PROTOCOL SUMMARY

A randomized, double-blind, placebo-controlled trial of 5-hydroxytryptophan and creatine for SSRI or SNRI augmentation in treatment resistant depression associated with hypobaric hypoxia in females

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I. Introduction

We propose to determine if 8 weeks of dietary augmentation with oral 5g creatine monohydrate daily and 100 mg of 5-hydroxytryptophan (5-HTP) twice daily reduces hypoxia-related depressive symptoms measured by the 17-item Hamilton Depression Rating Scale (HAM-D) in women with SSRI- or SNRI-resistant depression, combined with the examination of changes in functional connectivity based on resting-state fMRI and changes in brain metabolism inferred from phosphorus-31 magnetic resonance spectroscopy. We further aim to examine whether response to the treatment is predicted by polymorphisms in tryptophan hydroxylase 2 or other genes affecting serotonin metabolism.

II. Background and Significance

A. Prevalence and Impact of Depression

Major depressive disorder (MDD) has a lifetime prevalence of over 16% (Kessler, Berglund et al. 2005) and is associated with significant personal and social costs, including lost work productivity (Birnbaum, Kessler et al. 2010), disability (Kessler, Barber et al. 1999, van der Voort, Seldenrijk et al. 2015), diminished quality of life, increased mortality (Markkula, Harkanen et al. 2016), and increased rates of suicide attempts (Malone, Haas et al. 1995) and completed suicides (Angst, Angst et al. 1999, Blair-West, Cantor et al. 1999). Although MDD is often regarded as a single disorder, it may encompass a variety of different etiologies with overlapping symptoms and signs (Fakhoury 2015, Haase and Brown 2015). There are significant regional variations in rates of MDD and suicide (Weissman, Bland et al. 1999), suggesting that environmental factors may contribute to the pathogenesis of MDD and suicide in some cases (Tempier, Meadows et al. 2008, Lopizzo, Bocchio Chiavetto et al. 2015).

The prevalence of MDD in the United States varies widely. Mental Health America surveyed all 50 states from 2002-2006 (Mark, Shern et al. 2007) and found the annual prevalence of major depressive episodes varied from 7.3% (South Dakota) to 10.1% (Utah). Suicide rates also vary. The District of Columbia had the lowest age-adjusted annual rate, at 5.3/100,000, while Alaska had the highest, at 23.1/100,000. Utah, the state with the highest prevalence of depression, recorded an annual suicide rate of 17.1/100,000. Apart from Alaska and West Virginia, the states with the highest annual suicide rates were clustered in the intermountain west; these included Arizona, Colorado, Idaho, Montana, Nevada, New Mexico, Utah, and Wyoming. More recently, the Substance Abuse and Mental Health Services Administration (SAMHSA) reported substantial regional variations in the annual (2014) prevalence of adults having serious thoughts of suicide, from a low of 3.3% (Connecticut) to a high of 4.9% (Utah) (SAMHSA 2015). South Dakota continued to have the lowest annual prevalence of major depressive episodes at 5.3%, while Maine had the highest at 8.2%, and Utah was second at 8.0% (SAMHSA 2015).

B. Depression, Suicide, and Altitude

The clustering of high rates of depression and suicide in the intermountain west suggests that altitude may play a role in these conditions (Young 2013).. The intermountain states have higher mean altitudes than the rest of the country. The mean altitude of Utah, for instance, is 1860m, while the mean altitude of Connecticut is 150m (Carpenter and Provorse 1996). There is a substantial body of evidence supporting this connection (Table 1). The National Survey on Drug Use and Health (NSDUH) from SAMHSA for 2004-2006 encompassed 203,870 responders in 345 substate regions and included data regarding the frequencies of serious psychological distress and respondents having at least one major depressive episode in the previous year. Comparison of this data with each substate region's mean altitude demonstrated a correlation with both the incidence of serious psychological distress ($r = 0.18$; $p = 0.0005$) and the percentage of people having at least one major depressive episode in any of the study years ($r = 0.27$; $p < 0.0001$) (DelMastro, Hellem et al. 2011).

Altitude of residence is also correlated with suicide risk. Cheng *et al.* (Cheng, Yakobi et al. 2002) first observed that states in the mountain west had higher suicide rates, even after controlling for other risk factors like poverty, access to psychiatric care, and population density. They later demonstrated that suicide rates in the 50 capital counties in the United States were strongly correlated with altitude ($r = 0.75$, p <

0.0001) (Cheng, Mendenhall et al. 2005), and that suicide rates in all 3060 US counties were correlated with altitude even after controlling for county median income and population density (Brenner, Cheng et al. 2006).

Further evidence to support this hypothesis was provided by Haws *et al*. (Haws, Gray et al. 2009), who evaluated the correlation between each states' peak altitude, the altitude of its capital city, and suicide rates from 1990-1994, as reported by the CDC. After adjusting for age, race, and sex, suicide rates remained highly correlated with both peak altitude ($r = 0.62$, $p < 3.9E-06$) and capital city altitude ($r = 0.74$, $p < 3.4E-09$). Even more recently, a study of suicide rates and all-cause mortality in all 2584 U.S. counties, using CDC mortality data from 1979 to 1998, demonstrated that although all-cause mortality decreased with increasing altitude ($r = -0.31$, $P < 0.001$), suicide rates increased with altitude ($r = 0.50$, $p < 0.001$), even after controlling for demographic factors like age, gender, race, median household income, and population density (Brenner, Cheng et al. 2011). The study also noted a threshold effect, with a dramatic increase in suicide rates occurring at between 610m-914m. Similarly, a study of 8,871 suicide deaths recorded by the National Violent Death Reporting System (NVDRS) in 2006 in 15 states divided them into low altitude, middle altitude, and high altitude groups. 83.4% were at low altitude, 11.8% were at middle altitude, and 4.8% were at high altitude. However, the suicide rate adjusted for population distribution (as most of the population lives near sea level) was 17.7/100,000 at high altitude, 11.9/100,000 at middle altitude, and only 5.7/100,000 at low altitude (Betz, Valley et al. 2011). Finally, an analysis of 35,725 completed suicides from the NVDRS from 2005-2008, representing 922 U.S. counties, demonstrated that altitude of residence was a significant, independent predictor of suicide in bipolar disorder, and that individuals with bipolar disorder committed suicide at the highest mean altitude compared to persons with unipolar depression, schizophrenia, or anxiety disorders (Huber, Coon et al. 2014).

Socioeconomic factors, such as rates of gun ownership, poverty, and social isolation, might seem to explain the association between suicide and altitude, but efforts to control for these in the above studies reveal that altitude is an independent risk factor. The authors of (24) noted that high-altitude suicide victims differed from low- and middle-altitude victims with respect to race, depressed mood, suicide attempts, substance use disorders, violence, firearm-related suicide, employment, and several other variables. They did not, however, adjust for these variables in comparing suicide rates between regions. In (23), the positive correlation with altitude held for both firearm-related ($r = 0.40$, $p < 0.001$) and non-firearm-related ($r = 0.31$, $p < 0.001$) suicides, suggesting regional variations in rates of firearm ownership do not explain differences in suicide risk. In another recent study, U.S. suicide rates between 1979 and 1998 were strongly correlated with altitude ($r = 0.51$, $p < 0.001$) even after controlling for rates of gun ownership and population density. Again, rates of both firearm-related and non-firearm-related suicides were correlated with altitude (Kim, Mickelson et al. 2011).

C. Hypobaric Hypoxia and Depression

One possible link between altitude and depression/suicide is relative hypobaric hypoxia (lower blood oxygen concentration due to lower inhaled oxygen) (Young 2013). The effective concentration of inspired oxygen is represented as its partial pressure (PIO2), which is calculated as PIO2 = FIO2 x (Pb – 6.3kPa), where FIO2 is the fraction of inspired oxygen, Pb is the barometric pressure, and 6.3kPa is the partial pressure of water vapor at 37°C (Rahn and Otis 1949). FIO2 is effectively constant at 20.9%, irrespective of altitude (Peacock 1998), but barometric pressure decreases with altitude in a curvilinear fashion (Lilienthal, Riley et al. 1946). Accordingly, PIO2 also decreases with altitude (Grocott , Martin et al. 2009). At sea level, PIO2 is approximately 19.6kPa (Peacock 1998). Salt Lake City (the authors' location) has an average altitude of around 1370m (USGS 2016) and an average PIO2 of approximately 16.6kPa. Similarly, Denver, Colorado, has an average altitude of 1610m (USGS 2016) and an average PIO2 of 16.1kPa.

Lower PIO2 can contribute to lower arterial oxygen concentrations (PaO2). Although there are physiologic adaptations to altitude both acutely and chronically (Peacock 1998), these are neither universal nor complete (Grover, Weil et al. 1985). A comparison of arterial blood oxygen concentrations in healthy non-smokers residing at sea level (n=97) and at 1400m (n=243) demonstrated that the average PaO2,

weighted for the age distribution of the samples, was 12.7kPa at sea level and 9.9kPa at 1400m (Crapo, Jensen et al. 1999). Thus, increased altitude can contribute to reduced PaO2, and thus to reduced brain oxygen levels.

Animal studies also imply that hypoxia is associated with depression. In rats, one week of simulated high altitude (equivalent to 3048m) produces increased immobility and decreased time to first prolonged immobility in the forced swim test (FST), both of which are regarded as depression-like behavior (Bogdanova, Abdullah et al. 2014). This effect may be sex-dependent: when male and female rats were housed at four different simulated altitudes (6096m, 3048m, 1370m, and sea-level) only females exhibited increases in depression-like behavior (Kanekar, Bogdanova et al. 2015).

D. Hypoxia and Altered Serotonergic Signaling

Several biological pathways may mediate the connection between hypoxia and increased risks of depression and suicide. One pathway may involve changes in serotonergic signaling. Reductions in serotonin levels, serotonin metabolite levels, and changes in specific serotonin receptors (including 5HT1a and 5HT2a) have been found in the circulating platelets, cerebrospinal fluid (CSF), and postmortem brain tissues of suicide victims (Pandey 2013). Alterations in serotonin signaling are also clearly implicated in the pathogenesis of major depressive disorder (Risch and Nemeroff 1992, Owens and Nemeroff 1994, Owens and Nemeroff 1998, Haase and Brown 2015, Kohler, Cierpinsky et al. 2016). Hypoxia could affect brain serotonin levels because the synthesis of serotonin is oxygen-dependent. Chronic hypoxia alters the synthesis and metabolism of neurotransmitters such as serotonin, dopamine, and norepinephrine in a brain region-dependent manner. Simulated altitudes up to 7000m for 24 hours do not affect norepinephrine turnover, can increase dopamine turnover (Prioux-Guyonneau, Cretet et al. 1979) and are associated with up to a 30% reduction in serotonin turnover (Prioux-Guyonneau, Mocaer-Cretet et al. 1982). In rodents, housing at simulated high altitude for at least one week is associated with increased brain levels of dopamine and norepinephrine but decreased levels of serotonin, particularly in the frontal cortex (Ray, Dutta et al. 2011).

Human data regarding the effects of hypoxia on serotonin and other monoamines are limited but still suggestive. Post-mortem brain levels of serotonin and 5-hydroxyindoleacetic acid (5HIAA), which is the major metabolite of serotonin, are generally lower in smokers than in non-smokers (Benwell, Balfour et al. 1990). Similarly, in patients with a variety of psychiatric conditions, smoking is associated both with lower levels of serotonin, as measured by fenfluramine challenge or cerebrospinal fluid levels of 5HIAA (Malone, Waternaux et al. 2003).

Hypobaric hypoxia could reduce serotonin synthesis by affecting the activity of tryptophan hydroxylase (Kumar 2011). Serotonin synthesis begins with dietary tryptophan, a common amino acid. Tryptophan is converted to 5-HTP by tryptophan hydroxylase, and 5-HTP is converted to serotonin by aromatic acid decarboxylase (Boadle-Biber 1993). Tryptophan hydroxylase 2 (TPH2) is the primary isoform present in the central nervous system, and mediates the rate-limiting step in central serotonin synthesis (Walther, Peter et al. 2003, Hasegawa and Nakamura 2010). TPH2 has a high affinity for oxygen (Katz 1980), and its affinity for tryptophan is reduced at lower oxygen tension (Katz 1981), suggesting its activity is oxygen-dependent. In rats, exposure to 10% atmospheric oxygen for 14 days reduces tryptophan hydroxylase activity in the dorsal and median raphe nuclei, as measured by the accumulation of 5-HTP (Poncet, Denoroy et al. 1997).

Relative serotonin depletion due to hypoxia could be associated with resistance to standard antidepressants. Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed antidepressants, accounting for over 80% of the market in the US (Lin, Erickson et al. 2011) and 63% of the European market (Bauer, Monz et al. 2008). SSRIs prevent reuptake of serotonin and thereby increase its synaptic availability (Fuller and Wong 1977). When serotonin production is inhibited, however, the efficacy of SSRIs may be compromised. Mice with low serotonin synthesis secondary to a polymorphism in TPH2 exhibit increased depression-like behavior (Osipova, Kulikov et al. 2009), and their responsiveness to citalopram and paroxetine in the FST is significantly reduced (Kulikov, Tikhonova et al. 2011). They also exhibit worsened reduction in tissue serotonin levels when exposed to fluoxetine, which is not

observed in wild-type mice; this reduction can be prevented by administration of 5-HTP (Siesser, Sachs et al. 2013). Furthermore, these mice exhibit increased social defeat stress and reduced improvements in social interaction in response to fluoxetine (Sachs, Ni et al. 2015). Antidepressants with broader activities may treat depression related to serotonergic deficits more effectively than SSRIs, however, as rats exposed to simulated high altitude exhibit a response to desipramine in the FST, but do not respond to fluoxetine (Kanekar, Bogdanova et al. 2015).

In principle, supplementation with 5-HTP, which bypasses the oxygen-dependent step in serotonin production, could correct alterations in serotonin metabolism associated with altitude. 5-HTP was explored as a potential antidepressant in the 1970s and 1980s. It crosses the blood-brain barrier easily, elevates brain serotonin levels, and shows antidepressant efficacy in clinical trials (Birdsall 1998, Turner, Loftis et al. 2006). It therefore represents a promising treatment for altitude-associated depression.

E. Safety Profile and Toxicity of 5-HTP in Adults

The most common adverse effects associated with 5-HTP supplementation are gastrointestinal (GI) problems, which include nausea, vomiting, and diarrhea. Less common side effects include insomnia, palpitations, and headache (BYERLEY, JUDD et al. 1987). (Cangiano, Ceci et al. 1992)conducted a double blind placebo study with 20 obese female patients that were given 300 mg three times daily. This study demonstrated significant decreases in carbohydrate craving and food intake, which resulted in weight loss for patients treated with 5-HTP. At this higher dose they reported initial nausea in patients but not significant enough for subjects to drop out of study. This and other studies indicate that GI side effects are dose-dependent and mostly occurred in patients taking 5-HTP alone. These effects are moderate and usually disappear once steady dosage is reached. (BYERLEY, JUDD et al. 1987).

An additional concern of the use of 5-HTP, especially if combined with typical antidepressants, is an increased risk for serotonin syndrome, a potentially fatal condition involving high blood pressure, delirium, tremor, hyperreflexia, and other abnormalities caused by excessive blood serotonin levels (Sternbach 1991). However, the clinical literature to date does not support this concern and clinical studies generally suggest that 5-HTP is safe, even if combined with other antidepressants. In addition, since both chronic hypoxic diseases and living with hypobaric hypoxia are connected to low brain serotonin levels (Katz 1982), the likelihood of serotonin syndrome is low in those with these hypoxic conditions. To our knowledge, no human cases of serotonin syndrome have been reported in association with 5-HTP, either as a single therapy or as a combination with other medications.

Another important safety consideration involving the use of 5-HTP, which is a derivative of L-tryptophan, is the historical association between L-tryptophan and eosinophilia-myalgia syndrome (EMS) (FDA 1998). Eosinophilia-myalgia syndrome is marked by the development of flu-like constitutional symptoms, muscle aches, and eosinophilia. The association led the FDA to ban the sale of L-tryptophan in 1991. Subsequently, however, the development of EMS in association with L-tryptophan was related to contaminants in the supply produced by a particular manufacturer (Michelson, Page et al. 1994). The FDA lifted the ban on L-tryptophan in 2001 (FDA 2001). Nevertheless, occasional case reports of EMS in association with L-tryptophan and, less frequently, 5-HTP, have been made (Allen, Peterson et al. 2011) (NEMSN 2014) and the NIH continues to urge that 5-HTP be used “cautiously” because of the risk of EMS associated with 5-HTP has not been well established (NIH 2014)

F. Altered Brain Bioenergetics in Depression and Hypoxia

A second potential link between altitude and depression is the effect of hypobaric hypoxia on brain bioenergetics. Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) demonstrates reduced total nucleotide triphosphate (β-NTP) concentrations, and in some studies, higher phosphocreatine (PCr) concentrations in adults with depression when compared to healthy volunteers (Moore, Christensen et al. 1997, Volz, Rzanny et al. 1998, Iosifescu, Bolo et al. 2008). This pattern, which is more common in females than males (Renshaw, Parow et al. 2001), suggests that some depressed patients have increased brain energy stores that are not being accessed efficiently to support cerebral activity. This specific pattern of altered energy metabolism is associated with an increased likelihood of treatment response to both SSRIs and

triiodothyronine. Administration of triiodothyronine is associated with reductions in brain PCr and increases in β-NTP (Iosifescu, Bolo et al. 2008). Brain phosphocreatine levels are also disrupted in the frontal lobes of adolescents with bipolar depression (Shi, Kondo et al. 2012).

Proton MRS (^{1}H-MRS) studies suggest similar alterations in brain metabolites in depression (Ende, Demirakca et al. 2006, Caverzasi, Pichiecchio et al. 2012). Patients with first-episode depression exhibit reduced n-acetylaspartate (NAA) to creatine ratios in several brain areas, including prefrontal white matter (Wang, Jia et al. 2012, Jia, Zhong et al. 2015) and prefrontal cortex (Sozeri-Varma, Kalkan-Oguzhanoglu et al. 2013), and exhibit progressive decreases in the ratio of NAA to creatine in pregenual anterior cingulate cortex with continued illness (Tae, Kim et al. 2014). Similar abnormalities are also found in geriatric depression (Chen, Chiang et al. 2009) and bipolar depression (Zhong, Wang et al. 2014), though levels of other brain metabolites, such as choline, may differ between unipolar and bipolar depression (Shi, Forrest et al. 2014). There is one published report of ^{1}H-MRS in adolescents with depression, which also indicates alterations in brain metabolism (Steingard, Yurgelun-Todd et al. 2000).

Hypobaric hypoxia could directly promote changes in brain metabolism that lead to depression. Chronic hypoxia clearly leads to reductions in energy reserve in other tissues with high energy demands, such as cardiac and skeletal muscle (Murray 2016), which can be demonstrated using ^{31}P-MRS (Holloway, Cochlin et al. 2011, Jameel, Hu et al. 2014). This has been demonstrated specifically for hypobaric hypoxia, as prolonged exposure to high altitude is associated with deficits in cardiac adenosine triphosphate (ATP) to PCr ratios (Holloway, Montgomery et al. 2011). In animal models, chronic hypoxia has been shown to lead to reductions in the levels of several metabolites, including PCr and NAA (Raman, Tkac et al. 2005). Similarly, hypobaric hypoxia alters mitochondrial dynamics in the hippocampi of rats (Jain, Prasad et al. 2015), and is associated with neurodegeneration and apoptosis of hippocampal pyramidal cells (Maiti, Singh et al. 2007).

In humans, chronic hypoxia related to COPD is associated with reduced brain PCr concentrations, suggesting a shift from oxidative phosphorylation to glycolysis (Mathur, Cox et al. 1999). Chronic hypobaric hypoxia also has lasting effects on multiple aspects of cognition, which may indicate widespread bioenergetic deficits (Muthuraju and Pati 2014). Evaluation of 40 age- and gender-matched healthy individuals residing at 1370m (Salt Lake City, UT) or at 7m (Belmont, MA or Charleston, SC) showed that residents at 1370m have reduced total creatine (creatine + phosphocreatine) in the anterior cingulate cortex compared to those at sea level (Renshaw P 2012). This suggests that altitude alone can alter brain metabolism in healthy subjects.

In many studies, antidepressants produce normalization of brain NAA/creatine ratios, including in drug-naïve patient with first-episode depression (Gonul, Kitis et al. 2006, Wang, Jia et al. 2012) and post-stroke depression (Huang, Chen et al. 2010). Accordingly, supplementation with creatine could benefit depression and other psychiatric conditions (D'Anci, Allen et al. 2011, Allen 2012). Creatine has a role in high-energy phosphate transfer during skeletal muscle contraction and neuronal activity via the creatine kinase reaction (Wyss, Felber et al. 1998). The enzyme creatine kinase catalyzes the reversible reaction of creatine and ATP, forming PCr and ADP (McLeish and Kenyon 2005). The intracellular creatine/PCr ratio plays an important role in maintaining an adequate supply of energy, in the form of cellular ATP (Wallimann, Wyss et al. 1992). PCr may be viewed as a reservoir of "high-energy phosphate," which is able to supply ATP, the primary energy source in cells, on demand. Consequently, creatine plays a significant role in energy homeostasis of cells with intermittently high-energy requirements (McLeish and Kenyon 2005). Creatine supplementation improves the function of the creatine kinase/PCr system by increasing cellular creatine and PCr levels, improving the rate of ATP re-synthesis, and maintaining cellular-energy homeostasis (Jost, Van Der Zee et al. 2002).

In rats, chronic creatine supplementation reduces depression-like behavior (immobility in the FST), though only in females (Allen, D'Anci et al. 2010). Clinical trials in humans provide evidence for creatine's effect on brain function. Rae et al. (2003) showed that increasing oral creatine intake resulted in a significant positive effect on both working memory and intelligence in vegetarian young adults. The administration of oral creatine monohydrate also alters brain creatine, PCr, and β-NTP (Lyoo, Kong et al. 2003). In a small pilot study, 5 adolescent females with SSRI-resistant MDD were treated with 4g of creatine daily for 8

weeks; at baseline, depression ratings were inversely related to brain β-NTP levels. Creatine supplementation in this group increased brain PCr levels (Kondo, Sung et al. 2011). Adolescent females with SSRI-resistant depression treated with 2, 4, or 10g of creatine daily for 8 weeks in addition to their baseline antidepressant exhibit graded increases in brain PCr concentrations that correlate with improvements in mood (Kondo, Forrest et al. 2016). In a large, placebo-controlled study of creatine augmentation of escitalopram, creatine was related to significant improvements in depression scores compared to placebo, in treatment-naïve adult females with MDD (Lyoo, Yoon et al. 2012). In this study, response to creatine was associated with normalization of brain rich club hub network connections as well as increases in prefrontal NAA concentrations compared to patients receiving escitalopram plus placebo (Yoon, Kim et al. 2015). Reductions in brain phosphocreatine levels are also found in female methamphetamine users, who are more likely to exhibit depression than male counterparts (Hellem, Lundberg et al. 2015), and supplementation with creatine, even in the absence of an antidepressant, improves brain PCr levels and depression scores in women with depression and methamphetamine dependence (Hellem, Sung et al. 2015). To our knowledge, only one study of creatine augmentation in depression has found no effect, and it was small (n = 14) and recruited subjects who may have been less ill as they were required to have failed to respond to only 3 weeks of antidepressant treatment (Nemets and Levine 2013).

G. Safety Profile and Toxicity of Creatine in Adults

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 (Poortmans and Francaux 1998, Poortmans and Francaux 1999, Mihic, MacDonald et al. 2000, Gualano, Ugrinowitsch et al. 2008). 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 (Shao, Martin et al. 2008). 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 (Shao, Martin et al. 2008). Studies conducted in athletes and military personnel indicate a substantial safety level of both short- and long-term creatine supplementation in healthy adults (Poortmans and Francaux 1999, Robinson, Sewell et al. 2000, Bennett, Bathalon et al. 2001, Greenwood, Kreider et al. 2003, Greenwood, Kreider et al. 2003, Kreider, Melton et al. 2003). 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 (Pritchard and Kalra 1998, Koshy, Griswold et al. 1999). Literature reviews and expert consensus panels have concluded there is no evidence supporting an association between creatine and renal disease (Poortmans, Auquier et al. 1997, Terjung, Clarkson et al. 2000, Farquhar and Zambraski 2002, Yoshizumi and Tsourounis 2004).

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 (Harris, Söderlund et al. 1992). In response to the concerns regarding creatine and renal toxicity, Poortmans conducted studies of the effect of creatine supplementation on renal function, showing that short-term supplementation does not alter glomerular filtration rate (Poortmans and Francaux 1998), and that chronic supplementation of up to five years' duration did not impair renal function in healthy athletes (Poortmans and Francaux 1999).

(Schilling, Stone et al. 2001)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 (Bender, Samtleben et al. 2008). Interestingly, the participants who received creatine performed better on the depression subscale of the Unified Parkinson Disease Rating Scale (Bender, Koch et al. 2006).

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.

H. Synergistic Effects of 5-HTP and Creatine

Serotonin and creatine are processed separately in the brain, and deficits in these chemicals lead to distinct clinical problems. Therefore, we believe that treatment with a combination therapy, which could correct both deficits, would have a synergistic effect in the treatment of hypoxia-related depression and possibly other forms of treatment-resistant depression. Thus, we propose to investigate antidepressant efficacy of dietary 5-hydroxytryptophan (5-HTP) and creatine used as augmenting agents for patients with SSRI-resistant depression, as a means to restore the brain neurotransmitter and metabolic imbalances linked to chronic hypoxia.

I. Gender Differences in Depression

Globally, depression is among the most common disorders affecting females throughout their lives, and is the leading cause of disability among women between the ages of 15 and 44 years (Lopez 2006). Women are more than twice as likely to be diagnosed with depression as men (Bebbington, Dunn et al. 1998), potentially due to gender-based variations in biological, psychological and socioeconomic factors. Biological factors potentially involved include female hormones and brain neurochemistry. Significant differences are seen in serotonin synthesis in the human brain between healthy adult men and women (Nishizawa, Benkelfat et al. 1997): the mean rate of serotonin synthesis is 52% lower in females vs. males. With low basal serotonin in the brain, women are likely to be more vulnerable to hypoxia-induced reduction in brain serotonin, implying that women exposed to chronic hypoxia (altitude, smoking, COPD or asthma), may be more susceptible to depression than men.

The Utah Department of Health published a report on depression and antidepressant use in Utah (Health 2010). Using health insurance claims from 899,323 Utah residents, antidepressant use was examined, and over six million pharmacy claims from 2009 were reviewed. Of interest, they reported that 84,000 individuals were prescribed antidepressants, resulting in antidepressants being the most widely prescribed medication in the state. When comparing antidepressant utilization by gender, researchers noted that women are prescribed antidepressants more than twice as often as men.

J. Functional Connectivity in Depression

The variability in patient-reported and clinician-assessed measures in depressive symptomatology make the identification of reproducible biomarkers of depression and antidepressant response essential. It has also been hypothesized that the pathogenesis of MDD may, in some cases, involve disruption of cortical regulation of emotional processing (Mayberg 1997, Phillips, Drevets et al. 2003). As noted above, abnormalities in structural connectivity have been noted in depression and these abnormalities may be improved by antidepressant treatment (Yoon, Kim et al. 2015). A potential limitation of the measurement of structural connectivity in depression is that it may underestimate changes in the behavior of neural circuits, as these may not always correlate with structural alterations. Likewise, improvements in neural circuit function associated with antidepressant response could, in principle, precede structural changes. Accordingly, measurements of functional connectivity—correlations in the activity of interconnected brain regions—could be a valuable tool for assessing antidepressant response. Task-based functional magnetic resonance imaging (fMRI) has been used extensively in the study of depression and has generally confirmed the relationship between depression and decreased fronto-limbic activation. The assessment of resting-state functional connectivity has certain advantages relative to task-based protocols, in that it may yield more general (as opposed to task-specific) results, is less demanding for ill patients, and has more ready

application to clinical settings. Resting-state functional connectivity MRI (fcMRI) has been shown to correlate with structural connectivity (Greicius, Supekar et al. 2009) and has been used to investigate the behavior of cortical networks in major depressive disorder. Although results are somewhat inconsistent, in general resting-state fcMRI studies have pointed to reduced activity in fronto-limbic networks related to the pathophysiology of MDD, and in particular to reduced connectivity between the medial prefrontal cortex (MPFC) and amygdala and the anterior cingulate cortex (ACC) and amygdala (Anand, Li et al. 2005, Anand, Li et al. 2007, Anand, Li et al. 2009, Cullen, Gee et al. 2009, Veer, Beckmann et al. 2010, Wang, Hermens et al. 2012). Depression is also associated with specific increased functional connectivity between hubs of the default mode network, specifically the medial prefrontal cortex to posterior cingulate cortex (Greicius, Flores et al. 2007, Zeng, Shen et al. 2012), a finding that dovetails with hyperactivation of default mode network (particularly subgenual anterior cingulate cortex) that may be associated with increased rumination in depression (Gotlib, Sivers et al. 2005, Beauregard, Paquette et al. 2006, Yang, Simmons et al. 2009). There is also early evidence that treatment-resistant depression is specifically associated with disruption of thalamo-cortical circuits (Lui, Wu et al. 2011). Recent work suggests that antidepressant response is associated with normalization of fronto-limbic connections, including a strengthening of connections between the ACC and amygdala (Cullen, Klimes-Dougan et al. 2016). Some early studies (Zhang, Tang et al. 2016) indicate that alterations in functional connectivity associated with antidepressant response are also correlated with changes in neuronal metabolites, such as glutamate, in brain regions implicated in these altered circuits, providing a neurochemical correlate of changes in circuit activity.

K. Gene x Environment Interactions in Depression

Hypobaric hypoxia related to high altitude of residence could interact with genetic differences that affect serotonin metabolism or brain energy regulation to produce treatment resistant depression. Mice with low serotonin synthesis secondary to a polymorphism in tryptophan hydroxylase 2 (TPH2) exhibit increased depression-like behavior (Osipova, Kulikov et al. 2009), and their responsiveness to the SSRIs citalopram and paroxetine in the FST is significantly reduced (Kulikov, Tikhonova et al. 2011). They also exhibit worsened reduction in tissue serotonin levels when exposed to fluoxetine, which is not observed in wild-type mice; this reduction can be prevented by administration of 5-HTP (Siesser, Sachs et al. 2013). Furthermore, these mice exhibit increased social defeat stress and reduced improvements in social interaction in response to fluoxetine (Sachs, Ni et al. 2015). Although the effects of hypobaric hypoxia on antidepressant response have not been studied directly, other populations with reduced serotonin levels exhibit reduced SSRI response. Polymorphisms in TPH2 that could alter the efficiency of serotonin production have been associated with unipolar depression (Zill, Baghai et al. 2004, Zhang, Gainetdinov et al. 2005, Van Den Bogaert, Sleegers et al. 2006) and bipolar disorder (Harvey, Gagne et al. 2007, Lin, Chao et al. 2007, Cichon, Winge et al. 2008, Roche and McKeon 2009), and are thought to affect suicide risk in major depressive disorder (Zill, Buttner et al. 2004, Zhou, Roy et al. 2005, De Luca, Hlousek et al. 2006, Yoon and Kim 2009). They may also predict non-response to conventional antidepressants (Tsai, Hong et al. 2009, Lim, Won et al. 2014). Conceivably, TPH2 polymorphisms could also augment the contribution of high altitude to the development of depression.

L. Preliminary Data

We conducted a pilot study of 5-HTP and creatine as augmenting agents for SSRI/SNRI-resistant major depressive disorder in 15 women. The study was open-label and there was no placebo control. Subjects were evaluated at a screening visit and at a baseline visit one week later before treatment, and then received study drug for 8 weeks. There were also two follow-up visits after treatment discontinuation at weeks 10 and 12. The primary outcome measure was change in HAM-D scores over the 8 weeks of treatment. The mean HAM-D score averaged over the screening and baseline visits was 18.6 (SD=1.9), and the mean HAM-D score at week 8 was 7.5 (SD=4.5), with an average mean difference of 11.5 ($t=-8.2$, $p < 0.00001$). If antidepressant response is defined as at least a 50% reduction in HAM-D score, then 10/15 (67%) of subjects were responders. Assuming a 40% placebo response rate, this result is significant ($z =$

2.1, p = 0.03). We believe that this preliminary data suggests that creatine and 5-HTP may represent effective augmenting agents for antidepressant-resistant major depressive disorder.

In the pilot study treatment with 5-HTP and creatine was well tolerated. There were no suicide attempts and no subjects exhibited acute worsening of suicidal ideation. Two subjects were lost to follow-up. One subject was withdrawn from the study by investigators because of concern for serotonin syndrome, but after thorough clinical evaluation was deemed not to meet criteria for serotonin syndrome. There were no adverse events attributable to the study medications. One subject received a diagnosis of medullary sponge kidney during the study period, but this is a familial condition (Leung 2014) and unlikely to be related to study participation.

III. Specific Aims

Aim 1: To describe changes in HAM-D scores over the course of 8 weeks of creatine and 5-HTP supplementation in women compared to placebo. A clinical trial of creatine augmentation in SSRI-treated females with major depressive disorder found that creatine significantly improved HAM-D scores compared to placebo (Lyoo, Yoon et al. 2012). There are also reports of preclinical studies demonstrating antidepressant effects of creatine in female, but not male rats (Allen, D'Anci et al. 2010). Therapeutic administration of 5-HTP has also been shown to be beneficial in the treatment of depression (Birdsall 1998). We hypothesize that subjects' HAM-D scores will improve over 8 weeks with 5-HTP + creatine supplementation compared to placebo.

Aim 2: To identify functional neuroimaging correlates of treatment response in the aforementioned study. Resting-state functional magnetic resonance imaging can be used to demonstrate alterations in brain connectivity in depression and to describe how they evolve with antidepressant treatment. To that end, we will examine the correlation between clinical response in the study proposed and changes in functional connectivity over 8 weeks. We hypothesize that abnormalities in functional connectivity associated with depression will normalize over 8 weeks of treatment in a fashion correlated with clinical improvement in those receiving active treatment, but will remain unchanged in those receiving placebo.

Aim 3: To identify neurochemical correlates of treatment response in the aforementioned study. We will examine the correlation between clinical response in the aforementioned subjects and changes in frontal cortical energy metabolism over 8 weeks, as measured by phosphorus magnetic resonance spectroscopy (^{31}P-MRS). We hypothesize that deficits in frontal cortical energy metabolism will normalize over 8 weeks of treatment in a fashion correlated with clinical improvement in those receiving active treatment.

IV. Methods

A. Eligibility Criteria

36 women with treatment-resistant depression will be randomly assigned in a 1:1 ratio to either 5-HTP/creatine or placebo for eight weeks, and an additional 18 participants will be recruited as healthy controls for neuroimaging purposes. Table 1 outlines eligibility criteria. Table 2 provides a list of exclusionary medications.

Using purposive sampling, treatment-resistant depressed females will be recruited for this trial. Females will be considered for participation if they are between the ages of *25-40 years*, and meet the operational definition of depression as a *Hamilton Depression Rating Score (HAM-D) of 16 or greater.* An age range of 25-40 was chosen based on considerations of participant ability to consent, declining overall physical health with advanced age, and to minimize heterogeneity in brain structure and connectivity associated with both early adult remodeling and age-related degeneration.

Participants will be regarded as having treatment-resistant depression if they have a HAM-D score at baseline of $\geq$ 16 and meet DSM-IV criteria for a major depressive episode, and have been taking a therapeutic dose of any FDA-approved selective serotonin reuptake inhibitor (SSRI) or serotonin norepinephrine reuptake inhibitor (SNRI) for at least 8 weeks. This a more liberal definition of "treatment-resistant depression" than is often utilized in the psychiatric literature, where "treatment-resistant

depression is generally taken to mean the absence of significant clinical response after 2 trials of antidepressants from different pharmacological classes (Berlim and Turecki 2007). However, treatment resistance understood as failure to achieve remission after a single adequate antidepressant trial is sufficient to address our primary hypothesis.

Participants will be eligible for inclusion if they have been adequately adherent to any FDA-approved SSRI or SNRI at standard doses for at least 8 weeks. Participants will also be eligible if they are using bupropion (wellbutrin) as a cotherapy to SSRI or SNRI medication. Eligible SSRIs and dose ranges include citalopram 20mg-40mg per day, escitalopram 10mg-30mg per day (Roseboom and Kalin 2013), fluoxetine 20mg-80mg per day (Zahajszky, Rosenbaum et al. 2009), fluvoxamine 100mg-300mg per day (Aboujaoude and Koran 2009) sertraline 50mg-200mg per day (Block, Yonkers et al. 2009), or paroxetine 20mg-50mg per day (Schatzberg and Nemeroff 2009). Eligible SNRIs include venlafaxine 75mg-300mg per day (Rudolph, Fabre et al. 1998), duloxetine 30-120mg per day (Mallinckrodt, Prakash et al. 2006), desvenlafaxine 50mg- 100mg per day (Thase, Kornstein et al. 2009), and levomilnacipran 40-120mg per day (Citrome 2013). Adequate adherence will be assessed according to participant report and is defined as completion of at least 75% of scheduled doses. To be eligible, participants should have been receiving the baseline SSRI dose for at least 4 weeks but may have had dose adjustments prior to that period.

Individuals who meet criteria for any psychiatric condition apart from major depressive disorder on the SCID-I will not be invited to participate, with the exception of subjects who meet criteria for anxiety disorders (e.g., social anxiety disorder, generalized anxiety disorder, specific phobias, panic disorder, agoraphobia, obsessive-compulsive disorder, or post-traumatic stress disorder). Individuals with substance use disorders will not be included 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. In our pilot study we had excluded subjects if they were *diabetic* because of concern that creatine might influence insulin production, but a recent study has suggested that creatine is safe in diabetes and may even improve glycemic control (Gualano, de Salles Painneli et al. 2011). Accordingly, subjects with diabetes will not be excluded.

Since gastrointestinal discomfort has been anecdotally reported in some individuals taking creatine and 5-HTP, females with *gastrointestinal diseases, such as colitis (e.g. infectious colitis, ulcerative colitis, Crohn's disease, and ischemic colitis) or diverticulitis*, will be excluded. Individuals with a diagnosed *seizure disorder* will also be excluded, because in one report, the authors suggest that creatine might be implicated in seizure activity, although the reported seizure activity was associated with hypoglycemia, secondary to cardiac arrest (Haller, Meier et al. 2005). Moreover, the seizure events were reported through the Food and Drug Administration's MedWatch System, but did not undergo a formal review.

Current suicide risk identified by administration of the Columbia Suicide Severity Rating Scale (C-SSRS) (Posner, Brent et al. 2008) will exclude an individual's participation. Individuals with a previous diagnosis of *serotonin syndrome* will not be included for participation. Although there is no observational evidence to our knowledge that a history of serotonin syndrome increases future risk for that disorder, in theory subjects' physiologies (including central and peripheral serotonin metabolism, underlying cardiovascular conditions, and/or drug metabolism) may predispose them to that condition, suggesting previous development of the syndrome increases risk. Accordingly, subjects with a history of diagnosed serotonin syndrome or current evidence of serotonin syndrome will be excluded.

Individuals with a history of *pulmonary disease* will also be excluded from the study, given the possible association between SSRI use and idiopathic pulmonary hypertension (Fox, Azoulay et al. 2014). Given the association between antidepressant use and the development of QT prolongation and torsade des pointes (Vieweg, Wood et al. 2009), individuals with evidence of QT prolongation (QTc > 500ms) on EKG or a history of cardiac disease will be excluded. Likewise, individuals with a history of *major autoimmune or rheumatologic illness (rheumatoid arthritis, psoriatic arthritis, lupus, mixed connective tissue disease, ankylosing spondylitis, fibromyalgia, dermatomyositis, polymyositis, and related conditions)* will be excluded from the study. This is because of the historical association between the use of L-tryptophan and the development of eosinophilia-myalgia syndrome (an inflammatory condition marked by high eosinophil

concentrations and muscle aches). Exclusion of participants with preexisting rheumatological conditions will minimize confounds and facilitate the detection of any new cases of EMS. Individuals with a history of EMS will also be excluded. Finally, to minimize confounds, individuals with pre-existing eosinophilia (absolute eosinophil count $\geq$ 500/uL) will be excluded.

Individuals being treated with *any non-SSRI/SNRI antidepressant (TCA, MAO-I, alpha receptor antagonist, serotonin receptor modulator and norepinephrine-dopamine reuptake inhibitor) as a monotherapy will not be included.* We will still allow the inclusion of patients taking bupropion as a adjunctive antidepressant, and similarly will allow the inclusion of subjects taking trazodone as a sleep aid, up to 200mg at bedtime. This exception will be made because the likelihood of serotonin syndrome in patients taking adjunctive trazodone is small, and because subjects with depressioin frequently require treatments for insomnia. We will exclude subjects taking any antipsychotic, any mood stabilizing medication, or other antidepressant augmenting agent (including 5-HTP, creatine, l-methylfolate, tianeptine, s-adenosyl methionine). Individuals taking any additional serotonergic medications (i.e., medications known to have been implicated in the development of serotonin syndrome) will not be included. These medications are listed in table 2 ("Excluded medications and drugs") below, which is adapted from (Boyer and Shannon 2005). Note that this table includes medications (e.g., mood stabilizers like lithium) that are also excluded from the study for other reasons. Although many of these medications are commonly prescribed and the exclusion of participants taking them may significantly impact recruitment, it is necessary to increase the margin of safety in the study by reducing the likelihood of new-onset serotonin syndrome.

Participants who are currently 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.

B. Study Withdrawal Criteria

A participant will be withdrawn from the study if she experiences intolerable or clinically significant side effects to creatine and/or 5-HTP (including the development of serotonin syndrome or eosinophilia-myalgia syndrome), 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 or changes the baseline antidepressant. 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.

Table 1. Eligibility Criteria

INCLUSION CRITERIA
Female gender, ages 25-40 years inclusive Current diagnosis of Major Depressive Disorder identified by the SCID-I Current HAM-D_{17} score of $\geq$ 16 Adequate adherence to any FDA approved SSRI or SNRI for at least 8 weeks Right-handed
EXCLUSION CRITERIA
Any non-MDD and non-anxiety psychiatric diagnosis, as identified by the SCID-I

History of or current diagnosis of renal disease, such as chronic renal failure, acute renal failure or end stage renal disease Current colitis or diverticulitis History of or current pulmonary disease History of cardiac disease or QTc > 500ms History of fibromyalgia, lupus, eosinophilia-myalgia syndrome, dermatomyositis, polymyositis, rheumatoid arthritis, psoriatic arthritis, mixed connective tissue disease, ankylosing spondylitis, or other related rheumatological condition History of or current seizure disorder Current serious suicide risk identified by the Columbia Severity Suicide Rating Scale Current treatment with an antipsychotic, mood stabilizer, or non-SSRI antidepressant except for bupropion as an augmenting agent Positive pregnancy test, pregnancy, failure to use adequate birth control method Previous diagnosis of serotonin syndrome or evidence of serotonin syndrome Use of any excluded drugs or medications including serotonergic drugs or medications (Table 2) Pre-existing eosinophilia (absolute eosinophil count ≥ 500/uL) Contraindications to MRI: ferromagnetic implants, implanted devices, claustrophobia
STUDY WITHDRAWAL CRITERIA
Intolerable or clinically significant side effects to creatine or 5-HTP Hospitalization for suicidal ideation/suicide attempt Positive pregnancy test, pregnancy Incarceration Initiation of any excluded psychotropic medication (antipsychotic, mood stabilizer or antidepressant) Discontinuation of FDA approved SSRI or incomplete adherence to SSRI Initiation of any excluded serotonergic medications Evidence of serotonin syndrome Evidence of eosinophilia-myalgia syndrome

Table 2. Excluded medications and drugs

Amphetamines (including dextroamphetamine, methamphetamine)
Cocaine
MDMA (Ecstasy)
Amphetamine derivatives (including fenfluramine, dexfenfluramine, phentermine)
Levodopa, Carbidopa-levodopa
Meperidine
Tramadol
Pentazocine
Dopamine-norepinephrine reuptake inhibitors (except for bupropion as an augmenting agent)
Tricyclic antidepressants (TCAs) (including amitriptyline, amoxapine, clomipramine, desipramine, doxepin, imipramine, maprotiline, nortriptyline, protriptyline, trimipramine)
St. John's wort (Hypericum perforatum)

5-HT3 receptor antagonists (including dolasetron, granisetron, ondansetron, palonosetron)
Metoclopramide
Valproate
Carbamazepine
Sibutramine
Dextromethorphan
Cyclobenzaprine
LSD
Ergot derivatives (ergotamine, methylergonovine)
Monoamine oxidase inhibitors (including phenelzine, tranylcypromine, isocarboxazid, moclobemide, selegiline, rasagiline, linezolid, isoniazid, tedizolid, methylene blue, procarbazine, Syrian rue
Lithium
Serotonin modulators (except for trazodone up to 200mg at bedtime)

C. Procedures

1. Overview of procedures

All procedures performed by study personnel are research-related. 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. Table 3 outlines the schedule of study procedures for clinical participants. 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 or a bachelor's level research assistant with training in the specific measures used. Physical examinations and laboratory 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 participants 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 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. Subjects will be separately consented for blood sample storage for later genetic testing and will be allowed to opt-out of this procedure while still participating in the other aspects of the study.

To determine if an individual is eligible for study participation, a screening visit will be conducted. Initially, a HAM-D_{17} will be administered to determine if the patient exhibits depressive symptoms that are sufficiently severe for inclusion in the study. Next, the SCID-I will be administered to confirm a diagnosis of a current major depressive episode. In addition, subjects will be screened for other DSM-IV-TR disorders using the SCID-I to assess exclusion criteria. Afterward, the MADRS, C-SSRS, BAI, YMRS, and CGI-S will be administered. All participants will receive a pregnancy test unless they report a history of menopause, hysterectomy, tubal ligation or are currently using an intrauterine device (IUD) as a form of

contraception. Study subjects will receive a baseline BUN/creatinine (to assess for renal insufficiency), CBC (to screen for pre-existing eosinophilia), vitals, and a baseline focused physical. Subjects who meet other screening criteria will also have blood drawn to be stored for subsequent genetic testing.

An EKG will be performed to evaluate the QT interval and to screen for other evidence of cardiac disease. They will also be screened for evidence of serotonin syndrome using the Hunter Criteria. A complete medical and psychiatric history (focused on illness duration, length of current treatment, treatment history, and history of medical illnesses that preclude participation, e.g., renal disease, pulmonary disease, rheumatologic disease, diabetes, gastrointestinal disease, pregnancy, seizure disorder, serotonin syndrome) will be completed.

After the screening visit, participants will have a baseline visit after one week, prior to the start of treatment. At the baseline visit, the HAM-D_{17}, MADRS, C-SSRS, BAI, YMRS, and CGI-S will be administered. Vital signs will be checked. Participants will again be evaluated for serotonin syndrome using the Hunter Criteria. Baseline ^{31}P-MRS and resting state fMRI will be completed as described below. After initiation of treatment, subjects will be reevaluated at 1, 2, 4, 6, and 8 weeks ($\pm$5 days). At each these visits, the HAM-D_{17}, MADRS, C-SSRS, BAI, YMRS, and CGI-S will be administered again. Follow-up ^{31}P-MRS and resting state fMRI will be completed at week 8. Vital signs, focused physical exam, collection of urine for pregnancy screening if indicated, recording of concomitant medications, self-reported drug use, medication compliance assessment (utilizing a visual analogue scale), study medication dispensing, and screening for treatment-related adverse effects will also be undertaken. Screening for treatment-related adverse effects will include application of the Hunter Criteria for serotonin syndrome (Dunkley, Isbister et al. 2003) as well as evaluation for symptoms of eosinophilia-myalgia syndrome. If subjects exhibit symptoms of eosinophilia-myalgia syndrome, a CBC with automated differential will be obtained. Finally, participants will be seen for follow up visits at 2 weeks and 4 weeks ($\pm$5 days) after completion of 5-HTP/creatine supplementation. Measure at these visits will include the HAM-D_{17}, MADRS, C-SSRS, BAI, YMRS, CGI, DESS, vitals, focused physical exam, and evaluation for serotonin syndrome and EMS. Again, if symptoms of EMS are present, a CBC with automated differential will be obtained.

Once entered into the study, depressed subjects will be randomized to receive either creatine monohydrate and 5-HTP or placebo using a random-digit method that is based on computer-generated numbers. Block randomization using a SAS code, PROC PLAN with Block, will be used to ensure equal treatment allocation within each block. 18 participants (50% of the trial's clinical subjects) will be randomized to placebo and 18 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 8 weeks of treatment and 4 weeks of follow-up. We anticipate an attrition rate of 20% based on our pilot study (where attrition was 13%), and so will plan to recruit a total of 36 subjects. 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.

Eighteen healthy, age- and gender-matched controls will participate in the neuroimaging portions of the study only as well as screening measures to verify their eligibility to serve as controls. Healthy controls will receive the same battery of psychiatric tests at baseline to verify the absence of any major psychiatric condition. They will receive ^{31}P-MRS and resting state fMRI at the baseline visit and 8 weeks later. Pregnancy tests will be completed at both the baseline and week 8 visits unless subject reports menopause, hysterectomy, tubal ligation or is currently using an intrauterine device (IUD). A general physical exam and medical history will be completed at the screening visit. Study procedures for healthy controls are detailed in Table 4.

2. Screening for Serotonin Syndrome

Given the potential development of serotonin syndrome in patients taking multiple serotonergic medications (in this study, an SSRI plus 5-HTP), monitoring for serotonin syndrome is an important element of participant safety. There are no widely-used standardized scales for the assessment of serotonin syndrome, but algorithms for the detection and diagnosis of the condition exist (Dunkley, Isbister et al.

2003). As noted above, subjects will have vital signs performed at each visit. Participants will also be asked about symptoms of serotonin syndrome at each visit. In our pilot study, a physician completed a thorough physical exam for each patient to screen for these symptoms, but no cases of serotonin syndrome were required and this approach was deemed to be inefficient. Accordingly, in the currently proposed study, we will have a physician on call during each patient visit to complete a physical exam and further assessment for serotonin syndrome if there are vital sign abnormalities or symptoms (severe diarrhea or nausea, sweating, shakiness, stiffness, confusion) that are suggestive of serotonin syndrome. In such cases, the clinician will assess for ocular clonus, mydriasis, diaphoresis, rigidity, hyperreflexia, and clonus, and will evaluate the likelihood of serotonin syndrome using the Hunter Criteria. Finally, at the baseline visit subjects will receive an information sheet describing signs and symptoms of serotonin syndrome in lay terms and providing instructions on how they should seek further evaluation if those symptoms are evident.

3. Screening for Eosinophilia-Myalgia Syndrome

No standardized measures for the detection of eosinophilia-myalgia syndrome (EMS) exist. In this study, participants will be informed about the symptoms of EMS, and will be provided with an information sheet describing the symptoms of EMS in lay terms and providing instructions on how they should seek further evaluation if those symptoms arise. They will be asked about symptoms that may be indicative of EMS (especially myalgias) at each treatment and follow-up visit. At the screening visit, a CBC with automated differential will be performed to screen for pre-existing eosinophilia. Although in principle participants could receive a complete blood count at each visit after the initiation of treatment to screen for the development of EMS, to our knowledge there is no published data to support this intervention and the presence of eosinophilia in the absence of clinical symptoms is not likely to be specific for EMS. In our pilot study, subjects received a physical exam at each visit to screen for symptoms of EMS, but this was found to be an inefficient measure. Accordingly, in the currently proposed study a physician will be on call during each patient visit to complete a physical exam and further assessment for EMS if there are vital sign abnormalities or symptoms (rash, other skin changes, hair loss, muscle aches, joint pain, shortness of breath) that are suggestive of EMS.

Table 3. Study Procedures for Clinical Subjects

	Screening	**Baseline**	**Treatment**					**Follow-up**	
Week	-1	0	1	2	4	6	8	10	12
SCID-I	x								
HAM-D	x	x	x	x	x	x	x	x	x
MADRS	x	x	x	x	x	x	x	x	x
C-SSRS	x	x	x	x	x	x	x	x	x
BAI	x	x	x	x	x	x	x	x	x
CGI-S	x	x	x	x	x	x	x	x	x
YMRS	x	x	x	x	x	x	x	x	x
DESS								x	x
Medical History	x								
Diet History Questionnaire II	Given to patient after screening for home completion								
Hunter criteria		x	x	x	x	x	x	x	x
Screening for EMS			x	x	x	x	x	x	x

Side-effect screening			x	x	x	x	x		
Pregnancy test**	x	x	x	x	x	x	x		
BUN/Creatinine	x								
CBC with differential	x		x*	x*	x*	x*	x*	x*	x*
Blood draw for DNA storage	x								
EKG	x								
Vitals	x	x	x	x	x	x	x	x	x
Focused physical exam	x		x*	x*	x*	x*	x*	x*	x*
Review of current medications	x	x	x	x	x	x	x		
^{31}P-MRS		x					x		
Resting State fMRI		x					x		

* = only if clinically indicated by presence of adverse symptoms or symptoms suggestive of EMS
** = unless subject reports menopause, hysterectomy, tubal ligation or is currently using an intrauterine device (IUD)

Table 4. Study Procedures for Healthy Controls

	Screening	**Baseline**	**Follow-up**
Week	-1	0	8
SCID-I	x		
HAM-D	x		
MADRS	x		
C-SSRS	x		
BAI	x		
CGI-S	x		
YMRS	x		
Medical History	x		
Pregnancy test	x	x	x
^{31}P-MRS		x	x
Resting State fMRI		x	x

4. Study Drug Dosing

We propose creatine monohydrate and 5-hydroxytryptophan supplementation of approximately 18 female subjects with treatment resistant depression (power analysis calculation is described in statistical analysis section), along with approximately 18 subjects treated with placebo. Subjects enrolled in the active treatment arm will be treated with 5 grams daily of 8and 100 mg twice daily of 5-HTP for 8 weeks. Recommended creatine and 5-HTP doses are based on doses that are most commonly used in clinical trials or in historical practice. However, with natural products it is often unclear what the optimal doses are to balance safety and efficacy. The following example doses have been used orally in adults (age $\geq$ 18):

a. Creatine

- For aging: 0.3g/kg for five days, followed by 0.07g/kg for 79 days (Chilibeck, Chrusch et al. 2005)
- Cholesterol reduction: 25g per day for five days followed by 5-10g daily (Earnest, Almada et al. 1996)
- Chronic obstructive pulmonary disorder: 5.7g three times per day for two weeks, followed by 5.7g per day (Fuld, Kilduff et al. 2005), and 0.3g/kg per day for seven days followed by 0.07g/kg daily for seven weeks (Faager, Soderlund et al. 2006)
- Congestive heart failure: 20g for five days (Andrews, Greenhaff et al. 1998)
- Depression: 3-5g daily for four weeks (Roitman, Green et al. 2007, Kondo, Sung et al. 2011, Lyoo, Yoon et al. 2012)

A dose of five grams was selected because that was the dose given in the only published RCT of creatine as a treatment option for depression in adult females. Five grams of creatine was well tolerated with few reported side effects in the depression RCT, and in addition, the authors noted a decrease in depression and anxiety rating scores with five grams of augmentation creatine (Lyoo, Yoon et al. 2012).

b. 5-HTP

- Depression: 50 mg-300mg daily for 35 days (Sano 1972)
- Fibromyalgia: 100 mg daily for 30 days (Puttini and Caruso 1992)
- Obesity: 900 mg daily for 12 weeks (Cangiano, Ceci et al. 1992)
- Chronic headaches:600 mg daily for 6 months (Titus, Davalos et al. 1986)
- Insomnia: 600 mg daily (Wyatt, Zarcone et al. 1971)

A dose of 100 mg twice daily of 5-HTP was selected because this is equivalent to the average total daily dose of 200mg given as treatment option for depressed patients. We have elected to provide patients with twice daily dosing because the half-life of 5-HTP is estimated to be 4.3 ± 2.8h (Westenberg, Gerritsen et al. 1982) . In previous studies, 200mg given as a single daily dose was well tolerated with few side effects reported (Sano 1972, Alino, Gutierrez et al. 1976). We have elected to provide the split dose of 100mg twice daily to decrease the likelihood of serotonin syndrome and other adverse effects, especially given that we propose to study participants already taking an SSRI.

5. Measures

We plan to use the following instruments for data collection:

- Structured Clinical Interview for DSM-IV (SCID-I)
- Hamilton Depression Rating Scale (HAM-D)
- Montgomery-Asberg Depression Rating Scale (MADRS)
- Columbia Suicide Severity Rating Scale (C-SSRS)
- Clinical Global Impressions Scale (CGI) severity of illness subscale (CGI-S)
- Beck Anxiety Inventory (BAI
- Young Mania Rating Scale (YMRS)
- Discontinuation-Emergent Signs and Symptoms checklist (DESS)
- Serotonin Syndrome Screening-Hunter Criteria
- Eosinophilia-Myalgia Syndrome Screening
- Eosinophilia-Myalgia Participant Information Sheet
- Serotonin Syndrome Participant Information Sheet
- Diet History Questionnaire II (past month with portion sizes)

The following paragraphs discuss the internal consistency, test-retest reliability, interrater reliability, content validity, construct validity, and criterion-related validity of these tests. Demographic data, including age, race, ethnicity, marital status, income, and years of education will also be collected. History of

antidepressant treatment, current medications, medical history (history of seizures, history of serotonin syndrome), and substance use will be collected via participant self-report. Self-report drug use data will include use yes or no, and if yes, amount used and route of administration. Finally, side effects will be monitored using a checklist of medication side effects and the collection of vital signs (temperature, pulse, blood pressure) at each visit.

a. Structured Clinical Interview for DSM-IV-TR Axis I Disorders

The SCID-I is a semi-structured clinician-administered interview used to determine Axis I disorders (First, Spitzer et al. 1997). A search of the literature resulted in reports of good reliability. Relevant to the sample we plan to recruit, adequate test-retest reliability kappas were reported in a sample of substance abusers. Specifically, kappa = 1.00 was reported for the current stimulant abuse or dependence section of the SCID-I and kappa = 0.70 was reported for the lifetime stimulant abuse or dependence section of the SCID-I (Peters, Greenbaum et al. 1998). Also relevant to the sample we are interested in, interrater reliability values of 0.81 for the depression module and 0.78 for the substance use disorder module of the SCID-I were reported for a sample of depressed women (Bromberger, Kravitz et al. 2005). To our knowledge, there have not been any published reports of internal consistency on the SCID-I for depression or substance use disorders, and therefore, we will calculate Cronbach's alpha for the depression module and the substance use disorders' module.

Validity of the SCID-I can be evaluated in terms of how well it reflects the DSM-IV diagnostic criteria, although, this can be complex because the SCID-I is typically used as the "gold standard" to which other measures are compared (First, Spitzer et al. 1997). Extensive pilot and field-testing suggest good content validity of the SCID-I in mixed-age samples. A summary of the criterion-related validity studies indicate there is a high level of correspondence between SCID-I determined diagnoses and other variables including clinical symptoms of disorders.

b. Hamilton Depression Rating Scale

The HAM-D is a semi-structured clinician-administer depression rating scale. It is the most widely used rating scale for depression (Bagby, Ryder et al. 2004). The original HAM-D consisted of 17-items, and later it was revised to a 21-item scale with four items (diurnal variation, paranoid ideation, obsessive/compulsive symptoms, and depersonalization/derealization) added to it that are not summated in the total score (Iannuzzo, Jaeger et al. 2006). The 17-item HAM-D ($HAM\text{-}D_{17}$) consists of 17 items that are rated on three- or five- point scales and it will be used in the proposed study.

Adequate internal consistency of the HAM-D has been reported with Cronbach's alpha ranging from 0.46 – 0.92 (Bagby, Ryder et al. 2004). Cronbach's alpha of 0.46 was reported from a study evaluating depression severity in the elderly with physical illness, and the author concluded that the $HAM\text{-}D_{17}$ is an unreliable tool for measuring depression in their population of interest (Hammond 1998). Test retest reliability is satisfactory at the item level with Pearson's r reported $\geq$ 0.70 in the majority of published studies (Bagby, Ryder et al. 2004). Finally, reports of interrater reliability have shown acceptable agreement among raters, with kappa values of 0.81 – 1.00 (Iannuzzo, Jaeger, Goldberg, Kafantaris & Sublette, 2006). The use of a structured interview guide has been shown to increase interrater reliability from a Pearson r as low as 0.39 without the structured interview guide to a Pearson r of 0.78 with the structured interview guide (Bagby, Ryder et al. 2004).

Validity of the HAM-D has been extensively evaluated, and findings suggest that it is a highly valid instrument for defining and measuring depression in adult patients with mental illness (Bagby, Ryder et al. 2004).

In the area of content validity, a concern regarding the HAM-D capturing the current operationalized definition of depression has been raised. The HAM-D items are based on the DSM criteria for depression, and the DSM has been revised at least three times since the inception of the HAM-D. However, the core items of depression on the DSM remain unchanged, and similarly, the core items of the HAM-D have remained the same for 40 years. Fourteen separate instruments have been used to evaluate convergent validity, and several of the studies reported correlations $\geq$ .70, demonstrating adequate

convergent validity. To measure discriminant validity, investigators attempted to detect depression in patients with medical conditions rather than psychiatric disorders. Their findings noted that positive predictive power was very low and variable even though sensitivity, specificity, and negative predictive power were large and rather consistent. Also, studies have been conducted comparing predictive validity of the HAM-D with other scales measuring depression, such as the Beck Depression Inventory (BDI) and Zung Self-Rating Depression scale, and the HAM-D was found to be more sensitive to change than other scales (Bagby, Ryder et al. 2004).

c. Montgomery-Asberg Depression Rating Scale

The Montgomery-Asberg Depression Rating Scale (MADRS) is a 10-item clinician-administered depression rating scale designed to be used in conjunction with the HAM-D, with greater sensitivity to antidepressant-induced symptomatic improvement (Montgomery and Asberg 1979). Each question has four response choices, rated 0, 2, 4, and 6 respectively. In contrast to the HAM-D, the MADRS emphasizes psychological as opposed to somatic symptoms of depression. Although data regarding the psychometric properties of the MADRS is limited, it is widely used in clinical trials (Zimmerman, Chelminski et al. 2004, Williams and Kobak 2008). Nevertheless, the MADRS is deemed to have high interrater reliability even when used by non-clinicians, with a Pearson r of 0.89-0.97 (Montgomery and Asberg 1979). The internal consistency of the MADRS is judged to be high, with r = 0.95 for all items. The external validity of the MADRS is also deemed to be good, with Pearson coefficients of 0.7 with the Raskin Depression Scale (RDS) (Maier, Heuser et al. 1988, Maier, Philipp et al. 1988) and between 0.8 and 0.9 with the HAM-D (Müller, Himmerich et al. 2003).

d. Columbia Suicide Severity Rating Scale

The C-SSRS is a semi-structured clinician-administered scale to assess for suicidality. There are two forms of the C-SSRS: a baseline scale and a past week scale. The baseline scale is used to establish lifetime and current suicide attempts or ideation, and the past week scale evaluates sucidality over a period of seven days (Posner, Brown et al. 2011). Reports of internal consistency in studies of adults with depression and/or other psychiatric concerns have shown acceptable internal reliability (Cronbach's alpha (α) = 0.73 for baseline and past week). In a study of adolescents with a history of a recent suicide attempt, internal consistency of the C-SSRS was noted to be high, $\alpha = 0.937$ for the initial visit and $\alpha = 0.946$ for the past week visit (Posner, Brown et al. 2011).

Three separate studies of adults or adolescents with psychiatric diagnoses evaluated convergent and divergent validity. Pearson's r was calculated from comparing components on the C-SSRS with other instruments measuring similar constructs. These results indicated strong convergent validity, with the C-SSRS baseline moderately correlated with the Scale for Suicide Ideation (r = 0.52), and the C-SSRS past week scale moderately correlated with the suicide item on the Montgomery Asberg Depression Rating scale (MADRS) (r = 0.69) (Posner, Brown et al. 2011). The past week C-SSRS was compared with depression items on the MADRS and BDI, and the C-SSRS demonstrated strong divergent validity when items on the MADRS and BDI did not overlap with items on the C-SSRS except with the suicide items (Posner, Brown et al. 2011). Finally, an assessment of predictive validity, using the Columbia Suicide History Form and the suicide evaluation board classifications, demonstrated that baseline C-SSRS ratings significantly predicted suicide attempts (Posner, Brown et al. 2011)

e. Clinical Global Impression Scale

The CGI was developed for use in drug trials in schizophrenia by the National Institute of Mental Health (Guy 1976). It involves an overall clinician-determined assessment of functioning that takes into account all clinical information, such as the patient's history, social circumstances, symptoms, behavior, and functional limitations related to symptom severity. The CGI includes three subscales: Severity of Illness (CGI-S), Global Improvement (CGI-I) and Efficacy Index. The CGI-S rates the severity of the patient's illness on a 7-point scale ranging from 'Normal' = 1 to 'Extremely Ill' = 7, based on the clinician's experience with other patients with the same condition. The CGI has been shown to correlate strongly with

other widely-accepted research scales such as the HAM-D, MADRS (Bandelow, Baldwin et al. 2006) PANSS, and BPRS (Leucht, Kane et al. 2006).

f. Beck Anxiety Inventory

The Beck Anxiety Inventory (BAI) is a 21-item self-report instrument for assessing the severity of anxiety. Each item offers four response choices scored from 0 to 3, with a higher score representing a more severe experience of the symptom (Beck, Epstein et al. 1988). Adequate internal consistency has been reported in samples of nonpsychiatric patients, inpatient psychiatric patients and outpatient psychiatric patients with Cronbach's alpha ranging from 0.83 to 0.95 (De Ayala, Vonderharr-Carlson et al. 2005). Test-retest reliability is satisfactory with a mean reliability estimate of 0.66 and time intervals between administrations ranging from 7 to 112 days with an average of 32 days (De Ayala, Vonderharr-Carlson et al. 2005). Relevant to how the BAI will be administered for this study, a one-week test-retest estimate of 0.75 has been reported (Beck, Epstein et al. 1988). Since the BAI is a self-report tool, there are no reports of interrater reliability.

With regard to content validity, the BAI addresses the majority of DSM symptoms of generalized anxiety in addition to 11 out of 13 of the DSM symptoms of panic disorders (Beck, Epstein et al. 1988). Findings from studies of construct validity indicate acceptable convergent and discriminate validity. For convergent validity, correlations in the range of 0.47 to 0.71 between the BAI and the Cognition Checklist Anxiety Subscale, the State Trait Inventory, the Anxiety Subscale of the Symptom Checklist-90-Revised, and the Hamilton Rating Scale for Anxiety and anxiety diaries have been. Studies of discriminant validity have shown that the BAI has a linear relationship with the Beck Depression Inventory (De Ayala, Vonderharr-Carlson et al. 2005), but factor analysis results indicate that the Beck Depression Inventory items load on different factors than BAI items (Beck, Epstein et al. 1988).

Given the frequent correlation between anxiety and depression, anxiety data is being collected as a possible confounding variable.

g. Young Mania Rating Scale

As the use of antidepressant medications can promote the development of manic and hypomanic episodes in persons with previously undetected/undeclared bipolar disorder (Altshuler, Post et al. 1995, Goldberg and Truman 2003), it is important to screen for symptoms of mania in clinical trials using novel antidepressants. The Young Mania Rating Scale (YMRS) is an 11-item clinician-administered scale that detects symptoms of mania and which gives a rating of the severity of manic episodes. Interrater reliability is high, with a Pearson correlational coefficient of 0.93 for the overall test, with scores for individual items varying from 0.66 to 0.92. The YMRS score also correlates highly with global measures of severity and length of hospital stay (Young, Biggs et al. 1978)

h. Discontinuation-Emergent Signs and Symptoms Checklist

The abrupt discontinuation of serotonergic agents can produce a discontinuation syndrome marked by a variety of constitutional symptoms, such as problems with balance, diarrhea, fatigue, myalgias, and sleep disturbances. Psychological symptoms include anxiety and/or agitation, crying spells, and irritability (Schatzberg, Haddad et al. 1997). The Discontinuation-Emergent Signs and Symptoms checklist (DESS) is a 43-item clinician-administered scale designed to detect symptoms related to SSRI discontinuation, based on retrospective review of patients' symptoms (Rosenbaum, Fava et al. 1998). Although there is limited data regarding the psychometric properties of the DESS, it is widely used in clinical studies where the possibility of symptoms related to SSRI discontinuation may arise, or to compare the relative severity of discontinuation syndromes between different drugs (Judge, Parry et al. 2002, Baldwin, Montgomery et al. 2007).

i. Diet History Questionnaire II

The Diet History Questionnaire II (DHQ-II) is a 153-item self-report survey assessing typical dietary intake among 30 USDA foot groups for the past year, including portion sizes. The DHQ-II allows

quantitative estimation of USDA food-pyramid serving sizes. It is available in year-long and month-long forms in versions that do and do not inquire about portion sizes. We will use the web-based month-long version with portion sizes to enable quantitative estimates of subjects' intake in the month before study participation. The DHQ-II has been shown to have an average correlation across food groups, of 0.6 with serial 24-hour diet recall, with the lowest correlation for eggs, at 0.42, and the highest at 0.84 for milk (Millen, Midthune et al. 2006). A login to the survey will be given to subjects after the screening visit, to be completed at leisure before the baseline visit. As the nature of the information is not time-sensitive and the survey is long, if subjects fail to complete the measure before the baseline visit, they will be allowed to return it at any point later in the study. Survey results will be analyzed using the National Cancer Institute's Diet*Calc software, which generates quantitative estimates of nutrient intake from food frequency and portion size reports.

6. Imaging Methods and Data Analysis

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

MRI scans will be conducted twice: at the baseline visit, and following 8 weeks of treatment with study drug. 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) (Provencher 1993), 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% (Provencher 2001).

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.

c. Resting-state fcMRI

Recent advances in acquisition for BOLD resting-state functional connectivity analysis afford unprecedented opportunities to achieve single-subject diagnostics using extended acquisition protocols (Shah, Cramer et al. 2016). We will use the Siemens Prisma MRI scanner which allows multiband, multiecho BOLD acquisitions that can acquire 3 independent echo time measurements for each TR facilitating Independent Component Analysis removal of motion effects while achieving high temporal-resolution acquisition (Kundu, Inati et al. 2012, Kundu, Brenowitz et al. 2013, Olafsson, Kundu et al. 2015). We have optimized acquisition parameters for the CMRR Multiband/Multiecho BOLD sequence using TR=1517 ms, spatial resolution 2.0 x 2.0 x 2.0 mm, 1770 Volumes per 15 minute acquisition, and will perform 2 acquisitions for 30 minutes total BOLD scan time at each imaging session.

7. Sample storage for genetic testing

There is currently limited information regarding genetic polymorphisms that could interact with environment to increase the risk of depression, although we hypothesize that polymorphisms affecting serotonin synthesis and energy metabolism may contribute. Likewise, there is to our knowledge no information regarding genetic polymorphisms that could contribute to response to 5-HTP or creatine. In principle, participants could be screened for common SNPs associated with major depression, and the association of these SNPs with treatment response and/or changes in neuroimaging markers could be examined. The Illumina PsychArray, for example, is a commercially-available microarray that includes approximately 510,000 SNPs associated with common psychiatric disorders, and could be used to conduct such screening. However, it is unlikely that a small study such as the one proposed would provide sufficient power to correlate differences in treatment response with genetic differences in the absence of clear clinical changes in the treatment group or significant differences in neuroimaging markers. Accordingly, prospective genetic testing of all study participants is likely to be inefficient. Instead, we propose to generate a DNA repository that can be used for subsequent genetic testing if other study results suggest that it may be fruitful.

a. Resources for bio-specimen collection, storage, and handling

Blood from study participants will be collected at the baseline study visit in conjunction with routine laboratory testing as described above. Samples will be transported by study staff to the University of Utah Center for Clinical and Translational Science (CCTS) Translational Technologies and Resources core facility for DNA extraction. This core uses the Qiagen Autopure LS automated DNA extractor, a highly reliable and repeatable DNA extraction method. All samples have been stored in -80 freezers housed in the

University of Utah Psychiatry Department lab. Freezers are equipped with automatic alarm systems. This lab has a computerized database indexed by anonymous sample ID number. The database can store freezer location, amount, concentration, and quality of each sample.

b. Laboratory, DNA extraction, storage, quality control, preparation

DNA for this study will be made at the University of Utah Clinical and Translational Science Center (CCTS) DNA facility using standard, automated procedures as described above. DNA is housed in the Molecular Genetics Laboratory in the Department of Psychiatry, University of Utah School of Medicine. In addition, DNA quality assurance, Whole Genome Amplification (WGA), and DNA preparation for assays will be done in this laboratory. The laboratory consists of a total of 1400 square feet of modern lab space in two adjoining rooms. The labs are fully stocked with all necessary equipment and supplies to carry out DNA purification, Whole Genome Amplification, DNA quality control and DNA storage. The DNA is stored in three locked -80^0 C upright freezers, that are monitored 24/7 by an auto alarm system. The lab is also equipped with a tissue culture room containing a Baker SterilGard Biological hood, two Forma $C0_2$ incubators and all necessary supplies needed to grow cells for DNA extraction. Other equipment in the lab consists of, four centrifuges, three balances, four microscopes, one 4^0 refrigerator, one -20^0 freezer, a chemical hood, and an autoclave. The lab has unlimited access to a NanoDrop ND-1000 Spectrophotometer to quantify and qualify all DNA samples. All equipment is on a yearly preventative maintenance schedule to ensure that is always in good working order and calibrated. The lab has two password-protected computer work stations that are connected to the University of Utah secure servers, so that all sample data, sample inventories and information are securely stored and backed up routinely.

c. Consent for genetic sample storage

As noted previously, subjects will be consented separately for DNA sample storage and possible genetic testing, using a separate informed consent document, and may opt out of this procedure while still participating in the remainder of the study. To protect patient privacy, samples will be identified with an anonymous sample ID number, which is linked to identifying information only through a secure, encrypted, password-protected database. Any genetic results obtained subsequently will be stored only under sample ID numbers and without identifying information.

d. Disclosure of incidental findings

If genetic testing is ultimately conducted, we will not communicate routine study results to subjects.

e. Closure of sample repository and destruction of samples

If the investigators elect not to pursue genetic testing for study participants involving stored DNA, samples will be destroyed according to routine biohazard disposal protocols.

V. Data Analysis

1. Treatment with 5-HTP and creatine will be associated with significant improvements in HAM-D scores, compared to placebo in depressed subjects.

In our preliminary data, HAM-D scores had significant decreases following 8-weeks of open-label 5-HTP and creatine treatment with estimated parameters, $\triangle$scores = 11.1, ρ (correlation coefficient) = 0.4, and SD = 5.8 over 9 time points. Also, based on a prior recommendation for minimal detectable points = 7 for HAM-D in randomized control trials with effect size = 0.875 (Moncrieff and Kirsch 2015), a sample size of 30 depressed subjects (18 active vs. 18 placebo treatments) will have 82% power using a linear mixed model with first-order autoregressive covariance structure.

2. Brain chemical parameters using phosphorus (P-31) spectroscopy will demonstrate that treatment with 5-HTP and creatine will be superior to placebo in restoring frontal lobe PCr and nucleoside triphosphate.
Following 8-weeks of oral creatine, adult female methamphetamine users had significantly increased PCr levels (Hellem, Sung et al. 2015). The standardized effect size was 0.920. Also, an open-label creatine supplementation in depressed adolescents demonstrated significantly increased PCr levels following creatine administration (Kondo, Sung et al. 2011). 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 n = 54 (36 depressed and 18) will have 84% power to detect the repeated measures treatment effects.

3. Resting state functional connectivity measures will show significant improvements in fronto-limbic functional connectivity after 8 weeks of treatment with 5-HTP and creatine in depressed subjects.
This hypothesis will examine a relatively new question using the resting state fMRI to evaluate the improved fronto-limbic functional connectivity between the medial prefrontal cortex and posterior cingulate, amygdala and anterior cingulate cortex and amygdala, following 8-weeks of 5-HTP and creatine supplementation. A recent analysis in our laboratory determined that we can achieve single subject reproducibility of functional connectivity metrics of 0.16 Fisher-transformed correlation units (Shah, Cramer et al. 2016). If we conservatively set the effect size = 8% and SD = 9% of mean, a sample size of 30 depressed subjects (18 treatments and 18 placebo) and 18 healthy controls will achieves 80% power to detect significant treatment effects with the repeated measures design.

VII. Administrative Procedures

A. Study Drug Administration

Study 5-HTP will be acquired in bulk from Fuller Enterprises and will be encapsulated by University of Utah Investigational Drug Services (IDS). 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 in two parts: creatine monohydrate powder to allow a dose of 5 grams per day and tablets containing 5-hydroxytryptophan (5-HTP) 100mg. Participants will be instructed to take both medications together with food. They will be instructed to stir creatine into a non-carbonated drink such as water, juice, coffee, or tea. Participants will be directed to take both medications daily for eight weeks. At each study visit, study medication adherence will be assessed. Participants will be asked to maintain empty bottles of creatine and 5-HTP and return them at weekly visits. Participants will be informed to store both medications at room temperature.

Throughout the treatment phase of the study, participants will be asked to continue their baseline antidepressant at the initial recorded dose. Antidepressants will not be provided by the study but subjects who require renewal of their prescriptions may have a prescription written by the study physician.

B. Drug Storage

Under the supervision of Dr. Renshaw (IND holder), Hana Sabic 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

Clinical participants will be compensated a total of $235 in cash to account for travel expenses and time. Compensation will be given using the schedule below. Per-visit compensation is based on the estimated time for each visit (at least one hour) and is judged not to represent an undue inducement. Study participants may choose to skip being compensated at some visits and then be compensated in lump sums.

1. $25 - Screening
2. $65 - Baseline visit (includes $50 compensation for MRI)
3. $15 - Treatment phase visits (weeks 1, 2, 4, 6)
4. $65 – Treatment week 8 (includes $50 compensation for MRI)
5. $10 – Follow-up visits (weeks 10, 12) (total $20)

Healthy MRI controls will receive compensation on the above schedule for screening, baseline, and Week 8 visits only, for a total of $155.

E. Recruitment

Subjects will be recruited through radio advertisements broadcast on local radio networks. We will also contact outpatient providers in the Department of Psychiatry to apprise them of the study so that they can refer patients (no referral fees will be offered), and these providers will be sent digital copies of the flyer via email.

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.

G. 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.

VI. 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. Participants may also experience discomfort when providing urine for pregnancy tests. 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 herself or others, they will be referred for appropriate care. If the participant is hospitalized for worsening illness, they will be withdrawn from the study. Participants may experience gastrointestinal discomfort as a result of taking creatine or 5-HTP. Participants will be encouraged to take both medications with food to minimize this risk. Finally, as detailed above, supplementation with 5-HTP increases the risks of eosinophilia-myalgia syndrome and serotonin syndrome. Participants will be counseled about these risks, informed of the signs and symptoms of these conditions, 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.

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.

B. Data Safety and Monitoring

The principle investigator and study coordinatory will perform monitoring of case report forms on a continuous basis. The study will also convene a data safety monitoring board.

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.

2. Data Safety Monitoring Board and Plan

The Data Safety and Monitoring Board (DSMB) and monitoring plan were created in compliance with the University of Utah Institutional Review Board's (IRB's) *Investigator Guidance on Data Safety & Monitoring* (Version L1907), the IRB's *Guidance on Ongoing Data & Safety Monitoring*, and the *NIH Policy for Data and Safety Monitoring*.

a. Components of the Monitoring Plan

This plan provides specific mechanisms for monitoring Adverse Events (AEs) and the safety of study participants, as well as the integrity and completeness of the data. The components of the plan are:

- The DSMB membership and qualifications
- A description of the review process
- A description of the reports to be produced by the DSMB
- A description of the information contained in the DSMB reports
- A schedule of how and when the DSMB will meet
- The "Stopping Criteria" for the study
- The definition of "Adverse Events" and "Unanticipated Problems"
- A description of how and when the DSMB will report its findings to the IRB, the FDA and the research team

b. Data Safety Monitoring Board

The study will be conducted in compliance with National Institutes of Health (NIH) requirements for ensuring the safety of study participants and the validity and integrity of data. The Data Safety Monitoring Board (DSMB) for the study will consist of the following individuals:

Dr. Paul Carlson. Dr. Carlson is an expert on the neuroimaging of dementia and the management of mood disorders, and cares for patients at the University Neuropsychiatric Institute. He has experience in the conduct of clinical trials in dementia and mood disorders and is well qualified to serve on the DSMB.

Dr. Deborah Bilder. Dr. Bilder has devoted her career to the research and treatment of autism spectrum disorders, and serves as the Medical Director of the Autism Spectrum Disorder Clinic and the Neurobehavior HOME Program at the University of Utah. Dr. Bilder's long-standing involvement in conducting innovative and sound clinical research makes her an excellent candidate to serve on this board.

The DSMB will serve as an independent body; the DSMB members are not investigators participating in the study.

1.1 Functions of the Data and Safety Monitoring Board

The DSMB will function as an independent body charged with monitoring the safety of study participants, and ensuring that the scientific goals of the study are met. To support these goals, the DSMB will review data and safety monitoring reports.

1.2 Monitoring of Safety Data by the DSMB and the Research Team

The study team will provide the following information to the DSMB for its review on an annual basis: Serious Adverse Events (SAEs), Unanticipated Adverse Events (UAEs), Adverse Events (AEs), Unanticipated Problems (UPs), study retention rates, reasons for early study withdrawal, and abnormal laboratory values that are determined to be related to study participation. The DSMB will have access to any additional data it deems necessary to fulfill its mission. The study team will respond promptly to any DSMB data requests.

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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