PROJECT TITLE: **The effects of alcohol consumption on central adiposity and testosterone following weight loss in obese, pre-menopausal women**

INVESTIGATORS: Principal Investigators: John W. Apolzan PhD and Ursula A. White PhD
Co-Investigator: Corby K. Martin PhD
Medical Investigator: Frank L. Greenway M.D.

I. BACKGROUND AND SIGNIFICANCE

Alcohol (i.e. ethanol) is one of the most widely used recreational substances by humans and is consumed regularly by much of the U.S. population. Despite the high prevalence of alcohol intake, the metabolic health effects associated with use have not been firmly established. There is a paucity of data from longitudinal studies in humans that examine the metabolic response to routine alcohol consumption in a randomized controlled trial (RCT).

II. STUDY RATIONALE AND OBJECTIVES

This is a novel pilot study to examine, for the first time, the effects of ethanol consumption on fat distribution and testosterone during weight loss in a RCT. Findings from this study would provide insight into an interesting and unanswered question -- does routine alcohol intake exert unfavorable health effects despite the expected beneficial outcomes of caloric restriction and weight loss? This research may provide new knowledge of the metabolic outcomes resulting from alcohol intake and pathways that may be involved leading to potential new therapeutic targets of treatment.

The *objective* of the proposed study is to enroll women with obesity that will undergo a controlled, energy restricted feeding intervention to test the effects of chronic ethanol consumption on adipose distribution and circulating testosterone during weight loss. Women will be randomized to an ethanol-free control group or an ethanol-consuming group, and all will consume 30% energy-restricted diets.

III. STUDY POPULATION AND ELIGIBILITY CRITERIA

a. Subjects
   - We will enroll 12 participants (N=7 (Ethanol group) and N=5 (Control group)) using the inclusion and exclusion criteria described below. The Recruitment Core at Pennington Biomedical will recruit the women via listserv emails, community flyers, and health promotion events. We will recruit an ethnically diverse group based on the demographics of Baton Rouge.

b. Inclusion criteria
   - Pre-menopausal women only
   - 21-40 years of age
   - BMI 27-50 kg/m^2 (+ 0.5 will be accepted)
   - Must practice and be willing to continue to practice appropriate birth control (defined as a method which results in a low failure rate, i.e., less than 1% per year, when used consistently and correctly, such as double barrier methods [male condom with spermicide, with or without cervical cap or diaphragm], implants, intrauterine contraceptive devices, tubal ligation (surgically sterile), abstinence, or in an established relationship with a vasectomized or same sex partner) during the entire duration of the study

- Must be willing to adhere to all study procedures, including consumption of all study foods and beverages and attendance at all study visits
- Must be willing to eat at least one meal at PBRC 3 times per week on weekdays (excluding holidays)
- Must be willing to consume alcohol
- Must be willing to abstain from alcohol for 8-weeks if randomized to non-alcohol control group.
- Must be a daily, or almost daily drinker, defined as typically consuming at least 8 drinks per week, but no more than 4 per day
- Must be willing to undergo an overnight alcohol test (at home) of ethanol at the proposed dose before enrollment in the study
- Must have access to a device that can be used for video monitoring of compliance (i.e. Skype)
- Must be willing to have your blood stored for future research

c. Exclusion criteria
- Non-drinkers of alcohol
- Habitual binge drinkers, as defined by the consumption of ≥ 4 standard drinks per day or ≥ 28 drinks per week.
- Self-reported alcoholics or a history of alcoholism
- Have a 1st degree relative with alcoholism
- Any attendance or inpatient stay for alcohol or drug treatment
- Display any characteristics of current or future substance abuse disorders
- Presence of any psychiatric, behavioral, or medical disorder that, in the opinion of the PIs, Co-I, or MI, may interfere with study participation, the ability to adhere to the protocol, or has the potential for increased substance abuse
- Prescription medications that interact with alcohol intake
- Abnormal screening laboratory safety tests
- Smokers
- Diagnosis of Type 1 or 2 diabetes mellitus, cancer, or major organ disease
- Serious digestive disorders
- Conditions that affect metabolism or body weight (i.e. uncontrolled thyroid conditions, bariatric surgery, pregnancy, breastfeeding)
- Partial and/or full hysterectomy
- Hormonal pharmaceutical contraceptives including oral contraception (birth control pills), injectables (Depo-Provera), or the patch (Xulane)
- PCOS
- Use of medications that affect body weight or metabolism (i.e. atypical antipsychotics, weight loss medications).
- Not willing to store biospecimens for future use

IV. EXPERIMENTAL DESIGN AND METHODS

All study visits and clinic procedures will be conducted over the course of 1 year at Pennington Biomedical Research Center (PBRC) by trained nurses and research staff in accordance with standard operating and quality control procedures approved by PBRC's Institutional Review Board (IRB). This is a between-subjects parallel study design. All women will undergo a 30% energy restriction for 8-weeks and will be randomized to an ethanol-consuming group or a non-ethanol control group at the start of the study.

Table 1: Schedule of Study Events					
Tests	SV 1	Pre-Rand Visit	CV1	Intervention wk 1- wk 8	CV 2 (wk 8)
Informed Consent/Lifestyle Interview	X				
Height (SV1 only)/Metabolic Weight/BMI, Waist and Hip Circumference, Pulse, and Blood Pressure	X		X		X
Alcohol-related questionnaires (The Short Alcohol Dependence Data, SASSI, MAST)	X			X	
Pedometer Distributed/Collected		X		X (Wk8)	
Food Record/Washout Instructions		X			
Food Record Compliance Check			X		
Medical History/ Physical/ Medications	X				
Medications/Adverse Event Report			X	X(wkly)	X
Fasting Blood Draw*	X†		X*		X*
Questionnaires (retrospective VAS)			X		X
MRI, ^{1}H-MRS, FibroScan			X		X
Physical Activity (steps/day)			X		X
Alcohol Tolerance Test		X			
Randomization			X		
Controlled Feeding Dietary Intervention				X	
Body Weight				X	

CV, Clinic Visit; SASSI, Substance Abuse Subtle Screening Inventory; MAST, Michigan Alcoholism Screening Test; VAS, Visual Analogue Scale / † Chem 15, HCG / *Chem 26, insulin, free and total testosterone and archives

Study Visits

Screening Visit 1 (S1)

SV1 will be conducted the morning after a ~10-hr overnight fast. After acknowledging understanding of the study and all procedures, subjects will sign an informed consent. Demographics, vital signs, and medical history will be assessed. A physical exam will be administered. Anthropometric characteristics (i.e. height, metabolic weight, BMI, blood pressure, waist and hip circumference) will be measured. A blood sample will be collected for laboratory safety tests and menstrual cycle status will be determined.

A semi-standardized Lifestyle (diagnostic) Interview will be administered by trained behavioral staff (Master's Level Psychologists) and overseen by clinical psychologist and Co-Investigator Corby Martin, Ph.D. The interviewer will investigate any current or lifetime prevalence of substance use and will identify and exclude for psychological co-morbidities that preclude participation in the study. Substance Abuse Subtle Screening Inventory (SASSI) and Michigan Alcoholism Screening Test (MAST) questionnaires will be administered. All

participants will be screened and excluded for the presences of diagnosable mood and/ or personality disorders that would interfere with the ability to adhere to protocol. Structured Clinical Interviews (SCID-5 and SCID-5 PD) are semi structured guides to interview that enhance and standardize diagnosis or evaluation of various psychological disorders. The SCID 5 evaluates DSM 5 diagnoses, including mood disorders, psychotic symptoms, psychotic disorders substance use, etc.). The SCID 5 PD is an evaluation of 10 different personality disorders including: Avoidant Personality Disorder, Dependent Personality Disorder, Obsessive-Compulsive Personality Disorder, Paranoid Personality Disorder, Schizotypal Personality Disorder, Schizoid Personality Disorder, Histrionic Personality Disorder, Narcissistic Personality Disorder, and Antisocial Personality Disorder. Test scores from the questionnaires will be thoroughly analyzed by Dr. Corby Martin and his team followed by a more detailed discussion with the subject. Participants that display any characteristics of substance abuse or mental disorders will be excluded. The interview will also ascertain barriers to participation in the study, including participants' willingness to commit to the study, potential scheduling conflicts, support from household members to participate in the study, motivation and challenges facing the participant, details of the randomization and intervention, and other study-related issues of importance. This information will be used to identify and exclude from enrollment participants who will not be capable of completing the study. The results from the diagnostic interview will be used to provide an overall eligibility assessment for each candidate. A multidisciplinary team consisting of behavioral experts, dietitians, clinical staff, Medical Investigator (Frank Greenway, MD), and study staff will discuss and approve a candidate for official admission to the study. If eligible, the participants will undergo a Pre-Randomization Visit, which will include a mandatory one-night test of ethanol consumption at the proposed dose to assess alcohol tolerance.

Pre-Randomization Visit

Before enrollment in the study, all women will be required to undergo an overnight alcohol tolerance test at the required ethanol dose. Participants will receive the required ethanol dose at the pre-randomization visit along with instructions for consumption. Participants will be monitored via video chat technology (Skype) by study staff from the time the person starts drinking to 1-2 hours after they have finished drinking the alcohol. For minor adverse events experienced by the subject, such as headache, nausea, diarrhea or other similar issue, that are conveyed to the study staff, one should call the medical investigator. In the unlikely event that the person experiences serious adverse events and becomes agitated and destructive, or otherwise being out of control and a danger to herself and others, or if the person becomes so sedated that she is no longer responding to conversation in the audio portion of the Skype and is becoming unconscious, these would be indications to call 911. Women will be instructed to do a one-week alcohol-free washout. Women will be administered a dietary record and step log (pedometer provided) and asked to return these completed items at Clinic Visit 1. Pending adherence to the overnight alcohol test, the one-week ethanol-free washout, and the return of a dietary record, participants will undergo Clinic Visit 1.

Clinic Visit (CV) 1 and 2

At CV1 and 2, women will report to PBRC after an overnight fast during the luteal phase of the menstrual cycle. Anthropometric characteristics will be measured. Body composition scans (MRI, ^{1}H-MRS, and FibroScan) and blood draws (Chem 26, insulin, testosterone (free and total)) will be performed. A sample of blood (serum and plasma) will be collected for archive. The appetite questionnaire will be administered. Participants will be randomized to the control or ethanol group and begin the dietary intervention.

8-Week Dietary Intervention

Participants will be provided all meals that are prepared by the PBRC metabolic kitchen (5-day meal rotation) and given both verbal and written instructions as to the consumption of the

food. On weekdays, at least one meal (i.e. breakfast, lunch, or dinner) will be consumed at PBRC 3 times per week, (excluding holidays), whereas the other weekday meals will be packaged for takeout. In addition, alcohol, weekend, and holiday meals will be packaged for takeout. Participants' energy requirements will be determined from their estimated resting metabolic rate (RMR), calculated via the Mifflin-St. Jeor formula, with an activity factor of 1.5. The energy requirements will then be multiplied by 0.70 so that all participants will restrict energy needs by 30% compared to weight maintenance. Each group (control and ethanol) will have the same 30% reduction in calories and will consume the same percentage of each traditional macronutrient (20% protein, 50% carbohydrate, and 30% fat). The ethanol group will consume a 30% energy restriction diet that will also include ~2.5 standard drinks, or 35 grams of ethanol, administered as 80-proof distilled spirits (e.g. 80 proof gin, rum, vodka, whiskey, or tequila). In the United States, one "standard" drink contains roughly 14 grams of pure alcohol. Caloric differences between the spirits will be accounted for by being administered on specific days of the meal rotation so that the appropriate mixers are provided. The remaining calories (Mifflin-St. Jeor formula, with an activity factor of 1.5 multiplied by 0.70 minus the ~240 kcals from ethanol) will be consumed as 20% protein, 50% carbohydrate, and 30% fat. The control group will consume these ~240 kcal as included in the aforementioned macronutrient percentages (20% protein, 50% carbohydrate, and 30% fat).

Participants will be asked to have an in-clinic check in once per week when coming for their in-house meal. At this check a metabolic weight will be obtained, adverse events and changes in medication will also be assessed. The ethanol group will be also be administered the alcohol-related questionnaires.

Instructions for Alcohol Consumption

The alcohol will be treated with additional oversight and will be purchased, logged, handled, and dispensed by the PBRC pharmacist. Women randomized to the ethanol group will be provided detailed verbal and written instructions regarding the consumption of alcohol. Participants will be provided with a list of the specific types of alcohols that are permitted. Measured doses of alcohol will be prepared by the PBRC pharmacist. Sealed, individual bottles (~7) of alcohol will be dispensed to the participant once per week (Monday; excluding holidays) through PBRC clinic. Each bottle will contain a pre-measured dose of alcohol, and participants will drink one bottle per day of the week. The subject will be instructed to consume the alcohol over the course of a couple hours at home (not strictly before bedtime) so as not to interfere with important daily tasks and to avoid periods where it is necessary to drive. She will be given general information about alcohol and informed of the effects of its use on the body, including impaired coordination, judgment, alertness, and diminished quality of sleep. The participants will be warned of the risks associated with performing daily activities and operating vehicles or other machinery after alcohol ingestion. Subjects will also be provided information about the warning signs of alcohol dependency.

Dietary and Alcohol Compliance

Compliance with consuming the study meals and alcohol will be monitored. The consumption of select meals and the ingestion of all alcoholic drinks will be monitored by study staff via video technology (e.g. Skype, cellular phone device) designed to quantify dietary compliance. A daily diary will be completed by the women each day to document the time and amount of the study alcohol consumption. This diary will be returned at the Monday clinic visit (excluding holidays). Participants will also complete a food and alcohol consumption log at the daily study visits. Subjects will be instructed to report if any additional food, beverages, and alcoholic drinks were consumed. Subjects who are under-compliant (<85%) or over-compliant (>100%) will be counseled by study staff on the importance of compliance. Repeated (i.e. >2 events) under- or over-compliance will result in the participant being dropped from the study.

Body weight will be monitored weekly during the intervention. All collected data will be documented. Collectively, these methods will be implemented to enforce dietary compliance during the intervention.

Participant Safety

Subjects in the ethanol group will be closely monitored and frequently assessed throughout the study to identify any signs of alcoholism. A questionnaire (The Short Alcohol Dependence Data Questionnaire) will be administered weekly to evaluate the possibility of alcohol dependency. We will also re-administer the SASSI and MAST questionnaires. Study staff will verbally correspond with the participants weekly to assess any adverse events and ascertain any undesirable changes in mood or daily routine that may be associated with alcohol consumption. The Medical Investigator will have oversight through the duration of the study.

If any participant exhibits signs of alcohol dependency issues during the study based on self-report or the weekly questionnaires, a more comprehensive assessment (i.e. interview) by trained study staff and the medical investigator will be done. The subject will be withdrawn from the study and referred to a substance abuse center for treatment.

At the end of the intervention, participants will be contacted (phone) by trained study staff at 2 weeks and 4 weeks after completion of the study in order to assess if the women have acquired any alcohol abuse and/or dependency issues. If the subject exhibits any alcohol-related issues, she will be referred to a substance abuse center for treatment.

Study Procedures

Blood Draws: Blood will be collected at screening (Chem15, HCG), as well as at Clinic Visit 1 and Clinic Visit 2 (Chem26 + insulin, testosterone (free and total)). Blood (serum and plasma) will also be archived at the CV1 and CV2 time points.

Magnetic Resonance Imaging (MRI): scABD AT and VAT volumes will be defined and quantified with magnetic resonance imaging (MRI) using a 3.0T scanner (GE, Discovery 750w) by obtaining ~581 images from the dome of the liver to the pubic symphysis. Images will be analyzed by a single trained analyst.

^{1}H-Magnetic Resonance Spectroscopy (MRS): Intrahepatic lipid will be measured by ^{1}H-MRS on a 3.0T whole body imaging and spectroscopy system (GE, Discovery 750w) with a commercially available ^{1}H body coil. IHL content will be determined with jMRUI (Java-Based Magnetic Resonance User Interface), and IHL peak areas will be expressed relative to the peak area of an external phantom of known constant concentration (peanut oil signal).

FibroScan: Liver assessments will be obtained using a FibroScan ultrasound transient elastography device. The participant lies supine, a lubricating gel is applied to the skin at the level of the liver, and a hand-held probe is pressed against the gel as is done in a traditional liver ultrasound exam. Ultrasound images of the liver are obtained, guided by the FibroScan user interface. From these images, quantitative measurements of liver fat content and stiffness are obtained and uploaded to the study database. The procedure takes ~20 minutes.

The FibroScan procedure can identify clinically-significant levels of liver fibrosis and/or steaosis. A result deemed significant by the medical investigator of this study will trigger a clinically actionable incidental finding.

Radioimmunoassay (RIA): Testosterone levels will be measured by standard radioimmunoassay reagent sets.

Physical Activity: We will collect physical activity data with a pedometer to determine the number of steps per day.

Appetite Questionnaire: Retrospective Visual Analogue Scale (RVAS) will be used to measure average ratings of appetitive sensations that participants experienced over the past week (17,18). This validated method of collecting VAS data has been found to be consistent with daily assessments of satiety.

V. POTENTIAL RISKS AND BENEFIT ASSESSMENT

The known risks, inconveniences, or side effects from the proposed procedures in the project are shown in Table 2.

Table 2. Potential Risks and Efforts to Minimize the Risks to Human Subjects

Procedure	Potential Risks	Efforts to Minimize the Risks
Ethanol (alcohol) Consumption	Alcohol intake is associated with a variety of health risks, including high blood pressure, liver disease, impaired cognitive function, weakened immune system, and certain types of cancers. Other risks include drowsiness, malaise, mood and behavior changes, depression, headaches, impaired coordination and judgment, and the potential for addiction and dependency. Drinking alcohol also increases the risk of short-term harms, including motor vehicle accidents and injuries.	Before enrolling in the study, several measures will be conducted to minimize risks including the following: stringent inclusion/exclusion criteria; SASSI and MAST substance abuse screening questionnaires, a diagnostic interview by a trained behavioral psychologist, and an overnight alcohol test. Additional safeguards and alcoholism screening tools will be implemented frequently throughout the duration of the study. To reduce the risks of alcohol-related harms, participants will be instructed to consume the alcohol over the course of a couple hours at home (not strictly before bedtime) and not while driving or operating machinery. Participants will also report any adverse events or undesirable changes in mood or behavior throughout the duration of the study. Participants will be monitored for non-compliance, as the consumption of additional alcoholic drinks beyond what is required for the study may exacerbate the risks associated with alcohol use.
Blood Draws (Fasting)	Bruising, bleeding, pain, and infection pose minimal risks.	Trained phlebotomists and personnel will use sterile technique.

Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS)	There are no known biological risks associated with magnetic resonance scanning. There is a minimal risk for side effects, such as feelings of claustrophobia or muscle-skeletal discomfort from lying partially in the magnet for up to 45 minutes. There may also be loud, unpleasant noise. The magnetic field can move any metallic objects and disrupt the function of certain electronic devices that are inside the body. The long-term risk of exposure to a magnetic field is unknown, but considered extremely low.	Pillows will be used to ensure comfort during the testing. Earplugs or headphones are provided to reduce the noise. Certain implants, electronic devices, or any other foreign metallic objects in the body will not be permitted.
FibroScan	There may be minor discomfort from the application of the gel and pressure on the skin from the Fibroscan probe. However, there are no known health risks associated with the FibroScan.	
Confidentiality of Data	Taking part in this research may involve providing information that one considers confidential or private. There is a slight risk that data could be revealed inappropriately or accidentally	Study researchers and staff will take steps to protect data that is collected. Efforts, such as coding research records, keeping research records secure and allowing only authorized people to access research records, will be made to keep the data safe. The SCID-5, SCID-NP, SASSI, and MAST data will not be stored in Medical Records. Rather, this information will be stored in locked file cabinets in the Ingestive Behavior Laboratory, which is under the directorship of Dr. Martin,who will supervise the delivery of these instruments and, in collaboration with the Medical Investigator, Dr. Greenway, interpret the instruments.
Archive of Biological (Blood) Sample	The primary risk to participants who have blood banked for future research is the risk of loss of confidentiality and/or privacy. These blood samples will be stored indefinitely. The samples may be given to other investigators for future research as well. The future research may not take	For privacy and confidentiality, the biospecimens will be labeled with a unique series of letters and numbers. The research done with the specimens may help to develop new products in the future, or may be used to establish a cell line or test that could be

	place at Pennington Biomedical Research Center and may not be reviewed by Pennington Biomedical Research Center's Institutional Review Board.	patented or licensed. Subjects will not receive any financial compensation for any patents, inventions or licenses developed from this research. Storage and disposal of biospecimens will be conducted in a manner conforming to the appropriate care and handling of biological specimens as outlined through the Institutional Biohazard Committee Guidelines.

Protection Against and Minimizing Risks. While there are some potential risks for the proposed study, the design makes every attempt to prevent the possibility of any adverse events by the activities listed in Table 2, last column. Monitoring by the experienced investigators and professionals involved in the study will minimize all potential risks. By mitigating the potential risks, the risks that are associated with the proposed procedures are less than the potential benefits to be gained by the participants.

Potential Benefits to Human Subjects.

Benefits. Subjects will undergo an 8-week, 30% calorie-restricted feeding intervention that will result in substantial weight loss and potential improvements in metabolic health. Participants will also receive important information about their health (i.e. adipose tissue distribution and insulin sensitivity).

Compensation for participation. After completion of the study, participants will be compensated $250. Subjects that do not complete the entire study will be compensated $50 for the successful completion of CV1, $100 for the successful completion of the intervention, and $100 for the successful completion of CV2.The total $250 is determined according to standard policies for procedures done at PBRC.

Data Collection, Storage and Subject Confidentiality.

Protection of Subject Privacy and Stored Data. Protection of subject privacy will be accomplished by a variety of stringent security measures. Physical examinations will occur in an enclosed, private area. All health history and exam results will also be discussed in a private area. All medical records will be stored in locked areas, and access to these areas is limited to the clinic support staff, the Director of the clinical facilities, PIs at PBRC, and authorized designees of the PI. Volunteer medical records will be filed according to an assigned volunteer ID number. Further, the SCID-5, SCID-NP, SASSI, and MAST data will not be stored in Medical Records. Rather, this information will be stored in locked file cabinets in the Ingestive Behavior Laboratory, which is under the directorship of Dr. Martin who will supervise the delivery of these instruments and, in collaboration with the Medical Investigator, Dr. Greenway, interpret the instruments. This process is in place to prevent the unintended release of psychological information should a copy of a participant's medical records be requested. The only people who will know that these patients are research participants are members of the research team. No information about them, or provided by them during the research, will be disclosed to others without their written permission, except if it is necessary to protect their rights or welfare (for example, in case of injury or emergency care), or if it is required by law. When the results of the research are published or discussed in conferences, no information will be included that would reveal the identity of the individuals. Participants will be identified by codes when the data gathered in this procedure is presented or published. Authorized representatives of the NIH may

need to review records of individual participants. As a result, they may see their name; but they are bound by rules of confidentiality not to reveal the participants' identity to others. Electronic data is stored in a secured Microsoft SQL database. Only authorized IT personnel in Computing Services have access to the database.

Database Protection. Electronic data storage is secured with password protection and similarly restricted with only the PI and authorized persons having access to databases containing confidential clinical records, i.e. those containing name or other identifying information. Electronic communication will involve only unidentifiable information.

Participant confidentiality. PBRC complies with the federal 1996 Health Insurance Portability and Accountability Act (HIPAA). Specifically, PBRC protects the privacy and confidentiality of medical records and information contained in medical records of persons who are subjects of research projects, including all protected health information (PHI) as defined by the HIPAA privacy Regulations. PHI of research subjects and the use or disclosure of such information is governed by PBRC research policies as well as Common Rule, FDA regulations, and other applicable laws.

PBRC and study PIs (the persons chiefly responsible for the record) protect the privacy of research subjects and their PHI collected during a research project. PBRC will not use or disclose existing PHI or PHI created during a research project, unless the:

- Subject signs both (a) a HIPAA Authorization for use and disclosure of PHI using an approved Authorization Form or other form containing all the elements of legally effective HIPAA authorization; and (b) the informed consent to participate in research form approved by IRB; or
- IRB grants a waiver to the requirement of obtaining a signed HIPAA authorization Form, or
- IRB approved protocol uses properly de-identified PHI

All participants are assured of their anonymity and confidentiality both verbally and in the informed consent.

Data and Safety Monitoring Plan (DSMP)

- Description – The DSMP has been developed to provide ongoing oversight and monitoring of the proposed study to ensure and maintain:
    a) The scientific validity and integrity of the data so that new unbiased scientific knowledge is obtained
    b) Protocol compliance
    c) The safety of the participants (The study staff will remain in contact with the participants throughout the study. The participants will have the PIs' and study coordinator's work telephone number and the after-hours number to allow participants to contact them in case of an emergency.)

- Data and Safety Monitoring committee - A local group of scientists at the Pennington Biomedical Research Center (PBRC) will perform the Data and Safety Monitoring (DSM). It includes:
    a. Members of the Investigator team:
        i. The PIs: Ursula White, Ph.D. and John Apolzan, Ph.D.
        ii. The study coordinator
        iii. The Medical Investigator: Frank Greenway, M.D.
        iv. The Bio-Statistician: Robbie Beyl, Ph.D.
    b. Independent individuals who are not directly involved in the study:
        i. Independent Monitor: TBD

- Meeting structure - These meetings will be open sessions, at which all committee members will be present. The main focus will be on accrual, protocol compliance, review of lab data and review of all AEs/SAEs. No outcome results will be discussed except after completion of the data collection.
    a. The DSM committee will meet once every 3 months. (Note: An ad hoc meeting will be held with Dr. Greenway in case of a serious adverse event or death.)
    b. Minutes will be recorded by the study coordinator and will be forwarded to all members of the committee.
    c. The Independent Monitor will:
        1. Identify any deficiencies in the DSM plan and advise solutions
        2. Prepare a report reflecting the results of the reviews and discussion by the DSM committee.

- Data Quality and Management - The PI or study staff will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance.

- Safety Monitoring
    a) *Study risk assessment* - A description of the anticipated risks (i.e. adverse events) related to the procedures and efforts to minimize the associated risks are shown in Table 2 and will be included in the consent form.
    b) Definitions

        - Adverse events (AE) are defined as expected adverse events if they are grade 2 and above in severity and unexpected side effect/events of any severity category. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.) or any combination of these.

        - Serious Adverse Event (SAE) is any adverse event that results in one or more of the following outcomes:
            - Death
            - A life-threatening event
            - Inpatient hospitalization or prolongation of existing hospitalization
            - A persistent or significant disability/incapacity
            - A congenital anomaly or birth defect
            - Important medical event based upon appropriate medical judgment

    c) Plan for AE detection and documentation
    Flexible monitoring via interactive contacts
        - The study staff will remain in contact with the participants throughout the study. The participants will have the PI, Medical Investigator, and study coordinator's work telephone number and the after-hours number to allow participants to contact them with questions or in case of an emergency related to the alcohol.

    Fixed visits
        - At the start and end of the study, participants will come to the PBRC when routine biochemical blood tests are performed to exclude major pathology. In addition, the Medical Investigator or a designated physician's assistant will meet with the participants monthly when they

come to give blood for measurement of liver enzymes. Any out of range lab values from these tests will be recorded and reported to the PI.

AE documentation by the study coordinator

- Any event that is reported to either the PI or his designated research associates by the subject or medical staff caring for the subject which meets the criteria for an adverse event will be documented as such and graded as to its attribution (alcohol relatedness) and severity.
- Any serious adverse event that is fatal, immediately life-threatening or permanently (or significantly) disabling and requires hospitalization will be separately documented.

d) Plan for AE reporting to IRB

Planned reporting at the regular DSM meetings.

- All AE will be reported to the DSM committee every six months. In addition, the lab results from each new subject and any abnormal liver enzyme values will be reported after consultation with our pharmacist who has the randomization codes.

Serious AE reporting.

- All serious adverse events will be immediately reported to the PIs. The PIs and designated research team will generate a serious adverse event report. The report will include a description of the event, when and how it occurred, as well as any official chart records or documentation to corroborate the event.
- The serious adverse events will be reported to the Medical Investigator at an expedited ad hoc meeting.
- The report will be then immediately forwarded to the IRB (<5 days of the event).

e) Annual safety reports. Based on the documentation from the DSM committee meetings compiled by the Independent Monitor, the study coordinator will prepare a report. A statement reflecting the results of the reviews with the Independent Monitor will be sent to the IRB. This report will include a list of adverse events and will address: (1) whether adverse event rates are consistent with pre-study assumptions; (2) reasons for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data is needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely.

f) Stopping Rules - This study will be stopped prior to its completion if: (1) the intervention is associated with adverse effects that call into question the safety of the intervention; (2) difficulty in study recruitment or retention will significantly impact the ability to evaluate the study endpoints; (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) other situations occur that might warrant stopping the trial. Data on subject accrual and completion rates will be synthesized and evaluated annually to determine if the study should be terminated. These data will be reviewed by the study PIs, with consultation from the study biostatistician.