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Study Protocol with Statistical Analysis Plan

Official Title:	Pilot study of creatine monohydrate as an augmenting agent for electroconvulsive therapy in persons with major depressive disorder
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I. Introduction

We propose to determine if augmentation of electroconvulsive therapy (ECT) utilized for the treatment of major depressive disorder (MDD) with daily oral creatine will lead to an accelerated response to treatment, an overall increase in response rate, and will protect against cognitive adverse effects associated with ECT. We propose to conduct a two-arm, parallel, randomized, double-blinded, placebo-controlled trial, with a treatment group receiving 20 g oral loading dose of creatine for 1 week starting the day before initiating ECT, followed by 5 g oral creatine daily for roughly five weeks, including the approximately three-week ECT treatment course and a two-week follow-up period. Response to treatment will be assessed using the Quick Inventory of Depressive Symptomatology (QIDS) at each treatment and the 17-item Hamilton Depression Rating Scale (HAM-D17) at the end of each week.

II. Background and Significance

A. Prevalence and Impact of Depression

MDD has a lifetime prevalence of over 16%[1] and is associated with significant personal and social costs, including lost work productivity [2], disability [3, 4], diminished quality of life, increased mortality [5], increased rates of suicide attempts [6] and completed suicides [7, 8]. The financial impact of depression in the United States is significant, with an estimated economic burden of individuals with MDD of approximately $210.5 billion dollars annually, including direct costs, suicide-related costs, and workplace costs [9]. Although MDD is often regarded as a single disorder, it may encompass a variety of different etiologies with overlapping symptoms and signs [10, 11]. An additional complication of MDD is the high risk of disease recurrence; the presence of two or more chronic medical conditions, female gender, never having been married, activity limitation, and less contact with family are all significant predictors of MDD persistence [12].

MDD can be challenging to treat clinically, especially due to its propensity to be treatment-resistant. Treatment-resistant Depression (TRD) is currently defined as failure to achieve remission after two or more adequate pharmacologic trials [14, 15]. Current literature suggests that the majority of individuals with MDD do not reach and subsequently maintain a fully remitted state [16]. Furthermore, results from the STAR-D trial indicate that the overall likelihood of failure to achieve remission is increased with increasing number of failed medication trials [17]. At this time, the adjunctive use of atypical antipsychotics has been well-investigated, but less is known about alternative adjunctive agents [16].

Current clinical guidelines for the treatment of depression establish basic principles for establishing a treatment plan, preparing for potential need for long-term treatment, and assessment of remission. For moderate major depression, first line treatment includes antidepressant monotherapy and psychotherapy. For severe major depression, antidepressant therapy can be augmented with an antipsychotic or ECT [18]. Due to the overall disease burden of unmanaged MDD, the prevalence of treatment resistant depression, high rates of primary treatment failure, the additional study of alterative adjunctive therapy is both appropriate and potentially impactful.

B. Efficacy of ECT in Treatment of MDD

ECT is considered to be a first line treatment for depression with psychotic features, but it is also often used to treat patients with treatment-resistant depression (TRD) [19]. According to a 2015 metanalysis, approximately one third of patients with MDD do not respond to ECT, with failed medication trials and longer depressive episodes being the strongest predictors of poor response [20]. For treatment-resistant depression, the overall response rate is approximately 58%, compared to a 70% response rate in patients without TRD [20]. When assessing the overall

efficacy of ECT, both remission and response are used, although remission is more frequently used in clinical practice. In general, response has been defined as a 50% decrease in baseline depression screening scores, while remission is defined as a score <7 on the HAM-D17, or <10 with the 24-item HAM-D [19].

Assessments of remission in MDD after ECT treatment suggest that chronic depression, medication resistance, longer episode duration, and younger age are all statistically significant predictors of non-remission [21]. Remission rates in ECT tend to be robust; data from the CORE trial suggested an overall remission rate of 87%, which further delineates into 95% remission rate with the presence of psychotic features and 83% without [22]. Again, however, rates of response and remission with ECT are lower for patients with treatment-resistant depression, who may make up the majority of patients receiving ECT in clinical samples.

Although ECT is an effective treatment for MDD, up to one third of patients experience significant memory loss and other adverse cognitive effects after receiving ECT [23]. Strategies to limit ECT's effects on memory, such as altering electrode placement (e.g., from bitemporal to bifrontal), ensuring days off between treatments, and modulating pulse width, amplitude, and frequency, may all have some benefit[24]. Still, cognitive complaints remain one of the most significant side-effects of ECT, and concerns about these effects represent a major reason that patients who could benefit from ECT choose not to pursue it. [23]. Nootropic agents, thyroid hormone, and donepezil have all been studied to mitigate the cognitive side effects, but no consistent benefit has been shown and no current adjunctive medication is recommended [24].

C. ECT Augmentation

In order to maximize the efficacy of ECT in the treatment of MDD, several studies have analyzed the benefit of augmentation with a variety of antidepressants, anesthetic agents, and nutritional supplements. The most thoroughly studied has been adjunctive ketamine administration with ECT, although findings have been inconsistent. A metanalysis of RCTs investigating adjunctive ketamine and ECT in 2019 did not find that ketamine improves the efficacy of ECT when compared to other anesthetic agents, although it was suggested that ketamine could lead to improvement of depressive symptoms in the acute phases of ECT when used in combination [25]. There was no improvement in depressive symptoms with ketamine augmentation by the end of the ECT series [25]. The effect of ketamine on the neurocognitive side effects associated with ECT remains unclear, but no clear benefit has been shown [25]. In addition, the long-term efficacy and safety of ketamine use in ECT is unknown, particularly in the setting of maintenance treatment.

Many secondary agents have been studied in conjunction with ECT therapy, including caffeine sodium benzoate (CSB), hyperventilation, and methylxanthines [26]. However, these agents have primarily been studied for their potential to lower seizure threshold or increase overall seizure duration during ECT, without clear effects on overall ECT efficacy or symptom improvement apart from effects on seizure characteristics [26]. Less is understood about the effects of nutritional supplementation, such as folate, thyroid hormone, tryptophan, or S-adenosylmethionine (SAM-e). A case study from 2015 demonstrated improvement in response to ECT after folate supplementation [27].

There is some research that has demonstrated associations between response/remission in ECT and serum levels of various vitamins and essential nutrients. A 1994 study examining the association between serum 5-methyltetrahydrofolate (5-MeTHF) levels and ECT response did not demonstrate any significant association between ECT response and serum 5-MeTHF levels, although low serum 5-MeTHF levels were positively correlated with depression symptom severity [28]. A pilot study has further analyzed serum levels of vitamin B12, folate, S100B, homocysteine, and procalcitonin in patients undergoing ECT, and found that decreased vitamin B12 and folate levels in conjunction with elevated homocysteine and S100B levels lead to increased sensitivity to ECT, as evidenced by increased remission rates [29]. Thyroid hormone has been studied in

the setting of ECT therapy, both as an adjunctive treatment for depressive symptoms and as an agent to reduce neurocognitive deficits associated with ECT and has been demonstrated to reduce ECT associated amnesia and promote a decrease in depressive symptoms [30]. Still, thyroid hormone has not been widely used in clinical practice, possibly because of concerns about adverse effects.

D. Creatine and Depression

There is a growing body of literature surrounding the use of standardized, pharmaceutical-grade nutrients (nutraceuticals) as augmentation therapy in the setting of treatment resistant depression. A 2016 metanalysis concluded that adjunctive use of SAM-e, l-methylfolate, omega-3, and vitamin D with antidepressant therapy leads to a reduction in depressive symptoms, while isolated studies showed a similar effect with the use of creatine, folinic acid, and an amino acid combination [31].

Creatine is a naturally-occurring organic acid that is known to play a role in brain energy homeostasis and is hypothesized to be involved in the pathophysiology of depression via altered energy metabolism [31]. Oral creatine supplementation has been shown to increase cerebral phosphocreatine levels, which is hypothesized to shift cerebral creatine kinase activity, leading to increased ATP production [32-34]. Early literature suggests that creatine may have an antidepressant effect when used as adjunctive therapy in MDD, due to its role in altering brain bioenergetics [35]. Creatine has been shown to lead to an earlier treatment response in patients treated with escitalopram, with positive response to therapy as early as 2 weeks after beginning treatment [34]. To date, no studies have investigated the use of creatine as an adjunctive therapy to ECT.

Inadequate tissue bioenergetic functioning is thought to be related to disease pathology that affects predominately organs that are comparatively highly metabolically active, like the brain, liver, heart, and skeletal muscle [36]. Because creatine supplementation has the potential to increase bioenergetic stores, it may produce an antidepressant response by enabling synaptogenesis, increasing connectivity between frontal cortical regions and the amygdala, or enhancing frontal cortical functioning. ECT is thought to produce an antidepressant effect largely by promoting synaptogenesis in frontal cortical regions via alterations in the activity of NMDA and AMPA receptors, leading to upregulations in BDNF. Accordingly, creatine has the potential to augment the efficacy of ECT by increasing bioenergetic stores available for synaptogenesis. There is also some evidence to suggest that creatine has activity at NMDA receptors, functioning as a neuromodulator that is released in response to electrical stimulation [37]. Its activity at NMDA receptors is potentially another explanation for its antidepressant effect.

In a mouse model study of hyperhomocystinemia, a condition that leads to impaired creatine kinase activity, creatine supplementation was shown to have neuroprotective effects, with suggestion of memory improvement [38]. Other mouse model studies have shown that creatine can generate improvement of spatial memory, when compared to a traditional diet, as well as improve learning and mitochondrial function[39, 40]. Several human studies have also demonstrated that creatine supplementation is associated with multiple cognitive improvements, including effects on attention, mood, working and long-term memory, and mental fatigue [41-45]. These data suggest that creatine is a potential approach for addressing the cognitive side effects associated with ECT therapy.

E. Creatine Safety and Toxicity

Retrospective and prospective studies in humans have found no evidence for long-term or short-term significant side effects from creatine supplementation taken at recommended doses [46-49]. Most controlled studies of creatine report an absence of side effects or report no differences in the incidence of side effects between creatine and placebo [50]. Mihic and colleagues (2000) have demonstrated that creatine loading increases fat-free mass, but does not affect blood pressure or plasma creatinine in adult men and women.

Reports in the popular media of links between creatine use and muscle strains, muscle cramps, heat intolerance, and other side effects are not supported by the medical literature [50]. Studies conducted in athletes and military personnel indicate a substantial safety level of both short- and long-term creatine supplementation in healthy adults [51-56]. Concerns about high-dose creatine's association with renal toxicity are based exclusively on two published case reports; in one of the cases the patient had a documented pre-existing kidney condition [57, 58]. Literature reviews and expert consensus panels have concluded there is no evidence supporting an association between creatine and renal disease [59-62].

Concern has been raised regarding creatine's potential for adverse effects on the kidneys and renal system, in part because creatine supplementation can increase urinary creatine and creatinine excretion [63]. In response to the concerns regarding creatine and renal toxicity, Poortman's conducted studies of the effect of creatine supplementation on renal function, showing that short-term supplementation does not alter glomerular filtration rate [47], and that chronic supplementation of up to five years' duration did not impair renal function in healthy athletes [49].

[64] conducted a retrospective study of participants who had been taking oral creatine from 0.8 to 4 years, at an average dose of 9.7 grams per day. Data was collected on 65 health-related variables. These included a complete blood count, 27 serum chemistries, and anthropometric data including vital signs and % body fat. On all 65 variables, group means fell within the normal clinical range. The authors concluded that that long-term creatine supplementation does not result in adverse health effects.

Evidence to date suggests that even aged, debilitated, medically fragile patients are able to tolerate creatine supplementation. Bender and colleagues studied elderly patients with Parkinson's Disease who had received either placebo or four grams/day of creatine for two years. They found no differences between the creatine and placebo groups in laboratory markers of renal dysfunction [65]. Interestingly, the participants who received creatine performed better on the depression subscale of the Unified Parkinson Disease Rating Scale [66].

No strong evidence exists linking creatine supplementation and gastrointestinal discomfort. These reports remain anecdotal, as there are no documented reports of creatine over placebo resulting in stomach concerns.

III. Specific Aims

Aim 1: To evaluate the effect of creatine supplementation on response to ECT over the course of 6 weeks among persons with MDD. We hypothesize: 1) that creatine will increase response rates to ECT compared to placebo, as determined by a > 50% reduction in HAM-D17 scores earlier in treatment course as compared to placebo; 2) that creatine supplementation will increase remission rates (HAM-D17 < 7) in persons receiving ECT for MDD, compared to placebo; and 3) that creatine supplementation will reduce the time to response and remission among ECT recipients compared to placebo.

Aim 2: To evaluate the effect of 6 weeks of creatine supplementation on ^{31}P-MRS neuroimaging data among persons with MDD. We hypothesize that creatine supplementation of persons with MDD receiving ECT will promote increases in frontal cortical concentrations of phosphocreatine (and phosphocreatine to total phosphorus ratios) relative to baseline, while placebo supplementation will have no impact on phosphocreatine levels.

Aim 3: To determine if creatine supplementation throughout an ECT course leads to decreased cognitive adverse effects of ECT as measured by changes in the Montreal Cognitive Assessment (MoCA) score, compared to placebo. We hypothesize that creatine augmentation during ECT therapy will be protective against cognitive adverse effects, as measured by changes in subjects' MoCA scores between baseline and 8 weeks, when compared to placebo.

IV. Methods

A. Eligibility Criteria

Participants with a diagnosis of Major Depressive Disorder with moderate to severe symptoms will be randomly assigned in a 1:1 ratio to either ECT with creatine augmentation or ECT with placebo augmentation or 6 weeks. Individuals must meet criteria for major depressive episode for a duration of at least 2 months in order to participate. Participants must be greater than 18 years of age and considered to be a good candidate for ECT based on clinical assessment. Individuals who are pregnant or breast-feeding or who possess any other contraindication to ECT will not be invited to participate. Participants can receive ECT treatments in either inpatient or outpatient setting at the University Neuropsychiatric Institute.

Individuals who meet diagnostic criteria for other psychiatric conditions apart from major depressive disorder (including Bipolar I, Bipolar II, or personality disorders) will not be invited to participate. Individuals with substance use disorders will be excluded because substance use disorders typically confound the diagnosis of depression and can contribute to treatment resistance. Individuals will not be considered for study participation if they have *renal disease* because to date it cannot definitively be stated if short and long-term creatine usage is or is not harmful to the kidneys. Appropriate renal function will be determined based on normal creatinine clearance, determined by routine laboratory work (basic metabolic panel).

Participants who are already undergoing electroconvulsive therapy (ECT) or transcranial magnetic stimulation (TMS) for the treatment of depression, or who have completed a course of ECT within a month of the baseline visit, will not be invited to participate given the possibility of confounding treatment effects as well as increased seizure risk. Individuals currently undergoing psychotherapy remain eligible to participate.

Participants who have implanted ferromagnetic hardware, implanted electronic devices, or retained ferromagnetic materials from surgery or injuries will not be invited to participate as these represent contraindications to MRI. Likewise, individuals who are unable to tolerate confinement in the MRI scanner will not be invited to participate.

Individuals who lack capacity to consent to treatment or to participate in the study will be excluded. Patients who are hospitalized involuntarily will also be excluded. Individuals demonstrating active psychosis or any other clinical characteristic making them inappropriate candidates for treatment will be excluded. Patients with pre-existing neurologic condition, any major neurocognitive disorder, or known traumatic brain injury will not be invited to participate.

B. Study Withdrawal Criteria

A participant will be withdrawn from the study he/she experiences intolerable or clinically significant side effects to creatine, hospitalization for suicidal ideation or suicide attempt, develops a positive pregnancy test or gives other evidence of pregnancy, is incarcerated, initiates any excluded medication, or discontinues ECT before completion of the treatment series (e.g. because of adverse effects, lack of efficacy, or loss to follow-up). In addition, the principal investigator retains the right to withdraw participants from the study without their permission, in the event they are unwilling or unable to maintain adherence to the research protocol.

C. Procedures

1. Overview

All procedures performed by study personnel are research-related, but will be performed in addition to routine care (see Table 1). None of the study activities will be considered standard of care. There will be no cost to study subjects for their participation. Participants will be compensated for their time and travel. Study visits will be supervised by a board-certified/board-eligible psychiatrist or psychiatry resident and will be conducted either by a board-certified/board-eligible psychiatrist, psychiatry resident, or an at least baccalaureate degree level research assistant with training in the specific measures used. Laboratory and other study interpretation will be conducted by a board-certified/board-eligible psychiatrist. Consent will be obtained before any study procedures are initiated. Potential participants will be informed of the study and offered a consent form to review. They will be encouraged to discuss study participation with their relatives. If a potential participant expresses interest in study participation, the informed consent process will be conducted. After the informed consent process, individuals will be offered time to consider study participation and to ask questions. Subjects will have the opportunity to discuss the study with a study team member in a setting free of coercion. The language of the informed consent form is written at a level easily understood by the subject and

Study Timeline	
Week	Procedures
Visit 1	Eligibility screening: MINI, HAM-D17, MOCA, QIDS, Labs, CGI, BSS, ATRQ
Visit 2	QIDS
	^{31}P-MRS Scan 1
	Start creatine 20g per day or placebo
	Start ECT (treatments 1 up to 3) as per routine
Week 2 (Subjects may begin to complete ECT, moving to follow-up phase)	Creatine 5g per day or placebo
	Continuing ECT (treatments 3 or 4 through 6 or 7) as per routine
	QIDS before each ECT
	HAM-D17 , CGI, BSS, MOCA at end of week
Week 3 (Most subjects will complete ECT, moving to follow-up phase)	Creatine 5g per day or placebo
	Continuing ECT (treatments 3 or 4 through 6 or 7) as per routine
	QIDS before each ECT
	HAM-D17 , CGI, BSS, MOCA at end of week
	^{31}P-MRS Scan 2 (if ECT complete)
Week 4 (Almost all subjects will complete ECT, moving to follow-up phase)	Creatine 5g per day or placebo
	Continuing ECT (treatments 3 or 4 through 6 or 7) as per routine
	QIDS before each ECT
	HAM-D17, CGI, BSS, MOCA at end of week
	^{31}P-MRS Scan 2 (if ECT completed this week)
Follow-up phase: 2 weeks (to start after completion of ECT or after week 4, whichever comes first)	Creatine 5g per day or placebo (for two weeks)
	HAM-D17, CGI, BSS, MOCA two weeks after completion of ECT series
	^{31}P-MRS Scan 2 (if not yet completed)

any questions asked by the subject will be answered honestly and free of bias. A specific meeting time will be set up between a study team member and the participant where the entire informed consent document will be carefully explained in its entirety. The length of the meeting will be designed so there is the necessary amount of time for all questions to be answered.

To determine if an individual is eligible for study participation, a screening visit will be conducted. Initially, a HAM-D17 will be administered to determine if the patient exhibits depressive symptoms that are sufficiently severe for inclusion in the study. Next, the Mini International Neuropsychiatric Interview (MINI) will be administered to confirm a diagnosis of a current major depressive episode. Study subjects will receive a baseline basic metabolic panel (BMP) to assess for renal insufficiency, and vitals (these may have been obtained in the course of ongoing clinical care). The QIDS, Antidepressant Treatment Response Questionnaire (ATRQ), Clinical Global Impression (CGI), Beck Suicide Scale (BSS), and a MoCA will be administered. Each participant will be assessed for history of ECT therapy, current medications, and any other medical history.

Once entered into the study, depressed subjects will be randomized to receive either creatine or placebo using a random-digit method that is based on computer-generated numbers. Block randomization created by Investigational Drug Services (IDS) will be used to ensure equal treatment allocation within each block. 50% of the trial's clinical subjects will be randomized to placebo and the other 50% to active treatment. The study will be conducted as a double-blind trial, with neither participants nor research staff aware of participant assignment. Except in cases of medical emergency, the double-blind will not be "broken" until recruitment is closed and the final participant has completed 6 weeks of treatment and 4 weeks of follow-up. The blind will be broken following the culmination of the study or at the request of a medical professional dealing with a medical emergency in a case in which it would help a study participant.

2. Electroconvulsive Therapy

Participants will be recruited from a population of patients who have already been referred for ECT and who have had initial clinical assessments to determine whether ECT is indicated. As this study is an add-on to standard clinical care only, routine ECT procedures will be followed, though the ECT service (attending psychiatrist, anesthesiologist) will be notified of subject participation. In general, subjects will, after being medically cleared for ECT (psychiatric exam, physical exam, EKG, laboratory studies if indicated), received bifrontal ECT every other day for between 6 and up to roughly 14 treatments, with a total duration of treatment lasting between two and four weeks. Anesthesia for ECT is provided by a board-certified anesthesiologist and comprises the use of methohexital, midazolam, etomidate, or ketamine, as indicated, and at the discretion of the anesthesiologist. Participants may receive interventions designed to augment the likelihood of a seizure being achieved, such as a caffeine infusion, hyperventilation, or other techniques, at the discretion of the treating ECT psychiatrist and anesthesiologist. After ECT, participants are monitored for recurrent seizure/status epilepticus and for vital sign abnormalities for roughly thirty minutes, then released to home (if outpatient) with 24 hour per day supervision by adult family members, or else escorted back to the inpatient unit, where they again receive 24 hour per day supervision by unit staff. Prior to each treatment, subjects complete a Quick Inventory of Depressive Symptoms (QIDS) to assess their overall burden of depressive symptoms. The exact duration of treatment/number of treatments is determined by the treating ECT psychiatrist, based on clinical response. The study will record all pertinent variables related to ECT as noted in the clinical record, including number of treatments, QIDS score, number of seizures per treatment session, seizure duration, adverse effects, augmentation strategies, anesthesia type, and vitals.

2. Drug Dosing

Participants who have been assigned to the creatine arm of the trial will receive a 20g loading dose daily for 1 week starting as soon as possible before ECT begins and after completion of the

^{31}P-MRS; this will be administered in 4 divided doses of 5g each. Participants will then receive 5 g creatine daily throughout the course of ECT therapy (~3 weeks), with continuation for an additional 2 weeks after the completion of the acute series of ECT, again at 5g daily (thus, up to 5 weeks total supplementation with creating 5g per day, depending on the length of ECT) Placebo recipients will receive an inert, relatively tasteless powder matched to creatine (e.g., glucose). Creatine doses are based on doses that have previously been shown to be safe and efficacious [67-69].

3. Measures

We plan to use the following for determining participant baseline and data collection:

- Hamilton Depression Rating Scale (HAM-D17) (at baseline, the end of week 1, the end of week 2, the end of week 3, and two weeks after completion of the ECT series)
- Quick Inventory of Depressive Symptomatology (QIDS) (at baseline and prior to each ECT session)
- Antidepressant Treatment Response Questionnaire (ATRQ) (at baseline)
- Mini International Neuropsychiatric Interview (MINI) (at baseline)
- Clinical Global Impressions Scale (CGI) illness improvement subscale (CGI-I) (at baseline, week 1, week 2, week 3, and two weeks after completion of the ECT series)
- Beck Suicide Scale (BSS) at baseline, week 1, week 2, week 3, and two weeks after completion of the ECT series)
- Montreal Cognitive Assessment (MoCA) at baseline, week 1, week 2, week 3, and two weeks after completion of the ECT series)

4. Imaging

a. Magnetic Resonance Imaging (Siemens 3T MRI system)

MRI scans will be conducted twice: after the baseline visit and prior to initiating ECT, and following completion of the ECT series (i.e., after ~3 weeks, and during the 2 week post-ECT follow-up period). The 3.0 Tesla Siemens Prisma whole-body clinical scanner (Siemens Medical Solutions, Erlangen, Germany) located within the University Neuropsychiatric Institute (UNI) will be used to acquire this data. Participants will first undergo a routine anatomic MRI protocol, which includes MRI images acquired in the axial and coronal planes. Specifically, the anatomic scan protocol consists of a T1 weighted structural scan (MP2RAGE), and double-echo T2 weighted scan, and a Fluid Attenuated Inversion Recovery scan (FLAIR). The purposes of the MR anatomic screening session include screening subjects for gross structural abnormalities and acquiring images for use in brain cortical thickness measurements. Anatomic MRI examinations will be performed with Siemens 64 channel head coil. After localization, anatomical imaging will be obtained using a T1-weighted, sagittal oriented 3D-Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence (TR/TE/TI 5000/2.93/700 ms, matrix 256x256, FOV 256x256 mm, flip angle 4 degree, slice thickness 1.0 mm, slab 176 mm, bandwidth 240 Hz/pixel). Axial proton-density and T2 weighted images will be acquired to screen for brain structural abnormalities using 2D Double echo T2 weighted turbo spin echo (TSE) sequence (TR 7110 ms, TE 28/84 ms, FOV 240x210, slice thickness 3 mm, flip 150°, bandwidth 179 Hz/pixel). FLAIR sequence (TR/TE/TI 8000/90/2500 ms, slice thickness 5 mm, FOV 240x168, voxel size 0.8x0.6x5.0 mm, bandwidth 200 Hz/pixel, turbo factor 13) will be used to detect juxtacortical-cortical lesions. All anatomic MRI images will be read by a board-certified, CAQ neuroradiologist to screen for structural abnormalities.

b. Measurement of *In-Vivo* Brain Chemistry Using Phosphorus-31 Magnetic Resonance Spectroscopy (^{31}P-MRS)

Phosphorus spectroscopy data will be acquired on the same Siemens 3T system. We aim to keep

the duration of each MRSI examination at or under 25 minutes. A 3D-MRSI sequence with elliptically weighted phase-encoding will be used to collect ^{31}P-MRSI data to minimize T2 signal decay. Acquisition parameters will be: data matrix size 16x16x8; TR 2000 ms; tip-angle 90 degree for hard RF pulse; Rx bandwidth ±1 kHz; complex-points 1024; readout duration 256 ms; pre-acquisition delay 0.3ms; FOV 240x240 mm^2 ; 16 NEX.

c. Spectral Analysis of ^{31}P-MRS Data

Spectroscopy will be analyzed using Liner Combination of Model Spectra (LCModel) [70], which analyzes an *in vivo* spectrum as a linear combination of model in vitro spectra from individual metabolite solutions. This model is fully automatic and user independent. A nearly model-free constrained regularization method is used for convolution and baseline. For quantification, absolute metabolite concentrations (institutional units) will be estimated using the unsuppressed water signal as an internal concentration reference. Also, total creatine levels will be used as a denominator for calculating the relative concentration for the comparison with previous reports. The standard Siemens libraries of model metabolite spectra provided with LCModel will be used in the basis set. The metabolites from the basis set will include alanine, aspartate, creatine, gamma-amino butyric acid, glucose, glutamine, glutamate, glycerophosphocholine, glutathione, myo-inositol, scyllo-inositol, lactate, N-acetylaspartate, N-acetylaspartylglutamate, phosphocholine, phosphocreatine, phosphoethanolamine, and taurine. For the reliability of detection, the Cramer-Rao lower bounds (CRLB) will be determined: the acceptable upper limit of estimated standard deviations will be set at 20% [71].

Post processing of ^{31}P-MRS data will be conducted using jMRUI software (jMRUI v. 4.0, European Community) with the AMARES algorithm (Advanced Method for Accurate, Robust and Efficient Spectral fitting of MRS data with use of prior knowledge). Before fitting the FID (Free-induction-decay) data, a Hamming filter will be applied to reduce signal contamination from neighboring voxels, with apodization of 10 Hz line broadening. Fourier transformation, frequency shifts correction, and zero-order/first order phase correction as well as baseline correction will be applied. The structural image-processing tool FSL (FMRIB Software Library, Release 4.1, University of Oxford) will be used to account for gray matter, white matter, and cerebrospinal fluid (CSF), in order to correct the partial volume effects on metabolite concentrations. The MRS grid will be positioned over the images in an identical fashion between baseline and treatment scans for each participant. The peak area for each ^{31}P-MRS metabolite will be calculated as a percentage of the total phosphorus signal.

V. Statistical Plan and Sample Size Determination

Aim 1: *To evaluate the effect of creatine supplementation on response to ECT over the course of 6 weeks among persons with MDD.*

As this is a pilot study, we have limited information regarding the potential magnitude of the effect of creatine supplementation on HAM-D scores in patients receiving ECT. Based on a prior recommendation that one assume a minimum detectable score of 7 for the HAM-D in randomized control trials, with a moderate effect size of 0.875 [72], we estimate that a sample size of 30 depressed subjects (15 active vs. 15 placebo) will have ~82% power to detect a significant difference between groups using a linear mixed model with first-order autoregressive covariance structure.

Aim 2: *Aim 2: To evaluate the effect of 6 weeks of creatine supplementation on ^{31}P-MRS neuroimaging data among persons with MDD.*

Following 8 weeks of oral creatine, adult female methamphetamine users had significantly increased PCr levels [73]. The standardized effect size was 0.920. Also, a study involving open-label creatine supplementation in depressed adolescents demonstrated significantly increased

PCr levels following creatine administration [68]. The change of PCr levels was approximately 6.2% of the mean with 9.6% SD. Therefore, if we set the anticipated minimum detectable effect size (MDES) = 9% with SD = 10% for this project, a total sample size of 30 (15 active vs. 15 placebo) would have ~80% power to detect the anticipated effect using a repeated measures design.

VI. Administrative Procedures

A. Study Drug Administration

Study creatine will be acquired in bulk and will be allocated into dosage packets by University of Utah IDS. Study drug will be dispensed after the baseline visit for participants eligible to continue in the study. Study drug will be dispensed as creatine monohydrate powder to allow a dose of 20g per day in four divided doses, and later 5 grams per day as a single dose. Participants will be instructed to take medication with food. They will be instructed to stir creatine into a non-carbonated drink such as water, juice, coffee, or tea. They will be instructed to take medication daily prior to beginning ECT therapy (to complete 20 g loading dose for one week) and to continue to take 5 g daily for the duration of ECT and for two additional weeks following completion of the acute ECT series. At each study visit, study medication adherence will be assessed and participants will be asked to maintain empty bottles of creatine and return them at follow-up visits. Participants will be informed to store both medications at room temperature.

B. Drug Storage

Under the supervision of Dr. Renshaw (IND holder), Dr. Brent Kious will be responsible for the safe storage of study medication. The study drug will be stored at room temperature in a locked room with access limited to those individuals authorized by Dr. Renshaw.

C. Drug Accountability

We will maintain an inventory that includes a signed account of study drug received, dispensed to and returned by each participant. At the conclusion of the study, we will conduct and document final drug supply (used and unused) inventory and reconciliation. An explanation will be required in the event of discrepancies. A copy of the final inventory will be provided to Dr. Renshaw (IND holder) at the end of the study.

D. Compensation

There will be no cost to study subjects for their participation. Participants will be compensated for their time and travel according to the following schedule:

Week 1:
- Visit 1a (Screening): $25
- Visit 1b (Imaging): $50

Week 2:
- Visit 2 (Follow-up): $15

Week 3:
- Visit 3a (Follow-up): $15
- Visit 3b (Imaging, *if ECT completed this week*): $50

Week 4:
- Visit 4a (Follow-up): $15
- Visit 4b (Imaging, *if ECT completed this week and imaging not already done*): $50

Weeks 5-6 (Follow-up phase):
- Visit 5a (Follow-up): $15
- Visit 5b (Imaging, *if not already completed*): $50
- Visit 6a (Follow-up): $15

Visit 6b (Imaging, *if not already completed*): $50

Total compensation per subject will be *up to* $200 depending on duration of participation and completion of various study elements.

E. Recruitment
Subjects will be recruited by direct contact through the electroconvulsive therapy service, on either an inpatient or outpatient basis, by Dr. Kious, Dr. Mickey, or their designees.

F. Facilities
A private office at the University Neuropsychiatric Institute will be used for screenings and subsequent study visits. The office has clinical equipment available, including a blood pressure machine, thermometer, and a scale to measure weight. Neuroimaging procedures will be conducted in the MRI suite at the University Neuropsychiatric Institute.

Data Collection and Management
Study data will be recorded on Case Report Forms (CRFs). Completed CRFs will be filed in participant binders, stored in a locked office, with access limited to research personnel. Data from CRFs will be entered into REDCap, a secure, web-based application for building and managing online surveys and databases. No identifiable data will be entered in REDCap. Forms that have missing or inconsistent data will be recorded in the database; however, a "missing data" code (-99) will be entered in place of each piece of missing or inconsistent data.

VII. Protection of Human Subjects
A. Risks to Study Participants
During the screening visit, participants may become emotionally upset when asked about their psychiatric history including suicide attempts, or physical and sexual abuse. It is possible that a participant's illness could worsen during the study. This could be related or unrelated to the study. If the participant's illness worsens to the point that they are a danger to themselves or others, they will be referred for appropriate care. As outlined above, there are no long or short-term side effects associated with creatine use. Patient's renal function will be assessed with baseline Basic Metabolic Panel and monitored as indicated throughout study. No gastrointestinal concerns are anticipated, but patients will be instructed to take medication with food to minimize possible risk. Participants will be counseled about potential risks and provided with information sheets detailing the symptoms of these disorders in lay terms and describing steps to take for further evaluation if indicated, and will be screened for both conditions at each visit after the initiation of treatment.

Although one of the safest procedures administered under general anesthesia, there are some known adverse effects of ECT therapy, including:

- Aspiration pneumonia
- Fracture
- Dental and tongue injury
- Headache
- Nausea
- Myalgias
- Impaired cognition

The mortality rate for ECT is less than 1 per 100,000 treatments, making this outcome unlikely. In addition, mortality is primarily related to underlying coronary artery disease. Participants will be screened for these diseases prior to undergoing the study.

MRI/MRS scans do not use ionizing radiation like x-rays or CT scans. Instead, magnetic fields and radio waves are used to take the pictures. There are no known risks related to MRI

scans – other than the risk of injury when metallic objects are brought into the scanning room by mistake. Serious injury can occur during an MRI scan to persons who have:

- Cardiac (heart) pacemakers.
- Metal clips on blood vessels (also called stents).
- Artificial heart valves.
- Artificial arms, hands, legs, etc.
- Brain stimulator devices.
- Implanted drug pumps.
- Cochlear (ear) implants.
- Ocular (eye) implants or known metal fragments in eyes.
- Exposure to shrapnel or metal fillings
- Other metallic surgical parts.
- Orthodontic braces on the teeth.
- Body jewelry or piercings that cannot be removed for the scan.
- Certain tattoos with metallic ink
- Certain transdermal (skin) patches such as NicoDerm (nicotine for tobacco dependence),
- Transdermal scopolamine or Ortho-Evra (birth control)

If the participants have any such devices, or has had a surgery where metal devices were placed in their body, they cannot take part in the study unless cleared for MRI scanning by the surgeon who implanted the medical device(s).

Serious risks exist if ferromagnetic objects (things that stick to magnets) are brought into the scanning area. These items can become dangerous flying objects, and are not allowed near the MRI scanner. The FDA has approved the 3T scanner for routine clinical studies. The FDA has decided that MRI machines of 8T or less do not pose a risk. Although the scans we are using in this study have no known risks, there could be ill effects that are delayed, such that they have not yet been recognized by the FDA. The brain scans do not cause pain. Apart from the scanner noise, the participant will not know the scan is taking place. Inside the scanner, some people experience claustrophobia (fear of being in small spaces), dizziness, headaches, or a metallic taste in the mouth. Some people experience double vision or see flashing lights. These symptoms are temporary, and will stop when the participant leaves the scanner. The participant may feel cramped inside the scanner. There is a mirror placed inside the scanner so the subject can see his or her face, and look out into the scanning room. The technologist will be able to hear the participant at all times. Very rarely, someone having an MRI scan feels a tingling in his or her back. This is due to the magnetic field changing quickly during the scan. The precautions taken will avoid all the known risks related to MRI scans. The participant can stop the scan at any time.

Data Safety and Monitoring

The principle investigator, psychiatry resident, and study coordinator will perform monitoring of case report forms on a twice-yearly basis.

1. Adverse events

Unanticipated problems and adverse events that are related to the research, or which place participants at greater-then-expected risk, will be reported to the Institutional Review Board (IRB) within ten working days of the event. *Unanticipated problems* involving risk to participants or others are defined as any incident, experience or outcome that meets the following criteria:

- Unforeseen (not expected by the researcher or the research participant) given the research procedures and the subject population being studied;
- Related or probably related to participation in the research, or if the event or problem probably or definitely affects the safety, rights and welfare of current participants; and

- Suggests that the research places participants or others at a greater risk of harm (includes physical, psychological, economic or social harm) than was previously known or recognized.

  An *unexpected adverse event* is any adverse event occurring in one or more participants participating in a research protocol, whose nature, severity, or frequency is not consistent with either:
- The known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in the protocol related-documents (i.e. protocol, investigational brochure, consent form, or product labeling).
- The expected natural progression of any underlying disease, illness or medical condition of the subject experiencing the adverse event.

The Principal Investigator will make the initial determination if an unexpected, adverse event is related or unrelated to the investigational drug or a clinical or research procedure. An adverse event is "related to the research" if in the opinion of Dr. Kious or Dr. Renshaw, it was more likely than not related to the investigational agent or intervention.

C. Reporting to the U.S. Food and Drug Administration (FDA)

(a) Terms and Definitions:

Associated with the use of the drug. There is a reasonable possibility that the experience may have been caused by the investigational drug being studied.

Disability. A substantial disruption of a person's ability to conduct normal life functions and activities of daily living (ADLs).

Life-threatening adverse drug experience: Any adverse drug experience that places the participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred.

Serious adverse drug experience: An adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include: allergic bronchospasm requiring treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected adverse drug experience: Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure only referred to "elevated hepatic enzymes" or "hepatitis." Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure only listed "cerebral vascular accidents." "Unexpected," as used in this definition, refers to an adverse drug experience that has not been

previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

(b) Review of Safety Information:

The Principal Investigator will promptly review all information relevant to the safety of the drug obtained or otherwise received from any source, foreign or domestic, including information derived from any clinical or epidemiological investigations, animal investigations, commercial marketing experience, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities that have not already been previously reported to the FDA.

(c) IND Safety Reports (IND#125637):

(1) *Written reports*

(i) As the IND holder, Dr. Renshaw shall notify the FDA in a written IND safety report of:

(A) Any adverse experience associated with the use of the drug that is both serious and unexpected; or

(B) Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification shall be made as soon as possible and in no event later than 15 calendar days after the sponsor's initial receipt of the information. Each written notification may be submitted on FDA Form 3500A or in a narrative format (foreign events may be submitted either on an FDA Form 3500A or, if preferred, on a CIOMS I form; reports from animal or epidemiological studies shall be submitted in a narrative format) and shall bear prominent identification of its contents, i.e., "IND Safety Report." Each written notification to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND. If FDA determines that additional data are needed, the agency may require further data to be submitted.

(ii) In each written IND safety report, Dr. Renshaw shall identify all safety reports previously filed with the IND concerning a similar adverse experience, and shall analyze the significance of the adverse experience in light of the previous, similar reports.

(iii) The study team will send an annual report 60 days within the “study may proceed” date.

(2) *Telephone and facsimile transmission safety reports.* Dr. Renshaw shall also notify FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but in no event later than seven calendar days after the event. Each telephone call or facsimile transmission to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND.

(3) *Reporting format or frequency.* FDA may request that Dr. Renshaw submit IND safety reports in a format or at a frequency different than that required under this paragraph.

(d) Follow-Up:

(1) Dr. Renshaw shall promptly investigate all safety information received by it.

(2) Follow-up information to a safety report shall be submitted as soon as the relevant information is available.

(3) If the results of Dr. Renshaw's investigation show that an adverse drug experience not initially determined to be reportable is so reportable, he shall report such experience in a written safety report as soon as possible, but in no event later than 15 calendar days after the determination is made.

(4) Results of Dr. Renshaw's investigation of other safety information shall be submitted, as appropriate, in an information amendment or annual report to the FDA.

D. Reporting to the University of Utah Institutional Review Board (IRB)

The study team will strictly observe the University of Utah IRB policy that requires researchers to submit reports of events that may represent unanticipated problems (UPs) involving risks to participants and others, including unexpected, research-related adverse events. Reports will be submitted to the IRB as soon as possible after the principal investigator learns of the event, but in all cases within 10 working days. Late reports will be accompanied by a written explanation from the principal investigator as to why the report is tardy. The following will be reported promptly by the principal investigator to the IRB:

- Unexpected, research-related adverse events;
- Breached of confidentiality or privacy that involves real or potential risk such as unauthorized use or disclosure of protected health information (PHI);
- New information indicating a change to the risks or benefits of the research, such as:
  - Reports that indicate that frequency or magnitude of harms or benefits may be different than initially presented to the IRB;
  - Publications that show that the risks or potential benefits of the research may be different than initially presented to the IRB;
  - Changes in FDA labeling or withdrawal from IND status or marketing of a drug, device, or biologic used in the research protocol;
- Incarceration of a participant, because this study is not approved to enroll prisoners;
- Complaints from participants or others involved in the research that indicate unexpected risks;
  or complaints that cannot be resolved by the research team;
- Warning or determination letters issued by any funding agency or regulatory body including the Office of Human Research Protections (OHRP), the Department of Health and Human Services (DHHS), or the Food and Drug Administration (FDA).
- Protocol Deviations**,** if they are:
  - Intended to eliminate apparent immediate hazard to a research participant;
  - Harmful (i.e. caused harm to participants or others, or placed them at increased risk of harm – including physical, psychological, economic, or social harm).
  - Possible *Serious Non-Compliance* (defined as an act or omission to act that resulted in increased physical, psychological, safety, or privacy risk that compromised the rights and welfare of research participants) – such as deliberate or repeated failure to obtain prior review and approval of new protocols and on-going human participants research by the IRB, or deliberate or repeated failure to obtain or document informed consent from human participants, or deliberate or repeated omission of a description of serious risks of the experimental therapy when obtaining informed consent, or deliberate or repeated failure to limit administration of the investigational drug or device to those participants under the

investigator's supervision, or deliberate or repeated failure to maintain accurate study records, report changes to the research, or report unanticipated problems posing risk to subjects or others to the IRB, or deliberate or repeated failure to comply with the conditions placed on the study by the University, the IRB, sponsor, or the FDA.

- Possible *Continued Non-Compliance* (defined as a pattern of repeated actions or omissions to act that suggests a future likelihood of recurrence and that indicates a deficiency in the ability or willingness to comply with Federal regulations, or the policy, requirements, and determinations of the University of Utah IRB governing human subjects research) – such as consistently late submission of continuing review or items that require prompt reporting, repeated failure to comply with IRB requirements for completion of human subjects training before initiating study procedures, repeated failure to submit the required documents to the IRB, repeated refusal to comply with IRB requests, or repeated failure to submit progress reports.

E. Record Retention

In keeping with 21CFR312.57, study records will be maintained for at least two years after the drug is approved by the FDA or after shipment and delivery of the drug for investigational use has ceased and the FDA has been notified.

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