daily urine ketone test	1	10	daily	total of 70
Ketone finger prick	1	10	Up to 5x/week	max of 50
Ketone blood test #3	1	10	1	10
Ghrelin blood test #3	1	10	1	10
Liver function, FFA, platelets/coagulation blood test #2	1	10	1	10
Week #2 – Total Compensation up to				**$650**
Week 3 Study Procedures				

KD/SA Randomization Week	10	100	1	100
Urine Drug Screen prior to MRI/NPT	1	10	1	10
Saliva Drug Screen prior to MRI/NPT	1	10	1	10
Urine pregnancy if female who can become pregnant prior to MRI/NPT	1	10	1	10
MRI session #3 (structure, functional reactivity, functional connectivity, MRS)	10	100	1	100
NPT including questionnaires Session #2 (Personality Measures will not be repeated)	13	130	1	130
Sleep Profiler #5	10	100	1	100
Sleep Profiler #6	10	100	1	100
™GENEActiv #2 (entire week of wear)	10	100	1	100
Ratings for Mood, Sleep, and Alcohol Craving	7	70	daily	total of 70
Daily urine ketone test	1	10	daily	total of 70
Ketone finger prick	1	10	Up to 5x/week	max of 50
Ketone blood test #4	1	10	1	10
Ghrelin blood test #4	1	10	1	10
Liver function, FFA, platelets/coagulation blood test #3	1	10	1	10
Week #3 – Total Compensation up to				**$880**
Potential Week 4 Study Procedures				
♦KD/SA Randomization Week	10	100	1	100
Week #4 – Total Compensation up to				**$100**
Total Compensation to all subjects up to paid on discharge				**$ 2540**
Telephone Follow-Ups				
Week 1 post discharge	1	10	1	10

Week 2 post discharge	1	10	1	10
Week 4 post discharge	1	10	1	10
Week 8 post discharge	1	10	1	10
Week 12 post discharge	1	10	1	10
			Total Compensation for F-Ups	**50**
			Total Compensation to all subjects up to paid on discharge	**$ 2590**

♦ Note that if KD continues into Week 4, subjects will be compensated.

Compensation will be paid to subjects on day of discharge, and separately for each follow-up call. Compensation will be prorated for parts completed if subjects do not complete the study. If a subject is withdrawn from the study for non-compliance, he/she will be paid for procedures completed up to the point of withdrawal. If needed, subjects will be provided with a taxi paid for by NIH. *If needed, MRI/MRS scans may be repeated due to excessive head motion or equipment failure, for which participants will be paid an additional $100 per MRI session. The maximum number of scans in the protocol is 5. If patients need to repeat other study procedures, they will be reimbursed for this according to the same inconvenience units for these procedures; e.g., $100 for 1 night of sleep profiler, $100 for GENActiv, $10 per urine ketone test.

NIH employees or staff who participate during work hours must have permission from their supervisor. NIH employees or staff must either participate outside of work hours or take leave in order to receive compensation.

23. References

See end of document.

24. Attachments/ Appendices

a. **Eligibility checklists**
Uploaded to iRIS.

b. **Recruiting advertisements**
Uploaded to iRIS.

c. **AUD medications list**
Uploaded to iRIS for IRB review.

d. **Eligibility Exclusion Medications checklist**
Uploaded to iRIS for IRB review.

e. **Study Day Medications checklist**

Uploaded to iRIS for IRB review

25. Consent Forms

- AUD consent uploaded to iRIS

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